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An extract from the culture of a thermophilic *Staphylococcus aureus* strain suppresses allergic inflammation in the airways *in vivo* and degranulation of mast cells and basophils *in vitro*

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ABSTRACT

Aim. To study the anti-allergic effects of ruzam, an extract from the culture of a thermophilic *Staphylococcus aureus* strain, in an *in vivo* model of asthma and its influence on degranulation of mast cells and basophils *in vitro*.

Materials and methods. Allergic asthma in guinea pigs was reproduced by two intraperitoneal injections of ovalbumin followed by a series of inhalations of this antigen for 1.5 months. Ruzam (6 μg / kg) or a reference drug (sodium cromoglycate, 3 mg / kg) was administered daily via a nebulizer during the last 6 days of immunization. One day after completion of inhalations with ovalbumin and compared drugs, changes in the airways were assessed using cytological, morphometric, and histologic methods. Rabbit blood basophils and rat peritoneal mast cells were used to determine the effect of ruzam on IgE-independent degranulation induced by the compound 48 / 80 *in vitro*. The effect of ruzam was compared with that of hydrocortisone hemisuccinate. Basophils from the blood of ovalbumin-sensitized guinea pigs were used to evaluate the effect of the drug on IgE-dependent degranulation induced by ovalbumin. Granules of mast cells and basophils were detected by alcian blue staining to calculate the degranulation index.

Results. In the asthma model, ruzam reduced the degree of airway obstruction by increasing the bronchoalveolar lavage volume returned and suppressed neutrophilic and eosinophilic inflammation, while mobilizing other effector cells of the anti-pathogen immunity (lymphocytes and macrophages). Ruzam has proven to have a stronger anti-allergic effect than sodium cromoglycate by several parameters. At concentrations of 8.4–840 μg / ml, ruzam inhibited degranulation of mast cells and basophils, induced by the compound 48 / 80, equally to hydrocortisone hemisuccinate (10^{-3} M). At concentrations of 280 and 420 μg / ml, ruzam dose-dependently inhibited ovalbumin-induced degranulation of basophils in sensitized guinea pigs.

Conclusion. The anti-allergic effect of ruzam was confirmed in test systems *in vivo* and *in vitro*. We speculate here that the TLR2 signaling pathway may be involved in biological and pharmacological effects of this drug.

Keywords: ruzam, *Staphylococcus aureus*, thermophilic strain, asthma model, allergic inflammation, mast cells, basophils, degranulation

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Экстракт из культуры термофильного штамма *Staphylococcus aureus* подавляет аллергическое воспаление в дыхательных путях *in vivo* и дегрануляцию тучных клеток и базофилов *in vitro*

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РЕЗЮМЕ

Цель – изучить противоаллергическое действие рузама – экстракта из культуры термофильного штамма *Staphylococcus aureus* – на модели астмы *in vivo*, а также его влияние на дегрануляцию тучных клеток и базофилов *in vitro*.

Материалы и методы. Аллергическую астму у морских свинок воспроизводили двумя внутрибрюшинными инъекциями овальбумина с последующей серией ингаляций этого антигена в течение 1,5 мес. Рузам (6 мкг/кг) или референс-препарат (кромогликат натрия, 3 мг/кг) вводили ежедневно с помощью небулайзера в течение последних 6 сут иммунизации. Через 1 сут после завершения ингаляций овальбумина и сравниваемых препаратов оценивали изменения в дыхательных путях с помощью цитологических, морфометрических и гистологических методов. Для определения влияния рузама на IgE-независимую дегрануляцию, индуцированную соединением 48/80 *in vitro*, использовали базофилы крови кроликов и перитонеальные тучные клетки крыс. Эффект рузама сравнивали с таковым гидрокортизона гемисукцината. Базофилы крови сенсибилизированных овальбумином морских свинок использовали при оценке действия препарата на IgE-зависимую дегрануляцию, индуцированную овальбумином. Гранулы тучных клеток и базофилов для расчета индекса дегрануляции окрашивали с помощью альцианового синего.

Результаты. На модели астмы рузам снижал степень обструкции дыхательных путей, повышая объем возврата бронхоальвеолярного смыва, и подавлял нейтрофильное и эозинофильное воспаление, при этом мобилизуя другие клетки-эффекторы противоинфекционного ответа (лимфоциты и макрофаги). По ряду критериев противоаллергической эффективности рузам превосходил кромогликат натрия. Рузам в концентрациях 8,4–840 мкг/мл ингибировал дегрануляцию тучных клеток и базофилов, вызванную соединением 48/80, в той же степени, что и гидрокортизона гемисукцинат (10^{-3} М). Рузам (280 и 420 мкг/мл) дозозависимо подавлял индуцированную овальбумином дегрануляцию базофилов сенсибилизированных морских свинок.

Заключение. Подтверждено противоаллергическое действие рузама в тест-системах *in vivo* и *in vitro*. Выдвинута гипотеза о TLR2-опосредованном характере биологических/фармакологических эффектов препарата.

Ключевые слова: рузам, *Staphylococcus aureus*, термофильный штамм, модель астмы, аллергическое воспаление, тучные клетки, базофилы, дегрануляция

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Structural components and products of microorganisms, primarily bacteria, have long been investigated to find bioactive substances with a potentially wide spectrum of pharmacological effects [1]. In this regard, development of bacterial immunomodulators is one of the most promising research areas; its viability has been proven in clinical trials and practical healthcare. [2, 3]. Ruzam, developed by a group of Russian scientists, belongs to this class of drugs. It is an extract from the culture of a thermophilic *Staphylococcus aureus* strain. Ruzam has been successfully used in the treatment of allergy for three decades [4, 5]. The effectiveness of this drug in the treatment of pollinosis, bronchial asthma, allergic rhinitis, atopic dermatitis, urticaria and angioedema, and latex, food, and insect allergies, as well as in the prevention of respiratory infections has been shown [4].

Currently, ruzam is approved for clinical use in the form of a solution for subcutaneous injections. Successful results of preclinical and clinical trials of nasal and orally inhaled forms of ruzam in the treatment of allergic respiratory diseases are quite encouraging [6].

Previous studies on the anti-allergic effect of ruzam showed that the drug is capable of suppressing ovalbumin-induced inflammation in sensitized animals *in vivo* and inhibiting IgE-dependent and IgE-independent degranulation of mast cells and basophils in test systems *in vitro.* The materials of this study were presented by A.G. Chuchalin et al. back in 2003, but only as a fragment of the review article. [5]. These data are of great interest in the context of development and implementation of new dosage forms of ruzam. Besides, they explain the pharmacological effects of the drug identified in clinical trials [4]. The above encouraged to describe in detail the results of earlier experiments and subject them to additional mathematical processing and interpretation in the light of emerging immunological paradigms.

MATERIALS AND METHODS

Ruzam is a complex of lipoproteins derived from a culture of the thermophilic *Staphylococcus aureus* strain C2. The drug was provided for testing by Ruzam-M (Russia). Allergic respiratory inflammation was reproduced *in vivo* according to the method proposed by P.A. Hutson et al. [7] and modified as described previously [8]. Ovalbumin (Sigma-Aldrich, USA) at a dose of 10 mg / kg was intraperitoneally administered twice with a 7-day interval to male and female guinea pigs weighing 300–400 g. Then the animals inhaled (Pari LC Plus nebulizer (Pari GmbH, Germany)) 1 ml of the ovalbumin solution once every 4 days for 1.5 months. The concentration of ovalbumin was gradually raised from 0.1 to 1%. Ruzam at a dose of 6 μg / kg or sodium cromoglycate at a dose of 3 mg / kg as a reference listed drug were administered via the same nebulizer in the form of an aqueous solution at a dose of 1 ml daily during the last 6 days of immunization in the animal groups “Ovalbumin + ruzam” and “Ovalbumin + cromoglycate”, respectively. In the “Ovalbumin” group, sterile water was inhaled via the nebulizer in the last days of immunization in the same mode and volume. Each inhalation lasted 180 seconds.

Aerosol particles characteristics were evaluated using the aerodynamic particle sizer APS 3300 (TSI, USA) and the cascade impactor with a subsequent fluorometric analysis of the selected samples. The average volumetric flow rate was 0.46 ml / min, the mass median aerodynamic diameter was 6.2 μm, the respirable fraction of ruzam or sodium cromoglycate was 38.5 and 39.8%, respectively. Twenty-four hours after the inhalation of the last dose of ovalbumin, changes in the

airways were assessed using cytological, morphometric, and histologic methods. Bronchoalveolar lavage fluid (BALF) was collected under hexenal anesthesia injected intraperitoneally through the endotracheal cannula by double instillation of 10 ml sterile 0.9% NaCl solution heated up to 37 °C into the lungs.

Then the BALF volume returned was evaluated. The absolute number of cells per 1 ml (cytosis) in the BALF after centrifugation at 200 g for 10 min was determined. The number of neutrophils, eosinophils, macrophages, and lymphocytes was counted in Romanowsky-stained smears [9]. The density of bronchus-associated lymphoid follicles (whitish plaques with the diameter of 3–5 mm protruding above the bronchial mucosa) was assessed in the macroslides under the magnifying glass using the ocular measuring grid proposed by G.G. Avtandilov [8].

For histologic studies, lung tissues were fixed by Carnoy's solution and embedded in paraffin. The 4–5-μm sections were stained with hematoxylin and eosin to identify eosinophils, neutrophils, macrophages, lymphocytes, and histiocytes, or with toluidine blue (pH 2.0) to determine the number of mast cells. In both cases, cells were counted at 400x magnification. Mast cell degranulation was assessed in points: 1 point – the entire cytoplasm was densely filled with dark purple granules, 2 points – vacant areas in the cytoplasm were noted; sometimes separate granules were located near the cell, 3 points – vacant areas accounted for 50–70% of the cytoplasm, the nucleus was bare, the granules were located loosely; 4 points – vacant areas accounted for more than 70% of the cytoplasm (granulolysis). The degranulation index (DI) was calculated by the formula:

$$DI = \Sigma (i \times n_i) / \Sigma n_i,$$

where i is the degree of degranulation in points, and n_i is the number of cells with i degree (%) [10].

Peritoneal mast cells from Wistar rats were obtained as described previously [11].

Basophils were isolated as part of a leukocyte suspension from the blood taken from the heart of rabbits or guinea pigs by two-stage sedimentation. At the first stage, the blood was diluted with ethylenediaminetetraacetic acid (Sigma-Aldrich, USA); at the second stage, it was diluted with a citrate-containing liquid [12]. The effect of ruzam on IgE-independent degranulation of mast cells and basophils *in vitro* was evaluated at final concentrations of 8.4, 84, and 840 μg / ml. Hydrocortisone 21-hemisuccinate sodium salt (Sigma-Aldrich, USA) at concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M was used as a reference listed drug. Degranulation was induced by the compound 48 / 80 (Sigma-Aldrich, USA) (1 μg / ml), which is a polymer that causes degranulation of mast cells and basophils and liberates histamine [13].

The effect of ruzam on IgE-dependent degranulation of basophils *in vitro* was assessed as described previously [12]. The leukocyte suspension (10^4 cells / ml) was isolated from the heart blood of guinea pigs, which 1 month before were sensitized by the intraperitoneal injection of 10 μg of ovalbumin (Sigma-Aldrich, USA) with the aluminum hydroxide adjuvant (100 mg of gel) according to the method [14]. The suspension of leukocytes was incubated in a medium with ruzam (280 and 420 μg / ml) or without the drug (control) for 15 minutes at 37°C and 5% CO_2. Then a solution of ovalbumin at a final concentration of 0.35% was added to the culture for 10 minutes to induce degranulation of basophils. The reaction was stopped by adding cooled salt solution. This cell suspension was centrifuged at 100 g for 7 min. Slides for microscopy were prepared from the sediment. They were fixed and stained according to the method [15]. To detect basophil granules, 0.5% alcian blue (pH 1.0) was used. The nuclei were stained with safranin O 0.1% solution in 1% acetic acid. DI was calculated according to the same method that was used in the histologic studies [10].

Treatment and control groups in *in vivo* studies included 5 guinea pigs each. In *in vitro* studies, cell culture triplets from each of the 3 animals were used for each concentration of ruzam and the reference listed drug as well as for controls.

Statistical data processing was performed using Statistica 18 (StatSoft Inc., USA). Independent and dependent samples were compared by quantitative characteristics using the Mann – Whitney and Wilcoxon tests, respectively. Quantitative data in tables and figures were presented as the mean and the standard deviation $M \pm SE$. The differences were considered statistically significant at $p < 0.05$. A trend toward statistical significance was noted at $0.05 \leq p < 0.1$.

RESULTS

Anti-allergic effects of ruzam in the model of ovalbumin-induced airway inflammation in guinea pigs

Two intraperitoneal injections of ovalbumin, and then a series of inhalations of this antigen to guinea pigs reproduced chronic allergic airway inflammation. In addition to morphological disorders, this inflammation

was characterized by a decrease in the BALF volume returned and an increase in the number of cells in the BALF by more than 1.5 times, mainly due to eosinophils and to a lesser extent due to neutrophils (Table 1). The number and proportion of lymphocytes did not change significantly. The absolute and relative number of macrophages decreased in comparison with the intact animals.

Table 1

The effect of ruzam and sodium cromoglycate on some parameters of bronchoalveolar lavage fluid in guinea pigs with ovalbumin-induced allergic airway inflammation, *M* ± *SE*

Parameter		Intact animals	Allergic airway inflammation		
			Ovalbumin	Ovalbumin + ruzam	Ovalbumin + cromoglycate
BALF volume returned, %		65.6 ± 6.3	50.0 ± 5.8*	62.9 ± 1.0#	50.7 ± 7.4
Cytosis, cells / ml		42.0 ± 9.0	68.0 ± 16.0*	58.0 ± 7.0	55.0 ± 19.0
Macrophages	cells / ml	32.0 ± 2.9	22.2 ± 2.4*	29.6 ± 2.4†	12.6 ± 3.0*
	%	76.6 ± 5.9	32.7 ± 3.5*	51.1 ± 5.2†	22.9 ± 5.5*
Lymphocytes	cells / ml	3.3 ± 0.4	4.3 ± 1.4	6.0 ± 1.3*†	2.7 ± 0.6
	%	7.8 ± 1.0	6.4 ± 1.7	10.4 ± 2.2*†	4.9 ± 1.1
Neutrophils	cells / ml	0.30 ± 0.05	1.8 ± 0.9*	0.6 ± 0.3#†	1.5 ± 0.5
	%	0.64 ± 0.13	2.7 ± 1.4*	1.0 ± 0.5#†	2.7 ± 0.1
Eosinophils	cells / ml	6.2 ± 2.0	39.5 ± 3.3*	21.6 ± 2.9*#†	38.4 ± 3.6*
	%	14.9 ± 5.0	58.1 ± 4.8*	37.7 ± 5.1*#†	69.8 ± 6.6*

* $p < 0.05$ compared with the intact animals; # $p < 0.05$ compared with the "Ovalbumin" group; † $p < 0.05$ compared with the "Ovalbumin + cromoglycate" group

Daily ruzam inhalations for 6 days at the final stage of allergic inflammation modeling increased the BALF volume returned. This parameter was equal to that in the intact guinea pigs (Table 1). Sodium cromoglycate did not change the BALF volume returned.

A trend toward a decrease in cytosis in BALF was revealed in both groups of animals treated with either ruzam or sodium cromoglycate. However, the number of eosinophils and neutrophils reduced significantly only in the "Ovalbumin + ruzam" group.

Sodium cromoglycate contributed to an even greater decrease in the number of macrophages in BALF, whereas with ruzam inhalations, the number of these cells rose. As a result, this parameter in the "Ovalbumin + ruzam" group did not differ from that in the intact animals, but was more than twice higher than in the "Ovalbumin + cromoglycate" group (Table 1).

The number of lymphocytes in BALF tended to increase with ruzam and to decrease with sodium cromoglycate. As a result, the absolute and relative number of lymphocytes in the animals receiving ruzam was about twice as high as in the guinea pigs after reference drug inhalation.

Immunization of the guinea pigs with ovalbumin led to significant morphological changes in the airways, a combination of which can be characterized as bronchitis and obstructive emphysema. Dystrophic epithelial changes and extensive areas of desquamation were detected. In the bronchial lumen, in addition to desquamated epithelial cells, large numbers of eosinophils and neutrophils and single macrophages were present. Diffuse focal polymorphonuclear leukocyte (eosinophil and neutrophil), lymphocyte, and histiocyte infiltration was noted in the interalveolar septa. The number of polymorphonuclear leukocytes per field of view increased more than two-fold in comparison with the intact animals (Fig. 1). A statistically unconfirmed trend toward a decrease in the number of mast cells per field of view was revealed; degranulation parameters in these cells did not change significantly. The density of bronchus-associated lymphoid tissue increased approximately two-fold (Fig. 2).

The tested and reference listed drugs in the model of allergic airway inflammation approximately equally reduced eosinophil and neutrophil infiltration in the lungs: following ruzam inhalation, the number of polymorphonuclear leukocytes in the interalveolar septa decreased by 3.6 times, and after sodium cromoglycate – by 3 times (Fig. 1). No significant differences between the groups of guinea pigs receiving the compared drugs were revealed for this parameter: in both cases, the number of polymorphonuclear leukocytes decreased to the same level as in the intact animals.

Inhalations with ruzam or sodium cromoglycate did not significantly change the number of mast cells and their degranulation parameters in the lungs of the ovalbumin-sensitized guinea pigs. Besides, neither of them had any effect on the density of bronchus-associated lymphoid follicles (Fig. 2).

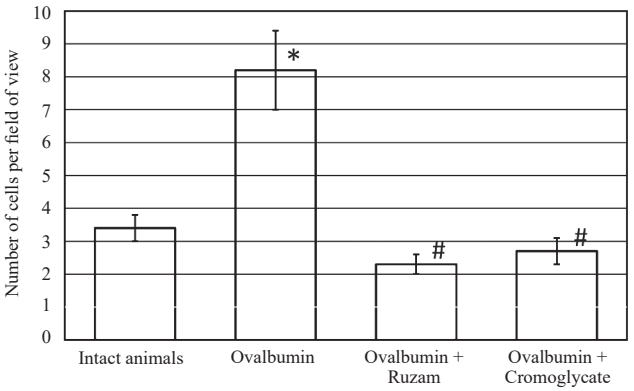

Fig. 1. The effects of ruzam and sodium cromoglycate on the number of polymorphonuclear leukocytes in the interalveolar septa in the guinea pigs with ovalbumin-induced allergic airway inflammation, $M \pm SE$: * $p < 0.05$ compared with the intact animals; # $p < 0.05$ compared with the "Ovalbumin" group

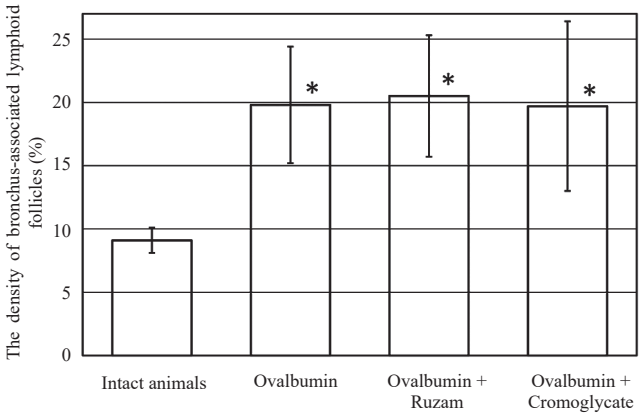

Fig. 2. The effects of ruzam and sodium cromoglycate on the density of bronchus-associated lymphoid follicles in the ovalbumin-sensitized guinea pigs, $M \pm SE$: * $p < 0.05$ compared with the intact animals

The effect of ruzam on mast cell and basophil degranulation in vitro

The compound 48 / 80 in the test systems *in vitro* quite expectedly increased DI in rat peritoneal mast cells by 6 times and in rabbit blood basophils by 2 times in comparison with that in the intact cells (Table 2).

Ruzam in a wide range of concentrations (8.4 – 840 μg / ml) reduced the index of 48 / 80-induced mast cell degranulation in rats more than two-fold (Table 2). However, the effect has not proven to be dose dependent. The reference listed drug, hydrocortisone 21-hemisuccinate, has shown to inhibit degranulation only at the highest concentrations used (10^{-3} M).

Similar patterns were revealed when evaluating the effect of ruzam and the reference listed drug on degranulation of rabbit blood basophils induced by the histamine liberator (Table 2). Ruzam at all the concentrations used reduced DI by 1.5 times, while hydrocortisone 21-hemisuccinate demonstrated a comparable effect only at the concentration of 10^{-3} M.

The addition of ovalbumin into the culture of basophils obtained from the blood of guinea pigs previously immunized with this antigen doubled DI in these cells compared with that in unstimulated basophil cultures that served as controls in this test system (Fig. 3).

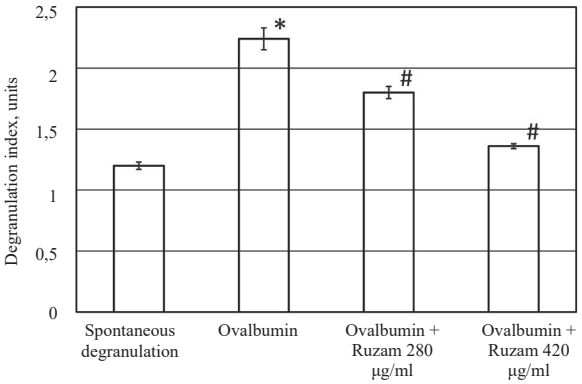

Fig. 3. The effect of ruzam on ovalbumin-induced degranulation of basophils in the ovalbumin-sensitized guinea pigs *in vitro*, $M \pm SE$: * $p < 0.01$ compared with spontaneous degranulation; # $p < 0.01$ compared with ovalbumin-induced degranulation without additional effects

Table 2

The effects of ruzam and hydrocortisone 21-hemisuccinate on *in vitro* degranulation of mast cells and basophils induced by the compound 48 / 80, $M \pm SE$

Options for influencing the cultured cells			Degranulation index, units	
			Rat peritoneal mast cells	Rabbit blood basophils
Intact cells			0.40 ± 0.05	0.70 ± 0.05
Cells stimulated by the compound 48/80	No additional effects		2.40 ± 0.10*	1.40 ± 0.10*
	Ruzam	840 μg / ml	1.10 ± 0.10*#	0.90 ± 0.05#
		84 μg / ml	1.10 ± 0.10*#	1.00 ± 0.05
		8.4 μg / ml	1.12 ± 0.10*#	0.95 ± 0.05
	Hydrocortisone 21-hemisuccinate	10^{-3} M	1.10 ± 0.05*#	0.80 ± 0.05#
		10^{-4} M	1.28 ± 0.05*	1.25 ± 0.05*
		10^{-5} M	1.50 ± 0.05*	1.65 ± 0.05*

* $p < 0.01$ compared with the intact cells; # $p < 0.01$ compared with the cells stimulated by the compound 48 / 80 without additional effects

Ruzam dose-dependently suppressed ovalbumin-induced degranulation of basophils in the sensitized animals. At the same time, at both concentrations used (280 and 420 μg / ml), the drug reduced DI to a level that was not significantly different from the control values.

DISCUSSION

The morphological and cytological changes in the model of allergic airway inflammation observed in our study are generally consistent with the results of similar studies in ovalbumin-induced asthma models [16, 17]. At the same time, when determining the number of mast cells in the lung tissue and their DI, we did not reveal significant differences compared with the intact animals. We even revealed a slight trend toward a decrease in the number of these cells, which partly contradicts previously published data on expansion of mast cells in the peribronchial tissue in patients with asthma [18] and animals with chronic allergic airway inflammation induced by ovalbumin [16].

Despite the fact that mature mast cells reside in tissues for a long time and are able to withstand repeated cycles of degranulation [19], some authors point out the possibility of temporary depletion of the population of these cells following intense degranulation [20, 21]. We conducted a histologic study of the lungs 24 hours after inhalation of the last challenging dose of ovalbumin, which *a priori* should cause massive and rapid degranulation of mast cells in the airways. This, in turn, could lead to a short-term decrease in the number of these cells in the lung tissue, masking or leveling their expansion in other, longer periods.

This limitation of the reproduced model did not allow us to evaluate the effectiveness of ruzam and the reference listed drug (sodium cromoglycate) by changes in the number of mast cells in the lung tissue and their DI *in vivo*. However, it did not reduce the informative value of assessing the anti-allergic effects of the compared pharmacological substances by other important criteria.

Ruzam increased the BALF volume returned, which indicated reduction of bronchial obstruction caused by allergic inflammation. Sodium cromoglycate was ineffective in this parameter.

A fundamentally different nature of the effect of ruzam and the reference listed drug on the cellular composition of BALF was revealed in the model of ovalbumin-induced airway inflammation. A combination of cytological changes caused by the use of ruzam can be assessed as suppression of eosinophilic and neutrophilic inflammation with simultaneous mobilization of effector cells in the immune response against pathogens (lymphocytes and macrophages). The reference listed drug, on the contrary, did not change the number of polymorphonuclear leukocytes in BALF, but caused a trend toward a decrease in the number of lymphocytes and macrophages.

At the same time, in the context of using both drugs, similar-amplitude suppression of the eosinophil and neutrophil infiltration of the interalveolar septa in the lung tissue samples was found.

The development of allergic inflammation in the airways was accompanied by hyperplasia of bronchus-associated lymphoid tissue consisting of many inducible lymphoid follicles. These ectopic lymphoid formations, currently classified as tertiary lymphoid tissues, are clusters of immune cells that resemble secondary lymphoid organs in their follicular structure. Inducible lymphoid follicles are formed in peripheral non-lymphoid tissues in response to the effects of various triggers, including antigens [22].

In the model of allergic inflammation reproduced by us, the development of tertiary lymphoid organs in the bronchi was caused by repeated ovalbumin inhalations to sensitized animals. The fact that a 6-day course of inhalations with ruzam or sodium cromoglycate at the final stage of allergic inflammation modeling did not change the density of bronchus-associated lymphoid follicles in the guinea pigs is quite understandable. It is known that even a single exposure of the airways to antigenic stimuli induces the development of bronchus-associated lymphoid structures that exist for at least 4 weeks after antigen clearance, reaching a peak in their development on days 8–12 [22]. Obviously, a course of inhalations with ruzam or the reference listed drug in our study was too short to cause noticeable regression of lymphoid follicles, which had formed earlier under the influence of allergenic stimuli and had not completed a natural course of their evolution and involution. Presumably, longer and / or earlier use of ruzam in such a model *in vivo* could confirm the anti-allergic effects of the drug by this parameter as well.

Cross-linking of high-affinity IgE receptors (FcRI) on mast cells by allergens causes rapid release of proinflammatory mediators that stimulate not only smooth muscle contraction and mucus secretion [23, 24], but also fibroblast proliferation and collagen synthesis, which leads to airway remodeling in asthma

and fibrosis of other tissues susceptible to allergic inflammation [25–27].

Mast cells along with basophils play an essential role in IgE-dependent allergic reactions [28]. When the latter are degranulated, a large number of phlogogenic mediators are released, provoking the development of asthma, allergic rhinitis, urticaria, and many other, not only allergic, diseases [30].

Given the above as well as the limitations that we encountered when assessing the number and functional state of mast cells in the *in vivo* model, the data obtained in the *in vitro* test systems on the pronounced ability of ruzam to suppress degranulation of mast cells and basophils induced by the histamine liberator and degranulation of basophils induced by ovalbumin in the animals sensitized with this antigen are very valuable. Therefore, the studied drug directly blocked both IgE-dependent and IgE-independent mechanisms of mast cell and basophil degranulation. It is worth noting that ruzam was effective in suppressing degranulation of cells in three mammalian species, one of which (rabbit) did not belong to rodents. This seems inspiring in terms of extrapolating the results obtained from animals to humans.

The experimental data described in this study are consistent with the results of clinical trials of ruzam in the treatment and prevention of allergic diseases highlighted in the recent review [4]. Biological effects of the drug in the *in vivo* and *in vitro* models confirm the ability of ruzam to suppress type 2 (Th2) immune responses underlying the pathogenesis of most allergic diseases.

Considering the chemical nature of ruzam (the complex of bacterial lipoproteins), we believe that the drug implements its biological / pharmacological effects through Toll-like receptors (TLR) of innate immunity. TLR2 and its heterodimers TLR1 / TLR2 and TLR / TLR6 are the most likely molecular targets of ruzam in this case. TLR2-mediated signals can both activate and regulate immune responses depending on the nature and dose of ligands (lipopeptides, lipoteichoic acids, proteoglycans), the variant of homo- or heterodimerization of this receptor, the initial state of the body, and a number of other factors [31].

In the context of interpretation of the obtained data, the previously revealed ability of lipopeptides to suppress allergic inflammation via the TLR2 signaling pathway in ovalbumin-induced asthma models *in vivo* is of interest. This pathway contributed to T2→T1 polarization of the predominant immune response and potentiation of immunoregulatory mechanisms [32, 33]. Defective TLR2, on the contrary, aggravated ultrastructural, cytological, and molecular signs of ovalbumin-induced type 2 inflammation in the airways of sensitized animals [34]. In *in vitro* test systems, TLR2 ligands suppressed IgE-dependent [35, 36] and IgE-independent mast cell degranulation [37]. We consider testing the hypothesis on the key role of TLR2 and its heterodimers in pharmacological effects of ruzam promising for further research not only to refine the molecular mechanisms of its effect, but also to expand and optimize the scope and methods of its clinical use.

CONCLUSION

The anti-allergic effect of ruzam, the extract from the culture of the thermophilic *S. aureus* strain C2, was confirmed in the test systems *in vivo* and *in vitro*. In particular, in the model of ovalbumin-induced allergic asthma in guinea pigs, the drug reduced the degree of airway obstruction and the severity of neutrophilic and eosinophilic inflammation, while mobilizing effector cells of the immune response against pathogens (lymphocytes and macrophages). And in the *in vitro* models, ruzam suppressed both IgE-independent degranulation of mast cells and basophils induced by the histamine liberator and IgE-dependent degranulation of basophils in the sensitized animals. A hypothesis was put forward about the TLR2-mediated nature of the main biological and pharmacological effects of the drug identified in this study and described in other scientific papers.

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