



The Combined Effect of $\alpha 7n$ -Acetylcholine and $\beta 2$ -Adrenergic Receptors Agonists on the Implementation of the Cholinergic Anti-Inflammatory Pathway in Early Phase of Sepsis

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Abstract

Experiments on random-bred albino mice showed that application of $\beta 2$ ARs agonist (hexaprenaline sulfate, 1,5 $\mu\text{g}/\text{kg}$, a single dose) and $\alpha 7n$ AChRs agonist (GTS-21, 15 mg/kg, a single dose) cause a significant decrease in the mortality of mice from experimental sepsis (i. p., *E. coli* O157:H7) when it is modeling 2 h after using these drugs due to a decrease of the concentration of proinflammatory cytokines TNF- α , IL-1 β , and IL-6 (implementation of the cholinergic anti-inflammatory pathway). The combined use of $\beta 2$ ARs and $\alpha 7n$ AChR agonists determines their additive effect.

Keywords: Cholinergic Anti-Inflammatory Pathway; Sepsis; $\beta 2$ ARs Agonist; $\alpha 7n$ AChRs Agonist; Proinflammatory Cytokines

Introduction

Mortality from sepsis, depending on various factors, ranges from 12 to 60% of all deaths associated with diseases and their complications [1], and there is an increase in the number of cases of sepsis and the mortality rate from it [2].

Cholinergic stimulation, as we established in 1987 [3] and in subsequent studies, significantly reduces the mortality of albino mice from sepsis caused by intraperitoneal or intrapulmonary administration, respectively of *E. coli* and *P. vulgaris* [3-7]. Thus, the cholinergic anti-inflammatory mechanism has been discovered in 1987 [3], named «cholinergic anti-inflammatory pathway» in 2000 [8] after the research its implementation at the organismal, cellular and subcellular levels [4,5,8,9]. It should be noted that in 1995 it was proved the possibility of cholinomimetics for emergency activation of antimicrobial resistance of the organism in sepsis [4,5]. In the future, the study of the cholinergic anti-inflammatory pathway caused by the action of acetylcholine on $\alpha 7n$ -acetylcholine receptors ($\alpha 7n$ AChRs) cells of the monocyte-macrophage system (MMC), followed by inhibition of the production by the cells of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and reduced mortality from sepsis were devoted hundreds of articles various authors [6,8-15].

Reduced production of TNF- α , IL-1 β , IL-6 (anti-inflammatory effect occurrence) for cholinergic anti-inflammatory pathway is provided kinase JAK2, transcription factor STAT3, NF- κ B transcription factor) [8,13-17].

When the cholinergic anti-inflammatory pathway is realized, in addition to the excitation of $\alpha 7n$ AChRs [9,15,18,19], which cause the effects already mentioned, nAChRs activation of the brain substance of the adrenal glands and sympathetic ganglia occurs, which leads to the production of epinephrine and norepinephrine (NE), which activation of macrophage-monocytic system cell (MMS) adrenergic receptors and reduce the production of pro-inflammatory cytokines [19]. At this *n. vagus*, releasing acetylcholine (ACh) in the celiac ganglion, causes excitation of the spleen nerve, the action of NE through its efferent fibers on T lymphocytes, the production of ACh by these lymphocytes, activation of ACh of $\alpha 7n$ AChRs of MMS cells of the spleen [9,19]. Epinephrine and NE probably activating the adrenergic receptors of cells of the MMS (direct action) [19], $\beta 2$ -adrenergic receptors ($\beta 2$ ARs) of spleen T-lymphocytes (indirect effect) [10], cause the same effect as activation of $\alpha 7n$ AChRs, leading to reduction in the synthesis of proinflammatory cytokines by cells of the MMS [9,11,15].

Aim of the study

The aim of the study was to evaluate the combined action of $\beta 2$ -adrenergic and $\alpha 7n$ -acetylcholinergic receptors agonists in the implementation of the cholinergic anti-inflammatory pathway in sepsis in mice.

Materials and Methods

Experiments were carried out on random-bred albino mice of both sexes weighing 18 - 22 g. The control group of mice (control group 1, n = 8) received i. p. 2.0 ml isotonic sodium chloride solution (saline) at 2 h after subcutaneous injection saline (0.5 ml). A second group of mice (control group 2, n = 55) were injected subcutaneously with 0.5 ml of saline once. After 2 h after administration of saline mice received (i. p.) 2.5×10^9 CFUs diurnal culture of *E. coli* O157: H7 in 2.0 ml of saline (sepsis modeling) [3-5,20]. As used $\beta 2$ ARs selective agonist hexoprenaline sulfate (Nycomed) subcutaneously a single dose of 1.5 $\mu\text{g}/\text{kg}$ in 0.5 ml of saline (group 3; n = 35). The fourth group of mice were injected an $\alpha 7n$ AChRs agonist GTS-21 [3-(2,4 - dimethoxy benzylidene)-anabaseine dihydrochloride] (Sigma-Aldrich) subcutaneously, 15 mg/kg, a single dose [21]. The fifth group of mice received a combined effect of $\beta 2$ ARs selective agonist hexoprenaline sulfate (1.5 $\mu\text{g}/\text{kg}$) and $\alpha 7n$ AChRs agonist GTS-21 (subcutaneously, a single dose of 15 mg/kg. Preparations (groups 3-5) were administered to mice 2 h before sepsis modeling.

Mortality in mice from experimental peritonitis was evaluated 4 and 24 h after the administration of 2.5×10^9 CFUs diurnal culture of *E. coli* O157:H7 in 2.0 ml of saline (i. p.).

The concentrations of TNF- α , IL-1 β , and IL -6 were measured in mice blood of all groups (groups 1 - 5) using by ELISA (My Bio Source) according to manufacturer's instructions (4 and 24 h after the sepsis modeling). To determine the concentration of proinflammatory cytokines used monoclonal antibodies My Bio Source (cat. N - MBS494184, MBS494492, MBS335516 for TNF- α , IL-1 β , and IL-6, respectively). Blood for analysis was collected from the retro-orbital sinus. The data processed statistically using Student's t test.

Results

The use of $\beta 2$ ARs agonist hexoprenaline sulfate and $\alpha 7n$ AChRs agonist (GTS-21), as well as their combination 2 hours before the sepsis modeling, caused a decrease ($p < 0.05$) mortality after 4 h compared with control group 2 (sepsis), respectively, in 2.13; 2.91 and 4.61 times ($p < 0,05$) ($p < 0,05$), respectively (Table 1) (by 19,3; 23,9 и 28,5%), and after 24 h - in 1.38; 1.59 и 3.15 times (by 25,2; 33,8 and 62,0%) ($p < 0,05$), respectively (table 1).

Series of Experiments	Term study of mortality after the introduction of <i>E. coli</i> , h	
	4	24
Sepsis (control group 2, n = 55)	36,4 \pm 6,5	90,9 \pm 3,9
$\beta 2$ ARs agonist hexoprenaline sulfate (group 3, n = 35)	17,1 \pm 6,3*	65,7 \pm 8,0*
$\alpha 7n$ AChRs agonist (GTS-21) + sepsis (group 4; n = 40)	12,5 \pm 5,1*	57,1 \pm 8,4*
$\beta 2$ ARs agonist + $\alpha 7n$ AChR agonist (GTS-21) + sepsis (group 5; n = 38)	7,9 \pm 4,4*	28,9 \pm 7,6**

Table 1: Effects of $\beta 2$ -adrenoreceptors agonist (hexoprenaline sulfate, 1,5 $\mu\text{g}/\text{kg}$), $\alpha 7n$ -acetylcholine receptors agonist (GTS-21, 15 mg/kg) and their combined effect on mortality of mice from sepsis (i.p., *E. coli* O157:H7), % (M \pm m) * - $p < 0,05$ as compared to control (group 2); ** - $p < 0,05$ as compared to control (group 2) and group 3 and 4.

A similar effect was caused by $\beta 2$ ARs agonist (hexoprenaline sulfate). There was no significant difference in mortality of mice between the parameters in these groups when using $\beta 2$ ARs and $\alpha 7n$ AChRs agonists 4 and 24 h after the sepsis modeling (groups 3 and 4). The combined action (group 5) of $\beta 2$ ARs and $\alpha 7n$ AChRs agonists caused a greater effect than the isolated effect of drugs.

The concentrations of TNF- α , IL-1 β and IL-6 cytokines significantly increased in the blood of mice 4 h after the sepsis modeling of (control group 2) compared to control group 1 (intact animals), respectively, in 17.8; 19.5 and 57.7 times ($p < 0.05$), after 24 h, the concentrations of these proinflammatory cytokines significantly decreased, exceeding the parameters of group 1 in 1.4 ($p > 0.05$), 4.5 and 8.2 times ($p < 0.05$), respectively (Table 2).

The obtained experimental data indicate that $\beta 2$ ARs agonist reduced the concentrations of TNF- α , IL-1 β and IL-6 in blood 4 h after sepsis modeling (group 3) in comparison with the parameters of control group 2 (sepsis without drugs), respectively, 3,8; 3,3 and 11.2 times ($p < 0.05$). In this case, the concentration of proinflammatory cytokines in the blood significantly ($p < 0.05$) exceeded the corresponding parameters of control group 1. The concentrations of TNF- α , IL-1 β and IL-6 24 h after sepsis modeling decreased compared to these parameters after 4 h, remaining below the values of group 2 in 1.3 ($p > 0.05$), 3.1 and 3.0 times ($p < 0.05$), respectively.

Series of experiments	TNF α		IL1 β		IL-6	
	4	24	4	24	4	24
Sepsis (control group 1)	34 \pm 5 (8)	38 \pm 6 (9)	26 \pm 4 (8)	28 \pm 5 (8)	33 \pm 6 (8)	25 \pm 4 (8)
Sepsis (control group 2)	606 \pm 84 ^a (8)	55 \pm 8 ^c (5)	507 \pm 68 ^a (8)	125 \pm 21 ^{ac} (5)	1905 \pm 243 ^a (7)	205 \pm 34 ^{ac} (5)
β 2ARs agonist -(hexaprenaline sulfate) + sepsis (group 3)	160 \pm 28 ^{ab} (7)	43 \pm 8 ^c (7)	155 \pm 20 ^{ab} (7)	41 \pm 7 ^{abc} (7)	170 \pm 29 ^{ab} (7)	69 \pm 12 ^{abc} (5)
α 7nAChR agonist (GTS-21) + sepsis (group 4)	179 \pm 23 ^{ab} (6)	36 \pm 7 ^{bc} (6)	174 \pm 18 ^{ab} (6)	57 \pm 7 ^{abc} (6)	205 \pm 25 ^{ab} (6)	59 \pm 8 ^{abc} (6)
β 2ARs agonist + α 7nAChRs agonist (GTS-21) + sepsis (group 5)	93 \pm 10 ^{abd} (7)	40 \pm 6 ^c (7)	84 \pm 9 ^{abd} (7)	20 \pm 3 ^{abcd} (7)	87 \pm 9 ^{abd} (7)	32 \pm 4 ^{abcd} (7)

Table 2: Effects of β 2-adrenoreceptors agonist (hexoprenaline sulfate, 1,5 μ g/kg), α 7n-acetylcholine receptors agonist (GTS-21, 15 mg/kg) and their combined effect on concentrations of proinflammatory cytokines in the blood of mice after sepsis modeling (i.p., E. coli O157:H7), pm/ml % (M \pm m)

Note. 4 and 24 - time after sepsis modeling, h; in parentheses is the number of mice; a -p <0.05 compared with control (group 1); b-p <0.05 compared with the corresponding parameter in sepsis (control group 2); c -p <0.05 compared with parameter after 4 h; d - p < 0.05 compared with parameters with isolated exposure to β 2ARs and α 7nAChRs agonists.

The concentrations of TNF- α , IL-1 β and IL-6 in the blood of mice after application of the α 7nAChR GTS-21 agonist 4 hours after sepsis modeling (group 4) decreased compared to the parameters of control group 2, respectively, in 3.4; 2.9 and 9.3 times (p < 0.05). There was a reduction of concentration of TNF- α , IL-1 β and IL-6 cytokines 24 h after sepsis modeling compared with the corresponding values after 4 h, remaining below the values of group 2, respectively, 1.6; 2.2 and 3.5 times (p < 0.05).

There was no significant difference of concentrations of TNF- α , IL-1 β and IL-6 in the blood of mice when using β 2ARs and α 7nAChRs agonists after modeling sepsis (groups 3 and 4).

The concentrations of TNF- α , IL-1 β , and IL-6 in the blood of mice 4 h after sepsis modeling (group 5) decreased compared to the values of control group 2 (sepsis) with the combined action of β 2ARs and α 7nAChRs agonists, respectively, in 6.5; 6.0 and 21.9 times (p < 0.05). The blood concentrations of these cytokines after 24 h significantly decreased compared to values after 4h, and compared with the parameters of group 2 their concentrations were lower in 1.4 (p > 0.05), 6.2 and 6.4 times, respectively (p < 0.05). The contents of proinflammatory cytokines in groups 3, 4, and 5 was statistically significant (p < 0.05) higher than the corresponding values of control group 1 after 4 h after sepsis modeling.

The proinflammatory cytokines after the use of β 2ARs and α 7nAChRs agonists in sepsis (groups 3 and 4) decreased to a lesser extent (p < 0.05) than with their combined effect (group 5). So, the combination of β 2ARs and α 7nAChRs agonists 4 h after the sepsis modeling reduced the concentrations of TNF- α , IL-1 β and IL-6 in the blood of mice compared to the isolated action of these preparations, respectively, in 1.8; 1.8; 2.0 times (p < 0.05) compared with group 3 and 1.9; 2.1; 2.4 times (p < 0.05) compared with group 4. This suggests that the additive effect of these drugs (β 2ARs and α 7nAChRs agonists) in the implementation of the cholinergic anti-inflammatory pathway is noted.

Discussion

The data obtained suggest that the α 7nAChRs agonist (GTS-21) due to the implementation of the cholinergic anti-inflammatory pathway [6,22] leads to a decrease in mortality from sepsis [3,4,5] due to a decrease of MMS cell production of pro-inflammatory cytokines [23,24].

The literature data [6,10,25] suggest that the additive effect of β 2ARs and α 7nAChRs agonists (reduction in mortality from sepsis) is associated with a decrease of the concentrations of proinflammatory cytokines in the blood by hexaprenaline sulfate and GTS-21 due to activation of the cholinergic anti-inflammatory pathway and adrenergic mechanisms. Excitation of nAChRs of the adrenal

glands and sympathetic ganglia causes activation of MMS cell adrenergic receptors by epinephrine and NE and suppression of the cytokines TNF- α , IL-1 β and IL-6 [19,24,25]. The described effects are enhanced by a decrease in the synthesis of proinflammatory cytokines by the $\alpha 7$ nAChRs agonist (GTS-21), acting directly on $\alpha 7$ nAChRs of MMS cells [6,23,25,26].

It is known that monocytes and macrophages have β ARs, and their activation usually leads to anti-inflammatory effect [19] due to inhibition of the nuclear transcription factor NF- κ B [27]. Mechanisms of the reduction of synthesis of proinflammatory cytokines by the action of an agonist $\beta 2$ ARs (action on MMS cells) currently not well understood, but research results are inconsistent [18,19].

Conclusion

1. The application of $\beta 2$ ARs and $\alpha 7$ nAChRs agonists (hexaprenaline sulfate and GTS-21) cause a significant decrease in the mortality of mice from experimental sepsis (i.p., *E. coli* O157:H7) when it is modeling 2 h after using these drugs due to a decrease of the concentration of proinflammatory cytokines TNF- α , IL-1 β , and IL-6 (implementation of the cholinergic anti-inflammatory pathway).
2. The combined use of $\beta 2$ ARs and $\alpha 7$ nAChRs agonists determines their additive effect.

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