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STUDY OF ANTI-INFLAMMATORY ACTIVITY AND ACUTE TOXICITY INDICATORS OF NEW SILVER SALT OF PYRAZOLE-3-CARBOXAMIDE

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ИЗУЧЕНИЕ ПРОТИВОВОСПАЛИТЕЛЬНОЙ АКТИВНОСТИ И ПОКАЗАТЕЛЕЙ ОСТРОЙ ТОКСИЧНОСТИ НОВОЙ СЕРЕБРЯНОЙ СОЛИ ПИРАЗОЛ-3-КАРБОКСАМИДА

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Objective. To study the anti-inflammatory activity and some toxicological indicators of the new derivative of pyrazole-3-carboxamide SSP.
Materials and methods. To assess the biological activity of the compound, its anti-inflammatory effect was studied in the model of acute inflammatory edema caused by sub-plantar injection of carrageenan solution into a rat`s hind leg. The duration effect was assessed by the intensity of suppression of the inflammatory re-

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sponse in relation to the control level. In order to study the safety of the substance, the local irritant effect was determined when applied cutaneously in the experiments on rats. The severity of the irritant effect was assessed by the erythema degree, the amount of edema and the increase in local skin temperature. In addition, acute toxicity of the substance under study was determined. The test compound and reference drugs were applied to the skin.

Results. The study of acute toxicity of the compound SSP and the reference drug nystatin when applied cutaneously to rats showed that LD_{50} of both substances was more than 2500,0 mg/kg. Indicators of the degree of local irritating effect of the drugs demonstrate its absence. At the same time, the studied SSP compound has no anti-inflammatory effect administered either orally or cutaneously.

Conclusions. The data obtained allow us to classify the new silver salt of pyrazol-3-carboxamide SSP as a class 5 non-toxic substance. The studied SSP compound has no local irritant effect and does not demonstrate anti-inflammatory activity in the carrageenan inflammation model.

Keywords. Silver salt of pyrazol-3-carboxamide, acute toxicity, carrageenan inflammation model, local irritant effect, nystatin, nimesulide.

Цель. Изучить противовоспалительную активность и некоторые токсикологические показатели нового производного пиразол-3-карбоксамидов SSP.

Материалы и методы. Для оценки биологической активности соединения исследовали его противовоспалительное действие на модели острого воспалительного отека, вызванного субплантарным введением в заднюю лапу крысы раствора каррагенина. Эффект длительности оценивали по выраженности торможения воспалительной реакции по отношению к контрольному уровню. С целью изучения безопасности вещества определяли местно-раздражающее действие в экспериментах на крысах при накожном нанесении. Выраженность раздражающего действия оценивали по степени эритемы, величине отека и увеличению местной температуры кожи. Кроме того, определялась острая токсичность изучаемого вещества. Исследуемое соединение и препараты сравнения наносились на кожу.

Результаты. Исследование острой токсичности соединения SSP и препарата сравнения нистатина при накожном нанесении крысам показало, что $ЛД_{50}$ обоих веществ составила более 2500,0 мг/кг. Показатели, позволяющие оценить степень местно-раздражающего действия препаратов, свидетельствуют о его отсутствии. При этом исследуемое соединение SSP не проявляет противовоспалительной активности ни при пероральном, ни при накожном применении.

Выводы. Полученные данные позволяют отнести новую серебряную соль пиразол-3-карбоксамида SSP к 5-му классу практически нетоксичных веществ. Соединение SSP не проявляет местно-раздражающего действия и не обладает противовоспалительной активностью на модели каррагенинового воспаления.

Ключевые слова. Серебряная соль пиразол-3-карбоксамида, острая токсичность, каррагениновая модель воспаления, местно-раздражающее действие, нистатин, нимесулид.

INTRODUCTION

Superficial mycoses that are caused by dermatophytes and also yeast fungi are an important public health problem affecting different age groups of the population [1–4]. Topical agents are more often used for the treatment of this pathology. They are the most popular on the pharmaceutical market and account for more than 90 % of sales of all antimycotics [5].

In this regard, the search for new compounds with antifungal activity, which can potentially be used for topical therapy of mycoses, is an actual direction of pharmacy. The fact that the substance has additional pharmacological effects, such as anti-inflammatory activity, may contribute to the

acceleration of the recovery process during this pathology.

The most important characteristic of the supposed pharmaceutical substance is the absence of negative effects on the human body, which is reflected by such indicators as acute toxicity, irritant effect.

The aim of the study is to investigate the anti-inflammatory activity and some toxicological indicators of a new pyrazole-3-carboxamide derivative SSP.

MATERIALS AND METHODS

The biological activity of a new silver salt of pyrazole-3-carboxamide, SSP was evaluated. It showed high antifungal activity in planktonic culture (pMIC501.0 mg/L, pMIC90 15.6 mg/L) and biofilm (sMIC50 11.8 mg/L) [6]. The studies were conducted in experiments on animals obtained from the nursery "Andreevka", Moscow region. The animals were kept in compliance with the rules of laboratory practice (GLP) and the Order of the Ministry of Health of the Russian Federation No. 199n dated 01.04.2016 "Rules of Good Laboratory Practice", as well as the terms of the guidelines for conducting preclinical studies of pharmaceuticals [7]. The conducted studies were approved by the Bioethics Commission of the Federal State Budgetary Educational Institution of Higher Professional Education PGFA of the Ministry of Health of the Russian Federation (protocol No. 7 of 20.01.2023).

Indomethacin ointment for external use produced by BIOSINTEZ PJSC (Russia), nimesulide produced by Ozon LLC (Russia) and nystatin produced by BIOSINTEZ PJSC (Russia) were used as reference pharmaceuticals.

Determination of acute toxicity of the compound at the dermal application was realized according to the guideline P 1.2.3156-13 "Assessment of toxicity and danger of chemical substances and their mixtures for human health ". The study included experiments on rodents (rats). Animals were randomly divided into groups by randomization. The absence of external signs of disease and homogeneity of groups by body weight (± 10 %) were considered as criteria for acceptable randomization.

The experimental medication in the suspension form in dimethyl sulfoxide and the comparison medication were applied on the skin in the back area using a dispenser in increasing doses according to Litchfield-Wilcoxon method. The fur of the animals was removed at the site of application 24 h before the beginning of the experiment. Control animals were dosed with diluent, which was dimethyl sulfoxide. Dosing was carried out according to the total mass of the sample. Groups of five animals of the same sex were used for the study of each medication dose. Besides, there were similarly sized groups of control animals, which were applied to comparison medications in the same way in an amount equivalent to the maximum tested dose of the medication.

The animals were observed for 14 days from the beginning of the experiment. Mor-

tality, time of animal death, symptoms of poisoning, daily observation of general condition and behavior, weighing, evaluation of feed and water consumption were recorded. The dead animals were autopsied and macroscopically examined. All intoxicated animals were euthanized in a CO_2 chamber and autopsied after 14 days. Macroscopic description and determination of mass ratios of internal organs and histologic studies were conducted.

Anti-inflammatory activity was determined on non-linear white rats weighing 180–250 g, both sexes, sexually mature – on the model of acute inflammatory edema caused by subplantar injection of 0.1 ml of 1 % aqueous solution of carragenin into the back paw of the rat at different routes of injection into the body [7]. The studied compound in the form of a suspension in dimethyl sulfoxide in the amount of 0.5 ml was applied on the skin of the foot of rats of the first group 1 h before injection of the phlogogenic agent. The second group of animals was injected orally at a dose of 50 mg/kg 1 h before carrageenan injection.

Animals that did not receive the medication served as a control. The increase in foot volume, indicating the development of edema, was evaluated oncometrically after 3 h after injection of the phlogogenic agent, which means at the peak of edema formation. The anti-inflammatory effect was evaluated by the expression of inhibition of the inflammatory reaction in relation to the control level. If this indicator was more than 30 %, the result was considered as positive.

The local irritant effect of the medication was studied in experiments on nonlinear white rats weighing 180–210 g, of both sexes, sexually mature – by dermal application. The studied substance was placed in an amount of 0.5 g in the form of a paste on a previously depilated skin area of about 6 cm2. These skin areas were covered with gauze attached with a plaster. The exposure period lasted 4 h, after which the substance was removed. The evaluation of the irritant effect was conducted for 14 days. The expression of irritant effect was evaluated by the degree of erythema, the amount of edema (thickness of the skin fold at the application site) and the increase of local skin temperature. The degree of erythema was evaluated using colorimetric rulers of S.V. Suvorov in points. Skin fold thickness was measured using a thickness gauge TR-1–10 in millimeters. Skin temperature was measured using an electrothermometer. Animals, to which the comparison medication was applied in the same way, were used as control.

Statistical analysis of the results was conducted using the following program package Statistica 8.0. The results were analyzed by variation statistics using the Fisher-Student method [8].

RESULTS AND DISCUSSION

Evaluation of the acute toxicity of the compound SSP and the comparison medication nystatin by dermal application on rats showed that the LD50 of both sub-

stances was more than 2500.0 mg/kg. The obtained data indicate that sex differences in the acute toxicity indices of the two substances do not appear. There were no lethal effects when applied it on the skin. The use of the compared compounds in doses higher than 2500.0 mg/kg was practically impossible.

Thus, the compound SSP and the comparison medication nystatin are practically equivalent (equitoxic) in terms of acute toxicity. The clinical picture of intoxication at their single use in maximum possible amounts is characterized by the absence of signs of toxic effect, changes in the general condition and behavior of animals. Species and sex differences were not observed in the course of intoxication. The clinical picture of intoxication, caused by both compared medications, is the same.

The results of body weight measurements of the animals that experienced intoxication when the medications were applied topically at maximum doses are presented in Table 1.

Dynamics of body weight of experimental animals in all groups does not significantly differ from control data. There are no statistically significant deviations in body weight of animals in the groups receiving the compared medications.

All animals that survived intoxication, as well as animals of the control group, were euthanized in a CO2 chamber at the end of the study. External examination of rats that were killed after 14 days from the beginning of the experiment, did not reveal any significant differences between the experimental animals and the control animals. All rats are of standard proportions, with average weight. Fur is shiny, clean looking, there are no bald areas. There is no discharge from natural orifices. Visible mucous membranes are pale, shiny, smooth. According to the data of autopsy and macroscopic examination dermal application of the

Table 1

Effect of the compared medications on body weight (g) of rats after a single dermal application, $M \pm m$

Observation time	Experimental group and sex					
	"Control"		"SSP"		"Nystatin"	
	male	female	male	female	male	female
Background	176.9 ± 4.0	175.8 ± 1.9	171.6 ± 3.6	177.0 ± 2.7	173.9 ± 3.4	179.2 ± 3.4
2nd day	179.9 ± 3.3	179.4 ± 3.0	173.3 ± 3.2	172.9 ± 3.1	168.6 ± 3.7	176.1 ± 3.5
7th day	187.1 ± 4.2	188.2 ± 3.2	189.3 ± 5.4	186.6 ± 3.3	183.6 ± 4.7	187.4 ± 2.8
14th day	193.9 ± 4.4	199.6 ± 3.6	193.4 ± 5.2	195.5 ± 2.8	191.7 ± 3.4	196.5 ± 2.7

compared preparations in the studied doses to rats of both sexes does not cause visible changes of internal organs, including endocrine glands, gastric and intestinal mucous membranes, as well as the brain.It was found in the course of histologic study that the skin and soft tissue material of laboratory animals showed focal epidermal sloughing foci with keratin, which is a result of mechanical impact in the framework of the experiment. Inflammatory cell infiltration in the underlying dermis in areas of epidermal sloughing is not detected in all examined samples. Single perivascular dermal mononuclear cells are normal unchanged elements of skin-associated lymphoid tissue (SALT-structures). Dystrophic, alterative and inflammatory changes were not revealed in the liver and kidneys of laboratory animals. Focal disorders of blood circulation in the form of full blood of capillaries of kidneys, liver are expressions of blood redistribution in the agonal period. The obtained data allow to refer to the compound SSP to the 5th class of practically non-toxic substances (GOST 32419-2022 “International Standard. Hazard classification of chemical products. General requirements").

The results of the anti-inflammatory activity of the compound are presented in Tables 2 and 3.

The injection of phlogogenic agent into animals causes a significant increase in foot volume by the third hour of observation, the increase reaches 104.8 %, this index was taken as a control value. The use of such a medication as “Indomethacin” ointment showed that the increase in carrageenan edema statistically significantly decreased, compared to control data, to 13.7 %, that is, the inhibition of the inflammatory response reaches 86.9 %. It is successfully used in practical medicine as an anti-inflammatory agent. Pre-application of the studied compound on the skin of the foot of rats causes inhibition of the inflammatory response, as compared to the

Table 2

Anti-inflammatory activity of the silver salt of pyrazole-3-carboxamide by dermal application, $M \pm m$

Compound	Volume gain of the foot after 3 h, %	Inhibition of reaction after 3 h, %
SSP suspension	94.32 ± 7.48, $p = 0.220$, $p' = 0.005$	10.0
“Indomethacin” ointment	13.7 ± 1.7, $p = 0.001$	86.9
Control group	104.8 ± 12.1	

Note: p – level of statistical significance in comparison with control data; p' – level of statistical significance in comparison with “Indomethacin” ointment.

Table 3

Anti-inflammatory activity of the silver salt of pyrazole-3-carboxamide during oral injection, $M \pm m$

Compound	Volume gain of the foot after 3 h, %	Inhibition of reaction after 3 h, %
SSP	103.1 ± 9.9, $p = 0.845$, $p' = 0.001$	1.65
Nimesulide	39.2 ± 2.9, $p = 0.001$	62.59
Control group	104.8 ± 12.1	

Note: p – level of statistical significance in comparison with control data; p' – level of statistical significance in comparison with nimesulide.

control data, only by 10 %, which is not a statistically significant result ($p > 0.05$).

It was found that under the action of the comparison compound nimesulide the growth of carrageenan edema was statistically significantly inhibited compared to the control data. The increase of the foot volume in rats is 39.2 % ($p < 0.001$), i.e. inhibition of the reaction reaches 62.59 %. At the same time the studied compound SSP does not show anti-inflammatory activity, the increase of inflammatory edema by the third hour of the experiment goes with the same intensity as in the control experiments.

In the process of studying the biological activity of SSP compound, the study of local irritant effect of the compound had a special importance. It was evaluated by the degree of erythema, the amount of edema (thickness of the skin fold at the application site) and the increase in local skin temperature. The results of the study are presented in Tables 4–6.

There is no increase in the thickness of skin folds during the dermal application of SSP and nystatin. Thus, it is established that the medications do not cause the development of edema.

Table 4

Skin fold thickness in rats after dermal application, $M \pm m$

Group	Thickness of skin fold in the area of medication injection, mm				
	Study terms, days				
	Control	1st	3rd	5th	14th
"SSP"	3.3 ± 0.2	3.4 ± 0.2	3.5 ± 0.3	3.5 ± 0.4	3.4 ± 0.2
"Nystatin"	3.4 ± 0.3	3.5 ± 0.3	3.6 ± 0.2	3.6 ± 0.3	3.4 ± 0.3

Table 5

Surface skin temperature in rats after dermal application, $M \pm m$

Group	Surface temperature *t*, °C				
	Study terms, days				
	Control	1st	3rd	5th	14th
"SSP"	37.6 ± 0.5	37.8 ± 0.5	37.7 ± 0.4	37.7 ± 0.6	37.7 ± 0.4
"Nystatin"	37.5 ± 0.3	37.5 ± 0.4	37.6 ± 0.3	37.8 ± 0.3	37.5 ± 0.3

Table 6

Severity of skin erythema in rats after dermal application

Group	Level of erythema in points				
	Study terms, days				
	Control	1st	3rd	5th	14th
"SSP"	0	1	0	0	0
"Nystatin"	0	0	0	0	0

The temperature of the skin areas at the application site did not increase when applying the SSP compound and nystatin. There were no statistically significant differences between the parameters and control data (at $p < 0.05$).

Analysis of the level of erythema showed that, in general, with both nystatin and the SSP compound, it was rated as zero, i.e., absent.

Conclusions

1. The obtained data allow to assign the new silver salt of pyrazole-3-carboxamide SSP to the 5th class of practically non-toxic compounds.

2. The compound SSP does not show local irritating effect.

3. The studied silver salt of pyrazole pyrazole-3-carboxamide does not show anti-inflammatory activity on the model of carrageenan inflammation.

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