RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Metabolomics in drugs research on zebrafish-based cardiotoxicity models: endothelial and mitochondrial dysfunction, oxidative stress

N.E. Moskaleva[1], P.M. Rezvanov[1], V.M. Samoylov[1], V.G. Varzieva[1], S.N. Baskhanova[1], V.V. Tarasov[1], E.A. Smolyarchuk[1], D.A. Kudlay[1, 2, 3], S.A. Appolonova[1]

[1] I.M. Sechenov First Moscow State Medical University (Sechenov University),
2 Bolshaya Pirogovskaya Str., Bldg 4, Moscow, Russia, 119435
[2] Lomonosov Moscow State University,
1 Leninskie Gory, Moscow, Russia, 119991
[3] State Research Center Institute of Immunology,
24 Kashirskoe Hwy, Moscow, 115522, Russia

E-mail: moskaleva_n_e@staff.sechenov.ru

Received 05 Jan 2025 After peer review 24 June 2025 Accepted 06 July 2025

The aim. To investigate the metabolic profile of zebrafish embryos when exposed to drugs with known risks of cardiotoxicity, such as acetaminophen, carbamazepine, salbutamol, ketorolac, bisoprolol, and metoprolol. The analysis is aimed at detecting changes in the level of amino acids (including branched chain BCAAs), products of carnitine metabolism (acylcarnitines) and related metabolic indices reflecting mitochondrial dysfunction, oxidative stress and disorders of the nitric oxide signaling pathway.

Materials and methods. Zebrafish embryos were incubated with the test substances in a concentration gradient (0.5–10×NOEC). A quantitative targeted metabolomics analysis was performed using high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) with a panel of 98 metabolites (amino acids, nitric oxide metabolism products, vitamins, nucleosides and acylcarnitines). The obtained concentrations of metabolites were compared with the control (0.1% DMSO). Statistically significant deviations were expressed as the ratio of concentration to control on a base 2 logarithmic scale (*log2FC*).

Results. Changes in concentrations of metabolites under the influence of cardiotoxic drugs were revealed. There was an accumulation of BCAAs (the sum of leucine, isoleucine, valine; log2FC≈0.5–2.2; p <0.05) compared with the control, as well as an increase in the level of acylcarnitines, indicating mitochondrial dysfunction: for example, metoprolol and bisoprolol caused an increase in the ratio of the sum of acylcarnitines to free carnitine by more than 4–6 times (*log2FC*=+3.8 for bisoprolol and -1.27 for metoprolol; p <0.01), as well as accumulation of long-chain acylcarnitines. Pronounced changes in indicators related to oxidative stress were noted: in the samples after exposure to beta-1 blockers (bisoprolol, metoprolol) and ketorolac, the concentration of methionine sulfoxide (by 80–130%, p < 0.01), the product of methionine oxidation, and the ratio of methionine sulfoxide/methionine increased, whereas when exposed to salbutamol, on the contrary, the level of methionine sulfoxide decreased (-120%, p <0.01), indicating a multidirectional effect on the oxidative status. Violations of the nitric oxide signaling pathway were reflected in an increase in the level of asymmetric dimethylarginine.

Conclusion. Each of the analyzed compounds produced a specific metabolic “imprint” in Zebrafish samples, reflecting the mechanisms of their cardiotoxicity. An increase in BCAA levels and related indicators indicates a violation of myocardial energy metabolism, the accumulation of long-chain acylcarnitines indicates incomplete beta-oxidation of fatty acids. An increase in the concentration of ADMA is associated with endothelial dysfunction, and an increase in methionine sulfoxide is associated with increased oxidative stress.

Keywords: cardiotoxicity; metabolomics; zebrafish; zebrafish; amino acids; acylcarnitines.

Abbreviations: CTx — cardiotoxicity; ADMA — asymmetric dimethylarginine; BCAA — the sum of branched-chain amino acids (valine+leucine+isoleucine); DMSO — dimethyl sulfoxide; FC (Fold Change) — the ratio of concentration in the sample to concentration in the control; NOEC — concentration having no effect; SDMA — symmetric dimethylarginine; GSG — a combination of amino acids involved in the synthesis of glutathione, glutamate/(serine+glycine); GABR — arginine total availability index, arginine/(citrulline+ornithine); ROS — reactive oxygen species.

For citation: N.E. Moskaleva, P.M. Rezvanov, V.M. Samoylov, V.G. Varzieva, S.N. Baskhanova, V.V. Tarasov, E.A. Smolyarchuk, D.A. Kudlay, S.A. Appolonova. Metabolomics in drugs research on zebrafish-based cardiotoxicity models: endothelial and mitochondrial dysfunction, oxidative stress. *Pharmacy & Pharmacology.* 2025;13(2):70-83. **DOI:** 10.19163/2307-9266-2025-13-2-70-83

Для цитирования: Н.Е. Москалева, П.М. Резванов, В.М. Самойлов, В.Г. Варзиева, С.Н. Басханова, В.В. Тарасов, Е.А. Смолярчук, Д.А. Кудлай, С.А. Апполонова. Метаболомное исследование кардиотоксичности лекарственных препаратов на зебрафиш в качестве модельного организма: эндотелиальная и митохондриальная дисфункция, окислительный стресс. *Фармация и фармакология.* 2025;13(2):70-83. **DOI:** 10.19163/2307-9266-2025-13-2-70-83

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2025-13-2-70-83

Метаболомное исследование кардиотоксичности лекарственных препаратов на зебрафиш в качестве модельного организма: эндотелиальная и митохондриальная дисфункция, окислительный стресс

Н.Е. Москалева[1], П.М. Резванов[1], В.М. Самойлов[1], В.Г. Варзиева[1], С.Н. Басханова[1], В.В. Тарасов[1], Е.А. Смолярчук[1], Д.А. Кудлай[1, 2, 3], С.А. Апполонова[1]

[1] Федеральное государственное автономное образовательное учреждение высшего образования «Первый Московский государственный медицинский университет имени И.М. Сеченова» Министерства здравоохранения Российской Федерации (Сеченовский Университет), Россия, 119435, г. Москва, ул. Большая Пироговская, д. 2, стр. 4
[2] Федеральное государственное бюджетное образовательное учреждение высшего образования «Московский государственный университет имени М.В. Ломоносова», Россия, 119991, г. Москва, Ленинские горы, д. 1
[3] Федерального государственного бюджетного учреждения «Государственный научный центр «Институт иммунологии»» Федерального медико-биологического агентства, Россия, 115522, г. Москва, Каширское шоссе, д. 24

E-mail: moskaleva_n_e@staff.sechenov.ru

Получена 05.01.2025 После рецензирования 24.06.2025 Принята к печати 06.07.2025

Цель. Исследовать изменения метаболомного профиля эмбрионов Данио-рерио (зебрафиш) при воздействии лекарственных препаратов с известными рисками кардиотоксичности — ацетаминофена, карбамазепина, сальбутамола, кеторолака, бисопролола и метопролола. Анализ нацелен на выявление изменений в уровне аминокислот (в том числе с разветвлённой цепью — BCAA), продуктов карнитинового обмена (ацилкарнитинов) и связанных метаболических индексов, отражающих митохондриальную дисфункцию, оксидативный стресс и нарушения сигнального пути оксида азота.

Материалы и методы. Эмбрионы Данио-рерио инкубировали с исследуемыми веществами в градиенте концентраций (0,5–10×NOEC). Проводился количественный целевой метаболомный анализ методом высокоэффективной жидкостной хроматографии — тандемной масс-спектрометрии (HPLC–MS/MS) с панелью из 98 метаболитов (аминокислоты, продукты обмена оксида азота, витамины, нуклеозиды и ацилкарнитины). Полученные концентрации метаболитов сравнивали с контролем (0,1% ДМСО). Статистически значимые отклонения выражали как отношение концентрации к контролю в логарифмическом масштабе по основанию 2 (*log2FC*).

Результаты. Выявлены изменения концентраций ключевых метаболитов под влиянием кардиотоксичных лекарственных препаратов. Наблюдалось накопление BCAA (сумма лейцин, изолейцин, валин; *log2FC*≈0,5–2,2; p <0,05) по сравнению с контролем, а также повышение уровня ацилкарнитинов, указывающее на митохондриальную дисфункцию: так, метопролол и бисопролол вызывали увеличение соотношения суммы ацилкарнитинов к свободному карнитину более чем в 4–6 раз (*log2FC*=+3,8 для бисопролола и –1,27 для метопролола; p <0,01), а также накопление длинноцепочечных ацилкарнитинов. Отмечены выраженные изменения показателей, связанных с оксидативным стрессом: в образцах после воздействия β1-блокаторов (бисопролол, метопролол) и кеторолака повышалась концентрация метионин-сульфоксида (на 80–130%, p <0,01) — продукта окисления метионина, и соотношение метионин-сульфоксид/метионин, тогда как при воздействии сальбутамола, напротив, уровень метионин-сульфоксида снижался (–120%, p <0,01), указывая на разнонаправленное влияние на окислительный статус. Нарушения сигнального пути оксида азота отразились в повышении уровня асимметричного диметиларгинина.

Заключение. Каждое из проанализированных соединений вызывало специфический метаболический «отпечаток» в образцах Данио-рерио, отражающий механизмы их кардиотоксичности. Повышение уровня BCAA и связанных показателей указывает на нарушение энергетического обмена миокарда, накопление длинноцепочечных ацилкарнитинов свидетельствует о неполном β-окислении жирных кислот. Рост концентрации ADMA ассоциирован с эндотелиальной дисфункцией, а увеличение метионин-сульфоксида — с усилением оксидативного стресса.

Ключевые слова: кардиотоксичность; метаболомика; Данио-рерио; зебрафиш; аминокислоты; ацилкарнитины

Список сокращений: КС — кардиотоксичность; ЛС — лекарственное средство; ADMA — асимметричный диметиларгинин; BCAA — сумма аминокислот с разветвлённой цепью (валин+лейцин+изолейцин); ДМСО — диметилсульфоксид; FC (Fold Change) — отношение концентрации в образце к концентрации в контроле; NOEC — концентрация, не оказывающая эффекта; SDMA — симметричный диметиларгинин; GSG — комбинация аминокислот, вовлечённых с синтез глутатиона, глутамат/(серин+глицин); GABR — индекс общей доступности аргинина, аргинин/(цитруллин+орнитин); АФК — активные формы кислорода.

INTRODUCTION

Cardiotoxicity (CT) of medicines remains a significant problem in modern pharmacology and clinical practice, as it is the reason for rejection at the stages of development and withdrawal of medicines at the post-marketing stage [1]. CT manifests itself as a result of a complex interaction of various molecular mechanisms of heart damage, such as oxidative stress (OS), mitochondrial and endothelial dysfunction, and impaired ion channel functions [2–5].

Metabolomic analysis provides the possibility of identifying biomarkers of myocardial damage. In particular, profiles of low molecular weight metabolites (amino acids, carnitine metabolism, etc.) in blood plasma reflect characteristic shifts in metabolism during toxic exposure [3–5]. For example, increased levels of branched-chain amino acids (BCAA: leucine, isoleucine, valine) are associated with impaired myocardial contractility [6–8], and the accumulation of long-chain acylcarnitines indicates impaired mitochondrial b-oxidation and energy balance [9–11]. An increased concentration of asymmetric dimethylarginine (ADMA), an inhibitor of NO synthase, correlates with impaired endothelial dysfunction and progression of heart failure [12, 13].

The assessment of CT of medicines is currently carried out on cellular systems using cardiomyocytes and on mammals [14, 15]. Modern methods in vitro screening provides a high level of throughput, but do not reflect the complex interactions occurring in an intact organism [15]. Studies on mammals have better predictive value in relation to clinical results, but have low throughput and high cost [15], which is the reason for the search for other biological models for studying organ toxicity of medicines.

Zebrafish (Danio rerio) demonstrate a high degree of metabolic conservatism and are used to assess the toxicity of various compounds due to genetic homology with mammals, transparency of embryos and high rate of development [16–18]. The most common method for studying CT using zebrafish as a model organism is to identify changes in heart rate or blood flow rate of embryos after incubation with the studied medicines [19].

It would be interesting to supplement the morphological data with information about the mechanisms of toxic action. The use of Danio rerio as a biological model provides the possibility of assessing the systemic effects of medicines in the conditions of an intact organism [20], allows identifying metabolomic markers associated with the mechanisms of the toxic effect of the substance on the myocardium [20], which can be used to identify organ toxicity in the development of new drugs.

THE AIM was to identify specific metabolomic changes that occur when Danio rerio embryos are exposed to various cardiotoxic medicines and to establish a relationship between these changes and the alleged mechanisms of toxicity.

MATERIALS AND METHODS

Reagents and materials

The following reagents and chemicals were used: deionized water (18.2 MΩ×cm) was obtained using a Millipore-Q system (Merck, Darmstadt, Germany), sodium chloride (NaCl), potassium chloride (KCl), calcium (II) chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), methylene blue and ascorbic acid (qualification “pure”) (Prime Chemical Group, Moscow, Russia); acetonitrile, methanol, formic acid “for HPLC”, sodium metabisulfite “reagent grade” (Sigma-Aldrich, USA). Also, salbutamol and bisoprolol fumarate (British Pharmacopoeia (BP) Reference Standard, EDQM, USA), acetaminophen, ketorolac tromethamine, cetirizine hydrochloride, metoprolol tartrate (Pharmaceutical Secondary Standard, Supelco, USA), carbamazepine (≥98% (HPLC), Sigma-Aldrich, USA) were used for the study.

E3 medium contained 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$ and 0.001% methylene blue. Substances were dissolved in dimethyl sulfoxide (DMSO) or water, and then diluted in E3 medium to obtain a concentration for incubation (the final concentration of DMSO was 0.1%).

Amino acid standards and compounds containing an amino group (Sigma-Aldrich, Germany) and isotopically labeled standards (Toronto Research Chemicals, Canada) were used for quantitative analysis. Internal isotopically labeled standards (MassChrom Internal Standard Mix, Chromsystems, Germany) were used for the analysis of acylcarnitines.

Conducting studies on Zebrafish

Wild-type Danio rerio were obtained and maintained at the Center for Biopharmaceutical Analysis and Metabolomic Research, Sechenov First Moscow State Medical University (Sechenov University). The fish belonged to the same population and were randomly distributed into spawning groups. The studies were

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-2-70-83

conducted with an automatically controlled 24-hour cycle (14 light and 10 dark time), and the temperature was set at 26±2°C. Unfertilized eggs and dead embryos were identified and separated on the 2nd day after fertilization, and live embryos were used for the study. Morphological pathologies, such as tail malformations, pigmentation levels, shape of the chord and somites, as well as heartbeat, were assessed using a light microscope (Leica DM2000, Germany).

Danio rerio embryos 4 days after fertilization (age up to 120 h) were used for the studies, therefore, approval by the Ethics Committee of any level was not required in the case of this study. Embryos were transferred to 12-well plates (20 embryos per biological sample in one well). Then, 5 mL of the testing medicine solution in E3 medium without methylene blue was added to each well. The concentrations of the medicine ranged from 0.5×NOEC to 10×NOEC (Table 1), 0.1% DMSO in E3 medium was added to the control group. NOEC was determined in acute toxicity experiments conducted in accordance with GOST 33774-2016 "Methods for testing chemical products that pose a danger to the environment. Acute toxicity to fish embryos". The plates were incubated for 4 h. To account for variability, each medicine was studied in 3 repeated incubations. The study design is shown in Figure 1.

Sample preparation for HPLC-MS/MS analysis

After incubation, embryos from each well (20 pcs. per sample) were transferred to 1.5 mL microcentrifuge tubes. The aqueous phase was removed and 10 µl of 13 mM aqueous solution of sodium metabisulfite was added to the sample to prevent oxidation of metabolites. Then, 500 µl of cold methanol with a mixture of internal standards was added, sonicated in an ice bath for 15 min and centrifuged (Centrifuge 5418R, Eppendorf AG, Germany) for 5 min at 16 900 rf at 4°C. After that, 450 µl of the supernatant was evaporated to dryness. The residue was reconstituted with 100 µl of methanol, diluted with 100 µl of deionized water, centrifuged for 10 min at 14,000 rpm on the same centrifuge and analyzed by HPLC-MS/MS.

HPLC-MS/MS analysis

HPLC-MS/MS analysis was performed using an Agilent 1290 Infinity II high-performance liquid chromatography system with an Agilent 6470A mass spectrometric detector (Agilent Technologies, USA). Chromatographic separation was performed using an Acquity UPLC BEH C18 analytical column (2.1×50 mm, 1.7 µm) from Waters Corporation, USA at 40°C. The mobile phase consisted of 0.1% aqueous solution of formic acid (A) and 0.1% solution of formic acid in acetonitrile (B), supplied in a gradient elution program at a flow rate of 0.5 mL/min. The total analysis time was 5.0 min. Samples were injected in a volume of 5 µl.

Mass spectrometric analysis was performed in multiple reaction monitoring mode after electrospray ionization. The mass spectrometry parameters were as follows: capillary voltage is 3500 W, gas temperature is 300°C, gas flow rate is 11 L/min. Compound-specific parameters (fragmentor, collision energy) and optimized for the best response of each analyte are published in Kozhevnikova et al. [9]. The initial data were processed using MassHunter Software (Agilent Technologies, USA).

Statistical analysis

Quantitative analysis of compounds containing an amino group was performed by the internal standard method in accordance with the calibration curve. MassHunter Software (Agilent Technologies, USA) was used to construct the calibration curve. The concentrations of acylcarnitines in the samples were determined by calculating the ratio of the peak areas of acylcarnitines and their internal standards (semi-quantitative method) using MassHunter Software (Agilent Technologies, USA).

Data from 3 repeated experiments were ranked and averaged. The Shapiro–Wilk test was used to assess the normality of the data distribution. In case of violation of normality in one of the groups, the non-parametric Mann–Whitney test was used to compare concentrations between the control and test groups. In the absence of deviations from normality, the Welch's t-test was used. Values at p <0.05 were considered significant. Statistical analysis was performed using STATISTICA version 10.0 software (USA).

To identify the trend of changes in the metabolomic profile, the ratio of the metabolite concentration in the test sample to the control (Fold Change) was calculated and expressed on a logarithmic scale to the base **2 (log2FC)** [21].

Metabolite ratios were also calculated [22–24]: GSG index — a combination of amino acids involved in glutathione synthesis — glutamate / (serine+glycine); BCAA — the sum of branched-chain amino acids — valine+leucine+isoleucine; GABR — index of total

arginine availability — arginine / (citrulline+ornithine); Fischer's index — the ratio of the sum of branched-chain amino acids to the sum of aromatic amino acids.

RESULTS

One of the main problems in the use of Danio rerio as a biological model in metabolomic studies remains high biological variability, which can complicate the interpretation of results and reduce reproducibility [25]. Using a strictly defined stage of development, standardization of diet and feeding time reduces metabolic fluctuations [25]. Combining 20 embryos into one biological sample eliminates variability, especially at early stages of development [25]. More reproducible results are also obtained if the ratios of metabolite concentrations in exposure groups compared to the control are interpreted.

Table 2 presents changes in the concentration of metabolomic markers (for the maximum concentration of 10×NOEC), characteristic of various CT mechanisms, which were observed under the influence of the studied substances. In the negative control (cetirizine), no significant shifts in metabolites were observed, the *log2FC* values are close to zero (p >0.05 for all), which confirms the absence of a cardiotoxic effect of this compound. In contrast, cardiotoxic substances caused significant changes in a number of markers. Based on the data obtained, it is possible to identify characteristic patterns of metabolomic changes. Thus, acetaminophen (at a maximum dose of ≈10×NOEC) led to a moderate increase in total BCAA and the ADMA / arginine ratio. Carbamazepine caused the most pronounced increase in ADMA (*log2FC*≈2.94) and the (ADMA / arginine) index — these shifts indicate a violation of the nitric oxide (NO) synthesis pathway and endothelial dysfunction under the action of carbamazepine. Salbutamol (β2-adrenomimetic), on the contrary, sharply reduced the level of L-arginine (–1.832) and ADMA (–1.409), but with an increase in the ADMA / arginine index. Salbutamol is also characterized by the highest increase in BCAA (log2FC≈1.38) with a decrease in acetylcarnitine (C2) and free carnitine (C0), which indicates an increase in carbohydrate catabolism and carnitine consumption. Ketorolac (NSAID) demonstrated the most significant accumulation of BCAA (*log2FC*≈2.18, p=0.001), which may indicate a developing energy imbalance and osmotic stress in cardiomyocytes. Bisoprolol and metoprolol (selective β1-adrenoblockers) caused similar metabolic shifts characteristic of a state of suppression of cardiac function: moderate increase in BCAA (≈+0.59 and +0.89), significant increase in ADMA (+1.32 and +0.61) against the background of a decrease in L-arginine (–1.25 and –1.14) — signs of endothelial dysfunction and a decrease in NO synthesis.

Figures 2–5 show the dose-dependent changes in the concentration of some markers. For example, for BCAA (Fig. 2), it is clear that cardiotoxic substances led to an increasing (with dose) increase in the total concentration. The increase in BCAA is especially noticeable when exposed to ketorolac and salbutamol — their curves increased sharply, reaching a 3–4-fold excess of the control at maximum drug concentrations. The ADMA / arginine ratio (Fig. 3) demonstrated differences in behavior: for carbamazepine, the ADMA / arginine ratio was proportional to the dose (an indicator of NO synthase suppression), whereas for ketorolac and salbutamol, the ratio increased sharply with the addition of a minimum amount of the drug (0.5×NOEC), and then did not change. The methionine sulfoxide/methionine ratio (Fig. 4) remained unchanged under the action of cetirizine and salbutamol (the latter even slightly reduced it), but increased with increasing concentrations of β1-blockers and ketorolac, reflecting an increase in oxidative stress in the heart tissue. Finally, the (C16+C18) / C0 ratio (Fig. 5) — an indicator of the accumulation of long-chain acylcarnitines — practically did not change under the action of acetaminophen, carbamazepine and salbutamol, but increased sharply for bisoprolol and metoprolol when transitioning to high concentrations. These graphical dependencies make the statistical data complete, confirming the presence of characteristic metabolic "fingerprints" in different mechanisms of CT.

DISCUSSION

The metabolomic profile of Danio rerio changed after exposure to cardiotoxic drugs, mimicking the metabolic consequences for the heart in mammals. According to literature data, an increase in the concentration of BCAA in human blood plasma is associated with a deterioration in myocardial contractility and a worsening prognosis in heart failure [9], and the accumulation of long-chain acylcarnitines correlates with the risk of developing arrhythmias and adverse outcomes in cardiomyopathies [26]. These data are also contain the obtained results: medicines known to cause energy deficiency in the myocardium (ketorolac,

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-2-70-83

β-blockers) [27] caused the greatest increase in BCAA and acylcarnitine levels, reflecting the inhibition of the tricarboxylic acid cycle and β-oxidation of fatty acids in cardiac tissue. A likely mechanism was the overload of mitochondria with fatty acids while simultaneously inhibiting their oxidation — in the case of β-blockers, due to a decrease in ATP demand against the background of bradycardia and a decrease in coronary blood flow, in the case of ketorolac, due to the suppression of mitochondrial biogenesis in chronic inflammation. This imbalance led to the accumulation of long-chain acylcarnitines, which can disrupt the functioning of ion channels and provoke arrhythmogenic effects [11].

Another important factor of CT is ED due to impaired NO signaling system [8, 9]. A Significant increase in ADMA (NO synthase inhibitor) levels under the influence of a number of medicines was observed, especially carbamazepine and β-blockers. It is known that ADMA competitively displaces L-arginine from the active center of endothelial NO synthase, reducing NO production and causing vasoconstriction and tissue ischemia [12]. An increase in ADMA concentration in plasma is considered a risk factor for ED and cardiovascular disorders [12]. In our studies, the increase in ADMA was accompanied by an increase in the ADMA / Arg index and a decrease in L-arginine levels (for example, with metoprolol), which indicates a possible depletion of NO-dependent vasodilation.

Salbutamol reduced the concentration of ADMA, probably by stimulating NO synthesis (adrenaline and β2-agonists increase eNOS activity [28, 29]). However, at the same time, a sharp drop in the level of L-arginine was observed, which together led to an imbalance in ADMA / Arg. Nevertheless, salbutamol increased the level of citrulline (a by-product of eNOS), which may indicate activation of NO synthesis; the decrease in arginine is probably associated with its redistribution to other pathways (for example, the ornithine cycle or creatine synthesis). In general, the data demonstrated that various mechanisms (an increase in ADMA, a deficiency of L-arginine, or their combination) contribute to a decrease in NO bioavailability under the action of toxic doses of medicines, which potentially leads to a deterioration in myocardial perfusion and increases its vulnerability to ischemia and arrhythmias [12].

Disorders of nitrogen metabolism of amino acids under the influence of toxicants also manifested in the accumulation of aromatic amino acids — phenylalanine, tyrosine, tryptophan — in almost all exposures. An increase in phenylalanine levels and a decrease in the tyrosine/phenylalanine ratio are typical for conditions of heart failure and may indicate a decrease in the activity of phenylalanine hydroxylase in the liver (for example, in hypoxia) [30]. The tyrosine/phenylalanine ratio increased significantly under the influence of β-blockers, which can be interpreted as enhanced conversion of phenylalanine to tyrosine in situ or increased breakdown of catecholamines (forming tyrosine) during prolonged β-blockade. In any case, the accumulation of phenylalanine — a marker of liver or kidney dysfunction — possibly reflects the systemic effect of medicines on the entire body, even with short exposure. Similarly, an increase in tryptophan may be associated with suppression of its metabolism via the kynurenine pathway, which is often observed in stressful conditions and inflammation.

OS is recognized as a key link in CT of many medicines [31], and in this study, it was observed under the influence of β-blockers — a significant accumulation of methionine sulfoxide indicates increased formation of reactive oxygen species (ROS) and oxidation of methionine. This is consistent with clinical data that overdose of selective β1-adrenoblockers leads to cardiogenic shock with tissue ischemia, provoking the release of free radicals and lipid oxidation [27]. Acetaminophen generates reactive metabolites in the liver [32, 33]; in the heart, its pro-oxidant effect was weak (there was only a tendency to decrease the level of methionine sulfoxide), possibly due to compensatory activation of antioxidant enzymes. Unexpectedly, a decrease in oxidation markers was observed with salbutamol — this phenomenon may be explained by the fact that acute β-adrenergic exposure activates protective pathways (for example, an influx of Ca2+ can stimulate the synthesis of NO, which has antioxidant properties, and also include the expression of NRF2-dependent antioxidant defense genes). It is more likely that salbutamol simply does not cause such a pronounced OS, since increased blood flow and metabolism in conditions of β-stimulation do not allow reduced equivalents to accumulate (maintaining a high level of NAD^+ / NADH). In turn, ketorolac moderately increased the concentration of methionine sulfoxide and taurine, which is consistent with data on nephropathies and cardiomyopathies caused by NSAIDs through the formation of ROS and depletion of cellular antioxidants [34].

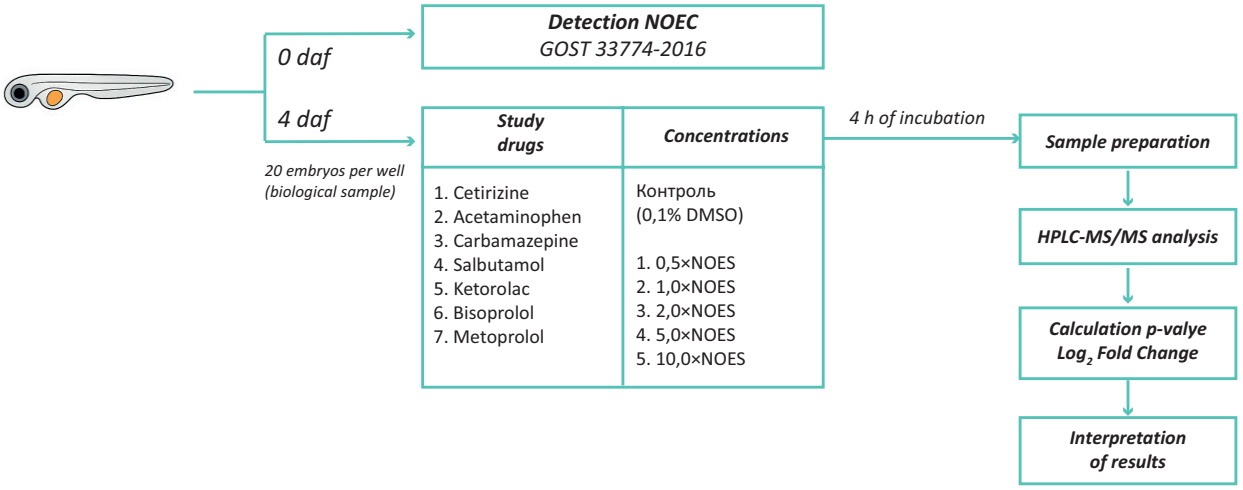

Figure 1 – Study design.

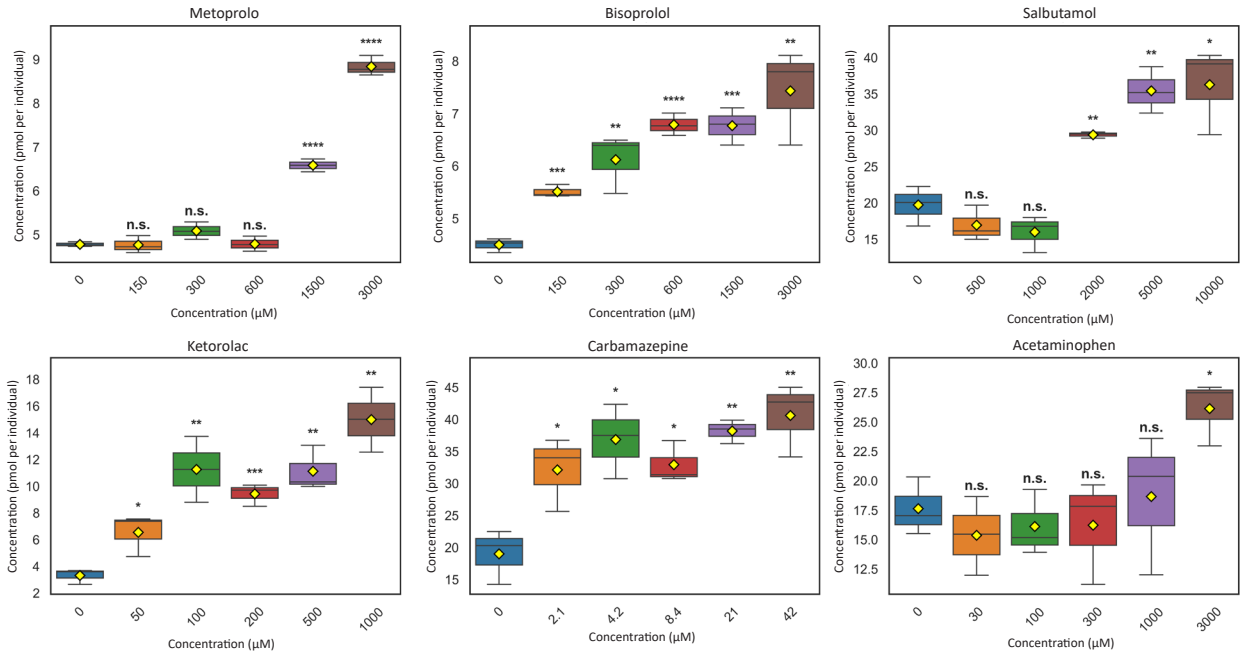

Figure 2 – Dependence of changes in the level of BCAA (pmol/individual) in Danio rerio embryos on the concentration of the studied drugs (μM).

Note: The median value is marked with a yellow rhombus, n.s. — p >0.05, * — p <0.05, ** — p <0.01, *** — p <0.001, **** — p <0.0001.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-2-70-83

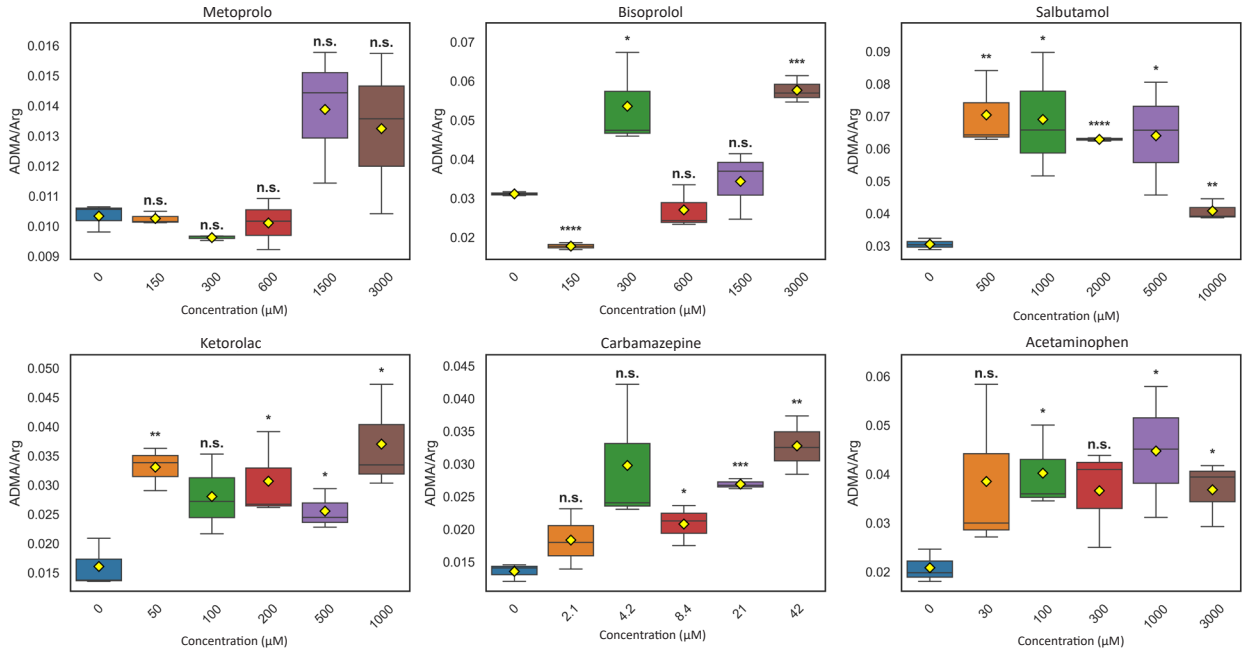

Figure 3 – Dependence of the ratio of ADMA / arginine concentrations in Danio rerio embryos on the concentration of the studied drugs (μM).

Note: The median value is marked with a yellow rhombus, n.s. — $p > 0.05$, * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, **** — $p < 0.0001$.

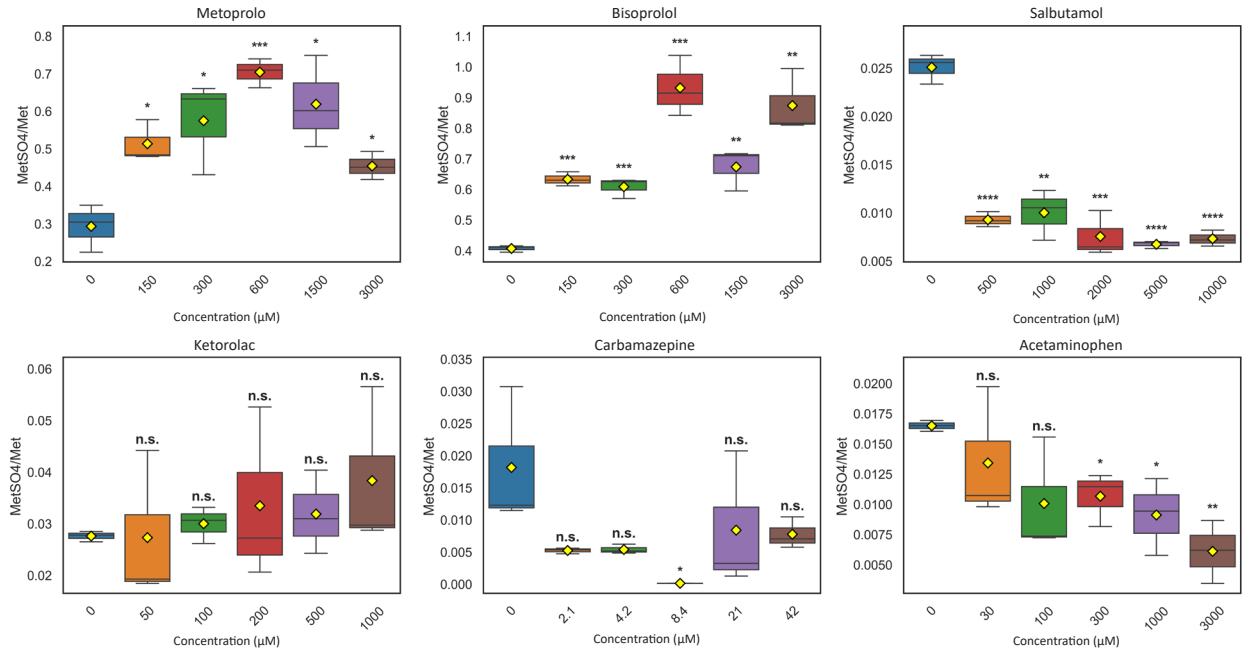

Figure 4 – Dependence of the ratio of methionine sulfoxide (MetSO4)/methionine concentrations in Danio rerio embryos on the concentration of the studied drugs (μM).

Note: The median value is marked with a yellow rhombus, n.s. — $p > 0.05$, * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, **** — $p < 0.0001$.

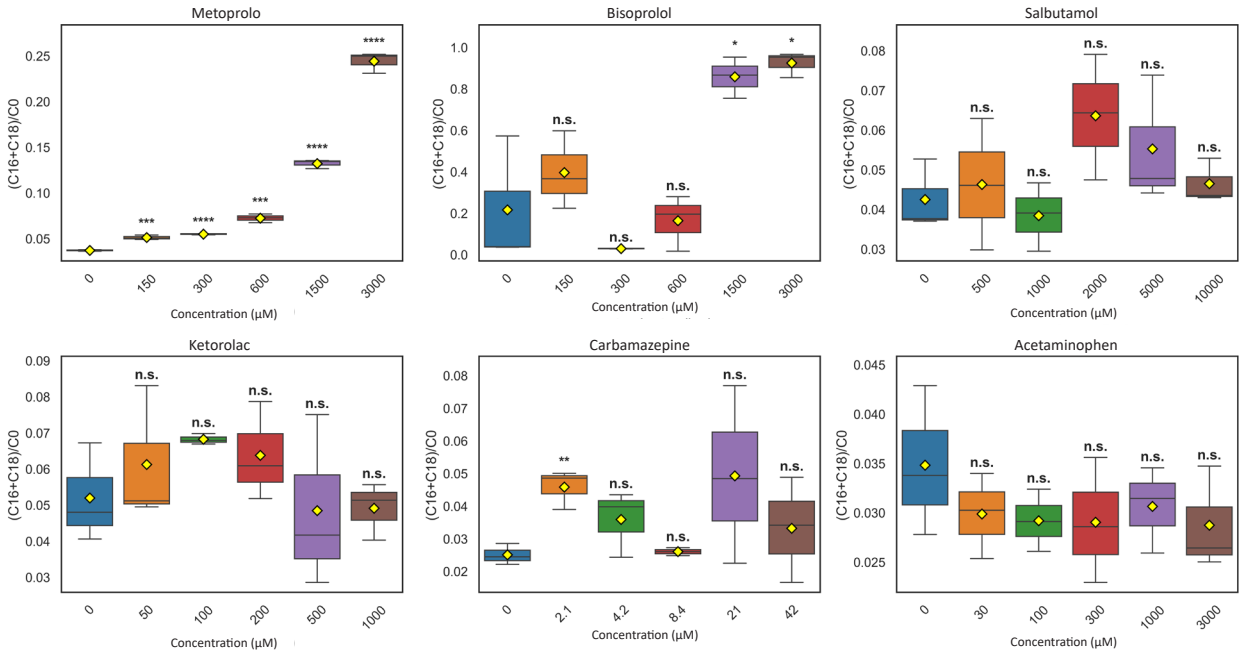

Figure 5 – Dependence of the ratio of (C16+C18)/C0 concentrations in Danio rerio embryos on the concentration of the studied drugs (μM).

Note: The median value is marked with a yellow rhombus, n.s. — $p > 0.05$, * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, **** — $p < 0.0001$.

Table 1 – Studied substances

Studied substances	Main targets / mechanism of action	Main manifestation of cardiotoxicity[1]	NOEC, μM	Concentrations, μM
Acetaminophen	COX–1/2 inhibitor	Sudden cardiac death (in overdose)	300	150, 300, 600, 1500, 3000
Carbamazepine	Na–channel blocker, enhancement of GABA–ergic transmission	Heart rhythm disorders	4,2	2,1, 4,2, 8,4, 21, 42
Ketorolac tromethamine	COX–2 inhibitor	Heart failure (chronically)	100	50, 100, 200, 500, 1000
Metoprolol (tartrate)	β1–adrenoblocker	Acute myocardial infarction (overdose)	300	150, 300, 600, 1500, 3000
Bisoprolol (fumarate)	β1–adrenoblocker	Heart failure (in high doses)	300	150, 300, 600, 1500, 3000
Salbutamol	β2–adrenomimetic	Tachyarrhythmia (in overdose)	1000	500, 1000, 2000, 5000, 10000
Cetirizine hydrochloride	H1–histamine receptor blocker	No cardiotoxicity	5	2,5, 5, 10, 25, 50

Note: COX — cyclooxygenase; GABA — gamma–aminobutyric acid.

[1] FDA's Adverse Event Reporting System (FAERS). – [Электронный ресурс]. – Режим доступа: https://www.fda.gov/drugs/surveillance/fdas–adverse–event–reporting–system–faers

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-2-70-83

Table 2 – *Log2 FC* values for measured metabolites (experiment *vs* control) under the influence of different compounds, when incubated at a concentration of 10×NOEC

Metabolite / Index	Cetirizine	Acetaminophen	Carbamazepine	Salbutamol	Ketorolac	Bisoprolol	Metoprolol
Branched–chain amino acids (BCAA)							
BCAA (Leucine + isoleucine + valine)	–0.233 (0.080)	0.568 (0.016)	**1.096 (0.006)**	**1.379 (0.001)**	**2.181 (0.001)**	0.590 (<0.001)	0.887 (<0.001)
Σ(Leucine + isoleucine)	–0.047 (0.732)	0.615 (0.030)	**1.359 (0.001)**	**1.113 (0.005)**	**2.522 (0.003)**	0.450 (0.002)	0.885 (<0.001)
Isoleucine	–0.216 (0.068)	0.521 (0.063)	0.753 (0.291)	**1.694 (<0.001)**	1.900 (0.012)	0.717 (0.001)	0.889 (<0.001)
Endothelial dysfunction, NO pathway							
Fisher index	–0.182 (0.080)	–0.428 (0.021)	–0.061 (0.841)	**1.164 (0.006)**	1.048 (0.013)	–0.868 (0.003)	–0.060 (0.145)
ADMA	–0.264 (0.255)	0.892 (0.061)	**2.943 (<0.001)**	**–1.409 (<0.001)**	–0.002 (0.996)	**1.318 (0.008)**	0.608 (0.037)
ADMA / Arg	0.280 (0.076)	0.821 (0.021)	**1.273 (0.002)**	0.418 (0.009)	1.204 (0.022)	0.141 (0.560)	0.357 (0.138)
Arginine	0.013 (0.913)	–0.246 (0.499)	**1.637 (0.003)**	**–1.832 (<0.01)**	–1.25 (0.012)	**1.190 (<0.01)**	**–1.137 (<0.01)**
Citrulline / Arg	0.529 (0.029)	0.144 (0.533)	**–0.937 (<0.001)**	**1.655 (0.003)**	1.245 (0.024)	–0.929 (0.263)	0.313 (0.597)
Homoarginine / ADMA	0.214 (0.599)	–0.803 (0.018)	–0.878 (0.058)	–0.390 (0.032)	–0.518 (0.339)	–0.279 (0.297)	–0.514 (0<048)
Homoarginine / SDMA	0.575(0.161)	0.284 (0.381)	1.405 (0.150)	–0.390 (0.018)	–0.371 (0.380)	0.974 (0.003)	–0.037 (0.615)
Homoarginine	–0.090 (0.820)	–0.252 (0.473)	**1.191 (0.002)**	**–1.809 (0.001)**	0.549 (0.020)	0.977 (0.001)	0.060 (0.388)
SDMA	**–0.648 (0.013)**	0.559 (0.058)	1.032 (0.249)	–1.422 (0.007)	**–0.150 (0.565)**	0.007 (0.828)	0.097 (<0.001)
GABR	0.006 (0.952)	–0.260 (0.281)	0.780 (<0.001)	**–1.345 (0.001)**	–0.823 (0.068)	**1.256 (<0.001)**	0.578 (0.010)
Oxidative stress							
Methionine	–0.842 (0.012)	0.500 (0.023)	0.825 (0.270)	0.207(–0.202)	0.556 (0.165)	–0.017 (0.856)	0.759 (<0.001)
Methionine sulfoxide	**–1.205 (0.001)**	–0.877 (0.055)	–0.279 (0.403)	**–1.579 (<0.001)**	0.927 (0.006)	0.700 (<0.001)	**1.389 (0.001)**
Methionine sulfoxide / Met	–0.352 (0.102)	**–1.434 (0.002)**	0.011 (0.963)	**–1.776 (<0.001)**	0.474 (0.304)	0.727 (0.003)	0.631 (0.019)
Glutamine	–0.998 (0.005)	–0.661 (0.035)	0.280 (0.418)	–0.733 (0.005)	0.038 (0.897)	**1.502 (0.003)**	–0.875 (0.001)
Glutamate	–0.441 (0.028)	–0674 (0.037)	0.011 (0.963)	–0.733 (0.005)	0.277 (0.453)	1.829 (0.016)	–0.815 (0.002)
Glutamine / Glut	–0.569 (0.044)	–0.027 (0.922)	0.213 (0.386)	–1.662 (0.003)	–0.192 (0.423)	–0.176 (0.666)	–0.060 (0.158
GSG	–0.433 (0.222)	–0.055 (0.466)	–0.233 (0.542)	**1.448 (0.007)**	0.251 (0.013)	1.109 (0.043)	0.834 (0.025)
Taurine	0.094 (0.475)	–0.321 (0.124)	0.017 (0.930)	–0.339 (0.224)	0.644 (0.016)	0.056 (0.657)	0.693 (0.002)
Mitochondrial dysfunction							
C0	0.454 (0.241)	–0.735 (0.01)	0.080 (0.329)	0.442 (0.081)	–0.394 (0.203)	**–1.243 (<0.01)**	**–1.866 (<0.01)**
C2	1.267 (0.049)	–0.607 (0.064)	**1.258 (0.004)**	0.322 (0.226)	0.429 (0.030)	1.366 (0.133)	**1.901 (<0.001)**
(C2+C3) / C0	1.009 (0.015)	–0.501 (0.090)	**1.205 (0.001)**	0.060 (0.830)	0.735 (0.007)	1.603 (0.076)	**2.394 (<0.001)**
C2 / C0	1.510 (0.108)	–0.748 (0.051)	1.347 (0.016)	0.623 (0.059)	–0.003 (0.992)	1.154 (0.215)	**1.304 (<0.001)**
(C16+C18) / C0	0.164 (0.420)	0.826 (0.021)	1.438 (0.056)	0.327 (0.014)	0.282 (0.122)	**3.809 (0.009)**	**1.268 (<0.01)**
Lipid metabolism							
C18:1 / C18	0.454 (0.241)	–0.735 (0.01)	0.080 (0.329)	0.442 (0.081)	–0.394 (0.203)	–1.243 (<0.01)	–1866 (<0.001)
(C16+C18:1) / C2	1.267 (0.049)	–0.607 (0.064)	**1.258 (0.004)**	0.322 (0.226)	0.429 (0.030)	1.366 (0.133)	**1.901 (<0.001)**
C16 / C0	1.009 (0.015)	–0.501 (0.090)	**1.205 (0.001)**	0.060 (0.830)	0.735 (0.007)	1.603 (0.076)	**2.394 (<0.001)**
C18:1 / C0	1.510 (0.108)	–0.748 (0.051)	1.347 (0.016)	0.623 (0.059)	–0.003 (0.992)	1.154 (0.215)	**1.304 (<0.001)**
Σ(acylcarnitines)/C0	0.164 (0.420)	0.826 (0.021)	1.438 (0.056)	–0.327 (0.014)	–0.282 (0.122)	**3.809 (0.009)**	**–1.268 (<0.01)**
C3 / C2	0.637 (0.067)	0.372 (0.062)	0.115 (0.781)	0.263 (0.233)	–0.188 (0.344)	–0.463 (0.063)	0.355 (<0.001)
C4 / C2	0.243 (0.290)	–0.020 (0.958)	0.348 (0.228)	0.221 (0.160)	–0.871 (0.002)	–0.625 (0.008)	**3.594 (<0.001)**
C8 / C10	–0.595 (0.082)	–0.414 (0.075)	0.190 (0.684)	0.493 (0.139)	0.248 (0.423)	0.552 (0.373)	–1.881 (0.331)
Σ(C2–C5)	–0.446 (0.007)	–0.022 (0.879)	0.381 (0.058)	–0.406 (0.043)	–0.001 (0.997)	**1.367 (0.008)**	**1.095 (<0.001)**
Σ(C6–C12)	–0.295 (0.011)	**–1.731 (0.001)**	0.295 (0.435)	0.138 (0.070)	–0.023 (0.873)	0.023 (0.876)	–0.120 (0.085)
Σ(C14–C18)	0.066 (0.247)	–0.937 (0.005)	0.523 (0.029)	0.488 (<0.001)	0.137 (0.345)	–0.352 (0.001)	**1.547 (<0.001)**
Aromatic amino acids							
Phenylalanine	–0.031 (0.633)	**1.115 (<0.01)**	**1.157 (<0.01)**	0.027 (0.887)	**1.129 (<0.01)**	**0.559 (<0.01)**	**0.617 (<0.01)**
Threonine	–0.501 (0.011)	0.043 (0.816)	–0.161 (0.232)	–0.546 (0.022)	–0.050 (0.855)	**1.128 (<0.01)**	**2.561 (<0.01)**
Tryptophan	–0.520 (0.047)	0.695 (0.015)	0.454 (0.623)	0.104 (0.443)	0.877 (0.026)	1.684 (<0.01)	1.032 (<0.001)
Tyrosine	0.302 (0.120)	1.078 (0.021)	1.306 (**<0.01**)	0.429 (0.033)	**1.287 (<0.01)**	**2.312 (<0.01)**	**1.364 (<0.01)**
Tyrosine / Phenylalanine (Tyr / Phe)	0.340 (0.170)	0.025 (0.715)	–0.025 (0.963)	0.149 (0.502)	0.105 (0.750)	1.758 (0.001)	0.738 (0.006)

Note: Data are presented as *log2 FC* (*p*–value). Changes with |*log2FC*| >1 at *p* <0.01 are highlighted in bold. C0 — carnitine; C2 — acetylcarnitine; C3 — propionylcarnitine; C4 — butyrylcarnitine; C5 — isovalerylcarnitine; C6 — hexanoylcarnitine; C8 — octanoylcarnitine; C10 — decanoylcarnitine; C12 — dodecanoylcarnitine; C14 — tetradecanoylcarnitine; C16 — hexadecanoylcarnitine; C18 — stearoylcarnitine; C18:1 — linoleylcarnitine

Table 3 – Relative contribution of the studied drugs according to three criteria (mitochondrial dysfunction, oxidative stress, impaired NO signaling pathway)

Substance	Mitochondrial dysfunction	Oxidative stress	Impaired NO signaling pathway
Cetirizine	–	–	–
Acetaminophen	+	–	+
Carbamazepine	+	–	+++
Salbutamol	+	–	++
Ketorolac	+++	++	++
Bisoprolol	+++	+++	+++
Metoprolol	+++	+++	+++

Note: "+++" — maximum, "++" — strong, "+" — moderate, "–" — no contribution.

Table 3 shows a comparative assessment of the contribution of each of the studied medicines to the development of key mechanisms of cardiotoxicity — mitochondrial dysfunction, oxidative stress, and disorders of the NO signaling pathway.

As it can be seen from Table 3, the most significant mitochondrial dysfunction was observed under the action of β1-adrenoblockers (bisoprolol, metoprolol) and ketorolac — these drugs caused the maximum accumulation of acylcarnitines and related indicators. Regarding OS, the same β1-adrenoblockers are in the lead (significant increase in methionine sulfoxide), ketorolac makes a slightly smaller contribution, while acetaminophen and salbutamol practically did not cause pro-oxidant effects. Impairment of the NO signaling pathway is most pronounced in carbamazepine (maximum increase in ADMA) and β1-adrenoblockers (significant increase in ADMA with a simultaneous decrease in arginine); ketorolac, acetaminophen, and salbutamol had a moderate effect on this pathway. Thus, systemic metabolomic analysis revealed a complex of interrelated mechanisms of CT in zebrafish embryos after exposure to drugs. The accumulation of ADMA and acylcarnitines in cardiac tissue reflects an energy imbalance.

Model adequacy

The use of zebrafish embryos to assess the cardiometabolic effects of medicines is justified by the high conservatism of the main metabolic and signaling pathways in fish and mammals. Despite some quantitative differences (for example, the intensity of oxidative metabolism in fish embryos is lower than in the heart of adult mammals), qualitative shifts in the metabolomic profile under the influence of toxicants demonstrate good reproducibility of patterns known in higher organisms. The data are consistent with the results of other studies. Thus, earlier cardiotoxic effects were associated with an increase in the level of BCAA and aromatic amino acids associated with metabolic overload of the heart [9, 35], as well as with the accumulation of long acylcarnitines as a precursor to the development of myocardial dysfunction [36, 37]. It is important to emphasize that zebrafish as an experimental model allows tracking these changes at early stages, even before the appearance of irreversible morphological disorders, which opens up the possibility of their use in screening for cardiotoxicity of new compounds.

Limitations of the study

The limitations of this study include the relatively short exposure time (4 h), which simulates acute effects but does not reflect possible compensatory-adaptive reactions with prolonged exposure. For example, with chronic administration of β-blockers, the mammalian body adapts by increasing the density of β-receptors, improving coronary blood flow, which softens metabolic shifts — in fish, similar mechanisms can also reduce toxicity. In addition, the concentrations of drugs we used (up to 10×NOEC) are high enough to detect sublethal effects; in a real clinical situation, such doses correspond to overdoses. However, it is in conditions of overdose that the mechanisms of toxicity manifest themselves "in their pure form", which was required to be demonstrated. In addition, it wasn't taken into account gender differences and the influence of hormonal background (embryos before sexual differentiation).

CONCLUSION

The study showed that metabolomics of Danio rerio embryos reflected the effects of cardiotoxic medicines, revealing specific changes in metabolic biomarkers. In particular, medicines with a risk of arrhythmia (salbutamol, carbamazepine) are characterized by the accumulation of BCAA and an imbalance of NO / ADMA; for cardiodepressants (selective β1-adrenoblockers) — pronounced Mitochondrial dysfunction with the accumulation of acylcarnitines and oxidative stress; for non-steroidal anti-inflammatory drugs (ketorolac) — a combination of moderately pronounced energy and oxidative shifts. At the same time, acetaminophen caused only minor metabolic changes, and the control antihistamine medicine cetirizine did not affect the parameters under consideration, which confirms the absence of cardiotoxicity in this model.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-2-70-83

FUNDING

The work was performed within the framework of the state assignment No. 124031100079-6. Research and development objective: "Development of digital models for predicting drug organotoxicity based on data from metabolomic analysis using an alternative biological model."

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Natalia E. Moskaleva — conducting the experimental part of the work, statistical processing of the results, preparation of a preliminary version of the manuscript; Viktor M. Samoylov — conducting the experimental part of the work, reviewing literary sources; Pavel M. Rezvanov — conducting the experimental part of the work, statistical processing of the results; Valeria G. Varzieva — conducting the experimental part of the work; Sabina N. Baskhanova — statistical processing of the results; Vadim V. Tarasov — approval of the final version of the manuscript; Elena A. Smolyarchuk — analysis of the literature sources and the results obtained in the work; Dmitry A. Kudlay — analysis of the results of the work with their interpretation and conclusions, approval of the final version of the manuscript; Svetlana A. Appolonova — development of the research concept, approval of the final version of the manuscript. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication).

REFERENCES

1. Onakpoya IJ, Heneghan CJ, Aronson JK. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. BMC Medicine. 2016;14:10. DOI: 10.1186/s12916-016-0553-2
2. Ferdinandy P, Baczkó I, Bencsik P, Giricz Z, Görbe A, Pacher P, Varga ZV, Varró A, Schulz R. Definition of hidden drug cardiotoxicity: paradigm change in cardiac safety testing and its clinical implications. Eur Heart J. 2018;40(22):1771–7. DOI: 10.1093/eurheartj/ehy365
3. Ussher JR, Elmariah S, Gerszten RE, Dyck JRB. The Emerging Role of Metabolomics in the Diagnosis and Prognosis of Cardiovascular Disease. J Am College Cardiolog. 2016;68(25):2850–70. DOI: 10.1016/j.jacc.2016.09.972
4. Singh A, Bakhtyar M, Jun SR, Boerma M, Lan RS, Su LJ, Makhoul S, Hsu PC. A narrative review of metabolomics approaches in identifying biomarkers of doxorubicin-induced cardiotoxicity. Metabolomics. 2025;21:68. DOI: 10.1007/s11306-025-02258-8
5. Rhee EP, Gerszten RE. Metabolomics and Cardiovascular Biomarker Discovery. Clin Chem. 2011;58(1):139–47. DOI: 10.1373/clinchem.2011.169573
6. Holeček M. Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements. Nutrition & Metabolism. 2018;15:33. DOI: 10.1186/s12986-018-0271-1
7. Gao C, Hou L. Branched chain amino acids metabolism in heart failure. Front Nutrition. 2023;10:1279066. DOI: 10.3389/fnut.2023.1279066
8. Kozhevnikova MV, Belenkov YN, Shestakova KM, Ageev AA, Markin PA, Kakotkina AV, Korobkova EO, Moskaleva NE, Kuznetsov IV, Khabarova NV, Kukharenko AV, Appolonova SA. Metabolomic profiling in heart failure as a new tool for diagnosis and phenotyping. Sci Rep. 2025;15(1):11849. DOI: 10.1038/s41598-025-95553-2
9. Kozhevnikova MV, Kakotkina AV, Korobkova EO, Kuznetsov IV, Shestakova KM, Moskaleva NE, Appolonova SA, Belenkov YN. Metabolomic Panel for the Diagnosis of Heart Failure with Preserved Ejection Fraction. Int J Mol Sci. 2025;26:2102. DOI: 10.3390/ijms26052102
10. Aitken-Buck HM, Krause J, Zeller T, Jones PP, Lamberts RR. Long-Chain Acylcarnitines and Cardiac Excitation-Contraction Coupling: Links to Arrhythmias. Front Physiology. 2020;11:577856. DOI: 10.3389/fphys.2020.577856
11. Kukharenko A, Brito A, Kozhevnikova MV, Moskaleva N, Markin PA, Bochkareva N, Korobkova EO, Belenkov YN, Privalova EV, Larcova EV, Ariani A, La Frano MR, Appolonova SA. Relationship between the plasma acylcarnitine profile and cardiometabolic risk factors in adults diagnosed with cardiovascular diseases. Clin Chim Acta. 2020;507:250–6. DOI: 10.1016/j.cca.2020.04.035
12. Sibal L, Agarwal SC, Home PD, Boger RH. The Role of Asymmetric Dimethylarginine (ADMA) in Endothelial Dysfunction and Cardiovascular Disease. Curr Cardiology Rev. 2010;6(2):82–90. DOI: 10.2174/157340310791162659
13. Markin SS, Ponomarenko EA, Romashova YA, Pleshakova TO, Ivanov SV, Bedretdinov FN, Konstantinov SL, Nizov AA, Koledinskii AG, Girivenko AI, Shestakova KM, Markin PA, Moskaleva NE, Kozhevnikova MV, Chefranova ZhYu, Appolonova SA. A novel preliminary metabolomic panel for IHD diagnostics and pathogenesis. Sci Rep. 2024;14:2651. DOI: 10.1038/s41598-024-53215-9
14. Brito-Martins M, Harding SE, Ali NN. beta(1)- and beta(2)-adrenoceptor responses in cardiomyocytes derived from human embryonic stem cells: comparison with failing and non-failing adult human heart. Br J Pharmacol. 2008;153(4):751–9. DOI: 10.1038/sj.bjp.0707619
15. Asnani A, Moslehi JJ, Adhikari BB, Baik AH, Beyer AM, de Boer RA, Ghigo A, Grumbach IM, Jain S, Zhu H; American Heart Association Council on Basic Cardiovascular Sciences; Cardio-Oncology Science Subcommittee of Council on Genomic and Precision Medicine and

Council on Clinical Cardiology; Council on Peripheral Vascular Disease; and Council on Arteriosclerosis, Thrombosis and Vascular Biology. Preclinical Models of Cancer Therapy-Associated Cardiovascular Toxicity: A Scientific Statement From the American Heart Association. Circ Res. 2021;129(1):e21–e34. DOI: 10.1161/RES.0000000000000473

16. Wang W, Gao X, Liu L, Guo S, Duan JA, Xiao P. Zebrafish as a Vertebrate Model for High-Throughput Drug Toxicity Screening: Mechanisms, Novel Techniques, and Future Perspectives. J Pharm Analysis. 2025:101195. DOI: 10.1016/j.jpha.2025.101195
17. Markin PA, Brito A, Moskaleva NE, Tagliaro F, La Frano MR, Savitskii MV, Appolonova SA. Short- and long-term exposures of the synthetic cannabinoid 5F-APINAC induce metabolomic alterations associated with neurotransmitter systems and embryotoxicity confirmed by teratogenicity in zebrafish. Comp Biochem Physiol C Toxicol Pharmacol. 2021;243:109000. DOI: 10.1016/j.cbpc.2021.109000
18. Markin PA, Brito A, Moskaleva NE, Tagliaro F, Tarasov VV, La Frano MR, Savitskii MV, Appolonova SA. Short- and medium-term exposures of diazepam induce metabolomic alterations associated with the serotonergic, dopaminergic, adrenergic and aspartic acid neurotransmitter systems in zebrafish (Danio rerio) embryos/larvae. Comp Biochem Physiol Part D Genomics Proteomics. 2021;38:100816. DOI: 10.1016/j.cbd.2021.100816
19. Dyballa S, Miñana R, Rubio-Brotons M, Cornet C, Pederzani T, Escaramis G, Garcia-Serna R, Mestres J, Terriente J. Comparison of Zebrafish Larvae and hiPSC Cardiomyocytes for Predicting Drug-Induced Cardiotoxicity in Humans. Toxicol Sci. 2019;171(2):283–95. DOI: 10.1093/toxsci/kfz165
20. Zakaria ZZ, Benslimane FM, Nasrallah GK, Shurbaji S, Younes NN, Mraiche F, Da'as SI, Yalcin HC. Using Zebrafish for Investigating the Molecular Mechanisms of Drug-Induced Cardiotoxicity. Biomed Res Int. 2018;2018:1642684. DOI: 10.1155/2018/1642684
21. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2001;98(9):5116–21. DOI: 10.1073/pnas.091062498
22. Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, Ochoa Gautier JB. Acquired Amino Acid Deficiencies: A Focus on Arginine and Glutamine. Nutr Clin Pract. 2017;32(1_suppl):30S–47S. DOI: 10.1177/0884533617691250
23. Rees CA, Rostad CA, Mantus G, Anderson EJ, Chahroudi A, Jaggi P, Wrammert J, Ochoa JB, Ochoa A, Basu RK, Heilman S, Harris F, Lapp SA, Hussaini L, Vos MB, Brown LA, Morris CR. Altered amino acid profile in patients with SARS-CoV-2 infection. Proc Natl Acad Sci U S A. 2021;118(25):e2101708118. DOI: 10.1073/pnas.2101708118
24. Masoodi M, Gastaldelli A, Hyötyläinen T, Arretxe E, Alonso C, Gaggini M, Brosnan J, Anstee QM, Millet O, Ortiz P, Mato JM, Dufour JF, Orešič M. Metabolomics and lipidomics in NAFLD: biomarkers and non-invasive diagnostic tests. Nat Rev Gastroenterol Hepatol. 2021;18(12):835–56. DOI: 10.1038/s41575-021-00502-9
25. Leonetti S, Herzog RI, Caprio S, Santoro N, Tricò D. Glutamate-Serine-Glycine Index: A Novel Potential Biomarker in Pediatric Non-Alcoholic Fatty Liver Disease. Children. 2020;7(12):270. DOI: 10.3390/children7120270
26. Da Silva KM, Iturrospe E, Bars C, Knapen D, Van Cruchten S, Covaci A, Van Nuijs ALN. Mass spectrometry-based zebrafish toxicometabolomics: a review of analytical and data quality challenges. Metabolites. 2021;11(9):635. DOI: 10.3390/metabo11090635
27. Moskaleva NE, Shestakova KM, Kukharenko AV, Markin PA, Kozhevnikova MV, Korobkova EO, Brito A, Baskhanova SN, Mesonzhnik NV, Belenkov YN, Pyatigorskaya NV, Tobolkina E, Rudaz S, Appolonova SA. Target Metabolome Profiling-Based Machine Learning as a Diagnostic Approach for Cardiovascular Diseases in Adults. Metabolites. 2022;12(12):1185. DOI: 10.3390/metabo12121185
28. Drygała S, Radzikowski M, Maciejczyk M. β-blockers and metabolic modulation: unraveling the complex interplay with glucose metabolism, inflammation and oxidative stress. Front Pharmacol. 2024;15:1489657. DOI: 10.3389/fphar.2024.1489657
29. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;33(7):829–837, 837a–837d. DOI: 10.1093/eurheartj/ehr30
30. Adding LC, Agvald P, Artlich A, Persson MG, Gustafsson LE. Beta-adrenoceptor agonist stimulation of pulmonary nitric oxide production in the rabbit. Br J Pharmacol. 1999;126(3):833–9. DOI: 10.1038/sj.bjp.0702369
31. Cheng ML, Wang CH, Shiao MS, Liu MH, Huang YY, Huang CY, Mao CT, Lin JF, Ho HY, Yang NI. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: diagnostic and prognostic value of metabolomics. J Am Coll Cardiol. 2015;65(15):1509–20. DOI: 10.1016/j.jacc.2015.02.018
32. Sabbatino F, Conti V, Liguori L, Polcaro G, Corbi G, Manzo V, Tortora V, Carlomagno C, Vecchione C, Filippelli A, Pepe S. Molecules and Mechanisms to Overcome Oxidative Stress Inducing Cardiovascular Disease in Cancer Patients. Life (Basel). 2021;11(2):105. DOI: 10.3390/life11020105
33. Ramachandran A, Duan L, Akakpo JY, Jaeschke H. Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives. J Clin Translat Res. 2018;4(1):75–100. DOI: 10.18053/jctres.04.201801.005
34. Bashir S, Morgan WA. Inhibition of mitochondrial function: An alternative explanation for the antipyretic and hypothermic actions of acetaminophen. Life Sci. 2023;312:121194. DOI: 10.1016/j.lfs.2022.121194
35. Hosohata K. Role of Oxidative Stress in Drug-Induced Kidney Injury. Int J Mol Sci. 2016;17(11):1826. DOI: 10.3390/ijms17111826
36. Fine KS, Wilkins JT, Sawicki KT. Circulating branched chain amino acids and cardiometabolic disease. J Am Heart Association. 2024;13:e031617. DOI: 10.1161/JAHA.123.031617
37. Truby LK, Regan JA, Giamberardino SN, Ilkayeva O, Bain J, Newgard CB, O'Connor CM, Felker GM, Kraus WE, McGarrah RW, Shah SH. Circulating long chain acylcarnitines and outcomes in diabetic heart failure: an HF-ACTION clinical trial substudy. Cardiovasc Diabetol. 2021;20(1):161. DOI: 10.1186/s12933-021-01353-z

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-2-70-83

AUTHORS

Natalia E. Moskaleva — Candidate of Sciences (Biology), Deputy Head of the Center for Biopharmaceutical Analysis and Metabolic Research at the Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-7309-8913. E-mail: moskaleva_n_e@staff.sechenov.ru

Pavel M. Rezvanov — graduate student, junior researcher at the Laboratory of Bioinformatics and Pharmacological Modeling of the Center for Biopharmaceutical Analysis and Metabolic Research at the Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0009-0006-8806-6902. E-mail: rezvanov_p_m@staff.sechenov.ru

Viktor M. Samoylov — 5th year student of the Faculty of Medical Biochemistry, laboratory technician at the Center for Biopharmaceutical Analysis and Metabolic Research at the Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0009-0009-5460-011X. E-mail: vmSamoilov@gmail.com

Valeria G. Varzieva — junior researcher technician at the Center for Biopharmaceutical Analysis and Metabolic Research at the Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-9067-8717. E-mail: varzieva_v_g@staff.sechenov.ru

Sabina N. Baskhanova — researcher at the Center for Biopharmaceutical Analysis and Metabolic Research at the Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0001-8716-1672. E-mail: baskhanova_s_n@staff.sechenov.ru

Vadim V. Tarasov — Doctor of Sciences (Pharmacy), Director of the Institute of Translational Medicine and Biotechnology, Vice-Rector for Scientific and Technological Development, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-9394-7994. E-mail: tarasov_v_v_2@staff.sechenov.ru

Elena A. Smolyarchuk — Candidate of Sciences (Medicine), Assistant Professor, Head of the Department of Pharmacology at the A.P. Nelyubin Institute of Pharmacy, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-2615-7167. E-mail: smolyarchuk_e_a@staff.sechenov.ru

Dmitry A. Kudlay — Doctor of Sciences (Medicine), Professor of the Department of Pharmacology at the A.P. Nelyubin Institute of Pharmacy, I.M. Sechenov First Moscow State Medical University (Sechenov University); Deputy Dean for Scientific and Technological Development of the Faculty of Bioengineering and Bioinformatics, Senior Researcher at the Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University; Leading Researcher at the Laboratory of Personalized Medicine and Molecular Immunology No. 71, State Research Center Institute of Immunology; Corresponding Member of the Russian Academy of Sciences. ORCID ID: 0000-0003-1878-4467. E-mail: kudlay_d_a@staff.sechenov.ru

Svetlana A. Appolonova — Candidate of Sciences (Chemistry), Head of the Center for Biopharmaceutical Analysis and Metabolic Research at the Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-9032-1558. E-mail: appolonova_s_a@staff.sechenov.ru