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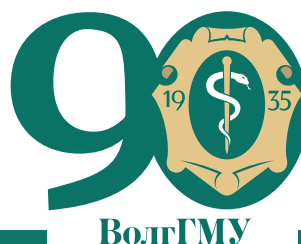
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Development of a method for *in vitro* cultivation of Corti's organ explants using polyethyleneimine

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The aim. To develop a method for *in vitro* cultivation of Corti's organ explants using polyethyleneimine (PEI) as an adhesive substrate. The study evaluated the potential of PEI as an effective alternative to existing substrates for *in vitro* cultivation of inner ear samples.

Materials and methods. Explants of Corti's organ from newborn mice (P1–P2) were used. Glass culture surfaces were treated with a PEI solution followed by treatment with fetal bovine serum. To assess the suitability of the developed explant model for ototoxicity studies, treatment with gentamicin was performed. Isoliquritigenin was used as an otoprotector. Morphological analysis of images obtained by confocal fluorescence microscopy was conducted. The survival of hair cells after staining with phalloidin labeled with Alexa Fluor 488 was evaluated. The magnitude of stereociliary angles of outer hair cells was assessed.

Results. PEI-coating provided reliable fixation of Corti's organ explants (up to 72 h) without signs of detachment from the culture surface. The samples maintained their attachment strength through two media changes and subsequent 16 washing cycles during fluorescent staining. Morphological analysis of the control group revealed no signs of toxic effects of PEI on hair cells. The organotypic model using PEI coating reproduced the classic pattern of gentamicin-induced ototoxicity with a basal-apical gradient of damage. For the first time, this model demonstrated the otoprotective effect of isoliquiritigenin (1 μ M) against gentamicin-induced ototoxicity (500 μ M), most pronounced in the middle turn of the cochlea for both inner and outer hair cells ($p < 0.0001$ for both comparisons with the gentamicin group).

Conclusion. The efficacy of PEI as an alternative substrate for cultivating inner ear samples has been demonstrated for the first time, showing optimal adhesive properties and no inherent toxicity. The developed method represents a promising approach for ototoxicity research and otoprotector screening.

Keywords: ototoxicity; otoprotection; hair cells; Corti's organ explant; polyethyleneimine; isoliquiritigenin

Abbreviations: PEI — polyethyleneimine; HBSS — Hank's balanced salt solution; FBS — fetal bovine serum; ILG — isoliquiritigenin; OHC — outer hair cells; IHC — inner hair cells.

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Разработка методики культивирования эксплантов органа Корти *in vitro* с использованием полиэтиленимина

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Цель. Разработать методику культивирования эксплантов органа Корти *in vitro* с использованием полиэтиленимина (PEI) в качестве адгезивного субстрата. В исследовании оценивалась перспективность применения PEI в качестве эффективной альтернативы существующим субстратам для культивирования образцов внутреннего уха *in vitro*.

Материалы и методы. В работе использовали экспланты органа Корти, полученные от новорождённых мышей (P1–P2). Стеклопластиковые поверхности обрабатывали раствором PEI с последующей обработкой фетальной бычьей сывороткой. Для оценки пригодности использования разработанной модели эксплантов в изучении ототоксичности проводили обработку гентамицином. В качестве отопротектора использовали изоликирителигенин. В исследовании проводился морфологический анализ изображений, полученных с помощью конфокальной флуоресцентной микроскопии. В результате оценена выживаемость волосковых клеток после окрашивания фаллоидином, меченным Alexa Fluor 488. Проведена оценка величины стереоцилиарных углов наружных волосковых клеток.

Результаты. PEI-покрытие обеспечило надежную фиксацию эксплантов органа Корти (до 72 ч) без признаков открепления от культуральной поверхности. Образцы сохраняли прочность прикрепления через два этапа смены культуральной среды и последующие 16 циклов промывки в процессе флуоресцентного окрашивания. Морфологический анализ контрольной группы не выявил признаков токсического воздействия PEI на волосковые клетки. Органотипическая модель с использованием PEI-покрытия позволила воспроизвести классическую картину гентамицин-индуцированной ототоксичности с базально-апикальным градиентом повреждения. Впервые с помощью данной модели продемонстрирован отопротекторный эффект изоликирителигенина (1 мкМ) против гентамицин-индуцированной ототоксичности (500 мкМ), наиболее выраженный в среднем отделе улитки как для внутренних, так и для наружных волосковых клеток ($p < 0,0001$ для обоих сравнений с группой гентамицина).

Заключение. Впервые показана эффективность PEI как альтернативного субстрата для культивирования образцов внутреннего уха, продемонстрировавшего оптимальные адгезивные свойства и с отсутствием собственной токсичности. Разработанная нами методика представляет перспективный подход для исследований ототоксичности и скрининга отопротекторов.

Ключевые слова: ототоксичность; отопротекция; волосковые клетки; эксплант органа Корти; полиэтиленимин; изоликирителигенин

Список сокращений: PEI — полиэтиленимин; HBSS — сбалансированный солевой раствор Хэнкса; FBS — раствор фетальной бычьей сыворотки; ИЛГ — изоликирителигенин; НВК — наружные волосковые клетки; ВВК — внутренние волосковые клетки.

INTRODUCTION

According to the World Health Organization (WHO), hearing impairments affect over 5% of the world's population, totaling approximately 466 million people [1]. The most common form of this pathology is sensorineural hearing loss [2]. Various factors can contribute to its development, such as prolonged exposure to intense noise, age-related changes, infectious and genetic diseases [3]. Additionally, drug-induced ototoxicity remains a frequent cause. Over 600 medications possess ototoxic effects to varying degrees [4]. Among the most ototoxic agents widely used in medicine are aminoglycoside antibiotics, platinum-based chemotherapy drugs, loop diuretics, macrolide antibiotics, and antimalarial agents [5, 6]. Despite the well-proven efficacy of the aforementioned agents in combating infections and malignant neoplasms, the use of these drugs is associated with a high risk of developing auditory and vestibular disorders. Nevertheless, in some cases, the benefits of their use outweigh the potential risks.

The risk of ototoxicity exists for all age groups. Although the main ototoxic drugs are well-known, the prevalence of this adverse reaction remains unexplored. The main diagnostic challenge is the late detection of ototoxicity, which typically occurs when severe hearing loss affecting speech frequencies has already developed. One of the reasons is the lack of vigilance regarding otological symptoms, as they do not pose a direct threat to the patient's life [7]. However, the consequences of such therapy can significantly reduce the patient's quality of life in the long term. In pediatric practice, ototoxicity is a very serious problem, as even moderate hearing loss can significantly impact speech and cognitive skill development, leading to difficulties in learning and social adaptation for affected children [8].

There are no approved otoprotectors to prevent hearing loss caused by aminoglycoside antibiotics. These drugs remain indispensable in the treatment of many dangerous diseases, such as tuberculosis, sepsis, cystic fibrosis, and neonatal infections. The incidence of ototoxicity after aminoglycoside therapy varies depending on dosage, route of administration, sensitivity of audiological tests, and other factors, averaging from 7% to 90%. Among aminoglycosides, the level of ototoxicity differs. For example, kanamycin exhibits a more pronounced cochleotoxic effect compared to streptomycin. Streptomycin has a

predominant vestibulotoxic effect with minimal impact on hearing [9].

Adequate experimental models are necessary to evaluate the efficacy of potential otoprotectors. To date, the use of organ of Corti explants is an indispensable approach in studying the ototoxic and otoprotective effects of substances. When working with explants, it is possible to a certain extent to model the real conditions of their existence. However, despite this, in a culture medium, an inevitable change in the phenotype of cultured tissue cells occurs. In this regard, cellular reactions may not fully correspond to the processes observed *in vivo*. This is the main limitation faced by researchers when using the organ of Corti explant culture model. Thus, the parameters of cell isolation and cultivation protocols can significantly influence experimental results [10].

One of the factors for successful *in vitro* cultivation is the nature of the adhesive substrate coating the surface of the culture vessel. Acting as an analog of the extracellular matrix, such a substrate must be biocompatible and ensure reliable sample fixation [11]. It is necessary for the samples to remain attached to the culture vessel, withstanding repeated washing steps that generate shear forces. The problem of detachment and "floating" during cultivation remains an important methodological challenge when working with inner ear explants [12]. Positively charged polymers such as poly-L/D-lysine, poly-L-ornithine, as well as purified molecules like collagen and laminin, are most commonly used for coating [12, 13]. Commercially available cell adhesives are also widely used [14]. Nevertheless, the search for more effective solutions to improve explant attachment and preserve their integrity during cultivation continues.

This work is based on the development of a method for *in vitro* cultivation of organ of Corti explants using polyethyleneimine (PEI) as an adhesive substrate. PEI has shown suitability and high adhesive activity in other *in vitro* systems [15, 16].

THE AIM of the study was to evaluate the prospect of using PEI as an effective alternative to existing substrates for working with inner ear specimens.

MATERIALS AND METHODS

Animals

Newborn C57BL/6 mice (P1–P2) were obtained and housed in the vivarium of the Institute of Gene Biology, Russian Academy of Sciences with constant access

to food and water. Air temperature was maintained at 22–24°C, and the light cycle (day / night) was 14 / 10 hours. Twelve male mice on days 1–2 after birth (P1–P2) were used in the experiments. Until the start of the experiment, the pups were kept with their mother and naturally nursed.

Animal experiments were conducted in accordance with ethical principles and regulatory documents recommended by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, as well as the Rules of Good Laboratory Practice, approved by Order of the Ministry of Health of the Russian Federation No. 199n dated January 4, 2016. The study protocol was approved by the Local Ethics Committee of the National Medical Research Center for Children's Health, protocol No. 05 dated May 15, 2025.

Surgical Instruments and Laboratory Glassware

Sterilization of microsurgical instruments intended for dissecting bone structures of the skull and opening the cochlear capsule, as well as coverslips (14 mm), was carried out by autoclaving. Aseptic treatment of auxiliary instruments and work surfaces was performed using a 70% aqueous solution of ethanol. Microdissection of the cochlear surface was performed using a microdissection knife (Fine Science Tools, No.10056-12) and Dumont #4 microforceps (Fine Science Tools, No.11241-30), Dumont No. 55 (Fine Science Tools, No.11295-51). For isolation of the entire cochlea and the organ of Corti from the temporal bone, preliminary preparation of sterile Petri dishes (Greiner Bio-One, No. 627160) was performed, into which chilled Hank's Balanced Salt Solution (HBSS) without calcium and magnesium ions (pH = 7.4; Gibco, Thermo Fisher Scientific, No.14185-052) was poured. To increase tissue viability during prolonged dissection, dishes with samples were stored on a cooled surface using a refrigerant throughout the procedure. To ensure reliable fixation of organ of Corti explants, coverslips (Ted Pella, No. 26023) were treated with PEI (Sigma-Aldrich, No. P3143). Coverslips were pre-placed in the wells of confocal dishes. In the first step, 200 µL of a 1 mg/mL PEI solution was applied to the coverslips, after which they were incubated for 1 hour at room temperature to create a layer that promotes explant adhesion. After this time, the coverslips were washed twice with deionized water to completely remove unbound polymer. In the final stage of substrate

preparation, for direct improvement of adhesive properties and enhancement of biocompatibility, each coverslip was coated with 200 µL of a 10% fetal bovine serum (FBS) solution in HBSS containing calcium and magnesium, followed by incubation for 2 hours.

Cochlea Isolation Procedure

For cochlea isolation, decapitation of the animals was performed, followed by placing the head in a sterile Petri dish. To prevent sample contamination, the external surface of the head was treated with a 70% aqueous solution of ethanol. After this, the head was transferred to a dissection dish containing 3 ml of chilled HBSS, where all subsequent manipulations were performed. In the next step, the skin and soft tissues covering the skull vault were removed using a surgical blade or spring scissors. Then, using the same surgical blade, the cranial cavity was carefully opened along the sagittal suture. After gaining access to the cranial cavity, a microsurgical spatula was used to carefully separate the brain from the bony structures of the skull and visualize the cochleae. Using a stereoscopic microscope (Olympus SZX7, Olympus Corporation, Japan, magnification 8× and 16×), the location of the otic capsules was determined. Using Dumont #4 microforceps and a microsurgical knife, synchondroses and sutures of the skull were dissected to isolate the cochleae [17].

Cochlea Surface Dissection

Throughout the cochlea surface dissection stage, Dumont #55 microforceps were used. The cartilaginous wall of the cochlear labyrinth was removed. Next, the organ of Corti with the stria vascularis was separated from the modiolus. The stria vascularis was carefully removed from the organ of Corti, and then the tectorial and Reissner's membranes were carefully removed. The isolated organ of Corti was divided into three equal parts using a microsurgical knife, corresponding to the basal, middle, and apical turns. The samples were transferred using a pipette and a 200 µL tip to a coverslip in a confocal dish with culture medium.

Organ of Corti Explant Culture

Explants were cultured in DMEM/F12 medium (Gibco, Thermo Fisher Scientific, No.11320033) supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, No. A5256701), 1× GlutaMAX (Gibco, Thermo Fisher Scientific,

No.35050061), and 134 μM ampicillin (Invitrogen, Thermo Fisher Scientific, No.11593-027). Samples were placed on coverslips pre-coated with a polyethyleneimine (PEI, 1 mg/mL) aqueous solution (Sigma-Aldrich, No.P3143). The position of the explants was monitored under a stereomicroscope (Olympus SZX7, Olympus Corporation, Japan) to achieve clear visualization of three rows of outer hair cells and one row of inner hair cells (at 56 $\times$ magnification). To improve explant adhesion to the coating, a brief medium removal method was employed: the culture medium was completely removed from the culture dish (Mattek, No.P35G-1.5-14-C). This step facilitated close apposition of the explant to the surface, after which the medium was immediately returned. Dishes with secured explants were incubated in a CO_2 incubator (37°C, 5% CO_2) for 24 h before the initiation of treatment with the test substances (Fig. 1).

Treatment of Explants with Test Substances

To prepare stock solutions, gentamicin (PanEco, No.A011) was dissolved in deionized water, and isoliquiritigenin (ILG) (98% purity, Xi'An YiyangBio-Tech Co., China) was dissolved in DMSO. Stock solutions were sterilized by filtration through a 0.22 μm pore size membrane filter and diluted in culture medium to the desired concentration. For single treatment of explants with the test substances, the culture medium was carefully removed from the culture dish containing the samples on the coverslip. Then, 2 mL of culture medium containing the test substances—gentamicin (500 μM) or gentamicin (500 μM) with ILG (1 μM)—was gently added to the dish, taking care to avoid detachment of the explants from the glass surface. Explants in the control group underwent a similar procedure but without treatment with the test substances. Explants from the control and experimental groups were incubated in a CO_2 incubator (37°C, 5% CO_2) for 24 h. The concentration of gentamicin (500 μM) causing loss of inner (IHC) or outer hair cells (OHC) was determined in previous studies [18]. It was also hypothesized that the concentration of ILG (1 μM) would induce an otoprotective effect, analogous to the neuroprotection we previously determined [19].

Phalloidin Staining of Explants and Hair Cell Visualization

About 24 hours after the treatment of Organ of Corti explants with the test substances, they were

stained with phalloidin (Invitrogen, Thermo Fisher Scientific, No. A12379) to visualize actin filaments in the stereocilia of inner and outer hair cells. Explants were fixed in 10% formaldehyde solution for 30 min, permeabilized with 0.1% Triton X-100 (Sigma-Aldrich, No.X100) in PBS (Sigma-Aldrich, No.P4417-100TAB) for 15 min, and blocked with 1% BSA (Sigma-Aldrich, No. A7906-10G) in PBS for 20 min at room temperature. Phalloidin staining of explants was performed by incubating the explants in the dye solution for 30 min at room temperature. Afterward, the samples were washed with PBS. Confocal microscopy was performed on a Nikon Eclipse Ti2 microscope using a Nikon S Plan Fluor ELWD 20 $\times$ /0.45 objective (Nikon Instruments Inc., Japan). Imaging was conducted using NIS-Elements software.

Counting of Hair Cells and Measurement of Stereociliary Angles

The number of inner and outer hair cells was counted in 100 μm segments of the basilar membrane. For each explant, analysis was performed in three randomly selected areas, after which the mean value per explant was calculated. Eight explants were used for each experimental group. No dead hair cells were observed in the rows of either inner or outer hair cells. To assess the ultrastructure of hair cells, stereociliary angles of outer hair cells were measured using ImageJ 1.54p Software. The angles of the V-shaped stereocilia bundles were measured by drawing two lines along the medial side of the bundles.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare data between groups. If statistically significant differences were found, pairwise comparisons of groups were performed using Tukey's post-hoc test. Data are presented as mean (M) $\pm$ standard deviation (SD). Results were considered statistically significant at $p < 0.05$.

RESULTS

Evaluation of PEI Coating Adhesive Properties

Explants were cultured for 72 h in DMEM/F12 medium containing 10% FBS, Glutamax (1 $\times$), and 134 μM ampicillin. The use of PEI as a coating addressed a key methodological challenge—reliable fixation of explants to the surface of culture vessels.

Due to the formation of a strong bond, the samples remained stably secured during incubation and multiple washes in the stages of medium exchange and fluorescent staining. In our work, phalloidin staining was demonstrated, which required approximately 16 washing cycles for one confocal dish. Explants remained stably secured throughout the entire cultivation period (up to 3 days). During the experiments, no displacement or floating of explants was observed, which not only prevented the loss of biological material but also minimized mechanical damage, ensuring the preservation of hair cells.

Effect of PEI Coating on Hair Cell Viability and Ultrastructural Organization

Hair cell viability and structural integrity were confirmed by the preservation of well-organized rows of both IHCs and OHCs, stained with phalloidin and AlexaFluor 488. A critically important finding was the absence of any signs of toxic effects of PEI on sensory hair cells. The vast majority of analyzed hair cells in the control group showed no signs of degeneration. Morphological analysis revealed unchanged cell ultrastructure. The characteristic V-shape of the cuticular plate remained intact, and the stereocilia retained their normal structure and orientation (Fig. 2A). This corresponds to the morphological norm, indicating the adequacy of the proposed cultivation model. To assess the spatial organization of stereocilia, the angles of their V-shaped bundles were measured in three anatomical turns of the cochlea (apical, middle, basal), each containing three rows of OHCs. Statistical analysis involved two stages. In the first stage, one-way ANOVA was used to establish the absence of significant differences between OHC rows (1, 2, 3) within each turn ($p > 0.05$ for all turns), indicating no difference in the angle values formed by the V-shaped stereocilia bundle within the turns (see Fig. 2A). In the second stage, after pooling data across rows, mean angle values between turns were compared. One-way ANOVA revealed statistically significant differences ($p < 0.0001$). Subsequent post-hoc analysis using Tukey's test confirmed the presence of statistically significant differences between all three turns ($p < 0.001$ for one and $p < 0.0001$ for the other two pairwise comparisons). Thus, we established a clear basal-apical gradient: the angle of the V-shaped stereocilia bundle systematically increased from the apical turn to the basal turn (Fig. 2B).

Gentamicin-induced Ototoxicity and the Otoprotective Effect of Isoliquiritigenin

Exposure to 500 μM gentamicin for 24 hours caused pronounced degenerative changes in organ of Corti explants compared to the control group (Fig. 3A, 3D, 3G). Gentamicin-induced toxicity manifested as massive loss of both IHC and OHC (Fig. 3B, 3E, 3H). In response to gentamicin, IHC loss occurred along a gradient from the basal to the apical cochlear turn, with IHCs of the basal turn being the most sensitive (see Fig. 3H) and IHCs of the apical turn being the least sensitive (see Fig. 3B). Confocal microscopy revealed significant disruption of hair cell morphology, with destruction of stereocilia and disintegration of V-shaped bundles noted. Quantitative analysis, performed by manual counting, confirmed a significant decrease in the number of hair cells upon gentamicin treatment compared to the control group cultured in a gentamicin-free medium. Combined application of 1 μM ILG and 500 μM gentamicin demonstrated a pronounced otoprotective effect. In the combined treatment group, the morphology of the sensory epithelium was substantially preserved compared to the group receiving only gentamicin in all cochlear turns. Preservation of the integrity of hair cell rows was observed (Fig. 3C, 3F, 3I). However, stereociliary bundles in the gentamicin and ILG group did not retain their characteristic V-shaped organization.

Quantitative assessment of hair cell survival showed that ILG increased the number of preserved cells, reducing the level of gentamicin-induced cell death. Figure 4 shows quantitative data for OHCs (Fig. 4A) and IHCs (Fig. 4B) for the apical, middle, and basal regions of the cochlea.

In the apical region, the number of OHCs in the control group was 13.0 ± 0.8 . Gentamicin treatment did not lead to significant OHC loss: in the "Gentamicin" group, their number was 11.1 ± 1.2 , and in the "Gentamicin + ILG" group, it was 13.6 ± 1.1 . At the same time, gentamicin caused a significant loss of IHCs down to 31.1 ± 1.7 ($p < 0.0001$ compared to control), however, in the ILG group, almost complete preservation of IHCs was observed (43.4 ± 2.8 ; $p < 0.0001$ compared to gentamicin).

In the middle region, the number of OHCs in the control group was 12.9 ± 2.0 . Gentamicin application caused rapid OHC death, reducing their number to 2.0 ± 1.3 ($p < 0.0001$). The protective effect of ILG was pronounced: in the "Gentamicin + ILG" group, the

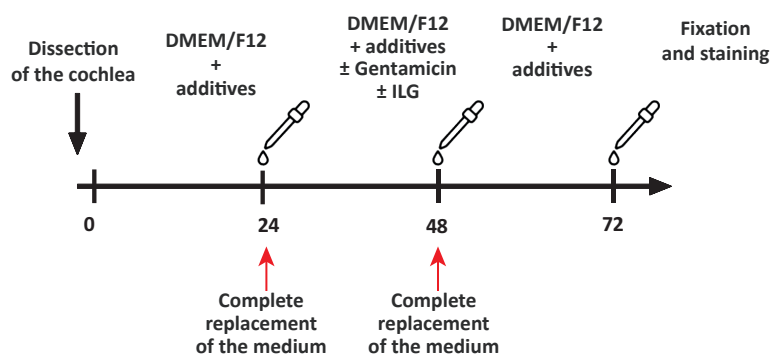


Figure 1 — Experimental timeline, showing the sequence and duration of various interventions in organ of Corti explant culture.

Note: The explant culture medium consists of DMEM/F12 nutrient medium and additives: 10% fetal bovine serum (FBS), GlutaMax, ampicillin. Every 24 hours, the samples were subjected to mechanical stress associated with changing the culture medium. The final fixation and staining step included 16 washing cycles of the samples. The total number of cycles was 18.

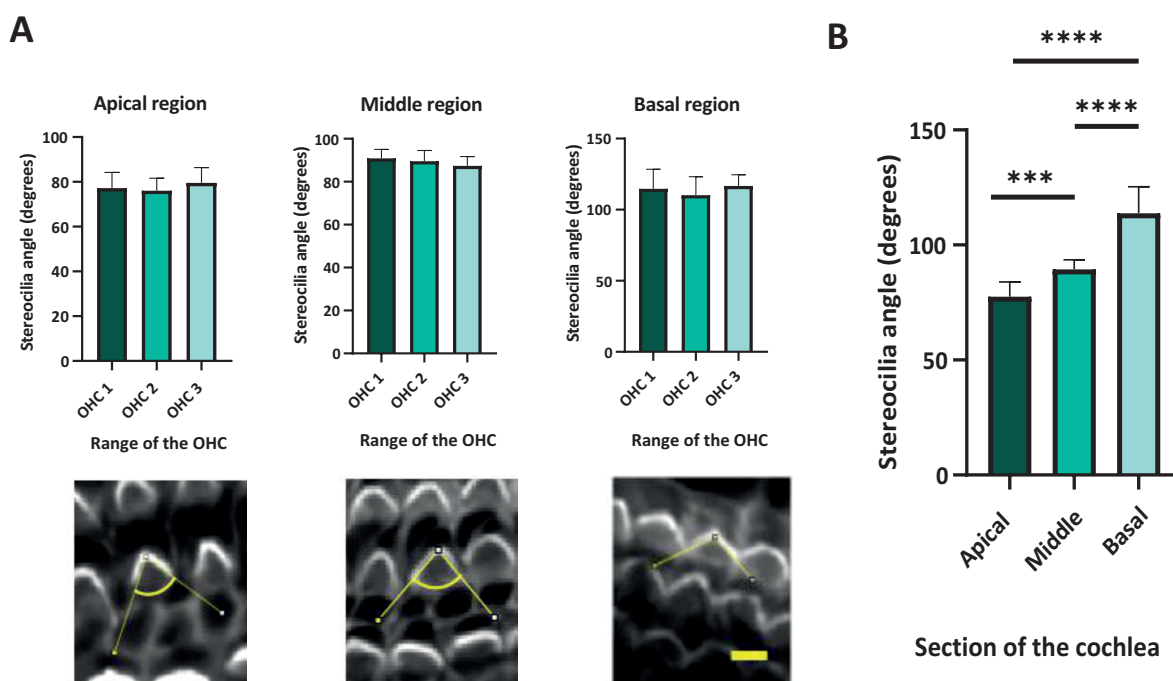


Figure 2 — Quantitative analysis of the stereocilia angle of outer hair cells.

Note: A, angle values within each compartment; B, comparison of mean values between compartments; *** $p < 0.001$; **** $p < 0.0001$; OHC, outer hair cells; OHC 1–3, rows of outer hair cells. Scale bar 5 μm .

number of OHCs was significantly higher, at 10.8 ± 1.0 ($p < 0.0001$ compared to gentamicin). A similar trend was observed for IHCs: control— 39.8 ± 7.1 , gentamicin— 16.5 ± 1.6 ($p < 0.0001$ compared to control), gentamicin+ILG— 30.0 ± 1.3 ($p < 0.0001$ compared to gentamicin).

The most pronounced damage was observed in the basal region. The number of OHCs in the control was 13.2 ± 0.7 . In the “Gentamicin” group, complete OHC loss was observed ($p < 0.0001$ compared to control), whereas with ILG application, cell survival was partially preserved (5.8 ± 1.6) ($p < 0.0001$ compared to the gentamicin group). The number of IHCs in the control

was 42.6 ± 3.1 . Gentamicin led to their complete death ($p < 0.0001$ compared to control), while in the “Gentamicin+ILG” group, 3.5 ± 1.3 IHCs were preserved ($p < 0.01$ compared to gentamicin).

Thus, the obtained data indicate that ILG at a concentration of 1 μM has the ability to protect the hair cells of the organ of Corti from the ototoxic effects of 500 μM gentamicin *in vitro*.

DISCUSSION

Hearing loss caused by ototoxic drugs can be both reversible and irreversible. Irreversible hearing loss develops in cases of hair cell and spiral ganglion neuron

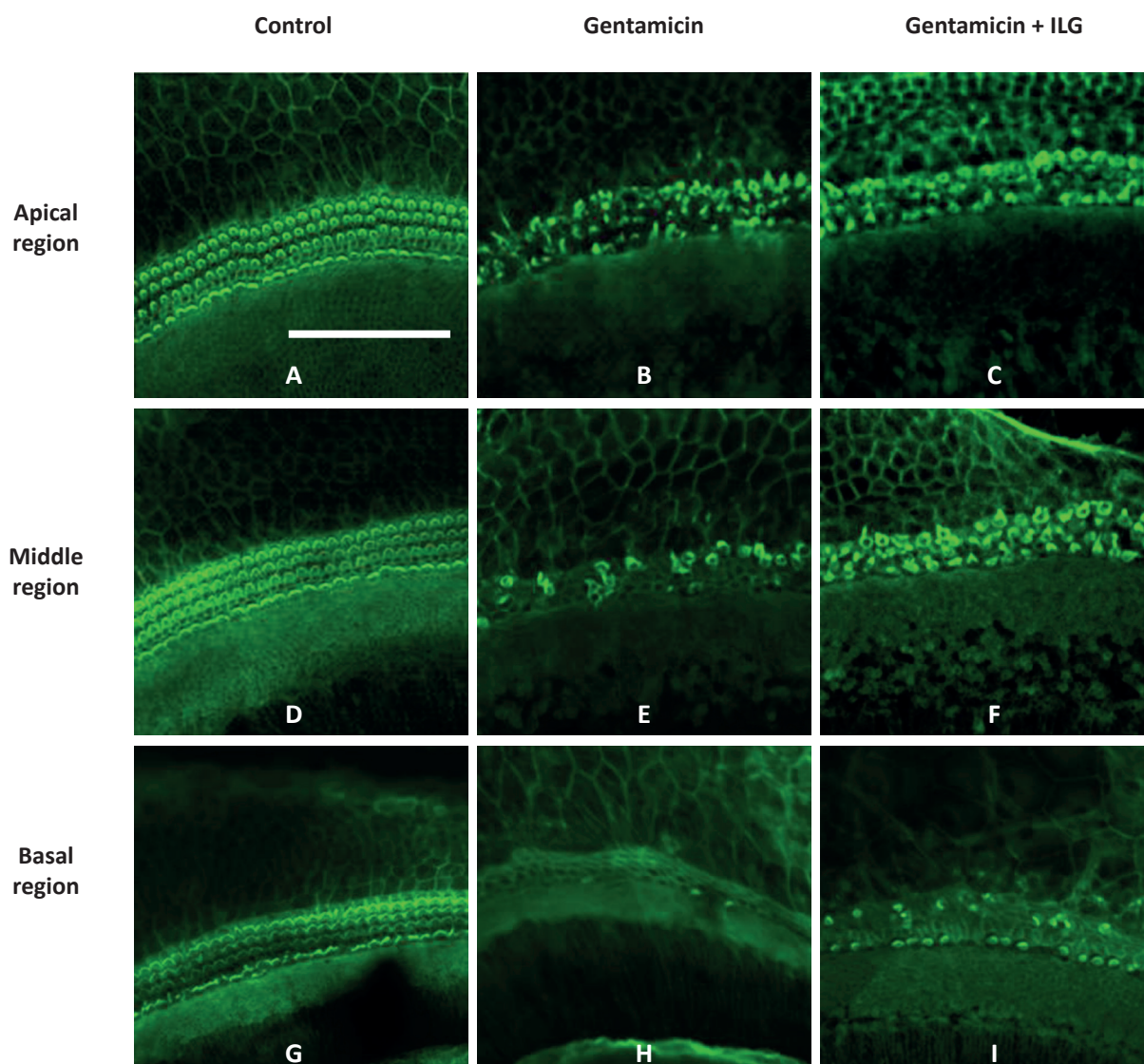


Figure 3 — Microscopic images of organ of Corti explants stained with phalloidin.

Note: Apical (A–C), middle (D–F), and basal (G–I) regions are shown. Gentamicin treatment (500 μ M) causes massive loss of inner and outer hair cells compared to the control. Combined application of gentamicin and isoliquiritigenin provides statistically significant preservation of hair cells. Scale bar 100 μ m.

death, which are incapable of regeneration in adult mammals. This is precisely why hearing impairment caused by platinum drugs and aminoglycoside antibiotics is irreversible [7]. The key pathways of inner ear cell death in the development of ototoxicity are not fully understood. Therefore, the mechanisms of ototoxicity are actively studied, and compounds with otoprotective properties are being sought [20, 21]. To date, the drug sodium thiosulfate remains the only clinically approved otoprotective agent worldwide specifically designed to prevent cisplatin-induced ototoxicity.

Over the past decade, significant progress has been made in the *in vitro* cultivation of mammalian inner ear hair cells, and various cultivation systems

have been published. The culture medium is a complex multicomponent system that mimics *in vivo* conditions for the auditory sensory epithelium. For this purpose, nutrient media with various additives, including growth factors, are used [13]. One of the problems in cultivating inner ear explants is preventing detachment and floating, which affects their integrity, response to experimental stimuli, and subsequent cell analysis [12]. Although natural matrix components (e.g., collagen, laminin, and fibronectin) or polyamino acid substrates (e.g., poly-*D/L*-lysine, poly-*D/L*-ornithine) are traditionally used for the adhesion of organ of Corti explants, the search for new alternatives remains relevant [13, 14]. In the present study, we show that synthetic PEI can also

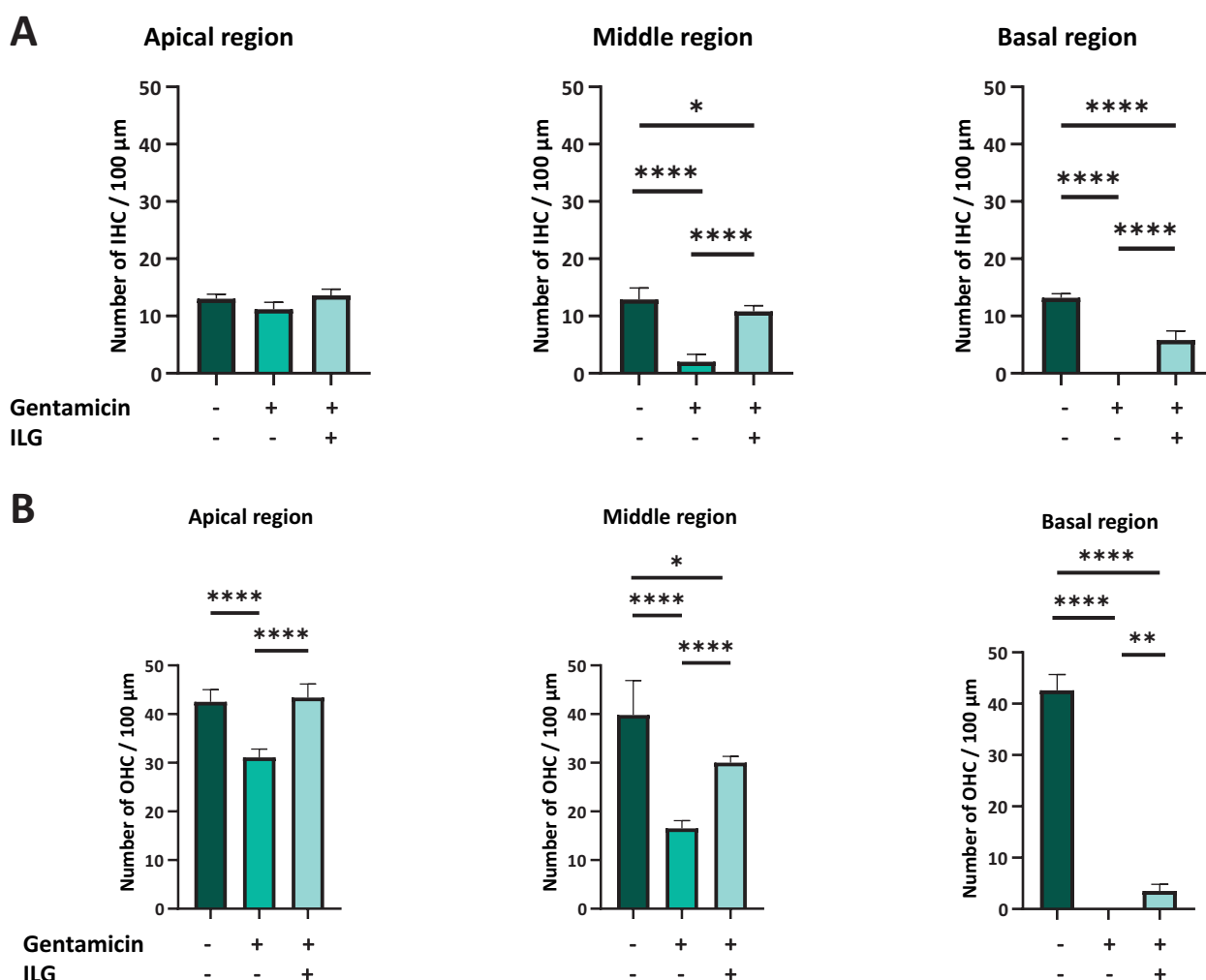


Figure 4 — Quantitative assessment of the protective effect of isoliquiritigenin against gentamicin-induced ototoxicity.

Note: A, inner hair cells, B, outer hair cells. The bar graph shows the number of hair cells in different experimental groups per 100 μ m of basilar membrane. Hair cell density was counted on segments of the basilar membrane 100 μ m in length. Statistical significance was determined using one-way ANOVA followed by Tukey's test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ between corresponding groups.

be successfully used as an adhesive coating for the cultivation of organ of Corti explants.

PEI is a synthetic cationic polymer with a high number of positive charges at physiological pH. Unlike poly-lysine and poly-ornithine, PEI does not contain peptide bonds, making it extremely resistant to proteolysis [22]. One of its distinguishing advantages is its strong adhesive activity, as shown in comparative studies with other attachment substrates [15]. The properties of PEI as a substrate for cell culture adhesion are well-studied, and it is widely used for this purpose. However, there are no reports to date on its use for the cultivation of organ of Corti explants. In our work, PEI successfully ensured the attachment of explants to the culture surface. It is important to show that PEI as a component of the culture system is not an additional damaging factor. In the context of

studying ototoxicity and otoprotection, it is important that the adhesive substrate itself does not affect cell survival and morphology throughout the cultivation period. In this case, damage and death of hair cells can be unequivocally attributed to the action of the investigated ototoxin. Hair cells in the control group maintained their morphological characteristics, assessed by the preservation of stereociliary bundles and the values of the stereociliary angles formed by them. There was no disorganization or fragmentation of stereociliary bundles. The morphological feature of a decrease in the stereociliary angle from the basal to the apical region corresponded to the norm [23]. This indicates that the PEI coating had no effect on the ultrastructural features of morphology or toxicity towards hair cells.

Our study demonstrates that the organotypic

culture optimized using PEI coating is applicable for studying ototoxicity and otoprotection. The behavior of organ of Corti explant cultures obtained according to the optimized protocol under conditions of gentamicin-induced ototoxicity was studied. Gentamicin is a standard approach for modeling aminoglycoside ototoxicity in organ of Corti explants *in vitro* and is used in studies of the otoprotective activity of substances [24]. In our experiment, a characteristic pattern of hair cell damage was observed, manifesting as a pronounced basal-apical gradient. Thus, the optimized organotypic experimental model correctly reproduces the typical characteristics of gentamicin-induced damage, which is a necessary condition for its use in pharmacological research.

Having confirmed that the organ of Corti explant model correctly reproduces gentamicin-induced ototoxicity, we applied it to study the potential otoprotective properties of ILG. Isoliquiritigenin (ILG) is a natural flavonoid that has attracted attention due to its broad biological activity, including anti-inflammatory and antioxidant properties. It exhibits neuroprotective properties, as previously established [19]. In the study by Y. Chen et al., it was shown that ILG has otoprotective properties in a model of cisplatin ototoxicity [25]. In the present study, it is shown for the first time that ILG also has a pronounced protective effect on the hair cells of the organ of Corti under conditions of gentamicin-induced damage. Aminoglycoside antibiotics increase ROS production when they enter hair cells through mechanotransduction channels, ultimately leading to lipid peroxidation and activation of pro-apoptotic hair cell death pathways [9]. Accordingly, a potential mechanism for the otoprotective effect of ILG in this case may be the reduction of oxidative stress in hair cells through the activation of the Nrf-2 signaling pathway [26]. Nrf-2 is responsible for cytoprotection by regulating the expression of numerous antioxidant

system proteins in the cell. Thus, ILG may represent a promising approach for preventing aminoglycoside ototoxicity.

Study Limitations

The present study utilized a relatively short-term organ of Corti explant culture (up to 72 hours). Therefore, further research is needed to evaluate the morphofunctional characteristics of hair cells during longer-term cultivation with a PEI-based adhesive coating. The expression level of ion channels and receptors under these culture conditions remains unclear, which is of fundamental importance for the interpretation of electrophysiological studies.

CONCLUSION

As a result of the conducted research, a method for *in vitro* cultivation of organ of Corti explants using PEI as an adhesive substrate has been developed. It has been shown for the first time that synthetic PEI is an effective substrate for reliable fixation of organ of Corti tissue explants. Its resistance to multiple washes has been demonstrated, which is critically important during cultivation and for preparing samples for fluorescent microscopy studies. Thus, the obtained results indicate that PEI represents a promising and effective alternative to traditionally used adhesive substrates for inner ear sample research. The developed method can be applied to solve current scientific problems, as confirmed by the model of gentamicin-induced ototoxicity and the evaluation of the otoprotective effect of ILG.

The developed model for culturing organ of Corti explants on a PEI coating creates a basis for solving a number of promising tasks. Possessing strong adhesive properties, this substrate can be effectively used in methods for studying ototoxicity, otoprotection, and regeneration of inner ear structures.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Eugene V. Polikarpov — conceptualization, methodology, investigation, validation, writing—original draft; Elena A. Smolyarchuk — formal analysis, administration; Ekaterina M. Grigorevskikh — validation; Elena Yu. Yaroshenko — investigation; Svetlana I. Pavlova — formal analysis; Dmitry A. Kudlay — writing—review & editing; Kirill V. Savostyanov —administration, fundraising; Zanda V. Bakaeva — conceptualization, methodology, investigation, formal analysis, writing—review & editing, scientific guidance. All the authors confirm their authorship compliance with the ICMJE international criteria (all the authors made a significant contribution to the conceptualization, conduct of the study and preparation of the article, read and approved the final version before publication).

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Updating approaches to the standardization of *Angelica archangelica* L. rhizomes and roots

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The aim. To update approaches to the standardization of *Angelica archangelica* rhizomes and roots, taking into account modern requirements, during a comprehensive study.

Materials and Methods. Morphological and anatomical features of *Angelica archangelica* rhizomes and roots were studied using light microscopy. Luminescence of tissues and a working standard sample of the coumarins eleutheroside B1 (7-O-glucoside of isofraxidine) were studied by luminescent microscopy, and vascular structures were studied by polarization microscopy. The profile of volatile compounds of lipophilic fractions of water-alcohol extract and essential oil was determined by gas chromatography with mass spectrometric detection (GC-MS). The total coumarins content was determined by UV spectrophotometry.

Results. Rhizomes and roots of *Angelica archangelica* of Altai origin were studied for the first time. The most significant results were obtained at the excitation wavelengths in the range of 330 to 400 nm — light blue luminescence of phloem cells and secretion of schizogenous cavities was observed, due to substances of a coumarin nature. The expediency of using a histochemical reaction with Sudan III solution, which reveals schizogenous cavities, secretion droplets, and cell walls of vascular elements, has been substantiated. For the diagnosis of specific conducting elements in *Angelica* raw material (powder), the use of polarization microscopy is recommended. Using GC-MS for lipophilic fractions of water-alcohol extract and essential oil, a chromatographic profile was established, including substances of both terpene and coumarin nature: prenylated coumarin osthol (up to 49.72 %) and epilobulol (25.61 %). The total coumarin content in the studied raw material was 1.50 ± 0.08 % calculated as coumarin.

Conclusion. A comprehensive approach to the analysis of *Angelica archangelica* rhizomes and roots, involving the use of both widely used (macroscopic, microscopic, spectrophotometric) and limited-use (luminescent, polarization microscopy, and GC-MS) methods, forms the basis for the development of a draft new pharmacopoeial article “*Angelica archangelica* Rhizomes and Roots.”

Keywords: *Angelica archangelica* L.; *Angelica archangelica* rhizomes and roots; light microscopy; luminescent microscopy; polarization microscopy; gas chromatography-mass spectrometry; water-alcohol extract; lipophilic fraction; essential oil; coumarins; terpenes; osthol; epilobulol; standardization

Abbreviations: BASs — biologically active substances; GC-MS — gas chromatography-mass spectrometry; PhM — pharmacopoeial monograph; MPRM — medicinal plant raw material; SPh RF XV ed. — State Pharmacopoeia of the Russian Federation XV edition; GPhM — general pharmacopoeial monograph; WSS — working standard sample; SS — standard sample; UV-light — ultraviolet light.

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Актуализация подходов к стандартизации дудника лекарственного (*Angelica archangelica* L.) корневищ и корней

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Цель. В ходе комплексного исследования актуализировать подходы к стандартизации дудника лекарственного корневищ и корней с учётом современных требований.

Материалы и методы. Морфолого-анатомические признаки дудника лекарственного корневищ и корней исследовали с помощью световой микроскопии. Люминесценцию тканей и рабочего стандартного образца кумариновой природы элеутерозида В1 (7-О-глюкозид изофраксидина) изучали люминесцентной микроскопией, сосудистые структуры исследовали поляризационной микроскопией. Профиль летучих соединений липофильных фракций водно-спиртового экстракта и эфирного масла определяли методом газовой хроматографии с масс-спектрометрической детекцией (ГХ-МС). Содержание суммы кумаринов определяли методом УФ-спектрофотометрии.

Результаты. Впервые изучены корневища и корни дудника лекарственного алтайского происхождения. Наиболее значимые результаты получены в диапазоне длин волн возбуждения 330–400 нм — наблюдали светло-голубую люминесценцию клеток флоэмы и секрета схизогенных вместилищ, обусловленную веществами кумариновой природы. Обоснована целесообразность применения гистохимической реакции с раствором судана III, выявляющей схизогенные вместилища, капли секрета и клеточные стенки сосудистых элементов. Для диагностики специфических проводящих элементов в сырье дудника (порошок) рекомендовано применение поляризационной микроскопии. Методом ГХ-МС для липофильных фракций водно-спиртового экстракта и эфирного масла установлен хроматографический профиль, включающий вещества терпеновой и кумариновой природы: пренилированный кумарин остол (до 49,72%) и эпиглобулол (25,61%). Содержание суммы кумаринов в исследуемом сырье составило 1,50±0,08% в пересчёте на кумарин.

Заключение. Комплексный подход к анализу дудника лекарственного корневищ и корней, заключающийся в использовании как широко распространённых (макроскопический, микроскопический, спектрофотометрический), так и ограниченных в применении (люминесцентная, поляризационная микроскопии и ГХ-МС) методов, служит основой для разработки проекта новой фармакопейной статьи «Дудника лекарственного корневища и корни».

Ключевые слова: дудник лекарственный; дудника лекарственного корневища и корни; световая микроскопия; люминесцентная микроскопия; поляризационная микроскопия; газовая хроматография с масс-спектрометрической детекцией; водно-спиртовой экстракт; липофильные фракции; эфирное масло; кумарины; терпены; остол; эпиглобулол; стандартизация

Список сокращений: БАВ — биологически активные вещества; ГХ-МС — газовая хроматография с масс-спектрометрией; ЛП — лекарственный препарат; ФС — фармакопейная статья; ЛРС — лекарственное растительное сырье; ГФ РФ — Государственная фармакопея Российской Федерации XV издания; ОФС — общая фармакопейная статья; РСО — рабочий стандартный образец; СО — стандартный образец; УФ-свет — ультрафиолетовый свет.

INTRODUCTION

Garden Angelica (*Angelica archangelica* L.; *A. archangelica*), widely known in folk medicine as “dyagil”, is a perennial herbaceous plant of the genus *Angelica* L. of the *Apiaceae* Lindl. family. In the Russian Federation, it is found in the European part, the Urals, Western Siberia, and the North Caucasus. It grows in moist places: along rivers, streams, banks of water bodies, at the edges of sedge bogs, in sparse forests, and in the forest-tundra. It is widespread in Europe. The species is listed in the Red Books of the Republic of Karelia, Tatarstan, and the Rostov and Saratov regions¹.

Issues concerning the morphological and anatomical characteristics of *A. archangelica* have been investigated by various groups of scientists: diagnostic features of leaves [1], fruits [2, 3], and herbs [4] have been identified. Within the scope of studying underground organs, the works of E.R. Grigoryan et al. [5] and F.A. Shakirova et al. [6] describe the external and microscopic features of whole, chopped, and powdered rhizomes and roots of *A. archangelica*. However, it should be noted that these sources lack descriptions of the results of luminescent and polarization analyses of histological features, which prevents the formation of a complete diagnostic picture of the raw material. Despite the fact that the work of F.A. Shakirova et al. [6] studied the luminescence of the tissues of *A. archangelica* rhizomes and roots. However, the formulation used, “pronounced fluorescence under ultraviolet light,” is subjective: the research materials do not specify the irradiation specifics (wavelength), the color of the fluorescence, or the tissue types exhibiting this luminescence. Furthermore, the researchers did not use reference samples to interpret the chemical nature of the luminescent agent.

The component composition of the essential oil and coumarins of the underground part of *A. archangelica* is of particular value for medical practice, as these biologically active compounds (BACs) exhibit a wide spectrum of pharmacological activity: antibacterial [7–9], antifungal [10], and anti-inflammatory [11, 12]. Using gas chromatography-mass spectrometry (GC-MS), species-specific components of the essential oil were identified: β -phellandrene, α -pinene, δ -3-carene, limonene [9, 13], and osthol [14–16]; coumarins identified include: osthol [7], angelicin, bergapten, imperatorin, oroselone, and methoxsalen [17–19, 21]. A liquid extract of garden angelica rhizomes and roots is part of the medicinal product (MP) “Iberogast” (Bayer Consumer Care AG, Switzerland), which is used to treat gastrointestinal diseases [20].

¹ Pimenov MG, Ostroumova TA. Umbelliferae of Russia; Moscow: Association of Scientific Publications of the KMC; 2012. 477 p. Russia

Garden angelica rhizomes and roots (*Angelicae archangelicae rhizomata et radices*) is an official medicinal plant raw material (MPRM) included in the pharmacopoeias of the USA, India, and the European Union. Specifically, the American Herbal Pharmacopoeia: Botanical Pharmacognosy—Microscopic Characterization of Botanical Medicines² provides descriptions of microscopic features for two species of angelica—*Angelica archangelica* L. and *Angelica sinensis* (oliv.) Diels. Monograph 01/2013:1857 “*Angelica archangelica* root” of the European Pharmacopoeia, 11th edition³, and “CANDA (Root)” of the Indian Pharmacopoeia, Volume 5⁴, present requirements that standardize the quality indicators of the MPRM of the specified plant. In the Russian Federation, GOST 21569-76 “Rhizomes and roots of garden angelica”⁵ is also in effect, standardizing the quality of raw materials intended for use in the food industry, including those supplied for export. Currently, the State Pharmacopoeia of the Russian Federation, XV edition (SPh RF XV ed.), lacks a pharmacopoeial monograph (PhM) for *A. archangelica* raw material.

The problem of falsification of powdered MPRM requires a comprehensive approach to control, where microscopic analysis remains a key tool. The most effective solution here is a combination of luminescent microscopy, GC-MS profiling, and spectrophotometry. The complementarity of morphological-anatomical and chemical indicators will ensure high identification accuracy and create significant obstacles to the potential falsification of the MPRM discussed in the article.

The data obtained during the study will form the basis for developing a draft of a new PhM “*Angelicae archangelicae* rhizomes and roots”.

THE AIM is to update approaches to the standardization of garden angelica rhizomes and roots through comprehensive research and in accordance with modern requirements.

MATERIALS AND METHODS

Study Object

Samples of *A. archangelica* rhizomes and roots, collected in 2024 in the foothills of the Krasnogorsky

² American Herbal Pharmacopoeia: Botanical Pharmacognosy — Microscopic Characterization of Botanical Medicines. *Angelica archangelica* L. Angelica Root. Radix *Angelicae archangelicae*. CRC Press; 2016. P. 234–236.

³ 01/2013:1857 «*Angelica archangelica* root» European pharmacopoeia 11.0.

⁴ CANDA (Root). The Ayurvedic pharmacopoeia of India Part – I. Volume V. P. 21–23. Available from: <https://www.ayurveda.hu/api/API-Vol-5.pdf>

⁵ GOST 21569-76 Rhizomes and roots of *Angelica officinalis*. Available from: <https://docs.cntd.ru/document/1200022507>. Russian

district of the Altai Territory (garden angelica rhizomes and roots of Altai origin), were used as the study object. Primary processing included cleaning the raw material from soil, washing with cold water, and drying at a temperature of approximately 45°C (artificial drying). The finished samples were purchased from the company IVA (Moscow, Russia) in 2025.

Macroscopic, Microscopic, Microchemical and Histochemical Studies

For the description and evaluation of the external characteristics of the raw material, the provisions presented in the general pharmacopoeial article (GPhM) GPhM.1.5.1.0006 "Roots, rhizomes, bulbs, tubers, cormels" were followed⁶. A Motic DM-39 stereomicroscope (Motic Xiamen, China) with magnifications of $\times 20$ and $\times 40$ and a built-in digital camera was used in the study.

When preparing samples, the requirements of GPhM.1.5.3.0003 "Microscopic and microchemical analysis of medicinal plant raw material and medicinal products of plant origin"⁷ and GPhM.1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations"⁸ were adhered to. Transverse sections of rhizomes and roots were prepared manually with a razor blade, as well as on a HM 325 Thermo FS microtome (Thermo Fisher Scientific, USA).

Identification of microscopic features, as well as micro- and histochemical studies, were performed according to GPhM.1.5.1.0006 "Roots, rhizomes, bulbs, tubers, cormels", GPhM.1.5.3.0003 "Microscopic and microchemical analysis of medicinal plant raw material and medicinal products of plant origin", and GPhM.1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations" using a Motik DM 1812 light microscope (Motic Xiamen, China)

with magnifications of $\times 40$, $\times 100$, $\times 400$, and a built-in digital camera. Micro-preparations were stained with a Sudan III solution to reveal lipophilic cytological features. Reagent preparation was carried out according to the methods described in GPhM.1.3.0001 "Reagents. Indicators"⁹.

Diagnosis of the conductive elements of the raw material was performed on a PLM-213 polarization microscope (LOMO, Russia) equipped with a ToupCam SCMO503100KPA video eyepiece (ToupTek Photonics, China); tissue luminescence was studied on an Altami LUM-2 luminescence microscope (Altami, Russia) with magnifications of $\times 100$ and $\times 400$, equipped with a Canon EOS 1200D camera (Canon Inc., Japan) and a high-voltage mercury lamp (HBO 100W) as a light source. Luminescence excitation was carried out through a filter system covering the following spectral range: blue excitation filter—420–550 nm, and ultraviolet filter—330–400 nm. As a reference sample, a working standard sample of eleutheroside B1 (7-O-glucoside of isofraxidin), isolated and provided by the scientific and educational center "Pharmacy" of Samara State Medical University, was used. Its luminescence was studied by irradiating air-dried crystals directly on a microscope slide.

For detailed microscopy of powder elements, alkaline sample preparation was used according to GPhM.1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations", method 2.

Extraction of Extract for GC-MS Analysis

Approximately 100 g of raw material, ground to a particle size passing through a sieve with openings of 2 mm in diameter, was placed in a 500 mL conical flask and extracted with a 60% ethanol in a volume sufficient to obtain a "mirror" using fractional maceration. The first infusion was carried out for 24 hours, and the subsequent 3 were for 12 hours with periodic shaking. The extracts were combined, allowed to settle, and then filtered through "red ribbon" filter paper (aqueous-alcoholic fraction). When choosing the extractant, its extraction capacity for the comprehensive extraction of a wide spectrum of BACs contained in the underground part of *A. archangelica* was considered: its ability to extract both low-polarity substances (of terpenoid nature) and the phenolic complex (coumarin derivatives).

Subsequently, liquid-liquid extraction with n-hexane and chloroform was performed, and

⁶ GPhM.1.5.1.0006 «Roots, rhizomes, bulbs, tubers, cormels». State Pharmacopoeia of the Russian Federation, XV ed. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-2/korni-kornevishcha-lukovitsy-klubniklubnelukovitsy/?sphrase_id=1215890. Russian

⁷ GPhM.1.5.3.0003 «Microscopic and microchemical analysis of medicinal plant raw material and medicinal products of plant origin». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-1/mikroskopicheskiy-i-mikrokhimicheskiy-analiz-lekarstvennogo-rastitelnogo-syrya-i-lekarstvennykh-sred/>. Russian

⁸ GPhM.1.5.3.0003.15 «Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-13/1/1-5/1-5-3/1-5-3-3/tekhnika-mikroskopicheskogo-i-mikrokhimicheskogo-issledovaniya-lekarstvennogo-rastitelnogo-syrya-i-l/>. Russian

⁹ GPhM.1.3.0001 «Reagents. Indicators». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-3/reaktivy-indikatory>

the corresponding fractions of substance sums were obtained, which were then subjected to chromatography. When selecting organic solvents, literature data confirming the optimal extraction capacity of these solvents for extracting volatile organic compounds [22–24] were used.

For this purpose, 10.0 mL of the aqueous-alcoholic fraction was placed in a 50 mL conical separatory funnel, and 5 mL of “extra pure” grade n-hexane (EKOS-1, Russia) was added and shaken for 2 min, then allowed to stand until complete phase separation. The hexane fraction was filtered through “red ribbon” filter paper into a 25 mL volumetric flask. Extraction with n-hexane was repeated 2 more times with 5 mL portions, each time filtering into the same volumetric flask. The volume of the solution in the volumetric flask was brought to the mark with n-hexane and mixed (hexane fraction). The chloroform fraction was obtained similarly.

Obtaining Essential Oil

Essential oil was obtained by hydrodistillation in accordance with GPhM.1.5.3.0010.15 “Determination of essential oil content in medicinal plant raw material and medicinal plant preparations”¹⁰ (method 2; 50 g of raw material, ground to a particle size passing through a sieve with openings of 2 mm in diameter, distillation time—3 hours).

GC-MS Analysis

The analysis was performed under conditions described previously [26, 27]. The qualitative composition of the extract and essential oil was determined in accordance with GPhM.1.2.1.2.0004 “Gas Chromatography”¹¹ on a “MAESTRO 7820” gas chromatograph (INTERLAB, Russia) with an Agilent 5975 mass spectrometric detector (Agilent Technologies Inc., USA).

Chromatographic conditions: HP-5MS capillary quartz column, 30 m×0.25 mm×0.25 μm (stationary phase: 5% diphenyl, 95% dimethylsiloxane), mobile phase—helium, flow rate—1 mL/min, column thermostat temperature—isothermal at 40°C for 5 min, heating to 80°C at a rate of 3°C/min, heating to 180°C at a rate of 4°C/min, heating to 280°C at a rate of 8°C/min, isothermal at 280°C for 15 min, evaporator

temperature—270°C, ion source temperature—150°C, quadrupole temperature—230°C, transfer line temperature—280°C, injection without splitting. Sample introduction method—autosampler, sample volume—1 μL.

Identification of volatile components was carried out by comparing linear retention indices and full mass spectra with data from the NIST 2.4 mass spectral library and literature data. During identification, only components with a mass spectral match coefficient above 90% were considered, which corresponds to standard scientific practice. The proportion of each component to the sum of all components was calculated by the internal normalization method based on the areas of the corresponding peaks in the chromatogram constructed by total ion current.

Quantitative Determination of Total Coumarins

For the quantitative determination of total coumarins, a spectrophotometric method was used in accordance with the requirements of GPhM.1.2.1.1.0003 “Spectrophotometry in the ultraviolet and visible regions”¹². Within the scope of standardizing MPRM containing a complex of phenolic compounds, spectrophotometric analysis remains relevant as a normalization method [27, 28] due to its justification in the context of high precision, speed, and satisfactory accuracy for establishing standards for the content of total BAC fractions [29].

The analysis was performed on an SF-56 Spectrophotometer (LOMO, Russia), included in the State Register of Measuring Instruments of the Russian Federation (No. 74729-19), using quartz cuvettes with a path length of 10 mm. Coumarin (Sigma-Aldrich, Germany, cat. No. C4261, purity ≥ 98%) was used as a standard sample (SS). Solutions were prepared using “extra pure” grade solvents (EKOS-1, Russia). Raw material moisture content was determined in accordance with the requirements of GPhM.1.5.3.0007.15 “Determination of moisture content in medicinal plant raw material and medicinal plant preparations”¹³ in a 2B-151 drying oven (Medlabtekhnik, USSR).

¹⁰ GPhM.1.5.3.0010.15 «Determination of essential oil content in medicinal plant raw material and medicinal plant preparations». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-14/1/1-5/1-5-3/opredelenie-soderzhaniya-efirnogo-masla-v-lekarstvennom-rastitelnom-syre-i-lekarstvennykh-rastitelnykh>. Russian

¹¹ GPhM.1.2.1.2.0004 «Gas Chromatography». State Pharmacopoeia of the Russian Federation, XV ed. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-2-khromatograficheskie-metody-analiza-gazovaya-khromatografiya/?sphrase_id=1215888. Russian

¹² GPhM.1.2.1.1.0003 «Spectrophotometry in the ultraviolet and visible regions». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-1-metody-spektralnogo-analiza-spektrofotometriya-v-ultrafioletovoy-i-vidimoy-oblastyakh/>. Russian

¹³ GPhM.1.5.3.0007 «Determination of moisture content in medicinal plant raw material and medicinal plant preparations». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-1/opredelenie-vlazhnosti-lekarstvennogo-rastitelnogo-syrya-i-lekarstvennykh-sredstv-rastitelnogo-prois/>. Russian

Standard Solution A of Coumarin SS.

Approximately 0.005 g (exact weight) of coumarin SS was placed in a 50 mL volumetric flask, dissolved in 96% ethanol, the volume of the solution was brought to the mark, and mixed.

Solution B of Coumarin SS. 2.0 mL of standard solution A was placed in a 25 mL volumetric flask, 2.0 mL of 1M sodium hydroxide solution was added, and the volume was brought to the mark with 70% ethanol and mixed.

Test Solution A. Approximately 1.0 g (exact weight) of raw material, ground to particles no larger than 1 mm, was placed in a 250 mL flask with a ground-glass stopper, and 80 mL of 96% alcohol was added. The flask was attached to a reflux condenser and heated in a boiling water bath for 30 min. After cooling to room temperature, the contents of the flask were quantitatively transferred through a "red ribbon" paper filter into a 100 mL volumetric flask. The residue in the flask was washed with the same alcohol, passing the washings through the same filter, and the volume of liquid in the flask was brought up to the mark with 96% alcohol. The contents of the flask were thoroughly mixed. Then, 25.0 mL of the resulting solution was evaporated to a volume of 2–3 mL, quantitatively transferred to a separatory funnel using 50 mL of purified water. The solution was acidified with 1M hydrochloric acid solution to pH 2–3 and extracted with ethyl acetate three times: first with 50 mL, then twice with 30 mL. The combined ethyl acetate extracts were filtered through 2 g of anhydrous sodium sulfate, evaporated to dryness on a rotary evaporator at 70°C, and the dry residue was dissolved in 70% alcohol and filtered into a 25 mL volumetric flask through a "red ribbon" filter.

Test Solution B. 1.0 mL of Test Solution A was placed in a 25 mL volumetric flask, 2.0 mL of 1M sodium hydroxide solution was added, the volume of the solution was brought to the mark with 70% alcohol, and mixed.

Reference Solution. 2.0 mL of 1 M sodium hydroxide solution was placed in a 25 mL volumetric flask, the volume of the solution was brought to the mark with 70% alcohol, and mixed.

After 10 min, the optical density of Test Solution B and the coumarin reference standard solution B was measured relative to the reference solution on a spectrophotometer at a wavelength of 335 nm in a 10 mm cuvette.

The content of coumarins, calculated as coumarin, in percent (X) was determined by the formula:

$$X = \frac{A \times a_0 \times 2 \times 100 \times 25 \times 25 \times P \times 100 \times 100}{A_0 \times 50 \times 25 \times a \times 25 \times 1 \times 100 \times (100 - W)} = \frac{A \times a_0 \times P \times 400}{A_0 \times a \times (100 - W)},$$

where A is the optical density of Test Solution B; A_0 is the optical density of coumarin reference standard solution B; a is the weight of the raw material, g; a_0 is the weight of the coumarin reference standard, g; W is the moisture content of the raw material, %.

The determination was performed in triplicate analytical replicates ($n = 3$). Results are expressed as the arithmetic mean $\pm$ standard deviation (SD).

The proposed method was validated in accordance with the requirements of GPhM.1.1.0012 "Validation of analytical methods"¹⁴ for the following characteristics: specificity, linearity, analytical range, accuracy, precision (repeatability and intermediate precision).

RESULTS

Macroscopic, Microscopic, Microchemical and Histochemical Analyses

Medicinal raw material "*Angelica archangelica* rhizomes and roots" is a collection of fragments of rhizomes in transverse and longitudinal sections with a rough, transversely wrinkled cork on the surface (Fig. 1F, 1G). The fragments vary in diameter from 2.5 cm to 5 cm and in height up to 2 cm. In cross-section, a brown cortical part with cavities in the parenchyma is visible, likely of rexygenic origin (Fig. 1D). The central part of the rhizome fragments (pith parenchyma) is light gray or light yellow. Upon detailed examination, the pith parenchyma has a radiate texture extending from the center (Fig. 1A, 1C). On some fragments, remnants of young cork and elements of the stem part of the plant are visible (Fig. 1I, 1H).

The raw material also contains large whole fragments of roots up to 15 cm in length and 1.5 cm in width. The surface of the roots is dark brown and has a wavy-wrinkled texture with pronounced attachment points of lateral roots (Fig. 1B).

Roots of secondary structure, described previously by other authors [5], have a pronounced wavy margin in cross-section due to rexygenic cavities—radial ruptures of the cortical parenchyma (Fig. 2A). The surface of the roots is covered with a dark brown (sometimes black) cork layer. In the cortical parenchyma, at low magnification, schizogenous cavities containing drops of yellow or light yellow secretion are visible. The cavities stain pink with Sudan III solution (Fig. 2B) and are better detected in cross-sections, including in powder fragments (Fig. 2C, 6C, 6E).

The wavy cambium zone also stains with Sudan III

¹⁴ GPhM.1.1.0012 «Validation of analytical methods». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/validatsiya-analiticheskikh-metodov/>. Russian

solution. At high magnification ($\times 400$), narrow-lumen cambial zone cells with brown protoplast contents are visible (Fig. 2D). The xylem part of the central cylinder is devoid of schizogenous cavities. It generally prevails over the secondary cortex. The xylem is represented by radially arranged groups of vessels, between which are located wide medullary rays, composed of uniform oval xylem parenchyma cells up to 10 cells thick (Fig. 2C). In the phloem parenchyma and medullary ray parenchyma, starch is present in the form of small simple grains, almost spherical in shape, up to $6\ \mu\text{m}$ in size.

Vessels in cross-section are of varying sizes with noticeably thickened cell walls, which stain pink with Sudan III solution (Fig. 2E). In longitudinal sections, three types of vascular elements are diagnosed: mainly scalariform, reticulate, and, less commonly, spiral vessels. It should be noted that the best contrast of vascular elements, including in powder, is achieved with polarized microscopy with a λ -light filter (Fig. 2F).

The species specificity of the medicinal raw material is closely related to the chemical composition of the producing plant. It is known that *A. archangelica* rhizomes and roots contain phenolic compounds of the coumarin group, which allows the luminescence characteristics to be interpreted as a pronounced diagnostic feature, additionally used to confirm the authenticity of the raw material.

During the examination of transverse sections of *A. archangelica* rhizomes and roots, the luminescence characteristics of their tissues were analyzed. Thus, the phloem parenchyma of the cortex, in the excitation range of 330–400 nm, exhibits a light blue luminescence, mainly due to the protoplasts of the cells (Fig. 3A). The oily secretion drops in the schizogenous cavities have a characteristic blue luminescence in this spectral region. It should be noted that the epithelial cells lining the cavities do not luminesce and are visually identified as a dark border against the luminescent elements of the parenchyma and the secretion filling the cavity lumens (Fig. 3A). In the excitation range of 420–550 nm, the phloem parenchyma and resinous secretion luminesce bright yellow (Fig. 3B).

The xylem part of the cross-section also has a characteristic bright blue luminescence in the excitation range of 330–400 nm due to the lignified cell walls of vessels and medullary ray cells. In the excitation range of 420–550 nm, the luminescence is weaker and yellow (Fig. 3C, 3D, 3E). The cork tissue in the visible spectrum is brown due to the polyphenol suberin.

The cork tissue cells are irregularly, almost

rectangularly shaped with remnants of protoplasts at the periphery (Fig. 3F). The light blue luminescence in the excitation range of 330–400 nm (Fig. 3G) and the dirty yellow luminescence in the excitation range of 420–550 nm (Fig. 3H) of the cork tissue cell walls are characteristic due to the presence of polyphenols (suberin), including simple phenolic compounds (Fig. 3D, 3E).

The epithelial cells of the cavities are thin-walled, rectangular in cross-section (Fig. 4A). The protoplasts of the epithelial cells are amorphous, light yellow, and stain pink when treated with Sudan III solution. The parenchyma around the cavity is composed of large, collapsed or wavy cells with noticeably thickened cellulosic cell walls compared to the thin-walled cells of the main parenchyma (Fig. 4B). To interpret the chemical nature of the luminescent agents, we analyzed the luminescence of the coumarin-based reference standard of eleutheroside B1 (Fig. 4F, 4G, 4H). It was found that the reference standard of eleutheroside B1 in the excitation range of 330–400 nm exhibits bright blue luminescence (Fig. 4G), similar to that of secretion drops in schizogenous cavities (Fig. 4C, 4D, 4E).

To test previously known and newly described characteristics of *A. archangelica* rhizomes and roots, model powders with particle sizes passing through sieves with mesh diameters of 0.5 mm and 0.25 mm were analyzed. Under a magnifying glass ($\times 20$, $\times 40$), the rhizome and root particles appear uniformly brown (Fig. 5A, 5B). When the dry powder is irradiated in the excitation range of 330–400 nm, the powder particles luminesce bright blue (Fig. 6A), characteristic of *A. archangelica* tissues described above. In the excitation range of 420–550 nm, the luminescence changes color to yellow (Fig. 6B).

Upon examination of a powder sample prepared according to the requirements of the SPH RF XV ed., fragments of phloem parenchyma with recognizable structures of schizogenous cavities are diagnosed (Fig. 5C). When treated with Sudan III solution, the secretion drops stain orange-pink. Additionally, upon irradiation of the powder in the excitation range of 330–400 nm, the secretion drops luminesce bright blue (Fig. 6C, 6E).

The powder also frequently contains vascular element fragments of varying sizes, mainly reticulate and spiral vessels (Fig. 5D). Under UV light in the excitation range of 330–400 nm, these fragments luminesce blue (Fig. 6D, 6F). Fragments of transverse sections of vascular elements are sometimes encountered. Typically, these are groups of vessels of different sizes (Fig. 5F).

It should be noted that the best contrast of denser powder elements is achieved using a polarizing microscope with a λ -light filter. In this case, vascular elements of various types are easily diagnosed in the general mass of weakly stained fragments by the bright staining of the dense cell walls (Fig. 5G, 5H).

Cork tissue fragments are easily diagnosed by the specific brown staining of the cell walls (Fig. 5E). Phloem parenchyma fragments are barely visible under normal light. In the excitation range of 330–400 nm, some fragments luminesce due to crystalline inclusions, presumably of coumarin origin (Fig. 6G, 6H, 6I).

Determination of Essential Oil Content

The essential oil content in *A. archangelica* rhizomes and roots was 0.09% calculated on a dry basis (with a raw material moisture content of 9.2%).

Gas Chromatography-Mass Spectrometry

GC-MS analysis allowed for the determination of the volatile organic compound profile of the lipophilic fractions of the aqueous-alcoholic extract and the essential oil of *A. archangelica* rhizomes and roots of Altai origin (Table 1).

The main group of biologically active substances in the hexane and chloroform fractions of the aqueous-alcoholic extract is represented by prenylated coumarins (49.94% and 44.42%, respectively), which constituted half of all identified components. The effective extraction of these coumarins with hexane is due to the prenylation process: an isoprene fragment ($-C_5H_9$) is attached to the coumarin nucleus at the C8 or C6 position, which increases lipophilicity and enhances their solubility in nonpolar solvents.

Furanocoumarins are present in the lipophilic fractions of the extract at 7.14% in hexane and 12.67% in chloroform. The hexane fraction also contains twice as much monoterpenoids (5.07% and 2.90%), sesquiterpenes (12.63% and 4.82%), sesquiterpenoids (9.58% and 3.49%), and significantly exceeds the chloroform fraction in monoterpene content (26.03% and 12.04%), which is reflected in the characteristic differences in the polarity of the extractants. The proportion of pyranocoumarins, methoxycoumarins, aromatic compounds, and fatty acids was minor (less than 2%) (see Table 1).

Analysis of the essential oil showed the dominant role of monoterpenes (46.2%), sesquiterpenes (43.5%), and sesquiterpenoids (35.23%). Monoterpenoids also constitute a significant portion—13.53%. Prenylated coumarins (osthol) are present in the essential oil (1.54%) due to their physicochemical properties. The absence of furanocoumarins, pyranocoumarins, and fatty acids is related to their high molecular weight and limited volatility during hydrodistillation (see Table 1).

According to the data presented in Table 1, the dominant component in the lipophilic fractions of the aqueous-alcoholic extract is the prenylated coumarin osthol. Its proportion averaged 49.72% in the hexane and 44.06% in the chloroform fractions of the total peak area of all compounds. Also among the leading components in the hexane and chloroform fractions were substances of terpene nature: α -pinene (7.29%, 4.24%); epiglobulol (5.42%, 2.04%); 3-carene (5.02%, 1.82%); D-limonene (3.82%, 1.46%); β -phellandrene (2.13%, 0.79%); bornyl acetate (1.99%, 0.78%); coumarin-based substances: oroselone (3.76%, 2.60%); angelicin (1.84%, 4.18%); oxypeucedanin (0.47%, 1.65%); isopinipenellin (0.38%, 2.48%), respectively.

In the essential oil, the dominant components were epiglobulol (25.61%), 3-carene (15.22%), bornyl acetate (5.89%), α -pinene (4.26%), D-limonene (3.67%), and osthol (1.54%) (see Table 1).

GC-MS chromatograms of volatile organic compounds of the lipophilic fractions of the aqueous-alcoholic extract and the essential oil of *A. archangelica* rhizomes and roots of Altai origin are presented in Figures 7–9.

Quantitative Determination of Total Coumarins

The content of total coumarins in *A. archangelica* rhizomes and roots of Altai origin, determined by spectrophotometric method, was $1.50 \pm 0.08\%$ calculated as coumarin. The analysis was performed in triplicate analytical replicates ($n = 3$), and the relative standard deviation (RSD) did not exceed 1.0%, indicating high reproducibility of the method.

The linearity range of the method was established in the concentration interval of coumarin from 2.0 to 20.0 $\mu\text{g/ml}$. The linear relationship is described by the equation: $y = 0.0482x \pm 0.0031$ with a coefficient of determination $r^2 = 0.9994$ and a correlation coefficient $r = 0.9997$ ($n = 10$), which confirms a high degree of linear dependence. The analytical range (application range) of the method was established in the same interval of 2.0–20.0 $\mu\text{g/mL}$, corresponding to 80–120% of the expected content of total coumarins in the test solution (normal concentration approximately 6.0 $\mu\text{g/mL}$). The accuracy of the method was confirmed by the “addition method” with an average recovery value of 98.9% ($n = 9$) in the range of 98.0–100.2%. Precision indicators met the requirements: the relative standard deviation for repeatability was 0.8% ($n = 6$), and for intermediate precision—1.5% ($n = 3$ days). Specificity was confirmed by the absence of significant absorption by ballast matrix components at 335 nm ($A \leq 0.02$).

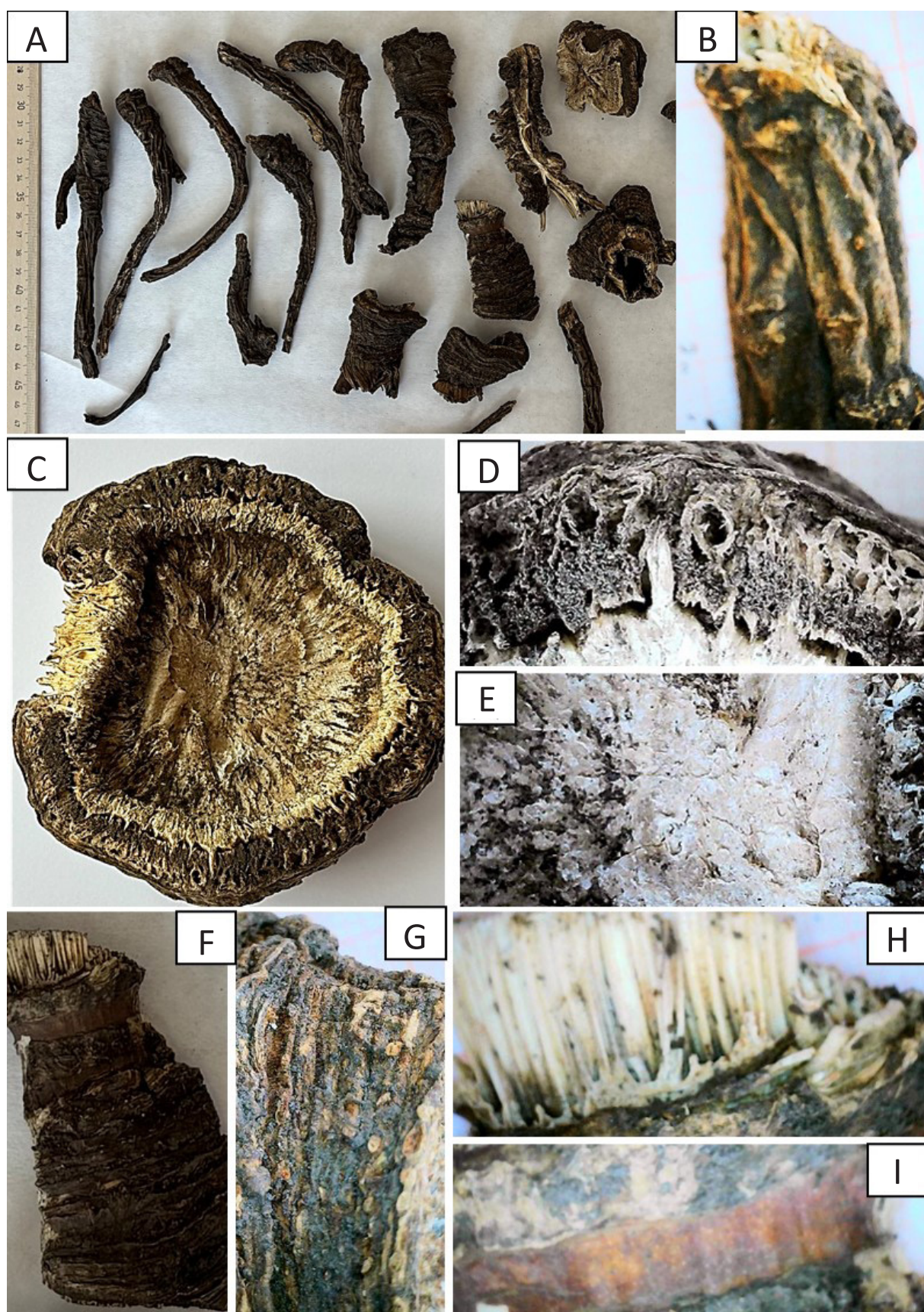


Figure 1 — Appearance and morphological features of *A. archangelica* raw material.

Note: A, collection of raw material fragments; B, wrinkled cork from the root surface; C, rhizome fragments in cross-section; D, cortical part of the rhizome; E, pith of the rhizome; F, rhizome surface fragment; G, rough transversely wrinkled cork of the rhizome; H, stem remnants; I, brown fragments of young cork.

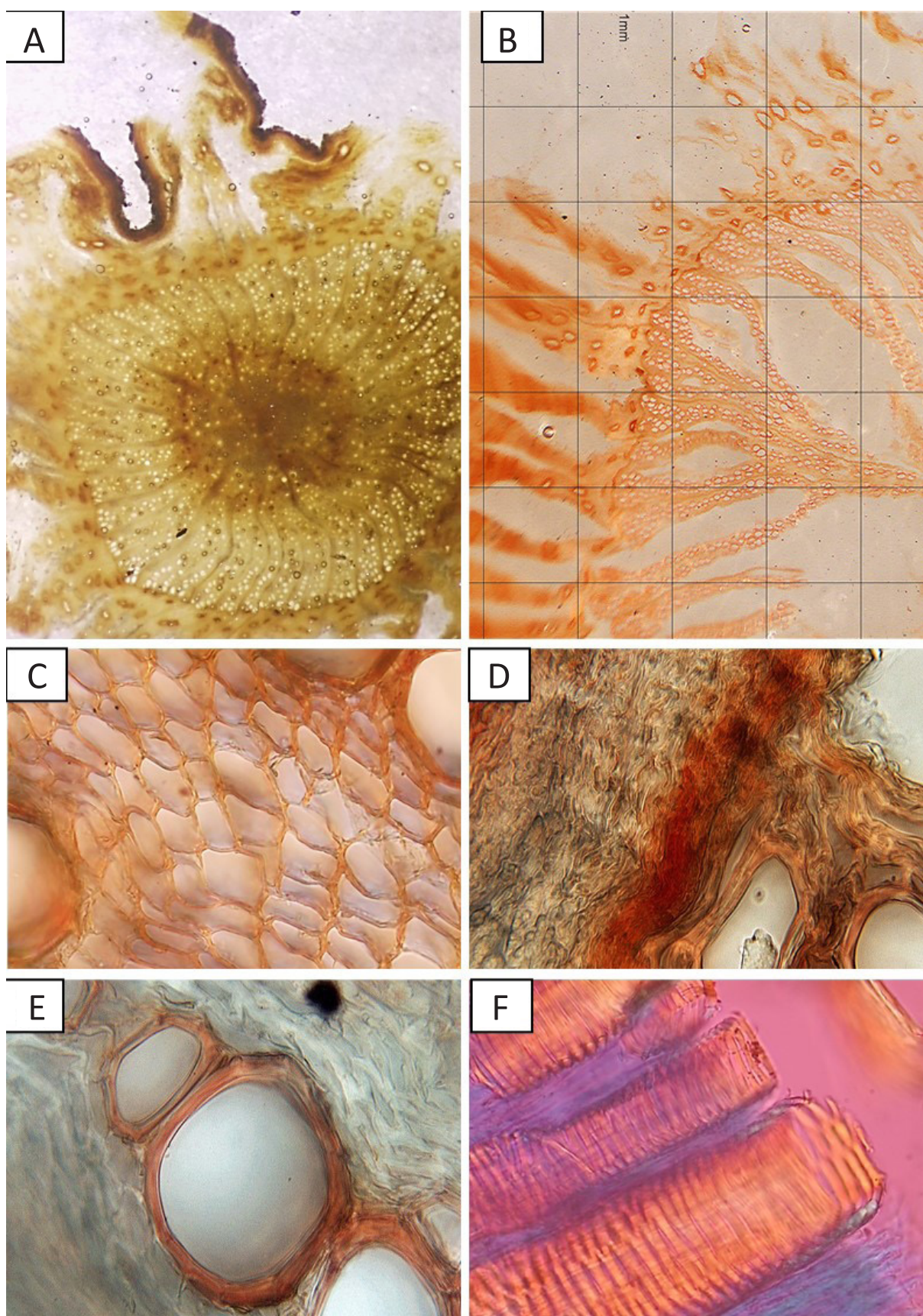


Figure 2 — Conducting tissues of *A. archangelica* rhizomes and roots.

Note: A, root cross-section, general view with a wavy edge (×40); B, fragment of xylem and phloem conducting elements. Stained with Sudan III solution (×40); C, ray parenchyma (×400); D, cambium fragment (×400); E, vascular elements in cross-section. Stained with Sudan III solution (×1000); F, fragment of reticulate vessels in polarized light with λ-filter (×400).

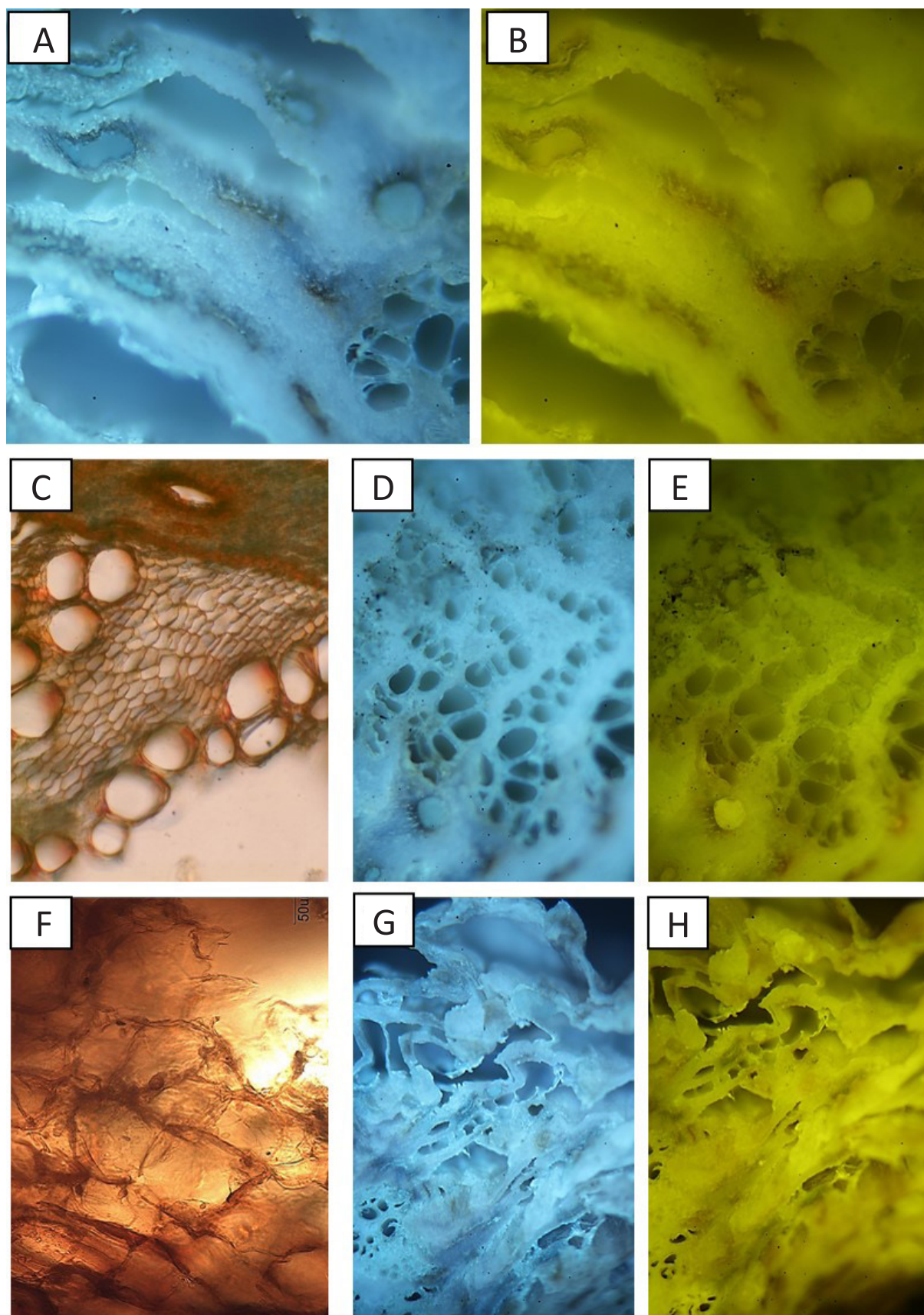


Figure 3 — Luminescence features of *A. archangelica* rhizome and root tissues.

Note: A, phloem in the excitation range of 330–400 nm ($\times 400$); B, phloem in the excitation range of $\lambda = 420\text{--}550$ nm ($\times 400$); C, xylem in visible light ($\times 400$); D, xylem in the excitation range of $\lambda = 330\text{--}400$ nm ($\times 400$); E, xylem in the excitation range of $\lambda = 420\text{--}550$ nm ($\times 400$); F, cork in visible light ($\times 400$); G, cork in the excitation range of $\lambda = 330\text{--}400$ nm ($\times 400$); H, cork in the excitation range of $\lambda = 420\text{--}550$ nm ($\times 400$).

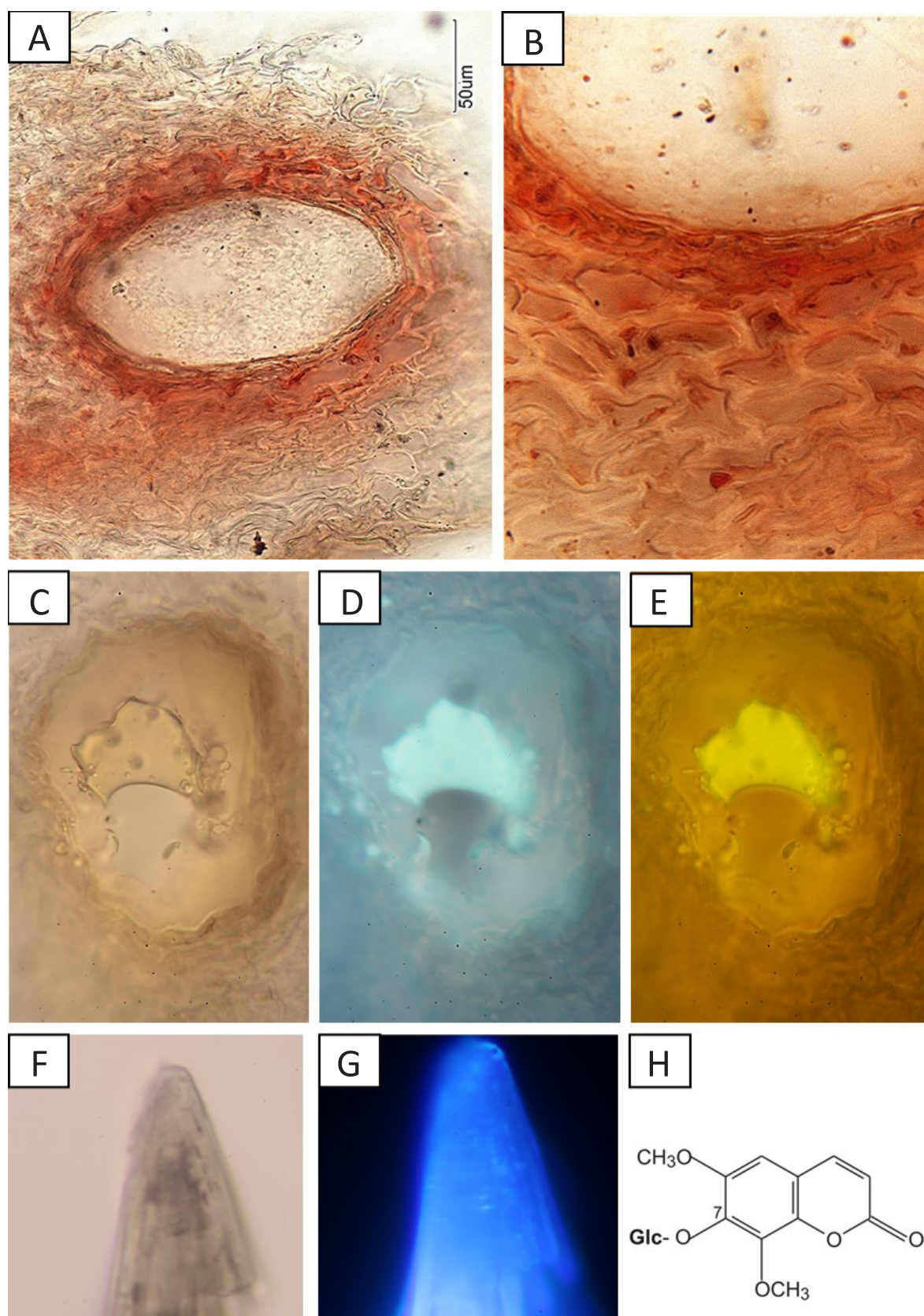


Figure 4 — Histological features of schizogenous cavities of rhizomes and roots of *A. archangelica*.

Note: A, staining of the schizogenous cavity with Sudan III solution ($\times 100$); B, cells of the epithelial layer and fundamental parenchyma around the cavity. Staining with Sudan III solution ($\times 400$); C, oily secretion of the cavity ($\times 100$); D, luminescence of the secretion in the excitation range $\lambda = 360-400$ nm ($\times 100$); E, luminescence of the secretion in the excitation range $\lambda = 420-550$ nm ($\times 100$); F, crystal of a standard sample ($\times 400$); G, luminescence of the standard sample in the excitation range $\lambda = 360-400$ nm ($\times 100$); H, structural formula of the standard sample of eleutheroside B1.

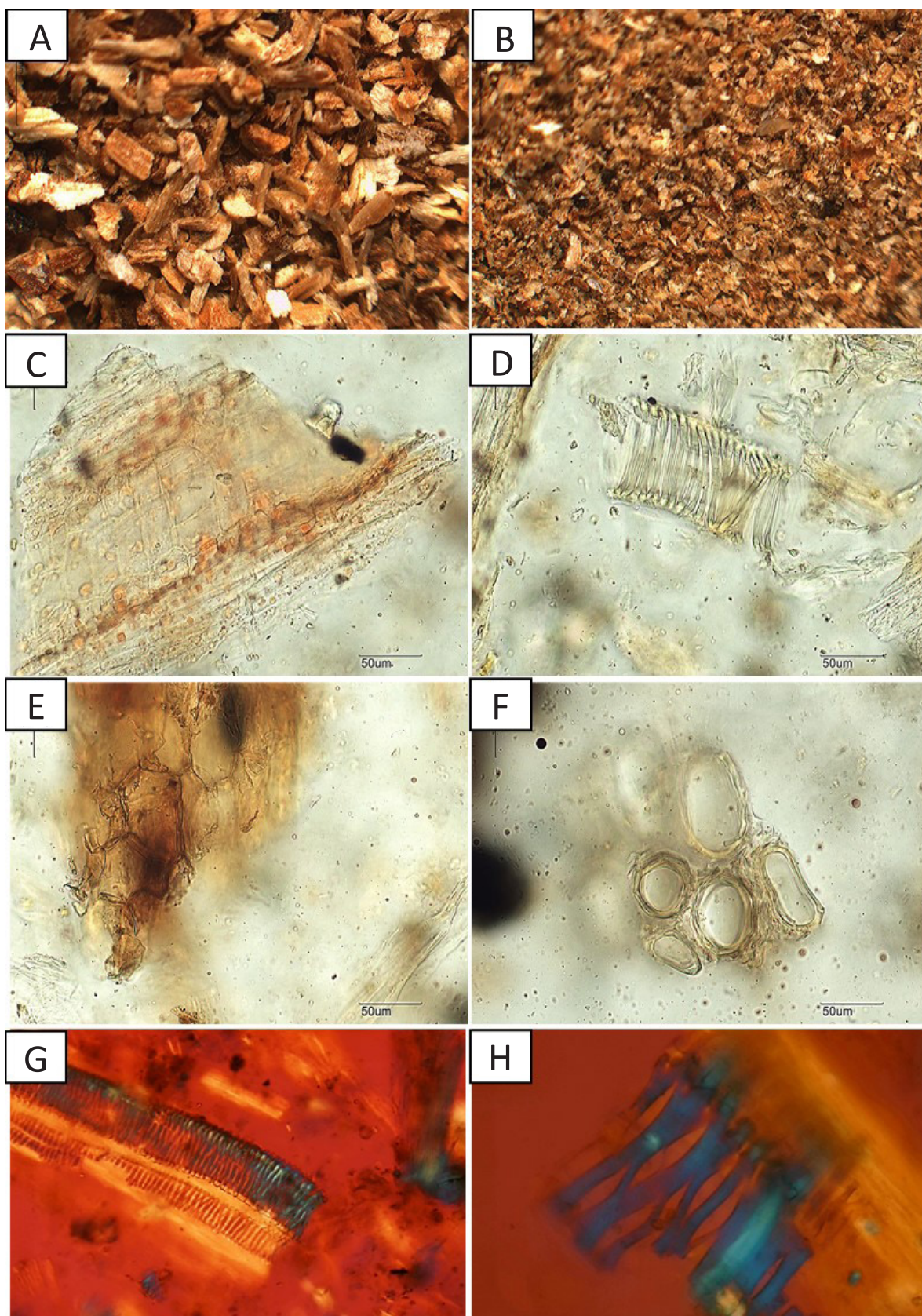


Figure 5 — Main diagnosable elements of the powder of rhizomes and roots of *A. archangelica*.

Note: A, powder sample with particle size passing through a 0.5 mm sieve (×40); B, powder sample with particle size passing through a 0.25 mm sieve (×40); C, fragment of a cavity with essential oil droplets (×400); D, fragment of a spiral vessel (×400); E, fragment of cork (×400); F, fragment of a group of vessels in cross-section (×400); G, fragment of reticulate vessels in polarized light λ-filter (×100); H, contrast of the cell wall of a fragment of reticulate vessels in polarized light λ-filter (×400).

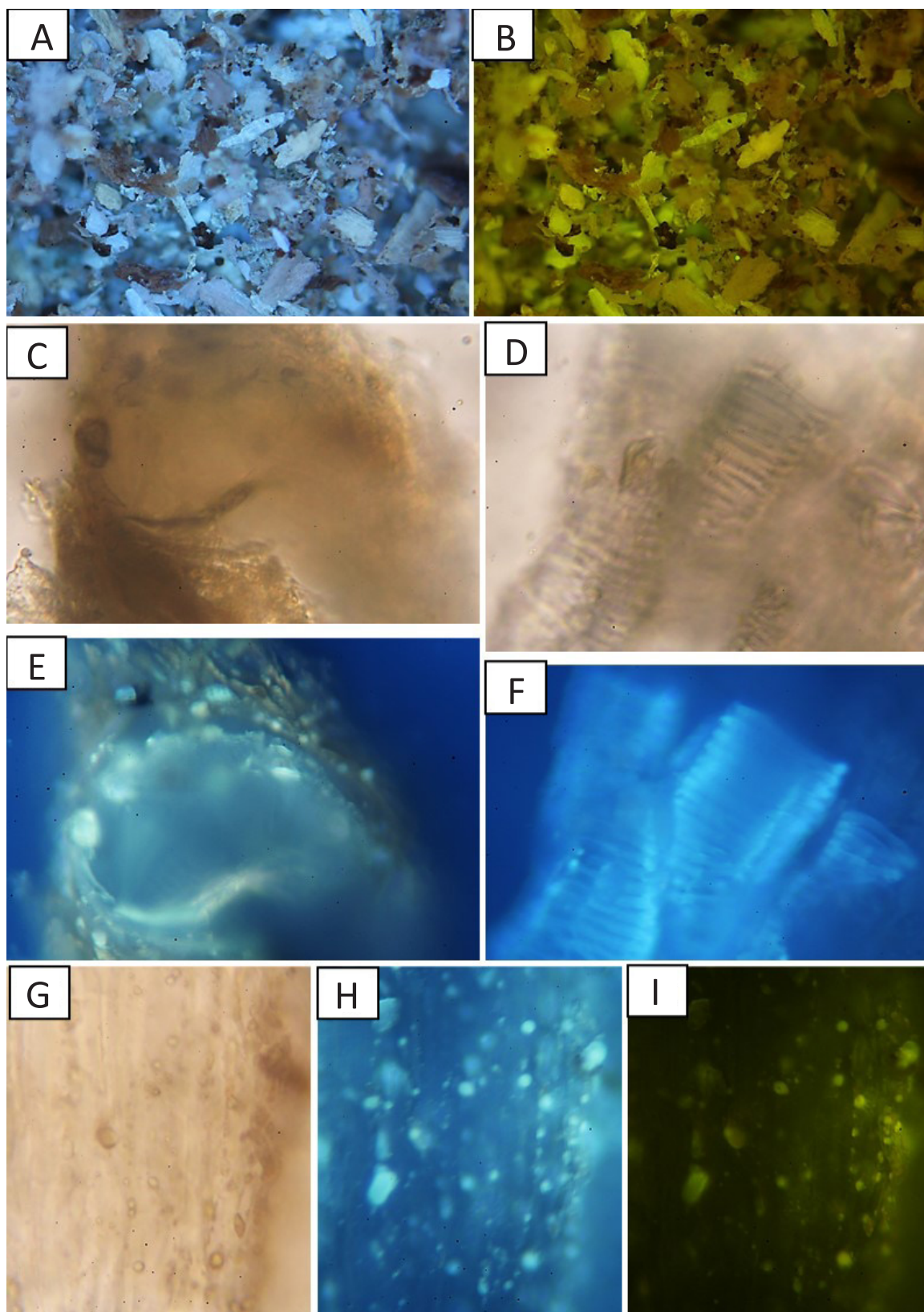


Figure 6 — Luminescence of elements of the powder of rhizomes and roots of *A. archangelica*.

Note: A, dry powder with particle size passing through a 0.25 mm sieve, luminescence in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 100$); B, dry powder with particle size passing through a 0.25 mm sieve, luminescence in the excitation range $\lambda = 420\text{--}550\text{ nm}$ ($\times 100$); C, fragment of a cavity with oil droplets in daylight ($\times 400$); D, fragment of reticulate vessels in daylight ($\times 400$); E, luminescence of a cavity with oil droplets in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 400$); F, luminescence of a fragment of reticulate vessels in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 400$); G, crystalline inclusions in the cells of the fundamental parenchyma ($\times 400$); H, luminescence of crystalline inclusions in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 400$); I, luminescence of crystalline inclusions in the excitation range $\lambda = 420\text{--}550\text{ nm}$ ($\times 400$).

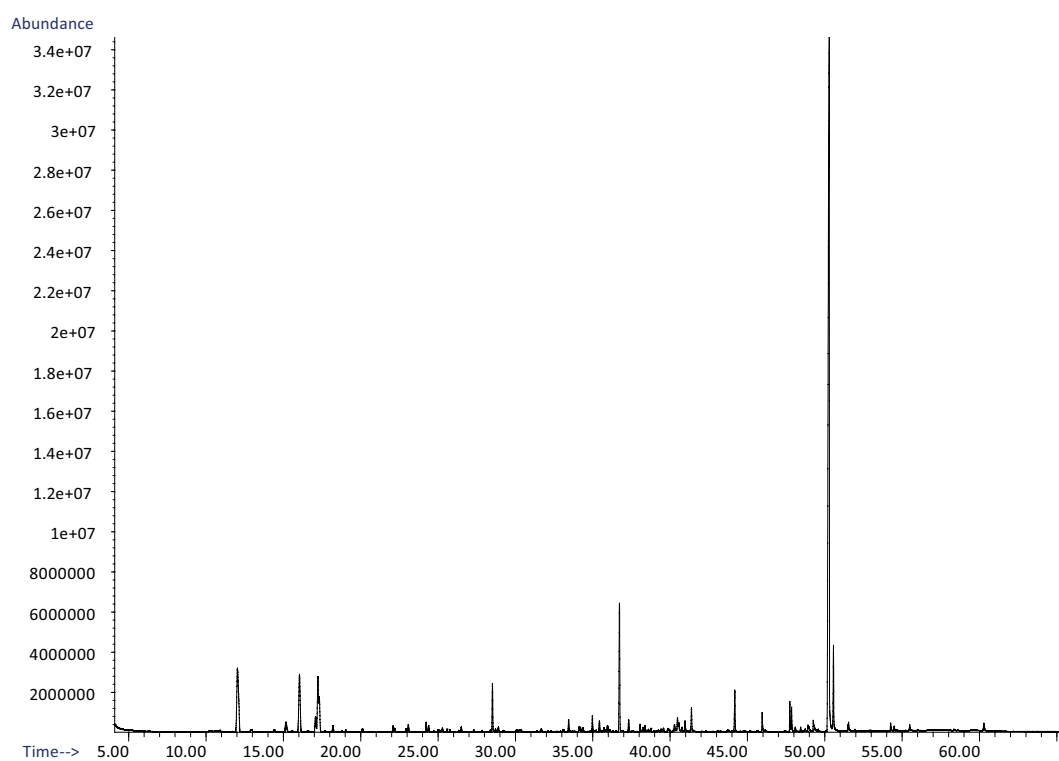


Figure 7 — GC-MS chromatogram of the hexane fraction of the aqueous-alcoholic extract from the rhizomes and roots of *A. archangelica*.

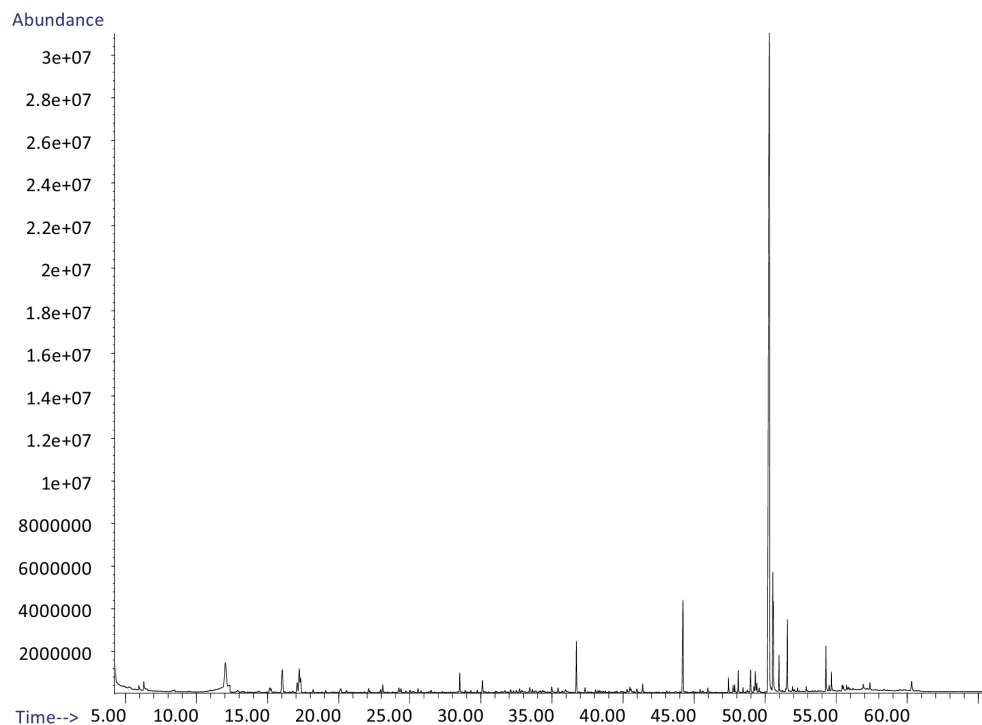


Figure 8 — GC-MS chromatogram of the chloroform fraction of the aqueous-alcoholic extract from the rhizomes and roots of *A. archangelica*.

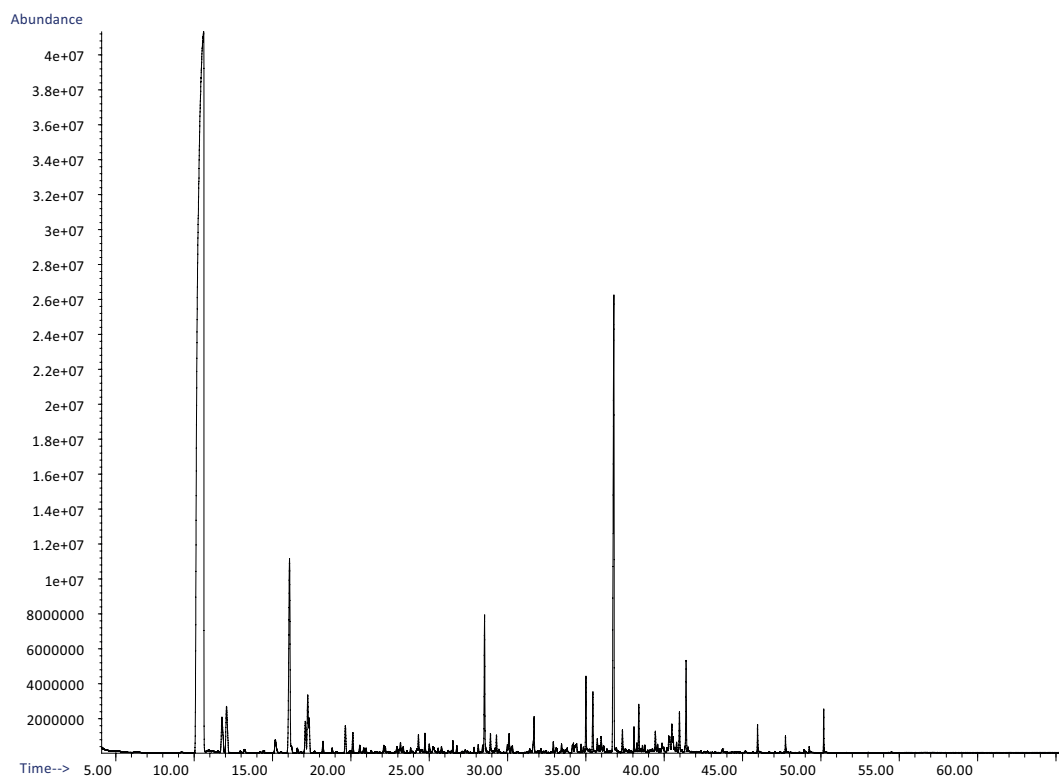


Figure 9 — GC-MS chromatogram of the essential oil from the rhizomes and roots of *A. archangelica*.

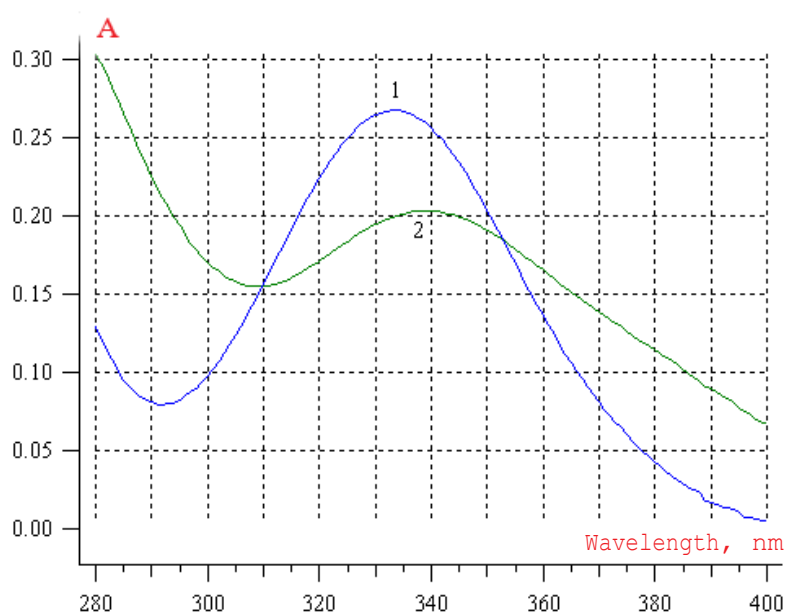


Figure 10 — Electron absorption spectrum of a coumarin standard solution and test solution B from the rhizomes and roots of *A. archangelica*.

Note: 1, coumarin standard, 2, test solution B.

Table 1 — Component composition of volatile organic compounds in hexane and chloroform fractions of water-alcoholic extract and essential oil from Altai origin *Angelica archangelica* rhizomes and roots

No	Retention time, min $\pm$ SD	Component	Detected in fraction from total area of all components, % $\pm$ SD		
			Hexane	Chloroform	Essential oil
1.	11,73 $\pm$ 0,163	α -Thujene, bicyclic monoterpene	0.1 $\pm$ 0.02	n.d.	3.43 $\pm$ 0.27
2.	12,042 $\pm$ 0,059	α -Pinene, bicyclic monoterpene	7.29 $\pm$ 0.58	4.24 $\pm$ 0.34	4.26 $\pm$ 0.34
3.	12,93 $\pm$ 0,049	Camphene, bicyclic monoterpene	0.29 $\pm$ 0.06	n.d.	0.19 $\pm$ 0.04
4.	13,184 $\pm$ 0,06	2,4(10)-Thuyadiene, bicyclic monoterpene	0.07 $\pm$ 0.01	n.d.	0.34 $\pm$ 0.07
5.	14,421 $\pm$ 0,04	β -Pinene, bicyclic monoterpene	0.25 $\pm$ 0.05	n.d.	0.21 $\pm$ 0.04
6.	15,167 $\pm$ 0,03	β -Myrcene, acyclic monoterpene	0.94 $\pm$ 0	0.3 $\pm$ 0.06	1.3 $\pm$ 0.1
7.	16,056 $\pm$ 0,07	3-Carene, bicyclic monoterpene	5.02 $\pm$ 0.4	1.82 $\pm$ 0.15	15.22 $\pm$ 1.22
8.	16,576 $\pm$ 0,026	α -Terpinene, monocyclic monoterpene	0.11 $\pm$ 0.02	0.05 $\pm$ 0.01	0.32 $\pm$ 0.06
9.	17,089 $\pm$ 0,023	ortho-Cymene, aromatic compound	1.08 $\pm$ 0.09	0.57 $\pm$ 0.06	2.14 $\pm$ 0.17
10.	17,238 $\pm$ 0,034	D-Limonene, monocyclic monoterpene	3.82 $\pm$ 0.31	1.46 $\pm$ 0.12	3.67 $\pm$ 0.29
11.	17,33 $\pm$ 0,03	β -Phellandrene, monocyclic monoterpene	2.13 $\pm$ 0.17	0.79 $\pm$ 0.08	1.92 $\pm$ 0.15
12.	17,673 $\pm$ 0,026	β -trans-Ocimene, acyclic monoterpene	0.09 $\pm$ 0.02	0.04 $\pm$ 0.01	0.12 $\pm$ 0.02
13.	18,211 $\pm$ 0,025	β -cis-Ocimene, acyclic monoterpene	0.39 $\pm$ 0.08	0.16 $\pm$ 0.03	0.63 $\pm$ 0.06
14.	18,799 $\pm$ 0,021	γ -Terpinene, monocyclic monoterpene	0.07 $\pm$ 0.01	0.04 $\pm$ 0.01	0.28 $\pm$ 0.06
15.	19,059 $\pm$ 0,049	Seidenone, monocyclic monoterpene ketone	0.15 $\pm$ 0.03	0.11 $\pm$ 0.02	0.1 $\pm$ 0.02
16.	19,912 $\pm$ 0,03	4-Carene, bicyclic monoterpene	0.04 $\pm$ 0.01	0.03 $\pm$ 0.01	0.15 $\pm$ 0.03
17.	20,12 $\pm$ 0,015	Terpinolene, monocyclic monoterpene	0.21 $\pm$ 0.04	0.17 $\pm$ 0.03	1.06 $\pm$ 0.08
18.	20,559 $\pm$ 0,025	para-Cymene, aromatic compound	0.03 $\pm$ 0.01	0.13 $\pm$ 0.03	0.45 $\pm$ 0.09
19.	22,101 $\pm$ 0,023	trans-para-2-Menthen-1-ol, monocyclic monoterpene alcohol	0.3 $\pm$ 0.06	0.19 $\pm$ 0.04	0.44 $\pm$ 0.09
20.	22,196 $\pm$ 0,019	Neo-allo-ocimene, acyclic monoterpene	0.18 $\pm$ 0.04	0.07 $\pm$ 0.01	0.29 $\pm$ 0.06
21.	22,942 $\pm$ 0,019	cis-2-para-Menthen-1-ol, monocyclic monoterpene alcohol	0.19 $\pm$ 0.04	0.17 $\pm$ 0.03	0.4 $\pm$ 0.08
22.	23,092 $\pm$ 0,025	Verbenol, bicyclic monoterpene alcohol	0.4 $\pm$ 0.08	0.36 $\pm$ 0.07	0.19 $\pm$ 0.04
23.	23,573 $\pm$ 0,017	para-Menthanone, monocyclic monoterpene	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01	0.14 $\pm$ 0.03
24.	24,238 $\pm$ 0,004	Verbenyl ethyl ether, bicyclic monoterpene ether	0.45 $\pm$ 0.09	0.24 $\pm$ 0.05	n.d.
25.	24,365 $\pm$ 0,105	para-Menta-1,5-dien-8-ol, monocyclic monoterpene alcohol	0.30 $\pm$ 0.06	0.2 $\pm$ 0.04	0.93 $\pm$ 0.11
26.	24,493 $\pm$ 0,022	Menthol, monocyclic monoterpene alcohol	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.21 $\pm$ 0.04
27.	24,729 $\pm$ 0,019	para-Cymenol-8, monocyclic monoterpene alcohol	0.06 $\pm$ 0.01	0.1 $\pm$ 0.02	0.94 $\pm$ 0.12
28.	25,017 $\pm$ 0,036	Krypton, monocyclic monoterpene ketone	0.17 $\pm$ 0.03	0.17 $\pm$ 0.03	0.60 $\pm$ 0.07
29.	25,267 $\pm$ 0,048	cis-Piperitol, monocyclic monoterpene alcohol	0.2 $\pm$ 0.04	0.13 $\pm$ 0.03	0.41 $\pm$ 0.08
30.	25,304 $\pm$ 0,011	Myrcenal, bicyclic monoterpene aldehyde	n.d.	n.d.	0.35 $\pm$ 0.07
31.	25,578 $\pm$ 0,013	Sabinol, monocyclic monoterpene alcohol	0.17 $\pm$ 0.03	0.20 $\pm$ 0.04	0.26 $\pm$ 0.05
32.	25,787 $\pm$ 0,019	Piperitol, monocyclic monoterpene alcohol	0.13 $\pm$ 0.03	0.11 $\pm$ 0.02	0.37 $\pm$ 0.07
33.	26,412 $\pm$ 0,021	3-Carene-2-ol, bicyclic monoterpene alcohol	n.d.	n.d.	0.13 $\pm$ 0.03
34.	26,514 $\pm$ 0,018	Thymyl methyl ether, aromatic ether	n.d.	n.d.	0.57 $\pm$ 0.05
35.	27,630 $\pm$ 0,012	Piperitone, monocyclic monoterpene ketone	n.d.	n.d.	0.10 $\pm$ 0.02
36.	28,407 $\pm$ 0,024	Myrthenyl acetate, bicyclic monoterpene ether	0.11 $\pm$ 0.02	0.05 $\pm$ 0.01	0.39 $\pm$ 0.08
37.	28,524 $\pm$ 0,023	Borneol acetate, bicyclic monoterpene ether	1.99 $\pm$ 0.16	0.78 $\pm$ 0.08	5.89 $\pm$ 0.47
38.	28,906 $\pm$ 0,011	Eucarvone, monocyclic monoterpene	0.27 $\pm$ 0.05	0.10 $\pm$ 0.02	1.11 $\pm$ 0.09
39.	30,307 $\pm$ 0,006	Carvyl acetate, monoterpene ester	0.09 $\pm$ 0.02	0.05 $\pm$ 0.01	0.41 $\pm$ 0.08
40.	31,405 $\pm$ 0,026	Cyclosativene, bicyclic sesquiterpene	0.07 $\pm$ 0.01	0.05 $\pm$ 0.01	0.3 $\pm$ 0.06
41.	32,11 $\pm$ 0,022	β -Elemene, monocyclic sesquiterpene	0.07 $\pm$ 0.01	0.16 $\pm$ 0.03	n.d.
42.	33,444 $\pm$ 0,011	α -Gurjunene, tricyclic sesquiterpene	0.58 $\pm$ 0.06	0.29 $\pm$ 0.06	0.47 $\pm$ 0.09
43.	33,695 $\pm$ 0,012	Sativene, bicyclic sesquiterpene	n.d.	n.d.	0.34 $\pm$ 0.07
44.	34,131 $\pm$ 0,023	β -Caryophyllene, bicyclic sesquiterpene	0.26 $\pm$ 0.05	0.08 $\pm$ 0.02	0.43 $\pm$ 0.09

No	Retention time, min $\pm$ SD	Component	Detected in fraction from total area of all components, % $\pm$ SD		
			Hexane	Chloroform	Essential oil
45.	34,204 $\pm$ 0,012	α -Campholenal, monocyclic monoterpenoid aldehyde	0.21 $\pm$ 0.04	0.08 $\pm$ 0.02	0.48 $\pm$ 0.10
46.	35,437 $\pm$ 0,019	Dihydroagarofuran, tricyclic sesquiterpenoid	0.48 $\pm$ 0.10	0.21 $\pm$ 0.04	2.63 $\pm$ 0.21
47.	35,724 $\pm$ 0,019	α -Murolene, bicyclic sesquiterpene	0.26 $\pm$ 0.05	0.12 $\pm$ 0.02	0.84 $\pm$ 0.09
48.	35,820 $\pm$ 0,043	α -Farnesene, acyclic sesquiterpenoid	0.11 $\pm$ 0.02	0.03 $\pm$ 0.01	0.45 $\pm$ 0.09
49.	35,958 $\pm$ 0,041	β -Bisabolene, acyclic sesquiterpene	0.52 $\pm$ 0	0.07 $\pm$ 0.01	1.18 $\pm$ 0.09
50.	36,126 $\pm$ 0,023	Cuparene, monocyclic sesquiterpene	0.15 $\pm$ 0.03	0.09 $\pm$ 0.02	0.59 $\pm$ 0.07
51.	36,745 $\pm$ 0,085	Epiglobulol, tricyclic sesquiterpenoid alcohol	5.42 $\pm$ 0.43	2.04 $\pm$ 0.16	25.61 $\pm$ 2.05
52.	36,877 $\pm$ 0,019	α -Longipinene, tricyclic sesquiterpene	0.07 $\pm$ 0.01	0.03 $\pm$ 0.01	0.22 $\pm$ 0.04
53.	36,944 $\pm$ 0,008	Humulene, monocyclic sesquiterpene	0.08 $\pm$ 0.02	0.03 $\pm$ 0.01	0.38 $\pm$ 0.08
54.	37,329 $\pm$ 0,013	Elemol, monocyclic sesquiterpene	0.55 $\pm$ 0	0.22 $\pm$ 0.04	1.31 $\pm$ 0.1
55.	38,373 $\pm$ 0,013	Caryophyllene oxide, bicyclic sesquiterpenoid	0.32 $\pm$ 0.06	0.12 $\pm$ 0.02	2.21 $\pm$ 0.18
56.	38,504 $\pm$ 0,016	Globulol, tricyclic sesquiterpenoid	0.11 $\pm$ 0.02	0.06 $\pm$ 0.01	0.32 $\pm$ 0.06
57.	39,431 $\pm$ 0,016	Bulnesol, bicyclic sesquiterpenoid alcohol	0.08 $\pm$ 0.02	n.d.	1.19 $\pm$ 0.10
58.	39,576 $\pm$ 0,007	γ -Eudesmol, bicyclic sesquiterpenoid alcohol	0.23 $\pm$ 0.05	0.08 $\pm$ 0.02	0.60 $\pm$ 0.05
59.	39,859 $\pm$ 0,009	α -Eudesmol, bicyclic sesquiterpenoid alcohol	0.22 $\pm$ 0.04	0.09 $\pm$ 0.02	0.73 $\pm$ 0.08
60.	39,960 $\pm$ 0,010	Agarospirol, bicyclic sesquiterpenoid alcohol	0.18 $\pm$ 0.04	0.06 $\pm$ 0.01	0.38 $\pm$ 0.08
61.	40,289 $\pm$ 0,011	α -Guaiene, bicyclic sesquiterpene	0.48 $\pm$ 0.10	0.20 $\pm$ 0.04	0.82 $\pm$ 0.09
62.	40,484 $\pm$ 0,016	Guaiol, bicyclic sesquiterpenoid alcohol	0.75 $\pm$ 0.08	0.19 $\pm$ 0.04	1.57 $\pm$ 0.13
63.	40,566 $\pm$ 0,015	β -Eudesmol, bicyclic sesquiterpenoid alcohol	0.51 $\pm$ 0.06	0.20 $\pm$ 0.04	0.93 $\pm$ 0
64.	41,384 $\pm$ 0,007	α -Bisabolol, monocyclic sesquiterpenoid alcohol	1.13 $\pm$ 0.09	0.40 $\pm$ 0.08	n.d.
65.	44,203 $\pm$ 0,069	Angelicin, furanocoumarin	1.84 $\pm$ 0.15	4.18 $\pm$ 0.33	n.d.
66.	47,858 $\pm$ 0,009	Hexadecanoic acid, ethyl ester, fatty acid ester	0.91 $\pm$ 0.08	0.29 $\pm$ 0.06	n.d.
67.	48,105 $\pm$ 0,023	Citropten, methoxycoumarin	0.25 $\pm$ 0.05	0.73 $\pm$ 0.08	n.d.
68.	48,441 $\pm$ 0,012	Xanthyletin, pyranocoumarin	0.18 $\pm$ 0.04	0.15 $\pm$ 0.03	n.d.
69.	48,948 $\pm$ 0,093	Methoxsalen, furanocoumarin	0.59 $\pm$ 0.06	0.91 $\pm$ 0.10	n.d.
70.	49,306 $\pm$ 0,023	Bergapten, furanocoumarin	0.10 $\pm$ 0.02	0.85 $\pm$ 0.09	n.d.
71.	49,581 $\pm$ 0,009	Phellopterin, prenylated coumarin	0.14 $\pm$ 0.03	0.17 $\pm$ 0.03	n.d.
72.	50,254 $\pm$ 0,160	Osthol, prenylated coumarin	49.72 $\pm$ 3.98	44.06 $\pm$ 3.52	1.54 $\pm$ 0.12
73.	50,570 $\pm$ 0,030	Orocelon, furanocoumarin	3.76 $\pm$ 0.3	2.60 $\pm$ 0.21	n.d.
74.	51,549 $\pm$ 0,046	Isopimpinellin, furanocoumarin	0.38 $\pm$ 0.08	2.48 $\pm$ 0.21	n.d.
75.	52,882 $\pm$ 0,025	Imperatorin, prenylated coumarin	0.08 $\pm$ 0.02	0.19 $\pm$ 0.04	n.d.
76.	54,268 $\pm$ 0,033	Oxypeucedanin, furanocoumarin	0.47 $\pm$ 0.09	1.65 $\pm$ 0.13	n.d.
77.	55,488 $\pm$ 0,011	Decursin, pyranocoumarin	0.48 $\pm$ 0.1	0.49 $\pm$ 0.10	n.d.
78.	56,002 $\pm$ 0,014	Selinidin, pyranocoumarin	0.24 $\pm$ 0.05	0.29 $\pm$ 0.06	n.d.
79.	60,298 $\pm$ 0,04	Dimethyl-2-oxo-2,8,9,10-tetrahydropyrano(2,3-f) chromene, pyranocoumarin	0.69 $\pm$ 0.08	0.91 $\pm$ 0.11	n.d.
By component classes: average values, % (number of components in the corresponding group of compounds in parentheses)					
Prenylated coumarins			49.94 (3)	44.42 (3)	1.54 (1)
Monoterpenes			26.03 (32)	12.04 (28)	46.2 (34)
Sesquiterpenes			12.63 (23)	4.82 (22)	43.5 (22)
Sesquiterpenoids			9.58 (12)	3.49 (11)	35.23 (11)
Monoterpenoids			5.07 (17)	2.90 (17)	13.53 (20)
Furanocoumarins			7.14 (6)	12.67 (6)	n.d.
Aromatic compounds			1.11 (2)	0.7 (2)	2.59 (2)
Pyranocoumarins			1.59 (4)	1.84 (4)	n.d.
Methoxycoumarins			0.25 (1)	0.73 (1)	n.d.
Fatty acids			0.91 (1)	0.29 (1)	n.d.

Note: SD, standard deviation; n.d., not detected.

Table 2 — Validation results of the spectrophotometric method for determining the sum of coumarins in *A. archangelica* rhizomes and roots

Validation parameter	Conditions / range	Acceptance criteria	Result
Specificity	Analysis of "blank" solution	$A \leq 0.05$	$A \leq 0.02$
Linearity	2,0–20,0 µg/mL (n=10)	$r \geq 0.990$	$r = 0.9997$
Analytical range	2.0–20.0 µg/mL (calculated as coumarin)	Corresponds to the linearity range; 80–120% of expected content in raw material	Range established and confirmed
Accuracy	Method of additions	98–102%	98.9%
Repeatability	$n = 6$	$RSD \leq 2.0\%$	$RSD = 0.8\%$
Intermediate precision	$n = 3$ days	$RSD \leq 2.0\%$	$RSD = 1.5\%$

The obtained validation results (Table 2) demonstrate the compliance of the method with the requirements set forth in regulatory documents and are characterized by satisfactory specificity, linearity, accuracy, and precision, which confirms the suitability of the proposed method for the purpose of pharmacopoeial standardization of *A. archangelica* medicinal raw material by total coumarin content. Figure 10 shows the absorption spectrum of the coumarin reference standard solution B and Test Solution B (maximum at 335 nm).

DISCUSSION

For the first time, morphological-anatomical and gas chromatographic studies of *A. archangelica* rhizomes and roots of Altai origin were conducted.

The results of the study of external and microscopic features are consistent with previously published data [5, 6]. For the first time, luminescence characteristics of anatomical structures of *A. archangelica* raw material were discovered in comparison with the reference standard of eleutheroside B1, which preliminarily allows for the interpretation of the localization of BACs. The most diagnostic in this case is the light blue luminescence of phloem cells and secretion in schizogenous cavities in the excitation range of 330–400 nm, attributed to coumarin-based substances. Histological features of the marker characteristic—schizogenous cavities—have also been clarified, which will allow for the identification of the object under study during acceptance control of medicinal raw material, for example, in comparison with *Angelica sylvestris* L. [30, 31]. Methods for diagnosing *Angelica archangelica* rhizome and root powder have been improved, and the expediency of using luminescent and polarized microscopy in the diagnosis of specific schizogenous cavities, vascular elements, and crystalline inclusions has been confirmed. Analysis of microscopic features of medicinal raw material

powder is a mandatory criterion for the acceptance of raw materials and quality control of drugs based on *Angelica archangelica* rhizomes and roots. The obtained data will allow for the development of the "Identification. Microscopic Features" section of the draft of pharmaceutical monograph "*Angelica archangelica* rhizomes and roots".

Previously identified in *A. archangelica* rhizomes and roots: prenylated coumarins—osthol [7], umbelliferone, imperatorin, isoimperatorin, fellopterin; hydroxycoumarins—umbelliferone; furanocoumarins—angelicin, bergapten, psoralen, xanthotoxin, oroselone, methoxsalen, xanthotoxol, archangelicin, pimpinellin, isopinipenellin [17–19, 21]. In raw material harvested in the Republic of Bashkortostan, the leading components were oroselone (71.36%), osthol (5.09%), bergapten (2.74%), methoxsalen (2.65%), angelicin (1.69%) [17]. The present study found that the leading component of the aqueous-alcoholic extract is osthol (49.72%). Apparently, it is the leading component of *A. archangelica* rhizomes and roots of Altai origin. For the first time, cypten, xanthelatin, decursin, and selinidin were identified here.

The content of essential oil in the underground part of *A. archangelica* varies significantly depending on the region of plant growth. For example, in the Republic of Bashkortostan—0.82% [32], near Mount Ozren, Serbia—0.10% [8], in the Ternopil region, Ukraine—0.13% [33], in the high-altitude regions of India in Potiwas (2200 m)—0.28%, Rudraprayag (3300 m)—0.33%, Uttarkashi (3500 m)—0.35% [34]. The essential oil content in the studied sample was 0.09%, which, according to monograph 01/2013:1857 "*Angelica archangelica* root" of the European Pharmacopoeia 11th edition, does not meet the normative value—not less than 2 mL/kg (0.2%). The low essential oil content may be due to the place and time of harvesting, climatic conditions, soil chemical composition, and other factors, as well as the drying temperature (45°C), which does not meet the requirements for essential oil-bearing raw materials

(40°C)^{15,16}, which must be considered when organizing the harvesting of medicinal raw materials.

In the study by A. Forycka et al., it is indicated that the essential oil of *A. archangelica* of Romanian, Finnish, and Serbian origin contains from 62 to 122 components [13]. In the Kemerovo region, Russian Federation, 75 components were found [15], and in Serbia—88 compounds. The largest part of the component composition of the essential oil is made up of monoterpenes and monoterpenoids [35, 36]. In the essential oil of *A. archangelica* growing in the Republic of Bashkortostan, sesquiterpenoids predominate, which are 2.6 times more than monoterpenoids [19]. In the present study, 62 components were identified in the essential oil of Altai *A. archangelica* (possible component volatilization), the basis of which is formed by monoterpenes (46.2%), sesquiterpenes (43.5%), and sesquiterpenoids (35.23%). Monoterpenoids were found in smaller quantities (13.53%).

Essential oil from the northern regions of Europe was characterized by a high level of α -pinene (65.3%), α -phellandrene (5.9–15.4%), and δ -3-carene (15.3%), while in samples from the south, its level was the lowest (0–0.2%); in the east and northeast, α -pinene was dominant (from 15.7% to 20.8%); in the northeast, high concentrations of β -phellandrene (13.5–16.9%), δ -3-carene (15.4–16.9%), limonene (8.0–9.0%), and sabinene (5.0–7.5%) were noted, whereas the concentration of the latter in the east was higher (5.9–14.85%); in the southeast, β -phellandrene (13.8–18.5%), α -pinene (11.4–15.0%), δ -3-carene (10.8–11.9%), α -cymene (6.8–10.6%), and α -phellandrene (5.9–8.6%) were present [13, 14]. The dominant components of the Siberian subspecies of *A. archangelica* are limonene (30.47%) and α -pinene (23.6%) [15]. The volatile organic compound profile of the essential oil of *A. archangelica* of Altai origin is most similar to that of flora from Lithuania and Serbia [14, 16], where the prevailing compounds are: α -pinene (19.4%, 29.7%), δ -3-carene (16.0%, 14.2%), β -phellandrene (15.4%), 15-pentadecanolide (14.9%), limonene (8.8%), and osthol (8.8%).

In the present study, the essential oil consists of 62 components, among which the leading ones are epiglobulol (25.61%), 3-carene (15.22%), bornyl acetate (5.89%), α -pinene (4.26%), and D-limonene (3.67%),

β -phellandrene (1.92%), osthol (1.54%). It is evident that, despite geographical differences, the essential oil of *A. archangelica* of Altai origin contains species-specific components (3-carene, α -pinene, D-limonene, β -phellandrene, osthol) characteristic of the essential oil of plants from the represented habitats, but in different proportions, which allows it to be considered authentic. Osthol is the only coumarin among *A. archangelica* that is present in the essential oil (1.54%), which is confirmed by literature data [14, 37] and can serve as a marker for confirming the authenticity of the essential oil from the rhizomes and roots of *A. archangelica*. Preliminarily, it can be stated that ortho-cymene, seudenone, 4-carene, neo-allo-ocimene, *para*-menthanone, sabinol, 3-carene-2-ol, thymyl methyl ether, eucarvone, α -gurjunene, α -campholenal, α -farnesene, epiglobulol, α -longipinene, globulol, guayol, bulnesol, agarospirol, and guayol have been identified for the first time in the essential oil of *A. archangelica* rhizomes and roots. The conducted analysis allows us to conclude that the profile of BACs of *A. archangelica* of Altai origin represents a distinct chemotype that requires individual quality assessment standards. The obtained data on the leading components of terpene and coumarin nature will allow for the development of sections on “Identification. Determination of major groups of biologically active compounds”.

The proposed method for quantitative determination of the sum of coumarins, calculated as coumarin, using a spectrophotometric method in the rhizomes and roots of *A. archangelica* is based on the specific chemical properties of coumarin and its derivatives, which contain a benzo- α -pyrone ring and intensely absorb light in the ultraviolet region of the spectrum. During the extraction of the sum of coumarins from the MPRM, 96% alcohol extraction was used, followed by liquid-liquid extraction. Ethanol extraction in this case ensures complete extraction of both free coumarins and their glycosidic forms. In the next step, the aqueous-alcoholic solution of the extract was acidified to pH 2–3, and triple extraction with ethyl acetate was performed, which effectively isolates neutral (molecular) and weakly acidic forms of coumarins from the polar matrix and, consequently, allows for the assessment of their quantitative transfer into the ethyl acetate phase. Ethyl acetate was chosen as the extractant due to its optimal polarity, ensuring high selectivity towards the target analytes and minimal co-extraction of ballast substances such as organic acids, sugars, and pigments. The obtained organic extract was evaporated to dryness, and the residue was dissolved in 70% alcohol

¹⁵ Rules for collecting and drying medicinal plants (collection of instructions); Ministry of Health of the USSR, Chief Pharmacy Department, All-Union. information bureau, etc. Moscow: Meditsina Publ.; 1985. 328 p. Russian

¹⁶ Kurkin VA. Pharmacognosy: a textbook for students of pharmaceutical universities (faculties). Kurkin VA; 6th edition, revised and expanded. Samara: OOO “Polygraphic association “Standard”; 2024. 1268 p. Russian

for subsequent spectrophotometric analysis. During spectrophotometric detection, alkaline hydrolysis of the lactone ring of coumarins causes ring opening, forming the corresponding phenolate anion, which is characterized by a bathochromic shift of the absorption maximum in the wavelength range of 330–340 nm. In the present work, the optical density was measured at a wavelength of 335 nm, which ensures high sensitivity and specificity for the determination of the sum of coumarins in an alkaline medium compared to measurement in a neutral medium. This sample preparation scheme ensures high reproducibility of results and minimizes background absorption that may be caused by accompanying components of the plant material.

It should be noted that the leading coumarin in the studied Altai origin raw material is osthol (see Table 1)—a prenylated coumarin derivative with a molecular mass different from coumarin (molecular mass of coumarin is 146.14 g/mol, molecular mass of osthol is 244.3 g/mol) and molar absorptivity. During the analysis, coumarin (1,2-benzopyrone) was used as a reference standard due to the absence of a certified for osthol, as it represents the basic chromophoric structure for the entire class of coumarins and is widely used in the standardization of MPRM containing coumarins. Consequently, a systematic error is introduced into the results of quantitative determination: the true content of osthol may differ from the obtained value due to differences in spectral characteristics and molecular masses of the standard and determined compounds.

The content of the sum of coumarins, determined by the spectrophotometric method in an alkaline medium at a wavelength of 335 nm, was $1.50 \pm 0.08\%$ calculated as coumarin. The obtained value is within the range characteristic for the rhizomes and roots of *A. archangelica*. According to literature data, the total coumarin content varies from 0.1% to 3.2% depending on the region of growth and harvesting conditions [17, 19, 38]. The result of 1.5% correlates with GC-MS data: with a water-alcoholic extract yield of ~20% and an osthol content of ~49% and 44% in the hexane and chloroform fractions, the calculated coumarin content is 1.5–2.0%, which confirms the reliability of the method. Nevertheless, in the absence of a certified RS for osthol, this approach remains acceptable for pharmacopoeial standardization purposes.

Study Limitations

The presented method for quantitative determination of the sum of coumarins by

spectrophotometry has a key limitation is a potential interference from other UV-active compounds, particularly phenolic carboxylic acids and flavonoid aglycones, which, like coumarins, can be extracted by ethyl acetate from the acidified aqueous phase. Since the final measurement is performed without chromatographic separation, the recorded optical density value at 335 nm may include contributions not only from coumarins but also from the indicated co-extracted substances, which can overestimate the quantitative determination results. Despite this limitation, the data obtained in the present study are valuable for the development of a pharmacopoeial monograph and can be supplemented in subsequent studies with an expanded sample size and the use of chromatographic methods for quantitative analysis.

Thus, the proposed method, based on spectrophotometric determination of the sum of coumarins, can be recommended for inclusion in the draft of pharmacopoeial monograph for RHM, provided that “calculated as coumarin” is clearly indicated and that results need to be confirmed by chromatographic methods when determining the content of specific compounds.

CONCLUSION

The conducted macroscopic, microscopic, microchemical, and histochemical examination allowed for the summarization of the diagnostic results of *A. archangelica* rhizomes and roots, as well as the supplementation of known structural features with new data on tissue polarization and luminescence. Morphologically, root surface fragments exhibit a wavy-wrinkled texture, while rhizome fragments are covered with a rough, transversely wrinkled cork. The luminescence of *A. archangelica* rhizome and root tissues is due to their complex chemical composition, mainly phenolic compounds. The most diagnostic is the light blue luminescence of phloem cells and the secretion of schizogenous cavities in the excitation wavelength range of 330–400 nm, attributed to coumarin-like substances. The expediency of using polarization microscopy in the diagnosis of specific conducting elements in powdered angelica raw material has been proven. The expediency of using the histochemical reaction with Sudan III solution, which diagnoses schizogenous cavities, secretion droplets, and vascular element cell walls, has been substantiated.

GC-MS analysis of the lipophilic fractions of the water-alcoholic extract and essential oil revealed a total of 79 components, including terpenes and coumarins. It was established that osthol is the leading compound in the coumarin class in the water-alcoholic extract

(up to 49.72%), while in the essential oil, epiglobulol plays a key role (25.61%). This distribution of dominant compounds from different BAC groups confirms the need for separate standardization of polar and non-polar fractions of RHM.

The content of the sum of coumarins determined by the spectrophotometric method was $1.50 \pm 0.08\%$ calculated as coumarin, which corresponds to literature

data and can be proposed as a standard in the draft pharmacopoeial monograph. Despite the indicated limitations related to the use of coumarins as a reference standard, the method demonstrates high precision and is suitable for analyzing the quality of RHM.

Thus, the present study contributes to the standardization of *A. archangelica* RHM and enhances the quality control of herbal preparations based on it.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Denis I. Shishkalov — investigation, writing—original draft, writing—review & editing; Ifrat N. Zilfikarov — conceptualization, administration, writing—review & editing, writing—review & editing; Vitaly M. Ryzhov — investigation, visualization, writing—review & editing; Tatyana K. Ryazanova — investigation, formal analysis, writing—review & editing; Timur A. Ibragimov — writing—original draft, writing—review & editing; Madina A. Abdurakhmanova — investigation, formal analysis, writing—review & editing; Vera V. Artemyeva — writing—review & editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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The relationship between the pharmacogenetics and pharmacokinetics of CFTR modulators in a population of children with cystic fibrosis

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The triple combination of elexacaftor / tezacaftor / ivacaftor is one of the key tools for targeted pharmacotherapy of cystic fibrosis. In the pediatric population, its use may be accompanied by a variable therapeutic response, which may be due to age-related pharmacokinetic features, as well as the influence of genetic polymorphism of biotransformation enzymes.

The aim. To analyze the pharmacokinetic parameters of elexacaftor, tezacaftor, ivacaftor, and lumacaftor in patients with cystic fibrosis, and to study the influence of various polymorphisms of cytochrome P450 genes (*CYP2C6*4*, *CYP2C19*2*, *CYP2D6*4*) and the *UGT1A1* gene on pharmacokinetics.

Materials and Methods. The prospective single-center study included 32 patients with cystic fibrosis aged 6 to 18 years, of whom 29 received the combination of elexacaftor / tezacaftor / ivacaftor, and 3 received the dual combination of ivacaftor / lumacaftor. Analyte concentrations in plasma were determined by HPLC-MS/MS at five time points within 12 hours after drug administration. Molecular genetic analysis was performed on total DNA isolated from whole blood leukocytes using the phenol-chloroform extraction method.

Results. Significant inter-individual variability in the pharmacokinetic parameters of all studied CFTR modulators was revealed. Normalized maximum concentrations of elexacaftor and ivacaftor were significantly higher in patients aged ≥ 12 years ($p = 0.027$ and $p = 0.046$, respectively). Carriage of the *CYP2C9*4* (GG) genotype, associated with slow metabolism, led to a statistically significant increase in the systemic exposure of elexacaftor ($p = 0.007$) and ivacaftor ($p = 0.034$). For the *CYP2D6*4* (AA) genotype, a significant increase in elexacaftor concentration 3 hours after administration was found ($p = 0.041$). Polymorphism of the *UGT1A1* gene, including genotypes associated with Gilbert's syndrome, did not have a significant effect on the total exposure of the studied drugs, while lower concentrations of tezacaftor and elexacaftor were noted at individual time points in carriers of the *28 allele.

Conclusion. The pharmacokinetics of CFTR modulators in children are characterized by high variability and depend on age and the polymorphism of genes encoding CYP2C9 and CYP2D6 isoenzymes. The obtained data justify the need for further research to optimize dosing, taking into account genetic and demographic factors.

Keywords: CFTR modulators; pharmacokinetics; pharmacogenetics; polymorphism of xenobiotic biotransformation genes

Abbreviations: CV — cystic fibrosis; FEV — forced expiratory volume in 1 second; PK — pharmacokinetics; TDM — therapeutic drug monitoring.

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Взаимосвязь фармакогенетики и фармакокинетики CFTR-модуляторов в популяции детей с муковисцидозом

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Тройная комбинация элексафтор/тезафтор/ивафтор является одним из ключевых инструментов таргетной фармакотерапии муковисцидоза. В педиатрической популяции ее применение может сопровождаться вариабельным терапевтическим ответом, в основе чего могут лежать возрастные особенности фармакокинетики, а также влияние генетического полиморфизма ферментов биотрансформации.

Цель. Проанализировать фармакокинетические параметры элексафтора, тезафтора ивафтора и лумафтора у пациентов с муковисцидозом, а также изучить влияние различных полиморфизмов генов цитохромов P450 (*CYP2C6*4*, *CYP2C19*2*, *CYP2D6*4*) и гена *UGT1A1* на фармакокинетику.

Материалы и методы. В проспективное одноцентровое исследование включено 32 пациента с муковисцидозом в возрасте от 6 до 18 лет, из них 29 получали комбинацию элексафтор/тезафтор/ивафтор, 3 — двойную комбинацию ивафтор/лумафтор. Концентрации анализов в плазме определяли методом ВЭЖХ-МС/МС в пяти временных точках в течение 12 ч после приёма препаратов. Молекулярно-генетический анализ проводили на тотальной ДНК, выделенной из лейкоцитов цельной крови с помощью метода фенол-хлороформной экстракции.

Результаты. Выявлена значительная межиндивидуальная вариабельность фармакокинетических параметров всех изученных CFTR-модуляторов. Нормированные максимальные концентрации элексафтора и ивафтора были значительно выше у пациентов в возрасте ≥ 12 лет ($p=0,027$ и $p=0,046$ соответственно). Носительство генотипа *CYP2C9*4* (GG), ассоциированного с медленным метаболизмом, приводило к статистически значимому повышению системной экспозиции элексафтора ($p=0,007$) и ивафтора ($p=0,034$). Для генотипа *CYP2D6*4* (AA) выявлено значимое повышение концентрации элексафтора через 3 ч после приёма ($p=0,041$). Полиморфизм гена *UGT1A1*, включая генотипы, ассоциированные с синдромом Жильбера, не оказывал значимого влияния на суммарную экспозицию изученных препаратов, при этом отмечены более низкие концентрации тезафтора и элексафтора в отдельных точках у носителей аллеля *28.

Заключение. Фармакокинетика CFTR-модуляторов у детей характеризуется высокой вариабельностью и зависит от возраста и полиморфизма генов, кодирующих изоферменты CYP2C9 и CYP2D6. Полученные данные обосновывают необходимость дальнейших исследований для оптимизации дозирования с учетом генетических и демографических факторов.

Ключевые слова: CFTR-модуляторы; фармакокинетика; фармакогенетика; полиморфизм генов биотрансформации ксенобиотиков

Список сокращений: МВ — муковисцидоз; ОФВ1 — объём форсированного выдоха за первую секунду; ФК — фармакокинетика; ТЛМ — терапевтический лекарственный мониторинг.

INTRODUCTION

Cystic fibrosis (CF) is a disease caused by mutations in the gene for the CF transmembrane conductance regulator (*CFTR*). The *CFTR* protein functions as a channel for chloride and bicarbonate ions, maintaining normal epithelial secretion in the respiratory system and other organs [1]. The most common mutation leading to CF is the deletion of phenylalanine at position 508 (F508del)¹. In Russia, 275 pathogenic variants of the *CFTR* gene have been identified, 26 of which are not present in international databases [2].

For a long time, CF therapy was limited to symptomatic approaches: physical airway clearance techniques, enzyme replacement therapy, and antibacterial therapy [3]. The effectiveness of these approaches was low, as evidenced by the high need for lung transplantation in CF patients [4]. The identification of the *CFTR* gene in 1989 facilitated the development of targeted therapy—*CFTR* modulators, small molecules that restore the function of the defective protein [5].

Ivacaftor, a *CFTR* potentiator, increases the probability of channel opening and is effective for mutations that impair its function (*G551D*, etc.) [6]. Initially approved for patients ≥ 6 years old with the *G551D* mutation, it is now approved for ages ≥ 1 month with any of 97 sensitive mutations [7]. The key outcome is a 78% reduction in mortality and an 89% reduction in lung transplants [8]. The combination of ivacaftor with lumacaftor, a corrector that enhances *CFTR* protein transport to the membrane, is approved for F508del homozygotes [3, 8]. Monotherapy with lumacaftor showed minimal efficacy, whereas the combination with ivacaftor demonstrated significant improvement in patients aged 2 years and older [9, 10].

The next stage was the introduction of tezacaftor, a next-generation corrector with a better safety profile and a lower risk of drug-drug interactions [11, 12]. The triple combination of elexacaftor / tezacaftor / ivacaftor, approved in 2019 for patients with at least one F508del mutation, is recognized as the most effective [3, 13]. A meta-analysis of 6 studies ($n = 1127$) confirmed a significant increase in forced expiratory volume in 1 second (FEV1) and a reduction in the frequency of pulmonary exacerbations with satisfactory safety [14]. In 2023, the combination received approval in the US and Europe for children aged 2 to 5 years [15].

Despite increased efficacy, the results

of triple therapy show high variability, and data in populations younger than 12 years are still limited [14]. The key reason is inter-individual differences in pharmacokinetics (PKs). The study by S.E.M. Vonk et al. (2025) revealed a significant spread in the area under the curve (AUC) values of the triple combination components between and within age groups: the highest exposure was recorded in children aged 6–11 years with a body weight of ≥ 30 kg, raising questions about the adequacy of dosing [16].

All components of the triple combination are metabolized by CYP3A4/5, have a $T_{\max}$ of 3–6 hours, $> 99\%$ protein binding, and are primarily excreted in feces [13]. A systematic review of 20 studies on the PK characteristics of *CFTR* modulators revealed significant variability in dose-normalized area under the curve (AUC_D) values. For elexacaftor, it ranged from 0.71 to 1.63 ($\mu\text{g/mL}\cdot\text{h}$)/mg, with the greatest variability observed in children younger than 6 years [17]. In studies of ivacaftor PK in adults, the dose-normalized trough concentration ($C_{\min-D}$) ranged from 0.0039 to 0.0063 ($\mu\text{g/mL}$)/mg, and in the pediatric population, it ranged from 0.004 to 0.013 ($\mu\text{g/mL}$)/mg [17]. Tezacaftor showed less variability in AUC in adult PK studies (ranging from 82.2 to 92.4 $\mu\text{g/mL}\cdot\text{h}$) compared to children (ranging from 0.86 to 1.34 ($\mu\text{g/mL}\cdot\text{h}$)/mg) [17]. Studies evaluating the PK of lumacaftor in children found greater variability in AUC₀₋₁₂ values in patients aged 2 to 5 years with higher weight (<14 kg and ≥ 14 kg) and receiving a higher dose (100 mg and 150 mg) [18]. Similar trends were observed in the results of an open-label Phase III study involving children younger than 2 years with CF, homozygous for F508del-*CFTR* [19].

Such differences in systemic exposure create a risk of overdose or sub-therapeutic concentrations. One reason for the variability in exposure parameters may be the polymorphism of genes encoding biotransformation enzymes, primarily cytochrome P450 isoforms. Carriage of UGT1A1 mutations (Gilbert's syndrome), associated with an increased risk of hepatotoxicity during *CFTR* modulator therapy, may also contribute [20, 21].

Optimization of *CFTR* modulator dosing regimens in the pediatric population remains a pressing issue, requiring PK studies to be conducted in parallel with the investigation of the potential role of genetic polymorphisms as predictors of PK changes.

THE AIM is to analyze the PK parameters of elexacaftor, tezacaftor, ivacaftor, and lumacaftor in patients with CF and to assess the influence of

¹ The register of patients with cystic fibrosis in the Russian Federation. The year 2023; Amelina EL, Kashirskaya NYu, Kondratyeva E.I, Krasovsky SA, Starinova MA, Voronkova AYU, Ginter EK, editors. Moscow: Publishing House "MEDPRACTICA-M"; 2025. DOI: 10.61726/1981.2025.26.16.001. Russian

CYP2C9*3, CYP2C19*2, CYP2D6*4, and UGT1A1 gene polymorphisms on them.

MATERIALS AND METHODS

Study Design

A prospective, single-center, observational study with an interventional PK cohort was conducted. The study period was from April 1, 2024, to September 23, 2024. A total of 32 patients aged 6 to 18 years were included in the study.

The study was approved by the Local Ethics Committee of the Research Centre for Medical Genetics (Protocol No. 1 dated March 18, 2024). Informed consent (in writing, including consent for participation in the study and for the publication of obtained data without identifying personal information of the study participant) was obtained from all participants, their parents, or legal guardians before any study-related procedures were performed.

Inclusion and Exclusion Criteria

Key inclusion criteria: children under 18 years of age with at least one F508del mutation or a mutation sensitive to the triple combination elexacaftor / tezacaftor / ivacaftor and receiving the combination elexacaftor / tezacaftor / ivacaftor, or ivacaftor / lumacaftor according to standard treatment protocols. As a result, 29 patients received elexacaftor / tezacaftor / ivacaftor. Doses of 100 / 50 / 75 mg were used in 24 patients, 50 / 25 / 37.5 mg in 4 patients, and 25 / 12.5 / 18.75 mg in 1 patient. Three patients received the ivacaftor / lumacaftor combination (2 of them at a dose of 125 / 100 mg and 1 at a dose of 125 / 200 mg).

Key exclusion criteria: concomitant use of drugs that are inhibitors or inducers of CYP and/or UGT enzymes within 14 days prior to blood collection, use of CFTR modulators for less than 6 days (the period specified by the manufacturer for achieving optimal effective concentration), and absence of a signed informed consent.

Concentrations of drugs

Concentrations of elexacaftor, ivacaftor, lumacaftor, and tezacaftor were determined in plasma samples at 5 time points (before the next dose of the drug—0 h, and 3 h, 6 h, 9 h, and 12 h after the start of administration). For each time point, 5 aliquots of blood plasma of at least 300 µL were obtained. The maximum concentration (C_{max}) over a 12-hour period after drug administration; the time to reach C_{max} in plasma (T_{max}) over a 12-hour period after drug administration; the

area under the “concentration-time” curve (AUC_{0-12}) after drug administration until the last measurement exceeding the limit of quantification, within 12 hours of observation; and dose-normalized C_{max} (C_{max}/D) and AUC (AUC_{0-12}/D) were evaluated.

Ivacaftor, tezacaftor, lumacaftor, and elexacaftor were obtained from Selleck Chemicals (14408 Sylvanfield Dr, Houston, TX 77014, USA). Hydrochloric acid, methanol (methyl alcohol), and acetonitrile were provided by Thermo Fisher Scientific (168 Third Avenue, Waltham, Massachusetts, 02451, USA), and formic acid by Panreac Química SLU (Carrer del Garraf, 2, 08211 Castellar del Vallès, Barcelona, Spain).

Software used in the work: Agilent Mass Hunter Workstation Software package, versions Data Acquisition v. B.08.02, Qualitative Navigator v. B.08.00, Qualitative Workflow v. B.08.00.

Concentrations of tezacaftor, ivacaftor, elexacaftor, and lumacaftor in plasma samples were determined by high-performance liquid chromatography (HPLC) with mass spectrometric (MS) detection in a single plasma sample. This method was developed by the authors and validated. Samples were analyzed on an Agilent 1200 liquid chromatograph (including a quaternary pump, mobile phase degasser, and column oven). An Agilent Polaris 3 C18-A column (5.0 × 50 mm, 5 µm) was used in the study. Separation was carried out at a column temperature of 40°C. The mobile phase consisted of two components: solution «A» (1 mL of concentrated formic acid diluted with deionized water to a total volume of 1 L) and solution «B» (1 mL of concentrated formic acid diluted with acetonitrile to a total volume of 1 L). Chromatographic separation was performed in gradient elution mode. The injection volume was 5 µL. Analysis was performed within 10 minutes. Under these conditions, the retention times (RT) of the chromatographic peaks of the analyzed compounds were: RT(tezacaftor) = 1.9 min; RT(lumacaftor) = 4.4 min; RT(ivacaftor) = 4.4 min; RT(elexacaftor) = 7.2 min (Fig. 1).

Mass spectra of tezacaftor, ivacaftor, lumacaftor, and elexacaftor were recorded in multiple reaction monitoring (MRM) mode on an Agilent TripleQuad LC/MS 6410 mass spectrometer (triple quadrupole type) after positive electrospray ionization. Nebulizer gas pressure was 35 psi. Drying gas flow rate was 10 L/min, ion source temperature was 350 °C. Fragmentor voltage was 135 V; collision cell voltages were: for elexacaftor—30 V, for ivacaftor—35 V, for lumacaftor—40 V, and for tezacaftor—20 V. The analytical range for the determination of target compound concentrations was 0.1–50 µg/mL.

Preparation of blood plasma samples was carried out by protein precipitation using a mixture of methanol and 0.1% hydrochloric acid solution. Plasma samples were thawed at room temperature. Then, 100 μ L of plasma was transferred to Eppendorf-type plastic tubes, 250 μ L of a mixture of methanol and 0.1% hydrochloric acid solution (in a 9 : 1 ratio) was added, mixed on a Vortex shaker, and incubated for 10 min. The samples were then mixed again. The resulting samples were centrifuged at 10,000 rpm for 10 min using an Eppendorf MiniSpin centrifuge (Germany). The supernatant was transferred to chromatography vials and placed in the chromatograph's autosampler for analysis.

Validation

The bioanalytical method was validated in accordance with the Rules for Conducting Bioequivalence Studies of Medicinal Products within the Eurasian Economic Union², the M10 Guideline on Bioanalytical Method Validation of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use³, and the Food and Drug Administration's Guidance for Industry on Bioanalytical Method Validation⁴ (2018).

Validation covered the following parameters: selectivity, lower limit of quantitation (LLOQ), response function and analytical range, accuracy, precision, matrix effect, and stability. Concentrations of tezacaftor, ivacaftor, lumacaftor, and elexacaftor in plasma were determined by direct calibration. The method's limit of sensitivity was 0.01 μ g/mL, LLOQ was 100 ng/mL, and the analytical range was from 0.1 μ g/mL to 50 μ g/mL. To construct the calibration curve within the analytical cycles of patient plasma sample analysis, calibration solutions with the following concentrations were used: 0.1 μ g/mL, 0.25 μ g/mL, 0.5 μ g/mL, 1 μ g/mL, 2.5 μ g/mL, 5 μ g/mL, 10 μ g/mL, 25 μ g/mL, and 50 μ g/mL. Calibration solutions and quality control solutions were prepared as follows: 10 μ L of the corresponding working solutions (diluted 10-fold with matrix) were added to 90 μ L of analyte-free plasma, followed by vortex mixing.

² Eurasian Economic Commission. Decision of the Eurasian Economic Commission Council No. 85. Rules for conducting bioequivalence studies of medical products within the framework of the Eurasian Economic Union. November 3, 2016.

³ ICH M10 on bioanalytical method validation – Scientific guideline. Available from: <https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation-scientific-guideline>

⁴ Bioanalytical Method Validation Guidance for Industry. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry>

Molecular Genetic Analysis

Molecular genetic analysis was performed on total DNA extracted from whole blood leukocytes using the standard phenol-chloroform extraction method (volume of 100 μ L to 200 μ L of whole-genome DNA). For 9 patients, nucleotide sequencing was performed by direct automated Sanger sequencing on an Applied Biosystems instrument (USA) according to the manufacturer's protocol. Analysis of extended *CFTR* gene rearrangements was performed on the same instrument using quantitative MLPA (multiplex ligation-dependent probe amplification) [22]. Sequencing data processing was carried out using the standard automated algorithm provided by Thermo Fisher Scientific (Torrent Suite™), as well as Gene-Talk and NGSDData software.

Data Analysis

Based on the obtained data on plasma concentrations of tezacaftor, ivacaftor, elexacaftor, and lumacaftor, PK parameters were calculated using a model-independent method (AUC_{0-12} ; AUC_{0-12-D} ; C_{max} ; C_{max-D} ; T_{max}). For each PK parameter, the minimum (min) and maximum (max) values, mean (M) and median (Me), 25% and 75% quartiles (Q1 ; Q3), coefficient of variation (CV), standard deviation (SD), and standard error of the mean (SE) were calculated. Statistical analysis, data visualization, and PK parameter calculation were performed using the R programming language version 4.4.1 (R Core Team) in the RStudio integrated development environment version 2024.09.0 Build 375 (Posit PBC) with additional use of the tidyverse package version 2.0.0 [23], ggplot2 version 3.5.1 [24], and PKNCA version 0.11.0 [25]. Analysis of significant differences between the PK parameters of the studied drugs was performed in two age groups (< 12 years and $\geq$ 12 years) using Welch's t-test. Differences were considered significant at $p < 0.05$.

RESULTS

Characteristics of the Study Population

A total of 32 patients were included in the study. The clinical and demographic characteristics of the study population are presented in Table 1.

Genetic Study

Genetic analysis was performed in the first stage. A "mild" genotype was identified in 15.63% (5 out of 32) of patients, and a "severe" genotype in 84.38% (27 out of 32). The analysis revealed that 37.5% (12 out of 32) of patients were homozygotes, with the

F508del/F508del variant observed in 91.67%. Heterozygotes were identified in 62.5%, among whom the proportion of *F508del/non-F508del* was 80% (16 out of 20) (Table 2).

The next stage of the study involved determining the concentrations of *CFTR* modulators in plasma samples obtained from patients (total number of blood plasma samples analyzed in the study is 154). When calculating PK parameters, the dose of each drug was calculated considering the patient's weight (mg/kg). PK parameters of tezacaftor, calculated from the analysis of plasma samples from 29 patients, are presented in Table 3. The average age of the population, including 29 patients, was 13.13 ± 3.0 (min = 6.6; max = 17.9) years, and the average body weight was 45.55 ± 13.45 (min = 19.5; max = 80.0) kg. In the analysis of patient subgroups <12 years ($n = 11$) and ≥ 12 years ($n = 18$), significant differences were found only for the drug dose (mg/kg), $p = 0.012$.

Individual PK curves of tezacaftor, constructed based on plasma concentration data from patients, are shown in Figure 2.

PK parameters of elexacaftor are presented in Table 4. Comparison of parameters in patient populations < 12 years ($n = 11$) and ≥ 12 years ($n = 18$) revealed a significantly higher $C_{\max}$ -D value in the second group ($p = 0.027$), despite these patients receiving a lower drug dose (mg/kg) ($p = 0.013$).

PK parameters of ivacaftor are presented in Table 5. Data were collected from 32 patients receiving the elexacaftor / tezacaftor / ivacaftor combination and from 3 patients receiving the ivacaftor/lumacaftor combination. As with elexacaftor, for ivacaftor, we found a significantly higher $C_{\max}$ -D value ($p = 0.046$) in patients ≥ 12 years old, along with a significantly lower drug dose (mg/kg) ($p = 0.041$).

Data on PK parameters of lumacaftor were obtained from plasma samples of 3 patients (ages: 14.3, 10.3, and 9.4 years) (Table 6). The small number of patients limits the analysis of the obtained data.

The next stage of the work is dedicated to assessing the influence of different genotypes on the PK parameters of *CFTR* modulators.

The values of AUC_{0-12} -D and $T_{\max}$ for different genotypes of the *CYP2C6**4 polymorphic variant of the *CYP2C6* gene ($M \pm SD$) are presented in Table 7. The results indicated that the GG genotype (associated with "slow" metabolism) leads to a statistically significant ($p < 0.05$) increase in systemic exposure to two of the three studied drugs (elexacaftor and ivacaftor).

The assessment of the quantitative content of tezacaftor ($\mu\text{g/mL}$) in blood plasma samples of CF

patients with different genotypes of the *CYP2C19**2 polymorphic variant of the *CYP2C1* gene did not reveal significant differences (Table 8).

The results of assessing the influence of different genotypes of the *CYP2D6**4 polymorphic variant of the *CYP2D6* gene on the content of elexacaftor ($\mu\text{g/mL}$) in blood plasma samples of CF patients are presented in Table 9. A significantly higher concentration of elexacaftor was noted at the 3-hour time point in the group of slow metabolizers (AA genotype).

The assessment of the influence of genotype (in Gilbert's syndrome) revealed a number of significant differences in the quantitative content of *CFTR* modulators (Table 10).

Analysis of PK parameters (Table 11) did not reveal a statistically significant influence of *UGT1A1* gene polymorphism (including variants causing Gilbert's syndrome) on the systemic exposure of the studied drugs (tezacaftor, elexacaftor, and ivacaftor).

DISCUSSION

The presented results were obtained in a population of 32 patients with CF aged 6.6 to 17.9 years. Genetic analysis confirmed the prevalence of the "severe" genotype (84.4% of patients are carriers of the *F508del* mutation), associated with the classic phenotype of the disease. The proportion of homozygotes for the *F508del* mutation was 34.4% ($n = 11$) of the total sample. The *F508del/non-F508del* variant was predominant among heterozygotes (80%). According to international data, the *F508del* mutation dominates among others: the frequency for patients in the USA is 79%, in Europe—59%, in Brazil—44% [26]. In the study by A. Orenti et al. (2023), it was demonstrated that for *F508del/non-F508del* heterozygotes compared to homozygotes, the prognosis of CF is less favorable: starting from childhood, they showed a significantly faster deterioration of respiratory function, assessed by the decrease in FEV1, a higher frequency of *Pseudomonas aeruginosa* infection, and higher mortality rates [27]. At the same time, it is important to note that over a 10-year period (from 2011 to 2021), a stable trend towards improvement in FEV1 indicators was observed in the general population of European CF patients: among children, the percentage of predicted value increased from 85% to 94.2%, and among adults—from 63.6% to 74.7% [28]. According to the authors, the greatest improvement was recorded in carriers of the *F508del* mutation in 2021, which is associated with the initiation of active use of the elexacaftor / tezacaftor / ivacaftor

combination. According to the British CF patient registry, the total proportion of CF patients for whom this drug combination is not suitable is only 8.6% (852 out of 9887)—these are patients without the F508del mutation [29]. A systematic review of studies conducted in various countries using national CF patient registries (57 included in the analysis) established that the elexacaftor / tezacaftor / ivacaftor combination was characterized by the most pronounced improvement in lung condition, despite some variability in results [30]. The noted variability may be a consequence of changes in the PK parameters of *CFTR* modulators, which justifies the importance of conducting PK studies in different patient populations.

Our comprehensive assessment of the PK parameters of elexacaftor, tezacaftor, ivacaftor, and lumacaftor revealed some influence of age in patients with CF on the PK parameters of the studied drugs. For elexacaftor and ivacaftor, a unidirectional trend was observed: patients aged ≥ 12 years had significantly higher $C_{\max}$ -D values ($p = 0.027$ for elexacaftor, $p = 0.046$ for ivacaftor), despite adolescents receiving significantly lower weight-based doses (mg/kg) of both drugs compared to the < 12 years group ($p = 0.013$ and $p = 0.041$, respectively). The obtained data indicate significant age-related differences in the pharmacokinetics of these *CFTR* modulators, likely due to changes in metabolic enzyme activity and volume of distribution during growth.

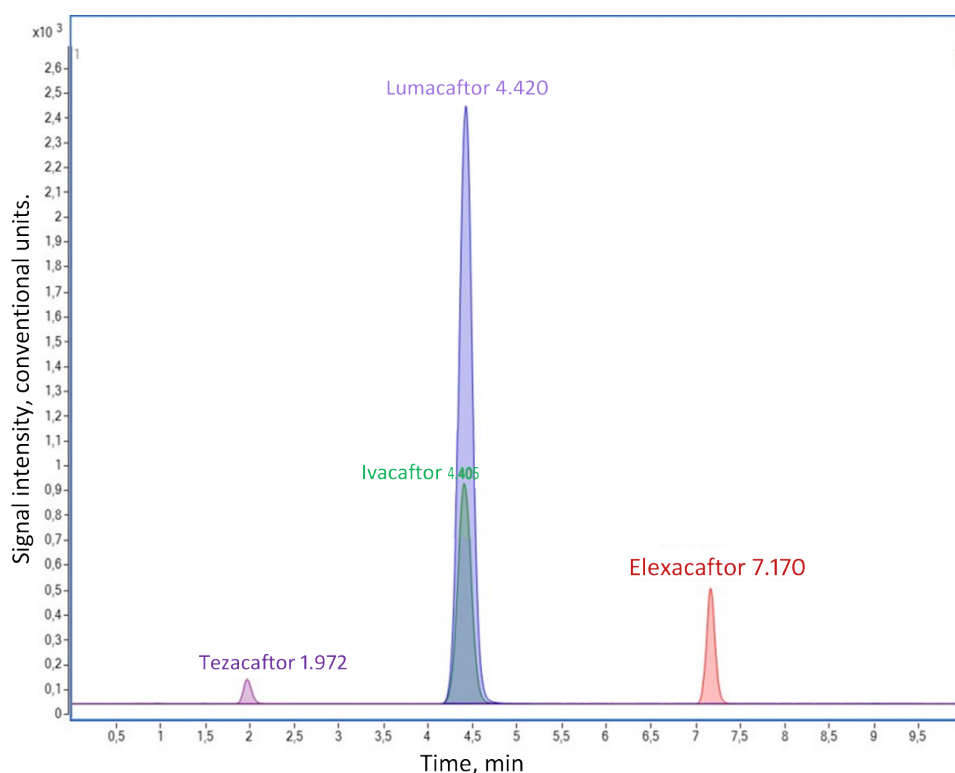


Figure 1 — Chromatographic-mass spectrometric profile of a plasma standard sample containing the analyzed compounds at a concentration of 1 $\mu\text{g/mL}$.

Table 1 — Clinical and demographic characteristics of the study population

Parameter	Value			
	M $\pm$ SD	min	max	n (%)
Age at the time of study participation, years	12.95 $\pm$ 2.97	6.60	17.90	—
Age at initial CF diagnosis, years	1.33 $\pm$ 2.11	0	15.0	—
Sex:	—	—	—	—
	Female			18(56.25)
	Male			14 (43.75)
Weight, kg	44.07 $\pm$ 13.45	19.5	80.0	—

Note: CF, cystic fibrosis.

**Table 2 — Structure of homozygotes and heterozygotes
(F508del/F508del, F508del/non-F508del, non-F508del/non-F508del)**

Homozygotes (n = 12)		Heterozygotes, total 20	
Type	n (%)	Type	n (%)
F508del/F508del	11 (91.67)	F508del/non-F508del:	16 (80.00)
R1066C/R1066C	1 (8.33)	F508del/2184insA	1 (6.25)
		F508del/406-1G->A	1 (6.25)
		F508del/3849+10KbC->T	1 (6.25)
		F508del/CFTRdele2,3	5 (31.25)
		F508del/L1335P	1 (6.25)
		F508del/L138ins	1 (6.25)
		F508del/S466X;R1070Q	2 (12.5)
		F508del/p.Tyr84X	1 (6.25)
		F508del/R553X	1 (6.25)
		F508del/2143delT	1 (6.25)
		F508del/S1196X	1 (6.25)
		non-F508del/ non-F508del	4 (20.00)
		3272-16T>A/CFTRdele9	1 (25)
		CFTRdele2,3/E92K	1 (25)
		CFTRdele2,3/4015	1 (25)
		G85E/CFTRdele2,3	1 (25)

Table 3 — Pharmacokinetic parameters of tezacaftor in the studied pediatric population (n = 29)

Parameter	Value			
	M ± SD	Me (Q1 ; Q3)	[min ; max]	Welch's t-test
AUC _{0-12'} , h×μg/mL (total population, n = 29)	83,43 ± 40,69	86.61 (54.41 ; 110.83)	[17.48 ; 208.11]	n/a
AUC _{0-12'} , h×μg/mL (population < 12 years, n = 11)	90,70 ± 50,25	90.73 (44.29 ; 116.1)	[27.9 ; 208.11]	t = 0.68, df = 15, p = 0.51
AUC _{0-12'} , h×μg/mL (population ≥ 12 years, n = 18)	78,99 ± 34,44	81.26 (56.1 ; 95.9)	[17.48 ; 144.2]	
AUC _{0-12—} D, h×μg/mL (total population, n = 29)	80,65 ± 42,79	81.28 (45.55 ; 104.3)	[22.32 ; 227.41]	n/a
AUC _{0-12—} D, h×μg/mL (population < 12 years, n = 11)	72,74 ± 36,76	74.40 (40.40 ; 112.62)	[22.32 ; 129.03]	t = 0.819, df = 25, p = 0.42
AUC _{0-12—} D, h×μg/mL (population ≥ 12 years, n = 18)	85,49 ± 46,42	83.38 (49.74 ; 102.62)	[22.72 ; 227.41]	
C _{max'} , μg/mL (total population, n = 29)	10,0 ± 4,78	10.21 (5.98 ; 12.56)	[1.83 ; 25.45]	n/a
C _{max'} , (population < 12 years, n = 11)	11,27 ± 5,79	11.38 (6.05 ; 13.92)	[4.78 ; 25.45]	t = 1.025, df = 15, p = 0.32
C _{max'} , (population ≥ 12 years, n = 18)	9,23 ± 4,02	10.03 (5.90 ; 11.62)	[1.83 ; 15.64]	
C _{max—} D, μg/mL (total population, n = 29)	9,64 ± 5,15	9.44 (5.35 ; 12.13)	[2.38 ; 28.36]	n/a
C _{max—} D, (population < 12 years, n = 11)	8,95 ± 4,04	7.56 (5.16 ; 12.44)	[4.06 ; 15.78]	t = 0.605, df = 26, p = 0.55
C _{max—} D, (population ≥ 12 years, n = 18)	10,06 ± 5,79	9.49 (7.14 ; 11.82)	[2.38 ; 28.36]	
T _{max'} , h (total population, n = 29)	3,62 ± 1,24	3.0 (3.0 ; 3.0)	[3.0 ; 6.0]	n/a
T _{max'} , h (population < 12 years, n = 11)	4,09 ± 1,51	3.0 (3.0 ; 6.0)	[3.0 ; 6.0]	t = 1.484, df = 15, p = 0.16
T _{max'} , h (population ≥ 12 years, n = 18)	3,33 ± 0,97	3.0 (3.0 ; 3.0)	[3.0 ; 6.0]	
Dose, mg/kg (total population, n = 29)	1,09 ± 0,31	1.11 (0.83 ; 1.30)	[0.41 ; 1.67]	n/a
Dose, mg/kg (population < 12 years, n = 11)	1,26 ± 0,25	1.25 (1.03 ; 1.52)	[0.93 ; 1.67]	t = 2.69, df = 25, p = 0.012*
Dose, mg/kg (population ≥ 12 years, n = 18)	0,98 ± 0,31	0.94 (0.76 ; 1.22)	[0.41 ; 1.47]	

Note: * is significant differences at p < 0.05; n/a, not applicable.

Table 4 — Pharmacokinetic parameters of elelexaftor in the studied pediatric population ($n = 29$)

Parameter	Value			
	M $\pm$ SD	Me (Q1 ; Q3)	[min ; max]	Welch's t-test
AUC _{0-12^h} h $\times$ μ g/mL (total population, $n = 29$)	102,01 $\pm$ 47,39	88.70 (68.70 ; 121.84)	[25.86 ; 228.34]	n/a
AUC _{0-12^h} h $\times$ μ g/mL (population < 12 years, $n = 11$)	99,93 $\pm$ 50,45	88.70 (71.59 ; 119.91)	[25.86 ; 228.34]	$t = 0.178$, $df = 20$, $p = 0.86$
AUC _{0-12^h} h $\times$ μ g/mL (population ≥ 12 years, $n = 18$)	103,28 $\pm$ 46,88	89.60 (62.87 ; 121.98)	[43.13 ; 198.6]	
AUC _{0-12^h} D, h $\times$ μ g/mL (total population, $n = 29$)	48,64 $\pm$ 21,14	41.07 (35.02 ; 63.98)	[10.34 ; 100.75]	n/a
AUC _{0-12^h} D, h $\times$ μ g/mL (population < 12 years, $n = 11$)	39,57 $\pm$ 16,43	38.54 (29.16 ; 49.16)	[10.34 ; 70.78]	$t = 2.031$, $df = 25$, $p = 0.053$
AUC _{0-12^h} D, h $\times$ μ g/mL (population ≥ 12 years, $n = 18$)	54,19 $\pm$ 22,17	47.93 (39.39 ; 68.12)	[25.06 ; 100.75]	
C _{max^h} μ g/mL (total population, $n = 29$)	10,26 $\pm$ 4,06	8.74 (7.20 ; 12.12)	[4.25 ; 21.91]	n/a
C _{max^h} (population < 12 years, $n = 11$)	9,9 $\pm$ 4,33	8.74 (6.91 ; 11.82)	[4.34 ; 20.95]	$t = 0.339$, $df = 23$, $p = 0.74$
C _{max^h} (population ≥ 12 years, $n = 18$)	10,49 $\pm$ 4,87	9.29 (7.48 ; 12.76)	[4.25 ; 21.91]	
C _{max^h} D, μ g/mL (total population, $n = 29$)	4,86 $\pm$ 1,97	4.22 (3.57 ; 6.09)	[1.73 ; 9.57]	n/a
C _{max^h} D, (population < 12 years, $n = 11$)	3,93 $\pm$ 1,39	3.73 (2.69 ; 4.85)	[1.73 ; 6.49]	$t = 2.342$, $df = 26$, $p = 0.027^*$
C _{max^h} D, (population ≥ 12 years, $n = 18$)	5,44 $\pm$ 2,08	4.80 (3.82 ; 6.63)	[3.22 ; 9.57]	
T _{max^h} h (total population, $n = 29$)	6,41 $\pm$ 3,28	6.0 (4.5 ; 9.0)	[0 ; 12]	n/a
T _{max^h} h (population < 12 years, $n = 11$)	6,55 $\pm$ 2,25	6.0 (6.0 ; 6.0)	[3.0 ; 12.0]	$t = 0.188$, $df = 26$, $p = 0.85$
T _{max^h} h (population ≥ 12 years, $n = 18$)	6,33 $\pm$ 3,83	6.0 (3.0 ; 9.0)	[0 ; 12.0]	
Dose, mg/kg (total population, $n = 29$)	2,18 $\pm$ 0,63	2.22 (1.67 ; 2.60)	[0.82 ; 3.33]	n/a
Dose, mg/kg (population < 12 years, $n = 11$)	2,52 $\pm$ 0,49	2.50 (2.06 ; 3.03)	[1.85 ; 3.33]	$t = 2.672$, $df = 24$, $p = 0.013^*$
Dose, mg/kg (population ≥ 12 years, $n = 18$)	1,97 $\pm$ 0,62	1.88 (1.52 ; 2.44)	[0.82 ; 2.94]	

Note: * is significant differences at $p < 0.05$; n/a, not applicable.**Table 5 — Pharmacokinetic parameters of ivacaftor in the studied pediatric population ($n = 32$)**

Parameter	Value			
	M $\pm$ SD	Me (Q1 ; Q3)	[min ; max]	Welch's t-test
AUC _{0-12^h} h $\times$ μ g/mL (total population, $n = 29$)	31,51 $\pm$ 23,80	25.31 (16.90 ; 36.83)	[3.33 ; 124.63]	n/a
AUC _{0-12^h} h $\times$ μ g/mL (population < 12 years, $n = 11$)	29,94 $\pm$ 30,82	20.27 (14.08 ; 36.32)	[3.33 ; 124.63]	$t = 0.276$, $df = 17$, $p = 0.780$
AUC _{0-12^h} h $\times$ μ g/mL (population ≥ 12 years, $n = 18$)	32,58 $\pm$ 18,42	27.24 (22.85 ; 48.37)	[5.15 ; 76.60]	
AUC _{0-12^h} D, h $\times$ μ g/mL (total population, $n = 29$)	19,55 $\pm$ 12,80	19.98 (8.74 ; 26.62)	[0.73 ; 51.51]	n/a
AUC _{0-12^h} D, h $\times$ μ g/mL (population < 12 years, $n = 11$)	14,97 $\pm$ 13,56	9.37 (5.37 ; 22.33)	[0.73 ; 51.51]	$t = 1.677$, $df = 23$, $p = 0.107$
AUC _{0-12^h} D, h $\times$ μ g/mL (population ≥ 12 years, $n = 18$)	22,69 $\pm$ 11,58	21.73 (13.89 ; 32.35)	[1.78 ; 45.96]	
C _{max^h} μ g/mL (total population, $n = 29$)	3,67 $\pm$ 2,49	3.25 (2.37 ; 4.33)	[0.41 ; 12.23]	n/a
C _{max^h} (population < 12 years, $n = 11$)	3,37 $\pm$ 2,93	2.76 (1.63 ; 3.95)	[0.41 ; 12.23]	$t = 0.533$, $df = 20$, $p = 0.600$
C _{max^h} (population ≥ 12 years, $n = 18$)	3,88 $\pm$ 2,20	3.44 (2.66 ; 5.23)	[0.67 ; 9.25]	
C _{max^h} D, μ g/mL (total population, $n = 29$)	2,28 $\pm$ 1,46	2.12 (1.10 ; 3.17)	[0.09 ; 6.35]	n/a
C _{max^h} D, (population < 12 years, $n = 11$)	1,67 $\pm$ 1,32	1.21 (0.85 ; 2.26)	[0.09 ; 5.06]	$t = 2.089$, $df = 27$, $p = 0.046^*$
C _{max^h} D, (population ≥ 12 years, $n = 18$)	2,70 $\pm$ 1,44	2.55 (1.57 ; 3.43)	[0.25 ; 6.35]	
T _{max^h} h (total population, $n = 29$)	5,34 $\pm$ 2,82	6.0 (3.0 ; 6.0)	[0 ; 12.0]	n/a
T _{max^h} h (population < 12 years, $n = 11$)	4,85 $\pm$ 1,52	6.0 (3.0 ; 6.0)	[3.0 ; 6.0]	$t = 0.926$, $df = 26$, $p = 0.363$
T _{max^h} h (population ≥ 12 years, $n = 18$)	5,68 $\pm$ 3,45	6.0 (3.0 ; 6.0)	[0 ; 12.0]	
Dose, mg/kg (total population, $n = 29$)	1,89 $\pm$ 0,94	1.72 (1.29 ; 2.17)	[0.61 ; 5.10]	n/a
Dose, mg/kg (population < 12 years, $n = 11$)	2,35 $\pm$ 1,16	1.88 (1.63 ; 2.46)	[1.39 ; 5.10]	$t = 2.217$, $df = 16$, $p = 0.041^*$
Dose, mg/kg (population ≥ 12 years, $n = 18$)	1,57 $\pm$ 0,62	1.50 (1.14 ; 1.97)	[0.61 ; 3.33]	

Note: * is significant differences at $p < 0.05$; n/a, not applicable.

Table 6 — Pharmacokinetic parameters of lumacaftor in the studied pediatric population ($n = 3$)

Patient ID	AUC_{0-12} , $h \times \mu g/mL$	AUC_{0-12-D} , $h \times \mu g/mL$	C_{max} , $\mu g/mL$	C_{max-D} , $\mu g/mL$	T_{max} , h	Dose, mg/kg
3	393.90	107.54	40.38	11.02	6	3.63
4	499.46	93.65	51.53	9.66	0	5.33
7	194.62	47.68	24.48	6.0	3	4.08
M	362.66	82.95	38.8	8.9	3	4.35
SD	154.8	31.33	13.59	2.6	3	0.88

Table 7 — Area under the pharmacokinetic curve in the 0–12 h interval, normalized to dose (AUC_{0-12-D}) time to maximum concentration (T_{max}) for different genotypes of the polymorphic variant $CYP2C6^*4$ of the $CYP2C6$ gene, $M \pm SD$

Parameter	Genotype			<i>p</i> -value
	AA (1) slow 1 (4.2%)	AG (2) 4 (16.7%)	GG (3) 19 (79.1%)	
Tezacaftor				
AUC _{0–12} –D	102.6	64.2 ± 33.7	87.3 ± 46.2	<i>p</i> _{2–3} = 0.056
T _{max}	3	3.7 ± 1.3	3.5 ± 1.5	<i>p</i> _{2–3} = 0.040*
Elexacaftor				
AUC _{0–12} –D	50.0	41.1 ± 11.2	52.1 ± 24.5	<i>p</i> _{2–3} = 0.007*
T _{max}	3	6.7 ± 2.9	6.5 ± 3.5	<i>p</i> > 0.05
Ivacaftor				
AUC _{0–12} –D	29.3	15.8 ± 11.7	21.1 ± 13.4	<i>p</i> _{2–3} = 0.034*
T _{max}	3	4.4 ± 2.1	6.0 ± 3.1	<i>p</i> > 0.05

Note: * is significant differences at $p < 0.05$.

Table 8 — Assessment of the quantitative content of tezacaftor ($\mu g/mL$) in plasma samples of patients with cystic fibrosis with different genotypes of the polymorphic variant $CYP2C19^*2$ of the $CYP2C19$ gene, $M \pm SD$

Time, h	Genotype			p -value
	AA (1) slow 1 (4.2%)	AG (2) 4 (16.7%)	GG (3) 19 (79.1%)	
0	2.1	2.4 ± 1.2	3.9 ± 2.8	$p > 0.05$
3	5.4	5.4 ± 2.4	11.3 ± 4.8	$p_{2-3} = 0.05$
6	12.7	4.8 ± 2.7	9.1 ± 3.7	$p > 0.05$
9	8.5	4.3 ± 2.6	6.9 ± 3.3	$p > 0.05$
12	5.5	3.3 ± 1.9	6.0 ± 3.1	$p > 0.05$

Table 9 — Assessment of the quantitative content of elexacaftor ($\mu g/mL$) in plasma samples of patients with cystic fibrosis with different genotypes of the polymorphic variant $CYP2D6^*4$ of the $CYP2D6$ gene, $M \pm SD$

Time, h	Genotype			p -value
	AA (1) slow 1 (4.2%)	AG (2) 7 (29.2%)	GG (3) 16 (66.6%)	
0	9.67	5.2 ± 3.7	8.4 ± 4.9	$p > 0.05$
3	11.1	7.6 ± 5.4	9.6 ± 4.7	$p_{2-3} = 0.041^*$
6	10.5	8.1 ± 4.9	10.7 ± 3.8	$p > 0.05$
9	10.1	7.7 ± 3.9	9.9 ± 4.2	$p > 0.05$
12	8.4	6.8 ± 3.2	9.2 ± 4.0	$p > 0.05$

Note: * is significant differences at $p < 0.05$.

Table 10 — Quantitative assessment of tezacaftor, elexacaftor, ivacaftor ($\mu\text{g/mL}$) in plasma samples of cystic fibrosis patients with different *UGT1A1* gene genotypes, $M \pm SD$

Time, h	Genotype		p-value
	1*/28*, 28*/28* (Gilbert's syndrome)—7 (29.2%)	1*/1* (3)—16 (66.6%)	
Tezacaftor			
0	2.2 ± 0.7	4.1 ± 2.9	p > 0.05
3	6.5 ± 2.9	11.5 ± 5.0	p = 0.044*
6	6.3 ± 3.5	9.5 ± 3.7	p > 0.05
9	4.4 ± 2.2	8.1 ± 2.2	p > 0.05
12	3.2 ± 1.7	6.5 ± 3.0	p > 0.05
Elexacaftor			
0	5.4 ± 2.6	8.4 ± 5.1	p > 0.05
3	6.2 ± 2.3	10.3 ± 5.1	p > 0.05
6	7.6 ± 2.6	10.9 ± 4.3	p = 0.030*
9	6.4 ± 1.7	10.5 ± 4.2	p > 0.05
12	5.8 ± 1.6	10.2 ± 4.1	p = 0.043*
Ivacaftor			
0	1.2 ± 0.9	3.1 ± 2.2	p > 0.05
3	4.4 ± 0.4	4.4 ± 2.7	p > 0.05
6	2.3 ± 1.0	4.1 ± 2.2	p > 0.05
9	1.4 ± 0.6	3.3 ± 2.0	p > 0.05
12	1.0 ± 0.5	3.0 ± 2.1	p = 0.032*

Note: * is a significant differences at $p < 0.05$.**Table 11 — Area under the pharmacokinetic curve in the 0–12 h interval, normalized to dose (AUC_{0-12-D}), and time to maximum concentration (T_{max}) for different genotypes of the *UGT1A1* polymorphic variant, $M \pm SD$**

Time, h	Genotype		<i>p</i> -value
	1*/28*, 28*/28* (Gilbert's syndrome)—7 (29.2%)	1*/1* (3)—16 (66.6%)	
Tezacaftor			
AUC _{0–12} –D	76.2 ± 61.7	82.6 ± 32.9	<i>p</i> > 0,05
T _{max}	3.7 ± 1.3	3.5 ± 1.5	<i>p</i> > 0,05
Elexacaftor			
AUC _{0–12} –D	45.5 ± 23.2	49.6 ± 20.7	<i>p</i> > 0,05
T _{max}	5.7 ± 3.2	6.8 ± 3.4	<i>p</i> > 0,05
Ivacaftor			
AUC _{0–12} –D	13.6 ± 10.8	22.7 ± 12.9	<i>p</i> > 0,05
T _{max}	4.4 ± 1.6	5.9 ± 3.2	<i>p</i> > 0,05

Our study found a high degree of variability in the PK parameters of *CFTR* modulators. For tezacaftor, the CV for AUC_{0-12} was 48.8%, and for C_{max} , it was 47.8%. Individual AUC_{0-12} values ranged from 17.48 to 208.11 $\text{h} \times \mu\text{g/mL}$ (a range of over 10-fold), and C_{max} ranged from 1.83 to 25.45 $\mu\text{g/mL}$ (a range of over 13-fold). Age analysis (< 12 years and ≥ 12 years) did not reveal differences in tezacaftor exposure parameters (AUC_{0-12} , C_{max} , as well as dose-normalized parameters) ($p > 0.05$). The only significant difference was a higher weight-based dose (mg/kg) in the < 12 years group (1.26 ± 0.25 vs. 0.98 ± 0.31 mg/kg, $p = 0.012$). Consequently, high inter-individual

variability in exposure for tezacaftor was determined, not directly related to age. The absence of significant differences for tezacaftor between age groups is consistent with published data indicating its predominantly linear PKs within the studied dose range [15, 16].

The variability in elexacaftor exposure is comparable to that of tezacaftor: CV% for AUC_{0-12} is 46.5%, and for C_{max} , it is 39.6%. The range of AUC_{0-12} values varied from 25.86 $\text{h} \times \mu\text{g/mL}$ to 228.34 $\text{h} \times \mu\text{g/mL}$ (an 8.8-fold range). Analysis of PK parameters in the < 12 and ≥ 12 years groups revealed several differences. For example, C_{max-D} was significantly

higher in children ≥ 12 years ($5.44 \pm 2.08 \mu\text{g/mL}$ vs. $3.93 \pm 1.39 \mu\text{g/mL}$, $p = 0.027$). For $\text{AUC}_{0-12\text{D}}$, the differences were borderline statistically significant ($54.19 \pm 22.17 \text{ h}\times\mu\text{g/mL}$ vs. $39.57 \pm 16.43 \text{ h}\times\mu\text{g/mL}$, $p = 0.053$), confirming the observed trend. Our findings suggest that patients aged ≥ 12 years exhibit higher elexacaftor exposure per unit dose compared to the < 12 years group.

In our study, ivacaftor demonstrated the highest inter-individual variability: CV% for $\text{AUC}_{0-12\text{D}}$ was 75.5%. The range of $\text{AUC}_{0-12\text{D}}$ values varied from $3.33 \text{ h}\times\mu\text{g/mL}$ to $124.63 \text{ h}\times\mu\text{g/mL}$ (a 37-fold range), and C_{max} ranged from $0.41 \mu\text{g/mL}$ to $12.23 \mu\text{g/mL}$ (a 30-fold range). Such high variability may be due to genetic polymorphism of the *CYP3A4* gene, responsible for ivacaftor metabolism, as well as differences in absorption. Similar to elexacaftor, statistically significant differences in $\text{C}_{\text{max-D}}$ were found for ivacaftor ($2.70 \pm 1.44 \mu\text{g/mL}$ in patients ≥ 12 years vs. 1.67 ± 1.32 in the < 12 years group, $p = 0.046$). This difference was observed despite a significantly lower weight-based dose in the older age group ($1.57 \pm 0.62 \text{ mg/kg}$ vs. $2.35 \pm 1.16 \text{ mg/kg}$, $p = 0.041$). Analysis of $\text{AUC}_{0-12\text{D}}$ generally supported a trend towards higher values in the ≥ 12 years group ($22.69 \pm 11.58 \text{ h}\times\mu\text{g/mL}$ vs. $14.97 \pm 13.56 \text{ h}\times\mu\text{g/mL}$), but the differences did not reach statistical significance ($p = 0.107$), which may be due to the high variability of the parameter.

Regarding the PK parameters of lumacaftor, our study was limited to only 3 patients, which did not allow for definitive conclusions.

Thus, it can be stated that the PK parameters of the studied *CFTR* modulators are characterized by extremely high inter-individual variability in exposure, justifying the expediency of therapeutic drug monitoring (TDM) for individualizing drug regimens [31]. High inter-individual variability in PK parameters was demonstrated in a population PK analysis of elexacaftor, tezacaftor, and ivacaftor (2025, 96 children aged 2 to 18 years) using one-compartment models. The authors showed that body weight significantly influenced apparent clearance and volume of distribution with allometric scaling. In children younger than 12 years weighing 30–40 kg, who received doses corresponding to adults (elaxacaftor 200 mg, tezacaftor 100 mg, ivacaftor 150 mg twice daily), higher exposure (AUC , C_{max} , C_{min}) was observed compared to adult patients with CF [15]. Another population PK study also confirmed that body weight is the primary covariate explaining the variability in PK parameters of tezacaftor and ivacaftor in the pediatric population [32]. According to a systematic review of 20 PK studies, the use of *CFTR* modulators in

children (ivacaftor, lumacaftor, tezacaftor, elexacaftor) is characterized by high inter-individual and intra-individual variability, primarily related to body weight and the maturity of enzymes metabolizing these drugs, as well as drug interactions and liver function [17].

Our pharmacogenetic analysis revealed a borderline but clinically significant difference in tezacaftor concentration at 3 hours post-dose between carriers of AG and GG genotypes of the *CYP2C19* polymorphic variant ($p = 0.05$). In GG homozygotes (“normal” metabolizers), the concentration at this time point ($11.3 \pm 4.8 \mu\text{g/mL}$) was more than double that in AG heterozygotes ($5.4 \pm 2.4 \mu\text{g/mL}$). This aligns with the significant difference in T_{max} for tezacaftor ($p = 0.040$) and suggests that *CYP2C19* genotype predominantly affects the early distribution phases of tezacaftor. The obtained data are consistent with a new understanding of the role of *CYP2C19* isoenzyme in tezacaftor metabolism, despite tezacaftor being primarily considered a *CYP3A4* substrate [33]. Analysis of the influence of *CYP2D6* gene polymorphism revealed several statistically significant differences. The GG homozygous genotype, associated with “poor” metabolism, was associated with significantly higher systemic exposure ($\text{AUC}_{0-12\text{D}}$) of elexacaftor ($p = 0.007$) and ivacaftor ($p = 0.034$) compared to AG heterozygotes. Hourly analysis of elexacaftor concentrations confirmed this trend—at 3 hours, the concentration in GG homozygotes was significantly higher than in AG ($9.6 \pm 4.7 \mu\text{g/mL}$ vs. $7.6 \pm 5.4 \mu\text{g/mL}$, $p = 0.041$). Our findings imply some involvement of *CYP2D6* in the metabolism of elexacaftor and ivacaftor, while published data from other researchers suggest no significant role of *CYP2D6* in the biotransformation of ivacaftor, which is mainly metabolized by *CYP3A* enzymes (*CYP3A4* and *CYP3A5*) [17, 34]. A similar pattern is characteristic of elexacaftor, which, according to published data, is primarily metabolized by *CYP3A4/5* [35].

A previously published study investigating the influence of various Phase I biotransformation enzyme genotypes on the efficacy and safety of *CFTR* modulators in patients with CF identified some significant associations [36]. It was found that patients with the AC genotype of the *CYP2C9*3* polymorphic variant showed improved body weight, height, and BMI after one year of *CFTR* modulator treatment compared to AA genotype carriers. Favorable effects regarding weight gain in patients with CF were observed in carriers of the GG genotype of the *CYP2D6*4* polymorphic variant. When examining the safety profile, the authors found that the GG genotype of *CYP2D6*4* polymorphism and the AA genotype of

*CYP2C19*2* polymorphism were associated with an increased frequency of adverse events, particularly elevated serum liver transaminases [36].

Contrary to expectations based on *UGT1A1*'s involvement in Phase II xenobiotic metabolism, the presence of alleles associated with Gilbert's syndrome (*28*28** and *1*28**) did not lead to a decrease in the metabolism rate of the studied drugs. On the contrary, at several time points (3 h for tezacaftor, 6 h and 12 h for elexacaftor, 12 h for ivacaftor), concentrations in the "normal" genotype *1*/1** group were paradoxically higher ($p < 0.05$). However, analysis of the integrated AUC_{0-12h} parameter showed these differences were nullified ($p > 0.05$). It can be hypothesized that the observed point differences are due to the small sample size and high variability (coefficient of variation in the Gilbert's syndrome group for tezacaftor AUC reached 81%), or the influence of unmeasured factors. Our data lead to the conclusion that *UGT1A1* polymorphism is not a key predictor of changes in the exposure of the triple combination, which is consistent with published data indicating the predominant metabolism of these drugs via the cytochrome P450 system. Despite published data not revealing a significant role for *UGT1A1* in CFTR modulator metabolism, the presence of Gilbert's syndrome is associated with a worse safety profile for this drug class. The work by E.K. Zhekaite et al. (2025) established that the incidence of adverse reactions associated with CFTR modulator use is significantly higher in patients with CF and Gilbert's syndrome (elexacaftor / tezacaftor / ivacaftor—30% vs. 10% in patients without Gilbert's syndrome; ivacaftor / lumacaftor—28% vs. 4%, $p < 0.05$ in both cases) [37].

Study Limitations

Several limitations should be considered when interpreting the results. The small sample size in subgroups of rare genotypes (particularly AA homozygotes for the *CYP2D6*4* polymorphic variant of the *CYP2D6* gene), which were excluded from comparative analysis, limits statistical power and prevents definitive conclusions for ultra-poor or ultra-rapid metabolizers. The authors did not assess the concentrations of the metabolites of the studied drugs, which might have provided more insight.

CONCLUSION

The conducted FC study revealed significant inter-individual variability in CFTR modulator exposure in children with CF. The lowest variability in parameters was recorded for ivacaftor, and the highest for elexacaftor. The high variability of the latter justifies the clinical significance of conducting TDM to optimize therapy and minimize the risk of adverse events or insufficient efficacy. An age-dependent exposure of elexacaftor and ivacaftor was established, indicating the need to consider age as a critical factor in developing personalized dosing regimens for triple combinations in children and adolescents with CF. The influence of genetic polymorphism of biotransformation enzymes on the exposure of ivacaftor and elexacaftor in the pediatric population justifies the expediency of further research aimed at developing algorithms for personalized dosing of CFTR modulators based on pharmacogenetic testing results.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Sergey K. Zyryanov — conceptualization, writing—review & editing; Elena I. Kondratyeva — investigation, writing—review & editing; Elena K. Zhekaite — investigation, анализ данных, writing—review & editing; Olga I. Butranova — formal analysis, writing—original draft, writing—review & editing; Pavel O. Bochkov — investigation, formal analysis. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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The role of pharmacogenetics in personalizing anticoagulant therapy: data from the Astrakhan region

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The metabolic characteristics of anticoagulants largely depend on the patient's genetic profile, determining the variability of pharmacological response to drugs and the risk of adverse reactions. Among the key genes encoding enzymes responsible for anticoagulant metabolism are *CES1*, *CYP2C9*, and *VKORC1*. They significantly influence the pharmacokinetics and pharmacodynamics of both indirect and direct oral anticoagulants, which dictates the effectiveness and safety of anticoagulant therapy. In clinical practice, the implementation of pharmacogenetic testing before prescribing anticoagulants will significantly reduce the risk of complications and improve therapeutic efficacy.

The aim. To assess the frequency of clinically significant polymorphisms of the *CYP2C9* (rs1799853), *VKORC1* (rs9912121), and *CES1* (rs2244613) genes in patients residing in the Astrakhan region.

Materials and Methods. The study sample consisted of 90 patients residing in the Astrakhan region who received inpatient treatment for confirmed coronavirus infection COVID-19 and were receiving anticoagulants. Venous blood collected in EDTA tubes was examined: diagnostic kits “*CYP2C9* gene Arg144Cys (rs1799853)” (NPF Sintol LLC, Russia), “*VKORC1* gene G1639A (G3673A) (rs9923231)” (NPF Sintol LLC, Russia), and “*CES1* gene C(-33)A (rs2244613) GenTest” (NMT LLC, Russia). After DNA isolation and amplification, blood samples were genotyped by PCR. The study was conducted with an assessment of Hardy–Weinberg equilibrium ($p > 0.05$).

Results. The results showed that the frequency of *VKORC1* gene polymorphisms occurred in 60 (66.7%) patients (95% CI: [56.96–76.44]; $p > 0.05$), *CES1* gene in 55 (61.1%) (95% CI: [51.03–71.17]; $p > 0.05$), and *CYP2C9* gene in 24 (26.7%) (95% CI: [17.56–35.84], $p > 0.05$).

Conclusion. The obtained results revealed a high frequency of distribution of polymorphic variants of genes associated with altered pharmacological response to anticoagulant therapy in patients residing in the Astrakhan region. This highlights the potential for implementing pharmacogenetic testing in the regional healthcare clinical practice for a personalized approach, optimized dosing, and reduced risks of adverse outcomes.

Keywords: pharmacogenetics; anticoagulants; warfarin; novel direct oral anticoagulants; *CES1*; *CYP2C9*; *VKORC1*

Abbreviations: ACT — anticoagulant therapy; ACDs — anticoagulant drugs; INR — international normalized ratio; NOACs — new oral anticoagulants.

Роль фармакогенетики в персонализации антикоагулянтной терапии: данные Астраханского региона

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Особенности метаболизма антикоагулянтов во многом зависят от генетических характеристик пациента — обуславливают вариабельность фармакологического ответа на препараты и риск возникновения побочных реакций. Одними из ключевых генов, кодирующих работу ферментов, отвечающих за метаболизм антикоагулянтов, являются *CES1*, *CYP2C9* и *VKORC1*. Они оказывают значительное влияние на фармакокинетику и фармакодинамику как непрямых, так и прямых пероральных антикоагулянтов, что определяет эффективность и безопасность антикоагулянтной терапии. В условиях клинической практики имплементация фармакогенетического тестирования перед назначением антикоагулянтов значительно снижает риск развития осложнений и повышает терапевтическую эффективность.

Цель. Оценка частоты встречаемости клинически значимых полиморфизмов генов *CYP2C9* (rs1799853), *VKORC1* (rs9912121) и *CES1* (rs2244613) у пациентов, жителей Астраханского региона.

Материалы и методы. Исследуемую выборку составили 90 пациентов, жителей Астраханского региона, получивших стационарное лечение в связи с подтверждённой коронавирусной инфекцией COVID-19, которым назначали антикоагулянты. Исследовали венозную кровь, собранную в пробирки с ЭДТА: диагностические наборы «Arg144Cys гена *CYP2C9* (rs1799853)» (ООО «НПФ Синтол», Россия), «G1639A (G3673A) гена *VKORC1* (rs9923231)» (ООО «НПФ Синтол», Россия) и «ГенТест *CES1* C(-33)A (rs2244613)» (ООО «НМТ», Россия). После выделения ДНК и амплификации образцы крови генотипировали посредством ПЦР. Исследование проведено с оценкой соблюдения равновесия Харди-Вайнберга ($p > 0.05$).

Результаты. В результате установлено, что частота полиморфизмов гена *VKORC1* встречается у 60 (66,7%) пациентов (ДИ 95%: [56,96–76,44]; $p > 0,05$), гена *CES1* — у 55 (61,1%) (ДИ 95%: [51,03–71,17]; $p > 0,05$), гена *CYP2C9* — у 24 (26,7%) (ДИ 95%: [17,56–35,84], $p > 0,05$).

Заключение. Полученные результаты выявили высокую частоту распределения полиморфных вариантов генов, ассоциированных с изменённым фармакологическим ответом на антикоагулянтную терапию, у пациентов — жителей Астраханского региона. Это акцентирует внимание на возможности внедрения в клиническую практику регионального здравоохранения фармакогенетического тестирования для персонализированного подхода, оптимизации дозирования и снижения рисков неблагоприятных исходов.

Ключевые слова: фармакогенетика; антикоагулянты; варфарин; новые прямые пероральные антикоагулянты; *CES1*; *CYP2C9*; *VKORC1*

Список сокращений: АКТ — антикоагулянтная терапия; АКП — антикоагулянтные препараты; МНО — международное нормализованное отношение; НОАК — новые пероральные антикоагулянты.

INTRODUCTION

In recent years, anticoagulant therapy (ACT) has become central to practical medicine due to its crucial role in the prevention and treatment of thromboembolic events. Impaired hemostasis and thrombosis, such as pulmonary embolism, systemic embolisms, venous thrombosis, atrial fibrillation, and acute coronary syndrome, ischemic stroke, are among the leading causes of mortality and disability worldwide, claiming 17.9 million lives annually^{1,2}. Venous thromboembolism affects approximately 10 million people globally, with an incidence of 1–2 cases per 1000 population per year, while atrial fibrillation affects around 60 million people, increasing the risk of ischemic stroke by 4–5 times [1, 2]. The COVID-19 pandemic highlighted a key pathophysiological link in severe disease progression: the development of hemocoagulation complications associated with increased blood clotting [3–5]. The hypercoagulation syndrome observed in patients with coronavirus

infection was accompanied by increased levels of D-dimer, fibrinogen, and vascular thrombosis, necessitating a re-evaluation of approaches to the prevention and therapy of thrombotic complications. Consequently, during the pandemic, anticoagulant drugs (ACDs) became an integral part of treatment for both hospitalized and outpatient patients, as reflected in the temporary methodological recommendations “Prevention, Diagnosis, and Treatment of New Coronavirus Infection (COVID-19)” V revision³ [6, 7]. ACD therapy plays a central role in clinical practice aimed at reducing cardiovascular diseases and improving prognoses in patients at high risk of adverse thrombotic events. Furthermore, a significant challenge is the selection of drug dosages, considering comorbid pathology of eliminating organs and systems, as well as clinically significant interactions with other medications.

For many decades, warfarin was the “gold standard” of ACT in the treatment of cardiovascular diseases. However, alongside its high efficacy,

¹ Healthcare in Russia. 2023: Statistical collection: Rosstat; Moscow, Z-46 2023. 179 p. Russian

² World Health Organization (WHO). Cardiovascular diseases. Available from: https://www.who.int/ru/health-topics/cardiovascular-diseases#tab=tab_1

³ Prevention, Diagnosis, and Treatment of New Coronavirus Infection (COVID-19): Interim Guidelines; Kraeva SA, Kostenko NA, Kamkin EG [et al.]. Version 3; Moscow: Ministry of Health of Russian Federation; 2020. 63 p. EDN: NKZMOV. Russian

warfarin use is associated with several limitations, such as a narrow therapeutic index, the need for constant monitoring of the international normalized ratio (INR), and multiple clinically significant drug interactions [8–10]. Under these circumstances, an individualized approach to warfarin dosing is crucial for achieving high efficacy and safety in ACT. The unpredictable pharmacokinetic and pharmacodynamic response to warfarin is determined, among other factors, by patients' genetic characteristics, which forms the basis for implementing pharmacogenetic strategies in ACD therapy [11, 12].

Currently, new oral anticoagulants (NOACs) such as dabigatran, apixaban, rivaroxaban, and edoxaban are actively used in clinical practice. These drugs have a more predictable pharmacological profile, do not require constant laboratory monitoring, are highly selective, and cause fewer clinically significant drug interactions [13, 14]. Overall, these advantages give NOACs benefits in many clinical situations, leading to their consideration as alternatives to warfarin. However, despite these advantages, individual sensitivity to them also affects the efficacy and safety of therapy, underscoring the need to study patients' pharmacogenetic, non-modifiable characteristics that influence the trajectory of pharmacological response.

For warfarin, the clinically significant genes are *CYP2C9* and *VKORC1*, polymorphisms of which lead to changes in the pharmacological response to the drug. The *CYP2C9* gene encodes an isoenzyme of the cytochrome P450 system involved in the metabolism of most drugs, including warfarin. The polymorphic variant of the *CYP2C9* gene, *CYP2C9*2*, rs1799853 (430C>T), is associated with reduced functional activity of the enzyme, which can lead to drug accumulation, increased therapeutic concentration, and excessive anticoagulation [15], undoubtedly requiring dose titration downwards.

The *VKORC1* gene, in turn, encodes vitamin K epoxide reductase, a key enzyme in vitamin K metabolism. It converts the oxidized form of vitamin to its reduced form, which is necessary for the γ -carboxylation of blood clotting factors. Warfarin inhibits *VKORC1*, leading to reduced synthesis of these factors and hypocoagulation. The mutation rs9923231 (-1639G>A) is associated with the need for a reduced warfarin dose to achieve the target INR level, due

to slowed enzyme activity and increased sensitivity to the drug because of reduced levels of activated vitamin K [16, 17]. This is most significant for drugs with a narrow therapeutic index, such as warfarin, where even a slight dosage change can lead to adverse effects.

Among the NOACs, dabigatran is one of the most frequently used for the prevention and treatment of thrombotic complications. Dabigatran is a prodrug, administered as the inactive compound dabigatran etexilate, which is converted to its active form in the body by specific esterase enzymes, namely carboxylesterase 1 (CES1) and carboxylesterase 2 (CES2). CES1, primarily expressed in the liver, performs the main function, while intestinal CES2 partially compensates for reduced CES1 activity. Figure 1 illustrates the metabolism of dabigatran etexilate under the action of CES1 and CES2 enzymes [18].

Currently, the study of *CES1* gene polymorphisms, which can alter enzyme activity and thus affect ACT efficacy and safety, is of particular interest. Despite dabigatran's predictable pharmacological profile and its use at therapeutic doses, *CES1* polymorphisms can influence its pharmacokinetic and pharmacodynamic parameters. Carriage of the polymorphic allele rs2244613 (1168-33A>C) is associated with reduced metabolic activity of the enzyme, leading to slower conversion of dabigatran etexilate to its active form, dabigatran, which can result in lower drug concentration in the blood and reduced anticoagulation [19].

To date, the most well-known and clinically significant is the automated algorithm for warfarin dose adjustment via WarfarinDosing.org, which considers polymorphic variants of *CYP2C9* and *VKORC1* genes that slow drug metabolism [20]. When using therapeutic dosages, patients have an increased risk of bleeding, but with genetic information, the dose is reduced, leading to an effective and safe anticoagulant effect.

The implementation of pharmacogenetic testing (PGT) approaches into practice is a promising direction in pharmacology and medicine. Identifying genetic markers that influence a patient's individual sensitivity to ACDs can serve as a foundational basis for developing personalized therapy algorithms aimed at increasing treatment efficacy and safety.

THE AIM is to assess the frequency of clinically significant polymorphisms in the *CYP2C9* (rs1799853), *VKORC1* (rs9912121), and *CES1* (rs2244613) genes in patients with COVID-19 who received inpatient treatment.

MATERIALS AND METHODS

Study Design

A single-center retrospective cohort study was conducted.

Ethics Approval

The study was conducted within the framework of a consortium between the Astrakhan State Medical University and the Russian Medical Academy of Continuous Professional Education "Ethnogenetic and Molecular Biological Basis of Complications After Post-COVID-19 Infection," approved by the Local Ethics Committee of the Astrakhan State Medical University, Protocol No. 3 dated December 30, 2021.

Study Duration

Patient recruitment was conducted from December 2022 to August 2023.

Study Conditions

The study was carried out at the multifunctional medical center of Astrakhan—a branch of the Astrakhan Regional City Clinical Hospital No. 3 named after S.M. Kirov, where patients received inpatient treatment for a confirmed diagnosis of coronavirus infection COVID-19.

Eligibility Criteria

Inclusion criteria: confirmed diagnosis of coronavirus infection COVID-19, moderate or severe form of the disease, presence of voluntary informed consent, age 18 years and older, permanent residence in the Astrakhan region, administration of anticoagulant therapy (ACT) as part of inpatient treatment.

Exclusion criteria: patient's refusal to sign informed voluntary consent to participate in the study, presence of absolute contraindications for ACT prescription, unconfirmed COVID-19 diagnosis, bleeding at the time of inclusion, age under 18 years; incomplete set of laboratory parameters used in

the analysis (coagulogram, biochemical and clinical blood test data), repeated entries of the same patient (duplicate cases) identified during re-analysis of the database. The sample included patients with and without concomitant cardiovascular pathology. In accordance with the temporary methodological recommendations, ACT was prescribed to all patients with moderate and severe COVID-19 without exception, due to the presence of COVID-associated coagulopathy and etiopathogenetic damage to the vascular endothelium [21, 22].

Study Methodology

For pharmacogenetic analysis, 3 mL of venous blood was collected into vacuum tubes with EDTA. Samples were stored according to regulatory documentation requirements at a temperature of -20°C in a vertical position on racks. DNA extraction was performed using the reagent kit "PROBA-RAPID-GENETIKA" (OOO "DNA-Tekhnologiya", Russia). Polymorphic variants of the *CYP2C9*, *VKORC1*, and *CES1* genes were identified by PCR using diagnostic kits "*CYP2C9* gene Arg144Cys (rs1799853)" (OOO "NPF Sintol", Russia), "*VKORC1* gene G1639A (G3673A) (rs9923231)" (OOO "NPF Sintol", Russia), and "*CES1* gene C(-33)A (rs2244613) GenTest" (OOO "NMT", Russia) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis of the results was performed using the StatTech v. 4.12.5 software (OOO "Stattech", Russia). The conformity of allele and genotype distributions to Hardy-Weinberg equilibrium was assessed using the χ^2 (chi-square) test. Normality of quantitative indicators distribution was checked using the Shapiro-Wilk test. Categorical variables were presented as n (%) with a 95% confidence interval, and quantitative variables as $M \pm SD$. The critical significance level was set at $p > 0.05$. Additionally, a comparison of the obtained polymorphism frequencies with data from domestic and foreign studies was conducted.

RESULTS

The study sample included 90 patients with a laboratory-confirmed diagnosis of coronavirus infection

COVID-19, presenting in moderate and severe forms. ACT was prescribed to all participants to prevent thromboembolic complications.

The distribution of patients by sex was characterized by a predominance of women—49 individuals (54.4%), while the number of men was 41 (45.6%). Patient age ranged from 24 to 70 years, with a mean age of 62.81 ± 11.19 years (Table 1).

A moderate form of COVID-19 was diagnosed in 78 (86.7%) patients, and a severe form in 12 (13.3%). The average duration of hospitalization was 12.06 ± 4.99 days. The average oxygen saturation level upon admission was $93.23 \pm 2.12\%$.

Analysis of laboratory parameters revealed pronounced signs of systemic inflammation and activation of the coagulation link of hemostasis. The average D-dimer level was 0.82 ± 3.28 $\mu\text{g/mL}$ (normal < 0.5 $\mu\text{g/mL}$), C-reactive protein— 74.12 ± 8.67 mg/L (normal < 5.0 mg/L), INR values reached the upper limits of normal— 1.16 ± 0.68 , while the average platelet count was within reference values— $237.43 \pm 155.10 \times 10^9/\text{L}$.

All patients (100%) received ACT at therapeutic doses. Heparin was used in all cases (100%); additionally, apixaban was prescribed to 13 patients (14.4%), and rivaroxaban to 7 patients (7.8%). NOAC therapy was recommended to all patients without exception (100%) after discharge from the hospital for 3 months.

The study of the prevalence of polymorphisms in the *CYP2C9*, *VKORC1*, and *CES1* genes showed a high frequency of their carriage among residents of the Astrakhan region. The assessment of allele and genotype distributions showed conformity to expected values according to Hardy–Weinberg equilibrium ($p > 0.05$). The obtained results indicate the genetic homogeneity of the study sample and confirm the reliability of the conducted analysis. Data on the prevalence of polymorphisms in the *CYP2C9*, *VKORC1*, and *CES1* genes in patients from the Astrakhan region are presented in Table 2.

Polymorphic variants of the *VKORC1* gene were found in 66.7% of patients (95% CI: [56.96–76.44], $p > 0.05$), and the *CES1* gene—in 61.1% (95% CI: [51.03–71.17], $p > 0.05$). *CYP2C9* gene polymorphism occurred less frequently—in 26.7% (95% CI: [17.56–35.84], $p > 0.05$).

For the *CES1* gene (rs2244613), analysis of genotype distribution showed a high prevalence of CC and AC genotypes (61.1%), associated with a significant decrease in enzyme metabolic activity, which can lead to failure to achieve therapeutic dabigatran concentrations in plasma and be accompanied by an increased risk of thrombotic events [19, 23, 24].

Analysis of genotype distribution for the *CYP2C9* gene (rs1799853) revealed that the most common genotype is CC, characterized by an unchanged rate of metabolic reactions. However, CT and TT genotypes were found in 26.7% of patients, which may indicate ACT inefficiency in every third patient. The presence of CT and TT genotypes demonstrates a reduced activity of the *CYP2C9* enzyme, responsible for warfarin metabolism. The presence of the T allele is associated with decreased drug clearance, increased INR at therapeutic doses, and an increased risk of hemorrhage [25, 26].

For the *VKORC1* gene, it was established that 60 (66.7%) patients had AA and GG genotypes, associated with slowed enzyme expression and high sensitivity to warfarin, which at standard doses can lead to major hemorrhages.

Analysis of these clinically significant gene polymorphisms showed that the study sample has a high proportion of carriers of genotypes affecting the pharmacokinetic and pharmacodynamic properties of anticoagulants. This distribution reflects the variability in metabolism and sensitivity to this group of drugs. Therapeutic doses may be inadequate for such patients, potentially leading to serious side effects.

Additionally, an assessment of the frequency of combinations of polymorphisms of the indicated genes was performed. The sample included patients with polymorphisms in one gene, as well as in combinations of two or all three genes.

Almost every second patient in the observation group (48.9%) has a combination of polymorphisms, meaning they have an atypical response to therapy, making the use of standard doses ineffective and unsafe. The detected high proportion of patients with multiple genetic predictors of altered drug metabolism indicates the significance of pharmacogenetic testing in the personalized selection of anticoagulants.

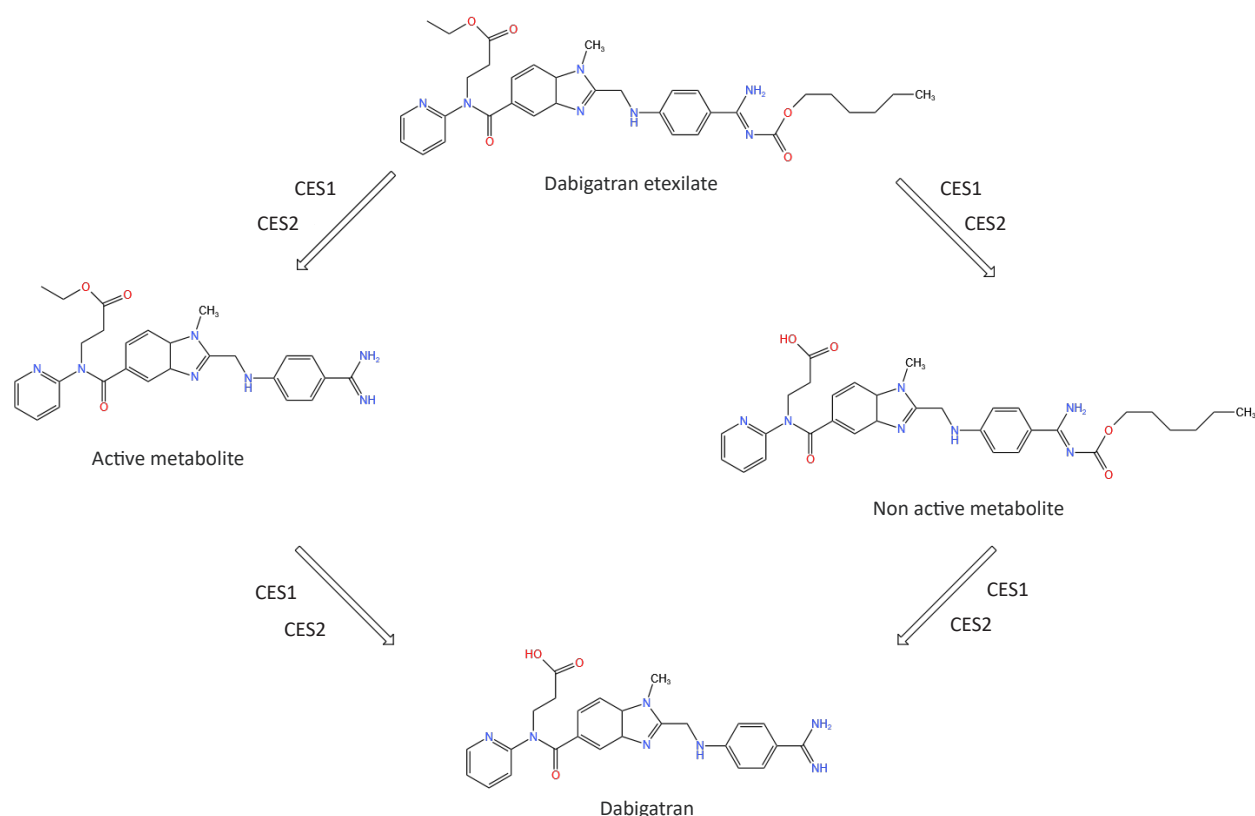


Figure 1 — Metabolism of dabigatran etexilate under the influence of CES1 and CES2 enzymes.

Table 1 — Clinical and demographic data of patients

Indicator	Value
Total number of patients, <i>n</i>	90
Women, <i>n</i> (%)	49 (54.4%)
Men, <i>n</i> (%)	41 (45.6%)
Age, M ± SD, years	62.81 ± 11.19
Moderate COVID-19, <i>n</i> (%)	78 (86.7%)
Severe COVID-19, <i>n</i> (%)	12 (13.3%)
Duration of hospitalization, M ± SD, days	12.06 ± 4.99
O ₂ saturation on admission, M ± SD, %	93.23 ± 2.12
D-dimer, M ± SD, µg/mL	0.82 ± 3.28
INR, M ± SD	1.16 ± 0.68
C-reactive protein, M ± SD, mg/L	74.12 ± 8.67
Platelets, M ± SD, ×10 ⁹ /L	237.43 ± 155.10
Anticoagulant prescription, M ± SD, <i>n</i> (%)	90 (100%)
heparin	90 (100%)
NOAC inpatient	20 (22.2%)
NOAC outpatient after discharge	90 (100%)
Lethal outcome, <i>n</i> (%)	6 (6.7%)

Note: INR, International Normalized Ratio; NOAC, Novel Oral Anticoagulants.

Table 2 — Results of the frequency assessment of carrier status for polymorphic alleles of *CES1*, *CYP2C9* and *VKORC1* genes in patients, calculated by Hardy-Weinberg equilibrium

Genotypes	Number of patients, <i>n</i> (%)	Confidence Interval	χ^2	<i>p</i>
<i>CES1</i> (rs2244613)				
CC	18 (20.0%)	[11.74–28.26]	0.97	0.32
AC	37 (41.1%)	[30.93–51.27]		
AA	35 (38.9%)	[28.83–48.97]		
<i>CES1</i> (rs2244613), alleles				
C	73 (40.6%)			
A	107 (59.4%)			
<i>CYP2C9</i> (rs2244613)				
TT	1 (1.1%)	[-1.05–3.25]	0.25	0.61
CT	23 (25.6%)	[16.58–34.62]		
CC	66 (73.3%)	[64.16–82.44]		
<i>CYP2C9</i> (rs1799853), alleles				
T	25 (13.9%)			
C	155 (86.1%)			
<i>VKORC1</i> (rs9923231)				
AA	27 (30.0%)	[20.53–39.47]	3.24	0.07
GA	33 (36.7%)	[26.74–46.66]		
GG	30 (33.3%)	[23.56–43.04]		
<i>VKORC1</i> (rs9923231), alleles				
A	87 (48.3%)			
G	93 (51.7%)			

Table 3 — Assessment of the frequency of occurrence of combinations of polymorphisms of *CES1*, *CYP2C9* and *VKORC1* genes in patients, residents of the Astrakhan region

Gene	Number, %	Total, %
Single polymorphism		
CES1	18 (20.0%)	40 (44.4%)
CYP2C9	2 (2.2%)	
VKORC1	20 (22.2%)	
Combinations of polymorphisms of two genes		
CES1 + VKORC1	21 (23.4%)	33 (36.7%)
CYP2C9 + VKORC1	8 (8.9%)	
CES1 + CYP2C9	4 (4.4%)	
Combinations of polymorphisms of three genes		
CES1 + CYP2C9 + VKORC1	11 (12.2%)	11 (12.2%)

To evaluate the obtained data in the context of global patterns, a comparative analysis of the frequency of polymorphisms in the *CYP2C9*, *CES1*, and *VKORC1* genes with data from international studies was conducted. It was found that the frequencies of *CES1* polymorphisms vary significantly across global populations: among African Americans, it is 75.5%; among Latin Americans—77%; among East Asians—40.4%; among Europeans—81.6%; among South Asians—59.2%.

The frequency of *VKORC1* gene variants also varies depending on the population: 10% among African Americans, 39%—Latin Americans, up to 89% in East Asian populations, 38% in

Europeans, and about 18% in South Asians. *CYP2C9* gene polymorphism is characterized by lower and more heterogeneous prevalence: African Americans—2.2%, Latin Americans—8.5%, East Asians—0.07%, Europeans—12.8%, and South Asians—4.6% [27].

DISCUSSION

The obtained data on the prevalence of polymorphisms in key anticoagulant (AKP) metabolism genes—*CYP2C9*, *CES1*, and *VKORC1*—among patients residing in the Astrakhan region differ from the results of genotype frequencies in other population groups of the Russian Federation.

In the European part of the Russian Federation, the prevalence of polymorphic variants of the *CYP2C9* gene is characterized by a relatively uniform distribution. In the Voronezh region, the frequency of the minor allele *CYP2C9*2* is 10.5%, and the allele *CYP2C9*3* reaches 6.7% [28]. Comparable indicators were recorded in Moscow, where the frequency of the *CYP2C9*2* allele is 9.7%, and *CYP2C9*3*—5.2%. The highest frequency of the *CYP2C9*2* allele among the studied regions of the European part of the country was noted in the Stavropol Krai and reaches 12%, while the prevalence of the *CYP2C9*3* allele is about 6% [29]. Similar values were found in St. Petersburg – the frequency of the *CYP2C9*2* allele is 11.11%, and *CYP2C9*3*—6.32% [30].

In the eastern regions of the Russian Federation, a change in the distribution structure of polymorphic variants of the *CYP2C9* gene is observed. In the Irkutsk region, the frequency of the *CYP2C9*2* allele decreases to 6.1%, while the frequency of *CYP2C9*3*, on the contrary, increases to 9.5%, which is one of the highest indicators among the studied regions [29]. In the Republic of Buryatia, the lowest prevalence of both minor alleles was found: the frequency of *CYP2C9*2* is 2.3%, and *CYP2C9*3*—1.7% [31]. In the Khabarovsk Krai, the frequency of the *CYP2C9*3* allele remains at 5.7% [32].

Analysis of the regional distribution of *CYP2C9* gene polymorphisms indicates significant geographical variability. The results obtained in the Astrakhan region show the highest frequency of *CYP2C9* gene polymorphisms, with this indicator reaching 26.7%, which exceeds the corresponding values obtained in other regions of the Russian Federation. This can likely be attributed to the multi-ethnic composition of the Astrakhan region.

The prevalence of the rs2244613 polymorphism of the *CES1* gene in the populations of the Russian Federation is characterized primarily by ethnic differences, with relative stability of indicators among Russian populations in different regions.

Among Russian residents of various territories, the frequency of the minor allele C of the *CES1* gene shows similar values: in Moscow, it is 28.4%, and in the Irkutsk region, it is 28.3%, indicating the absence of significant regional differences within the European part of Russia and Siberia [33, 34].

Higher values of this indicator are observed in the populations of the Republic of Dagestan. Among Avars, the frequency of allele C is 38.3%, among Dargins, it is 40.0%, while among Laks, it is 29.3%, which is comparable to the level in Russian populations [34].

For the rs9923231 polymorphism of the *VKORC1* gene in the populations of the Russian Federation, significant regional differences have been identified,

with an increase in the frequency of the minor allele A in the eastern part of Russia.

In the European part of Russia, the indicators remain relatively stable; for example, in Moscow, the frequency of allele A is 38.1% [26]. Among Chuvash, the frequency of allele A of the *VKORC1* gene (–1639G>A) was 48.60% [35].

The highest values were recorded among the indigenous peoples of Eastern and Western Siberia. In the Republic of Buryatia, the frequency of allele A is 85.3%, in the Republic of Sakha (Yakutia), it reaches 83.1%, among Selkups, it is 71.8%, and among Nenets, it is 61.0% [26].

The high frequency of clinically significant polymorphisms identified in the Astrakhan region, combined with the results of international studies, creates prerequisites for discussing the possibility of developing and implementing a regionally adapted protocol for conducting FGT before prescribing ACT in each subject of the Russian Federation.

Study Limitations

This study, despite the representativeness of the data and the identified trends, has several limitations that must be considered when interpreting the obtained results. The study has a relatively small sample size ($n = 90$), and patients were not stratified by ethnicity.

CONCLUSION

The conducted study revealed a high prevalence of polymorphisms in the *VKORC1*, *CES1*, and *CYP2C9* genes associated with altered sensitivity to ACT among patients residing in the Astrakhan region. The frequencies of the identified allelic variants were comparable to the data obtained in other subjects of the Russian Federation. A significant proportion of patients exhibited not only single but also combined polymorphisms, including combinations of two and three genes simultaneously, indicating potentially high variability in the pharmacokinetic profile of ACP in this population.

The dominance of combined genetic variants suggests an increased risk of hemorrhagic complications when using standard doses of ACP, especially in patients carrying multiple sensitive alleles. The totality of the obtained data underscores the necessity of implementing personalized approaches to ACT dosage selection and the use of pharmacogenetic testing before initiating treatment. Such an approach will enhance the safety and efficacy of therapy, minimize the risk of adverse reactions, and improve clinical outcomes in patients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Alexandra N. Romanova — formal analysis, writing—original draft; Bela I. Kantemirova — conceptualization, writing—review & editing; Olga V. Komarova, Natalia V. Timofeeva — formal analysis, visualization. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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Comparative analysis of bioequivalence, safety, and tolerability of semaglutide (tablets): results of an open-label randomized crossover comparative study

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The aim. To conduct a comparative evaluation of the physicochemical properties, biological activity, bioequivalence, and safety, including tolerability and immunogenicity, of the drug Semaltara® (semaglutide, tablets 3 and 14 mg (Biochemik, Russia) and the drug Rebelas® (semaglutide, tablets in dosages of 3 and 14 mg (Novo Nordisk A/S, Denmark) when administered to healthy volunteers.

Materials and Methods. To assess the bioequivalence of the investigational drug Semaltara® (semaglutide, tablets 3 and 14 mg (Biochemik, Russia) with a chemically synthesized active substance to the original (reference) drug Rebelas® (semaglutide, tablets in dosages of 3 and 14 mg (Novo Nordisk A/S, Denmark), an open-label randomized crossover

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comparative study ($n = 110$) was conducted. Patients were randomized into 4 groups: Group 1 ($n = 25$) — patients received the investigational drug Semaltara® and the comparator drug Rebelas® daily once a day at a dose of 3 mg (1 tablet) for 28 days; Group 2 ($n = 25$) — the comparator drug Rebelas® and the investigational drug Semaltara® daily once a day at a dose of 3 mg (1 tablet) for 28 days; Group 3 ($n = 30$) — the investigational drug Semaltara® and the comparator drug Rebelas® daily once a day at a dose of 14 mg (1 tablet) for 28 days; Group 4 ($n = 30$) — the comparator drug Rebelas® and the investigational drug Semaltara® daily once a day at a dose of 14 mg (1 tablet) for 28 days. The concentration of semaglutide was determined in serum samples using a pre-validated enzyme-linked immunosorbent assay (ELISA) method. The conclusion on bioequivalence of the compared drugs was made based on the assessment of 90 % confidence intervals for the ratios of geometric mean values of $C_{\max}$ and $AUC_{(0-t)}$ parameters of semaglutide in the original units of measurement.

Results. The results of the clinical study demonstrated the bioequivalence of the investigational drug and the comparator drug. The pharmacokinetic parameters of the drugs were comparable: Based on statistical analysis, the obtained 90 % confidence intervals for the ratio of $C_{\max,ss}$ and $AUC_{(0-t)ss}$ values of semaglutide 3 mg were 96.57–120.94 % and 94.37–120.06 %, respectively. For the 14 mg dosage, the 90 % confidence intervals for the ratio of $C_{\max,ss}$ and $AUC_{(0-t)ss}$ values of semaglutide were 91.79–109.68 % and 91.47–112.80 %, respectively. The tolerability of the drugs in volunteers was noted as good. No serious adverse events were registered during the entire study. Thus, the investigational drug Semaltara®, tablets, and the comparator drug Rebelas®, tablets, have a similar safety profile, including tolerability, at both the 3 mg and 14 mg dosages.

Conclusion. The conducted study demonstrated the bioequivalence and similar safety profile, as well as tolerability of the Russian drug Semaltara® (semaglutide 3 and 14 mg, tablets, Promomed LLC, Russia) in comparison with the foreign drug Rebelas® (semaglutide, tablets in dosages of 3 and 14 mg (Novo Nordisk A/S, Denmark).

Keywords: glucagon-like peptide-1; bioequivalence; pharmacokinetics; semaglutide; type 2 diabetes mellitus; physicochemical properties; safety profile; biological activity; tablets

List of Abbreviations: T2DM — type 2 diabetes mellitus; HbA1c — glycated hemoglobin; GLP-1 — glucagon-like peptide-1; BMI — body mass index; BP — blood pressure; SBP — systolic blood pressure; DBP — diastolic blood pressure; HR — heart rate; ECG — electrocardiography; AE — adverse event; SAE — serious adverse event; CI — confidence interval.

Сравнительный анализ биоэквивалентности, безопасности и переносимости семаглутида (таблетки): результаты открытого рандомизированного перекрестного сравнительного исследования

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Цель. Провести сравнительную оценку физико-химических свойств, биологической активности, биоэквивалентности и безопасности, включая переносимость и иммуногенность, лекарственного препарата Семальтара (семаглутид, таблетки 3 и 14 мг (АО «Биохимик», Россия) и препарата Ребелсас® (семаглутид, таблетки в дозировках 3 и 14 мг (Ново Нордиск А/С, Дания) при применении здоровыми добровольцами.

Материалы и методы. Для оценки биоэквивалентности исследуемого препарата Семальтара® (семаглутид, таблетки 3 и 14 мг (АО «Биохимик», Россия) с химически синтезированным активным веществом оригинальному (референтному) препарату Ребелсас® (семаглутид, таблетки в дозировках 3 и 14 мг (Ново Нордиск А/С, Дания) проведено открытое рандомизированное перекрестное сравнительное исследование ($n=110$). Пациенты путём рандомизации распределены на 4 группы: 1 группа ($n=25$) — пациенты получали исследуемый препарат Семальтара® и препарат сравнения Ребелсас® ежедневно 1 раз в сут в дозе 3 мг (1 таблетка) в течение 28 дней; 2 группа ($n=25$) — препарат сравнения Ребелсас® и исследуемый препарат Семальтара® ежедневно 1 раз в день в дозе 3 мг (1 таблетка) в течение 28 дней; 3 группа ($n=30$) — исследуемый препарат Семальтара® и препарат сравнения Ребелсас® ежедневно 1 раз в сут в дозе 14 мг (1 таблетка) в течение 28 дней; 4 группа ($n=30$) — препарат сравнения Ребелсас® и исследуемый препарат Семальтара® ежедневно 1 раз в день в дозе 14 мг (1 таблетка) в течение 28 дней. Концентрацию семаглутида определяли в образцах сыворотки крови с помощью предварительно валидированного метода иммуноферментного анализа (ИФА). Вывод о биоэквивалентности сравниваемых препаратов делали по оценке 90% доверительных интервалов для отношений средних геометрических значений параметров $C_{\max}$, $AUC_{(0-t)}$ семаглутида в исходных единицах измерения.

Результаты. Результаты клинического исследования продемонстрировали биоэквивалентность исследуемого препарата и препарата сравнения. Фармакокинетические параметры препаратов были сопоставимы между собой: По результатам статистического анализа полученные 90% доверительные интервалы для отношения значений $C_{\max}$, ss и $AUC_{(0-t)ss}$ семаглутида 3 мг составили 96,57–120,94% и 94,37–120,06%, соответственно. Для в дозировки 14 мг 90% доверительные интервалы для отношения значений $C_{\max}$, ss и $AUC_{(0-t)ss}$ семаглутида составили 91,79–109,68% и 91,47–112,80%, соответственно. Переносимость препаратов у добровольцев отмечена как хорошая. В течение всего исследования не зарегистрировано ни одного серьёзного нежелательного явления. Таким образом, исследуемый препарат Семальтара®, таблетки и препарат сравнения Ребелсас®, таблетки, имеют сходный профиль безопасности, включая переносимость как в дозировке 3 мг, так и в дозировке 14 мг.

Заключение. В ходе проведённого исследования показана биоэквивалентность и сходный профиль безопасности, а также переносимость российского препарата Семальтара® (семаглутид 3 и 14 мг, таблетки, ООО «Промомед», Россия) в сравнении с зарубежным препаратом Ребелсас® (семаглутид, таблетки в дозировках 3 и 14 мг (Ново Нордиск А/С, Дания).

Ключевые слова: глюкагоноподобный пептид-1; биоэквивалентность; фармакокинетика; семаглутид; сахарный диабет 2-го типа; физико-химические свойства; профиль безопасности; биологическая активность; таблетки

Список сокращений: СД 2 — сахарный диабет 2 типа; HbA1c — гликированный гемоглобин; ГПП-1 — глюкагоноподобный пептид-1; ИМТ — индекс массы тела; АД — артериальное давление; САД — систолическое артериальное давление; ДАД — диастолическое артериальное давление; ЧСС — частота сердечных сокращений; ЭКГ — электрокардиография; НЯ — нежелательное явление; СНЯ — серьёзное нежелательное явление; ДИ — доверительный интервал.

INTRODUCTION

According to the latest data from the NCD Risk Factor Collaboration (2022), the number of people with diabetes mellitus (DM) has reached 828 million, of whom more than 95% suffer from type 2 DM [1]. Statistics show that the prevalence of DM will continue to grow, however, this figure is likely higher due to varying estimates of current diabetes prevalence [1–3]. Type 2 DM is a complex, multisystem metabolic disorder characterized by high blood glucose levels resulting from progressive defects in insulin secretion or insulin resistance of tissues¹ [4]. Type 2 DM is a common and heterogeneous disorder characterized by varying degrees of beta-cell dysfunction and insulin resistance.

There is a strong link between obesity and type 2 DM, affecting pathways regulated by the central nervous system. These pathways control food intake and energy expenditure by integrating signals from peripheral organs and the environment [5]. It should also be noted that type 2 DM does not only affect older people; in recent years, a twofold or threefold increase in the incidence of type 2 DM in young people (< 40 years) has been observed [6].

Glucagon-like peptide-1 (GLP-1) receptor agonists are recently approved drugs for the treatment of DM and obesity. GLP-1 has physiological effects on various organs. Like incretin, it enhances glucose-dependent insulin secretion by pancreatic β -cells, promotes β -cell neogenesis, inhibits β -cell apoptosis, and suppresses glucagon secretion by α -cells (shown in rodent studies). Furthermore, GLP-1 affects other tissues and organs, including the stomach—slowing gastric emptying, the heart—exerting cardioprotective effects, as well as adipose tissue and skeletal muscles—improving glucose uptake, and also acts centrally on hypothalamic neurons, inducing satiety [7–9]. GLP-1 receptor agonist therapy suppresses appetite, leading to weight loss, which in turn has a broad impact on improving patients' quality of life and reducing the risk of cardiovascular and renal complications [10]. Various GLP-1 receptor agonists are approved for the treatment of type 2 DM, including exenatide, liraglutide, lixisenatide, dulaglutide, and semaglutide. These drugs are characterized by subcutaneous administration, although an oral form of semaglutide is also available [10]. Scientific studies have shown that

GLP-1 analogs improve glycemic control in patients with type 2 DM and additionally contribute to a reduction in systolic blood pressure (BP). Long-acting preparations of this class are associated with more effective glucose reduction and exhibit fewer gastrointestinal side effects compared to their short-acting counterparts [11–14].

Scientific developments in recent years have allowed for a detailed study of the role of incretin hormones in regulating carbohydrate metabolism in the human body and their effect on β -cells, as well as the creation of a new direction in pharmacotherapy. One of the representatives of incretin mimetics is semaglutide [15, 16].

Semaglutide reduces total body weight and fat mass by decreasing energy intake. The mechanism of action of semaglutide contributes to appetite reduction by enhancing satiety signals and weakening hunger signals, as well as improving control over food intake volume and reducing cravings for it. As a consequence, insulin resistance decreases due to weight loss. In addition, semaglutide reduces the preference for high-fat foods [17, 19].

The company LLC “PROMMOMED RUS” has developed a drug with the active substance semaglutide, Semaltara®, in tablet form.

For the semaglutide (tablets), the reference product is Rybelsus® (INN: semaglutide), tablets—an original drug from Novo Nordisk A/S, registered in Denmark, where the level of pharmaceutical market regulation requirements is not lower than that established in the EAEU.

In the reference product Rybelsus®, the active substance semaglutide is obtained by a biotechnological method from recombinant DNA of *Saccharomyces cerevisiae*, whereas in the investigated product Semaltara®, the active substance is obtained by chemical synthesis [17].

Current international requirements allow the use of an accelerated registration procedure for drugs such as semaglutide without conducting comparative efficacy studies, considering them redundant [18].

To establish equivalence, it is sufficient for the applicant to demonstrate using modern analytical methods (HPLC-MS, NMR, etc.) that the active ingredient in the proposed synthetic peptide is the same as in the reference drug—comparable physicochemical characteristics, biological activity,

¹ International Diabetes Federation. IDF Diabetes Atlas 10th Edition; 2021. Available from: <https://diabetesatlas.org/>

and an identical impurity profile². According to FDA guidance (ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin, 2021), information on the safety and efficacy of the original drug, in the case of a similar dosage form, route of administration, dosage, and conditions of use, is sufficient to establish equivalence of the drug³.

Thus, conducting a bioequivalence study is relevant and applicable for confirming the reproducibility of the investigated semaglutide drug⁴.

THE AIM is to conduct a comparative analysis of the physicochemical properties, biological activity, bioequivalence, and safety, including tolerability and immunogenicity, of the medicinal product Semaltara® (semaglutide, tablets 3 mg and 14 mg (JSC "Biokhimik", Russia) and the reference product Rybelsus® (semaglutide, tablets in dosages of 3 mg and 14 mg (Novo Nordisk A/S, Denmark) used to healthy volunteers.

MATERIALS AND METHODS

Study Design

A single-center, open-label, randomized, crossover bioequivalence study with multiple administrations of the study drug and reference drug was conducted.

The total duration of the study for each volunteer was no more than 71 days.

The study included the following stages:

1. Screening—no more than 14 days.

2. Randomization.

3. Period I—volunteers in groups 1 and 3 received the study drug Semaltara® (T1 or T2), volunteers in groups 2 and 4 received the reference drug Rybelsus® (R1 or R2). The duration of the period was no more than 28 days (outpatient visits on days 1, 15, 25, 26, 27; hospitalization in the evening on day 27 at least 10 hours before drug intake for up to 26 hours after drug intake on day 28, blood sampling within 24 hours after drug intake on day 28).

4. Period II—volunteers in groups 1 and 3 received the reference drug Rybelsus® (R1 or R2), volunteers in groups 2 and 4 received the study drug

Semaltara® (T1 or T2). The duration of the period was no more than 28 days (outpatient visits on days 29, 43, 53, 54, 55; hospitalization in the evening on day 55 at least 10 hours before drug intake for up to 26 hours after drug intake on day 56, blood sampling within 24 hours after drug intake on day 56).

5. Completion of study participation.

Considering the variability data of semaglutide drugs, a crossover design with multiple administrations of the study drug / reference drug in each period is provided. Given that steady-state concentration of semaglutide is achieved after 4–5 weeks of once-daily administration, each period includes a duration of 28 days of study drug / reference drug intake [6].

Considering that the study involved the investigation of steady-state concentration of the drug with multiple administrations, a washout period is not provided in the present study.

A graphical scheme of the study design is presented in Figure 1.

Study subjects and eligibility criteria

The study was conducted with healthy male and female volunteers aged 18 to 65 years inclusive, who met all inclusion criteria and none of the exclusion criteria.

Inclusion criteria: signing the informed consent form ICF, as well as the ability and willingness to comply with all Protocol requirements; women of non-childbearing potential and men aged 18 to 65 years inclusive at the time of signing the ICF; body mass index (BMI) 25–30 kg/m² inclusive; verified diagnosis of "healthy" based on standard clinical, laboratory, and instrumental examination methods; hemodynamic parameters: systolic blood pressure (SBP) 100–130 mmHg, diastolic blood pressure (DBP) 60–90 mmHg, heart rate (HR) 60–90 bpm, respiratory rate (RR) 16–20 breaths/min; negative results for human immunodeficiency virus (HIV), syphilis, hepatitis B (HBsAg), and hepatitis C (HCV); negative results for alcohol, psychotropic, and narcotic substance use; willingness to abstain from alcohol consumption during study participation. Women of non-childbearing potential (history of hysterectomy, bilateral oophorectomy, tubal ligation, infertility, absence of menstruation for 12 consecutive months prior to screening), and men; the absence of treatment upon diagnosis of "infertility" prior to study enrollment, as well as planned treatment throughout the study and for

² ANDAs for certain highly purified synthetic peptide drug products that refer to listed drugs of rDNA origin. Available from: <https://collections.nlm.nih.gov/catalog/nlm:nlmuid-9918350982606676-pdf>

³ Ibid.

⁴ National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 56843331, Semaglutide. Retrieved May 15, 2023. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Semaglutide>

2 months after its completion (both standard, surgical, and non-traditional or experimental treatments); consent of male volunteers to use reliable methods of contraception (abstinence, barrier method (condom or occlusive cap (diaphragm or cervical / vault cap)) or dual barrier method of contraception (condom or occlusive cap (diaphragm or cervical / vault cap) plus spermicide (foam / gel/ film / cream / suppository)) throughout their participation in the study and for 2 months after its completion.

Exclusion Criteria: history of allergies, drug intolerance; hypersensitivity to semaglutide or any of the excipients of the study drug / reference drug, to therapeutic proteins; women of childbearing potential; use of medicinal products: history of semaglutide use; other analogs of human GLP-1—within less than 6 months prior to screening; medicinal products with a significant impact on hemodynamics and/or liver function (barbiturates, omeprazole, cimetidine, etc.)—within less than 2 months prior to screening; other medicinal products (including those used for weight loss), including herbal and homeopathic preparations, vitamins and/or dietary supplements (DSs)—within less than 4 weeks prior to screening; inability to insert a cubital heparinized catheter; difficulty swallowing tablets; tendency for frequent bowel movements, diarrhea within less than 2 weeks prior to screening /at the time of screening / randomization; any difficulties with blood collection in history or any vasovagal episodes during blood collection that, in the opinion of the investigator, could significantly interfere with blood sampling for the study; donation or loss of blood (≥ 450 mL of blood or plasma) within less than 3 months prior to screening; planned donation of blood / plasma/blood components or surgical intervention during study participation; any deviations from normal values in laboratory and/or instrumental examination; history of severe cardiovascular, bronchopulmonary, endocrine, central nervous system, gastrointestinal diseases, malignant oncological or hematological diseases; gastrointestinal surgery (except appendectomy) in history; history of medullary thyroid carcinoma, including familial; history of multiple endocrine neoplasia type 2; diabetes mellitus; history of pancreatitis; severe depression, suicidal thoughts or behavior, including in history; acute infectious diseases or symptoms of ARVI within less than 4 weeks prior to screening; special diet (e.g., vegetarian, vegan, low-salt, low-calorie, etc.) within less

than 2 months prior to screening procedures; special lifestyle (night work, extreme physical exertion) within less than 2 months prior to screening procedures; alcohol consumption within 72 hours prior to any screening procedures; history of consuming more than 10 units of alcohol per week (1 unit of alcohol is equivalent to 500 mL of beer, 200 mL of wine, or 50 mL of spirits) or history of alcoholism, drug addiction, substance abuse; smoking more than 10 cigarettes per day at the time of screening; undergoing piercing, tattooing /tattooing procedures within less than 1 month prior to screening or planning to undergo them during study participation; inability or incapacity to comply with necessary contraceptive measures; pregnancy (positive pregnancy test in women) or breastfeeding period; mental, physical, and other reasons that prevented the volunteer from adequately assessing their behavior and correctly following the study Protocol; participation in another clinical study within less than 3 months prior to screening; other reasons that, in the opinion of the investigator, prevented the volunteer from participating in this study.

Exclusion Criteria: the principal investigator could exclude a volunteer from the study for the following reasons: volunteer's refusal to participate in the clinical study; the investigator decided that the volunteer needed to be excluded in the volunteer's best interest; erroneous enrollment of a volunteer who did not meet the inclusion criteria and/or met the exclusion criteria; positive test for alcohol, psychotropic and/or narcotic substances; variability (positive pregnancy test in women); missed two or more blood draws for pharmacokinetic parameter determination consecutively within one study period or three blood draws for pharmacokinetic parameter determination within one study period; volunteer's use of vitamins, DS, drugs, including herbal and/or homeopathic, except for the study drug / reference drug; development of any diseases or conditions during the study that make further participation impossible; volunteer refuses to cooperate, is undisciplined, does not comply with the study participation rules.

Randomization Procedure

Each volunteer who met all inclusion criteria and did not meet any exclusion criteria was assigned a randomization number according to the randomization plan prepared for this study in the WinPepi 11.65 program (module ETCETERA 3.26) using

a random number generation method. The volunteer's randomization number was entered by the investigator into the Clinical Trial Participant Registration Log for screening / randomization. If a volunteer prematurely discontinued study participation, their randomization number was not reused, and the volunteer could not subsequently return to the study.

Study conditions and duration

The study was conducted from August 9, 2024, to March 21, 2025, at the Research Center of the Clinical Hospital No. 3 (Yaroslavl, Russia). The total duration of the study for each volunteer was no more than 71 days (including the screening period). All randomized volunteers completed the study fully in accordance with the approved Study Protocol.

Description of Procedures and Methodology

Drug Administration

A total of 110 volunteers who met the inclusion criteria and had no exclusion criteria were randomized into 4 groups:

3 mg Dosage

Group 1 ($n = 25$) received the study drug Semaltara® (T1) in Period I of the study and the reference drug Rybelsus® (R1) in Period II (T1R1 sequence). The drugs were administered orally once daily at a dose of 3 mg (1 tablet) for 28 days.

Group 2 ($n = 25$) received the reference drug Rybelsus® (R1) in Period I of the study and the study drug Semaltara® (T1) in Period II (R1T1 sequence). The drugs were administered orally once daily at a dose of 3 mg (1 tablet) for 28 days.

14 mg Dosage

Group 3 ($n = 30$) received the study drug Semaltara® (T2) in Period I of the study and the reference drug Rybelsus® (R2) in Period II (T2R2 sequence). The drugs were administered orally once daily at a dose of 14 mg (1 tablet) for 28 days.

Group 4 ($n = 30$) received the reference drug Rybelsus® (R2) in Period I of the study and the study drug Semaltara® (T2) in Period II (R2T2 sequence). The drugs were administered orally once daily at a dose of 14 mg (1 tablet) for 28 days.

Volunteers took the study drug / reference drug orally in the morning on an empty stomach, at least 30 minutes before food and additional fluids/drinks. Tablets should be swallowed whole, without chewing, dissolving, crushing, or breaking them, with 120 mL of still water.

Sample preparation and collection

Blood sampling times for pharmacokinetic parameter assessment:

Day 1: no more than 15 minutes before administration of the study drug / reference drug (corresponds to time 0 h in Tables 59–62; this point is not included in pharmacokinetic profile construction).

Days 25 and 53: no more than 15 minutes before administration of the study drug / reference drug (corresponds to time 576 h in Tables 59–62 and time -72 h in pharmacokinetic profiles).

Days 26 and 54: no more than 15 minutes before administration of the study drug / reference drug (corresponds to time 600 h in Tables 59–62 and time -48 h in pharmacokinetic profiles).

Days 27 and 55: no more than 15 minutes before administration of the study drug / reference drug (corresponds to time 624 h in Tables 59–62 and time -24 h in pharmacokinetic profiles).

Days 28 and 56: no more than 5 minutes before administration of the study drug / reference drug and then at 10 min, 20 min, 30 min, 40 min, 50 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 12 h, 24 h after administration of the study drug / reference drug on days 28 and 56 (correspond to times 648 h, 648.17 h, 648.33 h, 648.5 h, 648.67 h, 648.83 h, 649 h, 649.5 h, 650 h, 651 h, 652 h, 654 h, 660 h, and 672 h in Tables 59–62 and times 0 h, 0.17 h, 0.33 h, 0.5 h, 0.67 h, 0.83 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 12 h, and 24 h in pharmacokinetic profiles).

Thus, 35 blood samples were collected for each volunteer (15 mL each) for pharmacokinetic parameter assessment. A total of 3850 blood samples were collected for pharmacokinetic parameter assessment in 110 volunteers, assuming each volunteer completed the study. 7700 blood samples were transferred to the analytical center for pharmacokinetic parameter assessment (aliquots A and B) in 110 volunteers.

During the screening, blood was collected in a volume of no more than 25 mL for clinical, biochemical, and serological analyses, determination of glycated hemoglobin, and blood glucose level determination using a glucometer.

During the hospital stage, upon discharge from the inpatient facility (in periods I and II), blood was collected for clinical and biochemical analyses, determination of glycated hemoglobin, and blood glucose level determination using a glucometer, with a total volume not exceeding 15 mL in each period.

Analytical Method

Pharmacokinetic parameters were assessed based on semaglutide concentration in serum by HPLC using a triple quadrupole tandem mass spectrometric detector. For this purpose, the following pharmacokinetic variables were calculated: $AUC_{(0-t)ss}$ is area under the “concentration–time” curve during the dosing interval at steady state (corresponding to AUC_{0-24} on Days 28 and 56); $C_{max, ss}$ is maximum serum drug concentration at steady state (corresponding to C_{max} on Days 28 and 56); $C_{min, ss}$ is minimum serum drug concentration at steady state (corresponding to C_{min} on Days 28 and 56); $T_{max, ss}$ is time to reach maximum serum drug concentration at steady state.

Additional pharmacokinetic calculations could be performed if necessary.

Sample Preparation

Sample preparation was performed by protein precipitation using a methanol–acetonitrile mixture (70 : 30). To 100 μ L of serum sample placed in 2 mL Eppendorf centrifuge tubes, 10 μ L of semaglutide working standard solution was added, followed by 300 μ L of precipitant. The mixture was vortexed for 10 sec and then centrifuged for 15 minutes at 12,300 g. Subsequently, 320 μ L of the supernatant was transferred to a well of a 96-well plate and placed in the chromatograph autosampler.

Chromatographic Separation and Detection Conditions

Chromatographic separation and detection were performed on a Nexera XR high-performance liquid chromatograph and an LCMS-8040 triple quadrupole tandem mass spectrometric detector. Raw data processing was carried out using LabSolutions Ver. 5.91 software (Shimadzu Corp., Japan). Chromatographic separation and detection conditions are presented in Tables 1 and 2.

Method Validation

The developed method was validated according to the following parameters: selectivity, system suitability, matrix effect, calibration curve, accuracy, precision, recovery, lower limit of quantification, sample transfer, short-term stability (“bench-top” and “post-preparative”), stability upon three freeze-thaw cycles, stability of stock and working standard solutions, long-

term analyte stability in matrix, and absence of sample dilution effect.

Within 1–3 validation cycles, the method was fully validated in December 2024. The confirmed analytical range of the method was **1.00–300.00 ng/mL** in serum for semaglutide.

During the analytical stage of the study according to protocol No. 13101-SR, version 1.0 dated June 07, 2024, “Open randomized cross-over comparative study of pharmacokinetic parameters, bioequivalence, safety, and tolerability of the drug WTBC13101 (PROOMED RUS LLC, Russia) in healthy volunteers,” a discrepancy was found between the obtained results and the expected concentration range, based on which the analytical range of the method for semaglutide was selected. In this regard, a decision was made to change the analytical range of the method for semaglutide such that the LLOQ does not exceed 5% of the minimum C_{max} value.

In December 2024, as part of the 4 validation cycle, partial validation of the method for quantitative determination of semaglutide in human serum was conducted to expand the analytical range for semaglutide towards reducing the method’s LLOQ from 1.00 ng/mL to 0.50 ng/mL (Rules for conducting bioequivalence studies of medicinal products within the Eurasian Economic Union, approved by Decision No. 85 of the Council of the Eurasian Economic Commission dated 03.11.2016, Annex VI, Sections 2 and 3, paragraphs 48 and 50). The following validation parameters were assessed: selectivity, calibration curve, accuracy, precision, lower limit of quantification. The confirmed analytical range of the method was **0.50–300.00 ng/mL** in serum for semaglutide.

Bioequivalence Assessment

Bioequivalence of the studied drugs was assessed based on semaglutide concentration in serum. The conclusion on the bioequivalence of the compared drugs was formed using an approach based on the assessment of 90% confidence intervals (CIs) for the ratios of the geometric mean values of $AUC_{(0-t)ss}$ and $C_{max, ss}$ parameters of semaglutide in original units of measurement. The 90% CI limits were rounded to two decimal places. The drugs were considered bioequivalent if the limits of the estimated confidence interval for $AUC_{(0-t)ss}$ and $C_{max, ss}$ of semaglutide were within 80.00–125.00%.

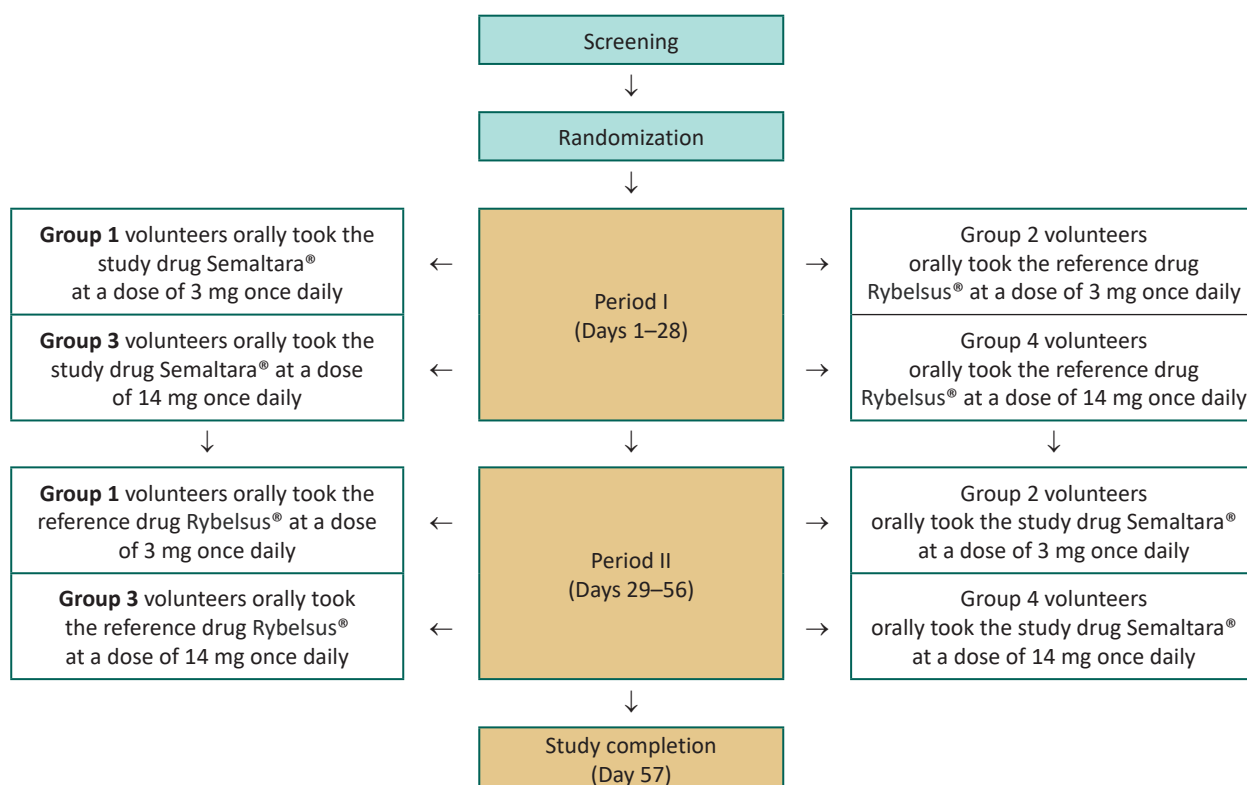


Figure 1 — Study Design.

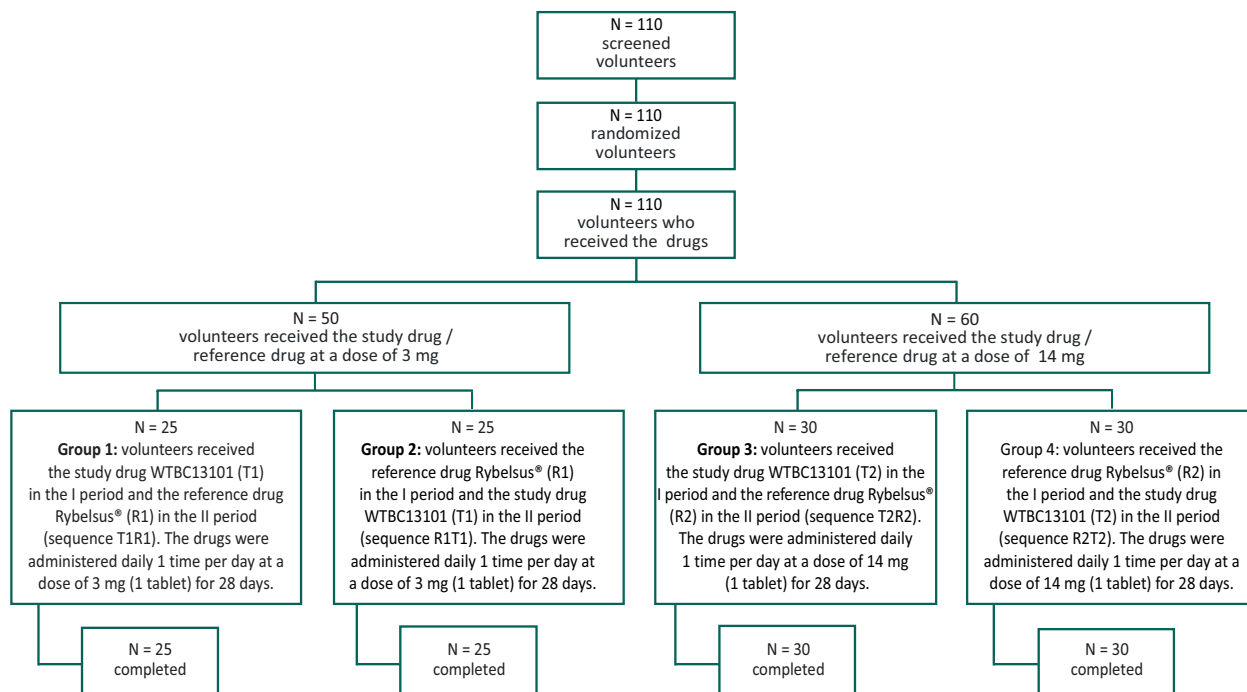


Figure 2 — Distribution of Volunteers.

Table 1 — Chromatographic conditions

Chromatographic conditions			
Column		Phenomenex Luna C18, 100A, 50×2.00 mm, 5 μm	
Pre-column		Phenomenex C18, 4×3.0 mm	
Column thermostat temperature		50°C	
Mobile phase	Eluent A	0.3 % formic acid solution in water (by volume)	
	Eluent B	0.3 % formic acid, 7% ethyl acetate, 5% methanol in acetonitrile (by volume)	
Gradient of the mobile phase	Time, min	% content of eluent B	Flow rate of the mobile phase, mL/min
	0.00	5.0	1.50
	0.50	5.0→100.0	
	1.31	100.0	
	1.99	100.0	2.00
	2.00	100.0	
	2.05	5.0	
	2.50	5.0	1.50
	2.70	100.0	
	3.00	100.0	
	3.01	100.0	2.00
	3.99	100.0	
	4.00	100.0	
	4.05	5.0	1.50
	5.50	5.0	
Volume of the injected sample		20 μL	
Retention time of semaglutide		about 1.1 min	
Liraglutide retention time		about 1.1 min	
Time of registration of the chromatogram		0.00–5.50 min	

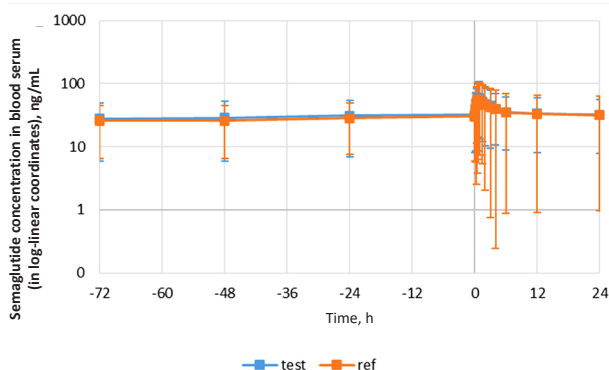
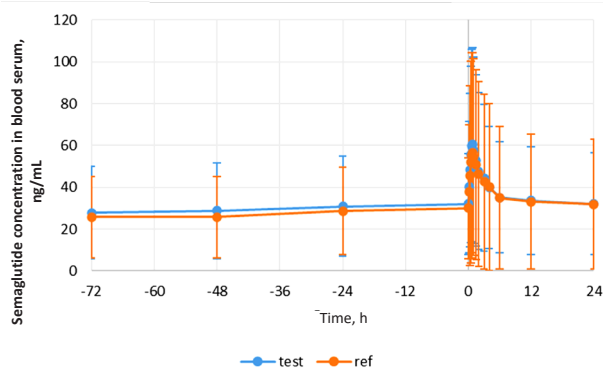
Table 2 — Mass spectrometric detection conditions

Mass spectrometric detection conditions							
Parameters of an electrospray ionization							
Sheath gas	Auxiliary Gas	Heating unit	Desolvation line	Needle voltage		Ionization mode	
				SEM	LIR	SEM	LIR
3 L/min	20 L/min	400 °C	250 °C	5.0 κB	5.0 κB	+	+
Semaglutide detection conditions, m/z							
1029.10→1302.30		1028.90→1109.65		1029.10→1237.60		1029.40→1359.10	
Liraglutide detection conditions, m/z z							
938.35→1128.30			938.35→1063.75				

Table 3 — Average values of the main pharmacokinetic parameters for the study drug and the reference drug

Parametr	Dose 3 mg		Dose 14 mg	
	Semaltara® (T1)	Rybelsus® (R1)	Semaltara® (T2)	Rybelsus® (R2)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C _{max, ss} , ng/mL	51.30 ± 48.06	47.36 ± 52.55	171.10 ± 77.70	171.90 ± 97.43
C _{min, ss} , ng/mL	20.81 ± 18.70	19.77 ± 23.38	107.57 ± 57.92	105.42 ± 66.01
T _{max, ss} , h	0.74 ± 0.23	0.74 ± 0.29	0.79 ± 0.17	0.81 ± 0.12
AUC _{(0–t)_{ss}} , ng×h/mL	670.43 ± 649.14	628.67 ± 818.19	3246.81 ± 1593.14	3226.51 ± 1974.19
ln(C _{max, ss})	3.88 ± 0.69	3.80 ± 0.67	5.12 ± 0.46	5.12 ± 0.52
ln(AUC _(0–t))	6.47 ± 0.73	6.40 ± 0.74	8.07 ± 0.54	8.05 ± 0.62

3 mg Dosage



14 mg Dosage

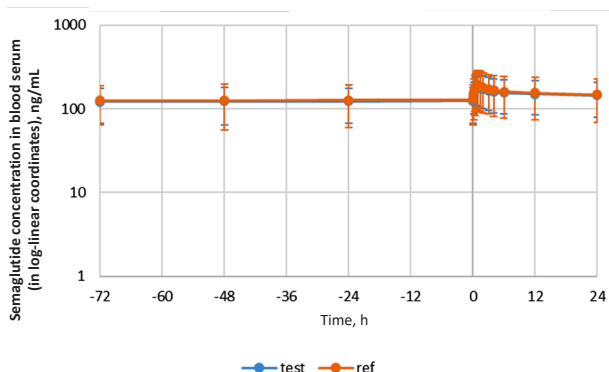
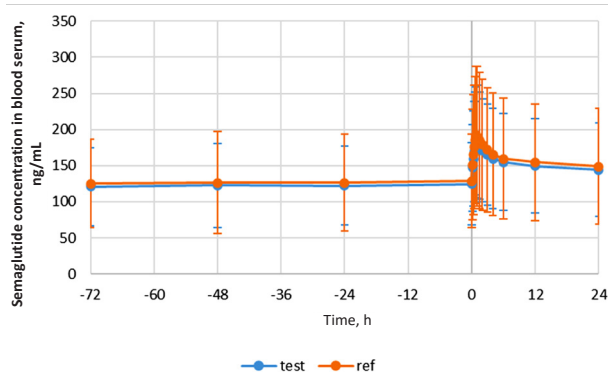


Figure 3 — Pharmacokinetic profiles of semaglutide drugs by concentration and time.

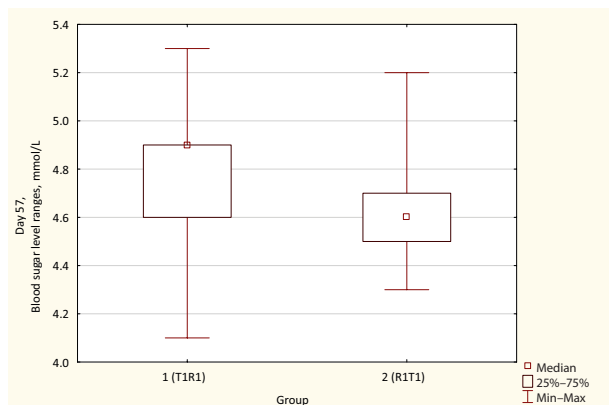
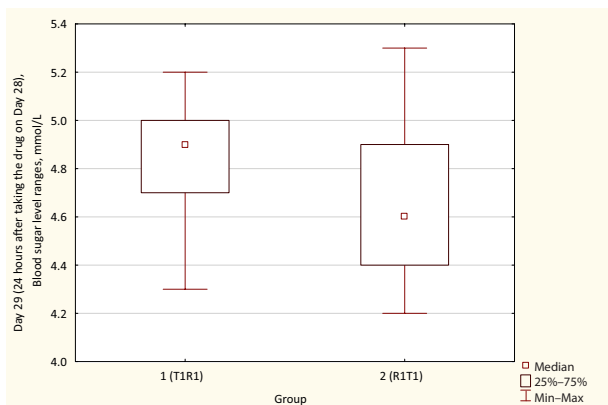
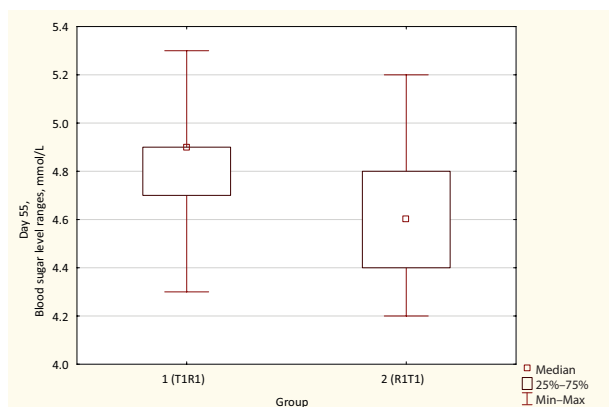
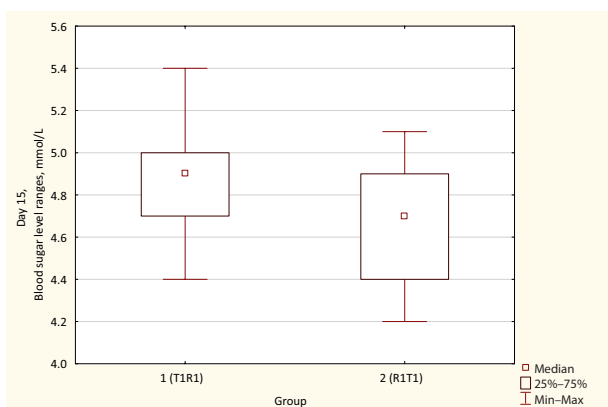


Figure 4 — Comparative analysis of blood glucose levels at hospital and outpatient stages in volunteers.

Safety and Tolerability Assessment

To assess the safety and tolerability of the study drug semaglutide, the following criteria were evaluated:

- Frequency and severity of adverse events (AEs) recorded based on deviations from normal laboratory test results, physical examination, assessment of vital signs, and ECG.
- Number of cases of premature discontinuation from the study due to the development of AEs and/or serious adverse events (SAEs), including those related to the study drug / reference drug.

The investigator's assessment of the overall tolerability of the study drug / reference drug was performed using a Likert scale.

Ethics Approval

The study complied with the ethical principles set forth in the Declaration of Helsinki as revised, the Good Clinical Practice rules of the Eurasian Economic Union, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH E6 GCP R2) Good Clinical Practice guidelines, and other applicable legislation. The clinical study protocol was approved by the Ethics Council of the Ministry of Health of Russia (Extract from the minutes of meeting No. 363 of the Ethics Council of the Ministry of Health of the Russian Federation dated July 23, 2024), as well as by the Local Independent Ethics Committee at the Research Center of the Clinical Hospital No. 3 (extract from the minutes of meeting No. 213 dated August 29, 2024).

Statistical Analysis

Sample Size Calculation

The required number of participants was calculated based on data on intra-individual variability coefficients (CV_{intra}) of key pharmacokinetic parameters in similar studies. The Assessment report Rybelsus [1] presents data indicating that CV_{intra} for $AUC_{(0-t)ss}$ does not exceed 33%. The sample size calculation was performed using PASS 11 software (USA). Considering that the 90% CI was 80.00–125.00%, $AUC_{(0-t)ss}/C_{max, ss}$ was 33%, $\alpha = 0.05$, study power was 80%, and the expected T / R ratio was 0.95, at least 46 healthy volunteers were required to fully complete the study and be included in the statistical analysis. Accounting for potential

dropouts during the study, 50 healthy volunteers were randomized into the 3 mg dosage study (25 volunteers per group) and 60 healthy volunteers (30 volunteers per group) into the 14 mg dosage study. Thus, the total number of randomized volunteers was 110.

Statistical Analysis

Pharmacokinetic parameters, statistical analysis of safety and tolerability indicators, and results reporting were performed using statistical packages (StatSoft Statistica version 13.3, and R project (version 4.1.3, GPL-2/GPL-3 license) with the “bear” extension, version 2.9.1⁵). For all pharmacokinetic parameters, the following statistical parameters were calculated: arithmetic mean, geometric mean, standard deviation of the mean, coefficient of variation, median, minimum and maximum values, range. Statistical analysis was conducted assuming a log-normal distribution for $C_{max, ss}$ and $AUC_{(0-t)ss}$, and a normal distribution for other pharmacokinetic parameters. After logarithmic transformation, these parameters were analyzed using analysis of variance (ANOVA).

The conclusion on the bioequivalence of the compared drugs was made using an approach based on the assessment of 90% CI for the ratios of the geometric mean values of $AUC_{(0-t)ss}$ and $C_{max, ss}$ parameters of semaglutide in original units of measurement. The 90% CI limits were rounded to two decimal places. The drugs were considered bioequivalent if the limits of the estimated confidence interval for $AUC_{(0-t)ss}$ and $C_{max, ss}$ of semaglutide were within 80.00–125.00%.

For all safety and tolerability indicators collected during the study, descriptive statistics are presented as mean and standard deviation (Mean $\pm$ SD), 95% confidence interval for the mean (M [95% CI]), median (Me), interquartile range [Q1 ; Q3], minimum (min) and maximum (max), or as frequencies and percentages (n , %) depending on the variable type.

For the analysis of frequency indicators, proportions were compared using the two-sided exact Fisher's test (or the χ^2 (“chi-squared”) test, if all expected values in the contingency table cells for this analysis were 5 or more). For the comparison of continuous quantitative indicators, the Student's t-test (in case of normal distribution) or the Mann-Whitney

⁵ R project. bear (BE/BA for R) v2.9.6. Available from: <https://www.pkpd168.com/bear>

U test was used (in case of distribution different from normal).

Differences were considered statistically significant at $p < 0.05$.

RESULTS

Bioequivalence and comparability of safety and tolerability profile

Population

The study included 110 male and female volunteers who received the study drugs / reference drugs at doses of 3 mg and 14 mg. The mean age of volunteers in the population was 46.82 ± 8.93 years (range 23 years to 64 years), body weight was 83.26 ± 8.62 kg (range 66.50 kg to 97.20 kg), height was 172.91 ± 7.18 cm (range 162 cm to 189 cm), and the mean BMI was 27.78 ± 1.30 kg/m² (range 25.05 kg/m² to 29.83 kg/m²).

Among the volunteers who received the study drugs / reference drugs at doses of 3 mg and 14 mg, there were 58 (52.73%) male volunteers and 52 (47.27%) female volunteers.

Pharmacokinetics and bioequivalence

3 mg Dose

Based on the statistical analysis, the obtained 90% CIs for the ratio of $C_{\max,ss}$ and $AUC_{(0-\tau),ss}$ values of semaglutide were 96.57–120.94% and 94.37–120.06%, respectively. The obtained confidence intervals fall within the limits established by regulatory documentation and the Study Protocol (80.00–125.00% for $C_{\max,ss}$, $AUC_{(0-\tau),ss}$), indicating that the bioequivalence of the study drugs at a dose of **3 mg** in this sample of subjects is statistically proven (Table 3).

The obtained intra-individual variation coefficients for the pharmacokinetic parameter $C_{\max,ss}$ were 34.46%, and for the parameter $AUC_{(0-\tau),ss}$ they were 37.03%.

The statistical power of the study for the pharmacokinetic parameter $C_{\max,ss}$ was 80.32%, and for the pharmacokinetic parameter $AUC_{(0-\tau),ss}$ it was 74.70% (Table 3).

14 mg Dose

Based on the statistical analysis, the obtained 90% CIs for the ratio of $C_{\max,ss}$ and $AUC_{(0-\tau),ss}$ values of semaglutide were 91.79–109.68% and 91.47–112.80%, respectively. The obtained confidence intervals fall within the limits established by regulatory documentation and the Study Protocol (80.00–125.00% for $C_{\max,ss}$, $AUC_{(0-\tau),ss}$), indicating that the bioequivalence of the study drugs at a dose of **14 mg** in this sample of subjects is statistically proven (see Table 3).

The obtained intra-individual variation coefficients for the pharmacokinetic parameter $C_{\max,ss}$ were 29.74%, and for the parameter $AUC_{(0-\tau),ss}$ they were 35.28%.

The statistical power of the study for the pharmacokinetic parameter $C_{\max,ss}$ was 93.92%, and for the pharmacokinetic parameter $AUC_{(0-\tau),ss}$ it was 85.44%.

Figure 3 presents the averaged pharmacokinetic profiles over time (in linear and log-linear transformations, with and without standard deviations) after administration of the study drug and the reference drug at doses of 3 mg and 14 mg. As can be seen from the compared curves, the concentration-time relationship for the compared drugs is practically indistinguishable.

Complete Blood Count

As a result of the comparative analysis of clinical blood count parameters, no intergroup differences were found, except for differences in “Leukocytes, 10⁹/L” ($p = 0.0073$) at the outpatient stage on day 29 between groups 1 and 2, “Hemoglobin, g/L” ($p = 0.0270$), “Erythrocytes, 10¹²/L” ($p = 0.0114$), “Platelets, 10⁹/L” ($p = 0.0032$), “Color Index/MCH, pg” ($p = 0.0324$) at the outpatient stage on day 29 between groups 3 and 4, “Hemoglobin, g/L” ($p = 0.0313$), “Hematocrit, %” ($p = 0.0371$), “Erythrocytes, 10¹²/L” ($p = 0.0042$), and “Platelets, 10⁹/L” ($p = 0.0040$) at the inpatient stage on day 57 between groups 3 and 4. Differences in these parameters were deemed clinically insignificant because all volunteers had these parameters within the reference ranges.

Urinalysis

Based on the comparative analysis of urinalysis parameters, no significant differences were found, except for differences in “pH, units” ($p = 0.0245$) at the outpatient stage between groups 1 and 2, and “pH, units” ($p = 0.0239$) at the inpatient stage between groups 1 and 2. Differences in these parameters were deemed clinically insignificant because all volunteers had them within the reference ranges.

Biochemical Blood Analysis

Based on the comparative analysis of biochemical blood analysis parameters, no intergroup differences were found, except for differences in “AST, U/L” ($p = 0.0478$) at the outpatient stage, “Total Bilirubin, μmol/L” ($p = 0.0456$), and “AST, U/L” ($p = 0.00298$)

at the inpatient stage, between groups 1 and 2. Differences in these parameters were deemed clinically insignificant because all volunteers had these parameters within the reference ranges.

Differences were also found in "Total Cholesterol, mmol/L" ($p = 0.0443$) at the inpatient stage between groups 3 and 4. Differences in these parameters were deemed clinically insignificant because all volunteers had these parameters within the reference ranges.

Blood Glucose Level

Based on the comparative analysis of blood glucose level parameters, no intergroup differences were found, except for differences in "Blood Glucose Level, mmol/L" ($p = 0.0126$) at the outpatient stage on day 15, "Blood Glucose Level, mmol/L" ($p = 0.0132$) at the outpatient stage on day 29 (24 hours after drug administration on day 28), "Blood Glucose Level, mmol/L" ($p = 0.0031$) at the outpatient stage on day 55, and "Blood Glucose Level, mmol/L" ($p = 0.0032$) at the inpatient stage on day 57, between groups 1 and 2. Differences in these parameters were deemed clinically insignificant because all volunteers had these parameters within the reference ranges.

Blood Pressure Parameters

Based on the comparative analysis of vital signs, no intergroup differences were found between groups 1 and 2, except for differences at the outpatient stage on day 55 for SBP ($p = 0.0340$), on day 1 (0 h, before randomization) for DBP ($p = 0.0363$), on day 43 for RR ($p = 0.0337$), and at the inpatient stage: on day 56 for DBP ($p = 0.0103$), and on day 27 for RR ($p = 0.0100$). Differences in these parameters were deemed clinically insignificant because all volunteers had these parameters within the normal range. No AEs were recorded for these parameters.

Based on the comparative analysis of vital signs, no intergroup differences were found between groups 3 and 4, except for differences at the outpatient stage on day 29 (4 h) for DBP ($p = 0.0272$), on day 53 for DBP ($p = 0.0276$), on day 15 for HR ($p = 0.0159$), on day 53 for HR ($p = 0.0019$), on day 53 for temperature ($p = 0.0292$), on day 1 (0 h, before randomization) for RR ($p = 0.0495$), on day 1 (4 hours after drug administration) for RR ($p = 0.0183$), on day 29 (4 hours after drug administration) for RR ($p = 0.0431$), and at the inpatient stage: on day 27 for HR ($p = 0.0252$).

Differences in these parameters were deemed clinically insignificant because all volunteers had these parameters within the normal range. No AEs were recorded for these parameters.

Safety and tolerability

Safety and tolerability were assessed for all 110 volunteers who received a dose of the drug.

In groups 1 and 2 (3 mg dose), no cases of AEs were recorded. In groups 3 and 4 (14 mg dose), nausea was reported as an AE in 6 volunteers.

A total of 6 AEs were recorded in 6 volunteers during the study: 3 AEs were noted in 3 volunteers in group 3 (T2), and 3 AEs were noted in 3 volunteers in group 4 (R2).

All AEs recorded during the study were of mild severity. According to the investigators, the causal relationship with the use of the study drug / reference drug was assessed as "probable" in all cases. Analysis of the frequency of AE outcomes in volunteers showed that by the end of the study, "recovery without sequelae" was observed in all cases. In all cases of volunteers with recorded AEs, no treatment was administered.

All volunteers completed the study fully in accordance with the approved study protocol.

The investigator's assessment of the overall tolerability of the study drug / reference drug was conducted using a Likert scale. Tolerability of the study drug / reference drug at a dose of 3 mg was assessed as "good" in 100% of cases. Tolerability of the study drug / reference drug at a dose of 14 mg was also assessed as "good" in 90% of cases and "satisfactory" in 10% of cases. Tolerability of the study drug / reference drug at a dose of 14 mg on day 1 was assessed as "good" in 90% of cases and "satisfactory" in 10% of cases. On days 29 (24 hours after drug administration on day 28 and 4 hours after drug administration) and day 57, tolerability was assessed as "good" in 100% of cases.

No SAEs were identified during or after the study. There were no deaths.

No cases of pregnancy in study participants / sexual partners of study participants were recorded during or after the study.

Thus, the study drug Semaltara®, tablets (T), and the reference drug Rybelsus®, tablets (R), have a similar safety profile, including tolerability, at both the 3 mg and 14 mg doses.

DISCUSSION

The structure of semaglutide resembles native human GLP-1 with three key modifications: 1) substitution of alanine with alpha-aminoisobutyric acid at position 8, rendering it resistant to DPP-4 degradation; 2) addition of an 18-carbon chain to lysine at position 26, enhancing albumin binding and increasing half-life; 3) substitution of lysine with arginine at position 34, improving stability [20]. These modifications create a potent, long-acting GLP-1 analog. Oral administration of semaglutide faces bioavailability challenges due to its size and susceptibility to enzymatic degradation. Co-administration with SNAC improves absorption by buffering gastric pH, reducing pepsin activity, and increasing the lipophilicity of semaglutide itself. This allows semaglutide to cross the gastric membrane and enter the systemic circulation, functioning similarly to the subcutaneous form [21, 22].

Unlike most oral drugs, semaglutide is absorbed in the stomach, and its absorption is dependent on the amount of *N*-(8-[2-hydroxybenzoyl]amino) caprylate sodium (SNAC). Studies indicate that 300 mg of SNAC provides the highest plasma levels of semaglutide. Semaglutide and SNAC concentrations are highest near the absorption site in the stomach, decreasing significantly beyond 3 cm. This supports the local buffering effect of SNAC and the need for co-formulation and co-development of the drug product [21]. Oral semaglutide has lower bioavailability and higher inter-individual variability compared to the subcutaneous form, necessitating higher doses to achieve comparable results [23].

Clinical trial program results showed that oral doses of semaglutide 7 mg and 14 mg led to more pronounced improvements in Hb_{A1c} levels compared to sitagliptin, while the 3 mg dose did not show clinically significant benefit [24]. Food, rather than water, significantly reduces semaglutide absorption [21, 25]. Therefore, patients should fast before taking the medication and refrain from eating for at least 30 min after administration [21].

Semaglutide is metabolized via proteolytic cleavage by DPP-4 and neutral endopeptidases, followed by beta-oxidation of its fatty acid side chain [26]. The breakdown products are excreted in urine and feces, indicating renal and hepatic impairment [26, 27].

Pharmacokinetic studies show that oral semaglutide in combination with 300 mg SNAC has

similar pharmacokinetics to the subcutaneous form, irrespective of the presence of renal or hepatic insufficiency [23, 28].

Numerous clinical studies, including the SUSTAIN and PIONEER programs, have been conducted to evaluate the efficacy and safety of injectable and oral semaglutide in patients with diabetes. The SUSTAIN clinical trial program included Phase IIIA randomized controlled trials (SUSTAIN 1–5) to assess the efficacy and safety of weekly subcutaneous semaglutide administration in various groups of patients with type 2 DM, from drug-naïve individuals to those on oral antidiabetic drugs and/or insulin [29–33]. Additionally, SUSTAIN 7 compared semaglutide with dulaglutide, and SUSTAIN 6 focused on cardiovascular safety [34, 35]. The primary outcome in SUSTAIN 1–5 was the change in Hb_{A1c} from baseline to end of treatment. Semaglutide demonstrated significant and sustained reductions in Hb_{A1c} across all studies, ranging from -1.2% to -1.5% at a dose of 0.5 mg and -1.5% to -1.8% at a weekly dose of 1 mg. In SUSTAIN 7, semaglutide reduced Hb_{A1c} by -1.5% and -1.8% at doses of 0.5 mg and 1 mg, respectively, while dulaglutide reduced it by -1.1% and -1.4% at doses of 0.75 mg and 1.5 mg, respectively.

SUSTAIN 8 compared semaglutide (1.0 mg weekly) to canagliflozin (300 mg daily) in 788 patients over 52 weeks. Semaglutide resulted in a greater reduction in Hb_{A1c} (-1.5% vs. -1.1%) and more significant weight loss (-4.7 kg vs. -3.2 kg), demonstrating its superior efficacy in glycemic control and weight management [36].

SUSTAIN 9 evaluated the efficacy of semaglutide in 302 patients on insulin over 30 weeks. Hb_{A1c} levels decreased by -0.8% with semaglutide compared to placebo (+0.1%), and weight decreased by -2.0 kg compared to +0.2 kg with placebo. This study confirmed the role of semaglutide as an effective add-on therapy for patients on insulin [37].

SUSTAIN 10 compared semaglutide (1.0 mg weekly) to liraglutide (1.2 mg daily) in 577 patients over 30 weeks. Semaglutide provided a greater reduction in Hb_{A1c} (-1.7% vs. -1.0%) and more significant weight loss (-5.8 kg vs. -1.9 kg), highlighting its superior efficacy among GLP-1 receptor agonists [38].

Building on the success of injectable semaglutide trials, various clinical studies have been conducted to evaluate the efficacy and safety of oral semaglutide, providing valuable insights into its role as an innovative treatment option for patients with type 2 DM.

A Phase I study by C. Granhall et al. assessed

the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple doses of oral semaglutide and the absorption enhancer SNAC in healthy volunteers and individuals with type 2 DM [39]. In a single-ascending dose study, various combinations of oral semaglutide (2–20 mg) and SNAC were tested to optimize absorption. Results showed that oral semaglutide exposure increased in a dose-dependent manner, with 300 mg of SNAC being the optimal dose. AEs, primarily gastrointestinal disorders and headaches, were mild but more frequent in the semaglutide group compared to placebo [39]. In a multiple-ascending dose study, 107 subjects (healthy and with type 2 DM) were given semaglutide (up to 40 mg) in conjunction with SNAC. Oral semaglutide exposure was similar in healthy individuals and patients with type 2 DM, leading to a significant reduction in Hb_{A1c} (1.5%) and body weight (up to 7.2 kg) after 10 weeks ($p < 0.001$). Most adverse events were mild, with gastrointestinal disorders being the most common [39]. Further pharmacokinetic studies found no consistent pattern of change in semaglutide exposure in patients with renal impairment, except for those with severe renal impairment, where exposure was lower [23].

A Phase II study by M. Davies et al. evaluated the efficacy of oral semaglutide compared to subcutaneous semaglutide for glycemic control in 632 patients with type 2 DM [40]. Participants were randomized into 9 groups, including various doses of oral semaglutide (2.5 mg to 40 mg), placebo, and subcutaneous semaglutide. The primary endpoint, Hb_{A1c} reduction, was achieved: with oral semaglutide 40 mg, the reduction was 1.8% compared to 0.3% in the placebo group ($p < 0.001$). Hb_{A1c} reduction was dose-dependent across all oral semaglutide groups, ranging from 0.4% to 1.6%. At 26 weeks, more participants achieved an Hb_{A1c} level below 7% with oral semaglutide, with rates increasing from 44% in the 2.5 mg group to 90% in the 40 mg group ($p < 0.001$ for all). All groups receiving oral semaglutide also experienced significant weight loss compared to placebo, with the 40 mg dose leading to a reduction of 6.9 kg ($p < 0.001$). No notable difference in weight loss was observed between the oral semaglutide 20 mg and 40 mg groups and the subcutaneous semaglutide group.

The global Phase III clinical trial program for oral semaglutide, PIONEER, comprises 12 clinical trials and involves 9542 patients with type 2 DM. The PIONEER program used two criteria for efficacy assessment:

the treatment policy criterion, which includes patients regardless of drug discontinuation or rescue medication use, and the study drug efficacy criterion, which assumes all patients continued treatment without rescue medication.

PIONEER 1, completed in February 2020, evaluated the safety and efficacy of oral semaglutide versus placebo in patients with type 2 DM treated with diet and exercise alone [41]. Participants (mean age 55 years, Hb_{A1c} = 7.0–9.5%) were randomized to receive 3 mg, 7 mg, or 14 mg of oral semaglutide or placebo for 26 weeks. All semaglutide doses significantly reduced Hb_{A1c} compared to placebo ($p < 0.001$). More participants achieved an Hb_{A1c} value $< 7\%$ with semaglutide (55.1%, 68.8%, 76.9%) compared to placebo (31%) ($p < 0.001$ for all). Reductions in fasting plasma glucose and body weight were more pronounced with semaglutide, particularly at the 14 mg dose, which showed a weight loss difference of -2.3 kg ($p < 0.001$). Higher doses also significantly increased lipase levels and heart rate.

In the PIONEER 2, oral semaglutide (14 mg once daily) was compared with empagliflozin (25 mg once daily) for 52 weeks in patients with type 2 DM with Hb_{A1c} levels from 7.0% to 10.5%, all of whom were taking metformin (≥ 1500 mg/day). At 26 weeks, oral semaglutide showed a greater reduction in Hb_{A1c} (-1.3%) compared to empagliflozin (-0.9%, $p < 0.0001$), with 47.4% of patients achieving an Hb_{A1c} level of $\leq 6.5\%$ vs. 17.2% in the empagliflozin group. These effects were maintained up to and including 52 weeks. Oral semaglutide also resulted in a significantly greater reduction in body weight ($> 10\%$) at both 26 weeks (12.5% vs 6.8%) and 52 weeks (15.0% vs 7.8%, $p = 0.0028$), confirming its superiority regarding Hb_{A1c} and weight reduction [42].

In the 78-week PIONEER 3, oral semaglutide (3 mg, 7 mg and 14 mg) was compared with sitagliptin (100 mg) in 1864 patients with type 2 DM not controlled by metformin, with or without sulfonylurea (mean age 58 years, Hb_{A1c} 7.0–10.5%). At 26 weeks, Hb_{A1c} reduction was more significant with oral semaglutide 14 mg (-1.3%) compared to sitagliptin (-0.8%), with 55% of patients in the 14 mg group achieving an Hb_{A1c} level of $< 7.0\%$ ($p < 0.001$). Fasting plasma glucose also improved with semaglutide 14 mg (-30.5 mg/dL) compared to sitagliptin (-15.4 mg/dL). By 78 weeks, semaglutide demonstrated a more significant reduction in fasting plasma glucose and body weight:

30% of the 14 mg group achieved $\geq 5\%$ weight loss by 26 weeks ($p < 0.001$) compared to 10% with sitagliptin, and 33% versus 12% by 78 weeks [24].

The PIONEER 4 was the first to compare oral semaglutide with another GLP-1 receptor agonist, subcutaneous liraglutide, in patients with type 2 DM treated with metformin, with or without an SGLT-2 inhibitor (mean age 55 years, Hb_{A1c} 7.0–9.5%). Dose escalation was performed in both groups. At 52 weeks, oral semaglutide showed a greater reduction in Hb_{A1c} (-1.6%) compared to liraglutide (-1.1% , $p = 0.0002$) and placebo (-0.2% , $p < 0.0001$). Furthermore, 43% of patients taking oral semaglutide achieved an Hb_{A1c} level of $\leq 6.5\%$, compared to 33% with liraglutide ($p = 0.0084$) and 4% with placebo ($p < 0.0001$). Oral semaglutide also led to greater weight loss than liraglutide or placebo, with 14% and 16% of patients achieving $\geq 10\%$ weight loss at 26 and 52 weeks, respectively. Semaglutide showed a more significant reduction in fasting plasma glucose compared to placebo ($p < 0.0001$), although no statistically significant difference was observed compared to liraglutide [43].

The PIONEER 5 and PIONEER 6 evaluated the efficacy of oral semaglutide in patients with type 2 DM and renal impairment, and assessed its cardiovascular safety in patients with type 2 DM with established cardiovascular disease, chronic kidney disease, or high cardiovascular risk.

The PIONEER 7 compared the safety and efficacy of flexible-dose oral semaglutide and sitagliptin in patients with type 2 DM ($Hb_{A1c} = 7.5\text{--}9.5\%$). The study included 504 participants randomized to receive either dose-adjusted oral semaglutide (3 mg, 7 mg, or 14 mg) or sitagliptin 100 mg. At 52 weeks, 58% of patients in the semaglutide group achieved an Hb_{A1c} level of $< 7\%$ compared to 25% in the sitagliptin group ($p < 0.0001$). A significantly greater weight loss was observed with semaglutide, averaging -2.9 kg compared to -0.8 kg with sitagliptin ($p = 0.0065$). Fewer patients required additional therapy with semaglutide (3.2%) compared to sitagliptin (15.9%, $p < 0.0001$). Semaglutide outperformed sitagliptin in both Hb_{A1c} reduction and weight loss [44].

The PIONEER 8 evaluated the efficacy, safety, and tolerability of oral semaglutide (3 mg, 7 mg, 14 mg) compared to placebo in patients with type 2 DM receiving stable insulin therapy. At 26 weeks, semaglutide significantly reduced Hb_{A1c} by -0.6% ,

-0.9% , and -1.3% for the 3 mg, 7 mg, and 14 mg doses, respectively, compared to -0.1% in the placebo group ($p < 0.0001$). A greater proportion of patients achieved an Hb_{A1c} level of $< 7\%$ with semaglutide (58.4% at the 14 mg dose) compared to the placebo group (6.8%). Weight loss was dose-dependent, with a maximum reduction of -3.7 kg at the 14 mg dose ($p < 0.0001$). Symptomatic rescue medication use was less frequent in the semaglutide groups, and fewer patients required additional glucose-lowering agents compared to the placebo group. Additionally, semaglutide improved fasting lipid profiles and led to a slight increase in heart rate [45].

The PIONEER 9 evaluated the safety and efficacy of oral semaglutide versus subcutaneous liraglutide in Japanese patients with type 2 DM. The study included 243 patients randomized to receive oral semaglutide (3 mg, 7 mg or 14 mg), liraglutide (0.9 mg), or placebo. At 26 weeks, oral semaglutide resulted in Hb_{A1c} reductions of -1.1% , -1.6% , and -1.8% compared to -1.4% with liraglutide and -0.4% with placebo. At 52 weeks, an Hb_{A1c} level of $< 7.0\%$ was achieved by 80% of patients taking semaglutide 14 mg, compared to 49% with liraglutide. Greater weight loss was observed with semaglutide 14 mg (-2.8 kg) than with liraglutide ($+0.4$ kg) or placebo (-1.0 kg). Heart rate slightly increased in all GLP-1 receptor agonist groups [46].

In the PIONEER 10, an open-label, 52-week trial in Japan, oral semaglutide (3 mg, 7 mg, 14 mg) was compared to subcutaneous dulaglutide (0.75 mg) in patients with type 2 DM whose blood sugar was not controlled by a single oral antihyperglycemic agent. After 52 weeks, semaglutide 14 mg led to a greater reduction in Hb_{A1c} (-1.8%) compared to dulaglutide (-1.3%). An Hb_{A1c} level of $< 6.5\%$ was achieved by 21%, 43%, and 58% of patients taking semaglutide at doses of 3 mg, 7 mg, and 14 mg, respectively, vs. 41% of patients taking dulaglutide. Semaglutide 14 mg also resulted in greater weight loss (-1.9 kg), while dulaglutide caused weight gain ($+1.1$ kg) [47].

The PIONEER 11 evaluated the efficacy and safety of oral semaglutide versus placebo in a predominantly Chinese population with type 2 DM inadequately controlled by diet and exercise alone. The study was conducted across 52 centers, with 521 participants randomized to receive oral semaglutide (3 mg, 7 mg and 14 mg) or placebo for 26 weeks. The primary endpoint was the change in Hb_{A1c} , and the secondary endpoint was body weight. Semaglutide

significantly reduced Hb_{A1c} compared to placebo for all doses, with reductions ranging from -1.0% to -1.5% ($p < 0.001$). Weight loss was also significant for the 7 mg and 14 mg doses. Adverse events, primarily gastrointestinal, were more frequent in the semaglutide group but were mostly mild to moderate in severity. Similar results were observed in the Chinese subpopulation, which comprised 74.9% of the study participants [48].

The PIONEER 12 compared oral semaglutide with sitagliptin in Chinese patients with type 2 DM inadequately controlled by metformin for 26 weeks. Oral semaglutide (3 mg, 7 mg, and 14 mg) resulted in significantly greater reductions in Hb_{A1c} and body weight compared to sitagliptin. Hb_{A1c} reductions were -0.2%, -0.7%, and -1.0% for the semaglutide doses, while body weight reductions were -0.9 kg, -2.3 kg, and -3.3 kg. Most AEs with semaglutide were mild to moderate gastrointestinal disorders. Similar results were noted in the Chinese subpopulation [49]. However, it is likely that oral semaglutide demonstrated safety and non-inferior efficacy compared to subcutaneous semaglutide and other GLP-1 agonists in clinical trials. As the first oral GLP-1 receptor agonist, it offers an alternative for patients with type 2 DM who are apprehensive about injectable medications but struggle to achieve target Hb_{A1c} levels with existing oral agents.

In summary, the listed studies have demonstrated the efficacy and safety of oral semaglutide across diverse patient populations, including those with type 2 DM and various comorbidities, treatment regimens, and geographic regions. The SUSTAIN and PIONEER clinical trial programs provide extensive evidence of semaglutide's efficacy and safety, regardless of its administration route, whether

injectable or oral. The SUSTAIN studies showed significant reductions in Hb_{A1c} and weight loss with notable superiority over references such as dulaglutide and liraglutide. Semaglutide consistently outperformed alternative treatments in improving glycemic control, particularly in patient groups ranging from those with cardiovascular risk factors to those on insulin therapy. The PIONEER program further validated the effectiveness of oral semaglutide, offering a promising alternative to injectable therapy, especially for patients unwilling to use injections but needing to achieve glycemic targets. Both programs underscore semaglutide's potential to enhance treatment adherence, lower Hb_{A1c} levels, and promote weight loss across various patient profiles, thereby solidifying its role as a key advancement in type 2 DM management.

Study Limitations

The small sample size, due to the single-center design of the study, significantly increases the probability of underestimating the real clinical effect during the statistical interpretation of the results. Continuation of this clinical study, as well as conducting additional clinical and observational studies involving a larger number of patients and an increased observation period, will enhance the representativeness of the results of semaglutide use.

CONCLUSION

Thus, the clinical study conducted confirmed the bioequivalence and demonstrated a similar safety profile, including tolerability, of the study drug Semaltara®, tablets, and the reference drug Rybelsus®, tablets, at both 3 mg and 14 mg dosages.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Alexander S. Ametov, Larisa A. Balykova — conceptualization, data analysis, writing—review & editing; Igor E. Shokhin — investigation, data analysis, visualization; Kira Ya. Zaslavskaya — investigation, data analysis, visualization, writing—original draft; Ekaterina A. Rogozhina — methodology, investigation, data analysis;

Tatiana G. Bodrova — investigation data analysis, writing—original draft; Larina A. Zorina — conceptualization, data analysis, writing—original draft; Victoria S. Scherbakova — data analysis, writing—original draft; Denis V. Kurkin — data analysis, writing—original draft; Ksenya N. Koryanova — data analysis, writing—original draft; Ekaterina S. Mishchenko — working with data, writing—original draft; Maxim L. Maksimov — data analysis, writing—original draft; Alexander V. Matveev — data analysis, writing—original draft; Larisa I. Shcherbakova — data analysis, writing—original draft; Irina N. Dyakova — data analysis, writing—original draft; Evgeny D. Kozlov — data analysis, writing—review & editing; Dmitry N. Andreev — conceptualization, writing—original draft; Yuri G. Kazaishvili — conceptualization, writing—original draft; Sergey M. Noskov — conceptualization, writing—original draft. Petr A. Bely — conceptualization, working with data, writing—original draft.

All the authors confirm their authorship compliance with the ICMJE international criteria (all the authors made a significant contribution to the conceptualization, conduct of the study and preparation of the article, read and approved the final version before publication).

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Evaluation of the Effect of Semaglutide on Certain Cardiovascular Risk Factors in Overweight or Obese Healthcare Workers. The “Start with Yourself” Program: A Post-registration, Single-center, Prospective Study of Routine Clinical Practice

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The aim. To evaluate the effect of semaglutide on certain cardiovascular risk factors in patients with obesity.

Materials and methods. The study included 50 individuals: men and women aged 18 to 65 years, patients with overweight (BMI ≥ 27 and < 30) and obesity (BMI ≥ 30), combined with at least one comorbidity (prediabetes or type 2 diabetes mellitus), arterial hypertension, dyslipidemia, obstructive sleep apnea syndrome, or cardiovascular disease. Patients received subcutaneous semaglutide (Velgia®) once a week. Clinical examination was performed, including: interview, blood pressure measurement, anthropometric data assessment (height, weight, BMI, waist circumference, and hip circumference), cardiovascular risk assessment using the SCORE scale, biochemical blood analysis (including lipid profile), and adverse event registration.

Results. A significant decrease in body weight, BMI, waist circumference, and hip circumference was observed after 12 weeks of semaglutide therapy. By 24 weeks, a sustained reduction in these indicators continued. After 6 months of semaglutide use, positive changes in the lipid profile were identified: a decrease in total cholesterol by 8.27 % due to a decrease in non-HDL cholesterol, LDL cholesterol by 12.73 %, triglycerides by 13.09 %, and the atherogenic index by 1.83 %. Velgia® therapy for 6 months led to a reduction in the calculated 10-year cardiovascular risk according to the SCORE2 scale by 18.6 %. Analysis of anthropometric and biochemical blood data showed a significant decrease in indicators, indicating a reduced risk of cardiovascular events in patients with obesity.

Conclusion. During semaglutide therapy, no clinically significant changes were observed in liver function parameters (ALT, AST, ALP, bilirubin), pancreas (amylase), kidneys (creatinine, urea, GFR), and systemic inflammation markers (CRP). However, a significant decrease in transaminase activity (ALT, AST) and blood uric acid concentration was noted. The registered adverse events (primarily gastrointestinal: nausea, epigastric discomfort) were temporary and moderate in nature. No cardiovascular events were observed during the use of semaglutide.

Keywords: semaglutide; diabetes mellitus; obesity; glucagon-like peptide-1 receptor agonist; atherosclerosis; prediabetes

Abbreviations: BMI — body mass index; TC — total cholesterol; HDLs — high-density lipoproteins; LDLs — low-density lipoproteins; ALT — alanine aminotransferase; AST — aspartate aminotransferase; ALP — alkaline phosphatase; GFR — glomerular filtration rate; CRP — C-reactive protein; T2DM — type 2 diabetes mellitus; GLP-1 RAs — glucagon-like peptide-1 receptor agonists; MACE — major adverse cardiovascular events; CVD — cardiovascular disease; SBP — systolic blood pressure; DBP — diastolic blood pressure; FPG — fasting plasma glucose; MI — myocardial infarction.

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Оценка влияния препарата семаглутид на некоторые факторы сердечно-сосудистого риска у медицинских работников с избыточной массой тела или ожирением. Программа «Начни с себя»: пострегистрационное одноцентровое проспективное исследование рутинной клинической практики

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Цель. Провести оценку влияния препарата семаглутид на некоторые факторы сердечно-сосудистого риска у пациентов с ожирением.

Материал и методы. В исследование включено 50 человек: мужчины и женщины в возрасте от 18 до 65 лет, пациенты с избыточной массой тела (ИМТ $\geq$ 27 и <30) и ожирением (ИМТ $\geq$ 30), в сочетании с хотя бы одним сопутствующим заболеванием (предиабет или сахарный диабет 2 типа), артериальная гипертензия, дислипидемия, синдром обструктивного апноэ во сне или сердечно-сосудистые заболевания. Пациентам вводился препарат семаглутид (Велгия®) подкожно 1 раз в неделю, проводилось клиническое обследование, включающее: опрос, измерение артериального давления, оценку антропометрических данных (рост, масса тела, ИМТ, окружности талии и окружности бёдер), оценка сердечно-сосудистого риска по шкале SCORE, проведение биохимического анализа крови (в т.ч. липидограммы), регистрация нежелательных явлений.

Результаты. Отмечено достоверное снижение массы тела, ИМТ, окружности талии и бёдер через 12 нед терапии препаратом семаглутид. К 24 нед продолжилось устойчивое снижение данных показателей. Через 6 месяцев применения препарата семаглутид выявлены позитивные изменения в липидном спектре: снижение общего холестерина на 8,27% за счёт снижения холестерина не-ЛПВП, холестерина ЛПНП — на 12,73%, тиреоглобулина — на 13,09%, индекса атерогенности — на 1,83%. Терапия препаратом Велгия® в течение 6 мес привела к снижению расчётного 10-летнего сердечно-сосудистого риска по шкале SCORE2 на 18,6%. При анализе данных антропометрии, биохимического анализа крови отмечено достоверное снижение показателей, что свидетельствует о снижении риска сердечно-сосудистых событий у пациентов с ожирением.

Заключение. На фоне терапии препаратом семаглутид не наблюдалось клинически значимых изменений в показателях функции печени (АЛТ, АСТ, ЩФ, билирубин), поджелудочной железы (амилаза), почек (креатинин, мочевины, СКФ) и маркеров системного воспаления (СРБ). При этом отмечено достоверное снижение активности трансаминаз (АЛТ, АСТ) и концентрации мочевой кислоты в крови. Зарегистрированные нежелательные явления (преимущественно со стороны ЖКТ: тошнота, дискомфорт в эпигастрии) носили временный и умеренный характер. Сердечно-сосудистых событий в период применения препарата семаглутид не отмечено.

Ключевые слова: семаглутид; сахарный диабет; ожирение; агонист рецепторов глюкагоноподобного пептида-1; атеросклероз; предиабет

Список сокращений: ИМТ — индекс массы тела; ОХС — общий холестерин; ХС — холестерин; ЛПВП — липопротеины высокой плотности; ЛПНП — липопротеины низкой плотности; АЛТ — аланинаминотрансфераза; АСТ — аспартатаминотрансфераза; ЩФ — щелочная фосфатаза; СКФ — скорость клубочковой фильтрации; СРБ — С-реактивный белок; СД2 — сахарный диабет 2 типа; арГПП-1 — агонисты рецепторов глюкагоноподобного пептида-1; MACE — риск серьезных сердечно-сосудистых событий; ССЗ — сердечно-сосудистые заболевания; САД — систолическое артериальное давление; ДАД — диастолическое артериальное давление; ГПН — глюкоза плазмы натощак; ОИМ — острый инфаркт миокарда

INTRODUCTION

Obesity is a chronic disease characterized by the excessive accumulation and dysfunction of adipose tissue [1]. Obesity is a significant risk factor for many comorbid conditions, primarily type 2 diabetes mellitus (T2DM), cardiovascular diseases, as well as a range of oncological, gastrointestinal, and musculoskeletal diseases, and psychological problems [2]. An increased risk of cardiovascular disease (CVD) is the most common cause of mortality in patients with obesity [3].

In the Russian Federation, as of 2016, the proportion of individuals with overweight was 62.0%, and with obesity, it was 26.2% [4]. Based on a survey of a representative sample of the population aged 35–74 years residing in 15 regions of the Russian Federation within the framework of the “ESSE-RF” study, 3 phases of prospective studies have been conducted [5]. The third survey (“ESSE-RF3”) showed the prevalence of overweight (BMI) at 44.0% and 33.7%, obesity at 30.0% and 39.5%, and abdominal obesity at 30.9% and 55.1% among men and women, respectively [6].

In patients with existing obesity, comorbid pathology progresses: cardiometabolic disorders (T2DM, hypercholesterolemia, ischemic heart disease, stroke, hypertensive disease) and non-metabolic diseases (gastroesophageal reflux disease, non-alcoholic fatty liver disease, malignant neoplasms, musculoskeletal damage, sleep-wake disorders, depressive disorders), which significantly leads to a reduction in average life expectancy [7].

The low efficacy of obesity treatment, despite financial costs, necessitates a review of existing approaches and the development of new treatment methods and medications based on a deeper understanding of the pathogenetic basis of obesity [8].

Traditional therapeutic approaches demonstrate limited effectiveness in terms of weight loss and cardiovascular outcomes in the long term. The emergence and implementation of incretin-based drugs in clinical practice have ushered in a new era in the treatment of metabolic diseases. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) and, subsequently, dual and multi-agonists, exert a complex effect on key pathophysiological links of T2DM and obesity [9–11].

Semaglutide is the latest GLP-1 RA, approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the long-term treatment of obesity in 2021. The approval of semaglutide at a dose of 2.4 mg per week for weight loss is based on the results of the STEP (1–8) research program [12]. Furthermore, the STEP 4

study demonstrated the positive effect of long-term semaglutide treatment for weight loss [13].

In the treatment of obesity, it is crucial to define individual goals for each patient, going beyond simple weight reduction [14]. The SELECT trial proved that the drug at a dose of 2.4 mg reduces the risk of major adverse cardiovascular events (MACE) by 20% in patients with obesity and established cardiovascular disease, but without diabetes [15]. These results allowed for the registration of a new indication for semaglutide is the reduction of cardiovascular event risk in patients with obesity.

Thus, studying the effect of semaglutide on key cardiovascular risk factors in patients with obesity is relevant.

PROMED Group (Russia) has developed the “Start with Yourself” program to support doctors and care for their own health and well-being, which is directly related to patient health. This study was conducted within the framework of this program.

THE AIM is to evaluate the effect of semaglutide (Velgia®) on certain cardiovascular risk factors in patients with obesity.

MATERIALS AND METHODS

Study Design

A post-registration, single-center, prospective study with minimal intervention in routine clinical practice was conducted.

Study Conduct and Duration

The study was conducted from May 5, 2025, to January 31, 2026, at the National Research Ogarev Mordovia State University and the Republican Clinical Hospital No. 4 (Saransk, Republic of Mordovia, Russia). The total duration of the study for each volunteer was no more than 171 days (including the screening period). All patients completed the study fully in accordance with the approved Study Protocol.

Study Subjects and Eligibility Criteria

The study “Efficacy and Safety of the Russian Analog of Long-Acting Human GLP-1 Drug Velgia® (Semaglutide) in Routine Clinical Practice in the Republic of Mordovia: “Republican Program for Prevention of Risks of Atherosclerosis-Mediated Cardiovascular Diseases and Diabetes Mellitus in Healthcare Workers with Overweight (BMI 27–30) or Obesity (BMI ≥ 30)” included 50 individuals.

Inclusion Criteria: Men and women aged 18 to 65 years, patients with overweight (BMI ≥ 27 and < 30) and obesity (BMI ≥ 30), combined with at least one

concomitant disease (prediabetes or T2DM, arterial hypertension, dyslipidemia, obstructive sleep apnea syndrome, or cardiovascular diseases), voluntary informed consent to participate in the study.

Exclusion Criteria: Age younger than 18 years and older than 65 years, oncological diseases, severe decompensated pulmonary, hepatic, or renal pathology.

Methodology

Investigator physicians screened patients in accordance with the inclusion / exclusion criteria. Anthropometry was performed (measurement of height, body weight, waist circumference, hip circumference), BMI was calculated; complaints and medical history were collected; a physical examination was conducted (including measurement of BP, HR); cardiovascular risk was calculated (SCORE2-Diabetes / SCORE / SCOREII index) using the MDCalc calculator; quality of life was assessed according to EQ 5D-3L questionnaires; biochemical blood analysis results were assessed (fasting plasma glucose, insulin, HOMA-IR index, Hb_{A1c} , creatinine with eGFR calculation, bilirubin, urea, C-reactive protein, uric acid, ALT, AST, ALP, total cholesterol, LDL, HDL, triglycerides), performed using an AU480 automated biochemical analyzer (Beckman Coulter, USA).

Patients received semaglutide (Velgia®) subcutaneously once a week, regardless of the time of day, in the abdomen, thigh, or upper arm, irrespective of food intake. The initial dose was 0.25 mg once a week, and the dose was increased over 16 weeks, starting from 0.25 mg up to a maintenance therapeutic dose of 2.4 mg once a week. Participation in the study lasted 24 weeks from Visit 1. In total, there were 6 visits in the study, including screening, dose titration, treatment, and follow-up. During each visit, the investigator completed the patient case report form and paper-based patient questionnaire forms. Any adverse events detected during the study were documented by the investigator in an adverse event report.

Ethics Approval

The study protocol was approved at a meeting of the Local Ethics Committee of the Medical Institute, National Research Ogarev Mordovia State University (Protocol No. 137 dated February 28, 2025).

Statistical Analysis

Statistical analysis was performed using Jamovi 2.6.44 Software. Normality of distribution was assessed using the Shapiro-Wilk test. For normal distribution,

means (M) and standard error of the mean (m) were evaluated. For non-normal distribution, median (Me) and interquartile range [Q1; Q3] were evaluated. The significance of differences was determined by the paired Student's t-test for normal distribution, and by the Wilcoxon test for non-normal distribution. Comparison of groups for qualitative characteristics was performed using the chi-square test. The critical level of statistical significance was taken as $p < 0.05$.

RESULTS

Population

The study group included 50 patients: 4 men (8%) and 46 women (92%) aged 22–65 years (mean age 46.06 ± 1.42 years) at the time of signing informed consent, with confirmed overweight and obesity. The duration of obesity or overweight ranged from 5 to 35 years, with an average of 15.36 ± 1.08 years at the time of inclusion in the study (Table 1).

Only 8 (16%) patients had no concomitant pathology. 24 (48%) had more than two concomitant diseases. The structure of concomitant pathology was as follows: arterial hypertension—23 (46%) patients, T2DM—8 (16%), prediabetes—8 (16%), musculoskeletal diseases—6 (12%), hypothyroidism—5 (10%), 2 (4%) each—iron deficiency anemia, chronic pancreatitis, varicose veins of the lower extremities, chronic heart failure, urolithiasis, chronic pyelonephritis—1 (2%) (see Table 1).

Smoking as an additional cardiovascular risk factor was noted in 5 patients (10%). At Visit 1, cardiovascular risk was assessed as low in 11 (22%) patients, moderate—in 7 (14%), high—in 20 (40%), and very high—in 12 (24%) subjects. The 10-year CVD risk (SCORE) was $4.85 [2.00; 9.13]\%$.

The mean systolic blood pressure (SBP) at the time of inclusion in the study was 127.74 ± 1.82 mmHg, and diastolic blood pressure (DBP) was 80.34 ± 1.28 mmHg.

Fasting plasma glucose (FPG) was 5.80 ± 0.22 mmol/L, and glycated hemoglobin (Hb_{A1c}) was $5.30 \pm 0.18\%$.

Alanine aminotransferase (ALT) activity was 32.90 ± 2.57 U/L, and aspartate aminotransferase (AST) was 24.09 ± 1.35 U/L. In 80% of cases, ALT activity exceeded reference values, and in 8% of cases, AST elevation was detected.

In 18% of cases, hyperuricemia was detected, and in 2% of cases, elevated urea was found (mean levels were 315.02 ± 11.38 mmol/L and 5.53 ± 0.17 mmol/L, respectively). Bilirubin and amylase levels did not exceed reference values. The mean bilirubin level was 9.95 ± 0.44 μ mol/L, and amylase was 38.57 ± 1.79 U/L.

Elevated C-reactive protein (CRP) was noted in

14% of patients. The median CRP in the study group was 1.65 [0.20; 3.35].

The mean glomerular filtration rate (GFR), calculated using the CKD-EPI formula, was 82.39 ± 1.91 mL/min/1.73 m². GFR less than 90 mL/min/1.73 m² was observed in 64% of subjects, and less than 60 mL/min/1.73 m² in 8%.

Lipid profile abnormalities were recorded in the majority of the examined patients. The most common deviations were hypercholesterolemia and elevated low-density lipoprotein cholesterol (LDL-C) levels.

Hypercholesterolemia was detected in 32 (64%) patients with a mean total cholesterol (TC) level of 5.57 ± 0.15 mmol/L. Elevated LDL-C was observed in 34 (68%), with a mean value reaching 3.85 ± 0.14 mmol/L. Hypertriglyceridemia was diagnosed in 16 (32%), with a mean triglyceride level of 1.91 ± 0.17 mmol/L. Hypo-alpha-lipoproteinemia (decreased HDL-C) was noted in 17 (34%) patients with a mean value of 1.19 ± 0.03 mmol/L. The atherogenic index was elevated in more than half of the patients (56%, $n = 28$) and averaged 3.82 ± 0.40 .

Dynamics of anthropometric parameters in patients

A significant decrease in body weight, BMI, waist circumference, and hip circumference was observed after 12 weeks of semaglutide therapy. By 24 weeks, a sustained reduction in these parameters continued.

After 12 weeks of therapy, body weight decreased by an average of 6.65 kg, and after 24 weeks, by 12.0 kg, which corresponded to 6.71% and 11.3% respectively. BMI decreased by 2.50 kg/m² and 4.33 kg/m² respectively. The average reduction in waist circumference was 4 cm by 12 weeks and 7.65 cm by 24 weeks, and hip circumference decreased by 4 cm and 9 cm respectively (Table 2).

As early as 12 weeks of semaglutide therapy, amidst significant changes in body weight, a redistribution of patients towards less severe obesity categories was recorded: the number of patients with overweight increased to 5 (10.2%), while the number of subjects with class III obesity decreased by half compared to baseline, amounting to 20.4% ($n = 10$) (Figure 1).

By 24 weeks of semaglutide use, the total proportion of patients with any degree of obesity was 75% ($n = 36$). Further improvement in anthropometric parameters was observed: normalization of body weight (BMI < 25 kg/m²) was achieved in 2 (4.2%) patients, and another 9 (18.8%) who initially had obesity moved into the overweight group. The most pronounced reduction was noted in the severe obesity groups: the proportion of patients with class II obesity

decreased by 7%, and with class III obesity by 23.2% relative to baseline values (see Figure 1). The obtained data indicate a dose-dependent and time-dependent effect of the drug, most pronounced in patients with severe forms of obesity. The use of semaglutide for 24 weeks allowed for body weight reduction sufficient to move out of the obesity category in every fourth patient ($n = 12$).

Dynamics of blood pressure

Initially and throughout 6 months of semaglutide therapy, patients maintained stable and well-controlled blood pressure levels. Systolic blood pressure (SBP) from the Visit 4 and diastolic blood pressure (DBP) by the Visit 6 showed a significant decrease and corresponded to target values, including patients with arterial hypertension (Figure 2).

Dynamics of blood biochemical parameters

Initially, transaminase activity exceeded reference values but did not exceed a 3-fold increase from normal: ALT in 10 patients (20.8%) and AST in 4 (8.3%). After 6 months of semaglutide use, ALT activity exceeded normal levels in only 2 (4%, $p < 0.05$), and AST activity was within normal limits in all patients ($p < 0.05$). Transaminase levels significantly decreased: ALT from 32.65 ± 2.63 U/L to 22.09 ± 1.36 U/L—a 32.4% reduction, and AST from 24.10 ± 1.39 U/L to 18.40 ± 0.72 U/L—a 23.7% reduction (Table 3).

The levels of alkaline phosphatase, bilirubin, amylase, urea, and creatinine after 6 months of semaglutide use did not differ from baseline values and remained within their respective reference ranges. The mean levels were: alkaline phosphatase 157.68 ± 6.44 U/L, bilirubin 10.75 ± 0.69 μmol/L, amylase 42.60 ± 2.98 U/L, urea 5.07 ± 0.19 mmol/L (see Table 3).

Initially, elevated CRP was detected in 6 patients, and by the end of the study, in 5 patients.

Uric acid levels significantly decreased from 314.23 ± 11.66 μmol/L to 281.58 ± 11.23 μmol/L (an 11.4% reduction, $p < 0.05$), and the number of patients with hyperuricemia decreased from 9 (18.7%) to 5 (10.4%) (see Table 3).

The mean glomerular filtration rate (GFR), calculated using the CKD-EPI formula, did not change compared to baseline and was 82.87 ± 1.73 mL/min/1.73 m² (99.9% of baseline, $p = 0.9$). GFR between 60–89 mL/min/1.73 m² was observed in 68.8% (baseline 66.6%, $p = 0.8$), and less than 60 mL/min/1.73 m² in 2.1% (baseline 8.3%, $p = 0.16$) (Table 3).

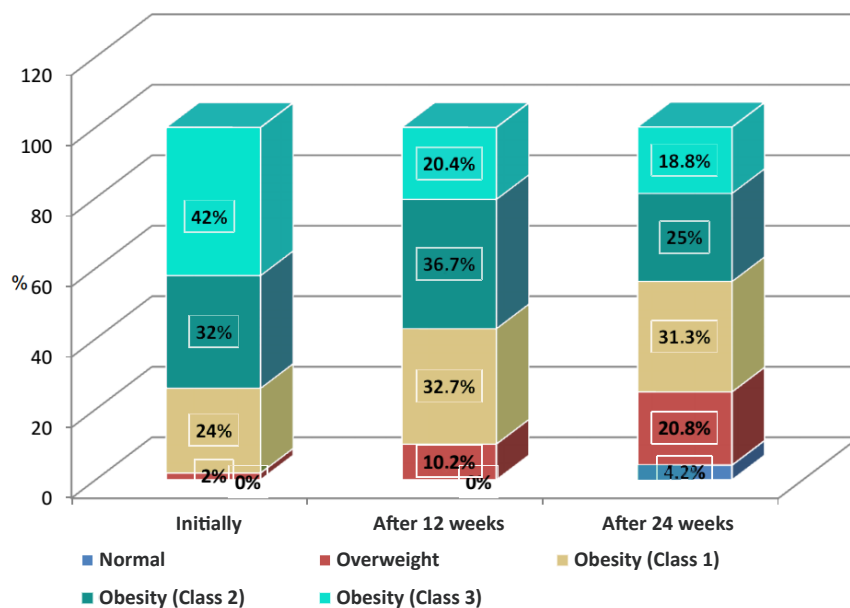


Figure 1 — Dynamics of obesity by body mass index during treatment with semaglutide.

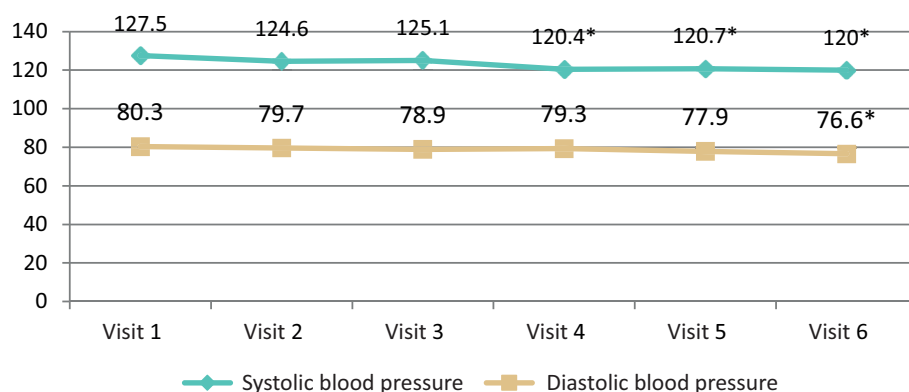


Figure 2 — Dynamics of systolic and diastolic blood pressure during treatment with semaglutide.

Note: * is differences are statistically significant at $p < 0.05$.

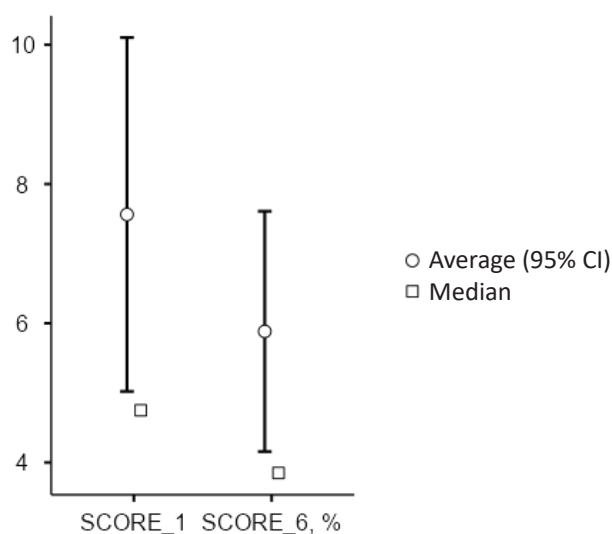


Figure 3 — Cardiovascular risk by SCORE after 6 months of therapy with semaglutide ($n = 48$).

Note: missing values were excluded pairwise.

Table 1 — Clinical characteristics of the study group

Characteristic	Total (n = 50)
Age, (M ± m), years	46.06 ± 1.42
Sex	
Female, n (%)	46 (92%)
Male, n (%)	4 (8%)
Duration of obesity, (M ± m), years	15.36 ± 1.08
Body weight, (M ± m), kg	108.59 ± 2.99
Body mass index, (M ± m), kg/m ²	39.06 ± 0.84
Waist circumference, (M ± m), cm	113.32 ± 1.95
Hip circumference, (M ± m), cm	124.89 ± 1.63
Overweight, n (%)	1 (2%)
Obesity, n (%)	49 (98%)
	Grade I 12 (24%)
	Grade II 16 (32%)
	Grade III 21 (42%)
Comorbidities, n (%):	
Type 2 Diabetes Mellitus	8 (16%)
Prediabetes	8 (16%)
Arterial Hypertension	23 (46%)
Musculoskeletal disorders	6 (12%)
Hypothyroidism	5 (10%)
Iron deficiency anemia	2 (4%)
Chronic pancreatitis	2 (4%)
Varicose veins of the lower extremities	2 (4%)
Chronic heart failure	2 (4%)
Urolithiasis	2 (4%)
Chronic pyelonephritis	1 (2%)

Table 2 – Dynamics of anthropometric parameters in patients receiving semaglutide at 12 and 24 weeks of therapy

Characteristic	At 12 weeks (n = 49)	At 24 weeks (n = 48)
Body weight, kg	6.65 [5.15 ; 9.30]	6.71 ± 0.500
Body mass index, kg/m ²	2.50 [1.80 ; 3.20]	4.33 ± 0.268*
Waist circumference, cm	4.00 [1.00 ; 7.00]	7.65 ± 0.927*
Hip circumference, cm	4.00 [1.00 ; 7.00]	9.00 [6.50 ; 12.0]*

Note: * is differences are statistically significant at $p < 0.05$.**Table 3 — Blood parameters after 6 months of semaglutide treatment (n = 48)**

Characteristic	Visit 1	Visit 6	% of baseline value	p (t-test)
ALT, U/L	32.65 ± 2.63	22.09 ± 1.36	67.6	0.0005*
AST, U/L	24.10 ± 1.39	18.40 ± 0.72	76.3	0.0004*
Alkaline phosphatase, U/L	148.6 ± 6.10	157.68 ± 6.44	106	0.3
Bilirubin, μmol/L	9.88 ± 0.44	10.75 ± 0.69	108.8	0.3
Amylase, U/L	38.18 ± 1.84	42.60 ± 2.98	111.5	0.2
Creatinine, μmol/L	78.03 ± 1.61	79.10 ± 1.21	101.3	0.6
Urea, mmol/L	5.48 ± 0.17	5.07 ± 0.19	95.5	0.12
Uric acid, μmol/L	314.23 ± 11.66	281.58 ± 11.23	89.6	0.04*
C-reactive protein, mg/L	1.65 [0.20 ; 3.35]	0.10 [0.057 ; 0.575]	6	0.002*
GFR, mL/min/1.73 m ² :	82.97 ± 1.91	82.87 ± 1.73	99.9	0.9
GFR ≥ 90, n (%)	12 (25%)	14 (29.1%)	3.9	0.8
GFR 60–89, n (%)	32 (66.6%)	33 (68.8%)	1.8	0.8
GFR < 60, n (%)	4 (8.3%)	1 (2.1%)	6.2	0.16

Note: missing values were excluded pairwise, * is differences are statistically significant at $p < 0.05$.

Cardiovascular Risk Assessment

Initially, cardiovascular risk in the study group was assessed as low in 11 (22%) patients, moderate in 7 (14%), high in 20 (40%), and very high in 12 (24%).

When calculated using SCORE, the 10-year cardiovascular disease risk in the baseline group was 4.85 [2.00; 9.13]%. After 6 months of semaglutide therapy, it decreased by 20.6% to 3.85 [1.00; 7.45]% ($p < 0.001$) (Figure 3).

No cardiovascular events were registered during the study period.

After 6 months of semaglutide use, a number of positive changes in the lipid profile were noted. A significant decrease in TC by 8.27% ($p < 0.05$) was observed, attributed to a reduction in non-HDL cholesterol ($p < 0.05$). LDL-C decreased by 12.73% ($p < 0.05$), and thyroglobulin by 13.09% ($p < 0.05$). Additionally, a decrease in the atherogenic index by 1.83% was noted. HDL-C data were statistically insignificant, with no negative trend observed.

Safety Assessment

Adverse events that could be associated with the use of the investigational drug at a dose of 0.25 mg of semaglutide were reported by 18 patients (36%). The majority of patients, 9 (18%), reported nausea, predominantly when consuming fatty foods. Heartburn, diarrhea, feeling of fullness, and joint pain were reported by 4% of patients for each symptom. In 2% of cases, upper abdominal pain, constipation, diarrhea, leg weakness, and lower back pain were observed.

No cardiovascular events (acute myocardial infarction, unstable angina, acute cerebrovascular accident, hypertensive crises requiring hospitalization) were registered among patients who completed the 6-month study.

DISCUSSION

The conducted study showed that 6-month therapy with Velgia® in patients with obesity (mean BMI 39.06 kg/m²) and high comorbidity (84% had concomitant diseases) led to a significant reduction in body weight, improvement in metabolic profile, and reduction in cardiovascular risk. After 24 weeks, body weight decreased by 12.0 kg (11.3%), BMI decreased by 4.33 kg/m², and waist circumference by 7.65 cm. The proportion of patients with class III obesity halved (from 42% to 19.5%), and 4.2% achieved normal body weight. These data are consistent with the

STEP 1 study [12], where body weight reduction over 68 weeks was 14.9%, however, our study demonstrates the achievement of a pronounced effect already in the medium term.

A significant decrease in TC by 8.27% ($p < 0.001$), LDL-C by 12.73% ($p < 0.001$), and triglycerides by 13.09% ($p < 0.05$) was recorded. The atherogenic index decreased by 1.83% with no negative trend in HDL-C. The calculated 10-year cardiovascular risk using the SCORE2 scale decreased by 18.6% (from 7.01% to 5.9%), and the proportion of patients with very high risk decreased from 25% to 16.7%. Over 6 months of observation, not a single cardiovascular event was registered, which, considering the initially high (42%) and very high (25%) risk, confirms the cardioprotective potential of the therapy. These results correlate well with the data from the SELECT study [15] in patients with obesity without diabetes.

Semaglutide is widely used for the treatment of T2DM and is gaining attention due to its cardioprotective properties [16]. As shown in the clinical study by M.N. Kosiborod et al. [17], semaglutide can reduce the risk of cardiovascular death and worsening of condition in patients with heart failure. Specific mechanisms may be related to semaglutide's ability to attenuate inflammatory responses, preserve mitochondrial function, reduce oxidative stress, and inhibit cellular apoptosis [18–22]. The NOD-like receptor protein 3 (NLRP3) inflammasome is associated with cardiac damage, such as myocardial ischemia and fibrosis [23–25]. The study by W. He et al. [26] demonstrated that semaglutide can improve impaired mitophagy caused by transverse aortic constriction (TAC) and suppress NLRP3 inflammasome activation, thereby reducing cardiac hypertrophy. This finding reveals a link between semaglutide and the NLRP3 pathway, highlighting its pronounced anti-inflammatory effect. Further verification in a diabetic model showed that semaglutide treatment attenuates inflammation-induced cardiac damage by increasing RKIP expression and suppressing the TBK1 / NF- κ B signaling pathway. These effects are confirmed by improved cardiac function and reduced biomarkers of myocardial injury [19], providing a theoretical basis for expanding the clinical application of semaglutide.

A recent clinical study showed that semaglutide improves cardiovascular outcomes by enhancing vascular endothelial function and reducing

inflammation, independent of weight loss [27]. In a model of obesity-related cardiac damage, its anti-inflammatory effect was confirmed. Semaglutide significantly reduced body weight in obese mice while suppressing the expression of genes associated with inflammation and oxidative stress (such as S100A8, S100A9, and Cxcl2) in neutrophils, thereby reducing cardiac damage [18, 28]. Clinical studies show that the anti-inflammatory properties of semaglutide may prevent atrial fibrosis and alleviate symptoms of atrial fibrillation in individuals with obesity [29]. When used with antihypertensive and lipid-lowering drugs, semaglutide reduced high-sensitivity CRP levels by 37.8% [30]. These data support the potential application of semaglutide in treating inflammation-induced heart damage.

Furthermore, semaglutide has demonstrated a protective effect against myocardial damage caused by acute myocardial infarction (AMI), coronary artery perfusion impairment, and ischemia-reperfusion injury [31, 32]. Key mechanisms include, but are not limited to, mitigating cellular damage, modulating metabolism and vascular function, and inhibiting apoptosis. For instance, in experimental animal models of AMI, semaglutide not only significantly reduced myocardial edema, necrosis, and fibrous tissue hyperplasia but also effectively lowered residual post-infarction risks by modulating energy metabolism, reducing blood glucose, lipids, and body weight [31, 33]. In Yorkshire pigs with myocardial ischemia, semaglutide treatment significantly enhanced AMPK, AKT, and eNOS activation and expression, improved vascular diastolic function, and increased coronary perfusion in ischemic areas [41]. Previous studies have confirmed that the AMPK / AKT / eNOS signaling pathway plays a critical role in regulating vasodilation and stimulating angiogenesis [18, 34], which may be one of the key mechanisms by which semaglutide alleviates myocardial ischemia. Additionally, semaglutide exerted cardioprotective effects by inhibiting ferroptosis [32] and activating the PKG/PKC ϵ /ERK1/2 signaling pathway [22], thereby reducing cellular apoptosis and infarct size. Meanwhile, subsequent clinical studies have further confirmed that the combined use of GLP-1RAs (such as semaglutide) and SGLT-2 inhibitors after percutaneous coronary intervention can increase myocardial salvage in the peri-infarct period, highlighting its potential value for clinical application. Clinical studies show that semaglutide

significantly reduces the risk of myocardial infarction and heart failure compared to tirzepatide and has a numerical advantage over it in reducing MACE [35]. Another important mechanism by which semaglutide improves cardiovascular health is by enhancing cardiac metabolic function. Myocardial damage is primarily associated with mitochondrial dysfunction and impaired glucose and lipid metabolism, leading to the accumulation of toxic lipids [36–38]. For example, semaglutide can improve mitochondrial function through the PI3K / AKT / CREB5 / NR4A1 axis, thereby mitigating myocardial damage caused by TAC [39]. Recent studies suggest that semaglutide can reduce Slc27a2 expression and decrease cardiac damage by enhancing lipid metabolism and reducing lipid accumulation in cardiac cells. Although Slc27a2 levels are higher in the blood of obese individuals [40], confirmation in human cardiac tissues is lacking due to ethical limitations. Despite this, studies strongly support the cardioprotective effects of semaglutide in obesity. The work by H. Yan et al. [31] and C. Stone et al. [41] further demonstrated in models of AMI and chronic cardiac injury, respectively, that semaglutide modulates glucose and lipid metabolism to optimize myocardial energy utilization. Thus, the mitochondrial protective and metabolic regulatory effects of semaglutide underscore its potential therapeutic value in both acute and chronic cardiac injury, opening promising prospects for future CVD treatment.

As research progresses, opportunities arise for developing personalized treatment plans tailored to individual patient characteristics. Creating algorithms that consider biomarkers, genetic variations, and metabolic profiles can help clinicians optimize drug selection, dosage, and combination therapy. This approach aims to enhance treatment efficacy while minimizing side effects and reducing the financial burden on patients. Furthermore, dynamic monitoring of cardiac and renal function, as well as metabolic changes, will allow for treatment adjustments—such as altering dosage and combination therapy regimens—based on the evolving pathophysiological state throughout the disease course [16]. This strategy is particularly promising for optimizing the management of patients with multiple comorbidities.

For the production of the medicine Velgia®, Promomed Group uses the solid-phase peptide synthesis (SPPS) method. This allows for process automation and precise reproduction of the desired

peptide structure, which negates the risks of changes in the resulting substance and, consequently, adverse immune reactions during drug administration.

In 2025, the unique semaglutide in the Russian Federation, Velgia® Eco, was introduced, which does not contain preservatives, including phenol and benzyl alcohol. The drug is supplied in single-use devices, eliminating the need for protection of the solution from microbial contamination. A simpler composition is particularly important for sensitive patients, as it reduces the additional component load during multi-month use and lowers the likelihood of adverse reactions associated with excipients. Such a composition is of fundamental importance in patients with concomitant liver and kidney diseases, where the accumulation of certain preservatives is associated with additional risks. Velgia® Eco is available in single-use autoinjectors. This delivery form does not require needle attachment, manual dose setting, or assessment of injection completion. The simplicity of the procedure reduces the risk of user errors and helps to more accurately reproduce the prescribed treatment regimen [42].

Study Limitations

Although semaglutide may have therapeutic effects in patients with obesity and concomitant cardiovascular risk, future research should focus on understanding the mechanisms of semaglutide action depending on the subtype of cardiovascular disease. A thorough evaluation of the efficacy and safety of semaglutide in real-world patient populations with cardiovascular

risk is necessary through multicenter and long-term studies. In clinical practice, improved monitoring and personalized risk management are crucial for the effective use of semaglutide, which will help integrate semaglutide into precision CVD therapy.

CONCLUSION

A significant reduction in anthropometric parameters (body weight, body mass index, waist and hip circumference) was observed after 24 weeks of semaglutide therapy. During therapy, no clinically significant changes were observed in liver function parameters (ALT, AST, ALP, bilirubin), pancreatic function (amylase), renal function (creatinine, urea, GFR), and systemic inflammation markers (CRP). However, a significant decrease in transaminase activity (ALT, AST) and serum uric acid concentration was noted.

After 6 months of semaglutide use, positive changes in the lipid profile were identified: a decrease in total cholesterol by 8.27% due to a reduction in non-HDL cholesterol, LDL cholesterol by 12.73%, triacylglycerols by 13.09%, and the atherogenic index by 1.83%. During the 6-month follow-up, patients maintained stable target blood pressure levels. Semaglutide therapy for 6 months led to a 18.6% reduction in the calculated 10-year cardiovascular risk according to the SCORE2 scale. The registered adverse events, predominantly gastrointestinal, were moderate and short-term. No cardiovascular events were recorded during the period of semaglutide use. Thus, the use of Velgia® is promising in the pharmacotherapy of obesity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Larisa A. Balykova — conceptualization, data analysis, writing—review & editing; Vera V. Stolyarova, Darya A. Orlova — data analysis, investigation, visualization, writing—original draft; Svetlana V. Shokina, Ekaterina I. Yamashkina — data analysis, visualization, writing—original draft; Marina V. Esina, Tatiana A. Surina — investigation, data analysis; Larina A. Zorina, Victoria S. Scherbakova — data analysis, writing—review & editing; Kira Ya. Zaslavskaya, Petr A. Bely — conceptualization, writing—review & editing. All the authors confirm their authorship compliance with the ICMJE international criteria (all the authors made a significant contribution to the conceptualization, conduct of the study and preparation of the article, read and approved the final version before publication).

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Cefodizime: safety profile in patients with comorbidities

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The aim. To evaluate the safety profile of cefodizime in comparison with ceftriaxone and amoxicillin/clavulanic acid based on an analysis of current literature data.

Materials and methods. A literature search was conducted among randomized controlled trials, cohort studies, meta-analyses, and clinical guidelines for the period from 2021 to 2026 in the PubMed database.

Results. The analysis of publications and one clinical guideline revealed that the safety profile of cefodizime in comorbid patients is more favorable compared to ceftriaxone and amoxicillin / clavulanic acid. The use of ceftriaxone is associated with a dose-dependent and duration-dependent risk of developing biliary pseudolithiasis, cholestatic hepatitis, nephrolithiasis, and crystalluria, especially in patients with risk factors such as decompensated comorbidities, dehydration, starvation, and polypharmacy. Amoxicillin / clavulanic acid remains one of the leading causes of drug-induced liver injury, including cholestatic forms, and also demonstrates clinically significant interactions with vitamin K antagonists and direct oral anticoagulants. No convincing data on hepatotoxicity or nephrotoxicity or clinically significant interactions with oral anticoagulants were found for cefodizime, including in patients with hepatobiliary and urinary system pathology. However, the limited volume of clinical studies requires further investigation of its safety in larger cohorts.

Conclusion. Cefodizime, ceftriaxone, and amoxicillin / clavulanic acid are effective in comorbid patients; however, their safety profiles differ. Cefodizime is characterized by a lack of data on hepatotoxicity and nephrotoxicity and does not demonstrate clinically significant drug interactions with oral anticoagulants, unlike ceftriaxone and amoxicillin / clavulanic acid. At the same time, the limited number of clinical studies on cefodizime requires further investigation of its safety profile in larger cohorts of comorbid patients.

Keywords: cefodizime; antibacterial therapy; comorbidities; comorbidity; drug-induced liver injury; nephrotoxicity; oral anticoagulants; drug interactions

Abbreviations: ADR — adverse reaction; ESBLs — extended-spectrum beta-lactamases; PBP — penicillin-binding protein; DILI — drug-induced liver injury; NAFLD — non-alcoholic fatty liver disease; ALD — alcoholic liver disease; HLA — human leukocyte antigen; OACs — oral anticoagulants; DOAC — direct oral anticoagulants; AF — atrial fibrillation; INR — international normalized ratio.

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Цефодизим: профиль безопасности у пациентов с коморбидной патологией

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Цель. Оценить профиль безопасности цефодизима в сравнении с цефтриаксоном и амоксициллин/клавулановой кислотой на основе анализа актуальных литературных данных.

Материалы и методы. Поиск литературы проводили среди рандомизированных контролируемых исследований, когортных исследований, метаанализов и клинических рекомендаций за период с 2021 по 2026 гг. в базе данных PubMed.

Результаты. В результате анализа публикаций и одной клинической рекомендации выявлено, что профиль безопасности цефодизима у коморбидных пациентов более благоприятен по сравнению с цефтриаксоном и амоксициллин/клавулановой кислотой. Применение цефтриаксона сопряжено с дозозависимым и зависящим от длительности терапии риском развития билиарного псевдолиитаза, холестатического гепатита, нефролитаза и кристаллурии, особенно у пациентов с такими факторами риска, как декомпенсированные сопутствующие заболевания, дегидратация, голодание и полипрагмазия. Амоксициллин/клавулановая кислота остаётся одной из ведущих причин лекарственно-индуцированного поражения печени, включая холестатические формы, а также демонстрирует клинически значимые взаимодействия с антагонистами витамина К и прямыми пероральными антикоагулянтами. Для цефодизима не обнаружено убедительных данных о гепато- и нефротоксичности или клинически значимых взаимодействиях с пероральными антикоагулянтами, в том числе у пациентов с патологией гепатобилиарной и мочевыделительной систем. Вместе с тем ограниченный объём клинических исследований требует дальнейшего изучения его безопасности с участием больших когорт пациентов.

Заключение. Цефодизим, цефтриаксон и амоксициллин/клавулановая кислота эффективны у коморбидных пациентов, однако их профиль безопасности различается. Цефодизим характеризуется отсутствием данных о гепато- и нефротоксичности и не демонстрирует клинически значимых лекарственных взаимодействий с пероральными антикоагулянтами, в отличие от цефтриаксона и амоксициллин/клавулановой кислоты. В то же время ограниченное число клинических исследований цефодизима требует дальнейшего изучения его профиля безопасности в расширенных когортах коморбидных пациентов.

Ключевые слова: цефодизим; антибактериальная терапия; сопутствующие заболевания; коморбидность; лекарственное поражение печени; нефротоксичность; пероральные антикоагулянты; лекарственные взаимодействия

Список сокращений: НР — нежелательные реакции; БЛРС — β-лактамазы расширенного спектра; ПСБ — пенициллин-связывающие белки; ЛИПП — лекарственно-индуцированное поражение печени; НАЖБП — неалкогольная жировая болезнь печени; АБП — алкогольная болезнь печени; HLA — главный комплекс гистосовместимости человека; ФП — фибрилляция предсердий; ОАК — пероральные антикоагулянты; ПОАК — прямые пероральные антикоагулянты; МНО — международное нормализованное отношение.

INTRODUCTION

The increase in patients with polymorbidity and polypharmacy correlates with a higher incidence of adverse drug reactions during antimicrobial therapy. The most vulnerable are elderly patients

and those with chronic diseases of the liver, kidneys, cardiovascular, and respiratory systems, in whom even standard drug doses can be associated with an increased risk of adverse reactions (ARs) and drug interactions [1].

In this regard, the systematization of data on the safety profile of widely used β -lactam antibiotics in comorbid patients is of significant interest for practical healthcare [2].

Cefodizime and ceftriaxone, belonging to third-generation cephalosporins, as well as the combination drug amoxicillin / clavulanic acid, are widely used in the treatment of infections of various localizations in patients of therapeutic profile. Despite accumulated clinical experience, most available studies on the safety profile of these antibiotics were conducted in relatively homogeneous groups, without considering the pronounced comorbidity and polypragmasia characteristic of real clinical practice. This necessitates an analysis and comparison of published data on the frequency and structure of ARs of these drugs in patients with concomitant diseases [3, 4].

Lower respiratory tract infections are among the leading causes of mortality worldwide. According to the WHO, they rank 7 among the main causes of mortality in upper-middle-income countries and first in low-income countries¹. Urinary tract infections are the second most common pathology among all infectious diseases, affecting over 150 million people worldwide annually [5]. In Russia, 4.1 million medical consultations for urinary tract infections were registered in 2022 [6].

Community-acquired pneumonia, caused by viruses, as well as typical and atypical bacteria, remains one of the leading causes of mortality in patients of different age groups: in high-incidence countries, lethality is 9–17.5% [7]. The presence of concomitant diseases in patients with infectious and inflammatory pathology can affect both the course of the primary and comorbid processes, as well as the efficacy and safety of drug therapy [8].

Comorbidity is defined as the combination in one patient of two or more chronic diseases that are etiopathogenetically linked or coincide in their onset, regardless of the activity of each [9]. With age, the frequency of comorbid diseases increases: in individuals aged 65–74, it reaches 62%, and in patients aged 85 and older, it is 82% [8].

Patients with multiple concomitant diseases appropriately receive several medicines (adequate

polypharmacy). The burden of taking medicines increases the risk of side effects, which is a significant reason for hospitalization and prolonged hospital stays [9].

Cephalosporins are β -lactam antibiotics. Depending on the chronology of drug development and their antimicrobial properties, these antibiotics are divided into different generations, from first to fifth. Structurally, third-generation cephalosporins contain a six-membered dihydrothiazine ring connected to the β -lactam moiety. Substituents at C3, C4, and C7 are key factors in their antimicrobial activity. Furthermore, the carboxyl group at C4 must remain unchanged, and the acylamide side chain at C7 (7-aminocephalosporanic acid nucleus) plays a crucial role in the hydrophilic/hydrophobic properties of these compounds.

Third-generation cephalosporins are the most frequently prescribed group of antibiotics [10]. The first third-generation cephalosporin, cefotaxime, was developed in the late 1970s. Through improvements to the “parent” molecule, ceftriaxone and cefodizime have been created and introduced. These drugs differ from cefotaxime in that they have a longer half-life ($T_{1/2}$), allowing for once or twice daily dosing, which in turn improves patient adherence and reduces therapy costs [11].

These antimicrobial agents are characterized by a broad spectrum of activity, covering both Gram-positive and Gram-negative microorganisms. Their antibacterial effect is more pronounced against Gram-negative flora resistant to first and second-generation cephalosporins [10].

Currently, this advantage has been somewhat diminished due to the spread of extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* strains, even in community-acquired infections. However, Gram-positive microorganisms and a number of Gram-negative pathogens retain high sensitivity to this class of antibacterial agents, justifying their use in modern clinical practice [11].

Cefodizime belongs to the subclass of amino-2-thiazolyl-methoxyiminocephalosporins and shows significant structural similarity to cefotaxime and ceftriaxone. Several experimental studies have shown that, in addition to its antibacterial action, the drug has a regulatory effect on the inflammatory response

¹ World Health Organization (WHO). The top 10 causes of death. Available from: <https://www.who.int/ru/news-room/fact-sheets/detail/the-top-10-causes-of-death>

and stimulates the functional activity of various components of the immune system, allowing it to be considered a potential immunomodulator in infectious diseases [11, 12].

Being used at therapeutic doses, the pharmacokinetics of cefodizime remain linear. It may be more convenient to use than some other drugs in this class, as it can be administered once or twice daily [13, 14]. Cefodizime has several advantages for use in comorbid patients. This issue will be explored in detail in our article.

THE AIM is to evaluate the safety profile of cefodizime compared to ceftriaxone and amoxicillin / clavulanic acid based on an analysis of current literature data.

MATERIALS AND METHODS

Full-text publications were conducted in the PubMed database. Works studying cefodizime, ceftriaxone, and amoxicillin / clavulanic acid in the treatment of infectious and inflammatory diseases in polymorbid patients with hepatobiliary system pathology, urinary system pathology, or those taking oral anticoagulants from 2021 to 2026 were analyzed using the following keywords: “cefodizime”, “ceftriaxone”, “amoxicillin clavulanate”, “drug-induced liver damage”, “hepatotoxicity”, “cholelithiasis”, “nephrolithiasis”, “crystalluria”, “warfarin”, “direct oral anticoagulants” and their Russian equivalents.

As a result, 3325 sources were found. Works for which full text was not accessible, as well as those not directly related to the review topic, were excluded from the bibliography. Consequently, 58 scientific works were included in this review.

RESULTS AND DISCUSSION

Mechanism of Action of Cephalosporins

Cephalosporins inhibit the synthesis of the bacterial cell wall, specifically peptidoglycan. Peptidoglycans are the exoskeleton of bacteria, providing structural integrity and shape to cells and protecting them from lysis. These structures are cross-linked in the final stage of bacterial cell wall synthesis, forming peptidoglycan polymers with the help of membrane-bound enzymes (i.e., transpeptidases, carboxypeptidases, and endopeptidases), collectively known as penicillin-binding proteins (PBPs) [10].

Most bacteria possess at least one PBP, and they are the target for various β -lactam antibiotics such as cephalosporins, penicillins, carbapenems, and monobactams. The β -lactam ring structure of third-generation cephalosporins mimics the “D-Ala-D-Ala” fragment of the natural PBP substrate. Structural binding of cephalosporin antibiotics to the active site of PBPs in bacterial cell walls leads to the inhibition of their enzymatic activity and disruption of peptidoglycan synthesis, resulting in the inability to build a functional cell wall and subsequent death of bacterial cells due to osmotic lysis [10].

Spectrum of Activity of Cefodizime and Indications for Use

Cefodizime has a broad spectrum of antibacterial activity against Gram-positive and Gram-negative aerobes. Stable sensitivity is noted in *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Shigella sonnei*, *Yersinia enterocolitica*, and *Salmonella species*), whereas activity against *Citrobacter* (including *Citrobacter freundii*) and *Serratia marcescens* is noted as weak and variable; against *Enterobacter* spp., the drug, like most third-generation cephalosporins, is ineffective [14].

Cefodizime inhibited *Haemophilus influenzae* (ampicillin-sensitive and resistant), *Moraxella catarrhalis*, *Neisseria gonorrhoeae* (β -lactamase-producing and non-producing), and *Neisseria meningitidis*, but is inactive against *Pseudomonas aeruginosa* and *Xanthomonas maltophilia*. *Methicillin-sensitive strains of Staphylococcus aureus* are sensitive to the drug, whereas *Staphylococcus epidermidis* and *MRSA* remained resistant. *In vitro*, cefodizime effectively inhibited *Streptococcus agalactiae*, *S. pneumoniae*, and *S. pyogenes*, demonstrating activity comparable to first and second-generation cephalosporins [14].

In adult patients, the drug can be used for bacterial infections caused by susceptible microorganisms. Its use is of greatest clinical significance in patients with weakened body condition and/or reduced immune defense. The spectrum of indications includes uncomplicated lower urinary tract infections in women, other urinary tract infections, lower respiratory tract infections, and gonorrhea.

Dosage adjustment is necessary only in elderly patients with impaired renal function.

Side Effects

Cephalosporins are generally well-tolerated, but individuals with penicillin allergy may have an increased risk of allergic reactions due to the common β -lactam ring [15].

Generally, a higher frequency of cross-reactivity is observed with first-generation cephalosporins [15].

Ceftriaxone, while maintaining high clinical efficacy, is characterized by a more pronounced risk of drug-induced liver injury (DILI), including cholestatic forms and the development of biliary pseudolithiasis / cholelithiasis, as well as nephrotoxic complications manifesting as nephrolithiasis and crystalluria in predisposed patients. Concurrently, rare but clinically significant cases of severe cutaneous and systemic reactions (Stevens-Johnson syndrome, toxic epidermal necrolysis) and ceftriaxone-associated neurotoxicity in the form of encephalopathy have been described [10].

Cefodizime, in contrast, demonstrates a more favorable safety profile: according to available clinical data, the drug is generally well-tolerated upon parenteral administration, with no convincing evidence of clinically significant hepato- and nephrotoxicity, which allows it to be considered a preferred option in patients with an increased risk of hepatobiliary and renal complications of antibacterial therapy [14].

Comorbid Patients with Impaired Liver and Pancreatic Function

Pancreatitis, including both acute and chronic forms, is a primary cause of gastrointestinal morbidity and hospitalization [16].

Acute pancreatitis is a critical condition with high mortality, ranging from 20% to 40%, mainly due to systemic complications such as sepsis and multiple organ failure [17].

The global standardized incidence rate of pancreatitis in 2021 was 52.64 (95% CI: 45.57–60.42) per 100,000 people. The lowest incidence rate in individual countries was observed in Zambia (23.76, 95% CI: 19.15–29.79), and the highest in the Russian Federation (173.44, 95% CI: 142.20–204.01) [16].

It is estimated that in 2024, there were approximately 240 million people worldwide with

chronic hepatitis B virus infection, corresponding to 2.9% of the global population, as well as about 68 million people infected with hepatitis C virus².

According to available data, in 2024, approximately 1.1 million deaths from cirrhosis and liver cancer associated with hepatitis B virus were registered worldwide, which is 17% higher than in 2015: 240,000 people died from liver cirrhosis and liver cancer caused by hepatitis C virus (95% CI: 160,000–370,000), which is 12% less compared to 2015³.

It is estimated that hepatic steatosis affects 1.7 billion people worldwide and is one of the fastest-growing causes of chronic liver disease globally⁴.

According to the results of a meta-analysis conducted in 2023, the global prevalence of non-alcoholic fatty liver disease (NAFLD) has increased by 50.4% over the past 20 years. In the Russian clinical epidemiological study DIGER, the authors also note a significant increase in the frequency of NAFLD among the Russian population (+38.1% over 7 years of observation) [18].

Approximately 26% of individuals with alcohol abuse and up to 55% of patients with alcohol use disorder develop one of the clinical forms of alcoholic liver disease (ALD) [19].

About 10–20% of people with ALD develop liver cirrhosis. Thus, the prevalence of alcohol-related liver cirrhosis is estimated at approximately 0.3% in the general population, but increases to 12.9% in individuals with alcohol use disorder [20].

In 2023, according to available data, 3.15 million people suffered from alcoholic liver cirrhosis. 419,429 new cases were registered: despite the overall increase in disease prevalence, the age-standardized prevalence was 34.6 per 100,000 people, reflecting demographic shifts [21].

The epidemiology of chronic cholecystitis largely coincides with the epidemiology of gallstone disease. It is estimated that 10% to 20% of the world's population develops gallstone disease during their lifetime, with approximately 80% of cases remaining clinically asymptomatic [22].

² World Health Organization (WHO). Global hepatitis report 2026. Geneva; 2026. Available from: <https://whodc.mednet.ru/en/main-publications/infekzionnye-bolezni-i-borba-s-nimi/virusnyj-gepatit/4478/visit.html>

³ Ibid.

⁴ Seventy-ninth World Health Assembly — Daily update: 21 May 2026. World Health Organization; 2026. Available from: <https://www.who.int/news/item/21-05-2026-seventy-ninth-world-health-assembly---daily-update--21-may-2026> (accessed 21.05.2026).

Untreated acute cholecystitis is associated with a significant risk of complications and mortality, especially in the elderly patient group.

Gallbladder perforation, a potential complication of untreated cholecystitis, has a mortality rate of 30% [23].

Given the high prevalence of liver and biliary tract pathology in the population, it is crucial to assess the risk of hepatotoxic ARs when prescribing drug therapy.

Impact of Comorbidity on the Safety of β -Lactam Antibiotic Therapy

Numerous observational cohort studies confirm a direct correlation between the cumulative burden of chronic diseases and the frequency of ARs during β -lactam antibiotic therapy. In particular, an analysis of the North American population demonstrated a statistically significant association between high scores on the Charlson Comorbidity Index (CCI) and the probability of developing both typical (Type A) and idiosyncratic (Type B) ARs. Notably, the CCI comorbidity index has become part of the panel of independent predictors of ARs, alongside polypharmacy, geriatric status of the patient, and gender. Antibacterial agents were second only to anticoagulants in the hierarchy of pharmacological classes most frequently inducing ARs.

The most vulnerable cohort regarding drug-induced acute kidney injury when prescribing antimicrobial drugs are individuals of geriatric profile with pre-existing multimorbidity. Basic risk determinants include diabetes mellitus, essential arterial hypertension, systolic myocardial dysfunction, and chronic kidney disease. This risk is potentiated by concomitant therapy with drugs that modify the renin-angiotensin-aldosterone system (ACE inhibitors, angiotensin II receptor blockers) and diuretics. Dehydration acts as an additional trigger, exacerbating the prerenal vasoconstrictor component of nephrotoxicity [8].

Safety of Cefodizime, Ceftriaxone, and Amoxicillin / Clavulanic Acid in Patients with Hepatobiliary System Pathology

Parenteral administration of ceftriaxone causes biliary sludge (pseudolithiasis) in 3–46% of patients⁵ [24].

⁵ LiverTox: clinical and research information on drug-induced liver injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. Ceftriaxone. Updated 20 Dec 2021. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK548258/> (accessed 24.05.2026).

In most cases, it is asymptomatic and spontaneously resolves after discontinuation of the drug, but in rare cases, it leads to severe pancreatitis or cholecystitis requiring surgical intervention. In children, the risk is higher with high doses of ceftriaxone, prolonged therapy, fasting, and dehydration.

The mechanism involves the binding of the drug to calcium ions, leading to the formation of insoluble crystals in bile and urine. Interaction with calcium phosphate forms stable stone aggregates. Very rarely, idiosyncratic cholestatic hepatitis develops.

Clinical Trial Results and Patient Management Strategies

In most described cases, recovery was rapid, within 1–2 weeks, but some patients required cholecystectomy during the acute illness, and stones or sediment could persist for several months. The immunoallergic form of cholestatic hepatitis usually resolves quickly and is not associated with acute liver failure or chronic liver damage.

Probability Assessment: B (ceftriaxone is a highly probable cause of clinically significant liver injury and can also lead to the formation of bile sediment and “pseudolithiasis” caused by ceftriaxone crystallization in bile present in the gallbladder or biliary tract)⁶.

Bile acids and ceftriaxone may use the same transport pathway, so ceftriaxone secretion with bile can inhibit bile acid secretion. While ceftriaxone is 85–95% bound to albumin in blood, 45% is excreted unchanged in bile; therefore, the concentration of ceftriaxone in bile is 20–150 times higher than in serum [25, 26].

When the concentration of ceftriaxone in the gallbladder exceeds a threshold value, it is known to precipitate by binding to calcium ions secreted along with bile acids. Subsequently, the precipitated drug forms stones and can ultimately lead to ceftriaxone-induced biliary pseudolithiasis. The formation of this calcium-ceftriaxone complex is detected by ultrasound as a gallstone or bile sediment. Pseudobiliary stones are considered structurally unstable; therefore, they are easily excreted with bile [26].

T. Yassen et al. presented the results of a prospective study on the incidence and clinical outcomes of biliary pseudolithiasis associated with

⁶ Ibid.

ceftriaxone use. The study included 60 patients. In 11 of them, ultrasound revealed cholelithiasis (18.3%) [26]. In univariate analysis, cholelithiasis developed more frequently in patients requiring fasting and bed rest ($p < 0.001$). In multivariate analysis, the duration of fasting (OR, 4.44; 95% CI: 2.04–14.64; $p < 0.0001$) and the duration of bed rest (OR, 2.21; 95% CI: 1.25–4.26; $p = 0.008$) were significantly associated with pseudolithiasis and independent of each other. Furthermore, cholelithiasis appeared relatively early during the period of fasting and bed rest [26].

When studying potential risk factors using the FAERS database, data on 764 adverse events (AEs) related to the formation of stones in the hepatobiliary system during ceftriaxone therapy were analyzed to assess potential risk factors: age group, sex, dose. Sex was not identified as a risk factor for the development of ceftriaxone-induced hepatobiliary stones. However, male infants (under 1 year), boys, girls, male adolescents, and elderly women were potential risk factors for the development of ceftriaxone-induced hepatobiliary stones, based on $ROR_{025} > 1$ and $IC_{025} > 0$ criteria [26].

In a retrospective study involving 634 adult patients with common infectious diseases treated with ceftriaxone in a general hospital in Qatar, it was found that the use of ceftriaxone in combination with other drugs metabolized in the liver was associated with a significantly higher incidence of liver damage (19.7%) compared to data from other similar studies [27–29]. The inclusion criteria were as follows: a) hospitalized patients aged ≥ 18 years from January 2019 to December 2019 in an inpatient setting; b) patients who received intravenous ceftriaxone at a dose of ≥ 2 g/day for ≥ 2 consecutive days.

A retrospective analysis of hospitalized patients aged ≥ 65 years who received ceftriaxone at Innoshima-Ishikai Hospital between May 2022 and April 2025, conducted by T. Tagashira et al. [30], revealed that in patients aged ≥ 65 years, baseline ALT levels (≥ 11 U/L) and CRP levels (≥ 5.9 mg/dL) were associated with an increased risk of drug-induced liver injury during ceftriaxone use. Therefore, careful monitoring of liver function during and after ceftriaxone administration was recommended in this patient group. The study compared two groups: patients with and without liver dysfunction. Liver dysfunction developed in 19

patients, accounting for 18.1% (19/105). In the liver dysfunction group, ALT levels significantly increased after ceftriaxone administration. The median time to the onset of liver dysfunction was 8 days ([Q1–Q3] = 6.5–9.5 days). Furthermore, liver dysfunction developed within 8 days in 58% of patients. No significant differences were observed between the groups in terms of age, sex, duration of ceftriaxone administration, cumulative ceftriaxone dose, or concomitant medication use. The daily dose of ceftriaxone was 2 g/day in 101 patients (96% of the cohort). Significant differences were noted in ALT ($p = 0.001$), AST ($p = 0.046$), and CRP ($p = 0.009$) levels between the groups, while other laboratory parameters did not differ significantly [30].

Cephalosporins can cause self-limiting mixed / cholestatic hepatitis, occurring after a short course of therapy with a latency period of up to 3 weeks.

From January 1, 2004, to November 2, 2022, a total of 2347 cases of DILI were included in the DILIN study, of which 1854 were classified as probable, highly probable, or definite. Human leukocyte antigen (HLA) sequencing was performed, and its association with the risk of DILI was studied. As a result, 58 cases (3%) were associated with the use of various cephalosporins, including cefazolin ($n = 40$), cephalexin ($n = 4$), ceftriaxone ($n = 3$), cefdinir ($n = 3$), cefuroxime ($n = 3$), and five other individual drugs. Clinical manifestations included a self-limiting course with a mixed or cholestatic biochemical pattern without signs of hypersensitivity, occurring within 1–4 weeks. In the overall cohort, HLA-A*02:01 was significantly associated with an increased risk of cephalosporin-induced liver injury (OR: ~ 2.5 – 2.7 , $p < 0.0001$). The strongest association was observed with cefazolin, where the carriage rate was 85% compared to 38% in individuals with DILI caused by other drugs [15].

Results of a retrospective case-control study involving 185 children showed that the occurrence of pseudocholelithiasis (PL) was not significantly associated with ceftriaxone dosage. Furthermore, the rate of metabolism and excretion of ceftriaxone may play a key role in the development of PL. Serum analysis revealed distinct markers of liver and kidney function in the PL group, including lower levels

of total bile acids (PL(+) = 1.8 (0.85–3.79) $\mu\text{mol/L}$ vs. PL(-) = 3.9 (2.18–7.00) $\mu\text{mol/L}$, $p = 0.000$), adenosine deaminase (PL(+) = 14 (12–21) U/L vs. PL(-) = 19 (15–27) U/L, $p = 0.003$), and lactate dehydrogenase (PL(+) = 195 (180.5–268) U/L vs. PL(-) = 256 (207–313) U/L, $p = 0.003$), as well as higher creatinine levels (PL(+) = 57 (51.85–66.3) $\mu\text{mol/L}$ vs. PL(-) = 50.7 (39–62), $p = 0.021$) [31].

According to the VigiAccess resource⁷, ceftriaxone caused hepatobiliary system disorders, accounting for 1% of all AEs, with 4,596 cases reported, including: cholelithiasis—953 reports, cholestasis—360 reports, pseudocholelithiasis—219 reports, cholestatic hepatitis—160 reports, cholecystitis—146 reports, bile duct stones—57 reports, cholestatic liver injury—56 reports, acute cholecystitis—51 reports, biliary colic—47 reports, cholangitis—39 reports, biliary obstruction—10 reports.

Amoxicillin, both as monotherapy and in combination with the β -lactamase inhibitor clavulanic acid, is the most widely prescribed penicillin in Europe and many other countries [32, 33].

Amoxicillin is prescribed for infectious processes caused by microorganisms that do not produce β -lactamases. In clinical practice, the drug is used to treat respiratory tract diseases (upper and lower), urinary tract infections, *Helicobacter pylori* eradication, and bacterial infections of the skin and soft tissues.

Amoxicillin and amoxicillin / clavulanic acid are generally considered well-tolerated medicines with an established safety profile based on widespread global clinical use. However, amoxicillin / clavulanic acid combination should be prescribed with caution to patients with hepatic dysfunction and is not recommended for patients with a history of cholestatic jaundice / hepatic dysfunction [32].

ARs of the hepatobiliary system are an important issue in drug safety and pharmacovigilance, as they are a major cause of acute liver failure. Amoxicillin, both as monotherapy and in combination with clavulanic acid, has previously been described as a hepatotoxic agent in pediatrics [32].

In two previous population studies conducted in adult patients with DILI, the authors indicated that the amoxicillin / clavulanic acid combination demonstrates a higher risk of acute liver injury compared to the use of amoxicillin alone [34, 35].

The odds ratio (OR) for reports of serious “Hepatobiliary Disorders” for the combination drug amoxicillin / clavulanic acid compared to amoxicillin in the European Economic Area and the United Kingdom from 2020–2024 was 2.78 (95% CI 2.41–3.24) [32].

The hepatotoxic effect of amoxicillin is not a direct pharmacological effect but primarily an immunoallergic one [32]. This immune-mediated hypersensitivity leads to damage that is more often cholestatic or less commonly hepatocellular.

Polymorphism of *HLA* class I and II genes is considered a risk factor for the development of amoxicillin / clavulanate-induced liver injury, confirming the key role of the adaptive immune response in the pathogenesis of this complication [32].

A retrospective study analyzing AE reports in the global pharmacovigilance database VigiBase, registered in Switzerland from 2010 to 2020, showed that the amoxicillin / clavulanic acid combination was one of the most frequently suspected drugs in the development of severe drug-induced liver dysfunction (134—mention frequency of amoxicillin / clavulanic acid; 3.1%—of drugs; 6.6%—of individual AE reports). A total of 2042 individual AE reports were analyzed, including 10,646 drugs and 6,436 AEs.

Analysis using EudraVigilance data confirmed that the combination of amoxicillin and clavulanic acid is less safe than amoxicillin monotherapy, as the comparison of warnings about “hepatobiliary disorders” with the combination showed (OR = 2.78, 95% CI: 2.41–3.24) a stronger association between liver diseases and the use of amoxicillin and clavulanic acid-based drugs [32].

Data from a retrospective analysis of disproportionately using FAERS data for the period January 2010 to December 2023 showed that amoxicillin / clavulanic acid exhibited the most pronounced hepatotoxicity (OR = 4.56, 95% CI 4.12–5.03). The types of hepatotoxicity varied depending on the drug: cholestatic hepatitis was most common with amoxicillin / clavulanic acid [36].

Collectively, the available data suggest that elderly and senile patients exhibit increased vulnerability to DILI, largely attributed to age-associated decreases in hepatic blood flow, as well as changes in enzyme system activity and the clearance of drugs metabolized by the liver⁸. From a clinical-practical standpoint, age

⁷ VigiAccess. WHO global database of individual case safety reports. Available from: <https://www.vigiaccess.org/> (accessed 10.06.2026).

⁸ Clinical Guidelines. Medicinal liver damage; 2025. Available from: https://cr.minzdrav.gov.ru/view-cr/747_2. Russian

is a significant risk factor influencing the likelihood of developing drug-induced liver injury, as well as its type and phenotypic manifestation. Elderly patients predominantly experience cholestatic forms of DILI, along with a higher frequency of chronic progression compared to younger and middle-aged individuals.

Publications have described cases of DILI in elderly patients receiving ceftriaxone therapy [37, 38].

Direct randomized clinical trials specifically comparing cefodizime and ceftriaxone regarding the risk of biliary sludge / pseudolithiasis and hepatotoxicity in the PubMed database were not found; the available data only allow for indirect comparison of safety profiles based on clinical studies and review articles on cefodizime and ceftriaxone. Nevertheless, the totality of clinical and pharmacoepidemiological data indicates a higher risk of ceftriaxone-associated biliary complications and drug-induced liver injury compared to cefodizime, for which the hepato- and biliary safety profile appears more favorable.

Safety Profile of Cefodizime, Ceftriaxone, and Amoxicillin / Clavulanic Acid in Patients with Diseases of the Urinary System

A number of drugs can induce the development of nephrolithiasis and crystalline nephropathy; drug-induced stones account for 1% to 2% of all cases of urolithiasis [39].

Ceftriaxone is a poorly soluble drug with high urinary excretion, which promotes crystallization [39, 40].

T. Kimata et al. [41] conducted a case-control study involving 83 children with bacterial pneumonia aged 3 months to 8.9 years. The children were divided into two groups: 43 children receiving ceftriaxone (Group A) and 40 children receiving amoxicillin (Group B). Paired serum and urine samples were collected from patients in each group before and after treatment. No significant differences in demographic characteristics or blood biochemical parameters were found between the groups. However, the mean urinary calcium to creatinine ratio (uCa / Cr, mg/mg) was significantly higher in Group A patients than in Group B patients after treatment (0.19 vs. 0.09, $p < 0.001$), and analysis of paired urine samples showed that uCa / Cr significantly increased after treatment only in Group A

patients ($p < 0.001$). A weak, statistically insignificant correlation was found between ceftriaxone dose and uCa/Cr in Group A ($p = 0.10$, $r = 0.24$). The obtained results demonstrate that ceftriaxone can potentially significantly increase urinary calcium excretion, which, in turn, may be associated with ceftriaxone-associated urolithiasis or precipitate formation [41].

It should also be noted that transient hypercalciuria and increased excretion of oxalic and uric acids have been identified [42]; however, studies exist that confirm crystal formation with normal urinary calcium levels or other salts [43].

A. Louta et al. conducted a systematic review [39] that included 33 studies. Of these, 12 were case reports, 11 were prospective studies, 9 were retrospective studies, and only 1 was a randomized controlled trial. The systematic review aimed to analyze the known effects of ceftriaxone use in hospitalized pediatric patients, including an assessment of the likelihood of precipitate and stone formation in the biliary and urinary tracts, as well as an examination of the link with maternal history during pregnancy. The study results were analyzed to understand side effects and identify any predisposing factors.

According to the obtained results, the formation of urinary crystals after ceftriaxone administration is influenced by various risk factors, such as urinary stasis, family history, metabolic disorders (e.g., hypercalciuria), changes in urine pH, infection, environmental factors (e.g., high temperature), low urine output, high drug dosage, prolonged treatment, high urinary drug excretion, and concomitant therapy. The most common manifestation was kidney stone formation [43].

Although litholysis occurred early (by the end of the first week), cases of stone dissolution were noted to occur after 7 months [43].

The results of a systematic review and meta-analysis of 8 clinical studies ($n = 760$), which provided a pooled estimate of the incidence of ceftriaxone-induced urolithiasis in children, showed that the incidence of ceftriaxone-induced urolithiasis was 7% (95% CI 2–12%), with significant variability observed due to study design and diagnostic approach [25].

According to the VigiAccesse Database⁹, 2424 ARs related to kidney and urinary tract disorders

⁹ VigiAccess. WHO global database of individual case safety reports.

have been reported with ceftriaxone use, accounting for 1% of all reported ARs. The structure of ARs related to kidney and urinary tract disorders includes: nephrolithiasis—129 reports, crystalluria—31 reports, ureterolithiasis—24 reports, urinary stone—18 reports, bladder stone—13 reports, ureteral obstruction—6 reports, crystalline nephropathy—4 reports, urinary tract obstruction—4 reports, urethral stone—2 reports, urethral obstruction—2 reports, bladder obstruction—1 report.

Summary information on patients with urological pathology who have transurethral or ureteral catheters is presented in Table 2.

Ceftriaxone therapy can induce precipitation of the drug's calcium salts in the urinary tract. This phenomenon is predominantly observed in the pediatric population when high daily dosages (≥ 80 mg/kg) or cumulative doses exceeding 10 g are administered, especially in the presence of dehydration and immobilization. Microlithogenesis can be subclinical or manifest as ureteral obstruction with subsequent development of postrenal acute kidney injury. The phenomenon is fully reversible: crystal excretion and restoration of urodynamics occur after discontinuation of the antibiotic¹⁰.

Drug Interactions with Anticoagulants

Warfarin is the most widely used oral anticoagulant worldwide [44]. It is a vitamin K antagonist with a narrow therapeutic range, influenced by genetic polymorphism, drug interactions, and diet. Indications for the use of vitamin K antagonists include the following [45]: atrial fibrillation, acute coronary syndrome, heart failure, prosthetic heart valve, stroke, venous thromboembolism (VTE), pulmonary embolism, antiphospholipid syndrome.

Atrial fibrillation (AF) is the most common type of arrhythmia in adults, affecting approximately 59.7 million people worldwide as of 2019, an 111% increase since 1990 [46].

Over the past 20 years, the prevalence of AF has increased by 33%, and it is projected to increase by more than 60% by 2050. Age plays a crucial role in the factors contributing to AF development. Its prevalence is low (0.1%) in adults younger than 55 years, but increases to 5.9% in individuals aged 65 years and

older and to 9.0% in individuals aged 80 years and older [46].

AF is associated with an increased risk of stroke, so patients with AF are recommended to take oral anticoagulants (OACs) to reduce this risk [47].

The global prevalence and use of OACs increased from 42% in 2010 to 78% in 2018, respectively. Following the introduction of direct oral anticoagulants (DOACs), the proportion of OAC users worldwide nearly doubled [48]. Given the high prevalence of OAC use, it is important to assess the presence of clinically significant interactions between OACs and the antibacterial drugs being studied.

Numerous studies have convincingly confirmed an increased risk of serious bleeding in patients concurrently receiving antibiotics and warfarin [44, 49–51]. Cephalosporins and amoxicillin / clavulanic acid, despite not affecting the cytochrome P450 system, have shown a positive correlation with increased INR values.

However, one of the common complications associated with warfarin therapy is an increased risk of serious bleeding, especially when used concurrently with drugs that can affect its metabolism [44].

Specifically, antibiotics can influence the anticoagulant effect of warfarin through various mechanisms. Antibacterial drugs can induce or inhibit the activity of the CYP2C9 isoenzyme, which is crucial for warfarin metabolism. Furthermore, some antibiotics can disrupt the population of vitamin K-producing bacteria in the gut, further modulating warfarin's pharmacological response. As a result, these drug interactions can either enhance or weaken warfarin's efficacy, which can have potential clinical consequences [44].

Numerous cases have been described in the literature of the simultaneous use of amoxicillin or amoxicillin / clavulanic acid and warfarin, demonstrating an increase in the international normalized ratio (INR) and/or bleeding from 7 days after the start of amoxicillin to 9 days after discontinuation of amoxicillin therapy [44, 52, 53].

A prospective cross-sectional observational study conducted by M.I. Abdel-Aziz et al. [49] involving 120 patients investigated the effect of polypharmacy and high doses of amoxicillin / clavulanate on the intensity of the anticoagulant effect of warfarin in

¹⁰ Global hepatitis report 2026. Geneva: World Health Organization; 2026.

hospitalized patients. Potentially interacting drugs were classified according to their propensity to increase INR or bleeding risk. In 87.5% of patients receiving high doses of amoxicillin / clavulanic acid (10–12 g per day), INR values reached ≥ 4 , compared to 28.9% of patients receiving standard doses (up to 3.6 g per day) during hospitalization ($p \leq 0.001$). An increase in the number of potentially interacting drugs known to increase INR was a significant predictor of INR values ≥ 4 (OR = 2.5; 95% CI 1.3–4.7), and an increase in the number of potentially interacting drugs that increase bleeding risk was a significant predictor of bleeding episodes (OR = 3.1; 95% CI 1.3–7.3). This is likely due to the fact that most penicillins (with the exception of nafcillin and dicloxacillin) suppress intestinal microflora. This leads to a decrease in the population of vitamin K-producing bacteria, a deficiency of the latter, and consequently, an enhanced anticoagulant effect of warfarin [54].

A smaller number of intestinal bacteria producing vitamin K likely leads to a decrease in the amount of vitamin K-dependent plasma clotting

factors, enhancing the anticoagulant effect of warfarin [44].

Cephalosporins have been found to interact with warfarin, increasing the risk of hypoprothrombinemia, inhibiting P-glycoprotein, and altering gastrointestinal microflora [44].

A clinical case of hemarthrosis in an 85-year-old female patient with phlegmon during therapy with cephalosporin (ceftazoline) and warfarin, due to developed hypoprothrombinemia, has been described [55].

The mechanism of interaction between ceftriaxone and warfarin is currently not well understood. However, it is known that antibiotics can disrupt normal intestinal flora, which produces a significant amount of vitamin K. A small study in healthy volunteers showed that intestinal bacteria produce approximately 1.6 mg of menaquinones (bacterially synthesized vitamin K) [56].

Table 1 — Frequency of adverse reactions associated with impaired liver, biliary tract, and pancreatic function with the use of amoxicillin / clavulanic acid, ceftriaxone, cefodizime

Adverse Reaction	Amoxicillin / clavulanic acid ¹¹	Ceftriaxone ¹²	Cefodizime ¹³
ALP	uncommon	common	Rare
ALT	uncommon	common	rare
AST	uncommon	common	rare
Hepatitis	very rare	not identified	not identified
Cholestatic jaundice	very rare	not identified	not identified
Pancreatitis	Frequency unknown	The use of ceftriaxone is associated with rare episodes of pancreatitis, the pathogenesis of which may be due to the phenomenon of biliary pseudolithiasis obstruction. Although the initial patient cohort was characterized by high comorbidity and the presence of pre-existing risk factors for cholestasis (including parenteral nutrition), the trigger role of precipitates, consisting of calcium-ceftriaxone conglomerates, in the lumen of the bile ducts cannot be completely excluded.	not identified

Note: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

¹¹ Register of OHLP and LV of the EAEU. Amoxicillin+[Clavulanic acid] powder for solution preparation for intravenous administration, 500 mg+100 mg, 1000 mg+200 mg (vials), LP-No.(002949)-(RG-RU) dated Aug 07, 2023. Available from: https://lk.regmed.ru/Register/EAEU_SmPC

¹² Register of OHLP and LV of the EAEU. Ceftriaxone powder for solution preparation for intravenous and intramuscular administration, 1.0 g (vials) LP-No.(005496)-(RG-RU) dated May 17, 2024. Available from: https://lk.regmed.ru/Register/EAEU_SmPC

¹³ Register of OHLP and LV of the EAEU. Mizida-AG (Cefodizim) powder for solution preparation for intravenous and intramuscular administration, 2000 mg, LP-No.(010400)-(RG-RU) dated June 02, 2025. Available from: https://lk.regmed.ru/Register/EAEU_SmPC

Table 2 — Patients with urological pathology having transurethral or ureteral catheters

Pathology	Amoxicillin / clavulanic acid	Ceftriaxone	Cefodizime
Hematuria	very rare	rare	not identified
Glucosuria	not identified	rare	not identified
Precipitates in the urinary tract	In individuals with impaired renal excretion, the formation of crystals and the development of obstructive uropathy are observed extremely rarely, including acute renal failure.	The accumulation of crystalline conglomerates in the lumen of the urinary tract can initiate their obturation with subsequent development of postrenal acute renal failure.	not identified

Table 3 — Drug interactions of cefodizime, ceftriaxone, and amoxicillin / clavulanic acid with oral anticoagulants (vitamin K antagonists)

Interaction	Amoxicillin / clavulanic acid	Ceftriaxone	Cefodizime
Leukopenia	rare	common	frequency unknown
Thrombocytopenia	rare	common	frequency unknown
Coagulopathy	In patients receiving a combination of amoxicillin with clavulanic acid along with indirect anticoagulants, an increase in prothrombin time (increase in INR) has been reported in rare cases.	uncommon	frequency unknown
Interaction with anticoagulants (vitamin K antagonists)	Rare cases of increased International Normalized Ratio (INR) have been described in the literature in patients when acenocoumarol or warfarin was used concurrently with amoxicillin. When indirect (oral) anticoagulants are prescribed concurrently with a combination of amoxicillin and clavulanic acid, monitoring of relevant parameters is necessary, and a dose adjustment of anticoagulants may be required.	The risk of bleeding increases when vitamin K antagonists are used during ceftriaxone therapy. Blood coagulation parameters should be constantly monitored, and the dose of vitamin K antagonists should be adjusted if necessary.	not described

Note: INR, International Normalized Ratio.

Ceftriaxone is excreted in bile by approximately 33–67%, forming relatively high concentrations in the intestine¹⁴. This pharmacokinetic characteristic allows ceftriaxone to disrupt normal intestinal flora to a greater extent than other antibiotics primarily excreted by the kidneys, thereby causing a greater reduction in vitamin K synthesis.

In a retrospective study by L.M. Saum et al. [56], the increase in INR from baseline was compared in patients ($n = 120$) receiving long-term warfarin therapy and diagnosed with urinary tract infections, who were treated with ceftriaxone, a first-generation cephalosporin (cefazolin or cephalexin), or a penicillin (ampicillin / sulbactam, amoxicillin / clavulanic acid, piperacillin/tazobactam). It was found that the ceftriaxone group ($n = 27$) showed a statistically significantly higher peak INR compared to all other antibiotics studied (ceftriaxone—3.56, first-

generation cephalosporins—2.66, penicillins—2.98, ciprofloxacin—2.3; $p = 0.004$), as well as a statistically significantly greater degree of INR change (+1.19, +0.66, +0.8, +0.275; $p = 0.006$), and a statistically significantly greater percentage change in INR compared to ciprofloxacin (54.4% vs. 12.7%; $p = 0.037$) [56].

Ceftriaxone interacts with warfarin, increasing the INR in patients more than other commonly prescribed antibiotics for the treatment of urinary tract infections.

A summary of drug interactions between cefodizime, ceftriaxone, and amoxicillin / clavulanic acid with oral anticoagulants (vitamin K antagonists) is presented in Table 3.

A study conducted at Tikur Anbessa Hospital [57] found a moderate drug interaction between ceftriaxone and heparin (22.6%) and warfarin (6.7%). Bleeding or elevated INR was observed in 6 patients (1.9%), with one patient dying from bleeding.

Similar results were obtained in another retrospective study conducted in hospitals in Eastern

¹⁴ Register of OHLP and LV of the EAEU. Ceftriaxone powder for solution preparation for intravenous and intramuscular administration, 1.0 g (vials) LP-No.(005496)-(RG-RU) dated May 17, 2024.

Ethiopia [58], which analyzed the medical records of 271 patients. A total of 71 drugs were prescribed concurrently with ceftriaxone, most commonly metronidazole and tramadol. Ceftriaxone was most frequently used for postoperative prophylaxis ($n = 80$, 27.5%) and for the treatment of pneumonia ($n = 62$, 21.3%). Moderate drug interactions between ceftriaxone and unfractionated heparin ($n = 6$), warfarin ($n = 5$), and enoxaparin ($n = 1$) were found among concurrently prescribed drugs.

Review Limitations

The work performed has several important limitations that may affect the interpretation and generalization of the results.

Limitation of Design and Methodology. The authors conducted a non-systematic review, which is a major limitation of this study. Although the database we used is the most widely used in medicine, it does not fully cover all available information.

A significant number of studies were retrospective and had small patient samples. Large pharmacoepidemiological studies evaluating the long-term safety of cefodizime are lacking, and we also did not find studies that directly compared the safety profiles of the drugs studied.

CONCLUSION

It has been established that cefodizime, ceftriaxone, and amoxicillin / clavulanic acid are effective antibacterial drugs for the treatment of infections in patients with comorbid pathology. However, their safety profiles differ and should be considered when choosing therapy in high-risk patients.

Cefodizime demonstrated a favorable tolerability profile: no signals of hepatotoxicity or nephrotoxicity were noted in available clinical data, and no clinically significant drug interactions with oral anticoagulants were identified, unlike ceftriaxone and amoxicillin / clavulanic acid. These features make cefodizime a potentially preferred option in comorbid patients receiving anticoagulant therapy and/or having risk factors for VTE and kidney disease.

However, the volume of available clinical studies on cefodizime remains limited, which does not allow for a definitive determination of its place in the therapy of comorbid patients compared to the more studied ceftriaxone and amoxicillin / clavulanic acid. Further prospective randomized and observational studies, including patients with polymorbidity and polypharmacy, are needed for a more detailed assessment of the safety profile and confirmation of the obtained results.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Sergey K. Zyryanov — conceptualization, formal analysis, writing—original draft, writing—review & editing; Elena I. Kondratyeva — investigation, writing—review & editing; Elena A. Baibulatova — visualization, writing—original draft. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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