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The Combined Effect of STAT3 and NF-Kb Inhibitors (The Last Links of the Cholinergic Anti-Inflammatory Pathway) on Mouse Mortality and Blood Concentration of Proinflammatory Cytokines in Sepsis

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Research Article

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Abstract

Experiments on random-bred albino mice showed that the inhibitor of the transcription factor STAT3 (S3I-201) and the NF-Kb inhibitor (BAY 11-7082) significantly reduced the mortality of mice from experimental sepsis (intraperitoneal administration of E. coli) in 4 and 24 h after modeling by reducing blood levels of Proinflammatory cytokines TNFα, IL-1β, and IL-6. The combined actions of STAT3 and NF-Kb inhibitors (the last links of the cholinergic anti-inflammatory pathway) have an additive effect.

Keywords: Cholinergic Anti-Inflammatory Pathway; Proinflammatory Cytokines; Sepsis; STAT3 Inhibitor; NF-Kb Inhibitor

Introduction

Mortality from sepsis, depending on various factors, ranges from 12 to 60% of all deaths associated with diseases and their complications [1], and it is noted as an increase in the number of cases of sepsis, and mortality rate from it [2-5]. For the first time in 1987, it was found that cholinergic stimulation significantly reduces the mortality of albino mice from sepsis [6], and later proved the feasibility of using cholinomimetics for emergency activation of the body's antimicrobial resistance in sepsis [7]. The cholinergic anti-inflammatory mechanism was named after the study of its implementation on the organismic [6], cellular and subcellular levels in 2000 as the "cholinergic anti-inflammatory pathway" [6-8].

Currently, there is a great interest of researchers associated with the study of the possibility of reducing mortality in sepsis and various pathological processes by stimulating or inhibiting various elements of the cholinergic anti-inflammatory pathway [3-5,9,10], in particular, the possibility of achieving a therapeutic effect while inhibiting of NF-Kb and STAT3 transcription factor [9,11,12] (the last links of the cholinergic anti-inflammatory pathway) [8].

Aim of the Study

The aim of the study was to assess the effect of the inhibitor of the STAT3 transcription factor (S3I-201 drug) and the NF-Kb inhibitor (BAY 11-7082), as well as their combined effect (the last links of the cholinergic anti-inflammatory pathway) on the mortality of mice in the early phase of sepsis caused by experimental peritonitis, and the content of Proinflammatory cytokines TNF - α, IL-1 β, IL-6 in the blood.

Materials and Methods

The experiments were performed on random-bred albino mice of both sexes weighing 18-22g. The control group of mice (control group 1, n=8) received intraperitoneally 2.0ml of isotonic sodium chloride solution (saline) 10-15 minutes after the last intraperitoneal the introduction of 0.5ml 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=47) was injected for 4 days with 0.5ml of a 0.05% aqueous solution of DMSO (i.p., once daily). 30-45 min after administration of this solution, mice in this group received (i.p.,) 2.5×109 CFUs diurnal culture of *E. coli* in 2.0ml of saline (sepsis modeling) [4-7]. The third group (n=25) was administered (i.p., once daily) for 4days STAT3 inhibitor (S3I-201-2-Hydroxy-4-[[[[(4-methylphenyl) sulfonyl] oxy] acetyl] amino]-benzoic acid) (Sigma-Aldrich) at a dose of 5mg/kg in 0.5ml of a 0.05% aqueous solution of DMSO [11]. The fourth group (n=33) was administered (i.p., a single dose) the NF-Kb inhibitor BAY 11-7082-(E)-3-(4-Methylphenylsulfonyl)-2-propenenitrile-(Sigma-Aldrich) at a dose of 10 mg/kg in 0.5ml of a 1% aqueous solution of DMSO (Sigma-Aldrich) [5]. Prior to the administration of the NF-Kb inhibitor this group of mice received 0.5 ml of a 0.05% aqueous solution of DMSO for 3 days (i.p., once daily). In the 5th group (n=25) the STAT3 inhibitor (S3I-201) was administered in combination with the NF-Kb BAY inhibitor (11-7082). NF-Kb inhibitor was administered 10-15 minutes after the last injection of the STAT3 inhibitor. In groups 3 and 5, after 30-45 minutes after the administration of drugs, sepsis was modeled (in group 3, after 30-45 minutes after the last injection of the drug).

The mortality of mice (groups 2-3) was recorded after 4 and 24h after the sepsis modeling. The concentration of TNF-α, IL1β and IL-6 were measured in the blood plasma of all groups of mice (groups 1-5) using by ELISA (My Bio Source) 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-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 (program STATISTICA 6.0).

Results

The STAT3 inhibitor (S3I-201) caused a decrease in the mortality of mice 4 hours after the administration of the daily culture of *E. coli* (sepsis modeling) compared to the control group 2 (sepsis) by 2.4 times (by 28.1%-p<0.05), and after 24hours-by 1.4 times (by 24.9%-p<0.05). The NF-Kb inhibitor reduced mortality from sepsis after 4 and 24hours after its modeling compared with group 2, respectively, 2.0 times (p<0.05) (by 24.7%) and 1.8 times (by 35,4%) (p<0.05). The administration of the STAT3 inhibitor in combination with the NF-Kb inhibitor caused an additive effect. Thus, the mortality of mice compared with the control after 4 and 24hours after the administration of *E. coli* compared with the control (group 2) decreased, respectively, by 3.1 times (by 32.9%) (p<0.05) and by 2, 9 times (by 52.9%) (p<0.05) (Table 1).

Group (series of experiments)	Term study of Mortality After the Introduction of *E. coli*, h	
	4 h	24 h
2nd control group (sepsis; n=47)	48.9±7.3	80.9±5.7
3rd (STAT3 inhibitor - S3I-201; n=25)	20.0±8.0*	56.0±9.9*
4th (NF-Kb inhibitor BAY 11-7082; n=33)	24.2±7.5*	45.5±8.7*
5th (STAT3 inhibitor + NF-Kb inhibitor; n=25)	16.0±7.3*	28.0±9.0**

* -p<0.05 as compared to group 2); ** - p <0.05 in comparison with the control group 2 and group 3.

Table 1: Effect of STAT3 inhibitor (S3I-201, 5mg/kg, once daily for 4 days) and NF-Kb inhibitor (BAY 11-7082, 10mg/kg, and a single dose) and their combined effect on mice mortality in sepsis, % (**M**±*m*).

The combined effect of the drugs compared to mortality with only STAT3 inhibitor or NF-Kb inhibitor caused a statistically insignificant reduction of the studied parameter after 4 hours, respectively, by 1.3 times (by 4.0%-p>0.05) and by 1.5 times (by 8.2%-p>0.05), After 24 hours, a significant effect of the combination of drugs was observed compared with the effect of only the STAT3 inhibitor (a decrease in mortality by 2.0 times (28.0%-p <0.05), respectively. The obtained data show that the combined effect of the STAT3 and the NF-Kb inhibitors exceeds the effect of the STAT3 inhibitor, and insignificantly (p>0.05) the effect of the NF-Kb inhibitor.

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 to 19.7; 19.8 and 59.3 times (p<0.05), and after 24hours, the content of these cytokines compared to their level after 4h decreased, respectively, to 23.0; 4.4 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 3.2 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	ФНОα		ИЛ1β		ИЛ-6	
	4	24	4	24	4	24
Control group 1	49±6	38±5	31±5	33±6	35±7	25±4
Sepsis (control group 2)	966±105a	42±8c	615±78a	141±25ac	2077±262a	246±30ac
STAT3 inhibitor-S3I-201 (group 3)	174±23ab	34±7c	160±25ab	52±10 bc	286±32ab	81±13abc
NF-Kb inhibitor-BAY 11-7082 (group 4)	137±20ab	45±8c	141±26ab	60±9 abc	233±30ab	70±14abc
STAT3 inhibitor+ NF-kb inhibitor (group 5)	108±17ab d	50±8c	122±21ab	35±6 abc	191±27abd	45±7abcd

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 4h; [d] -p<0.05-in comparison with group 3.
Table 2: Effect of STAT3 inhibitor (S3I-201, 5mg/kg, once daily for 4 days) and NF-Kb inhibitor (BAY 11-7082, 10mg/kg, a single dose) and their combined effect on the concentration of Proinflammatory cytokines in blood of mice after modeling of sepsis, pg/ml (M±m; n=7-8).

The NF-Kb inhibitor (BAY 11-7082) 4 h after sepsis modeling reduced the blood levels of TNF-α, IL-1β and IL-6 (group 4) compared to the control group 2, respectively, by 7.0; 4.4 and 8.9 times (p<0.05). After 24 hours, the content of these cytokines compared with their level after 4 hours decreased, respectively, by 3.0; 2.4 and 3.3 times (p<0.05). The concentrations of IL-1β and IL-6 statistically significantly (p<0.05) exceeded those of the control group 1 by 1.8 and 7.8 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 3.5 times, respectively (p<0.05). The value of TNF-α was not significantly different from the levels in groups 1 and after 24h after the sepsis modeling.

The concentrations of TNF-α, IL-1β and IL-6 in group 5 (combined effect of STAT3 and NF-Kb inhibitors) compared with parameters of group 3 (effect of STAT3 inhibitor) 4h after sepsis modeling were lower respectively by 1.6 (p<0.05); 1.3 (p>0.05) and 1.5 times (p<0.05), and after 24h the levels of IL-1β and IL-6 were less than the corresponding values in the group of 3 by 1.5 (p>0, 05) and by 1.8 times (p<0.05).

The blood levels of TNF-α, IL-1β, and IL-6 in group 5 compared with those of group 4 (the effect of NF-Kb inhibitor) did not differ significantly (p>0.05) after 4 h after the sepsis modeling, and after 24 h IL-1β and IL-6 levels were less than the corresponding values in group 4 by 1.7 (p<0.05) and by 1.6 times (p>0.05).

The blood concentrations of TNF-α, IL-1β and IL-6 in group 5 with a combined effect of STAT3 inhibitor (S3I-201) and of NF-Kb inhibitor (BAY 11-7082) 4h after modeling sepsis compared with the control group 2 (sepsis without the use of drugs) decreased, respectively, by 8.9; 5.0 and 5.5 times (p<0.05). The content of cytokines IL-1β and IL-6 significantly (p<0.05) exceeded the corresponding parameters of group 1. The concentration of pro-inflammatory cytokines after 24h after modeling sepsis significantly decreased (p<0.05) compared with these parameters after 4h. The content of TNF-α did not differ from those in groups 1-4, and the levels of IL-1β and IL-6 remained lower than the values in group 2 by 4.0 and by 5.5 times (p<0.05), respectively.

The data obtained suggest that the combined action of STAT3 inhibitor and NF-Kb inhibitor (the last links of the cholinergic anti-inflammatory pathway) causes an additive effect.

Discussion

The cholinergic anti-inflammatory mechanism ("cholinergic anti-inflammatory pathway") [6,7,13-15], includes: acetylcholine m-acetyl cholinergic receptor type 1 (m1AChR) activation of the brain, modulating the immunoregulatory function of the vagus nerve [4,5,8,13]; excitation of efferent fibers n. vagus; effect of acetylcholine on α7n-acetylcholine receptors (α7nAChR) of cells of the macrophage-monocytic system (MMS) [3,5,15]. The occurrence of anti-inflammatory effect in cells of MMS is provided by JAK2 kinase; STAT3 transcription factor; NF-κB transcription factor [4,5,8,13,14]. These effects lead to a decrease in mortality from sepsis due to the reduction of the production of pro-inflammatory cytokines TNF-α, protein B1-HMGB1, macrophage-inflammatory protein-2-MIP-2, interleukins-IL-1β, IL-6 [5,7,8,13,14]. We have shown a decrease in the mortality of mice from sepsis after activation of α7nAChRs [3,4], m1AChRs [4], α2-adrenoreceptors (β2ARs) [5], inhibition of NF-κB factor [5], and also their combined action was evaluated.

The transcription factor NF-Kb modulates the synthesis of pro-inflammatory 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 [5].

Conclusion

The STAT3 inhibitor (S3I-201) and the NF-Kb inhibitor (BAY 11-7082) the last links of the cholinergic anti-inflammatory pathway-reduce the mortality of mice, the content of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in the blood in sepsis, and their combined action causes an additive effect.

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