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ISOLYQUIRITIGENIN AFFECTS PHAGOCYTES FUNCTIONS AND INCREASES MICE SURVIVAL RATE IN STAPHYLOCOCCAL INFECTION

E.A. Solenova, S.I. Pavlova

Chuvash State University n. a. I.N. Ulyanov
15, Moskovsky Ave., Cheboksary, Chuvash Republic, Russia, 428015

E-mail: elensoul@mail.ru

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The results of studying the effect of isoliquiritigenin on animal survival in the model of staphylococcal infection and the function of human and animal phagocytes are presented in this article.

The aim of the investigation was to study the effect of an isoliquiritigenin preliminary administration on the survival of animals against the background of staphylococcal infection, as well as on the function of phagocytes in mice and humans.

Materials and methods. To assess the survival of Balb/C mice, a model of infection caused by *Staphylococcus aureus J49 ATCC 25923* with the construction of Kaplan-Meier curves, was used. The effect on the phagocytes functions was studied by assessing the peptone-induced migration of phagocytes into the abdominal cavity of Balb/C mice, the absorption activity of phagocytes (neutrophils and monocytes) of human blood, as well as their production of reactive oxygen intermediates (ROIs) using a flow cytometry.

Results. It was found out that a preliminary triple intraperitoneal administration of isoliquiritigenin (30 mg/kg) increases the survival rate of Balb/C mice in staphylococcal infection caused by *Staphylococcus aureus J49 ATCC 25923.* At the same time, isoliquiritigenin dose-dependently activates the production of reactive oxygen intermediates by human neutrophils and monocytes without statistically significantly suppressing a phagocytic activity of monocytes and neutrophils against fluoresceinisothiocyanate-labeled *Staphylococcus aureus J49 ATCC 25923,* as well as peptone-induced migration of phagocytes into the abdominal cavity of mice.

Conclusion. Thus, a preliminary administration of isoliquiritigenin increases the survival rate of mice with staphylococcal infection and increases the production of reactive oxygen intermediates by phagocytes. The data obtained, can become the basis for further research of antibacterial and immunotropic effects of isoliquiritigenin in order to find new drugs for the treatment of staphylococcal infection.

Keywords: isoliquiritigenin; *Staphylococcus aureus;* innate immunity; phagocytosis; oxidative burst; phagocyte migration

List of abbreviations: ROI(s) – reactive oxygen intermediate(s)**;** DHR 123 – dihydrorhodamine 123**;** DMSO – dimethylsulfoxide**;** ISL – isoliquiritigenin; CFUs – colony-forming units**;** ConA – concanavalin A**;** NADP-oxidase – nicotinamide adenine dinucleotide phosphate oxidas**;** NTs – neutrophil traps**;** FITC – fluorescein isothiocyanate; PMA – phorbolmyristate acetate; phorbol-12 myristate-13-acetate; MTT – 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; OD – optical density

ИЗОЛИКВИРИТИГЕНИН ВЛИЯЕТ НА ФУНКЦИИ ФАГОЦИТОВ И ПОВЫШАЕТ ВЫЖИВАЕМОСТЬ МЫШЕЙ ПРИ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

Е.А. Солёнова, С.И. Павлова

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Чувашский государственный университет им. И.Н. Ульянова»
428015, Россия, Чувашская Республика, г. Чебоксары, пр-т. Московский, д. 15

E-mail: elensoul@mail.ru

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RESEARCH ARTICLE

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В данной статье представлены результаты изучения влияния изоликвиритигенина на выживаемость животных в модели стафилококковой инфекции и функции фагоцитов человека и животных.

Цель. Изучить влияние предварительного введения изоликвиритигенина на выживаемость животных на фоне стафилококковой инфекции, а также на функции фагоцитов мышей и человека.

Материалы и методы. Для оценки выживаемости мышей линии Balb/C использовали модель инфекции, вызванной *Staphylococcus aureus J49 ATCC 25923,* с построением кривых Каплан-Мейера. Влияние на функции фагоцитов изучали, оценивая пептон-индуцированную миграцию фагоцитов в брюшную полость мышей Balb/C, поглотительную активность фагоцитов (нейтрофилов и моноцитов) крови человека, а также продукцию ими активных форм кислорода с помощью проточной цитометрии.

Результаты. Установлено, что предварительное трехкратное внутрибрюшинное введение изоликвиритигенина (30 мг/кг) увеличивает выживаемость мышей Balb/C при стафилококковой инфекции, вызванной *Staphylococcus aureus J49 ATCC 25923*. При этом изоликвиритигенин дозозависимо активирует продукцию активных форм кислорода нейтрофилами и моноцитами крови человека, статистически значимо не подавляя фагоцитарную активность моноцитов и нейтрофилов в отношении флюоресцеинизотиоцианат-меченого *Staphylococcus aureus J49 ATCC 25923*, а также пептон-индуцированную миграцию фагоцитов в брюшную полость мышей.

Заключение. Таким образом, предварительное введение изоликвиритигенина повышает выживаемость мышей при стафилококковой инфекции и увеличивает продукцию активных форм кислорода фагоцитами. Полученные данные могут стать основой для дальнейшего исследования антибактериальных и иммунотропных эффектов изоликвиритигенина с целью изыскания новых лекарственных средств для лечения стафилококковой инфекции.

Ключевые слова: изоликвиритигенин; *Staphylococcus aureus*; врожденный иммунитет; фагоцитоз; кислородный взрыв; миграция фагоцитов

Сокращения: АФК – активные формы кислорода; ДГР 123 – дигидродамин 123; ДМСО – диметилсульфоксид; ИЛГ – изоликвиритигенин; КОЕ – колониеобразующие единицы; КонА – конканавалин А; НАДФН-оксидаза – никотинамидадениндинуклеотидфосфатоксидаза; НЛ – нейтрофильные ловушки; ФИТЦ – флюоресцеинизотиоцианат; ФМА – форбол-12-миристат-13-ацетат; МТТ – 3-(4,5-диметилтиазол-2-ил)-2,5-дифенил-тетразолиум бромид; OD – оптическая плотность.

INTRODUCTION

Recognition and elimination of microbial pathogens by a macroorganism occurs due to the activation of innate and adaptive immunity. Innate immunity prevents the introduction of microbes into tissues and is able to remove them before the mechanisms of the acquired immunity are activated. Innate immunological responses to the pathogen are almost instantaneous and are mainly based on the reactions of inflammation and phagocytosis, while the adaptive immunological response turns on only after a few days (optimally 7–14 days), since it requires proliferation and differentiation of lymphocytes.

A significant bacterial load in the model of acute bacterial infection causes death in laboratory animals during the first days of observation. This model of infection makes it possible to assess not only the antibacterial effects of the studied compounds, but also its influence on the functions of the effectors of innate immunity [1], among which phagocytes play an important role.

Polyphenolic compounds of higher plants (flavonoids) have a wide spectrum of biological activity, including antimicrobial and immunomodulatory effects [2]. For example, a parenteral administration of licorice root flavonoids increases the resistance of mice to acute staphylococcal infection at the doses that did not significantly affect the functions of innate immunity effectors [3], but prevented the activation and proliferative response of lymphocytes [4]. Dozens of different flavonoids have been isolated from licorice roots, one of the main of them being isoliquiritigenin (ISL). At various concentrations, ISL exhibits antibacterial properties, influences the proliferation of lymphocytes and their secretion of cytokines at the early stages of the immune response during staphylococcal infection in mice [2].

THE AIM of the investigation was to study the effect of isoliquiritigenin on the phagocytes functions in mice and humans as well as its effect against the background of staphylococcal infection in mice.

MATERIALS AND METHODS

Test agent

ISL (98% purity, Xi'An YiyangBio-Tech Co., China) was used as a test substance. In the experiments, a solution of ISL in dimethyl sulfoxide (DMSO, Panreac, Spain) was used so that *in vitro* the final concentration of the solvent in the test samples did not exceed 1%. Considering that the MIC of ISL against *S. aureus J49 ATCC 25923* is 64 μg/ml [5], ISL in the concentration range of 16–128 μg/ml was used for *in vitro* experiments. In the series of *in vivo* experiments, the ISL matrix solution in DMSO was diluted in phosphate-buffered saline (pH=7.4, PanEcoLLC, Russia), injected intraperitoneally in the volume of 0.5 ml as a true solution with a DMSO concentration of no more than 5%. Given the low toxicity and bioavailability of ISL [2], in *in vivo* experiments, ISL was injected intraperitoneally at the total dose of 30 mg/kg. The control samples / groups were injected with appropriate volumes / concentrations of solvent instead of ISL.

Bacterial strain and conditions for its cultivation

The cultivation of the *S. aureus J49 ATCC 25923* strain (Federal State Budgetary Institution Scientific

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Center for Expertise of Medicinal Products of the Ministry of Health of Russia (Moscow, Russia)), was carried out in Mueller-Hinton broth (Medicaplus LLS, Russia) at 37°C in glass aerated vials. For *in vitro* and *in vivo* experiments, dilutions for the cultivation and intraperitoneal administration to animals were prepared from an overnight bacterial culture in the middle log phase. To count colony forming units (CFUs), the optical density (OD) of the bacterial suspension was measured at 630 nm in a microplate photometer (ImmunoChem 2100, USA) using the McFarland standard, based on the following ratio: 1 optical unit OD630 = 8.5×108 CFUs/ml.

Experimental animals

Balb/C mice (males, 20–22 g, 6–8 weeks old) were obtained from the Research and Production Enterprise "Nursery of Laboratory Animals" of the Institute of Biology of the Russian Academy of Sciences (Pushchino, Russia). The animals were cared for and treated in accordance with the ARRIVE principles [6]. The animals were kept with free access to food and water. For the experiments, mice were randomly assigned to groups of 8. Withdrawal from the experiment was carried out without anesthesia by decapitation or cervical dislocation. When performing the experiments, the provisions of the Declaration of Helsinki (Brazil, 2013) were observed, the protocol of these experiments was approved by the ethical committee of Chuvash State University n. a. I.N. Ulyanov "(Protocol No. 20-04 dated April 17, 2020).

Obtaining blood from healthy volunteers

To determine the absorption activity of human blood phagocytes and the production of reactive oxygen intermediates by them, on the day of the experiment, the blood was taken into heparinized test tubes from healthy volunteers (60 people) aged 18–25 years after receiving voluntary informed consent. The protocol of these experiments had been approved by the ethics committee of the FSBEI HE "Chuvash State University n. a. I.N. Ulyanov" (Protocol No. 20–04 dated April 17, 2020).

Preparation of fluorescein isothiocyanate-labeled *S. aureus*

To inactivate S. aureus J49 ATCC 25923, an overnight bacterial culture was exposed in the water bath at 95ºC for 30-40 minutes, then it was centrifuged (1000 g, 25 min.) [7]. The killed precipitated bacteria were scoured once with a carbonate-bicarbonate buffer (0.1 M, pH 9.5), and a cell suspension was prepared with a bacterial concentration of 2×108 microbial bodies per ml. Fluorescein isothiocyanate (FITC) dissolved in DMSO, was added to the inactivated bacterial suspension at the rate of 0.05 mg per 108 bacteria, followed by incubation for 1 h at the room temperature without access to light. Then, the inactivated bacterial cells were scoured three times with phosphate-buffered saline by centrifugation (1000 g, 10 min), and a suspension of FITC-labeled bacteria was diluted to a concentration of 5×108 microbial bodies per ml. Aliquots were stored at –70°C.

Model of *S. aureus* infection in mice

A suspension of S. aureus in a phosphate-salt buffer was administered intraperitoneally 10^9 CFUs/per mouse. The day of infection was considered the zero day of the experiment. The survival of mice was evaluated every 6 hours on the first day and daily from the second day and for the next 20 days of the experiment. The experimental animals were injected with ISL before the infection (a total dose of 30 mg/kg, three times after 4 hours, intraperitoneally). As a reference, the animals of the control group were injected with the appropriate volumes and concentrations of the solvent.

Assessment of phagocyte chemotaxis

To assess the migration of phagocytes into the abdominal cavity, the authors were guided by the method proposed by Miyazaki [8]. The experimental animals were divided into 4 groups, which received the following: group 1 (negative control) – a sterile phosphate-salt buffer three times (0.5 ml, intraperitoneally); group 2 – a sterile peptone solution in a phosphate-salt buffer (3% – 3 ml, intraperitoneally); group 3 – a solvent three times (5% – 0.5 ml, intraperitoneally), then – a sterile peptone solution in a phosphate-salt buffer (3% – 3 ml, intraperitoneally); group 4-ISL (three times, intraperitoneally), then a sterile solution of peptone in a phosphate-salt buffer (3% – 3 ml, intraperitoneally). After 24 hours and 72 hours, the animals were withdrawn from the experiment and 20 ml of phosphate-salt buffer was injected intraperitoneally. After palpatory massaging of the abdomen, the rinsewaters were taken into plastic tubes for the subsequent centrifugation. The deposited cells were counted in Gorjaev's chamber. After that, the stimulation index was calculated as the ratio of the number of cells in the groups receiving peptone, to the number of cells in the negative control group.

Assessment of phagocyte activity absorption

The phagocyte activity absorption was assessed using a flow cytometry [7]. For this purpose, ISL was added to the samples of heparinized human blood at the concentrations of 16–128 μg/ml. The experimental samples were cultured for 30 minutes (t=37°C, ϕ=100%, CO_2=5%). Then, FITC-labeled *S. aureus* was added to the samples, and the incubation was continued for 30 minutes (t=37°C, ϕ=100%, CO_2=5%). After the incubation time for lysis of erythrocytes, the lysis solution (Backman Coulter, USA) was added and

incubated for 10 minutes. The samples were analyzed on a Cytomics FC500 flow cytometer (Backman Coulter, USA) and the phagocytic index (the number of phagocytes absorbing FITC-labeled bacteria to the total number of phagocytes) and the fluorescence intensity were calculated.

Assessment of producing reactive oxygen intermediates by phagocytes

The producing reactive oxygen intermediates by phagocytes was assessed using a flow cytometry [7]. To perform the test, PMA (0.1 μg/ml, samples with activated phagocytes) or 0.2% EDTA (control, non-stimulated samples of phagocytes <1% DMSO), phorbol-12-myristate-13-acetate (PMA)-induced "oxidative burst" were added to the samples of heparinized human blood, pre-incubated with ISL at concentrations of 16-128 μg/ml for 30 minutes (incubation conditions: t=37ºC; ϕ=100%; CO_2=5%), and incubated for 10 minutes. After the incubation, the fluorogenic substrate dihydrorhodamine 123 (GDR 123) was added to the samples and incubated for another 10 minutes. The lysed blood samples were analyzed on a Cytomics FC500 flow cytometer (Backman Coulter, USA) to determine the percentage of activated neutrophils and monocytes, as well as indicators of spontaneous and stimulated fluorescence intensity.

Statistical analysis

All experiments were performed in at least three repetitions. The data obtained were statistically processed using GraphPadPrism 8.4.0 Software. To assess the dynamics of the death of mice, Kaplan-Meier curves were constructed. The results obtained followed the law of normal distribution, were processed by the methods of variation statistics and were presented as the arithmetic mean (M) ± standard error of the mean (SEM). The significance of the differences between the groups in the experiments, was determined by the Student's test, conducting a pairwise comparison. The differences were considered significant at p <0.05, where p is the level of significance.

RESULTS

Effect of ISL on the survival rate of Balb/C mice infected with *S. Aureus J49 ATCC25923*

It was established that on the second day of the experiment, when infected with 109 CFU/per mouse, the beginning of death of the animals in both experimental groups was noted. In the control group, mortality increased more dynamically, and by the 4th day, the survival rate was only 17.0±7.6% (Fig. 1). In the group that received preliminary injections of ISL at the dose of 30 mg/kg, the survival rate was significantly higher, and on the 7th day of the experiment it was 67.0±16.5% (p <0.05).

Effect of ISL on peptone-induced migration of phagocytes into the abdominal cavity of mice

The migration of phagocytes into the abdominal cavity was assessed by calculating the stimulation index – the number of cells stimulated by intraperitoneal injection of peptone, relative to phosphate-buffered saline. The stimulation indices in mice treated with ISL and in control animals stimulated with peptone, did not differ significantly (Fig. 2). So, after 24 hours, the stimulation index in the control group was 2.4±0.1 vs 2.0±0.1 in the ISL-treated group; after 72 hours, the stimulation index values were characterized by values of 1.6±0.1 (control group) compared with 1.8±0.1 (the group receiving ISL).

Influence of ISL on the absorptive activity of human blood phagocytes

The study of the ISL influence on the absorption activity of phagocytes was carried out by the cytometric method. It was shown that the *in vitro* pretreatment of phagocytes with ISL does not lead to a significant change in the percentage of phagocytic neutrophils and monocytes compared to the control (Table 1).

In the control samples, the phagocytic index in neutrophils was 93.9±6.0%, and in monocytes it was 73.9±14.1%. At the ISL concentration of 128 μg/ml, an insignificant tendency towards a decrease in the phagocytic index of neutrophils (92.5±5.5%) and monocytes (64.1±13.7%) was observed. However, the assessment of the fluorescence intensity showed that, in comparison with the control, in the samples with the addition of ISL, there was an increase in the proportion of fluorescent neutrophils (184.8±44.8 vs 145.5±41.1) and monocytes (58.5±17.2 vs 64.1±18.1).

Effect of ISL on ROIs production by human blood phagocytes

The effect of ISL on reactive oxygen intermediates (ROIs) production by phagocytes was assessed without their activation by PMA (Table 2). Herewith, in the absence of ISL, the proportion of fluorescent neutrophils (3.9±1.8%) and monocytes (3.0±2.0%) was very low. Compared with the control values, the addition of ISL dose-dependently increased the proportion of fluorescent neutrophils at the concentrations of 128 μg/ml (100.0±0.1%; p<0.05), 64 μg/ml (99.6±0.5%; p <0.05), 16 μg/ml (34.7±8.9%; p<0.05), and at the concentrations of 128 μg/ml (86.2±11.7%; p<0.05), 64 μg/ml (47.2±18.7%; p <0.05) is the percentage of fluorescent monocytes (Fig. 3). At the same time, the neutrophil fluorescence intensity significantly differed from the control parameters (2.5±0.3 U) in the presence of 128 μg/ml ISL (6.6±1.6 U; p <0.05), 64 μg/ml (3.8±0.2 U; p<0.05), and monocytes – at 128 μg/ml (3.0±0.15 U; p <0.05).

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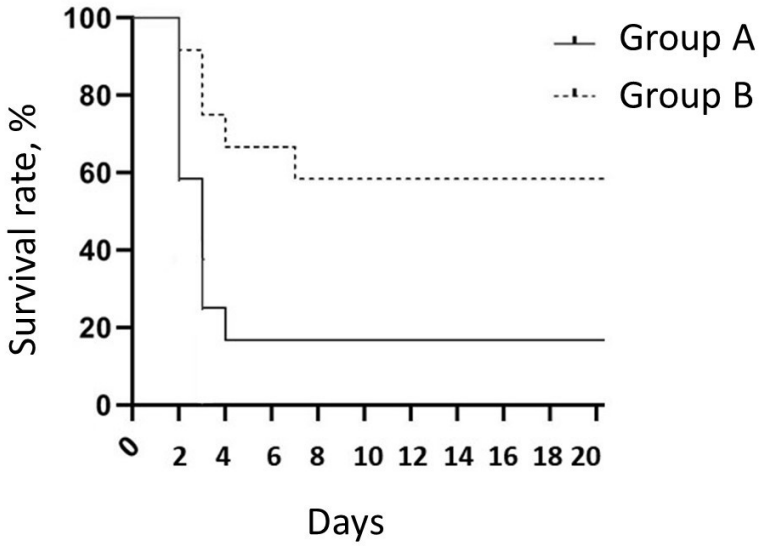

Figure 1 – Survival rate of Balb/C mice infected with *S. aureus J49 ATCC 25923*
Note: group A – control, 10^9 CFUs/per mouse; group B – preliminary injection of ISL (30 mg/kg), 10^9 CFUs/per mouse

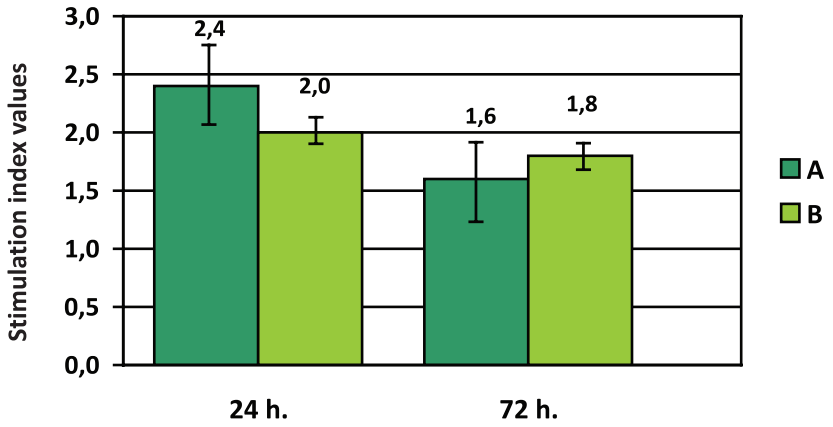

Figure 2 – Effect of ISL on the migration of phagocytes into the abdominal cavity of Balb/C mice
Note: A – control; B – ISL

Table 1 – Influence of ISL on the parameters of the phagocytic index (%) and fluorescence intensity (U) of neutrophils and monocytes of human blood

Phagocytes		Control	128 mkg/ml	64 mkg/ml	16 mkg/ml
Neutrophils	%	93,9±6,0	92,5±5,5	92,1±7,6	95,6±2,4
	U.	145,5±41,1	184,8±44,8 *	129,1±55,6	118,1±40,5
Monocytes	%	73,9±14,1	64,1±13,7	71,4±11,2	71,4±11,4
	U.	64,1±18,1	58,5±17,2 *	51,4±21,9	45,4±15,4

Note: * – reliable changes at p <0.05

Table 2 – Effect of ISL on production of ROIs by neutrophils and macrophages/monocytes of human blood

			Control	ISL		
				128 mkg/ml	64 mkg/ml	16 mkg/ml
Neutrophis	PMA activation	%	93.3±14.9	100.0±0.1	99.9±0.1	99.5±0.4
		Ед	11.3±3,7	15.8±3.0 *	14.8±4.2 *	14.1±5.2
	Without PMA activation	%	3.9±1.8	100.0±0.1 *	99.6±0.5 *	34.7±8.9 *
		Ед	2.5±0.3	6.6±1.6 *	3.8±0.2 *	2.1±0.2
Monocytes	PMA activation	%	44.5±27.2	97.8±1.8 *	88.1±7.8 *	62.7±17.7
		Ед	3.4±0.7	6.0±1.3 *	4.6±1.1*	4.0±1.0
	Without PMA activation	%	3.0±2,0	86.2±11,7 *	47.2±18.7 *	4.6±2.4
		Ед	2.3±0.5	3.0±0.15 *	2.3±0.2	2.4±0.7

Note: Unit (U) – units of fluorescence intensity (UFI); * – reliable changes at p <0.05

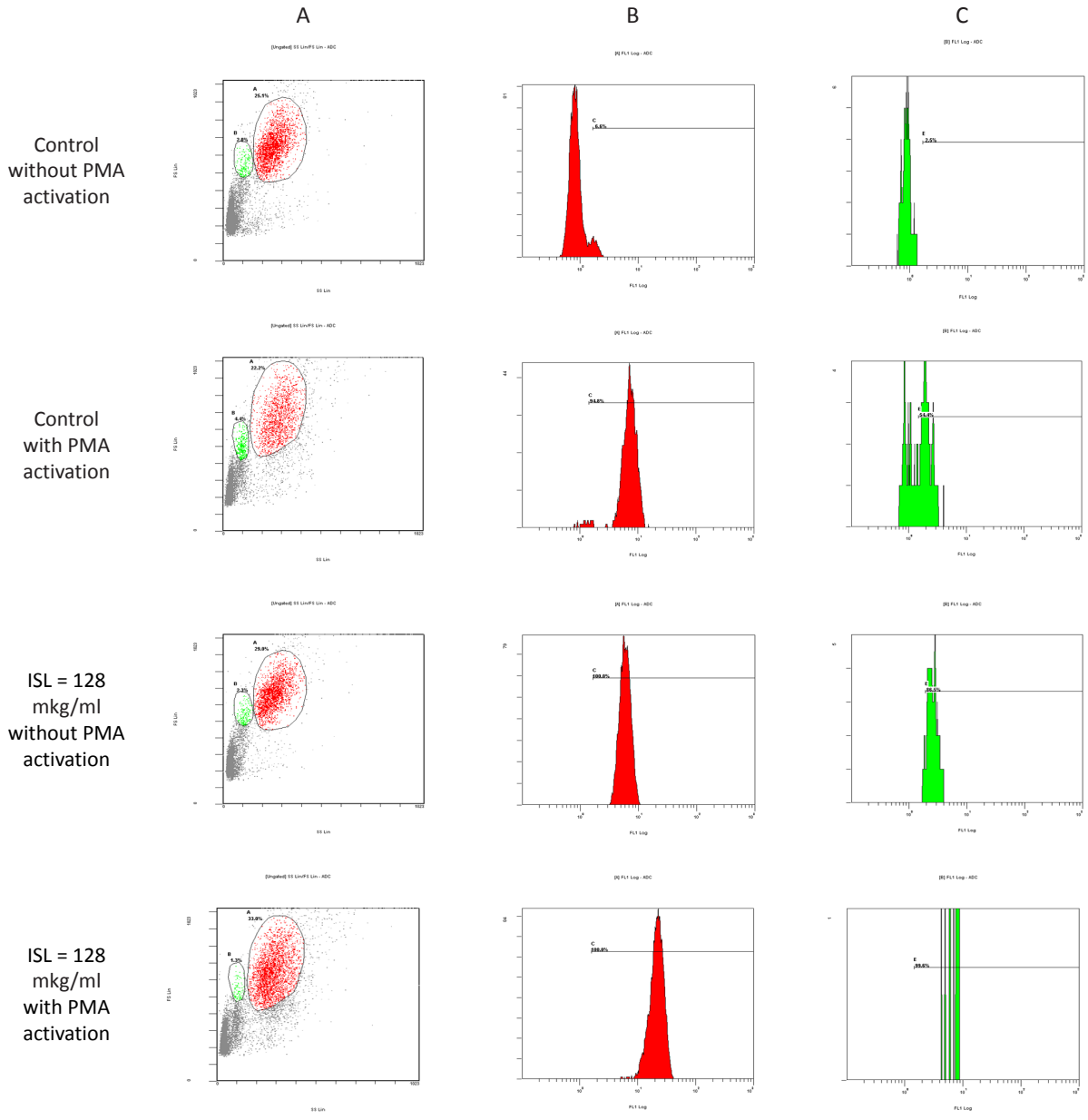

Figure 3 – Distribution of blood cells by lateral light scattering (A) and fluorescence histogram corresponding to the gates of neutrophils (red) and macrophages/monocytes (green) (B, C)

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The effect of ISL on the ability of human phagocytes to produce ROIs in response to the protein kinase C activator – PMA – has also been studied. In the control samples, the number of neutrophils producing ROIs was 93.3±14.9%, monocytes – 44.5±27.2% (Table 2). In the presence of ISL at the concentrations of 128 μg/ml, 64 μg/ml, and 16 μg/ml, an insignificant tendency towards a dose-dependent increase in the proportion of fluorescent neutrophils was observed: 100.0±0.1% (p= 0.07), 99.9±0, 1% (p = 0.07) and 99.5±0.4% (p=0.08), respectively (Fig. 2). Compared with the control samples (11.3±3.7 U) in the presence of ISL at the concentrations of 128 μg/ml (15.8±3.0 U, p <0.05) and 64 μg/ml (14.8±4.2 U, p<0.05), a significant increase in fluorescence intensity was noted, and at the concentration of 16 μg/ml, there was just a tendency to that (14.1±5.2 U, p = 0.09). When assessing the functions of monocytes in the presence of ISL at the concentrations of 128 μg/ml (97.8±1.8%, p<0.05), 64 μg/ml (88.1±7.8%, p <0.05), there was a dose-dependent significant increase observed in the number of fluorescent cells compared to the control values (44.5±27.2 U). The data on the increase in the proportion of fluorescent monocytes upon the exposure to ISL at the concentrations of 64–128 μg/ml were comparable with a dose-dependent increase in the degree of their fluorescence (Table 2).

DISCUSSION

The present research is devoted to the study of the ISL influence on some mechanisms of innate immunity (a phagocyte function) as possible factors affecting the survival in the early stages of bacterial infection. A parenteral administration of ISL before infecting the mice, increased the survival rate of the experimental animals in the model of staphylococcal sepsis and septic shock, when the animals died in the first 24–48 hours against the background of a pronounced bacterial load of 1.5×10^9 CFUs/per mouse [5]. In the present experiment, at the infectious dose of 10^9 CFUs/per mouse, the dynamics of the death rate was different (the animals died later), but the preliminary intraperitoneal injection of ISL was also effective and increased the lifespan of Balb/C mice.

The experiments have shown that ISL has an antibacterial activity [5]. However, the antibacterial activity is manifested at sufficiently high concentrations (64 μg/ml), the probability of reaching and maintaining of which in biological fluids/foci of infection *in vivo,* is low, especially since ISL has a rather short half-life [9, 10]. Taking into account these features, in the present work the emphasis was made on the study of the ISL influence on the functions of phagocytes.

Phagocytes are effector cells of innate immunity that provide the first line of defense against invasion of infectious pathogens. The main stages of phagocytic reactions are chemotaxis, absorption, killing and digestion of an infectious pathogen. The effect on chemotaxis was studied in a peptone-induced migration of phagocytes into the abdominal cavity of mice [8]. It was found that ISL has no suppressive effect on the migration of phagocytes both after 24 hours (chemotaxis of predominantly neutrophils) and after 72 hours (chemotaxis of predominantly macrophages).

The absorption of an infectious agent by phagocytes is realized through such mechanisms as: convergence of a phagocyte and a pathogen; establishing contact; preparation for dipping; circumfluence of the pathogen; membrane closure; absorption of an object. These stages were summarized using the cytometric method and FITC-labeled bacteria. The collection strain *S. aureus J49 ATCC 25923* was used as a bacterial agent for phagocytosis. In the studied concentration range (16–128 μg/ml) ISL did not reduce the proportion of neutrophils and monocytes able of absorbing bacterial cells. However, the fluorescence intensity of monocytes when exposed to certain concentrations of ISL, tended to decrease. Anyhow, this fact is not significant, since in high concentrations, ISL is able of realizing a direct antibacterial activity.

Thus, an intraperitoneal administration of ISL at the total dose of 30 mg/kg does not significantly affect the chemotaxis of neutrophils and macrophages in response to the standard migration activator peptone in Balb/C mice. It does not inhibit the absorption function of neutrophils and human blood monocytes against *S. aureus J49 ATCC 25923* in the concentration range of 16–128 μg/ml, either. It should be noted that at the indicated concentrations, ISL strongly inhibits the proliferation of mitogen-activated T-lymphocytes [5], which indicates that ISL exhibits selective suppressive properties in relation to adaptive immunity effectors, without affecting the main innate immune responses of phagocytes.

Killing and digestion of bacterial pathogens by phagocytes occurs due to the oxygen (ROIs production) and nitrogen metabolism. ROIs is a group of highly reactive oxygen-containing chemicals associated not only with pathological (chronic inflammation, pathological cell proliferation) [11], but also with physiological processes (survival, growth, cell proliferation and differentiation, an immune response) [12]. In particular, an important link in the implementation of innate immune responses is the launch of a massive ROI production ("oxidative burst") in phagocytes. It initiates the onset of the "oxidative burst" of nicotinamide adenine dinucleotide phosphate oxidase (NADP oxidase) (in phagocytes, the main isoform is type 2 NADP oxidase) [13], which can be activated in a signaling cascade associated with protein kinase C, phorbol esters, e.g., PMA [14]. In this regard,

the effect of ISL on the digestive ability of phagocytes was assessed by the production of ROIs by neutrophils and monocytes using the fluorogenic substrate of DHR 123, which interacts with ROIs to form fluorochrome rhodamine 123. The intensity of the "oxidative burst" was assessed using a flow cytometry. During the experiments, it was found that the addition of ISL (16–128 μg/ml) even to non-activated PMA cells dose-dependently increased the proportion of fluorescent neutrophils and monocytes, as well as the fluorescence intensity compared to the control samples, which indicates the accumulation of ROIs in phagocytes. The addition of ISL to PMA-activated phagocytes also increased ROIs production compared to the PMA-activation without ISL exposure. In this case, summation effects were observed in terms of fluorescence intensity. Thus, the sum of the fluorescence intensity of PMA-activated neutrophils (11.3±3.7 U) and neutrophils not activated by PMA, but incubated with ISL (128 μg/ml) (6.6±1.6 U), is comparable to the values of fluorescence intensity of PMA-activated neutrophils incubated with ISL at the dose of 128 μg/ml (15.8±3.0 U). A similar summation of the effect was found in monocyte samples.

On the other hand, flavonoids are well known as antioxidants that can reduce ROIs production by phagocytes. For example, resveratrol inhibits the activity of NADP oxidase, myeloperoxidase and, as a consequence, the formation of hypochlorous acid [15]. ROIs are involved in the implementation of one of the mechanisms of the innate immune response – the formation of "neutrophil traps" (NL) [16]. Neutrophils activated by bacteria *(S. aureus* and *E. coli)* or chemical substances (PMA) produce ROIs and form neutrophilic extracellular conglomerates, which are aimed at curbing bacterial dissemination from the focus of infection [17]. It has been demonstrated that flavonoids (epicatechin, catechin hydrate and rutin trihydrate, as well as luteolin, kaempferol) suppress the formation of ROI-dependent NTs [20, 21], and resveratrol improves lungs functions during acute respiratory tract infections or chronic inflammatory lungs diseases [22].

In some biological systems, as well as at high concentrations, natural polyphenols can demonstrate prooxidant properties [23–26]. Thus, ISL increases the production of ROIs in various tumor cells [27], which is considered as one of its antitumor mechanisms. Apparently, similar mechanisms of ROIs production with the participation of flavonoids, are possible in neutrophils and monocytes, as demonstrated in the present study in the samples with ISL. It is possible that this is important in realizing the antistaphylococcal effects of flavonoid compounds. However, one should also take into account the possibility of an irreversible damage to mitochondria, death of macrophages caused by an increase in ROI production and dissemination of absorbed bacteria during incomplete phagocytosis, which has been demonstrated in the study of resveratrol against mycobacteria [28].

CONCLUSION

It has been established that a preliminary intraperitoneal administration of ISL at the dose of 30 mg/kg to male Balb/C mice significantly increases the survival rate of animals in the model of the infectious process caused by *S. aureus J 49 ATCC 25923,* does not inhibit the chemotaxis of neutrophils and macrophages in Balb/C mice. It has also been revealed that ISL at the concentrations of 16–128 μg/ml does not statistically significantly affect the absorption of human peripheral blood by monocytes and neutrophils of *S. aureus J49 ATCC 25923*, but dose-dependently increases the number of phagocytes producing ROIs, as well as the intensity of the "oxidative burst" of activated neutrophils and human peripheral blood monocytes. Thus, these effects together can be considered as one of the possible mechanisms of the mice survival at the early stages of the development of staphylococcal infection in mice.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

E.A. Solenova – execution of experimental work, statistical processing of results, processing of research results, writing the text of an article; S.I. Pavlova – development of the concept and design of the study, management of experimental work, processing of research results, editing the text of the article.

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AUTHORS

Elena A. Solenova – Junior Researcher, Senior Lecturer of the Department of Pharmacology, Clinical Pharmacology and Biochemistry, Chuvash State University n. a. I.N. Ulyanov. ORCID ID: 0000-0001-6104-0864. E-mail: elensoul@mail.ru

Svetlana I. Pavlova – Doctor of Sciences (Medicine), the Head of the Department of Pharmacology, Clinical Pharmacology and Biochemistry, Chuvash State University n. a. I.N. Ulyanov. ORCID ID: 0000-0001-9976-7866. E-mail: flavonoid@yandex.ru