УДК 577.122.34.084.1:547.992.2:546.57-022.532
https://doi.org/10.20538/1682-0363-2021-4-71-78

The effect of coal-derived humic substances and their silver-containing bionanocomposites on arginine balance in peritoneal macrophages of intact mice

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ABSTRACT

Background. Antigen-presenting cells (APCs), especially macrophages, play an important role in the body defense against various pathogens. Their dysfunction and polarization are associated with most inflammatory and autoimmune diseases. The inflammatory process is regulated by activation and / or inhibition of genes differentially expressed by macrophages. Successful correction of inflammation leads firstly to elimination of inflammatory stimuli and then to remodeling and restoration of tissues and organs. It was experimentally confirmed that silver-containing bionanocomposites based on natural humic substances (HS) obtained from coal of different origin, as well as initial matrices of these HS, are capable of activating pro- and anti-inflammatory properties of macrophages.

Aim. To study cytotoxic, pyrogenic, and immunomodulatory properties (arginine balance) of initial HS samples and samples of silver nanoparticles ultradispersed in these HS matrices (HS-AgNPs) in the cell culture of peritoneal macrophages, as well as their effect on pro- and anti-inflammatory properties of APCs.

Materials and methods. Cultural and biochemical methods were used in the study.

Results. The study showed that the samples CHE-K, CHE-AgNPs, CHS-K, and CHP-K increased M1 macrophage polarization due to stimulation of the NO-synthase activity and inhibition of arginase. The samples CHI-K, CHI-AgNPs, CHP-AgNPs, and CHS-AgNPs modulated an alternative M2 or M2-like state of macrophage activation. At the same time, HS are not cytotoxic at effective concentrations, and three out of four studied samples did not contain pyrogenic impurities.

Conclusion. The use of HS and their silver-containing bionanocomposites, which have the ability to greatly affect the polarization of antigen-presenting cells, is a promising research area in correction of the inflammatory response for solving an important social and medical problem of treating chronic wounds.

Key words: coal-derived humic substances, silver nanoparticles, macrophage polarization, arginine balance.

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. This work was carried out with the financial support of the Russian Science Foundation (Grant No. 20-65-47052).

Conformity with the principles of ethics. Experiments using laboratory animals were carried out in accordance with the principles of humanity set out in the directives of the European Community (86/609/EEC) and the Decla-

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ration of Helsinki. The study was approved by the Bioethics Committee at Goldberg Research Institute of Pharmacology and Regenerative Medicine (Protocol No. 171052020 of 18.05.2020).

For citation: Trofimova E.S., Zykova M.V., Danilets M.G., Ligacheva A.A., Sherstoboev E.Yu., Grigorieva I.O., Mikhalev D.A., Tsupko A.V., Logvinova L.A., Perminova I.V., Belousov M.V. The effect of coal-derived humic substances and their silver-containing bionanocomposites on arginine balance in peritoneal macrophages of intact mice. *Bulletin of Siberian Medicine.* 2021; 20 (4): 71–78. https://doi.org/10.20538/1682-0363-2021-4-71-78.

Влияние гуминовых веществ угля и биокомпозиций с наночастицами серебра на их основе на баланс аргинина в перитонеальных макрофагах интактных мышей

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РЕЗЮМЕ

Введение. Антигенпрезентирующие клетки, особенно макрофаги, играют важную роль в защите организма от различных патогенов, их дисфункции, и поляризация связана с большинством воспалительных и аутоиммунных заболеваний. Воспалительный процесс жестко регулируется активацией и (или) ингибированием дифференциально экспрессируемых макрофагами генов. Успешная коррекция воспалительного процесса приводит к устранению воспалительных стимулов и далее ремоделированию и восстановлению тканей и органов. Экспериментально доказано, что биокомпозиции с наночастицами серебра на основе природных гуминовых веществ (ГВ) угля различного генеза, а также исходные матрицы данных ГВ способны активировать про- и противовоспалительные свойства макрофагов.

Цель. Исследование в культуре клеток перитонеальных макрофагов цитотоксических, пирогенных и иммуномодулирующих свойств (баланс аргинина) исходных образцов ГВ и образцов наночастиц серебра, ультрадиспергированных в данных матрицах гуминовых веществ (ГВ-AgNPs),а также их влияния на про- и противовоспалительные свойства антигенпрезентирующих клеток.

Материалы и методы. Использовались культуральные и биохимические методы.

Результаты. Показано, что образцы CHE-K, CHE-AgNPs, CHS-K, CHP-K за счет усиления активности NO-синтазы и ингибиции аргиназы способствуют поляризации перитонеальных макрофагов по классическому типу (M1). Образцы CHI-K, CHI-AgNPs, CHP-AgNPs и CHS-AgNPs модулируют альтернативный M2 или M2-подобный тип (M2-like state) активации макрофагов. При этом ГВ не цитотоксичны в эффективных концентрациях, а также три из четырех исследуемых образцов не содержат пирогенных примесей.

Заключение. Применение ГВ и серебросодержащих бионанокомпозиций на основе ГВ, обладающих способностью широко влиять на поляризацию антигенпрезентирующих клеток, является перспективным направлением исследований коррекции воспалительной реакции и, в частности, для решения острой социальной и медицинской проблемы – лечения хронических ран.

Ключевые слова: гуминовые вещества угля, наночастицы серебра, поляризация макрофагов, баланс аргинина.

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

Источник финансирования. Работа выполнена при финансовой поддержке РНФ (грант № 20-65-47052).

Соответствие принципам этики. Эксперименты с использованием лабораторных животных выполнялись с соблюдением принципов гуманности, изложенными в директивах Европейского сообщества (86/609/ЕЕС) и Хельсинкской декларации. Исследование одобрено биоэтическим комитетом НИИФиРМ им. Е.Д. Гольдберга (протокол № 171052020 от 18.05.2020).

Для цитирования: Трофимова Е.С., Зыкова М.В., Данилец М.Г., Лигачева А.А., Шерстобоев Е.Ю., Григорьева И.О., Михалёв Д.А., Цупко А.В., Логвинова Л.А., Перминова И.В., Белоусов М.В. Влияние гуминовых веществ угля и биокомпозиций с наночастицами серебра на их основе на баланс аргинина в перитонеальных макрофагах интактных мышей. *Бюллетень сибирской медицины.* 2021; 20 (4): 71–78. https://doi.org/10.20538/1682-0363-2021-4-71-78.

INTRODUCTION

It is known that antigen-presenting cells (APCs), especially macrophages (MPs), play an important role in the initiation of inflammation and pathogenesis of chronic (Crohn's disease, ulcerative colitis, asthma, allergies, atopic dermatitis, periodontosis) and autoimmune (rheumatoid arthritis, multiple sclerosis, diabetes mellitus, cardiovascular diseases, neurodegenerative disorders) diseases and cancer [1]. Macrophages are the first line of defense and, depending on the nature of the antigen (bacteria, viruses) or changes in the microenvironment (ischemia, necrosis, and apoptosis of cells), they are activated and take on various types of phenotypic and functional polarization [2].

The type of MP polarization determines the development of a specific immune response and activation of Th1, Th2, Th17, and Treg cells, which are further responsible for the pathological process and inflammatory response, systemic metabolism, hematopoiesis, vasculogenesis, and tissue homeostasis [3, 4]. Macrophages mainly exist in two different phenotypes: 1) M1 macrophages (classically activated or proinflammatory) through the expression of transcription factors, mainly nuclear factor-κB (NF-κB), produce proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-12, and IL-23; and 2) M2 (alternatively activated, anti-inflammatory) which are immunoregulatory cells producing anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGF-β) [1, 5–8].

The functions of M1 macrophages are traditionally associated with microorganism phagocytosis, microbicidal activity, induction of inflammation, and antitumor activity. M2 marcophages suppress inflammation and promote tissue repair and remodeling, homeostasis, and vasculogenesis. The M1/M2 balance determines the fate of the organ in conditions of inflammation or injury. In this case, MPs are very plastic, and one phenotype can repolarize into another [3]. During infection (inflammation), tissue-resident MPs initially exhibit the M1 phenotype; however, long-term development of this phase causes chronic inflammation, tissue damage, and loss of organ function. Under these conditions, suppression of inflammation and activation of M2 macrophages are extremely necessary [6, 9]. Thus, control and modulation of macrophage functions, their polarization, and a relationship between pro- and anti-inflammatory responses determine the outcome of inflammation and are necessary for managing inflammation and restoring organ functions [10].

Effective anti-inflammatory drugs are already available and a number of drugs of various origins are under development. The study of biocomposites based on humic substances (HS) and their silver-containing bionanocomposites possessing antimicrobial, anti-inflammatory, and wound healing properties for treatment of purulent, chronic non-healing wounds is innovative and has not been described in the world literature. First of all, the effect of these substances on the mechanisms of the anti-inflammatory response and formation of the immune response is unknown. The relevance of this kind of research is beyond doubt, since a lack of effective methods for treating chronic non-healing wounds leads to infection, and against the background of the existing problem of antibiotic resistance – to sepsis and death. Therefore, potentially effective and safe substances with antimicrobial and wound-healing properties should be carefully studied and introduced into clinical practice following confirmation of the therapeutic efficacy.

The aim of this study was to investigate the cytotoxic, pyrogenic, and immunomodulatory properties (arginine balance) of initial HS samples and samples of silver nanoparticles ultradispersed in these HS matrices (HS-AgNPs) in the cell culture of peritoneal macrophages, as well as their effect on pro- and anti-inflammatory properties of APCs.

MATERIALS AND METHODS

Test systems. In the experiments, we used conventional C57BL/6 mice (total of 80 heads) of both sexes at the age of 8–10 weeks, obtained from the Department of Experimental Biomodels at the Goldberg Research Institute of Pharmacology and Regenerative Medicine, the structural unit of Tomsk NRMC.

The substances under study were humic substances and silver-containing bionanocomposites based on them (HS-AgNPs), synthesized at the Laboratory of Natural Humic Systems of the Lomonosov Moscow State University, Chemistry Department. They were dissolved immediately before use in the culture medium. The synthesis of HS-AgNPs biomaterials was carried out by reducing silver ions in HS solutions (at a concentration of 15 g/l) using an $AgNO_3$ solution until the final concentration of silver nanoparticles was 20 mmol/l. The characteristics of the research objects are presented in Table 1.

Table 1

Experimental samples of coal-derived humic substances and silver-containing bionanocomposites based on them

Names of commercial samples of coal-derived HS	Sample code	
	HS (basic matrix)	HS with Ag nanoparticles (HS-AgNPs)
"Powhumus" (Humintech, Germany)	CHP-K	CHP-AgNPs
"Sakhalin humates", Russian Federation	CHS-K	CHS-AgNPs
"Irkutsk humates", Russian Federation	CHI-K	CHI-AgNPs
Humic substances, Genesis, Russian Federation	CHE-K	CHE-AgNPs

Cell preparation. From the cell suspension obtained by washing the abdominal cavity of mice with ice-cold sodium chloride solution, mature peritoneal MPs were isolated using the EasySep™Biotin Positive Selection Kit and antibodies specific to macrophage receptors, Anti-Mouse F4/80 Antibody (both Stem Cell, USA).

Cultivation conditions. MPs (2.5–3 × 10^6) were cultured for 48 hours (37°C, 5% CO_2, 100% humidity) in a complete culture medium (RPMI 1640 (Sigma, USA) with the addition of 10% fetal bovine serum (FBS) (Hyclone, UK), 20 mM of HEPES (Sigma, USA), 0.05 mM of 2-mercaptoethanol (Sigma, USA), 50 µg/ml of gentamicin (Sigma, USA), and 2 mM of L-glutamine (Sigma, USA) in 96-well plates in the presence of various concentrations of the studied samples or 0.1 µg/ml lipopolysaccharide (LPS) (Sigma, USA).

Study of the arginine balance and cytotoxicity. According to the attached protocols, the content of nitrites in the production of nitric oxide (NO) was determined in the supernatant by mixing the supernatant with the Griess reagent (Sigma-Aldrich, USA) in equivalent volumes, and the activity of arginase was measured in the cell lysate by the concentration of urea using a test system Urea-450 (Erba Lachema, Czech Republic). Cell proliferation was also assessed in the MP lysate, for which, 4 h before the end of cultivation, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT, Sigma, USA) was added to the wells at the final concentration of 200 µg/ml. The precipitate was dissolved with dimethyl sulfoxide (Sigma, USA). The absorption of solutions (units of optical density) was measured on a UNIPLAN PIKON enzyme immunoassay analyzer (AIFR-01, Pikon LLC, Russian Federation) at a wavelength of 540 nm. The nitrite concentration (µM) was calculated with a calibration curve constructed using standard sodium nitrite solutions. The amount of the enzyme catalyzing the formation of 1 µM urea per minute was taken as 1 unit of activity (U.A.) of arginase.

Determination of pyrogenicity. To determine the impurity of LPS – endotoxin – HS were treated for 1 h with the antibiotic polymyxin B (50 µg/ml), then the cells were added and incubated as described above.

Statistical processing was performed using Statistica 13.3 software, using one-way ANOVA, Dunnett's test, and Student's *t*-test, after checking for normality of distribution using the Shapiro – Wilk test (data distribution corresponds to normal), where M is the sample mean; m is the error of the mean; level of statistical significance of differences $p < 0.05$, and sample size $n \geq 5$ depending on the research method.

RESULTS AND DISCUSSION

It is known that humic substances are dark-colored nitrogen-containing organic compounds, the color intensity of which is directly proportional to the concentration of the sample [11]. Based on this, the effect of a native dark color of the HS samples on the spectrophotometric parameters of aqueous solutions was preliminary evaluated. It was shown that all HS samples at a concentration of 1 µg/ml did not affect the spectrophotometric parameters of MP supernatants even without the addition of the Griess reagent (Table 2). With an increase in the concentration to 10 µg/ml, only the CHS sample had an optical density 2.6 times higher than the control values, but at the concentration of 100 µg/ml, all samples were 2–15 times darker than the control.

It was found that at the same concentration of the studied substances, the optical density of the CHS-AgNPs sample was lower than the initial matrix of HS, while that of the samples CHP-AgNPs and CHI-AgNPs, on the contrary, was higher. Further, in the study of cell proliferation, it was shown that all the studied basic HS samples did not exhibit toxic effects in any of the concentrations used (Table 3). MP cultivation with the samples CHP-AgNPs, CHS-AgNPs, CHE-AgNPs, and CHP-AgNPs, starting from a concentration of 10 μg/ml, led to inhibition of cell proliferation by 2–3 times. Therefore, in the further work, in order to avoid false-positive results and pronounced toxic effects, when assessing the activating properties of the studied substances, concentrations of 1 and 10 μg/ml were used, and for the CHS sample – only 1 μg/ml.

Table 2

Effect of coal-derived humic substances and silver-containing bionanocomposites based on them on the optical density of macrophage supernatants (without Griess reagent), units of optical density, *M* ± *m*

Studied substance	Control (MP + medium)	MP + HS, μg/ml		
		1	10	100
CHP-K	119 ± 3	130 ± 4	135 ± 3	260 ± 8*♦
CHP-AgNPs		121 ± 3	128 ± 3	372 ± 3*♦■
CHS-K		132 ± 1	307 ± 6*●	1,777 ± 9*♦
CHS-AgNPs		130 ± 3	132 ± 4■	336 ± 3*♦■
CHI-K	127 ± 4	127 ± 2	135 ± 2	222 ± 2*♦
CHI-AgNPs		125 ± 1	133 ± 3	321 ± 5*♦■
CHE-K		139 ± 5	137 ± 1*	266 ± 4*♦
CHE-AgNPs		134 ± 2	137 ± 2	251 ± 2*♦

* differences compared with the control.
Note: ● – differences between concentrations of 1 and 10 μg/ml, ♦ – differences between concentrations of 10 and 100 μg/ml, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$, $n = 6$.

Table 3

Effect of various concentrations of coal-derived humic substances and silver-containing bionanocomposites based on them on the peritoneal macrophage proliferation of intact C57BL/6 mice, *M* ± *m*

Studied substance	Control 1 (MP + medium)	Control 2 (MP + LPS)	MP + HS		
			1	10	100
CHP-K	460 ± 9	425 ± 3	500 ± 8●	467 ± 8	512 ± 10●
CHP-AgNPs			494 ± 11	154 ± 6*●■	169 ± 6*●■
CHS-K	510 ± 4	451 ± 3	541 ± 11●	594 ± 9●	536 ± 8●
CHS-AgNPs			496 ± 9	169 ± 7*●■	164 ± 7*●■
CHI-K	282 ± 12	279 ± 7	291 ± 9●	308 ± 6●	340 ± 17*●
CHI-AgNPs			298 ± 13	197 ± 2*●■	208 ± 11*●■
CHE-K			264 ± 8●	260 ± 5	556 ± 10*●
CHE-AgNPs			294 ± 9	255 ± 2	273 ±10■

* – differences compared with Control 1.
Note: ● – differences between the parameter and Control 2, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$. The LPS concentration – 0.1 μg/ml, $n = 6$.

The study of the NO-activating properties of the initial HS matrices showed that the cultivation of MPs with the CHP-K and CHE-K samples led to an increase in the concentration of nitrites in comparison with the intact control by 1.2 (CHP-K) and 17 (CHE-K) times at the concentration of 1 μg/ml, and by 13 times – at the concentration of 10 μg/ml (Table 4). The basic matrices of the CHS-K and CHI-K samples, as well as none of the AgNPs samples, did not affect the secretory properties of MPs. The NO-activating effect of all substances was significantly lower than that of the mitogen-stimulated control.

It is known that consumption of L-arginine increases drastically in activated MPs. Molecular markers of M1 are NO synthase (iNOS), CD16, CD32, CD40, CD80, and CD86, while M2 macrophages are characterized by the activation of arginase-1 (Arg-1) and transglutaminase 2, macrophage surface marker CD36, receptor of transferrin CD71, CD163, mannose (MMR or CD206), CCL-22, and E-cadherin [1, 3, 7]. Classically activated MPs convert arginine into nitric oxide and citrulline with iNOS; alternatively activated MPs convert arginine into urea and ornithine by means of arginase. The study of the effect of the analyzed substances on the arginine balance in peritoneal MPs showed that an increase in the production of nitrites during the MP cultivation with the CHE-K (by 29 times) and CHE-AgNPs (by 1.5 times) samples was accompanied by a decrease in the arginase activity in cell lysates by 1.2 and 4 times, respectively, compared with the intact control (Table 5).

Table 4

Effect of different concentrations of coal-derived humic substances and silver-containing bionanocomposites based on them on the nitric oxide production by peritoneal macrophages of intact C57BL/6 mice, *M* ± *m*

Studied substance	Concentration	HS	Control 1 (MP + medium)	Control 2 (MP + LPS)
CHP-K	1	3.30 ± 0.13*●	2.66 ± 0.14	69.70 ± 0.18*
	10	34.63 ± 0.43*●		
CHP-AgNPs	1	2.77 ± 0.11●■		
	10	2.59 ± 0.07●■		
CHS-K	1	2.93 ± 0.06●	2.51 ± 0.15	65.93 ± 0.37*
CHS-AgNPs	1	2.77 ± 0.11●		
CHI-K	1	2.41 ± 0.06●		
	10	2.58 ± 0.13●		
CHI-AgNPs	1	2.81 ± 0.16●		
	10	2.87 ± 0.10●		
CHE-K	1	41.61 ± 0.28*	2.44 ± 0.17	37.89 ± 0.71*
	10	33.91 ± 0.71*		
CHE-AgNPs	1	2.53 ± 0.07●■		
	10	2.41 ± 0.07●■		

* differences compared with Control 1.

Note: ● – differences between the parameter and Control 2, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$. The LPS concentration – 0.1 μg/ml, $n = 6$.

Table 5

Effect of various concentrations of coal-derived humic substances and silver-containing bionanocomposites based on them on the activity of NO-synthase (nitrite production) and arginase (urea fermentation) in peritoneal macrophages of intact C57BL/6 mice, *M* ± *m*

Studied substance	Concentration, μg/ml	Nitrite concentration, μM	Urea fermentation, U.A.
Control 1 (MP + medium)	–	2.20 ± 0.22	53.64 ± 0.40
Control 2 (MP + LPS)	0.1	64.56 ± 0.67*	42.47 ± 0.46*
CHP-K	10	28.74 ± 0.72*●	52.85 ± 0.35●
CHP-AgNPs	10	2.49 ± 0.03●■	5.29 ± 0.82*●■
CHS-K	1	2.88 ± 0.19*●	33.81 ± 0.46*●
CHS-AgNPs	1	2.52 ± 0.05●	20.71 ± 0.56*●■
CHI-K	10	2.51 ± 0.07●	60.16 ± 0.45●
CHI-AgNPs	10	2.42 ± 0.04●	56.81 ± 0.74●
CHE-K	10	63.48 ± 0.30*	43.13 ± 0.35*
CHE-AgNPs	10	3.26 ± 0.11*●■	12.91 ± 0.51*●■

* – differences compared with Control 1.

Note: ● – differences between the parameter and Control 2, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$; $n = 5$ (nitrites) and $n = 10$ (arginase).

The CHP-K sample, against the background of a 13-fold increase in the nitrite concentration, did not affect the arginase activity, and the CHS-K, CHS-AgNPs, and CHP-AgNPs samples, not showing NO-activating properties, significantly increased urea fermentation relative to Control 1. The CHI-K and CHI-AgNPs samples did not affect the studied parameters.

The literature shows that extracts of plant origin may contain an admixture of endotoxin (LPS), which also causes an increase in the production of nitric oxide [12].

In order to assess the degree of purification of the studied substances from LPS, experiments were carried out using polymyxin B, which binds directly to endotoxin and, thus, blocks its stimulating effect. Table 6 shows that the antibiotic did not affect the NO-stimulating properties of the CHS-K and CHS-AgNPs, CHI-K and CHI-AgNPs, and CHE-K and CHE-AgNPs samples. During cultivation of the CHP-K and CHP-AgNPs samples with the antibiotic, the concentration of nitrites decreased by 1.3–1.7 times. The results obtained indicate the absence of endotoxin impurity and pyrogenic properties in the CHS-K and CHS-AgNPs, CHI-K and CHI-AgNPs, and CHE-K and CHE-AgNPs samples, and their presence in the CHP-K and CHP-AgNPs samples.

Table 6

The effect of polymyxin B on the NO-producing properties of coal-derived humic substances and silver-containing bionanocomposites based on them in peritoneal macrophages of intact C57BL/6 mice, *M* ± *m*

Studied substance, μg/ml		Control 1 (MP + medium)		Control 2 (MP + LPS)		MP + HS	
		polymyxin B		polymyxin B		polymyxin B	
		–	+	–	+	–	+
CHP-K	10	2.34 ± 0.03	2.36 ± 0.11	32.31 ± 0.41*	3.56 ± 0.11▲■	25.43 ± 0.02*●	14.93 ± 0.16▲■♦
CHP-AgNPs	10					2.64 ± 0.08●	1.91 ± 0.05▲■♦
CHS-K	1					3.63 ± 0.15*●	4.08 ± 0.09■
CHS-AgNPs	1					2.34 ± 0.08●	2.71 ± 0.06♦
CHI-K	10	2.48 ± 0.07	2.16 ± 0.13	36.63 ± 0.62*	2.72 ± 0.1▲■	2.78 ± 0.15●	2.53 ± 0.10
CHI-AgNPs	10					2.76 ± 0.07●	2.40 ± 0.08
CHE-K	10					45.30 ± 0.51*●	41.02 ± 0.54■♦
CHE-AgNPs	10					2.66 ± 0.06●	2.29 ± 0.06

* – differences compared with Control 1 without polymyxin.

Note: ▲ – differences between the parameter and incubation of each substance without polymyxin; ● – differences between the parameter and Control 2 without polymyxin; ■ – differences between the parameter and Control 1 with polymyxin; ♦ – differences between the parameter and Control 2 with polymyxin; level of statistical significance of differences $p < 0.05$. The polymyxin B concentration – 50 μM, LPS – 0.1 μg/ml, $n = 5$.

CONCLUSION

The studies have shown that the CHE-K, CHE-AgNPs, and CHS-K samples contribute to the polarization of antigen-presenting cells according to the classical type (M1) by increasing the activity of NO synthase and inhibition of arginase. The basic CHP-K matrix, which significantly enhances the NO-stimulating properties of cells against the background of stable arginase, can also be attributed to this type of substance. The functions of proinflammatory macrophages M1 are associated with phagocytosis, microbicidal activity, induction of inflammation and adaptive immune response, and antitumor activity and are accompanied by the secretion of Th1 cytokines.

On the contrary, the CHI-K and CHI-AgNPs samples did not affect the activity of NO-synthase and arginase of peritoneal MPs, which allows to consider these substances as activators of alternative, anti-inflammatory properties of M2 macrophages. The latter are aimed at formation of the extracellular matrix, repair and remodeling of tissues, suppression of inflammation, stimulation of vascular formation, apoptotic cell phagocytosis, and synthesis of anti-inflammatory cytokines (IL-10, TGF-β, IL-4, IL-1ra). The CHP-AgNPs and CHS-AgNPs samples that inhibit arginase activity but do not affect nitrite production can be attributed to the M2-like state polarization which has some, but not all, characteristics of M2 cells [3]. At the same time, HS are not cytotoxic at effective concentrations, and three out of four studied samples do not contain pyrogenic impurities.

Therefore, macrophages undergo various dynamic changes at each stage of wound healing. Firstly, M1 macrophages mediate tissue damage and initiate inflammatory reactions. Secondly, at the early stages of repair, infiltrating MPs exhibit the M2 phenotype to suppress acute inflammation, and then their depletion inhibits the formation of excessively vascularized and scar tissue. The use of silver-containing bionanocomposites based on HS, which have the ability to greatly affect the polarization of APCs, is a promising research area for solving an acute social and medical problem of treating chronic wounds.

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Authors contribution

Zykova M.V., Belousov M.V., and Sherstoboev E.Yu. substantiated the relevance of this work. Perminova I.V., Grigorieva I.O., Tsupko A.V., Mikhalev D.A., Logvinova L.A. and Zykova M.V. synthesized samples and carried out their standardization. Trofimova E.S., Danilets M.G., and Ligacheva A.A. developed an experiment, assessed the biological activity of the studied substances, and carried out cell culture studies. Danilets M.G. and Ligacheva A.A. carried out data processing. Trofimova E.S. and Danilets M.G. performed theoretical calculations. Danilets M.G., Trofimova E.S., Zykova M.V., and Belousov M.V. were involved in writing the text of the article. All authors participated in the discussion of the results.

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Received 01.09.2021
Accepted 05.10.2021