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Research Article

The Effects of α7n-Acetylcholine, β2-Adrenergic Receptors Agonists, NF-κB, STAT3 Inhibitors on the Implementation of the Cholinergic Anti-Inflammatory Pathway in Sepsis

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Abstract

Experiments on random-bred albino mice showed that the administration of α7n-acetylcholine receptors agonist (GTS-21, 15 mg/kg), β2-adrenoreceptor agonist (dexmedetomidine hydrochloride, 25 μg/kg), NF-κB inhibitor (BAY 11-7082, 10 mg/kg) and STAT3 inhibitor (S3I-201, 15 mg/kg) to mice 2h before sepsis modeling (i.p, 2.5 × 109 CFUs diurnal culture of *E. coli* O157:H7) caused a significant reduction of mouse mortality at 4 and 24h after *E. coli* injection due to a decrease in the proinflammatory cytokines TNF-α, IL-1β and IL-6 (implementation of a cholinergic anti-inflammatory pathway). The effects of the preparations used did not differ significantly.

Keywords: Cholinergic Anti-inflammatory Pathway; Sepsis; Proinflammatory Cytokines; α7n-Acetylcholine Receptor; β2-Adrenoreceptor; NF-κB Inhibitor; STAT3 Inhibitor

Introduction

Sepsis is a serious public health problem. Worldwide, the incidence of sepsis ranges from 20 to 30 million cases per year, with the frequency of lethality increasing [1-4]. From all deaths associated with diseases and their complications, mortality from sepsis, depending on various factors, ranges from 12 to 60% [5]. The lethality from septic shock, the most severe form of sepsis, continues to be above 50% [6]. We discovered the cholinergic anti-inflammatory mechanism in 1987 [7], named the "cholinergic anti-inflammatory pathway" after studying its implementation at the organismal, systemic, organ, cellular and subcellular levels in 2000 - 2002 [6-10]. We proved the possibility of using cholinomimetics for emergency activation of antimicrobial resistance in sepsis in 1995 [9,10]. The cholinergic anti-inflammatory pathway [3,6,11,12] is realized as a result of activation of acetylcholine m-acetylcholine receptors type 1 (m1AChRs) of the brain, modulating the immune regulating function of the vagus nerve; excitation of efferent fibers n. vagus; action of acetylcholine on α7n-acetylcholine receptors (α7nAChRs) cells of the monocyte-macrophage system. In the cells of this system, the onset of anti-inflammatory effect is provided by kinase JAK2; signal transducer and activator of transcription 3 (transcription factor) - STAT3; transcription factor NF-κB (nuclear factor kappa B, NF-kappa B). Activation of α7nAChRs of the monocyte-macrophage system by acetylcholine, influencing the function of these biochemical factors, leads to a decrease in the lethality from sepsis due to inhibition of production by the cells of the monocyte-macrophagal system of proinflammatory cytokines TNF-α, IL-1β, IL-6, B1-HMGB1 protein, macrophage-inflammatory protein- 2 - MIP-2 [3,4,8,11,13]. The similar effect is caused by adrenergic anti-inflammatory mechanism [3] (activation of epinephrine and norepinephrine of β2-adrenoreceptors - β2ARs - monocyte-macrophage system and spleen T-lymphocytes due to excitation of sympathetic ganglia and adrenal medulla by n-acetylcholine receptors) [3,8,14,15].

The comparative study of the effects of α7nAChRs, β2ARs agonists NF-kB and STAT3 inhibitors in sepsis is of current interest to develop methods to reduce mortality in this complication [3,4,16-19].

Aim of the Study

The aim of the study was to comparative evaluate the α7n-acetylcholine receptors (GTS-21) agonist, β2-adrenoreceptors agonist (dexmedetomidine hydrochloride), NF-κB inhibitor (BAY 11-7082) and STAT3 inhibitor (S3I-201) on the mice mortality after sepsis modeling caused by experimental peritonitis and the content of proinflammatory cytokines TNF-α, IL-1β, IL-6 in the blood.

Materials and Methods

Experiments were carried out on random-bred albino mice of both sexes weighing 18 - 22g. The control group of mice (control group 1, n = 9) received intraperitoneally 2.0 ml of isotonic sodium chloride solution (saline) 2h after the intraperitoneal administration of 0.5 ml of 0.05% aqueous solution of dimethyl sulfoxide - DMSO (Sigma-Aldrich) The second group of mice (control group 2, n = 160) was injected with 0.5 ml of a 0.05% aqueous solution of DMSO (i.p.) The mice in this group at 2h after administration of this solution received (i.p.) 2.5×10^9 CFUs diurnal culture of *E. coli* O157:H7 in 2.0 ml of saline (sepsis modeling) [1,7,9,20]. The third group of mice was given (i.p., *n* = 45) the α7nAChRs agonist GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride] (Sigma-Aldrich) at single dose of 15 mg/kg in 0.5 ml of a 0.05% aqueous solution of DMSO [21]. As the selective agonist of β2ARs (dexmedetomidine hydrochloride - Orion Pharma) was used, which was administered (i.p.) at single dose of 25 μg/kg [22] in 0.5 ml of a 0.05% aqueous solution of DMSO (group 4, n = 42). The fifth group (n = 38) was administered (i.p.) of NF-κB inhibitor BAY 11-7082 (Sigma-Aldrich) at single dose of 10 mg/kg in 0.5 ml of a 0.05% aqueous solution of DMSO [19]. The sixth group (n = 40) was administered (i.p.) STAT3 inhibitor (S3I-201 - 2-Hydroxy-4-[[[[(4-methylphenyl)sulfonyl]oxy]acetyl]amino]-benzoic acid) (Sigma-Aldrich) at single dose of 15 mg/kg in 0.5 ml of a 0.05% aqueous solution of DMSO [17]. The mice in groups 3 - 6 received at 2h after administration of α7nAChRs agonist, β2ARs agonist, NF-κB and STAT3 inhibitors received (i.p.) 2.5×10^9 CFU daily culture of *E. coli* O157:H7 in 2.0 ml saline (sepsis modeling).

The mortality of mice (groups 2 - 6) was recorded at 4 and 24h after the sepsis modelling the concentration of TNF-α, IL1β and IL-6 were measured in the blood plasma of all groups of mice (groups 1 - 6) using by ELISA (MyBioSoure) according to manufacturer's instructions (at 4 and 24h after the sepsis modeling). Determination the concentrations of proinflammatory cytokines used monoclonal antibodies MyBioSource (cat. N - MBS494184, MBS494492, MBS335516 for TNF-**α**, IL-1β and IL-6, respectively). Blood for research was taken from the retroorbital venous sinus. The obtained data were processed statistically using Student's t-test. Differences between the parameters were considered reliable at $p < 0.05$.

Results

The α7nAChRs agonist (GTS-21), β2ARs agonist (dexmedetomidine hydrochloride), NF-κB inhibitor (BAY 11-7082) and STAT3 inhibitor (S3I-201) caused a decrease in the mortality of mice 4h after the administration of the daily culture of *E. coli* (sepsis modeling) compared to the control group 2 (sepsis) by 2.5 times (by 30.0%), by 1.9 times (by 23.8%), by 1.7 times (by 21.1%) and by 1.5 times (by 17.5%) respectively ($p < 0.05$). The α7nAChRs agonist, β2ARs agonist, NF-κB and STAT3 inhibitors caused a decrease in the mortality of mice 24h after the sepsis modeling compared to the control group 2 (sepsis) by 1.4 times (by 24.7%), by 1.5 times (by 27.7%), by 1.7 times (by 32.5%) and by 1.3 times (by 17.5%) respectively ($p < 0.05$). The reduction in mortality under the different preparations (groups 3 - 5) was not significantly different ($p > 0.05$) (Table 1).

Table 1: Effects of α7n-acetylcholine receptors agonist (GTS-21, 15 mg/kg), β2-adrenoreceptor agonist (dexmedetomidine hydrochloride, 25 μg/kg), NF-κB inhibitor (BAY 11-7082, 10 mg/ kg), and STAT3 inhibitor (S3I-201, 15 mg/kg) on the mice mortality after sepsis modeling, % (M ± m).

Series of experiments	Term study of mortality after administration of *E. coli*, h	
	4	24
Sepsis (control group 2, n = 160)	50.0 ± 4,0	82,5 ± 3,0
α7nAChRs agonist (GTS-21) + sepsis (group 3; n = 45)	20,0 ± 6,0*	57,8 ± 7,4*
β2ARs agonist hexaprenaline sulfate + sepsis (group 4, n = 42)	26.2 ± 6.8*	54,8 ± 7.8*
NF-κB inhibitor BAY 11-7082 + sepsis (group 5, n = 38)	28,9 ± 7,5*	50,0 ± 8,2*
STAT3 inhibitor - S3I-201+ sepsis (group 6, n = 40)	32.5 ± 7.5*	65.0 ± 7.6*

* -$p < 0,05$ as compared to control (group 2).

The concentration of cytokines TNF-α, IL-1β and IL-6 after the sepsis modeling (control group 2) in the blood of mice after 4h compared with the control group 1 (intact animals), increased respectively by 18.5; 18.1 and 50.5 times ($p < 0.05$) and after 24h, the content of these cytokines compared to their level after 4h decreased, respectively, by 13.2; 4.8 and 8.4 times ($p < 0.05$). The content of IL-1β and IL-6 after 24h remained higher than in group 1 by 1.6 times ($p > 0.05$) and 4.9 times ($p < 0.05$), respectively, and the concentration of TNF-α in groups 1 and 2 did not differ significantly (Table 2).

The α7nAChRs agonist (GTS-21) at 4h after sepsis modeling reduced the blood levels of TNF-α, IL-1β and IL-6 (group 3) compared to the control group 2, respectively, by 3.6; 3.8 and 9.1 times

Table 2: Effects of α7n-acetylcholine receptors agonist (GTS-21, 15 mg/kg), β2-adrenoreceptor agonist (dexmedetomidine hydrochloride, 25 μg/kg), NF-κB inhibitor (BAY 11-7082, 10 mg/kg) and STAT3 inhibitor (S3I-201, 15 mg/kg) on the concentration of proinflammatory cytokines in blood of mice after sepsis modeling, pg/ml (M ± m; n=8-9)

Series of experiments	TNF-α		IL-1β		IL-6	
	4	24	4	24	4	24
Control group 1	42 ± 5	38 ± 6	35 ± 6	27 ± 7	45 ± 7	25 ± 5
Sepsis (Control group 2)	780 ± 90[a]	59 ± 9[c]	635 ± 73[a]	132 ± 25[ac]	2273 ± 263[a]	271 ± 38[ac]
α7nAChRs agonist (GTS-21) + sepsis (group 3)	151 ± 22[ab]	49 ± 9[c]	167 ± 30[ab]	54 ± 8[abc]	250 ± 33[ab]	82 ± 14[abc]
β2ARs agonist (dexmedetomidine hydrochloride) + sepsis (group 4)	169 ± 28[ab]	52 ± 8[c]	184 ± 32[ab]	64 ± 10[abc]	295 ± 35[ab]	105 ± 14[abc]
NF-κB inhibitor (BAY 11-7082) + sepsis (group 5)	129 ± 22[ab]	47 ± 7[c]	120 ± 24[ab]	52 ± 8[abc]	220 ± 29[ab]	75 ± 9[abc]
STAT3 inhibitor (S3I-201) + sepsis (group 6)	137 ± 30[ab]	43 ± 8[c]	155 ± 36[ab]	70 ± 11 [abc]	312 ± 38[ab]	94 ± 10[abc]

Note. 4 and 24 - time after sepsis modeling, h; in parentheses - number of animals; [a] -p <0.05 compared to control (group 1); [b]- p < 0.05 compared to the corresponding parameter of control group 2 (sepsis modeling); [c] -p <0.05 compared with parameter after 4 h.

(p < 0.05). The content of these cytokines after 24h compared with their level after 4h decreased, respectively, by 3.1; 2.4 and 3.3 times (p < 0.05). The concentrations of IL-1β and IL-6 after 24h statistically significantly (p < 0.05) exceeded those of the control group 1 by 2.0 and 3.3 times (p < 0.05), respectively and compared to the parameters of group 2, the content IL-1β and IL-6 were reduced by 2.4 and 3.3 times respectively (p < 0.05).

The β2ARs agonist (dexmedetomidine hydrochloride) at 4h after sepsis modeling decreased the blood concentrations of TNF-α, IL-1β and IL-6 (group 4) compared to the control group 2 by 4.6; 3.5 and 7.7 times respectively (p < 0.05). The blood levels of TNF-α, IL-1β and IL-6 after 24h compared with their level after 4h decreased, respectively, by 3.3; 2.9 and 2.8 times (p < 0.05). The concentrations of IL-1β and IL-6 after 24h were significantly higher (p < 0.05) than those of the control group 1 by 2.4 and 4.2 times (p < 0.05), respectively, and compared to the parameters of group 2, the blood concentrations of IL-1β and IL-6 were decreased by 2.6 times (p < 0.05).

The NF-κB inhibitor (BAY 11-7082) at 4h after sepsis modeling reduced the blood levels of TNF-α, IL-1β and IL-6 (group 5) compared to the control group 2, respectively, by 6.1; 5.3 and 10.3 times (p < 0.05). The content of these cytokines after 24h compared with their level after 4h decreased, respectively, by 2.7; 2.3 and 2.9 times (p < 0.05). The concentrations of IL-1β and IL-6 after 24h statistically significantly (p < 0.05) exceeded those of the control group 1 by 1.9 and 3.0 times (p < 0.05), respectively, and compared to the parameters of group 2, the content IL-1β and IL-6 were reduced by 2.5 and 3.6 times respectively (p < 0.05).

The STAT3 inhibitor (S3I-201) at 4h after sepsis modeling decreased the blood concentrations of TNF-α, IL-1β and IL-6 (group 6) compared to the control group 2 by 5.7; 1.9 and 7.3 times respectively (p < 0.05). The blood levels of TNF-α, IL-1β and IL-6 after 24h compared with their level after 4h decreased, respectively, by 3.2; 2.2 and 3.3 times (p < 0.05). The concentrations of IL-1β and IL-6 after 24h were significantly higher (p < 0.05) than those of the control group 1 by 2.4 and 4.2 times (p < 0.05), respectively, and compared to the parameters of group 2, the content IL-1β and IL-6 were reduced, respectively, by 1.9 and 2.9 times (p < 0.05).

The value of TNF-α was not significantly different from the levels in groups 1 - 6 at 24h after the sepsis modeling. The blood concentrations of proinflammatory cytokines TNF-α, IL-1β and IL-6 at 4 and 24h after sepsis modeling with the α7n-acetylcholine receptors agonist (GTS-21), β2-adrenoreceptor agonist (dexmedetomidine hydrochloride), NF-κB inhibitor (BAY 11-7082) and STAT3 inhibitor (S3I-201) administration (groups 3-6) were not significantly different from those of control group 2 (sepsis modeling without the use of preparations).

Discussion

The α7nAChRs agonist (GTS-21) due to the implementation of the cholinergic anti-inflammatory pathway [4,11,23] leads to a reduction in sepsis mortality [7,9,10,24] as a result of decrease of the production of proinflammatory cytokines by cells of the monocyte-macrophage system [3,4,13,24-26]. The β2ARs agonist (hexaprenaline sulfate) caused a similar effect. When β2ARs and α7nAChRs agonists were used, there was no significant difference in mouse mortality between the parameters in these groups (3 and 4) after sepsis modeling. The adrenergic mechanism (the action of the β2ARs agonist) is an important component in the implementa-

tion of the cholinergic anti-inflammatory pathway [3]. Monocytes and macrophages are known to have βARs, and their activation, results in reduced synthesis of proinflammatory cytokines due to impaired Ca^{2+} exchange and probably inhibition of the nuclear transcription factor NF-κB [5,14,27].

The reduction in mortality in mice after sepsis modeling under the influence of NF-κB inhibitor (BAY 11-7082) and STAT3 inhibitor (S3I-201) is due to a decrease in proinflammatory cytokine production (TNF-α, protein B1 - HMGB1, macrophage inflammatory protein-2 - MIP-2, interleukins - IL-1β, IL-6) by cells of the monocyte-macrophage system [4,11,24,28,29]. The transcription factor NF-κB modulates the synthesis of proinflammatory cytokines involved in the development of sepsis. Signalling pathways initiated by Toll-like receptors (TLR2 and TLR4) to which bacterial products, particularly *E. coli* lipopolysaccharide, bind lead to increased transcription of genes responsible for the expression of cytokines, chemokines, adhesion molecules, apoptotic factors and other mediators of sepsis-related inflammatory response [19].

The absence of statistically significant differences in mortality and blood concentrations of TNF-α, IL-1β, IL-6 in sepsis models under the action of α7nAChRs agonist (GTS-21), β2ARs agonist (dexmedetomidine hydrochloride), NF-κB inhibitor (BAY 11-7082) and STAT3 inhibitor (S3I-201) is due to the fact that these drugs were used in equivalent (equally therapeutic) doses [3,4,17,19,21,22,24,30]. Their effect is ultimately realized by reducing the production of proinflammatory cytokines [4,11,28,29].

Conclusion

The administration of α7n-acetylcholine receptors agonist (GTS-21, 15 mg/kg), β2-adrenoreceptors agonist (dexmedetomidine hydrochloride, 25 μg/kg), NF-κB inhibitor (BAY 11-7082, 10 mg/kg) and STAT3 inhibitor (S3I-201, 15 mg/kg) to mice 2h before sepsis modeling (i.p, *E. coli* O157:H7) caused a significant reduction of mouse mortality at 4 and 24h after *E. coli* injection due to a decrease in the proinflammatory cytokines TNF-α, IL-1β and IL-6 (implementation of the cholinergic anti-inflammatory pathway). The effects of the preparations used did not differ significantly.

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