DOI: https://doi.org/10.17816/EID108910

Доклиническое исследование токсикологического профиля нового соединения XC221GI

С.А. Суханова[1], О.В. Проскурина[1], Е.А. Джайн[2], А.А. Глобенко[2], М.И. Багаева[2], А.В. Рыдловская[3], В.Е. Небольсин[3]

[1] Всесоюзный научный центр по безопасности биологически активных веществ, Старая Купавна, Российская Федерация
[2] Валента Фарм, Москва, Российская Федерация
[3] Фарминтерпрайсез, Москва, Российская Федерация

АННОТАЦИЯ

Ключевой особенностью COVID-19 является интенсивное вирусиндуцированное воспаление в жизненно важных структурах организма и пространственно-временная дисрегуляция синтеза про- и противовоспалительных цитокинов и хемокинов, что выражается непредсказуемостью клинического течения и высоким риском формирования «цитокинового шторма». Состояние «цитокинового шторма» является патогенетической основой развития грозных осложнений, поэтому актуальной задачей ставится подбор эффективных и безопасных схем терапии, позволяющих управлять вирусиндуцированным воспалением в рамках упреждающей противовоспалительной терапии.

В работе представлена токсикологическая характеристика оригинального низкомолекулярного соединения XC221GI (1-[2-(1-метилимидазол-4-ил)-этил] пергидроазин-2,6-дион) по результатам доклинических исследований. Полученные результаты свидетельствуют об отсутствии у XC221GI токсического действия при многократном длительном применении. Все животные хорошо переносили введение соединения, доза без видимого нежелательного эффекта (NOAEL) составляла 30 мг/кг в сутки для собак и 450 мг/кг в сутки для крыс. Установлено отсутствие влияния XC221GI на форменные элементы крови, систему кроветворения и гемостаза, отсутствие у соединения цитотоксических, мутагенных, генотоксических и канцерогенных свойств, а также анафилактогенного и иммунотоксического действия.

Все известные данные позволяют классифицировать XC221GI как малотоксичное соединение и считать профиль безопасности нового соединения обоснованно благоприятным.

Ключевые слова: COVID-19; упреждающая противовоспалительная терапия; XC221GI; токсикология.

Как цитировать

Суханова С.А., Проскурина О.В., Джайн Е.А., Глобенко А.А., Багаева М.И., Рыдловская А.В., Небольсин В.Е. Доклиническое исследование токсикологического профиля нового соединения XC221GI // Эпидемиология и инфекционные болезни. 2021. Т. 26, № 5. С. 200–213. DOI: https://doi.org/10.17816/EID108910

Рукопись получена: 30.06.2022 **Рукопись одобрена:** 06.07.2022 **Опубликована:** 11.08.2022

ЭКО•ВЕКТОР

DOI: https://doi.org/10.17816/EID108910

Toxicity profile of the new compound XC221GI from pre-clinical studies

Svetlana A. Suhanova[1], Oksana V. Proskurina[1], Ekaterina A. Jain[2], Aleksander A. Globenko[2], Madina I. Bagaeva[2], Anastasia V. Rydlovskaya[3], Vladimir E. Nebolsin[3]

[1] All-Union Scientific Center for the Safety of Biologically Active Substances, Staraja Kupavna, Russian Federation
[2] Valenta Pharm Pharmaceutical Company, Moscow, Russian Federation
[3] Farminterprayses, Moscow, Russian Federation

ABSTRACT

The major feature of COVID-19 is intensive virus-induced inflammation in vital body organs and spatiotemporal dysregulation of pro- and anti-inflammatory cytokines and chemokines synthesis. All this leads to unpredicted clinical progression and high risk of "cytokine storm" development. The "cytokine storm" is the pathogenetic basis for further development of life-threatening complications. Thus, there is a huge need to select effective and safe approaches that allow to control virus-induced inflammation as a part of preventive anti-inflammatory therapy.

This article presents toxicological characteristics of the original low-molecular compound XC221GI (1-[2-(1-methylimidazole-4-yl)-ethyl]perhydroazin-2,6-dione) from pre-clinical studies.

The obtained results demonstrate that the XC221GI does not have any toxic effect in repeated long-term administration. The compound was well tolerated by all animals. The no-observed-adverse-effect level (NOAEL) was 30 mg/kg per day for dogs and 450 mg/kg per day for rats. There were no effects of XC221GI on blood count, hematopoiesis and hemostasis. As well as no cytotoxic, mutagenic, genotoxic, carcinogenic properties or anaphylactogenic and immunotoxic activity were revealed for XC221GI. All known data enable to classify XC221GI as a low toxic compound and consider its safety profile as reasonably favorable.

Keywords: COVID-19; preventive anti-inflammatory therapy; XC221GI; toxicology.

To cite this article
Suhanova SA, Proskurina OV, Jain EA, Globenko AA, Bagaeva MI, Rydlovskaya AV, Nebolsin VE. Toxicity profile of the new compound XC221GI from pre-clinical studies. *Epidemiology and Infectious Diseases.* 2021;26(5):200–213. DOI: https://doi.org/10.17816/EID108910

Received: 30.06.2022 **Accepted:** 06.07.2022 **Published:** 11.08.2022

BACKGROUND

The outbreak of coronavirus disease-2019 (COVID-19) rapidly became a significant medical problem, and in March 2020, it was declared an international emergency by the World Health Organization [1]. COVID-19 is characterized by intense virus-induced inflammation in vital body organs and spatiotemporal dysregulation of the synthesis of pro-/anti-inflammatory cytokines and chemokines, which is expressed in the clinical course unpredictably and increases a high risk of a cytokine storm. Cytokine storm is the pathogenetic basis for the development of severe complications such as thromboembolic events [2], acute respiratory distress syndrome [3], and multiple organ failure [4]. Several studies have established that clinical manifestations and instrumental signs of viral pneumonia can develop within 24–72 h from the onset of virus replication in the respiratory tract [5].

In this setting, the selection of effective and safe therapy regimens that enable the management of virus-induced inflammation within preemptive anti-inflammatory therapy is a relevant objective of practical public healthcare.

This paper presents the toxicological characteristics of the original low-molecular-weight compound 1-[2-(1-methylimidazol-4-yl)-ethyl]perhydroazine-2,6-dione (XC221GI) based on the results of preclinical studies. XC221GI (developed by Pharminterprices) has been actively studied since 2014 as an anti-inflammatory and antiviral agent for the treatment and prevention of diseases caused by respiratory viruses such as respiratory syncytial virus, influenza virus, rhinovirus, SARS-CoV-1, etc. The results demonstrate the unique pharmacodynamic profile of XC221GI, which can control the level of production of key inflammatory markers such as interleukin-6, interleukin-8, and C-X-C motif chemokine ligand 10. In this regard, developers had sufficient reasons to believe that XC221GI would have a favorable benefit–risk profile in the treatment of patients with COVID-19, which was confirmed in subsequent clinical studies.

This study aimed to evaluate the toxicological profile of XC221GI after single and repeated administrations and to examine its genotoxicity, carcinogenic potential, reproductive toxicity, allergenic effect, and immunotoxicity.

MATERIALS AND METHODS

All animal experiments were performed in compliance with the Good Laboratory Practice principles (GLP; GOST 33044-2014 "Principles of Good Laboratory Practice"[1], Order of the Ministry of Health of Russia dated April 1, 2016, N 199n "On approval of the Rules of Good Laboratory Practice"[2]) and other recommendations on keeping and using animals [6]. Laboratory animals were obtained from the nurseries of the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency of Russia, Charles River nursery (Germany), or research organization MediTox s.r.o. (Czech Republic).

Ethical considerations

The authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with the concerted author's recommendations (IAVES, 07/23/2010[3]). Research protocols were approved by relevant bioethics commissions (Conclusion No. 3/2016-1, 6/2016-1, 2/2017-1, 3/2016-3, 18/2016-2, 26/2016-1, 5-2016_1, 16/2016-1, 12/2016-2, 12/2016-4, 6/2016-2, 12/2016-3, and 73/2018).

Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 5) and Statistica v. 7 software package. For indicators, medians, arithmetic means, standard errors of arithmetic means, and standard deviations were calculated. Data for male and female animals were analyzed separately. For intergroup comparisons, the Kruskal–Wallis test with the Dunn post-test (with a small number of cases) or one-way analysis of variance with the Dunnett post-test (after confirming the normal nature of the distribution according to the Kolmogorov–Smirnov test and uniformity of the variance according to the Bartlett or F-test) was used. Pairwise comparisons using the *t*-test (with a normal distribution) or the Mann–Whitney test (with a non-normal distribution) were performed. Differences were considered significant at $p < 0.05$.

Single-dose toxicity study

Outbred (nonlinear) laboratory mice of both sexes (male body weight 21–25 g, female body weight 20–24 g) and outbred rats of both sexes (male body weight 195–205 g, female body weight 190–205 g) were used. The pharmaceutical substance was used as a solution, i.e., 1% solution of polysorbate-80 in distilled water was used as a vehicle. Mice ($n=6$ of each sex in the group) were injected intragastrically with XC221GI at a dose of 5000 mg/kg or vehicle. Rats in the intragastric dosing experiment ($n=4$ of each sex in the group) received 5000 mg/kg of XC221GI at a single-dose (the maximum dose that could technically be administered to mice or rats) or vehicle. Before the test compound administration, the body weight of the animals was measured; within 60 min after dosing, the condition of the animals was monitored with the assessment of signs of depression or excitation of the central nervous system, convulsions, tremors, and signs of changes in the activities of the autonomic nervous system. Mortality

[1] Interstate standard. Access mode: https://docs.cntd.ru/document/1200115791. Reference date: 02/15/2021.

[2] Access mode: https://docs.cntd.ru/document/420350679. Reference date: 02/15/02.2021.

[3] International Association of Veterinary Editors. Consensus Author Guidelines on Animal Ethics and Welfare for Veterinary Journals. [Internet]. Access mode: https://static1.squarespace.com/static/53e16b74e4b0528c244ec998/t/543acda6e4b094d04bcacacd/1413139878532/IAVE-AuthorGuidelines.pdf. Reference date: 02/15/2021.

was assessed throughout the day following the dosing. Surviving animals were followed up for 14 days after XC221GI administration, and examinations were performed two times a day. At the follow-up completion, all surviving animals were sacrificed by carbon dioxide inhalation, followed by exsanguination.

Repeated-dose toxicity studies

In the titration study, all animals received the test compound intragastrically. In all experiments, half of the animals were sacrificed the next day after the completion of dosing, whereas the remaining animals were sacrificed the next day after the end of the follow-up period. Body weight was measured weekly. Electrocardiography (ECG) using the Poly-Spectrum-8/V electrocardiograph (Neurosoft, Russia), non-invasive blood pressure (BP) measurement (MK-2000ST NP-NIBP Monitor, Muromachi Kikai CO, Ltd., Japan), and respiratory rate (RR) measurement were performed 14 days after the dosing and upon completion of the recovery period. Spontaneous motor activity of rats in the open field test was assessed on days 15 and 30 in a rectangular chamber [7]. On days 15 and 30, blood samples were also collected for clinical and biochemical tests, and urine samples were collected for the general analysis. In the clinical blood test, the erythrocyte count, mean corpuscular volume, hemoglobin level, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin, leukocyte count, white blood cell differential, and platelet count were evaluated using an automatic analyzer (BC-2800Vet, China). A biochemical blood test was performed using diagnostic kits (Olvex Diagnosticum, Russia), glucose levels were assessed using a glucometer (OneTouch Select, Switzerland); optical density was measured using a photometer (iMark, Bio-Rad, CA, USA); biochemical blood test parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase activity, as well as the levels of creatinine, urea, triglycerides, glucose, total protein, albumin, globulin, cholesterol, and albumin-to-globulin ratio. In the clinical urinalysis (DocUReader, Hungary), the specific gravity; pH; levels of bilirubin, ketone bodies, glucose, and protein; erythrocytes and leukocyte counts were evaluated.

Rats or rabbits were sacrificed by carbon dioxide inhalation, followed by exsanguination. In all experiments, pathomorphological studies evaluated the manifestations of the irritating effect of the test substance or the finished dosage form on the gastric mucosa.

Two-week toxicity study in rats. Male (body weight 220–250 g) and female (body weight 200–220 g) outbred rats were used. Twelve rats of each sex were distributed for the intragastric administration of XC221GI at a dose of 17.4 mg/kg, 87 mg/kg, or 174 mg/kg per day, or vehicle for 14 days. The selected doses of the test compound corresponded to an equivalent of a therapeutic dose (TD), 5 TDs, or 10 TDs for humans. Animals were examined twice daily to record mortality and evaluate signs of toxicity, behavioral changes, and general conditions. After sacrificing the rats, a pathomorphological study was performed with mass assessment and histological structure of the internal organs.

Thirty-day dosing toxicity study in rats. Outbred rats were used (male body weight 230–260 g, female body weight 200–230 g). The study methodology, XC221GI doses studied, number of animals in groups, and sex distribution were consistent with the design for the 2-week experiment. The duration of dosing and the recovery period were 30 days each.

Toxicity study with 3-month dosing in rats. Outbred rats of both sexes were used (male body weight 220–260 g, female body weight 210–250 g). The experiment design was identical to the methods of the experiment with the compound administered for 1 month, except for the duration of dosing (90 days). The recovery period was 30 days. BO assessment, ECG, RR measurement, open field test, and blood and urine sampling for analysis were performed on day 91 from the start of dosing and day 31 of the recovery period.

Toxicity study in rabbits. Studies were performed on Soviet Chinchilla rabbits of both sexes (body weight 2.25–3.3 kg). The design of the rabbit studies was consistent with the methodology used for the rat studies described above, except for behavioral and motor activity assessments, which were performed during clinical examinations without special techniques. In groups of 12 male and female rabbits, animals received XC221GI intragastrically at a dose of 9.1 mg/kg, 45.5 mg/kg, or 91 mg/kg per day (TD, 5 TDs, and 10 TDs, respectively). The vehicle was injected into the control group (12 male and female rabbits). Three series of experiments were conducted, when XC221GI was administered for different periods, namely, 14 days, 28 days with a recovery period of 28 days, and 3 months with a recovery period of 30 days.

Four-week dosing study in dogs. Dogs of both sexes (n=32, age 6–8.5 months, male body weight 8.4–14.4 kg, female body weight 7.6–13.3 kg) received XC221GI orally in the form of hard gelatin capsules at a dose of 6 mg/kg (3 female and 3 male dogs), 30 mg/kg (3 female and 3 male dogs), or 60 mg/kg per day (5 female and 5 male dogs) for 28 days, corresponding to TD, 5 TDs, and 10 TDs for humans. The control group (5 female and 5 male dogs) received hard gelatin capsules without active ingredients in the same amount as the highest-dose group. Two animals each from the control group and highest-dose group were used for the evaluation at the end of the 14-day follow-up period after cessation of the study compound administration. The condition of the animals was assessed two times a day during the period of XC221GI administration and then once a day during the follow-up period. An extended examination was performed 1 time a week. Ophthalmoscopy was performed before the start of XC221GI administration, after its termination, and at the end of the follow-up period. Before the administration of the test compound, 1 and 3 h after administration, and at the end of the follow-up period, the body temperature, RR, and BP were measured using a non-invasive method (S+Bmed VET, Germany). ECG

was measured using a SEIVA ECG Praktik Veterinary electrocardiograph (SEIVA, Czech Republic). Before XC221GI administration, at the end of the administration, and at the end of the follow-up period, blood samples were taken for clinical and biochemical tests (total protein, albumin, globulin, albumin-to-globulin ratio, glucose, total bilirubin, electrolytes, cholesterol, triglycerides, creatinine, alkaline phosphatase, transaminase, gamma-glutamyl transpeptidase, and lactate dehydrogenase) and to assess the blood coagulation system, which included determination of the total platelet count, activated partial thromboplastin (APTT), and prothrombin (PT) time. A Pentra 60 C+ hematologic analyzer (Horiba ABX, France) was utilized for the clinical analysis, and a Dimension Vista 500 analyzer (Siemens Healthcare Diagnostics, Germany) was used for the biochemical blood test. APTT and PT were measured with a coagulometer STart 4 (Diagnostica Stago, France). Urine was collected for general analysis before the drug administration, after its completion, and after the end of the follow-up period. Urinalysis was performed using an Urilyzer 100 Pro analyzer with Combi Screen PLUS test strips (Analyticon Biotechnologies AG, Germany).

For toxicokinetic studies, blood samples were taken from animals from the low-, medium-, and high-dose groups (3 male and 3 female dogs from each group) on days 1 and 28 of the XC221GI injection period immediately before and within 24 h after the XC221GI injection. To determine the plasma concentrations of XC221GI, a validated high-performance liquid chromatography technique with tandem mass spectrometry (HPLC-MS/MS) was applied using a Quattro Premier XE chromatography-mass spectrometric system (Waters, USA) and a Kinetex 1.7-μm HILIC 100 Å, 100×2.1 chromatographic column (Phenomenex, CA, USA); the lower limit of quantitative determination for XC221GI was 20 ng/mL, and 2 ng/mL for its metabolite XC221A (5-([2-(1-methyl-1H-imidazol-4-yl) ethyl]amino)-5-oxohexanoic acid). During the toxicokinetic study, the pharmacokinetic parameters of XC221GI and the XC221A metabolite formed by hydrolysis were determined. After the last dose of XC221GI, all animals from the low- and medium-dose groups, three animals from the control group, and three animals from the high-dose groups were sacrificed. The rest of the animals were sacrificed at the end of the follow-up period; intravenous barbiturates were administered. In all cases, a macroscopic pathological examination was performed. Animals, sacrificed after the follow-up period, were used for histopathological examination of the liver, kidneys, lungs, hematopoietic system, and reproductive system.

Mutagenic potential

Determination of potential XC221GI metabolites. XC221GI was incubated with human liver microsomes and rat liver microsomes for 5–45 min. Samples were taken from the incubation medium, and the amount of the remaining XC221GI was evaluated by HPLC-MS/MS (API6500, API6500+ and API4000, columns ACQUITY-BEH-C18, and Hypersil GOLD aQ). XC221GI was incubated with recombinant cytochrome P450 (CYP) isoenzymes to evaluate the enzyme potentially responsible for the compound metabolism. We used CYP1A2 (phenacetin as control), CYP2B6 (bupropion as control), CYP2C18 (paclitaxel as standard), CYP2C9 (diclofenac as standard), CYP2C19 (S-mephenytoin as control), CYP3A4 (midazolam as control), and CYP2D6 (bufuralol as control).

Ames test. In series 1 of the experiments, the Ames test was performed using *Salmonella typhimurium* strains TA97, TA 98, and TA100. Microorganisms were incubated with XC221GI in 1% polysorbate-80 solution. In experiments without metabolic activation, XC221GI was used at concentrations of 0.23, 2.27, 22.73, 227.27, or 2272.73 μg/mL; with metabolic activation, the compound was used at concentrations of 0.19, 1.85, 18.52, 185.19, or 1851.85 μg/mL. For metabolic activation, the S9 fraction of hepatocytes from male Wistar rats was used, which received Sovol intraperitoneally at a dose of 300 mg/kg 5 days before the sampling of liver cells. The incubation time was 48 h. The test was considered positive if the number of revertant colonies under the treatment of the test compound was ⩾2 times higher than that obtained for the negative control. In series 2 of experiments, *S. typhimurium* TA98, TA100, TA1535, and TA1537 *Escherichia coli* WP2 uvrA were used for the Ames test under conditions of metabolic activation in the presence of the S9 fraction of rat liver (inductor phenobarbital/β-naphthoflavone) and without metabolic activation. The study included a preliminary dissolution test, cytotoxicity test, initial test, and confirmatory mutation test. The selection of the analyzed concentration range was based on the results of the concentration range test and recommendations of the OECD[4] guidelines (OECD Test No. 471: Bacterial Reverse Mutation Test) for soluble non-toxic compounds. For these compounds, the maximum recommended test concentration is 5000 μg per dish. XC221GI was used at a concentration of 16, 160, 500, 1600, or 5000 μg per dish.

Test for the induction of chromosomal aberrations. Experiment 1 was performed on V79 Chinese hamster lung cells in accordance with OECD guidelines (OESD Test No. 473: *In Vitro* Mammalian Chromosome Aberration Test). For cytogenetic experiments, XC221GI concentrations of 250, 500, 1000, and 2000 μg/mL were chosen based on preliminary test results without metabolic activation and with metabolic activation in the presence of the S9 fraction of rat hepatocytes. Solutions of 0.4 μl/mL or 1.0 μL/mL of ethyl methanesulfonate or 5 μg/mL of cyclophosphamide were used as a positive control. At the end of incubation, cell cultures were treated with colchicine at a concentration of 0.2 μg/mL for 2.5 h before cell sampling.

The clastogenic and aneugenic potential of XC221GI was examined in an *in vitro* mammalian cell micronucleus test according to the OECD guidelines (OECD. Test No. 487: *In Vitro* Mammalian Cell Micronucleus Test). The study was

[4] OECD — Organisation for Economic Co-operation and Development https://www.oecd.org/

conducted on human peripheral blood lymphocytes with metabolic activation in the presence of the S9 fraction of the liver of male Wistar rats (inductor Aroclor 1254) and without it. Based on the results of the preliminary testing, three studied concentrations of XC221GI were selected, namely, 1, 2, and 5 mg/mL of culture. Water for injection was used as the vehicle. The experiment used a positive control (colchicine at a final concentration of 0.1 μg/mL, cyclophosphamide at a final concentration of 0.1 μg/mL) and a negative control (water for injection). Descriptive statistics methods were used to present the results.

To examine the effect of XC221GI on chromosome aberrations *in vivo*, we used CBA×C57BL/6 mice (body weight 20–22 g). At stage 1, animals (male mice, *n*=5 per group) received XC221GI once intraperitoneally at a dose of 2.86 mg/kg or 2000 mg/kg. Male mice (*n*=5 per group) from the positive control group were intraperitoneally injected with cyclophosphamide at a dose of 20 mg/kg, and the vehicle (1% solution of polysorbate-80) was used as a negative control. At stage 2 of the study, mice (5 male and 5 female mice per group) were intragastrically injected with XC221GI at a dose of 2.86 mg/kg per day or vehicle for 5 days. At both stages, 1 day after the dosing was completed, the mice were sacrificed by cervical dislocation, and 0.01 mL/g of 0.04% colchicine solution was injected 2–3 h before sacrifice. Bone marrow samples from the femur were collected, preparations were made from the sediment of the samples, stained with Romanovsky stain, and at least 100 metaphase plates were analyzed.

Alkaline elution test. The deoxyribonucleic acid (DNA) comet test was performed to assess genotoxic and carcinogenic potential. The experiment was conducted using male hybrids of the first generation of CBA×C57BL/6 mice (body weight 20–22 g). Animals (*n*=10 per group) received XC221GI once intragastrically in a 1% solution of polysorbate-80 at a dose of 36 mg/kg, 360 mg/kg, or 3600 mg/kg. The positive control group used mice that received cyclophosphamide intragastrically at a dose of 50 mg/kg once, and the vehicle-treated mice served as positive controls. Half of the animals were sacrificed by cervical dislocation 3 h after dosing, and the remaining animals were sacrificed after 18 h. The femurs were isolated, the epiphyzes were cut, and bone marrow cells were washed out with cold phosphate buffer with the addition of ethylenediaminetetraacetate (EDTA) at a concentration of 20 mmol/l and 10% dimethyl sulfoxide. The liver, kidneys, and spleen were isolated, and homogenates were prepared. Specimens of prepared cells were stained with SYBR Green (1:10,000 dilution in TE buffer with 50% glycerol), fluorescence microscopy was performed, and digital images of the specimens were obtained. Using the CASP 1.2.2 software, we analyzed the DNA content in the tail of DNA comets. Statistical analysis was performed by the Dunnett method.

Reproductive toxicity

Mature male and female rats were used in all experiments. XC221GI was administered intragastrically in a 1% solution of polysorbate-80 at a daily dose of 17.4 or 174 mg/kg, equivalent to TD and 10 TDs for humans. The control groups received the vehicle.

Effect on fertility. Female rats (*n*=20 per group) received the test compound or vehicle for 15 days (three estrous cycles), and they were placed in a cage with intact male rats for 10 days. Initially, during pregnancy and on day 20 of pregnancy, the body weight of the female rats was estimated. On day 20 of pregnancy, half of the animals were sacrificed, and the counts of the corpus luteum in the ovaries, implantation sites in the uterus, and number of dead and live fetuses were determined. The remaining female rats were followed up for a month after giving birth, and the development, physical condition, body weight dynamics, and offspring mortality were assessed. The fertility index was calculated as the ratio of the number of pregnant female rats to the number of female rats placed with male rats (%). In the experiment on male rats, animals (*n*=10 per group) received XC221GI or vehicle for 48 days, after which they were placed in the same cage with intact female rats. The number of dead and live fetuses in female rats was determined.

Influence on embryofetal development. Pregnant female rats (*n*=10 per group) received XC221GI or vehicle from days 1–19 of pregnancy. On day 20 of pregnancy, the female rats were sacrificed, the fetuses were removed, and the weight of the fetuses was estimated. Then, a pathomorphological study of the internal organs was performed according to Staples, and the skeleton was evaluated by the Dawson method with alizarin staining.

Influence on prenatal and postnatal development. Pregnant female rats (*n*=10 per group) received XC221GI or vehicle from day 6 of pregnancy until delivery. After birth, the number of live and dead rat pups was estimated. On days 7 and 21 after birth, the body weight of the F_1 offspring was measured, and signs of postnatal development disorders (timing of pinna detachment, appearance of lanugo, opening of eyes, and incisor eruption) were assessed. To assess the neurological development of the offspring, tests were performed for negative geotaxis, pendulum reflex, flipping on a plane, avoidance of a cliff, olfactory response, response to an acoustic stimulus, flipping in free fall, spontaneous motor activity, and the open field test.

Allergenic effect

The allergenic effect was examined on albino guinea pigs (body weight 250–300 g) and hybrids of the first generation of CBA×C57BL/6 mice. XC221GI was administered to guinea pigs at a dose of 15.5 mg/kg or 155 mg/kg, corresponding to the TD and 10 TDs for humans. Ovalbumine was used as a positive control. In guinea pigs, a test was performed for the induction of systemic and cutaneous anaphylaxis, immediate, and delayed-type hypersensitivity with cutaneous or intraconjunctival application. The Weigle anaphylactic index was calculated to assess systemic anaphylaxis. Delayed-type hypersensitivity was also assessed in mice [8].

Immunotoxicity

The experiments were performed on male hybrids of the first generation of CBA×C57BL/6 mice (body weight 20–22 g, *n*=10 per group). XC221GI at a dose of 28.6 mg/kg or 286 mg/kg per day or vehicle was administered intragastrically for 21 days. On the last day of dosing, a suspension of sheep red blood cells (2.5×10^8 cells) was administered intraperitoneally. After 7 days, the mice were sacrificed, and blood samples were collected, and the content of hemagglutinins (hemagglutination reaction with sheep red blood cells) and hemolysins (microtitration method using sheep red blood cells and guinea pig complement using the Takachi microtiter) in the blood serum was evaluated to assess the effect of XC221GI on the humoral part of the immune system. In another experiment with a similar design, animals were sacrificed on day 5 after immunization, blood was sampled from the spleen, in which phagocytic activity was assessed in a test with a tetrazolium stain, and resetting was evaluated to assess T- and B-cell immunity. To evaluate the effect on delayed-type hypersensitivity, animals received XC221GI at a dose of 28.6 mg/kg or 286 mg/kg per day or vehicle intragastrically for 10 days. On the last day of dosing, a suspension of sheep red blood cells (2.5×10^8 cells) was administered intraperitoneally. A challenging dose of sheep red blood cells (10^8 cells) was administered subplantarly after 5 days, and the foot pad thickness was assessed a day later.

RESULTS

Single-dose toxicity

At an intragastric dose of 5000 mg/kg, XC221GI did not cause death in mice, regardless of sex. Changes in animal behavior were not registered, and the next day after dosing, the condition of the mice remained within the normal range. Like mice, all rats survived after the intragastric administration of 5000 mg/kg of XC221GI. Dosing did not lead to the onset of external signs of toxicity, and the next day after administration, the condition of the animals remained within the physiological norm.

Repeated-dose toxicity

Dose titration study. In rat experiments, no signs of toxicity or other adverse effects were detected with a single intragastric administration of XC221GI in the dose range of 90–2000 mg/kg. Signs of XC221GI toxicity were not registered after repeated administrations of XC221GI to rats in the dose range of 18–450 mg/kg. Clinically significant changes in the erythrocyte and leukocyte counts and coagulogram were not noted. The biochemical blood test parameters also did not change clinically significantly. With a single oral administration of XC221GI to rats, the no-observed-adverse-effect-level (NOAEL) dose was 2000 mg/kg, whereas that with repeated oral administrations for 14 days was 450 mg/kg.

Research on rats

Two-week toxicity study. In the course of the daily intragastric administration of XC221GI at a dose of 17.4–87 mg/kg per day, all rats survived. No significant differences in vital signs were found compared with the data obtained in animals treated with the vehicle (p >0.05 in all cases of intergroup comparison). The body weight gains during the dosing period and recovery period were comparable in animals of all groups (p >0.05 in all cases of intergroup comparison). Among male and female animals, the indicators of ECG, BP, RR, and behavior in the open field test in the experimental groups were not significantly different from the data obtained in the case of vehicle administration. Parameters of the clinical blood test were comparable in animals of all groups both on days 15 and 30 of the study. The parameters of the general urine analysis both on days 15 and 30 of the study were comparable in rats of both sexes of all groups (p >0.05 in all cases of intergroup comparison). In the pathomorphological study, the studied structures of animals in the control and experimental groups conformed with the physiological norm. The mass of internal organs in male and female rats on days 15 and 30 was comparable in individuals of all groups (p >0.05 in an intergroup comparison).

Thirty-day dosing toxicity study. Following the administration of XC221GI intragastrically at a dose of 17.4–174 mg/kg per day to female and male outbred rats for 1 month, all animals survived. Throughout the dosing and recovery period, the general condition of the animals, their appearance, and feed and water intake remained satisfactory. The administration of the study compound did not lead to deviations in ECG, BP, RR, and behavior in the open field test. No differences in the clinical blood test and general urine test parameters were revealed. Pathologically, both male and female rats of the high-dose group showed an increase in relative liver mass on day 31 (p <0.05 compared with the parameter obtained in the vehicle administration). By the end of the recovery period, the liver weight of the high-dose group was comparable to that of the control group (p >0.05 in an intergroup comparison). No deviations in the histological presentation of the liver and other organs were revealed.

Three-month toxicity study. For 3 months of dosing, no animal death was registered. The effect of XC221GI on body weight gain was generally comparable with the results obtained in the 1-month experiment. No deviations in ECG, BP, and RR values were recorded during the experiment (p >0.05 in all cases of intergroup comparison). In male rats after 3 months of XC221GI administration, according to the results of the open field test, a significant dose-dependent increase in the total motor activity, number of crossed sectors, and racks was registered (p <0.05 in all cases compared with the indicator obtained in the control group). In female rats, XC221GI at a dose of 87 or 174 mg/kg per day caused a decrease in the overall motor activity and the number of crossed sectors (p <0.05 in all cases compared with the

indicator obtained in the control group). Regardless of sex, 1 month after the discontinuation of dosing, the parameters in the open field test recovered (p >0.05 in all cases of intergroup comparison). In the clinical blood analysis and urinalysis, the indicators remained comparable throughout the experiment. In male rats, XC221GI did not effect on biochemical blood parameters. In female rats after 3 months of dosing, a slight but significant increase was found in the serum ALT activity in the high-dose group and a decrease in the AST activity in the medium- and high-dose groups (p <0.05 in all cases of intergroup comparison). The pathomorphological study revealed only a significant increase in relative liver and thyroid mass in female rats in the high-dose group (p <0.05 vs. control rats), which resolved by the end of the recovery period. Changes in the mass of organs were not accompanied by deviations in their histological structure.

Research on rabbits

In rabbit studies with 2-week, 30-day, and 3-month dosing, regardless of the dose, XC221GI had no clinically significant effect on the general condition, appearance, body weight gain, and food and water intake among rabbits (p >0.05 in all cases compared with values obtained following vehicle administration). The indicators of ECG, RR, clinical and biochemical blood tests, and urinalysis remained comparable throughout the study (p >0.05 in all cases of intergroup comparison). The pathomorphological study did not reveal a clinically significant effect of the test compound on the mass and macroscopic and microscopic structure of the internal organs.

Toxicity and toxicokinetics study in dogs

No significant changes were detected in the body weight, food intake, and body temperature after XC221GI administration (p >0.05). Examination, including ophthalmoscopy, revealed no pathological changes. ECG, RR, and BP parameters did not differ significantly between the experimental and control groups (p >0.05). No clinically significant changes were found in the results of general and biochemical blood tests and general urinalysis. When assessing the mass of internal organs, no changes associated with the test compound were found. XC221GI did not cause macroscopic or histopathological changes in the liver and kidneys, which could indicate the presence of a toxic effect. On the part of the gastrointestinal tract and other organs, no macroscopic and microscopic changes were recorded. Thus, in dogs, NOEL dose was 30 mg/kg per day.

The results of the toxicokinetic analysis (Table 1) confirmed the level of systemic exposure to XC221GI,

Table 1. Toxicokinetic indicators XC221GI in beagle dogs treated XC221GI orally within 28 days

Group	XC221GI					
	6 mg/kg per day		30 mg/kg per day		60 mg/kg per day	
Number of animals	3 males	3 females	3 males	3 females	3 males	3 females
XC221GI after the initial dose administration on day 1						
C_{max}, ng/mL	1100±214	1079±170	4536±738	4363±789	6650±1430	7113±2164
t_{max}, h	0.7±0.3	0.7±0.3	0.4±0.1	0.5±0.0	0.7±0.3	0.6±0.4
K_{el}, h	1.2±0.2	1.2±0.3	0.3±0.1	0.3±0.1	0.3±0.3	0.2±0.0
$t_{1/2}$, h	0.6±0.1	0.6±0.1	2.3±0.9	2.5±1.1	3.8±2.4	3.0±0.3
AUC_{0-t}, ng*h/mL	1385±570	1343±561	10 462±3843	11 011±3820	12 607±3769	15 206±1645
$AUC_{0-\infty}$, ng*h/mL	1481±672	1439±672	10 573±4013	11 203±3910	12 782±3769	15 356±1602
MRT, h	1.2±0.3	1.2±0.3	2.4±0.9	2.9±1.4	2.7±1.2	2.8±0.5
XC221GI after the last dose administration on day 28						
C_{max}, ng/mL	1128±576	1109±567	4246±966	4734±328	5974±1314	9026±1937
t_{max}, h	0.7±0.3	0.7±0.3	0.7±0.3	0.4±0.1	0.8±0.3	0.4±0.1
K_{el}, h	1.0±0.9	1.0±0.5	0.4±0.2	0.3±0.1	0.3±0.1	0.2±0.1
$t_{1/2}$, h	0.8±0.4	0.8±0.4	2.0±1.1	2.1±0.4	3.5±1.9	4.0±2.2
AUC_{0-t}, ng*h/mL	1771±1290	1750±1317	9730±4612	9674±2679	14 102±3646	20 845±6933
$AUC_{0-\infty}$, ng*h/mL	1823±1288	1799±1317	9884±4616	9779±2665	14 289±3776	21269±7157
MRT, h	1.4±0.4	1.4±0.4	2.4±0.9	2.1±0.2	2.9±0.9	3.2±0.8

Note: C_{max} — maximum concentration; t_{max} — is the time to reach the maximum concentration; K_{el} — elimination constant; $t_{1/2}$ — half-life; AUC_{0-t} — the area under the pharmacokinetic curve "concentration-time" from the initial moment to the last determined concentration at the time point t; $AUC_{0-\infty}$ — the area under the pharmacokinetic curve "concentration-time" from the initial moment to infinity; MRT — mean retention time.

consistent with the results of the toxicity study, whereas the highest C_{max} values were recorded in the highest-dose group. The cumulation coefficients were calculated for XC221GI and XC221A separately for each dose. The cumulation ratios for XC221GI and XC221A after a 60 mg/kg dose were >1.25; therefore, cumulation was confirmed for both compounds at the highest-dose. The proportion of area under the pharmacokinetic curve (AUC) for the XC221A metabolite in relation to the proportion of AUC for XC221GI was calculated separately for each dose. The proportion of the concentration–time AUC from the initial time point to the last detectable concentration at time point t (AUC_{0-t}) of XC221A from AUC_{0-t} of XC221GI was the highest for a dose of 60 mg/kg (10.8%–16.76%) and the lowest for a dose of 6 mg/kg (4.6%–1.8%). Dose proportionality was calculated for C_{max}, AUC_{0-t}, and the concentration–time AUC from the initial point of time to infinity ($AUC_{0-\infty}$) on days 1 and 28 was confirmed based on C_{max} and AUC for XC221GI by analysis of variance (p <0.05) and coefficient of determination (R^2 >0.90). For C_{max} and AUC_{0-t}, no sex differences were revealed for any studied doses and days of drug administration (days 1 and 28).

Mutagenic potential

Determination of potential XC221GI metabolites. When XC221GI was incubated with liver microsomes for 45 min, the concentration ratios of the parent compound XC221GI and its hydrolytic metabolite XC221A were 0.09 and 0.08 when using rat liver microsomes and human liver microsomes, respectively.

When XC221GI was incubated with CYP isoenzymes, XC221GI had no inhibitory effect on CYP1A2, CYP2C9, CYO2C19, CYP2D6, CYP3A4, CYP2B6, or CYP2C8.

Ames test. In the Ames test without metabolic activation, the results were negative regardless of the strains of the microorganisms used. In experiments with metabolic activation of S9 by a fraction of rat hepatocytes, XC221GI at all the study concentrations did not increase the back mutation rate.

Tests for the induction of chromosomal aberrations. In experiments on V79 line Chinese hamster lung cells, no significant increase in the number of aberrant cells was noted when compared with those in the control experiments. Under metabolic activation at XC221GI concentrations of 1000 or 2000 μg/mL, the aberration rate was within the reference values (five aberrant cells without chromatid breaks/150 cells with a reference value of 2–5); however, the 95% confidence intervals exceeded slightly the control limits (4.11 and 4.34 aberrant cells excluding gaps/150 cells). No considerable significant differences were found in any cases (p >0.05). Polyploid or endoreduplicated metaphases were not registered in any experiment. XC221GI has been classified as a non-clastogenic substance based on test results.

After a single administration of XC221GI, the frequency of detection of aberrant metaphases was 0.6%, regardless of the compound dose, which was comparable with the results obtained with the vehicle administration (p >0.05 in an intergroup comparison). After cyclophosphamide administration, the incidence of aberrant metaphases was 27.2% (p <0.05 compared with vehicle administration).

With repeated intragastric administrations of XC221GI or vehicle to male rats, the incidence of aberrant metaphases was 0.6% (p >0.05 in an intergroup comparison). In female rats, after the vehicle administration, the indicator was 0.6%, and when dosing the test compound, it was 0.8% (p >0.05 in an intergroup comparison).

Micronucleus test. The micronucleus test on mammalian cells *in vitro* did not reveal a significant (p >0.05 when compared with control) increase in the proportion of binuclear cells with micronuclei in cell cultures for the studied concentrations of XC221GI. During repeated tests using the same concentrations of XC221GI, no increase in the proportion of aberrant cells was recorded, and no dose-dependent increase in the proportion of aberrant cells was revealed. Thus, XC221GI had no clastogenic or aneugenic effects on the culture of human peripheral blood lymphocytes.

Alkaline elution test. The results of the DNA comet test in mice after 3 h of exposure are presented in Table 2. During cyclophosphamide administration, a significant increase in the DNA content in the tail of DNA comets was noted in all cells, which indicated an increase in the incidence of DNA breaks (p <0.05 in all cases, compared with the indicator obtained after the vehicle administration).

Table 2. Content of DNA in the tail of DNA comets after 3 h of exposure, %

Group	Liver	Kidneys	Spleen	Bone marrow
Vehicle	5.55±0.49	5.52±0.41	5.18±0.37	5.79±0.51
Cyclophosphamide, 50 mg/kg	17.32±1.42†	15.18±1.61†	7.44±0.68†	15.74±1.74†
XC221GI, 36 mg/kg	5.83±0.50	6.58±0.54	6.33±0.61	6.65±0.35
XC221GI, 360 mg/kg	6.58±0.52	5.71±0.62	5.85±0.16	6.7±0.51
XC221GI, 3600 mg/kg	6.71±0.52	6.38±0.30	5.53±0.31	7.08±0.19

Note: Data are presented as averages and standard errors of the mean. † — differences are significant compared with the control group (p <0.05, Dunnett's test). DNA — dezoxyribonucleic acid.

No studied doses of XC221GI increased the incidence of DNA breaks, and the DNA content in the tail of DNA comets remained comparable with that obtained in the negative control group in all cell types (p >0.05 in all cases of intergroup comparison). When the exposure time was increased to 18 h, the results remained comparable.

Reproductive toxicity

Effect on fertility. The administration of XC221GI to female rats before mating did not affect the changes in the body weight of the animals. No changes in the frequency of post-implantation fetal death and changes in the fertility index were noted (p >0.05 when compared with the indicators obtained during the vehicle administration). In the postnatal period, no abnormalities of offspring development were noted, including the timing of pinna detachment, appearance of lanugo, tooth eruption, and opening of the eyes (p >0.05 when compared with the indicators obtained from the offspring of the control group). In female rats that mated with male rats treated with XC221GI, no increase in the incidence of pre- and post-implantation death of embryos was registered. The fertility index was comparable to that obtained with vehicle administration into male rats. An increase in the incidence of fetal impaired development was not recorded.

Influence on embryofetal development. The rates of body weight gain in female rats of the main groups did not differ from those in the control group (p >0.05 in the intergroup comparison). The incidence of pre-implantation fetal death and post-implantation mortality were comparable between the groups (p >0.05 in an intergroup comparison). No intrauterine development abnormalities were registered among the offspring of XC221GI-treated animals. The skeletal development in the offspring of XC221GI-treated female rats was comparable with that in the offspring of the control group.

Influence on prenatal and postnatal development. All rat pups were born alive; the average body weight was comparable in the offspring of female rats of all groups both on days 7 and 21 after birth (p >0.05 in an intergroup comparison). The terms of pinna detachment, appearance of lanugo, tooth eruption, and opening of the eyes did not differ in the offspring of the experimental and control groups (p >0.05 in an intergroup comparison). No significant differences were revealed in the neurological development of the offspring (p >0.05 in an intergroup comparison).

Allergenic effect

XC221GI did not induce anaphylactic shock in any guinea pig (Weigle score 0). In the active cutaneous anaphylaxis test, the diameter of the stain distribution zone after the intradermal administration of a challenging dose of XC221GI did not exceed 3 mm, which indicated a negative result. In guinea pigs, intraconjunctival or epicutaneous application of XC221GI did not result in delayed or immediate hypersensitivity reactions, and in mice, the administration of XC221GI did not cause delayed-type hypersensitivity.

Immunotoxicity

In mice, the intragastric administration of XC221GI at a dose of 28.6–286 mg/kg did not affect the formation of hemagglutinins and hemolysins in response to the administration of sheep red blood cells. In other experiments, XC221GI did not affect the intensity of the delayed-type hypersensitivity reaction, rosetting, and phagocytic activity of blood neutrophils.

Local irritative effect

In the course of toxicity studies with repeated intragastric administrations to rats or rabbits for 2 weeks to 3 months, no significant signs of irritation or damage to the gastric mucosa were revealed during autopsy.

DISCUSSION

The results obtained indicate that repeated long-term use of XC221GI has no toxic effects; therefore, the safety profile of XC221GI can be reasonably considered favorable.

The study of acute and chronic toxicities of XC221GI in five animal species (mice, rats, guinea pigs, rabbits, and dogs) showed that all studied animals tolerated the XC221GI administration well. Significant changes in the behavior of animals and their consumption of feed and water were not noted. Throughout the dosing and recovery periods, no animals died. *In vivo* experiments showed no signs of toxicity or other adverse effects after a single administration of XC221GI to outbred mice and rats of both sexes at a dose of 5000 mg/kg (the maximum dose for determining the toxicity class of chemicals in accordance with GOST 12.1.007-76[5]) and when repeated administration to rats, rabbits, and dogs of both sexes in studies with 2-week, 4-week, 30-day, and 3-month dosing of XC221GI in a wide dose range (6–174 mg/kg per day), which, taking into account interspecies conversion factors, corresponded to TD, 5 TDs, and 10 TDs in humans (the daily TD for a person is 200 mg).

According to the results of a clinical study of the pharmacokinetics of increasing doses of the drug with single and multiple oral administrations in healthy volunteers (NCT03459391), the systemic exposure of XC221GI when administered at a dose of 30 mg/kg per day to dogs exceeded that possible in humans by 10 times.

By the end of the recovery period, all indicators of clinical (erythrocyte count, hemoglobin level, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, hematocrit, leukocyte count, white

[5] Interstate standard. GOST 12.1.007-76. System of Operational Safety Standards. Harmful Substances. Classification and General Safety Requirements. Access mode: https://docs.cntd.ru/document/5200233. Reference date: 02/15/2021.

blood cell differential, and platelet count) and biochemical (ALT, AST, alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase, and levels of creatinine, urea, triglycerides, glucose, total protein, albumin, globulins, cholesterol, and albumin-to-globulin ratio, total bilirubin, and electrolytes) blood tests, coagulograms (total platelet count, APTT, and PT), and clinical urinalysis (specific gravity, pH, levels of bilirubin, ketone bodies, glucose, protein, erythrocytes, and leukocytes) were comparable in all groups of animals. ECG, BP, RR, and behavioral parameters in the open field test of the experimental groups were not significantly different from the data obtained during the vehicle administration.

In the pathomorphological study of the control and experimental groups, the macroscopic and histological structures of the internal organs conformed to the physiological norm. During the autopsy, no significant signs of irritation or damage to the gastric mucosa were detected, which indicated that the drug had no local irritating effects.

In a panel of standard *in vitro* and *in vivo* tests (Ames test, test for the induction of chromosomal aberrations, micronucleus test, and alkaline elution test), the absence of cytotoxic, mutagenic, genotoxic, and carcinogenic potential of XC221GI was confirmed in a wide range of doses.

XC221GI is not metabolized in the liver and does not affect the human liver cytochrome system.

In the analysis of the reproductive toxicity of XC221GI in mature male and female rats at a daily dose of 17.4 or 174 mg/kg, equivalent to TD and 10 TDs for humans, no changes in the incidence of post-implantation fetal death and fertility index and effects on embryofetal, prenatal, and postnatal development were detected.

In a study of immunotoxicity, XC221GI did not exert negative effects on the humoral (assessment of antibody production in animals) and cellular (induction of a delayed-type hypersensitivity reaction and T- and B-lymphocyte counts according to the rosette reaction) immunity links, and phagocyte activity (assessment of phagocytic and bactericidal activities of phagocytic cells of different localization). In a study of the allergenic effect of XC221GI at a dose equivalent to 1 and 10 TDs for humans, XC221GI did not cause delayed or immediate hypersensitivity reactions in the studied animals and did not show an anaphylactogenic potential.

Thus, the integral assessment of the toxicological profile enables to classify XC221GI as a low-toxic compound and to attribute it to the toxicity class IV in accordance with GOST 12.1.007-76[5].

The results of the study of the toxicological profile of XC221GI complement the known data on its pharmacodynamic activity and highlight the advantages of this drug when used as a preemptive anti-inflammatory therapy for COVID-19 in comparison with several standard COVID-19 therapy regimens [9]. These XC221GI benefits include a unique anti-cytokine profile to minimize excessive immune system suppression, a favorable metabolic safety profile compared with glucocorticosteroids and Janus kinase inhibitors (baricitinib and tofacitinib) (COVID-19 Treatment Guidelines, 2021), as well as a non-protein chemical structure, excluding immunological risks characteristic of monoclonal antibody preparations (tocilizumab, sarilumab, levilimab, and anakinra) [10–13].

CONCLUSION

In a series of studies of the acute and chronic toxicity in rodents and non-rodents, XC221GI did not demonstrate adverse effects on the gastrointestinal, cardiovascular, respiratory, nervous, and genitourinary systems. It also did not have a local irritating effect and did not cause signs of metabolic shifts in the internal environment of the body. No negative effects of XC221GI on blood corpuscles, hematopoietic system, and hemostasis were registered. The NOAEL value for XC221G1 was 2000 mg/kg after a single oral administration to rats, 450 mg/kg per day after oral administration to rats for 14 days, and 30 mg/kg per day after oral administration to dogs for 28 days. In a panel of standard *in vitro* and *in vivo* tests, XC221GI showed no cytotoxic, mutagenic, genotoxic, or carcinogenic properties. XC221GI did not have anaphylactogenic and immunotoxic effects, did not demonstrate allergenic properties, and did not have reproductive toxicity. Thus, the safety profile of the new compound XC221GI in repeated long-term use is reasonably favorable.

ADDITIONAL INFORMATION

Funding source. The work was funded by Pharmenterprises LLC (Russia) and JSC Valenta Pharm (Russia).

Competing interest. Employees of Pharmenterprises LLC (Russia) were responsible for the organization and conduct of research, scientific advice and review of the publication. Employees of JSC Valenta Pharm (Russia) participated in the data analysis and preparation of the publication.

Authors' contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published. S.A. Suhanova, O.V. Proskurina, A.V. Rydlovskaya, V.E. Nebolsin — search and analytical work when writing a review article; E.A. Jain, A.A. Globenko, M.I. Bagaeva — writing an article, the direction of the manuscript for publication.

Acknowledgement. The authors are grateful to Statendox LLC (Russia) for assistance in preparing the publication.

REFERENCES

1. Ruelens M, Wouters J. COVID-19 and the trials and tribulations of global health governance. *Bulletin of International Organizations*. 2021;16(2):70–98. (In Russ). doi: 10.37690/1811-0193-2020-4-24-33
2. Tang L, Yin Z, Hu Y, Mei H. Controlling cytokine storm is vital in COVID-19. *Front Immunol*. 2020;11:570993. doi: 10.3389/fimmu.2020.570993
3. Pavlikova EP, Agapov MA, Malakhov PS, et al. Mediastinal emphysema as a specific complication of COVID-19 (case report). *General Reanimatology*. 2021;17(2):4–15. (In Russ). doi: 10.15360/1813-9779-2021-2-4-15
4. Pasrija R, Naime M. The deregulated immune reaction and cytokines release storm (CRS) in COVID-19 disease. *Int Immunopharm*. 2021;90:107225. doi: 10.1016/j.intimp.2020.107225
5. Griffin DO, Brennan-Rieder D, Ngo B, et al. The importance of understanding the stages of COVID-19 in treatment and trials. *AIDS Rev*. 2021;23(1):40–47. doi: 10.24875/AIDSRev.200001261
6. National Research Council (US) Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press (US); 1996. doi: 10.17226/5140
7. Buresh Ya, Bureshova O, Houston DP. Methods and basic experiments on the study of the brain and behavior. Transl. from English by E.N. Pictortseva; ed. by A.S. Batuev. Moscow: Vysshaya shkola; 1991. 398 p. (In Russ).
8. Bunyatyan ND, Vasiliev AN, Verstakova OL, et al. Guidelines for conducting preclinical studies of medicines. Ed. by A.N. Mironov. Moscow: Grif i K; 2012. 944 p. (In Russ).
9. Van de Veerdonk FL, Giamarellos-Bourboulis E, Pickkers P, et al. A guide to immunotherapy for COVID-19. *Nat Med*. 2022;28(1):39–50. doi: 10.1038/s41591-021-01643-9
10. Philippova AV, Kolbin AS, Verbitskaya EV, et al. Safety issues of monoclonal antibodies used in rheumatology. *Good Clinical Practice*. 2019;(3):44–52. (In Russ.) doi: 10.24411/2588-0519-2019-10082
11. Kolbin AS, Kharchev AV. Safety of biological preparations and small molecules. Are there any differences? *Pediatric Pharmacology*. 2013;10(3):17–25. (In Russ).
12. Giezen TJ, Mantel-Teeuwisse AK, Leufkens HG. Pharmacovigilance of biopharmaceuticals: challenges remain. *Drug Saf*. 2009;32(10): 811–817. doi: 10.2165/11316550-000000000-00000
13. Strangfeld A, Eveslage M, Schneider M, et al. Treatment benefit or survival of the fittest: what drives the time-dependent decrease in serious infection rates under TNF inhibition and what does this imply for the individual patient? *Ann Rheum Dis*. 2011;70(11):1914–1920. doi: 10.1136/ard.2011.151043

СПИСОК ЛИТЕРАТУРЫ

1. Рюленс М., Ваутерс Я. COVID-19: испытания и потрясения для глобального управления в сфере здравоохранения // Вестник международных организаций. 2021. Т. 16, № 2. С. 70–98. doi: 10.17323/1996-7845-2021-02-05
2. Tang L., Yin Z., Hu Y., Mei H. Controlling cytokine storm is vital in COVID-19 // Front Immunol. 2020. Vol. 11. P. 570993. doi: 10.3389/fimmu.2020.570993
3. Павликова Е.П., Агапов М.А., Малахов П.С., и др. Эмфизема средостения — специфическое осложнение COVID-19 (клиническое наблюдение) // Общая реаниматология. 2021. Т. 17, № 2. С. 4–15. doi: 10.15360/1813-9779-2021-2-4-15
4. Pasrija R., Naime M. The deregulated immune reaction and cytokines release storm (CRS) in COVID-19 disease // Int Immunopharm. 2021. Vol. 90. P. 107225. doi: 10.1016/j.intimp.2020.107225
5. Griffin D.O., Brennan-Rieder D., Ngo B., et al. The Importance of understanding the stages of COVID-19 in treatment and trials // AIDS Rev. 2021. Vol. 23, N 1. P. 40–47. doi: 10.24875/AIDSRev.200001261
6. National Research Council (US) Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press (US), 1996. doi: 10.17226/5140
7. Буреш Я., Бурешова О., Хьюстон Д.П. Методики и основные эксперименты по изучению мозга и поведения / пер. с англ. Е.Н. Живописцевой; под ред. А.С. Батуева. Москва: Высшая школа, 1991. 398 с.
8. Бунятян Н.Д., Васильев А.Н., Верстакова О.Л., и др. Руководство по проведению доклинических исследований лекарственных средств / под ред. А.Н. Миронова. Москва: Гриф и К, 2012. 944 с.
9. Van de Veerdonk F.L., Giamarellos-Bourboulis E., Pickkers P., et al. A guide to immunotherapy for COVID-19 // Nat Med. 2022. Vol. 28, N 1. P. 39–50. doi: 10.1038/s41591-021-01643-9
10. Филиппова А.В., Колбин А.С., Вербицкая Е.В., и др. Вопросы безопасности лекарственных средств моноклональных антител, применяемых в ревматологии // Качественная клиническая практика. 2019. № 3. С. 44–52. doi: 10.24411/2588-0519-2019-10082.
11. Колбин А.С., Харчев А.В. Безопасность биопрепаратов и малых молекул. Существуют ли различия? // Педиатрическая фармакология. 2013. Т. 10, № 3. С. 17–25.
12. Giezen T.J., Mantel-Teeuwisse A.K., Leufkens H.G. Pharmacovigilance of biopharmaceuticals: challenges remain // Drug Saf. 2009. Vol. 32, N 10. P. 811–817. doi: 10.2165/11316550-000000000-00000
13. Strangfeld A., Eveslage M., Schneider M., et al. Treatment benefit or survival of the fi ttest: what drives the time-dependent decrease in serious infection rates under TNF inhibition and what does this imply for the individual patient? // Ann Rheum Dis. 2011. Vol. 70, N 11. P. 1914–20. doi: 10.1136/ard.2011.151043

AUTHORS' INFO

*** Ekaterina A. Jain**, Head of Preclinical research group,
JSC Valenta Pharm pharmaceutical company;
address: Ryabinovaya str., 26 buil. 10, Moscow, 121471, Russia;
ORCID: https://orcid.org/0000-0003-0283-8598;
e-mail: ekaterina.korsakova@valentapharm.com

Svetlana A. Suhanova, Cand. Sci. (Biol.);
ORCID: https://orcid.org/ 0000-0002-0548-4249;
e-mail: ssuhanova46@gmail.com

Oksana V. Proskurina, MD, Cand. Sci. (Med.);
ORCID: https://orcid.org/0000-0001-5718-3301;
e-mail: proskurina_ov@mail.ru

Aleksander A. Globenko, Medical director,
JSC Valenta Pharm pharmaceutical company;
ORCID: https://orcid.org/0000-0001-9295-2663;
e-mail: Aleksandr.Globenko@valentapharm.com

Madina I. Bagaeva, Medical advisor,
JSC Valenta Pharm pharmaceutical company;
ORCID: https://orcid.org/0000-0002-4577-1832;
e-mail: madina.bagaeva@valentapharm.com

Anastasia V. Rydlovskaya, Cand. Sci. (Biol.);
ORCID: https://orcid.org/0000-0003-0241-156X;
e-mail: rydlovskaya@pharmenterprises.ru

Vladimir E. Nebolsin, Cand. Sci. (Chem.);
ORCID: https://orcid.org/0000-0001-5939-9341;
e-mail: nv@pharmenterprises.ru

ОБ АВТОРАХ

*** Джайн Екатерина Александровна**, руководитель группы доклинических исследований АО «Валента Фарм»;
адрес: Россия, 121471, Москва, ул. Рябиновая, д. 26, стр. 10;
ORCID: https://orcid.org/0000-0003-0283-8598;
e-mail: ekaterina.korsakova@valentapharm.com

Суханова Светлана Алексеевна, к.б.н.;
ORCID: https://orcid.org/0000-0002-0548-4249;
e-mail: ssuhanova46@gmail.com

Проскурина Оксана Владимировна, к.м.н.;
ORCID: https://orcid.org/0000-0001-5718-3301;
e-mail: proskurina_ov@mail.ru

Глобенко Александр Александрович,
медицинский директор АО «Валента Фарм»;
ORCID: https://orcid.org/0000-0001-9295-2663;
e-mail: Aleksandr.Globenko@valentapharm.com

Багаева Мадина Ибрагимовна,
медицинский советник АО «Валента Фарм»;
ORCID: https://orcid.org/0000-0002-4577-1832;
e-mail: madina.bagaeva@valentapharm.com

Рыдловская Анастасия Владимировна, к.б.н.;
ORCID: https://orcid.org/0000-0003-0241-156X;
e-mail: rydlovskaya@pharmenterprises.ru

Небольсин Владимир Евгеньевич, к.х.м.;
ORCID: https://orcid.org/0000-0001-5939-9341;
e-mail: nv@pharmenterprises.ru

* Corresponding author / Автор, ответственный за переписку