



The Realization of the Cholinergic Anti-Inflammatory Pathway Under the Influence of $\alpha 7$ nAChRs Agonist and STAT3 Inhibitor in Sepsis

Pavel Franzevich Zabrodskii*

Saratov Medical University "REAVIZ", Saratov, Russian Federation

***Corresponding Author:** Pavel Franzevich Zabrodskii, Saratov Medical University "REAVIZ", Saratov, Russian Federation.

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Abstract

Experiments on random-bred albino mice showed that $\alpha 7$ n-acetylcholine receptors ($\alpha 7$ nAChRs) agonist (GTS-21) and STAT3 inhibitor (S31 - 201) lead to the realization of the cholinergic anti-inflammatory pathway (the reduce the mortality of mice, the blood concentrations of proinflammatory cytokines TNF- α , IL-1 β and IL-6 in sepsis (intraperitoneal of 2.5×10^9 CFUs diurnal culture of *E. coli*). The combined action of $\alpha 7$ nAChRs agonist and STAT3 inhibitor causes an additive effect.

Keywords: Cholinergic Anti-Inflammatory Pathway; Proinflammatory Cytokines; Sepsis; $\alpha 7$ n-Acetylcholine Receptors; 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 [1,2]. From all deaths associated with diseases and their complications, mortality from sepsis, depending on various factors, ranges from 12 to 60% [3,4]. For the first time in 1987, it was found that cholinergic stimulation significantly reduces the mortality of albino mice from sepsis [5], and later proved the feasibility of using cholinomimetics for emergency activation of the body's antimicrobial resistance in sepsis [6,7]. The cholinergic anti-inflammatory mechanism [5] was named after the study of its implementation on the organismic, cellular and subcellular levels in 2000 as the "cholinergic anti-inflammatory pathway" [5-9].

The cholinergic anti-inflammatory pathway [6-12], includes: acetylcholine m-acetyl cholinergic receptor type 1 (m1AChRs) activation of the brain, modulating the immunoregulatory function of the vagus nerve [9,10,13,14]; excitation of efferent fibers n. vagus; effect of acetylcholine on $\alpha 7$ nAChRs of the macrophage-monocytic

system (MMS) cells [12,14,15]. The occurrence of anti-inflammatory effect in cells of MMS is provided by JAK2 kinase (tyrosine-protein kinase JAK2); STAT3 transcription factor (STAT3 - signal transducer and activator of transcription 3); NF- κ B transcription factor (NF- κ B - nuclear factor kappa-light-chain-enhancer of activated B cells) [9-11,13,14]. These effects lead to a decrease in mortality from sepsis due to the reduction of the production of proinflammatory cytokines TNF- α , protein B1 - HMGB1, macrophage-inflammatory protein-2 - MIP-2, interleukins - IL-1 β , IL-6 [8-11,14].

It is of great interest to study the possibility of reducing mortality in sepsis and various pathological processes by stimulating or inhibiting various elements of the cholinergic anti-inflammatory pathway [13,15-17], in particular the possibility of achieving a therapeutic effect with activation of $\alpha 7$ nAChRs in combination with inhibition of the STAT3 transcription factor [16,18,19].

Aim of the study

The aim of the study was to assess the combined effect of the $\alpha 7$ n-acetylcholine receptors agonist and the NF- κ B inhibitor on the implementation of the cholinergic anti-inflammatory pathway in

early phase of sepsis (estimation of the mortality rate of mice in sepsis caused by experimental peritonitis and the blood content of proinflammatory cytokines TNF- α , IL-1 β , IL-6).

Materials and Methods

The experiments were performed on random-bred albino mice of both sexes weighing 18 - 22 g. The control group of mice (control group 1, $n = 8$) received intraperitoneally 2.0 ml of isotonic sodium chloride solution (saline) 10 - 15 minutes after the last intraperitoneal (i. p.) was administered of 0.5 ml of 0.05% aqueous solution of dimethyl sulfoxide - DMSO (Sigma-Aldrich), which was used daily for 4 days. The second group of mice (control group 2, $n = 50$) was injected for 4 days with 0.5 ml of a 0.05% aqueous solution of DMSO (i.p., once daily). 2 h after administration of this solution, mice in this group received (i. p.) 2.5×10^9 CFUs diurnal culture of *E. coli* in 2.0 ml of saline (sepsis modeling) [5-8,13,14,20,21].

The third group of mice ($n = 40$) was injected with $\alpha 7$ nAChR agonist GTS-21 [3-(2,4-dimethoxybenzylidene)-anabas Eine dihydrochloride] (Sigma-Aldrich) subcutaneously, 5 mg/kg, once daily for 4 days (in 0.5 ml of a 0.05% aqueous solution of DMSO), taking into account GTS-21 half-life period of 12 - 24 h [22].

The fourth group ($n = 25$) was administered (i.p., once daily) for 4 days STAT3 inhibitor (S3I-201 - 2-Hydroxy-4-[[[(4-methylphenyl)sulfonyl]oxy]acetyl]amino]-benzoic acid) (Sigma-Aldrich) at a dose of 5 mg/kg in 0.5 ml of a 0.05% aqueous solution of DMSO [18].

In the 5th group ($n = 30$) the $\alpha 7$ nAChR agonist (GTS-21) was administered (once daily for 4 days) in combination the STAT3 inhibitor (S3I-201). The STAT3 inhibitor was administered 10 - 15 minutes after the injection of the $\alpha 7$ nAChR agonist. In groups 3-5, after 2 h after the administration of drugs, sepsis was modeled. The mortality of mice (groups 2 - 3) was recorded after 4 and 24 h after the sepsis modeling. The concentration of TNF- α , IL1 β and IL-6 were measured in the blood plasma of all groups of mice (groups 1 - 5) using by ELISA (My Bio Soure) according to manufacturer's instructions. Determination the concentrations 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 research was taken from the retroorbital venous sinus. The data obtained were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p < 0.05$.

Results

The $\alpha 7$ nAChR agonist (GTS-21) caused a decrease of the mortality of mice after 4 h after the administration of the daily culture of *E. coli* (sepsis modeling) compared to the control group 2 (sepsis) by 2.29 times (by 22.5% - $p < 0.05$), and after 24 h - by 1.45 times (by 26.0% - $p < 0.05$). The STAT3 inhibitor (S3I-201) reduced mortality from sepsis after 4 and 24 h after sepsis modeling compared with group 2, respectively, by 2.00 times ($p < 0.05$) (by 20.0%) and by 1.53 times (by 30,0%) ($p < 0.05$). The administration of the $\alpha 7$ nAChR agonist in combination with the STAT3 inhibitor caused an additive effect. Thus, the mortality of mice compared with the control after 4 and 24 h after the administration of *E. coli* compared with the control (group 2) decreased, respectively, by 4.00 times (by 30.0%) ($p < 0.05$) and by 3,23 times (by 59.4%) ($p < 0.05$) (Table 1). It should be noted that the reduction of mice mortality from sepsis in the combined effect of the $\alpha 7$ nAChR agonist and the STAT3 inhibitor after 24 h after sepsis modeling compared to parameters of groups 3 and 4 was significant ($p < 0.05$), and in 4 h - statistically insignificant ($p > 0.05$), despite the difference of 2.0 times. The effects of the $\alpha 7$ nAChR agonist and the STAT3 inhibitor in estimating mouse mortality were practically the same.

Group (series of experiments)	Term study of mortality after the introduction of <i>E. coli</i> , h	
	4 h	24 h
2nd control group (sepsis; $n = 50$)	40,0 \pm 6,9	86,0 \pm 4,9
3rd ($\alpha 7$ nAChRs agonist GTS-21; $n = 40$)	17,5 \pm 6.4*	60,0 \pm 8.0*
4rd (STAT3 inhibitor - S3I-201; $n = 25$)	20.0 \pm 8.0*	56.0 \pm 9.9*
5th ($\alpha 7$ nAChRs agonist +STAT3 inhibitor; $n = 30$)	10.0 \pm 5,5*	26.6 \pm 8.1**

Table 1: Effect of $\alpha 7$ nAChRs agonist (GTS-21, 5 mg/kg, once daily for 4 days) and STAT3 inhibitor (S3I-201, 5 mg/kg, once daily for 4 days) and their combined effect on the mice mortality after sepsis modeling, % (M \pm m).* - $p < 0,05$ as compared to group 2); ** - $p < 0,05$ in comparison with the control (group 2) and groups 3; 4.

The concentration of cytokines TNF- α , IL-1 β and IL-6 after the sepsis modeling (control group 2) in the blood of mice after 4 h compared with the control group 1 (intact animals) increased respectively to 22.4; 17.1 and 51.9 times ($p < 0.05$), and the content

of these cytokines after 24 h (after the sepsis modeling) compared to their level after 4 h decreased, respectively, to 23.0; 4.4 and 8.4 times ($p < 0.05$). The content of IL-1 β and IL-6 after 24 h remained

higher than in group 1 by 3.6 times ($p < 0.05$) and 10.3 times ($p < 0.05$), respectively, and the concentration of TNF- α in groups 1 and 2 did not differ significantly (Table 2).

Series of experiments	ФНО α		ИЛ1 β		ИЛ-6	
	4	24	4	24	4	24
Control group 1	43 \pm 6	32 \pm 6	36 \pm 5	39 \pm 7	40 \pm 7	24 \pm 5
Sepsis (control group 2)	966 \pm 105 ^a	42 \pm 8 ^c	615 \pm 78 ^a	141 \pm 25 ^{ac}	2077 \pm 262 ^a	246 \pm 30 ^{ac}
$\alpha 7$ nAChRs agonist (GTS-21) (group 3)	143 \pm 17 ^{ab}	29 \pm 5 ^{bc}	163 \pm 19 ^{ab}	56 \pm 7 ^{abc}	214 \pm 21 ^{ab}	61 \pm 8 ^{abc}
STAT3 inhibitor (S3I-201) (group 4)	160 \pm 25 ^{ab}	36 \pm 7 ^c	170 \pm 26 ^{ab}	60 \pm 11 ^{bc}	294 \pm 33 ^{ab}	87 \pm 11 ^{abc}
$\alpha 7$ nAChR agonist + STAT3 inhibitor (group 5)	95 \pm 13 ^{abd}	27 \pm 6 ^c	98 \pm 16 ^{abd}	30 \pm 6 ^{bcd}	150 \pm 19 ^{abd}	43 \pm 7 ^{abce}

Table 2: Effect of $\alpha 7$ nAChRs agonist (GTS-21, 5 mg/kg, once daily for 4 days) and STAT3 inhibitor (S3I-201, 5 mg/kg, once daily for 4 days) and their combined effect on the concentration of proinflammatory cytokines in blood of mice after sepsis modeling, pg/ml ($M \pm m$; n=7-8).

Note. 4 and 24 - time after modeling of sepsis, h; a - $p < 0.05$ compared to control (group 1); b- $p < 0.05$ compared with corresponding parameter for sepsis (control group 2); c - $p < 0.05$ compared with parameter after 4 h; d - $p < 0,05$ - in comparison with groups 3; 4; e - $p < 0,05$ - in comparison with groups 4.

The $\alpha 7$ nAChRs agonist after 4 h after sepsis modeling reduced the blood levels of TNF- α , IL-1 β and IL-6 (group 3) compared to the control group 2, respectively, by 6.8; 3.8 and 9.7 times ($p < 0.05$). The blood content of these cytokines after 24 h, compared with their level after 4 hours decreased, respectively, by 4.9; 2.9 and 3.5 times ($p < 0.05$). The concentrations of IL-1 β and IL-6 (after 24 h after sepsis modeling) statistically significantly ($p < 0.05$) exceeded those of the control group 1 by 1.4 and 2.5 times ($p < 0.05$), respectively, and compared to the parameters of group 2 the content IL-1 β and IL-6 were reduced by 2.5 and 4.0 times, respectively ($p < 0.05$). The value of TNF- α was not significantly different from the levels in groups 1 and 2 after 24 h after the sepsis modeling.

The STAT3 inhibitor (S3I-201) 4 h after sepsis modeling reduced the blood levels of TNF- α , IL-1 β and IL-6 (group 4) compared to the control group 2, respectively, by 6.0; 2.4 and 7.1 times ($p < 0.05$). The concentrations of TNF- α , IL-1 β and IL-6 after 24 h after sepsis modeling compared with their level after 4 h decreased, respectively, by 4.0; 2.8 and 3.4 times ($p < 0.05$). The concentrations of IL-1 β and IL-6 after 24 h after sepsis modeling statistically significantly ($p < 0.05$) exceeded those of the control group 1 by 1.5 and 3.6 times ($p < 0.05$), respectively, and compared to the parameters of group 2, the content IL-1 β and IL-6 were reduced by 2.4

and 2.8 times, respectively ($p < 0.05$). The value of TNF- α was not significantly different from the levels in groups 1 - 3 after 24 h after the sepsis modeling. The blood content of TNF- α , IL-1 β and IL-6 in groups 3 and 4 were statistically no different.

The concentrations of TNF- α , IL-1 β and IL-6 in group 5 (combined effect of $\alpha 7$ nAChRs agonist and STAT3 inhibitor) compared with parameters of group 3 (effect of the $\alpha 7$ nAChRs agonist) after 4 h after sepsis modeling were lower respectively by 1.5 ($p < 0.05$); 1.7 ($p < 0.05$) and 1.4 times ($p < 0.05$), and after 24 h the levels of IL-1 β and IL-6 were less than the corresponding values in the group of 3 by 1.9 ($p < 0, 05$) and by 1.4 times ($p > 0.05$). The value of TNF- α was not significantly different from the levels in groups 1-4 after 24 h after the sepsis modeling.

The decrease of the proinflammatory cytokines concentrations at the combined effect of $\alpha 7$ nAChRs agonist and STAT3 inhibitor (group 5) compared with parameters of group 4 (effect of the STAT3 inhibitor) was reduced in the same way as in comparison with parameters of group 3 (effect of $\alpha 7$ nAChRs agonist).

The data obtained suggest that the combined action at the combined effect of $\alpha 7$ nAChRs agonist and STAT3 inhibitor causes an additive effect.

Discussion

The data obtained and the results described in numerous articles [8-10,13] suggest that the decrease in mice mortality from sepsis under the action of the $\alpha 7$ nAChRs agonist is due to a decrease in the concentration of proinflammatory cytokines (reduces the production of proinflammatory cytokines by macrophages and monocytes), in particular, TNF- α , IL-1 β and IL-6. Activation of $\alpha 7$ nAChRs of MMS cells with the participation of JAK2 kinase, NF- κ B and STAT3 transcription factor [9-11,13].

The NF- κ B transcription factor and STAT3 transcription factor modulates the synthesis of proinflammatory cytokines involved in development of sepsis. Signal pathways initiated by Toll-like receptors (TLR2 and TLR4) to which bacterial products bind, in particular, *E. coli* lipopolysaccharide, lead to enhanced transcription of genes responsible for expression of cytokines, chemokines, adhesion molecules, apoptotic factors and other mediators of inflammatory response associated with sepsis [8,14,23].

We have found that simultaneous stimulation of the $\alpha 7$ nAChRs agonist and STAT3 transcription factor inhibition significantly reduces the synthesis of proinflammatory cytokines TNF- α , IL-1 β and IL-6. The combined action at the combined effect of $\alpha 7$ nAChRs agonist and STAT3 inhibitor causes an additive effect. The data obtained by us can be used in the development of promising treatments for sepsis and other inflammatory diseases.

Conclusion

The combined effect of $\alpha 7$ n-acetylcholine receptors agonist (GTS-21) and STAT3 inhibitor (S31 - 201) reduce the mortality of random-bred albino mice, the blood concentrations of proinflammatory cytokines TNF- α , IL-1 β and IL-6 in sepsis - the realization of the cholinergic anti-inflammatory pathway. The combined action of $\alpha 7$ nAChRs agonist and STAT3 inhibitor causes an additive effect.

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