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Case Report

Biostatistical Analysis in Early Phase of Sepsis

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1. Abstract

Experiments on random-bred albino mice showed that application of β2ARs agonist (hexaprenaline sulfate, 1,5μg/kg, a single dose) and α7nAChRs agonist (GTS-21, 15 mg/kg, a single dose) cause a significant decrease in themortality of mice from experimental sepsis (i.p., E. coli O157:H7) when it is modeling 2 h after using these drugsdue toa decrease of the concentration of proinflammatory cytokines TNF-α, IL-1β, and IL-6 (implementation of thecholinergicanti-inflammatory pathway). The combined use of β2ARs and α7nAChR agonists determines their additive effect.

2. Keywords

Cholinergic anti-inflammatory pathway; Sepsis; β2ARs agonist; α7nAChR agonist; Proinflammatory Cytokines

3. Introduction

Mortality from sepsis, depending on various factors, ranges from 12 to 60% of all deaths associated withdiseases 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 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 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 ofacetylcholine on α7n-acetylcholine receptors (α7nAChRs) cells of the monocyte-macrophage system (MMC), followedby inhibition of the production by the cells of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and reduced mortalityfrom sepsis were devoted hundreds of articles various authors [6-15]. Reduced production of TNF-α,IL-1β, IL-6 (anti-inflammatory effect occurrence) for cholinergic anti-inflammatory pathway is provided kinase JAK2,transcription factor STAT3, NF-κB transcription factor) [8,13-17].

When the cholinergic anti-inflammatory pathway is realized, in addition to the excitation of α7nAChRs[9,15,18,19], which cause the effects already mentioned, nAChRs activation of the brain substance of the adrenal glandsand sympathetic ganglia occurs, which leads to the production of epinephrine and norepinephrine (NE), which activationof macrophage-monocytic system cell (MMS) adrenergic receptors and reduce the production of pro-inflammatorycytokines [19]. At this n.vagus, releasing acetylcholine (ACh) in the celiac ganglion, causes excitation of the spleennerve, the action of NE through its efferent fibers on T lymphocytes, the production of ACh by these lymphocytes,activation of ACh of α7nAChRs of MMS cells of the spleen [9,19]. Epinephrine and NE probably activating theadrenergic receptors of cells of the MMS (direct action) [19], β2-adrenergic receptors (β2ARs) of spleen T-lymphocytes(indirect effect) [10], cause the same effect as activation of α7nAChRs, leading to reduction in the synthesis ofpro-inflammatory cytokines by cells of the MMS [9,11,15].

4. Aim of the Study

The aim of the study was to evaluate the combined action of β2-adrenergic and α7n-acetylcholinergic receptorsagonists in the implementation of the cholinergic anti-inflammatory pathway in sepsis in mice.

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5. Materials and Methods

Experiments were carried out on random-bred albino mice of both sexes weighing 18-22g. The control groupof mice (control group 1, n = 8) received i.p. 2.0 ml isotonic sodium chloride solution (saline) at 2 h after subcutaneousinjection saline (0.5 ml). A second group of mice (control group 2, n = 55) were injected subcutaneously with 0.5 mlofsaline once. After 2 h after administration of saline mice received (i.p.) 2.5×109 CFUs diurnal culture of E. coliO157:H7 in 2.0 ml of saline (sepsis modeling) [3-5,20]. As used β2ARs selective agonist hexoprenaline sulfate(Nycomed) subcutaneously a single dose of 1.5 μg/kg in 0.5 ml of saline (group 3; n = 35). The fourth group of micewere injected an α7nAChRs agonist GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseinedihydrochloride] (Sigma-Aldrich) subcutaneously, 15 mg/kg, a single dose [21]. The fifth group of mice received a combined effect of β2ARs selective agonist hexoprenaline sulfate (1.5 μg/kg) and α7nAChRs agonist GTS-21 (subcutaneously, a single dose of 15mg/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 2.5×109CFUs diurnal culture of E. coli O157:H7 in 2.0 ml of saline (i.p.).The concentrations of TNF-α, IL-1β, and IL-6 were measured in mice blood of all groups (groups 1-5) using byELISA (MyBioSoure) according to manufacturer's instructions (4 and 24 h after the sepsis modeling). To determine theconcentration of proinflammatory cytokines used monoclonal antibodiesMyBioSource (cat. N -MBS494184,MBS494492, MBS335516 for TNF-α, IL-1β, and IL-6, respectively). Blood for analysis was collected from theretro orbital sinus. The date processed statistically using Student's t test.

6. Results

The use of β2ARs agonist hexoprenaline sulfate and α7nAChRs agonist (GTS-21), as well as their combination2 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 24h – in 1.38; 1.59 и 3.15 times (by 25,2; 33,8 and 62,0%) ($p<0, 05$), respectively (**Table 1**).

A similar effect was caused by β2ARs agonist (hexoprenaline sulfate). There was no significant difference inmortality of mice between the parameters in these groups when using β2ARs and α7nAChRs agonists 4 and 24 h afterthe sepsis modeling (groups 3 and 4). The combined action (group 5) of β2ARs and α7nAChRs agonists caused a greatereffect than the isolated effect of drugs.

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

Table 1: Effects of β2-adrenoreceptors agonist (hexoprenaline sulfate, 1,5μg/kg), α7n-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±m)

Series of experiments	Term study of mortality after the introduction of *E. coli*, h	
	4	24
Sepsis (control group 2, n = 55)	36,4±6,5	90,9±3,9
β2ARs agonist hexaprenaline sulfate (group 3, n = 35)	17,1±6,3*	65,7±8,0*
α7nAChRs agonist (GTS-21) + sepsis (group 4; n = 40)	12,5±5,1*	57,1±8,4*
β2ARs agonist +α7nAChR agonist (GTS-21) + sepsis (group 5; n = 38)	7,9±4,4*	28,9±7,6**

* – $p <0,05$ as compared to control (group 2); ** – $p<0,05$ as compared to control (group 2) and group 3 and4.

Table 2: Effects of β2-adrenoreceptors agonist (hexoprenaline sulfate, 1,5μg/kg), α7n-acetylcholine receptors agonist(GTS-21, 15 mg/kg) and their combined effect on concentrations of pro-inflammatory cytokines in the blood of mice aftersepsis modeling (i.p., E. coli O157:H7), pm/ml % (M±m)

Series of experiments	ФНОα		ИЛ1β		ИЛ-6	
	4	24	4	24	4	24
Sepsis (control group 1)	34±5 (8)	38±6 (9)	26±4 (8)	28±5 (8)	33±6 (8)	25±4 (8)
Sepsis (control group 2)	$606±8^{a}$ (8)	$55±8^{c}$(5)	$507±68^{a}$ (8)	$125±21^{ac}$ (5)	$1905±243^{a}$ (7)	$205±34^{ac}$ (5)
β2ARs agonist - (hexaprenaline sulfate) + sepsis (group 3)	$160±28^{ab}$ (7)	$43±8^{c}$ (7)	$155±20^{ab}$ (7)	$41±7^{abc}$ (7)	$170±29^{ab}$ (7)	$69±12^{abc}$ (5)
α7nAChR agonist (GTS-21) + sepsis (group 4)	$179±23^{ab}$ (6)	$36±7^{bc}$ (6)	$174±18^{ab}$ (6)	$57±7^{abc}$ (6))	$205±25^{ab}$ (6)	$59±8^{abc}$ (6)
β2ARs agonist +α7nAChRs agonist (GTS-21) + sepsis (group 5)	$93±10^{abd}$ (7)	$40±6^{c}$ (7)	$84±9^{abd}$ (7)	$20±3^{abcd}$ (7)	$87±9^{abd}$ (7)	$32±4^{abcd}$ (7)

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 withparameter after 4 h; d - $p <0.05$ compared with parameters with isolated exposure to β2ARs and α7nAChRs agonists.

The obtained experimental data indicate that β2ARs agonist reduced the concentrations of TNF-α, IL-1β and IL-6 in blood 4 h after sepsis modeling (group 3) in comparison with the parameters of control group 2 (sepsis withoutdrugs), respectively, 3,8; 3.3 and 11.2 times ($p <0.05$). In this case, the concentration of pro-inflammatory cytokines in theblood significantly ($p <0.05$) exceeded the corresponding parameters of control group 1. The concentrations of TNF-α,IL-1β and IL-6 24 h after sepsis modeling decreased compared to these parameters after 4 h, remaining below the valuesof group 2 in 1.3 ($p> 0.05$), 3.1 and 3.0 times ($p <0.05$), respectively.

The concentrations of TNF-α, IL-1β and IL-6 in the blood of mice after application of the α7nAChR GTS-21agonist 4 hours

after sepsis modeling (group 4) decreased compared to the parameters of control group 2, respectively, in3.4; 2.9 and 9.3 times ($p < 0.05$). There was a reduction of concentration of TNF-α, IL-1β and IL-6 cytokines 24 h aftersepsis 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-1β and IL-6 in the blood of mice when usingβ2ARs and α7nAChRs agonists after modeling sepsis (groups 3 and 4).

The concentrations of TNF-α, IL-1β, 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 β2ARs and α7nAChRs 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 4 h, and compared with the parameters of group 2 their concentrations werelower in 1.4 ($p>0.05$), 6.2 and 6.4 times, respectively ($p<0.05$). The contents of pro-inflammatory cytokines ingroups 3, 4,and 5 was statistically significant ($p<0.05$) higher than the corresponding valuesof control group 1 after4 h aftersepsis modeling.

The pro-inflammatory cytokines after the use of β2ARs and α7nAChRs 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 β2ARs and α7nAChRs agonists 4 h after the sepsis modeling reduced the concentrations of TNF-α, IL-1β and IL-6 in the blood ofmice compared to the isolated action of these preparations, respectively, in 1.8; 1.8; 2.0 times ($p < 0.05$) comparedwithgroup 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(β2ARs and α7nAChRs agonists) in the implementation of the cholinergic anti-inflammatory pathway is noted.

7. Discussion

The data obtained suggest that the α7nAChRs agonist (GTS-21) due to the implementation of the cholinergicanti-inflammatory pathway [6,22] leads to a decrease in mortality from sepsis [3,4,5] due to a decrease of MMS cellproduction of pro-inflammatory cytokines [23,24].

The literature data [6,10,25] suggest that the additive effect of β2ARs and α7nAChRs agonists (reduction inmortality from sepsis) is associated with a decrease of the concentrations of pro-inflammatory cytokines in the bloodbyhexoprenaline sulfate and GTS-21 due to activation of the cholinergic anti-inflammatory pathway and adrenergicmechanisms. Excitation of nAChRs of the adrenal glands and sympathetic ganglia causes activation of MMS celladrenergic receptors by epinephrine and NE and suppression of the cytokines TNF-α, IL-1β and IL-6 [19,24,25]. The described effects are enhanced by a decrease in the synthesis of pro-inflammatory cytokines by the α7nAChRs agonist(GTS-21), acting directly on α7nAChRs of MMS cells [6,23,25,26].

It is known that monocytes and macrophages have βARs, and their activation usually leads to anti-inflammatoryeffect [19] due to inhibition of the nuclear transcription factor NF-κB[27]. Mechanisms of the reduction of synthesisofpro-inflammatory cytokines by the action of an agonist β2ARs (action on MMS cells) currently not wellunderstood, butresearch results are inconsistent [18,19].

8. Conclusions

The application of β2ARs and α7nAChRs agonists (hexoprenaline sulfate and GTS-21) cause a significantdecreasein the mortality of mice from experimental sepsis (i.p., E. coli O157:H7) when it is modeling 2 h after usingthese drugs due to a decrease of the concentration of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 (implementationof the cholinergic anti-inflammatory pathway). The combined use of β2ARs and α7nAChRs agonists determines theiradditive effect.

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