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EXPERIMENTAL ARTICLES

Impact of Bacterial Autoregulatory Molecules (Homoserine Lactones and Alkylhydroxybenzenes) on the Oxidative Metabolism of the Cell Effectors of Natural Immunity

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Abstract—We investigated the impact of bacterial regulators homoserine lactones (HSLs) and alkylhydroxybenzenes (AHBs) (which are present in human fluids at pico- and nanomolar concentrations) on neutrophile oxidative metabolism. The HSL and AHB effects were determined using a test based on induced luminol-dependent chemoluminescence of neutrophiles in human peripheral blood. In this test, neutrophiles were preincubated with chemical analogs of bacterial autoregulators with different lengths of the hydrocarbon radical, such as HSL · HCl, C6- and C12-HSL, and C1-, C6-, and C12-AHB. We revealed that they suppressed the chemoluminescence and, accordingly, the oxidative metabolism of neutrophiles. This effect was more significant with HSLs than with AHBs. Within each of the two groups, the effect increased with an increase in the length of the hydrocarbon chain of the homologues.

High concentrations of long-chain autoregulators of both types produce a cytotoxic effect that is associated with apoptosis in the case of C12-HSL and with cell membrane damage in the case of C12-AHB. The effects of low HSL and AHB concentrations involve their protein-modifying properties and result in changes in the activities of neutrophile oxidative enzymes. To a lesser extent, these effects are due to the pro- and antioxidant activities of HSLs and AHBs, respectively. In light of the results obtained, the HSL and AHB effects are to be considered as a novel mechanism of regulating the activities of cell effectors of natural innate immunity. In symbiotic and parasitic systems, the mechanism involves the bimodal pattern of the effects of HSLs and AHBs that vary depending on their structure and concentrations.

Keywords: homoserine lactones, alkylhydroxybenzenes, neutrophiles, phagocytosis, reactive oxygen species, chemoluminescence

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Small-size autoregulatory molecules and intracellular communication systems based on them control a large number of events in the developmental cycle of microbial populations, including cell functional and structural differentiation [1]. For instance, homoserine lactones (HSLs) are a major class of autoregulators in gram-negative proteobacteria. They are responsible for quorum-dependent induction of the genes responsible for bioluminescence, antibiotic production, virulence, and biofilm formation [2, 3]. Alkylhydroxybenzenes (AHBs) [4] are another well-known group of autoregulatory molecules. They were detected in representatives of *Azotobacter, Pseudomonas, Micrococcus*, and other genera. AHBs perform the functions of adaptogens and hypometabolic and anabiotic (dormant) state inducers [5]. HSLs and AHBs are structurally similar to an extent (Fig. 1). Both groups have a polar lactone or resorcinol ring that is linked to a hydrocarbon radical of a variable length [4, 5]. Taken together, these features provide for the amphiphilic properties of these molecules.

Apart from microbial intra- and interspecies communication, HSLs and AHBs are likely to be involved in bioinformational interactions with higher organisms [6], due to the above peculiarities of the molecular organization. Additional prerequisites for these interactions include the formation of the autoregulators by a large number of representatives of the symbiotic and pathogenic microflora, the cross-species and polymodal pattern of the effects of the autoregulators, and their amphiphilic properties [1–5] enabling unrestricted translocation in the internal milieu of the human or animal organism. This is consistent with the detection of a number of HSL homologues in the saliva and lung tissue of cystic fibrosis patients at nano-, pico-, and femtomolar concentrations [7, 8] and of AHBs and the products of their metabolism in human blood plasma, urine, and fatty tissue at nano- and micromolar concentrations [9, 10].

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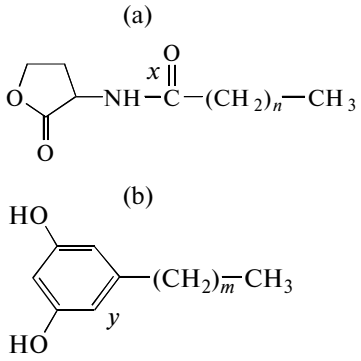

Fig. 1. Structural formulas of the tested HSL (a) and AHB (b) homologues, where $n = 0$, 4, and 10; $m = 0$, 5, and 11. Notes: *x*, the bond in the HSL · HCl molecule is missing; *y*, the hydrocarbon chain in the C6-AHB molecule is in the 4-*para* position.

Research on the biological impact of HSLs and AHBs in the above multicomponent systems revealed a number of immunomodulatory effects [4, 11–15]. As for the particularly well-characterized *Pseudomonas aeruginosa* autoregulator *N*-(3-oxododecanoyl)-L-homoserine lactone, a large number of its effects with respect to the cell mechanisms of adaptive and innate immunity were revealed. They are due to the suppression of lymphocyte proliferation and differentiation [11] and to changes in the functional activities of neutrophiles [12] and macrophages [13]. The AHB effects explored heretofore are predominantly due to alterations in molecular immunity factors, such as changes in the activity pattern of the bacteriolytic enzyme lysozyme and the inhibition of specific interactions in the antigen–antibody system [4, 14, 15].

In light of all the above, of special interest are comparative studies on the immunomodulatory properties of HSLs and AHBs, including the elucidation of the dependence of the effects of these autoregulators on their structure and the concentrations applied.

The goal of this work was to investigate the influence of a representative group of homologues of bacterial autoregulators of the homoserine lactone and alkylhydroxybenzene groups on the oxidative metabolism of human peripheral blood neutrophiles, which represents the main bactericidal system involving these innate immunity effectors [16].

MATERIALS AND METHODS

In these studies, we used chemical analogues of the microbial autoregulators HSLs and AHBs with a purity degree of 99.9%. The length of their hydrocarbon radicals was different (Fig. 1). The HSL homologues were the following commercial preparations: L-homoserine lactone hydrochloride (Sigma, United States) denoted as HSL · HCl herein, *N*-hexanoyl-DL-homoserine lactone (C6-HSL), and *N*-dodecanoyl-DL-homoserine lactone (C12-HSL) (Fluka, Germany). The tested AHB homologues included methylhydroxybenzene (C1-AHB) (Sigma, United States), hexylhydroxybenzene (C6-AHB) (Sigma, United States), and synthetic dodecylhydroxybenzene (C12-AHB) (Enamine, Ukraine). Immediately before the experiments, a series of aqueous solutions of the tested substances was prepared; in the case of C6- and C12-HSL and C12-AHB, the solution contained 5% ethanol. The tested concentrations were 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M; they corresponded to the HSL and AHB concentrations earlier detected in the biological fluids of humans and animals [7–10].

Human peripheral blood neutrophiles were isolated by centrifugation in a double ficoll–verografin gradient with densities of 1.077 g/cm^3 and 1.092 g/cm^3, respectively. They were collected from the interphase, washed with cold physiological saline, and resuspended in a volume of 199 medium [17] that equaled that of the plasma sample. The final concentration was 7×10^6/mL.

General assessment of the HSL and AHB influence on neutrophile oxidative metabolism during phagocytosis was carried out using the activated chemiluminescence test [18]. Luminescence intensity caused by the addition of the inducer (luminol) to the system was the quantitative criterion of reactive oxygen species generation. The neutrophile suspension (900 μL) was placed in quartz cuvettes. In the experimental systems, it was supplemented with 100 μL of the solutions of the tested substances (HSLs or AHBs); the control system contained 100 μL of water or 5% ethanol. The mixtures were incubated at 37°C for 60 min. Thereupon, 20 μL 0.015 M luminol (Sigma, United States) solution was added to the experimental and the control cuvette. Using a BLM8802M2K two-channel biochemiluminometer (Nauka Special Technology Design Bureau, Russia), the background luminescence of the cell suspensions was monitored for 100 s. The opsonophagocytosis assay was started by simultaneously supplementing the experimental and the control sample with 100 μL of the suspension of inactivated (by heating), blood serum-opsonized *Staphylococcus aureus* FDA 209P cells at a concentration of 10^9 CFU/mL. Luminol-dependent chemoluminescence (LDCL) was monitored for 15 min (amplifying the signal 50-fold). Thereupon, the ratio between the maximum LDCL intensity in the experimental and the control sample was calculated deducting the background luminescence value.

HSL and AHB cytotoxicity for neutrophiles was determined using the test with the trypan blue (TB) dye. Cell suspensions preincubated with various autoregulator concentrations at 37°C for 60 min were supplemented with an equal volume of 0.1% dye solution in 0.85% NaCl, incubated for 15 min, and counted in a Goryaev chamber. The cells were counted in 25 large square areas, and dead blue (TB+) and live unstained

Table 1. Intensity of induced luminol-dependent chemiluminescence of neutrophiles (% of the control value) after their preincubation with the autoregulators HSLs and AHBs

Autoregulatory factor concentration, M	Autoregulatory factors					
	HSL · HCl	C6-HSL	C12-HSL	C1-AHB	C6-AHB	C12-AHB
10^{-9}	46.09** ± 2.86	28.67** ± 1.83	18.61*** ± 0.93	88.49 ± 6.46	85.97 ± 6.02	55.18* ± 3.31
10^{-8}	38.20** ± 2.67	29.29** ± 1.90	16.71*** ± 0.89	84.89 ± 5.86	78.54 ± 5.81	51.87* ± 5.03
10^{-7}	30.54** ± 2.05	23.10** ± 1.50	13.69*** ± 0.73	62.46* ± 4.31	66.61 ± 4.46	48.75** ± 3.41
10^{-6}	29.86** ± 2.00	11.73*** ± 0.59	5.65*** ± 0.29	61.35* ± 4.23	60.54* ± 4.48	33.05** ± 2.31
10^{-5}	22.59** ± 1.47	7.66** ± 0.49	5.32*** ± 0.28	60.09* ± 4.03	50.76** ± 3.76	29.40** ± 1.19

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, relative to the control.

(TB–) cells were registered. The percentage of dead cells was determined as the (stained (TB+) cell number/total cell number) × 100% ratio. Apoptosis in C12-HSL- and C12-AHB-preincubated neutrophiles was monitored by measuring caspase-3 activity. The caspase-3 content was determined using the immunoenzyme assay with a Quantikine Human Active Caspase-3 test system (R&D Systems, United States). Using the erythrocyte osmotic resistance assay, the membranolytic activities of C12-HSL and C12-AHB were determined from the spectrophotometrically detectable hemoglobin release ($\lambda = 620$ nm) in hypotonic NaCl solutions within the 0.4–0.6% salt concentration range with a step of 0.05%.

The HSL and AHB effects on the oxidative processes were assessed in a model enzyme system containing 100 μL of autoregulator solution in one of the tested concentrations, 100 μL of 2×10^{-13} M horseradish peroxidase solution, 100 μL of 2×10^{-3} M hydrogen peroxide, 20 μL of 0.0015 M luminol, and 680 μL of 0.1 M sodium phosphate buffer (pH 8.6). To assess the pro- and antioxidant activities of HSLs and AHBs per se, all the above components except peroxidase were mixed in a BLM8802M2K biochemiluminometer cuvette. The background luminescence level was monitored for 20 s. Thereupon, peroxidase was added, and the intensity of the chemiluminescence "surge" was recorded for 60 s. To assess the protein-modifying activities of HSLs and AHBs with respect to the peroxidase per se, we preincubated the enzyme with HSL and AHB homologues at 37°C for 60 min, followed by placing the mixture in a cuvette with hydrogen peroxide and luminol in phosphate buffer; luminescence intensity was measured as described above. Based on the signal intensity values in the experimental (I_e and the control (I_c) cuvette, we calculated the relative chemiluminescence index (CLI) using the formula $CLI = (I_e - f_e)/(I_c - f_c)$, where f_e and f_c denote the respective background luminescence levels.

At least three repeats of all experiments were carried out. The statistical treatment of the results was performed using the Statistica 5.5 software package. The relative influence of specific mechanisms of action of HSLs and AHBs on the oxidative metabolism of neutrophiles was determined using the correlation analysis and analysis of variance (ANOVA) tools.

RESULTS

At the first stage of our research, we investigated the patterns of the HSL and AHB effects on the intensity of luminol-dependent chemiluminescence (LDCL) of human peripheral blood neutrophiles that is correlated with the activity of their oxygen-dependent bactericidal systems during phagocytosis. In general, the contact of neutrophile phagocytes with bacterial autoregulators resulted in suppressing their oxidative metabolism. The extent of this inhibition significantly varied depending on the structural features and the tested concentrations of the autoregulators (Table 1). Comparative analysis of the results revealed a number of patterns.

First, homoserine lactones caused a 2–10 times more intense LDCL suppression than alkylhydroxybenzenes with the same hydrocarbon radical length, when applied at the same concentrations under identical experimental conditions. This indicated that the structure of the polar groups attached to the HSL/AHB ring played an important role in terms of the effects produced by them. Second, LDCL inhibition significantly increased with an increase in the length of the nonpolar alkyl radical of the tested HSL or AHB homologue. This was consistent with the suggestion that hydrophobic HSL homologues with 11–13 carbon atoms in the side chain are predominantly characterized by immunosuppressive activities [19]. Third, the extent of LDCL inhibition increased with an increase in the dose of each of the tested HSL or AHB homologues. This pattern held within a relatively broad range of bacterial autoregulator concentrations, from 10^{-5} to 10^{-9} M, and probably at even lower concentrations in the case of HSLs. These concentrations corresponded to their detectable contents in the biological fluids and tissues of the human organism [7–10].

Table 2. Percentage of stained (TB+) neutrophiles (% of the total number) after 60 min incubation with various concentrations of HSLs and AHBs

Autoregulatory factor concentrations, M	Autoregulatory factors					
	HSL · HCl	C6-HSL	C12-HSL	C1-AHB	C6-AHB	C12-AHB
10^{-9}	4.1 ± 0.2	2.1 ± 0.1	2.9 ± 0.2	4.5 ± 0.3	2.9 ± 1.5	3.1 ± 1.6
10^{-8}	4.6 ± 0.3	5.4 ± 0.3	4.9 ± 0.3	3.4 ± 0.2	3.8 ± 0.2	4.4 ± 0.3
10^{-7}	6.8 ± 0.4	5.3 ± 0.3	5.0 ± 0.3	7.4 ± 0.4	4.9 ± 0.3	6.7 ± 0.4
10^{-6}	5.0 ± 0.3	6.0 ± 0.4	34.0* ± 7.7	10.5 ± 0.6	9.4 ± 0.6	8.5 ± 0.5
10^{-5}	79.8** ± 4.6	72.8** ± 3.1	92.1*** ± 0.7	72.1** ± 2.3	68.2** ± 2.9	68.6** ± 1.8

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, relative to the control.

In light of the results obtained, we raised the issue concerning the mechanisms of the inhibitory effects of HSLs and AHBs on the oxidative metabolism of neutrophile phagocytes. One possible mechanism implicated their direct cytotoxic activity.

In order to resolve the issue, we conducted a test with the trypan blue dye (TB) that selectively stains dead cells. The test revealed that the interaction between neutrophiles and HSLs and AHBs at the maximum tested concentration (10^{-5} M) yielded a large number of TB+ cells (Table 2). In particular, the effect of HSL homologues resulted in the death of 72.8 ± 3.1% ($P < 0.01$) to 92.1 ± 0.7% ($P < 0.001$) of the treated neutrophile phagocytes. However, decreasing the concentrations of the tested HSL homologues to 10^{-6} M caused a marked loss of their cytotoxicity that remained significant only with the long-chain homologue C12-HSL (34.0 ± 7.7%; $P < 0.05$). A similar result was obtained for the ANB group, where different homologues (10^{-5} M) resulted in emergence of 68.6 ± 1.8 ($P < 0.05$) to 72.1 ± 2.3% ($P < 0.01$) of TC+ cells while no statistically reliable cytotoxicity was observed in any of the variants at the homologue concentrations 10^{-6} M and lower.

Comparison of the influence of HSLs and AHBs on the oxidative metabolism of neutrophile phagocytes (LDCL) with the level of their cytotoxicity revealed a verifiable relationship between these two variables ($r = 0.604$; $P < 0.01$ and $r = 0.591$; $P < 0.05$, respectively). However, such relationships were not linear; they only manifested themselves in the presence of relatively high autoregulator concentrations in the phagocytosis system (Fig. 2a).

Further studies on the HSL/AHB cytotoxicity revealed several mechanisms involved in it. The activity of caspase-3, the key apoptosis enzyme, was detected in the extract of neutrophiles pretreated with C12-HSL but not C12-AHB. The C12-HSL effect manifested itself within the 10^{-5}–10^{-6} M concentration range. Dose-dependent caspase-3 expression occurred after 1 h of their co-incubation. By the fourth hour of incubation, maximum accumulation (23.3 ± 2.5 pg/mL) of this apoptosis marker was achieved (Fig. 3). The results obtained are in good agreement with the earlier data [20] on the apoptosis-inducing capacity of another long-chain HSL homologue, *N*-(3-oxododecanoyl)-L-homoserine lactone that forms part of the autoregulatory cascade of *P. aeruginosa* [20].

In contrast, C12-AHB that failed to cause a similar effect at concentrations of 10^{-6} M and higher, exhibited direct cytotoxic activity that was detected using the erythrocyte osmotic resistance test. While the lysis of only 4.22% of erythrocytes occurred in 0.6% NaCl solution, their pretreatment with 1.25×10^{-5} M and 5×10^{-5} M C12-AHB increased the lysed cell percentage to 22.01% and 59.87%, respectively. The membranotropic and membranolytic effects of AHBs [4, 21] can account for these data.

Other possible mechanisms of action of HSLs and AHBs in the phagocytosis system plausibly involve their oxidant properties and enzyme-modifying activity [3–5], which can potentially influence the oxidative metabolism of neutrophiles in the absence of cytotoxic effects. Two variants of these experiments were conducted using a cell-free enzyme system. The first variant was aimed at detection of the oxidant properties of HSLs and AHBs per se. In the second variant, their effects in terms of the catalytic activity of peroxidase were tested. The model enzyme peroxidase is a functional analogue of the myeloperoxidase of neutrophile phagocytes.

The experiments in which the oxidant properties of HSLs and AHBs were detected revealed significant differences between them (Table 3). C12-HSL and especially C6-HSL exhibited their prooxidant activity at concentrations of 10^{-5}–10^{-6} M, which manifested itself in an increase in induced chemiluminescence (ICL_1) to 1.55 ± 0.05–2.05 ± 0.05 ($P < 0.01$) in comparison to the control value (1.0). In contrast, the tested AHB homologues, particularly C1-AHB (10^{-5} M), caused a significant decrease in the ICL_1 values (down to 0.60 ± 0.03, $P < 0.01$). This is consistent with the idea that this group of biologically active substances possesses antioxidant properties [4, 5, 22]. However,

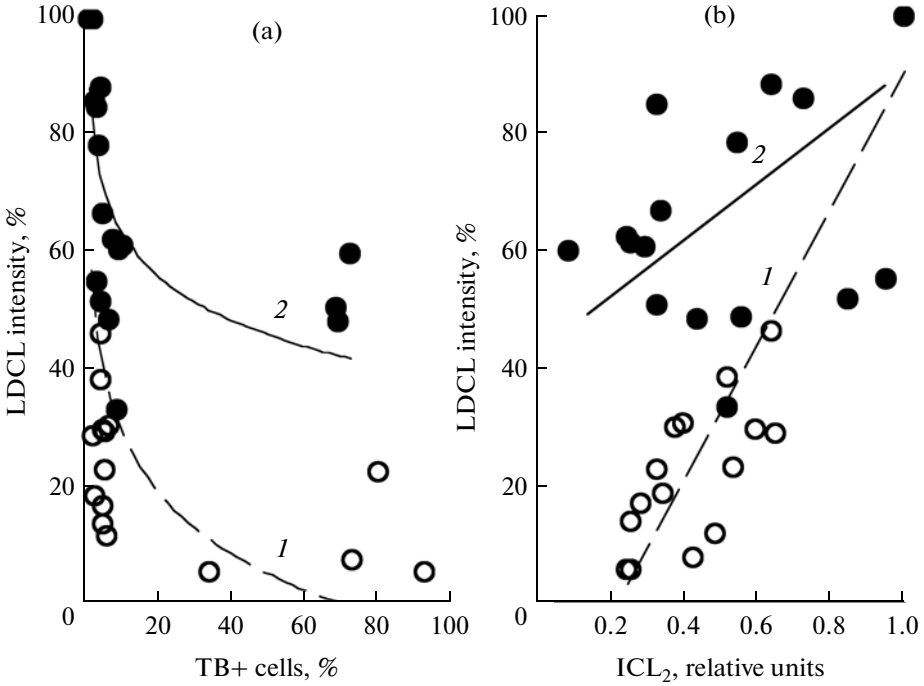

Fig. 2. Role of the cytotoxic effect (a) and the modification of enzyme activity (b) in the HSL and AHB effects on the total level of luminol-dependent chemiluminescence of neutrophile phagocytes (plotted on the vertical axis). Designations: *1* (white circles and dashed line), HSL effect; *2* (black circles and solid line), AHB effect.

in the tested system, the effects were only detectable within a narrow concentration range overlapping with the cytotoxicity range. Hence, the total contribution of the pro- or antioxidant activities of HSLs and AHBs to the level of oxidative metabolism in the neutrophile phagocytes treated with them was quite low. No significant correlations between the pro- or antioxidant activities of HSLs and AHBs per se (ICL_1) and the oxidative activity of neutrophiles (LCDL) were established.

In the second experimental variant, HSLs or AHBs were pretreated with peroxidase, the model enzyme. Measuring the chemiluminescence (ICL_2) of the model system containing peroxidase, hydrogen peroxide, and luminol revealed a decrease in ICL_2 in the system with peroxidase preincubated with the tested HSL and AHB homologues (Table 3). Their dose-dependent protein-modifying activity established earlier [14, 15] accounts for these data. Within the whole range of the tested AHB concentrations, ICL_2 decreased from 0.95 ± 0.06 to 0.09 ± 0.01 ($P < 0.001$) of the control value. This could be due to a decline in peroxidase catalytic activity. A more detailed characterization of the protein-modifying AHB effects with respect to peroxidase will be the subject of a separate contribution. Of particular importance in terms of this work was the detection of a positive correlation of the ICL_2 values with LCDL levels ($r = 0.687$, $P < 0.05$) measured at equivalent autoregulator concentrations (Fig. 2b). These data suggest that the inhibition of neutrophile oxidative metabolism is partly due to the protein-modifying effect of AHBs. Pretreatment of peroxidase with the tested HSL homologues also resulted in suppressing its catalytic activity. In the system with HSL homologue-pretreated peroxidase, a linear relationship between the final ICL_2 values and the corresponding LCDL levels in the cell-containing system was detected ($r = 0.749$; $P < 0.01$) (Fig. 2b).

DISCUSSION

The goal of this work was to examine a representative group of homologues of homoserine lactones (HSLs) and alkylhydroxybenzenes (AHBs), the bacterial autoregulators detected in the biological fluids of humans and animals, in terms of their capacity to influence the metabolic activity of neutrophile phagocytes from human peripheral blood. Standardized chemiluminescent assays were used in this work to lend more credibility to the comparative analysis of the HSL and AHB effects on the molecular and cell mechanisms of the oxidative activity of neutrophiles. The results obtained indicated that a number of homologues possess immunomodulating activity that is sufficient for the formation of network interaction patterns in symbiotic and parasitic systems. The effects of long-chain HSL and AHB homologues result in a marked inhibition of neutrophile oxidative metabolism that was determined by the suppression of induced luminol-dependent chemiluminescence

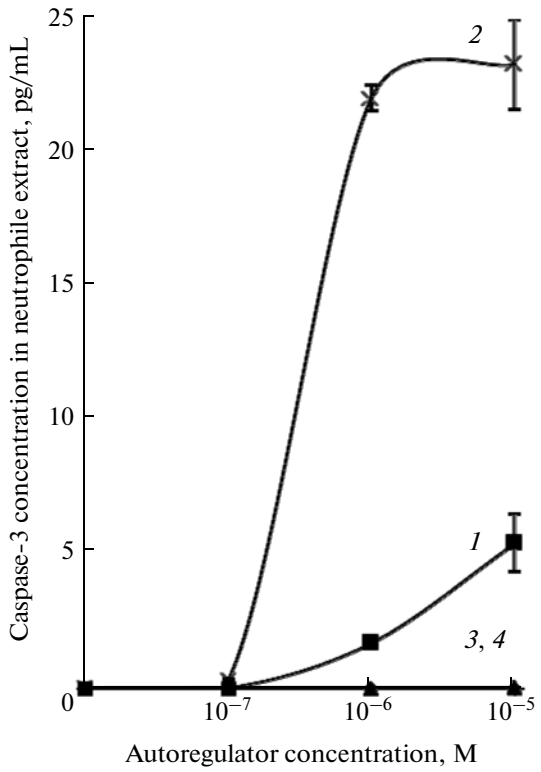

Fig. 3 Accumulation of caspase-3 in neutrophile extract after preincubation with C12-HSL for 1 (*1*) and 4 h (*2*) and C12-AHB for 1 (*3*) and 4 h (*4*).

(LDCL). Taking account of the important role of the oxygen-dependent bactericidal mechanisms in the antibacterial potential of neutrophiles [16], the biological effects of HSLs and AHBs revealed by us can be regarded as a manifestation of the immunosuppressive (anti-inflammatory) properties of bacterial autoregulators. This significantly contributes to our knowledge concerning their regulatory effects with respect to a wide range of molecular and cell factors involved in innate and adaptive immunity [11–15].

Research on the mechanisms of the effects of HSLs and AHBs detected in the phagocytosis system suggested that they are partially linked to the cytotoxic activity of the tested regulators, which increased with an increase in the length of their hydrocarbon chain and their concentration. It was more pronounced with HSLs. HSLs (exemplified by C12-HSL) induced cell death via apoptosis, whereas the cytolytic effects of AHBs (e.g., C12-AHB) were directly caused by their membranotropic activities [4, 5]. Generally, ANOVA revealed that the contributions of the cytotoxic properties of HSLs and AHBs to the inhibition of neutrophile oxidative metabolism was approximately equal, corresponding to 34.1% and 22.0%, respectively ($P < 0.01$) (Fig. 4). However, the cytotoxicity of the tested regulators only manifested itself at concentrations of 10^{-5} M and above. As for lower doses, both HSLs and AHBs failed to affect the integrity and viability of neutrophiles, which, nonetheless, were characterized by a low, partly inhibited, LCDL level.

Studies concerning the influence of bacterial regulators on reactive oxygen species (ROS) generation in model systems provided an explanation for the dose-dependent effects. One aspect of this influence involved the capacity of HSLs and AHBs to produce ROS or, conversely, to reduce their effective concentrations. In terms of pro-/antioxidant properties, HSLs and AHBs produced opposite effects that were, however, only moderately manifested with both regulators. Nevertheless, using other methods enabled obtaining high estimates of the antioxidant activity of AHBs, particularly of C1-AHB [4, 22, 35]. According to ANOVA, the effect of AHB antioxidant activity on neutrophile oxidative activity during phagocytosis was estimated to be 3.5% ($P < 0.05$), while the contribution of HSL pro-oxidant activity was statistically insignificant (0.8%; $P > 0.05$). Another aspect of the influence of HSLs and AHBs on neutrophile oxidative metabolism was considerably more important. The effect of both regulators on the catalytic activity of peroxidase, the central link of oxidative metabolic pathways, in a model system with peroxidase, H_2O_2, and luminol was due to their protein-modifying influence. The inhibition of peroxidase activity was more manifest with long-chain homologues of the tested bacterial autoregulators. As for C6- and C12-AHBs, they were expected to produce an inhibitory effect that was consistent with the previously revealed capacity of AHBs for nonspecific interactions with macromolecules of both enzymes and immune proteins. This interaction caused changes in their conformation and, accordingly, enabled AHBs to control the activities of these biopolymers [4, 5, 14, 15]. The contribution of the enzyme-modifying properties of long-chain homologues of this group of autoregulators to the suppression of the oxidative metabolism of neutrophiles was estimated to be 18.4% ($P < 0.01$) (Fig. 4). The reasons why C1-AHB possesses an analogous activity are more ambiguous. There is much evidence that it exhibits a pronounced stimulatory activity with respect to both simple and complex enzyme proteins [4, 5, 14, 24, 25]. The results obtained by us can be explained in light of data on the protein-modifying effect of polyphenols from *Ligustrum obtusifolium* leaves [26]. The authors of the work cited [26] established that the oxidized (quinone) forms of polyphenols completely inhibit amylase activity, while their unoxidized forms produce no inhibitory effect. We also revealed earlier that the modifying effects of AHBs vary depending on their oxidation degree [22, 23]. Based upon the C1-AHB effect on peroxidase in the luminol-dependent system, it seems very likely that there are two effects actually: (i) a decrease in ROS content due to the antioxidant activity of C1-AHB per se (as shown above) plus (ii) the inhibitory effect of the oxidized (quinone) form of C1-AHB generated by C1-AHB functioning as an ROS trap [23].

Table 3. Indices of the pro- and antioxidant properties of HSLs and AHBs per se (ICL_1) and their regulatory effects on peroxidase activity (ICL_2) in the peroxidase–H_2O_2–luminol system

Activity	Autoregulatory factor concentrations, M	Autoregulatory factors					
		HSL · HCl	C6-HSL	C12-HSL	C1-AHB	C6-AHB	C12-AHB
Oxidant properties of the tested agents (ICL_1)	10^{-9}	1.34 ± 0.08	1.11 ± 0.08	1.20 ± 0.07	1.14 ± 0.07	1.02 ± 0.08	1.11 ± 0.07
	10^{-8}	1.49* ± 0.07	1.21 ± 0.08	1.25 ± 0.08	1.40 ± 0.11	1.12 ± 0.07	1.18 ± 0.06
	10^{-7}	1.25 ± 0.06	1.28 ± 0.06	1.41* ± 0.08	1.36 ± 0.05	1.36 ± 0.09	1.07 ± 0.08
	10^{-6}	1.11 ± 0.06	1.55* ± 0.05	1.37* ± 0.08	1.23 ± 0.07	1.26 ± 0.07	1.12 ± 0.07
	10^{-5}	1.36 ± 0.10	2.05** ± 0.05	1.46* ± 0.09	0.60** ± 0.03	0.96 ± 0.06	1.18 ± 0.12
Peroxidase activity modulation (ICL_2)	10^{-9}	0.64* ± 0.04	0.65* ± 0.02	0.35** ± 0.02	0.95 ± 0.06	0.73 ± 0.04	0.95 ± 0.05
	10^{-8}	0.52* ± 0.03	0.60* ± 0.04	0.29** ± 0.02	0.33** ± 0.03	0.55* ± 0.04	0.85 ± 0.05
	10^{-7}	0.40* ± 0.03	0.54* ± 0.03	0.26** ± 0.02	0.25** ± 0.01	0.34** ± 0.02	0.56* ± 0.04
	10^{-6}	0.38** ± 0.03	0.49* ± 0.04	0.25** ± 0.02	0.26** ± 0.01	0.30** ± 0.02	0.52* ± 0.04
	10^{-5}	0.33** ± 0.02	0.43* ± 0.02	0.26** ± 0.01	0.01*** ± 0.01	0.33** ± 0.03	0.44* ± 0.03

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, relative to the control.

As for the HSL effects on peroxidase activity, there is evidence that long-chain HSL homologues can specifically interact with receptor proteins that are located on the neutrophile surface and involved in their chemotaxis [27]. We obtained data on the capacity of all HSL homologues to nonspecifically interact with hydrolases. In an analogy to AHBs, they cause changes in catalytic activity. Unlike AHBs, the HSL-caused activity changes are only inhibitory (unpublished). Presumably, peroxidase inhibition in the model system was due to HSLs' protein-modifying properties. According to ANOVA, HSLs' capacity to change the activity of the model enzyme system was 20.2% ($P < 0.05$) of the variability of the total LDCL level.

Analysis of ANOVA results also revealed that the factors considered in terms of ANOVA totally account for only 43 to 56% of the variability of neutrophile oxidative activity (based upon LDCL data). Unidentified reasons for the suppression of neutrophile oxidative metabolism in the presence of HSLs and AHBs may include their possible modifying effect on other enzyme proteins of the ROS-generating system, e.g., NADPH oxidase, as well as the HSL and AHB influence on myeloperoxidase release during neutrophile degranulation. These issues will be addressed in our further studies.

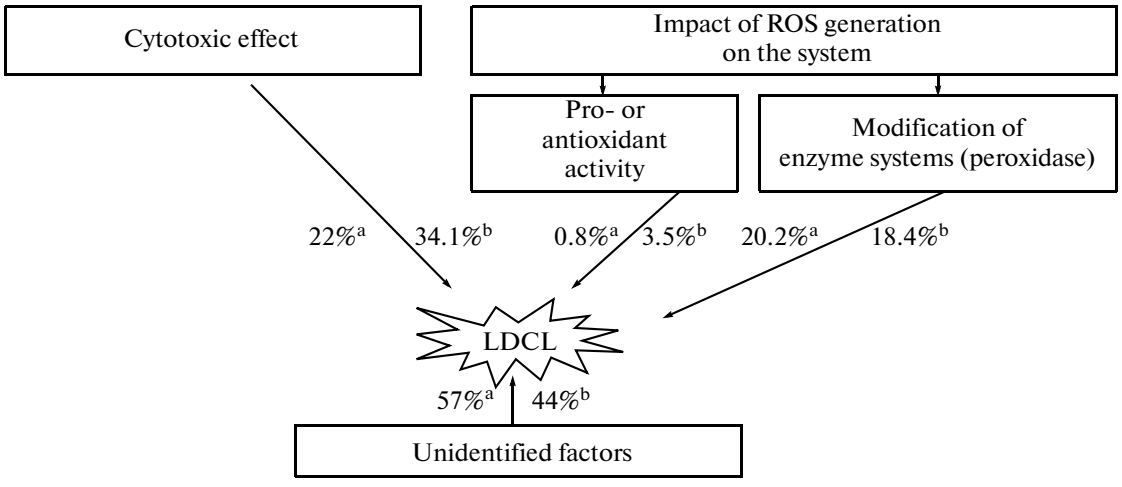

Fig. 4. ANOVA data (determination coefficients) concerning the contribution of specific mechanisms of action of HSLs (a) and AHBs (b) to the LDCL level of peripheral blood neutrophiles during phagocytosis.

Thus, the results obtained testify to a regulatory effect of bacterial autoregulators on neutrophile oxidative metabolism and point to a similarity between the biological activities of HSLs and AHBs. They also make it possible to elucidate the differences between their functional roles in symbiotic and parasitic systems. Altogether, this makes it possible to draw the guidelines for further studies and possible practical applications of the chemical analogs of HSLs and AHBs in medicine and biology. The multifunctional role of HSLs in heterologous biological systems is based on microbial population density control via quorum-sensing systems whose functioning is linked to the expression of virulence factors and biofilm formation [1–3] and manifests itself in disrupted functioning of neutrophile phagocytes and the induction of their apoptosis. Taken together, these properties give grounds for considering this group of autoregulators as a new factor of bacterial pathogenicity. This enhances the importance of research aimed at developing new methods of suppressing HSL formation and reception. They are currently regarded as the basis for a novel, alternative approach to controlling bacterial infections [28].

AHBs, the second group of tested autoregulators, display a prominent dose-dependent polymodal pattern of their effects, apart from the capacity for modifying neutrophile oxidative metabolism (as described above). Long-chain AHBs (C6- and C12-AHBs) exhibit growth-inhibiting activity [29]. Therefore, they can be envisaged as candidates for pharmaceutical preparations combining anti-inflammatory and antimicrobial properties. The short-chain C1-AHB possesses manifest adaptogenic [4, 5], radioprotective [22], and antioxidant [23, 30] properties which should be considered in terms of its immunomodulatory activity. The potential practical value held by chemical analogues of bacterial autoregulator molecules in medicine and biology is highlighted by the fact that these agents, particularly AHBs, are widely spread in plant products [5]. This accounts for some of the effects produced by traditional folk remedies and can help us harmonize the ratio between phytotherapy and the use of analogues of microbial multifunctional regulators.

Taken together, the results of our research enlarge our current knowledge concerning the possible involvement of small-size regulatory molecules in bioinformational interactions of microorganisms with higher organisms.

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