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Bioequivalence study of generic nirmatrelvir in healthy volunteers

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Nirmatrelvir is an antiviral drug that, in combination with ritonavir, is an effective agent for the etiotropic therapy of patients with mild to moderate COVID-19.

The aim of the study was to evaluate bioequivalence of the generic drug nirmatrelvir Arpaxel in combination with ritonavir and the original drug Paxlovid, which is a combination of nirmatrelvir/ritonavir, in a single dose administration to healthy volunteers.

Materials and methods. This research was an open-label, randomized, two-period crossover bioequivalence study. It included 2 periods, in each of which the volunteers received either a test drug (nirmatrelvir at the dose of 300 mg) in combination with ritonavir (100 mg), or a reference drug (a combination of nirmatrelvir 300 mg and ritonavir 100 mg), given as a single dose. A wash-out period between each of the administrations was 7 days. The blood sampling to determine the concentration of nirmatrelvir was carried out in the range from 0 to 36 h in each of the study periods. A nirmatrelvir concentration was determined by a validated HPLC-MS/MS method with a lower quantitation limit of 10 ng/mL. Bioequivalence was assessed by comparing 90% confidence intervals (CIs) for the ratio of geometric means of $AUC_{(0–36)}$ and C_{max} of the test drug and reference drugs with the established equivalence limits of 80.00–125.00%.

Results. In the study were included 68 healthy volunteers, 67 participants of which were included in the bioequivalence population. The pharmacokinetic parameters of the drugs were comparable to each other. The 90% confidence interval for the ratio of the geometric mean of the maximum drug concentration in the blood plasma and the area under the pharmacokinetic curve «concentration-time» from zero to the last blood draw within 36 hours of nirmatrelvir was 87.26–100.83 and 93.27–103.74%, which meets the criteria for assessing bioequivalence. The test drugs were well tolerated by the volunteers. The incidence of adverse events was similar for the test and reference drugs. No serious adverse events were recorded during the entire study.

Conclusion. As a result of this study, bioequivalence of the test and reference drugs has been established.

Keywords: COVID-19; bioequivalence; pharmacokinetics; nirmatrelvir; ritonavir; generic drug

Abbreviations: COVID-19 – a novel coronavirus infection; CI – confidence interval; AUC – area under the concentration-time curve; $AUC_{0–36}$ – area under the pharmacokinetic «concentration-time» curve from zero to the last blood sampling at which the concentration of the drug is equal to or higher than the lower limit of quantification within 36 hours; $AUC_{0–\infty}$ – area under the pharmacokinetic «concentration-time» curve, starting from the zero value of the time, extrapolated to infinity; C_{max} – the maximum concentration of the drug in the blood plasma; T_{max} – time to reach the maximum concentration; HPLC-MS/MS – high performance liquid chromatography with tandem mass spectrometry; AE/SAE – adverse event/serious adverse event; BMI – body mass index; PK – pharmakokinetics; ЭСG – electrocardiography.

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Исследование биоэквивалентности воспроизведенного препарата нирматрелвира у здоровых добровольцев

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Нирматрелвир представляет собой противовирусный препарат, который в сочетании с ритонавиром является эффективным средством для этиотропной терапии пациентов с COVID-19 легкого и среднетяжелого течения.

Цель. Оценить биоэквивалентность воспроизведенного препарата нирматрелвира Арпаксел в сочетании с ритонавиром и оригинального препарата Паксловид, представляющего собой комбинацию нирматрелвир/ритонавир, при однократном применении здоровыми добровольцами.

Материалы и методы. Данное исследование представляло собой открытое рандомизированное простое перекрестное исследование биоэквивалентности. Оно включало 2 периода, в каждом из которых добровольцы получали либо исследуемый препарат (нирматрелвир в дозе 300 мг) в комбинации с ритонавиром (100 мг), либо референтный препарат (комбинация нирматрелвира 300 мг и ритонавира 100 мг) однократно. Отмывочный период между каждым из приемов составил 7 сут. Отбор образцов плазмы крови для определения концентрации нирматрелвира производили в интервале от 0 до 36 ч в каждом из периодов исследования. Концентрацию нирматрелвира определяли валидированным методом ВЭЖХ-МС/МС с нижним пределом количественного определения 10 нг/мл. Для оценки биоэквивалентности проводили сопоставление 90% доверительных интервалов (ДИ) для отношения средних геометрических $AUC_{(0–36)}$ и C_{max} препаратов с установленными пределами эквивалентности 80,00–125,00%.

Результаты. В исследование были включены 68 здоровых добровольцев, из них в популяцию для оценки биоэквивалентности вошли 67 участников. Фармакокинетические параметры препаратов были сопоставимы между собой. Доверительный интервал 90% для отношения средних геометрических показателей максимальной концентрации препарата в плазме крови и площади под фармакокинетической кривой «концентрация-время» от нуля до последнего отбора крови в пределах 36 ч нирматрелвира составили 87,26–100,83 и 93,27–103,74%, что соответствует критериям оценки биоэквивалентности. Препараты исследования хорошо переносились добровольцами. Частота нежелательных явлений была схожей для исследуемого и референтного препаратов. В течение всего исследования не было зарегистрировано ни одного серьёзного нежелательного явления.

Заключение. В результате данного исследования была установлена биоэквивалентность исследуемого и референтного препаратов.

Ключевые слова: COVID-19; биоэквивалентность; фармакокинетика; нирматрелвир; ритонавир; воспроизведенный препарат

Список сокращений: COVID-19 – новая коронавирусная инфекция; ДИ – доверительный интервал; AUC – площадь под кривой «концентрация–время»; $AUC_{0–36}$ – площадь под фармакокинетической кривой «концентрация–время» от нуля до последнего отбора крови при котором концентрация препарата равна или выше нижнего предела количественного определения в пределах 36 ч; $AUC_{0–\infty}$ – площадь под фармакокинетической кривой «концентрация–время», начиная с нулевого значения времени, экстраполированная до бесконечности; C_{max} – максимальная концентрация препарата в плазме крови; T_{max} – время достижения максимальной концентрации; ВЭЖХ–МС/МС – высокоэффективная жидкостная хроматография с тандемной масс–спектрометрией; НЯ/СНЯ – нежелательное/серьезное нежелательное явление; ИМТ – индекс массы тела; ФК – фармакокинетика; ЭКГ – электрокардиография.

INTRODUCTION

Nirmatrelvir is an antiviral drug effective against SARS-CoV-2, the mechanism of action of which is realized by inhibiting the SARS-CoV-2 viral protease 3CLpro and blocking a virus replication [1–3]. To increase the systemic exposure of nirmatrelvir, it is used in combination with ritonavir, which is a strong inhibitor of CYP3A4 and significantly slows down the metabolism of nirmatrelvir [4]. Thus, in this combination, ritonavir acts as a pharmacokinetic enhancer (booster). Ritonavir did not shown own clinical efficacy against COVID-19.

The original nirmatrelvir Paxlovid[1] was developed by Pfizer, USA, and is available as a co-packaged combination of nirmatrelvir 150 mg tablets and ritonavir 100 mg tablets. The pharmacodynamic properties of nirmatrelvir[2] have been confirmed in preclinical and clinical studies [5–7]. *In vitro* data confirm the selectivity of nirmatrelvir against 3CLpro SARS-CoV-2 [8, 9]. During the clinical development, the efficacy of the nirmatrelvir/ritonavir combination in the treatment of mild to moderate COVID-19 was proven [10, 11].

The largest randomized placebo-controlled study (EPIC-HR) of the Paxlovid efficacy and safety was conducted with the participation of 2246 patients [12]. Based on the primary endpoint of this study, in the population of patients who had the symptoms onset no more than 3 days before the randomization, the incidence of COVID-19 – related hospitalization or death from any cause within 28 days was 0.717% (5/697) in the test drug group and 6.452% (44/682) in the placebo group. The difference between the groups was statistically significant (p <0.001). In addition, no deaths were reported in the test drug group, while 9 deaths (1.32%) were observed in the placebo group. It is also worth noting that in clinical studies, nirmatrelvir in combination with ritonavir was well tolerated and demonstrated a favorable safety profile.

In addition, the effectiveness of the nirmatrelvir/ritonavir combination has been confirmed by the data obtained in real clinical practice [13–16].

Despite the fact that the original drug containing the nirmatrelvir/ritonavir combination has been approved for use in many countries including the USA, Australia and Europe, it is currently not approved in Russia. The development and entry into the market of generic drugs of this combination will meet a high demand for the effective etiotropic therapy for COVID-19 in a pandemic [17–19].

The LLC "Drug Formulation" (R-Pharm group) has developed a generic drug of nirmatrelvir called Arpaxel; it contains 150 mg of nirmatrelvir in the dosage form of film-coated tablets. The packaging of the developed drug does not contain a co-packaged ritonavir drug, since its mono-drugs are commercially available. In order to confirm the bioequivalence of the developed generic and original drugs, this bioequivalence study has been conducted.

THE AIM of the study was to evaluate bioequivalence of the generic drug nirmatrelvir Arpaxel in combination with ritonavir and the original drug Paxlovid, which is a combination of nirmatrelvir/ritonavir, in a single dose administration to healthy volunteers.

MATERIALS AND METHODS

Study design

This Clinical Bioequivalence Study No. CJ051032185 was an open-label, randomized, two-period crossover study in healthy volunteers. The study design is shown in Fig. 1.

The study was conducted on the basis of the Clinical site LLC "Eco-Safety Scientific Research Center". The clinical stage of the study was carried out from November 2022 to January 2023.

The study fully complied with the ethical principles set out in the last revision of Helsinki Declaration, the rules of Good Clinical Practice of the Eurasian Economic Union, the rules of good clinical practice of the International Council for Harmonization (ICH E6 GCP R2), as well as other legislative acts applicable to this study. The clinical trial protocol was approved by the Ministry of Health of Russia (Permit No. 640 dated 2022 Nov 07) and the Ethics Council (extract from Protocol No. 317 dated 2022 Sep 06), as well as the local ethics committee at the research center (the extract from Protocol No. 262 dated 2022 Nov 17).

Objects of study

A total of 68 healthy volunteers were included in the study. Inclusion criteria were used: a male gender, the age of 18–45 years, a verified diagnosis of "healthy", a body mass index (BMI) 18.5–30 kg/m^2. The main exclusion criteria were the presence of chronic diseases of various organ systems, mental problems, hypersensitivity to the test drugs, lactose intolerance, the use of prohibited therapy drugs before the start of the study, or the presence of a positive PCR test for SARS-CoV-2. A volunteer was excluded from the study if he or she had withdrawn their formed consent, had received prohibited therapy, had grossly violated the requirements and procedures of the protocol, had experienced adverse events in which the volunteer's further participation in the study might be unsafe, or death. Before the start of the study, all participants familiarized themselves with the procedure for conducting the study and signed an informed consent form. A randomization into groups was performed in the ratio of 1:1 using the method of randomization envelopes.

[1] European Medicines Agency. Paxlovid 150+100 mg film-coated tablets: summary of product characteristics, 2022. Available from: https://www.ema.europa.eu/en/documents/product-information/paxlovid-epar-product-information_en.pdf.

[2] European medicines agency. Committee for Medicinal Products for Human Use. Assessment report EMA/95110/2022 – Rev. 1. Paxlovid. Available from: https://www.ema.europa.eu/en/documents/assessment-report/paxlovid-epar-public-assessment-report_en.pdf

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Drugs administration

The test drug was Arpaxel (film-coated tablets, 150 mg), in combination with Norvir® (ritonavir), film-coated tablets, 100 mg. The reference drug was the original drug nirmatrelvir/ritonavir – Paxlovid, film-coated tablets (150+100 mg). The volunteers were randomized to one of the groups – TR or RT. In group 1 (TR), the volunteers received the test drug in combination with ritonavir in period 1, and the reference drug in period 2. In group 2 (RT), the drugs were taken in the reverse order. The volunteers were given each of the drugs on an empty stomach as a single dose – 300 mg nirmatrelvir (2 tablets) and 100 mg ritonavir (1 tablet). The wash-out period between the doses of the drugs was 7 days. According to the literature data, the half-life ($T_{1/2}$) of nirmatrelvir administrated as a single dose in combination with ritonavir, is 6–7 h [20][3]. Thus, in order to minimize the risks of the first dose of the drugs affecting the nirmatrelvir pharmacokinetics (PK) in the 2nd period, the wash-out period should be at least 5 half-lives, i.e., at least 36 h.

The drugs were taken under the supervision of medical personnel. During the study, a special regimen of eating and drinking was kept to. The use of the drugs and dietary supplements that could have a pronounced effect on the hemodynamics or a liver function, could be inhibitors or inducers of CYP3A4 or P-glycoprotein, was forbidden. The drugs that increase the pH of the gastric juice, as well as a regular oral or patented use of other medicines and biologically active additives, were forbidden, too.

Sampling and sample preparation

To assess the concentration of nirmatrelvir in the volunteers' blood, the venous blood was sampled at the following time points: before taking the drug, then after 15, 30, 45 min, 1 h, 1 h and 15 min, 1 h and 30 min, 1 h and 45 min, 2 h, 2 h and 15 min, 2 h and 30 min, 2 h and 45 min, 3 h, 3 h and 15 min, 3 h and 30 min, 3 h and 45 min, 4 h, 4 h and 30 min, 5, 6, 8, 10, 12, 24 and 36 h after administration. The biosampling time points for the PK analysis were chosen in such a way as to obtain the most complete information in each of the of concentration-time curve. Given that the median time to reach the maximum concentration (T_{max}) of nirmatrelvir, according to the literature data, is 3 h [24], the chosen approach complies with the recommendations for choosing time points: at least at 3 points of the initial phase of increasing concentration, and at least at 5 points of the phase of decreasing concentration.

In case of the biosampling time deviation from the dew point, it was necessary to register its actual time. The actual time of biosampling was used to calculate pharmacokinetic parameters.

Blood sampling was carried out in test tubes containing K_2EDTA as an anticoagulant. After that, the samples were centrifuged in a Biosan LMC-3000 centrifuge (Biosan, Latvia) with an acceleration of 2000 g for 15 min to separate the plasma. The obtained samples were stored frozen at the temperature not exceeding – 65°C.

Analytical method

Nirmatrelvir plasma concentrations were determined by a validated high performance liquid chromatography with a tandem mass spectrometry (HPLC-MS/MS) method.

The sample preparation was performed by precipitating blood plasma proteins with chilled acetonitrile (LC/MS, Biosolve B.V., Netherlands) containing 0.1% formic acid. The HPLC system of the Sciex 5500 system (SCIEX, USA) and a hybrid triple quadrupole mass spectrometer with an electrospray ionization QTRAP 5500 (SCIEX, USA) were used for the study. Chromatographic separation was performed using a Waters Acquity BEH C18 Column, S-1.7 µm, 50×2.1 mm, chromatographic column in a gradient elution mode with a flow rate of 0.5 mL/min. A solution of ammonium formate (Sigma-Aldrich, USA) and formic acid (PA-ACS, Panreac, Spain) in water was used as mobile phase A, and a solution of formic acid in acetonitrile (HPLC-S, Biosolve B.V., Netherlands) was used as phase B). During the 1st minute of the analysis, 45% of phase A and 55% of phase B were injected. During the 2nd min, only phase B was injected, and at the end of the 2nd min and until the end of the analysis, 45% of phase A and 55% of phase B were injected. Ezetimibe was used as an internal standard. The retention time for nirmatrelvir was 0.5 min, for ezetimibe, it was 0.6 min. The total analysis time was 2.5 min. The lower limit of quantitation for nirmatrelvir was 10 ng/mL.

The data analysis was performed using Analyst 1.7.2 Software (SCIEX, USA). The analyte concentration was determined by the calibration curve of the dependence of the chromatographic peaks' areas of the analyte and the internal standard on the nominal concentration. To construct a calibration curve, a linear regression with the $1/x^2$ normalization was used. The correlation coefficient was not less than 0.99.

Safety assessment

The safety endpoints included the incidence and severity of all adverse events (AEs) and serious adverse events (SAEs). The incidence of grade 3–4 AEs, the incidence of the adverse events that led to an early termination of participation in the study, and the frequency of the AEs associated with study medications, were determined by CTCAE 5.0.

[3] WHO/PQT: medicines Guidance Document 28 April 2022 Notes on the design of bioequivalence study: Nirmatrelvir+ritonavir. URL: https://extranet.who.int/pqweb/sites/default/files/documents/BE_Nirmatrelvir_Ritonavir_28April2022.pdf

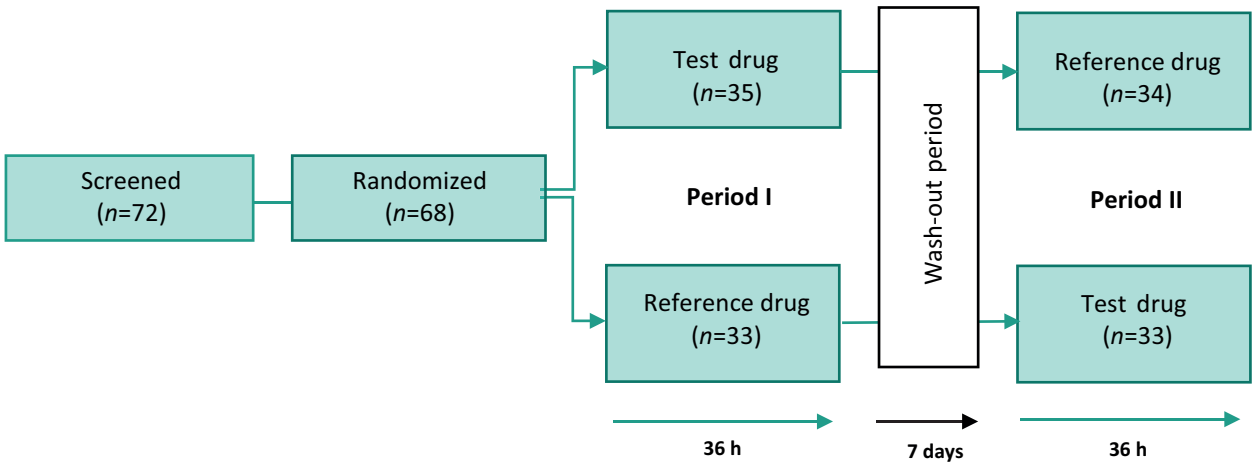

Figure 1 – Study design CJ051032185

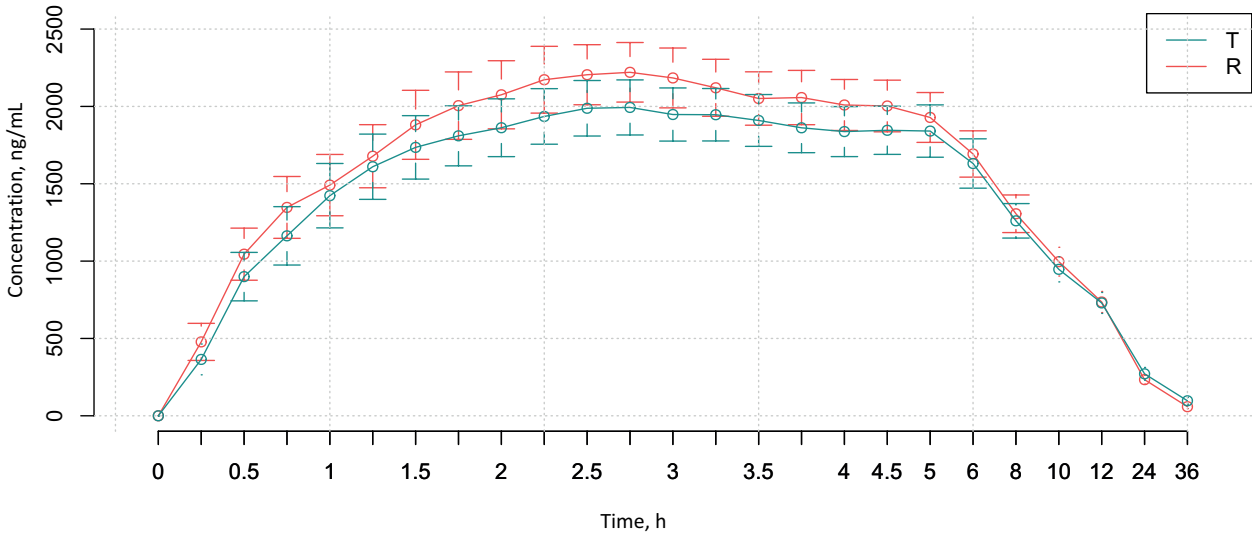

Figure 2 – Dynamics of nirmatrelvir concentration (mean±95% CI) after taking the test drug in combination with ritonavir (T) (*n*=68) and the reference drug Paxlovid (R) (*n*=67)

Note: R – reference drug, T – test drug+ritonavir.

Table 1 – Obtained values of pharmacokinetic parameters after taking test and reference drugs (N=68)

Pharmacokinetic parameters	Test drug (T) (*n*=68)	Reference drug (R) (*n*=67)
$AUC_{(0-36)}$, (ng/mL)*h	24 902.7±7 959.97	25 343.81±7 789.17
C_{max}, ng/mL	2 411.17±808.21	2 580.54±869
$AUC_{(0-\infty)}$, (ng/mL)*h	25 930.98±8 594.38	25 981.73±7 981.04
T_{max}, h	2.5 [1.7; 3.25]	2.25 [1.75; 3]
$T_{1/2}$, h	7.73±2.85	6.46±1.75

Note: *n* – the number of participants; C_{max} – maximum concentration; T_{max} – time to reach C_{max}; $T_{1/2}$ – half-life; $AUC_{(0-36)}$ – total area under the concentration-time curve in the time interval from 0 to 36 h; $AUC_{(0-\infty)}$ – area under the concentration-time curve in the time interval from 0 to infinity. All indicator values are presented as an arithmetic mean (standard deviation), except T_{max}, which presented as a median (minimum – maximum).

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Table 2 – Calculated 90% CI values for ratios of pharmacokinetic parameters of nirmatrelvir after taking test and reference drugs

Parameter	Geometric mean T/R ratio	Calculated values of 90% CI	CV_{intra} [1]
$AUC_{(0–36)}$	98.4	93.27-103.74	18.57
C_{max}	93.8	87.26-100.83	25.43

Note: CV_{intra} [1] – intra-individual coefficient of variability; CIs – confidence intervals; T – test drug + ritonavir; R – reference drug.

Table 3 – Summary of AEs frequency after taking each of the drugs

Adverse effect	Test drug (T) (N=68), *n* (%)	Reference drug (R) (N=67), *n* (%)	P[1] value
Laboratory and instrumental data			
Increase in blood bilirubin level	1 (1.47)	0 (0.00)	0.317
Decrease in platelet count	2 (2.94)	1 (1.47)	0.564
Increase in lymphocyte count	0 (0.00)	1 (1.47)	0.317
Hematopoietic system disorders			
Anemia	0 (0.00)	3 (4,41)	0.083
Nervous System Disorders			
Headache	0 (0.00)	1 (1.47)	0.317
Dysgeusia	7 (10.29)	4 (5.88)	0.317
Vascular disorders			
Hypertension	20 (29.41)	17 (25.00)	0.67
General disorders and reactions at the injection site			
Asthenia	0 (0.00)	1 (1.47)	0.317

Note: 1 is the level of significance when comparing the occurrence frequency of at least one specified adverse event (AE) after taking drugs (McNemar criterion). N is the number of subjects who took the specified drug or any of the drugs at least once. When calculating the frequency (%), the number of volunteers in the general population is taken as 100%. All AEs listed in the table belong to the 1st degree of severity.

For a close monitoring of safety in this study, a periodic evaluation of the vital signs, laboratory parameters (clinical and biochemical blood tests, urinalysis), a physical examination, ECG were performed. All the AEs were recorded from the start of the test drugs administration to the end of the follow-up. The AEs were coded using the Regulatory Dictionary of Medical Terms (MedDRA) [21].

Statistical analysis

The following parameters were used to calculate the sample size: the study power was 0.8 (80%); the level of statistical significance α was 0.05 (5%); according to literature sources, the intra-individual coefficient of the variation for nirmatrelvir C_{max} was 0.36 (36%). Taking into account the potential risk of dropping out from the study up to 25% of volunteers, 68 volunteers were planned to be included in the study.

A statistical analysis was performed using the freely distributed software package for a statistical analysis R-4.2.0 (R Foundation for Statistical Computing, Austria).

The nirmatrelvir pharmacokinetics was assessed using the following parameters: $AUC_{(0–36)}$ – the area under the concentration-time curve in the time interval from 0 to 36 hours; C_{max} – the maximum concentration; T_{max} – the time to reach C_{max}; $T_{1/2}$ – half-life; $AUC_{(0–\infty)}$ is the area under the "concentration-time" curve in the time interval from 0 to infinity.

The comparison of the drugs PK parameters was performed using the Wilcoxon test for linked samples. The frequencies of adverse events were compared using the McNemar test. Bioequivalence was assessed using the analysis of variance (ANOVA), which is a parametric method applied to log-transformed PK values (AUC and C_{max}). The statistical analysis also took into account various sources of variability that could affect the variables under study. The following parameters were used as fixed factors for constructing the ANOVA model: the drug use sequence; a period and drug; the subject of the study, nested in the sequence; cohort; the subject nested in the sequence nested in the cohort; the sequence nested in the cohort.

Based on the residual variation obtained from ANOVA, a 90% CI was calculated for the ratio of the geometric means of the logarithmically transformed baseline PK parameters ($AUC_{(0–36)}$ and C_{max}) calculated for test and reference drugs. To establish bioequivalence, the obtained CIs were compared with the prespecified bioequivalence limits, taken equal to 80.00–125.00%.

RESULTS

Population

A total of 68 male volunteers were included in the study: 35 in the TR group and 33 in the RT group. Since the hospitalization of all the 68 volunteers to the

clinical site for the study procedures was difficult, the volunteers were divided into 2 cohorts, which were included in the study 9 days apart. All the volunteers received at least one dose of test or reference drugs and were therefore included in the safety population. All the 68 volunteers were included in the pharmacokinetic analysis population. The bioequivalence population included 67 volunteers, since 1 volunteer from the TR group had been withdrawn from the study before the start of period 2 due to the withdrawal of an informed consent. All but one volunteer were white people, and that only volunteer belonged to asians. The mean age of the volunteers was 27.10 (±5.75) years, their body weight was 74.74 (±9.57) kg, and the BMI was 23.37 (±2.51) kg/m^2. Demographic and baseline characteristics of the volunteers did not differ between the groups.

Pharmacokinetics and bioequivalence

Pharmacokinetic parameters of nirmatrelvir after the administration of the test or reference drugs, are presented in Table 1. Fig. 2 is a graph of mean nirmatrelvir concentrations after studying the drugs.

After taking the test drug, $AUC_{(0–36)}$ was 24 902.7±7 959.97 ng*h/mL, and after taking the reference drug, it was 25 343.81±7 789.17 ng*h/mL. The maximum concentration of nirmatrelvir C_{max} was 2 411.17±808.21 and 2 580.54±869 ng/mL for the test and reference drugs, respectively. The values of the main pharmacokinetic parameters obtained after the use of the test and reference drugs, in general, were comparable between the drugs. Statistically significant differences between the drugs were observed in terms of $T_{1/2}$ (p=0.01), however, it should be noted that this indicator does not affect the assessment of the drugs bioequivalence, and there was no significant difference between the main pharmacokinetic parameters (C_{max} and AUC).

To assess the drugs bioequivalence, the $AUC_{(0–36)}$ parameter was used, since the obtained values of this indicator were more than 80% of the $AUC_{(0–\infty)}$ values. The calculated 90% CI for the ratio of geometric mean $AUC_{(0–36)}$ of the test and reference drugs was 93.27–103.74%. For the ratio of geometric mean C_{max} of the compared drugs, the 90% CI was 87.26-100.83%. The intervals obtained during the study, fully correspond to the established equivalence limit for $AUC_{(0–36)}$ and C_{max} – 80.00–125.00%, which clearly demonstrates the bioequivalence of the test and reference drugs (Table 2).

ANOVA results showed that the sources of variation such as drug differences, cohort, and period did not significantly affect the variables being evaluated. However, the sequence of the drug administration was found out to have a statistically significant effect on $AUC_{(0–36)}$ and $AUC_{(0–\infty)}$, and the cohort: period – on C_{max}.

When analyzing the results obtained, it was concluded that the statistically significant effect of the drug administration sequence and the period within the cohort identified during the analysis of variance, was due to external random factors and had no clinical significance.

Safety

In the study, a total of 61 AEs were reported in 43 volunteers. The data on AEs are presented in Table 3. The most common AEs (more than 5% in any of the groups) were dysgeusia and hypertension. Dysgeusia was observed in 7 (10.29%) volunteers after taking the test drug and in 4 (5.55%) volunteers after taking the reference drug. Hypertension was observed in 20 (29.41%) and 17 (25.00%) volunteers after taking the test and reference drugs, respectively. There were no significant differences between the compared drugs in the frequency of registration of adverse events (p >0.05). According to CTCAE 5.0, all the AEs registered during the study were classified as grade 1. No grade 2-5 AEs were registered.

According to the investigators, fewer than half of the reported AEs were related to the test drug. For the majority of AEs, the degree of association with the test drug was considered “doubtful”. The association of AEs with the drug use was established in 14 (20.59%) and 13 (19.12%) volunteers after taking the test drug in combination with ritonavir and the reference drug, respectively.

During the study, an association of AEs with the study medications was classified as “possible” for 1 case of anemia, 1 case of asthenia, and 14 cases of hypertension (7 after taking the test drug in combination with ritonavir and 7 after taking the reference drug). The drug association was classified as probable for 1 case of AE, a headache. The drug association was classified as certain for all 11 cases of AE dysgeusia. The frequencies of these events were comparable between the drugs. The reported AEs that the investigators considered drug-related, were consistent with the spectrum of adverse drug reactions of nirmatrelvir/ritonavir.

DISCUSSION

Nirmatrelvir has an antiviral activity against SARS-CoV-2 by inhibiting the main viral protease 3CLpro. The inhibition of 3CLpro by nirmatrelvir leads to the disruption of the polyprotein precursors processing, resulting in the termination of the viral replication [22, 23]. Due to the fact that nirmatrelvir is a substrate for the CYP3A4 enzyme, it is recommended to take it simultaneously with the pharmacokinetic enhancer ritonavir. In its turn,

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ritonavir is an inhibitor of CYP3A4, i.e., it reduces the rate of nirmatrelvir metabolism and thereby increases its systemic exposure [24, 25]. In Russia, ritonavir is registered as a part of combined drugs, as well as in the form of an independent drug. Due to the fact that ritonavir mono-preparations are commercially available, LLC "Drug Formulation" has developed a generic drug of nirmatrelvir, which does not include tablets with nirmatrelvir. In order to implement the state registration of the drug, a clinical bioequivalence study has been conducted.

The results of the clinical study showed that the PK parameters of the generic drug nirmatrelvir, when used together with ritonavir, are comparable to the PK parameters of the original drug nirmatrelvir/ritonavir. When conducting a statistical analysis of bioequivalence, the obtained 90% CI for the ratios of the geometric mean AUC and C_{max} nirmatrelvir values do not go beyond the specified intervals of 80.00–125.00% for these indicators, which confirms the bioequivalence of the test and reference drugs.

The data obtained as results of the study, are also consistent with the information available in the literature on the pharmacokinetics of the original drug nirmatrelvir/ritonavir. According to the data in the FDA fact sheet for healthcare providers on the original drug Paxlovid, as well as in the official instructions for the medical use of nirmatrelvir preparations placed in the State Register of Medicines, the geometric mean for nirmatrelvir C_{max} value after a single use on an empty stomach in combination with ritonavir is 2.21 mcg/mL, i.e., about 2 210 ng/mL. In the present study, the geometric mean C_{max} of the generic nirmatrelvir preparation practically did not differ from the literature data and was equal to 2274 ng/mL. The geometric mean $AUC_{(0-\infty)}$ of the original drug, according to the literature data, and the generic drug according to our study, are 23.01 (23 010 ng*h/mL) and 24 596 ng*h/mL, respectively. According to the literature data, the median T_{max} and mean $T_{1/2}$ of the original drug are 3.00 and 6.05 h, respectively. In this study, the generic drug showed similar values – 2.5 and 7.7 h for the median T_{max} and mean $T_{1/2}$.

The test and reference drugs were well tolerated by the volunteers. After taking the test and reference drugs, the incidence of AEs had no statistically significant differences. The AEs that were characterized by the investigators as drug-related were consistent with the published safety data for nirmatrelvir/ritonavir preparations. The most common adverse events, which occurred in more than 5% of participants, were dysgeusia and hypertension. According to the information specified in the instructions for the medical use of nirmatrelvir medicinal products, dysgeusia is a common (1–10%) adverse reaction when using these drugs. The hypertension AE is a common adverse reaction (1–10%) for ritonavir preparations in combination with nirmatrelvir/ritonavir used as a pharmacokinetic enhancer for nirmatrelvir.

CONCLUSION

In accordance with the protocol and requirements for confirming the bioequivalence of two drugs in agreement with international guidelines and legal requirements of the EAEU, the 90% CI obtained in this study for the ratio of the geometric mean $AUC_{(0-36)}$ and C_{max}, is fully within the range of 80.00–125.00%. Thus, it can be concluded that the test and reference drug nirmatrelvir are bioequivalent. It is also worth noting that the compared drugs showed a good tolerability and comparable safety profiles.

Based on the results of the bioequivalence study, Arpaxel was registered in the Russian Federation under the procedure for registering the drugs intended for use under the threat of occurrence and liquidation of emergencies.

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CONFLICT OF INTEREST

The clinical trial was organized by the sponsor JSC R-Pharm. The authors of the article Valentina G. Mozgovaya, Olga V. Filon, Anna V. Zinkovskaya, Antonina N. Dolgorukova, Elizaveta K. Khanonina, Vasily G. Ignatiev, Mikhail Yu. Samsonov are employees of JSC “R-Pharm”.

AUTHORS’ CONTRIBUTION

Rodion A. Oseshnyuk – research conducting; Anna Yu. Boroduleva, Pavel D. Sobolev, Ajyyna G. Nikiforova – analytical part development and validation; biosamples analysis; Svetlana A. Lesnichuk, Bair B. Garyaev, Anna A. Abramova – results analysis, text writing and editing; Valentina G. Mozgovaya – research design development, text writing and editing; Olga V. Filon – research design development, text writing and editing; Anna V. Zinkovskaya, Antonina N. Dolgorukova – statistical processing of research results; Elizaveta K. Khanonina – literary sources analysis, text writing and editing; Vasily G. Ignatiev, Mikhail Yu. Samsonov – aim setting, research design development.

REFERENCES

1. Joyce RP, Hu VW, Wang J. The history, mechanism, and perspectives of nirmatrelvir (PF-07321332): an orally bioavailable main protease inhibitor used in combination with ritonavir to reduce COVID-19-related hospitalizations. Med Chem Res. 2022;31(10):1637–46. DOI:10.1007/s00044-022-02951-6
2. Vangeel L, Chiu W, De Jonghe S, Maes P, Slechten B, Raymenants J, André E, Leyssen P, Neyts J, Jochmans D. Remdesivir, Molnupiravir and Nirmatrelvir remain active against SARS-CoV-2 Omicron and other variants of concern. Antiviral Res. 2022 Feb;198:105252. DOI:10.1016/j.antiviral.2022.105252
3. Marzi M, Vakil MK, Bahmanyar M, Zarenezhad E. Paxlovid: Mechanism of Action, Synthesis, and *In Silico* Study. Biomed Res Int. 2022 Jul 7;2022:7341493. DOI:10.1155/2022/7341493
4. Singh RSP, Toussi SS, Hackman F, Chan PL, Rao R, Allen R, Van Eyck L, Pawlak S, Kadar EP, Clark F, Shi H, Anderson AS, Binks M, Menon S, Nucci G, Bergman A. Innovative Randomized Phase I Study and Dosing Regimen Selection to Accelerate and Inform Pivotal COVID-19 Trial of Nirmatrelvir. Clin Pharmacol Ther. 2022 Jul;112(1):101–11. DOI:10.1002/cpt.2603
5. Vangeel L, Chiu W, De Jonghe S, Maes P, Slechten B, Raymenants J, André E, Leyssen P, Neyts J, Jochmans D. Remdesivir, Molnupiravir and Nirmatrelvir remain active against SARS-CoV-2 Omicron and other variants of concern. Antiviral Res. 2022 Feb;198:105252. DOI:10.1016/j.antiviral.2022.105252
6. Catlin NR, Bowman CJ, Campion SN, Cheung JR, Nowland WS, Sathish JG, Stethem CM, Updyke L, Cappon GD. Reproductive and developmental safety of nirmatrelvir (PF-07321332), an oral SARS-CoV-2 M^{pro} inhibitor in animal models. Reprod Toxicol. 2022 Mar;108:56–61. DOI:10.1016/j.reprotox.2022.01.006
7. Jeong JH, Chokkakula S, Min SC, Kim BK, Choi WS, Oh S, Yun YS, Kang DH, Lee OJ, Kim EG, Choi JH, Lee JY, Choi YK, Baek YH, Song MS. Combination therapy with nirmatrelvir and molnupiravir improves the survival of SARS-CoV-2 infected mice. Antiviral Res. 2022 Dec;208:105430. DOI:10.1016/j.antiviral.2022.105430
8. Greasley SE, Noell S, Plotnikova O, Ferre R, Liu W, Bolanos B, Fennell K, Nicki J, Craig T, Zhu Y, Stewart AE, Steppan CM. Structural basis for the *in vitro* efficacy of nirmatrelvir against SARS-CoV-2 variants. J Biol Chem. 2022 Jun;298(6):101972. DOI:10.1016/j.jbc.2022.101972
9. Owen DR, Allerton CMN, Anderson AS, Aschenbrenner L, Avery M, Berritt S, Boras B, Cardin RD, Carlo A, Coffman KJ, Dantonio A, Di L, Eng H, Ferre R, Gajiwala KS, Gibson SA, Greasley SE, Hurst BL, Kadar EP, Kalgutkar AS, Lee JC, Lee J, Liu W, Mason SW, Noell S, Novak JJ, Obach RS, Ogilvie K, Patel NC, Pettersson M, Rai DK, Reese MR, Sammons MF, Sathish JG, Singh RSP, Steppan CM, Stewart AE, Tuttle JB, Updyke L, Verhoest PR, Wei L, Yang Q, Zhu Y. An oral SARS-CoV-2 M^{pro} inhibitor clinical candidate for the treatment of COVID-19. Science. 2021 Dec 24;374(6575):1586–93. DOI:10.1126/science.abl4784
10. Wen W, Chen C, Tang J, Wang C, Zhou M, Cheng Y, Zhou X, Wu Q, Zhang X, Feng Z, Wang M, Mao Q. Efficacy and safety of three new oral antiviral treatment (molnupiravir, fluvoxamine and Paxlovid) for COVID-19: a meta-analysis. Ann Med. 2022 Dec;54(1):516–23. DOI:10.1080/07853890.2022.2034936
11. Drożdżal S, Rosik J, Lechowicz K, Machaj F, Szostak B, Przybyciński J, Lorzadeh S, Kotfis K, Ghavami S, Łos MJ. An update on drugs with therapeutic potential for SARS-CoV-2 (COVID-19) treatment. Drug Resist Updat. 2021 Dec;59:100794. DOI:10.1016/j.drup.2021.100794
12. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, Baniecki M, Hendrick VM, Damle B, Simón-Campos A, Pypstra R, Rusnak JM; EPIC-HR Investigators. Oral Nirmatrelvir for High-Risk, Nonhospitalized Adults with Covid-19. N Engl J Med. 2022 Apr 14;386(15): 1397–408. DOI:10.1056/NEJMoa2118542
13. Wong CKH, Au ICH, Lau KTK, Lau EHY, Cowling BJ, Leung GM. Real-world effectiveness of molnupiravir and nirmatrelvir plus ritonavir against mortality, hospitalisation, and in-hospital outcomes among community-dwelling, ambulatory patients with confirmed SARS-CoV-2 infection during the omicron wave in Hong Kong: an observational study. Lancet. 2022 Oct 8;400(10359):1213–22. DOI:10.1016/S0140-6736(22)01586-0
14. Wong CKH, Au ICH, Lau KTK, Lau EHY, Cowling BJ, Leung GM. Real-world effectiveness of early molnupiravir or nirmatrelvir-ritonavir in hospitalised patients with COVID-19 without supplemental oxygen requirement on admission during Hong Kong's omicron BA.2 wave: a retrospective cohort study. Lancet Infect Dis. 2022 Dec;22(12):1681–93. DOI:10.1016/S1473-3099(22)00507-2
15. Najjar-Debbiny R, Gronich N, Weber G, Khoury J, Amar M, Stein N, Goldstein LH, Saliba W. Effectiveness of Paxlovid in Reducing Severe Coronavirus Disease 2019 and Mortality in High-Risk Patients. Clin Infect Dis. 2023 Feb 8;76(3):e342–e349. DOI:10.1093/cid/ciac443. Erratum in: Clin Infect Dis. 2023 Mar 21;76(6): 1158–1159.
16. Dryden-Peterson S, Kim A, Kim AY, Caniglia EC, Lennes IT, Patel R, Gainer L, Dutton L, Donahue E, Gandhi RT, Baden LR, Woolley AE. Nirmatrelvir Plus Ritonavir for Early COVID-19 in a Large U.S. Health System: A Population-Based Cohort Study. Ann Intern Med. 2023 Jan;176(1): 77–84. DOI:10.7326/M22-2141
17. Yuan Y, Jiao B, Qu L, Yang D, Liu R. The development of COVID-19 treatment. Front Immunol. 2023 Jan 26;14:1125246. DOI:10.3389/fimmu.2023.1125246
18. Zhang JJ, Dong X, Liu GH, Gao YD. Risk and Protective Factors for COVID-19 Morbidity, Severity, and Mortality. Clin Rev Allergy Immunol. 2023 Feb;64(1):90–107. DOI:10.1007/s12016-022-08921-5
19. Lipsitch M, Krammer F, Regev-Yochay G, Lustig Y, Balicer RD. SARS-CoV-2 breakthrough infections in vaccinated individuals: measurement, causes and impact. Nat Rev Immunol. 2022 Jan;22(1):57–65. DOI:10.1038/s41577-021-00662-4
20. Reis S, Metzendorf MI, Kuehn R, Popp M, Gagyor I, Kranke P, Meybohm P, Skoetz N, Weibel S. Nirmatrelvir combined with ritonavir for preventing and treating COVID-19. Cochrane Database Syst Rev. 2022 Sep 20;9(9):CD015395. DOI:10.1002/14651858.CD015395.pub2
21. Große-Michaelis I, Proestel S, Rao RM, Dillman BS, Bader-Weder S, Macdonald L, Gregory W. MedDRA Labeling Groupings to Improve Safety Communication in

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2023-11-1-62-71

Product Labels. Ther Innov Regul Sci. 2023 Jan;57(1):1–6. DOI:10.1007/s43441-022-00393-1

22. Joyce RP, Hu VW, Wang J. The history, mechanism, and perspectives of nirmatrelvir (PF-07321332): an orally bioavailable main protease inhibitor used in combination with ritonavir to reduce COVID-19-related hospitalizations. Med Chem Res. 2022;31(10):1637-1646. DOI:10.1007/s00044-022-02951-6
23. Ullrich S, Nitsche C. The SARS-CoV-2 main protease as drug target. Bioorg Med Chem Lett. 2020 Sep 1;30(17):127377. DOI:10.1016/j.bmcl.2020.127377
24. Eng H, Dantonio AL, Kadar EP, Obach RS, Di L, Lin J, Patel NC, Boras B, Walker GS, Novak JJ, Kimoto E, Singh RSP, Kalgutkar AS. Disposition of Nirmatrelvir, an Orally Bioavailable Inhibitor of SARS-CoV-2 3C-Like Protease, across Animals and Humans. Drug Metab Dispos. 2022 May;50(5):576–90. DOI:10.1124/dmd.121.000801
25. Loos NHC, Beijnen JH, Schinkel AH. The Mechanism-Based Inactivation of CYP3A4 by Ritonavir: What Mechanism? Int J Mol Sci. 2022 Aug 30;23(17):9866. DOI:10.3390/ijms23179866

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