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RESEARCH OF GENERAL TOXICITY OF 2*H*-PIRANO[2,3-*c*]PYRIDINE DERIVATIVES

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The morbidity due to the infectious diseases remains on the high level and its treatment is one of the priorities of maintaining of health in Ukraine and in most of the world [1]. The leading role in infectious diseases treatment belongs to antibiotics [1 - 3]. The main negative phenomenon that stipulates the decrease in efficacy of antibacterial therapy is the continuously progressing resistance of the microorganisms to the antibiotics [1, 3, 4]. Antibiotic resistance is a global problem, that concerns all world countries [1, 4 - 6]. There are different approaches being developed in order to overcome the multiple resistance to antibacterial agents in all countries. One of the approaches is to search for therapeutical agents with new mechanisms of influence for treatment and monitoring of infections that are resistant to traditional therapy [1, 3, 4, 7, 8].

The problem of safety of potential therapeutical agents becomes even more important nowadays [2, 6, 9]. At present the data bank of registered serious adverse reactions contains almost 5 million notifications from all over the world, and there are more than 40 000 notifications in Ukraine since 1996 [2]. There is 2-3% of the general human population suffering from adverse reactions of medicinal preparations [2, 6]. The lethality due to the adverse reactions of medicines is on the 5-th place after heart and lung diseases, oncopathology and traumas [10]. The majority of undesirable influences of such agents could be foreseen and prevented according to the experimental studies data. The latter allow guaranteeing the safety of clinical trials and the following application of the agents in therapeutical practice in great measure [7].

In the search for highly active compounds with antimicrobial activity the most promising are synthetic derivatives of 2*H*-pyrano[2,3-*c*]pyridines and their heteroanalogs [11]. Our previous studies have proven that these compounds have a wide range and high efficacy of antimicrobial activity and that gram-positive and gram-negative microorganisms, as well as *Candida* fungi develop resistance towards them at slower pace 12, 13]. This report represents a fragment of the study of toxicity of 2*H*-pyrano[2,3-*c*]pyridines in order to create antimicrobial agents there of.

The aim of this study was to determine the potential general toxicity of 2*H*-pyrano[2,3-*c*]pyridines in experiments on laboratory animals (mice).

Material and methods. The study was carried out according to the requirements of the decree of Ministry of health of Ukraine № 944 of 14.12.2009 [14] and according to the existing methodical recommendations [7, 15].

The three most promising derivatives of 2*H*-pyrano[2,3-*c*]pyridines according to our data [12, 13], synthesized at Kharkov National pharmaceutical university at the organic chemistry department were: compound 1(1) – the derivative of 2-imino-3-*N*-aricarboxamides, compound 2(5) – the diaryl derivative and compound 5(7) – the ester derivative based on the diaryl derivative.

The study of general toxicity of 2*H*-pyrano[2,3-*c*]pyridine derivatives was carried out on 308 outbred white mice of both sexes, body mass 18 – 22 g, aged 2,0 – 2,5 months, that were housed in vivarium of State establishment «Mechnikov Institute for Microbiology and Immunology AMS of Ukraine». All animals that were used in the experiment were healthy without any physiological pathology. Before the start of the experiments the animals were quarantined, were given the standard rations and stable environment: relative humidity 50 – 60 %, air temperature 18 – 20 ºC, daylight regimen 12 C : 12 T. All manipulations with the animals were carried out according to the BST 42 1-88 "Laboratory animals. Technological process" and main conditions of the European Council Convention about protection of vertebral animals, that are used in the experiments and other scientific activities від 18.03.1986, EC Directive № 609 of 24.11.1986 and Ministry of Health of Ukraine № 281 of 01.11.2000.

In order to determine the parameters of acute toxicity of 2*H*-pyrano[2,3-*c*]pyridine derivatives the animals were given a single subcutaneous injection on the empty stomach in the morning with 1 ml of the corresponding compound diluted in polypropyleneglycole. The animals were divided randomly in 5 experimental groups, 8 animals in each. In course of the study different doses of experimental compounds were used – tolerable, toxic and lethal. Compound 1(1) was injected in the following dosages: 300, 600, 900, 1200, 1500 mg/kg of body mass, compound 2(5) – 100, 150, 300, 600, 1000 mg/kg body mass and compound 5(7) - 150, 300, 600, 1000, 1500 mg/kg of body mass. The control animals were injected with polypropyleneglycole. The main criterion of acute toxicity was death of the animals. The condition of the survived injected animals was observed for 14 days after the inoculation. The animal's clinical condition, motion behavior, other behavior characteristics were taken into account, as well as eating and drinking habits. The minimal doses haven't caused any toxic reactions in mice, the maximal dosages let to lethal result in all experimental animals. The surviving animals were observed for 14 days after the injections. The median lethal doses were calculated with the Kerber method [16].

In order to determine the threshold dosages for the repeated injections of 2*H*-pyrano[2,3-*c*]pyridine derivatives the compounds in 1,0 ml of polypropyleneglycole were injected once a day subcutaneously for 14 days. The duration of the injection course was determined with taking into account the foreseen potential application of 2*H*-pyrano[2,3-*c*]pyridine derivatives as antimicrobial agents. The studied compounds were injected in three dosages: relatively therapeutical (minimal), maximal for determination the expected toxicity level and intermediate between minimal and maximal dosages [7]. The dosage selection was based on the acute toxicity study and dosages were equaled to the corresponding shares of LD_{50}. According to the received dosages of compounds the experimental animals were divided into three groups, 8 animals in each: 1 group – 1/5 LD_{50}, 2 group - 1/10

LD_{50}, 3 group – 1/275 LD_{50}. The control animals were injected with 1,0 polypropyleneglycole. The experimental animals were observed for the next 30 days. In all the animals before the beginning of the experiment and on the 7, 14, 20 and 30 day after its completion specific and non – specific symptoms of intoxication, clinical condition and course of intoxication and its outcome were registered.

The determination of local irritating effect of 2*H*-pyrano[2,3-*c*]pyridine derivatives was carried out with the application of consecutive dosages that were significantly lower than LD_{50}, corresponding to 1/10 LD_{50}, 1/100 LD_{50} and 1/1000 LD_{50}. The quantity of animals in each statistical group in the study of irritating effect was 6 animals. The studied compounds were applied once a day on the exposed area of right side of the animal sized 2,5 × 2,5 cm for 7 days in the mentioned above dosages. The compounds were diluted in polypropyleneglycole. The effect was estimated according to observations for 5 and 15 minutes and 1 hour after application of the substances during the 7 days of the experiment. The irritating effect of the agents was determined visually. Such symptoms as hyperemia level, thickening of the skin fold and dermatitis symptoms were taken into account [17]. Dermatitis symptoms without crust and lesion development indicated a weak irritating effect. The marked irritating effect was characterized by crust development and such reactions as swelling, erythema, lesions, hair loss, change of pigmentation, et cetera. The strong local irritating effect was characterized by rapid swelling and hyperemia, hemorrhagic crusts development. The exposed skin area of the same size on the left side of the animal was used as a control. The animals were observed throughout the whole experiment.

The study results and discussion. The obtained data of the acute toxicity study of 2*H*-pyrano[2,3-*c*]pyridine derivatives have shown that, in case of single injection of the studied compounds in mice LD_{50} was in the range of 156,25 – 937,5 μg/kg. LD_{50} of compound 1(1) constituted 937,5 mg/kg of body mass, LD_{50} of compound 2(5) – 156,25 mg/kg of body mass and LD_{50} of compound 5(7) – 625,0 mg/kg of body mass, and animals' death was observed on the 1-3 day. After subcutaneous injection of the agents, usually 2(5), dosage dependent symptoms of acute poisoning were observed, which were characterized by listlessness, general weakness, the decrease in reaction to the irritants and general activity. The symptoms were interpreted as probable side effect of the studied compound on the sensor and somatomotor systems. Considering the administration way, the 2*H*-pyrano[2,3-*c*]pyridine derivatives were classified to the 4th toxicity class, i. e. low toxic agents [7]. The comparison of toxic effects of 2*H*-pyrano[2,3-*c*]pyridine derivatives it was established that compounds 1(1) and 5(7), median lethal doses of which were 937,5 mg/kg and 625,0 mg/kg of body mass, have the lower toxicity degree than the compound 2(5), lethal dose of which (156,25 mg/kg of body mass is nearing the limiting parameter of this class).

The study of influence of multiple injection of 2*H*-pyrano[2,3-*c*]pyridine derivatives was performed visually and also hematological parameters were observed. After 14-days' course of injections of compound in animals of 1 experimental group (1/5 LD_{50}) a medium level of suppression was observed, as well as symptoms of irritation at the injection site. One animal died on the 10th day. Other animals survived for 30 days of observation. The animals of the second research group (1/10 LD_{50}) after 14-days' course have shown a low level of suppression, 37,5 % of the animals had insignificant irritation on the injection site. The mentioned above deviations from normal condition remained throughout the whole observation period and returned to normal after the completion of the experiment on the 30th day. In the third group (1/275 LD_{50}) no suppression or irritation was observed. All animals of the group remained alive and healthy after the completion of the experiment. In the control group all animals survived and remained healthy after the injection of polypropyleneglycole.

The same reaction was observed in the corresponding groups that received compound 1(1).

The repeated injections of compound 2(5) there was medium suppression in 75 % of the mice, as well as listlessness and damaged coordination. In 37,5 % (3 animals) of this group a significant level of general suppression was observed, as well as damaged coordination, seizures, progressing respiratory failure. One mouse has died on the 7th day and another one – on the 20th day from asphyxia. All mice in the group had irritation and scratches on the injection site. The animals of the second experimental group (1/10 LD_{50}) after 20 injections of diluted compound 2(5) there was a weak suppression in 87,5 % of animals (7 mice). An insignificant irritation was observed in 62,5 % of the group (5 mice) on the injection site. One animal of the second research group has shown general medium suppression, listlessness, breathing failure and eventually death on the 20th day of the experiment. In the animals of the 3rd group (1/275 LD_{50}) no suppression, irritation or any external symptoms of toxicosis were observed. All animals were alive and healthy on the 30th at the end of the experiment.

No animal in the control group has shown any abnormality. All animals remained alive and healthy.

Body mass of the animals increased in the similar fashion in all groups. At the same time certain animals that received high dosages of compounds (first research groups) have shown unexpected delay in body mass gain. The hematologic test have shown that all studied compounds injected subcutaneously for 14 days did not influence the haemoglobin level and blood cells. The blood cell count parameters in research group correlated with the corresponding parameters in the control group and were in the normal range for this species. The variation of biochemical parameters of the blood in animals that have received multiple subcutaneous injections and solvent remained in the same range of physiological data. We have considered the obtained data as proof of the absence of adverse effect of the studied compounds on the haematological parameters and functional activity of inner organs. Lethal toxicity observed after injection of high dosages is probably connected with neurotoxicity, which needs to be elucidated further.

The study of local irritating effect of 2*H*-pyrano[2,3-*c*]pyridine derivatives have shown that application of solutions if studied compounds in all applied concentrations after 5, 15 and 60 minutes have not caused visible skin reaction. Only after application of compound 2(5) in dosage equivalent of 1/10 LD_{50} at the end of the 1st day visible marked weak local reaction in the one third of the animals. On the 4th day a weak local reaction in the form of external dermatitis was

observed in the half of the group and it was visible in all animals on the 6th day. At the end of the experiment application of compound 2(5) in the dosage equivalent of 1/10 LD_{50} has caused marked local irritation in the form of external dermatitis with swelling and crust formation, in other half of the group only weal local irritating effect was observed. Application of the dosage equivalents of 1/100 LD_{50} and 1/1000 LD_{50} of compound 2(5) its irritating effect was weak in half of the group. The skin applications of compound 5(7) in dosage 1/10 LD_{50} weak irritating effect was observed only on the 6-7th day in one third of exposed animals. Repeated applications of the compound in dosages 1/100 LD_{50} and 1/1000 LD_{50} have not caused irritation symptoms. Complete absence of local irritating effect was observed only in compound 1(1). Multiple application of the solvent (polypropyleneglycole) did not cause local irritation.

Conclusion. 1. According to the general toxicity parameters the researched 2*H*-pyrano[2,3-*c*]pyridine derivatives belong to the IV class of toxicity – low toxic agents.

2. The toxicity study of single and multiple injections the diaryl derivative was shown to be most toxic compared to the 2-imino-3-*N*-aricarboxamide and the ester based on the diaryl derivative.

3. According to the strength of the local irritating effect only 2-imino-3-*N*-aricarboxamide derivative has shown complete absence of irritating influence.

4. The data obtained in the study of general toxicity of 2*H*-pyrano[2,3-*c*]pyridine derivatives shows that the compound 1(1) – derivative of 2-imino-3-*N*-aricarboxamide could be acknowledged as most promising for further studies aimed for development of antimicrobial agents based there of.

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The aim of this study was to determine the potential general toxicity of 2*H*-pyrano[2,3-*c*]pyridines in experiments on laboratory animals (mice). The three most promising derivatives of 2*H*-pyrano[2,3-*c*]pyridines, synthesized at Kharkov National pharmaceutical university. The study of general toxicity of 2*H*-pyrano[2,3-*c*]pyridine derivatives was carried out on 308 outbred white mice of both sexes. According to the general toxicity parameters the researched 2*H*-pyrano[2,3-*c*]pyridine derivatives belong to the IV class of toxicity – low toxic agents. The data obtained in the study of general toxicity of 2*H*-pyrano[2,3-*c*]pyridine derivatives shows that the compound 1(1) – derivative of 2-imino-3-*N*-

aricarboxamide could be acknowledged as most promising for further studies aimed for development of antimicrobial agents based there of.

Key words: 2H-pyrano[2,3-c]pyridine derivatives, toxicity, local irritating effect.

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ВИЗНАЧЕННЯ ЗАГАЛЬНОТОКСИЧНОЇ ДІЇ ПОХІДНИХ 2*H*-ПІРАНО[2,3-*c*]ПІРИДИНІВ

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Метою роботи стало визначення потенційної загальнотоксичної дії похідних 2*H*-пірано[2,3-*c*]піридинів в експериментах на лабораторних тваринах (мишах). Досліджено три найбільш перспективні за нашими попередніми даними похідні 2*H*-пірано[2,3-*c*]піридинів, що були синтезовані в Харківському національному фармацевтичному університеті. Вивчення загальнотоксичної дії похідних 2*H*-пірано[2,3-*c*]піридинів проведено на 308 нелінійних білих мишах обох статей. Встановлено, що за параметрами гострої токсичності досліджені похідні 2*H*-пірано[2,3-*c*]піридинів належать до IV класу токсичності – малотоксичні речовини. За результатами вивчення загальнотоксичної дії похідних 2*H*-пірано[2,3-*c*]піридинів сполука 1(1) – похідна 2-іміно-3-*N*-арикарбоксамідів визнана найперспективнішою для подальших досліджень, спрямованих на створення на її основі протимікробного лікарського засобу.

Ключові слова: похідні 2Н-пірано[2,3-с]піридинів, токсичність, місцевоподразнююча дія.

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ИЗУЧЕНИЕ ОБЩЕТОКСИЧНОГО ДЕЙСТВИЯ ПРОИЗВОДНЫХ 2*H*-ПИРАНО[2,3-*c*]ПИРИДИНОВ

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Целью работы стало изучение потенциального общетоксичного действия производных 2*H*-пирано[2,3-*c*]пиридинов в экспериментах на лабораторных животных. Исследованы три наиболее перспективные производные 2*H*-пирано[2,3-с]пиридинов, синтезированные в Харьковском национальном фармацевтическом университете. Изучение общетоксичного действия проведено на 308 нелинейных белых мышах обоего пола. Установлено, что по параметрам острой токсичности изученные производные 2*H*-пирано[2,3-с]пиридинов относятся к IV классу токсичности – малотоксичные вещества. По результатам изучения общетоксичного действия производных 2*H*-пирано[2,3-*c*]пиридинов соединение 1(1) – производное 2-имино-3-*N*-арикарбоксамидов признано наиболее перспективным для дальнейших исследований, направленных на создание на ее основе противомикробного лекарственного средства.

Ключевые слова: производные 2Н-пирано[2,3-с]пиридинов, токсичность, местнораздражающее действие.