



## The Influence of Various Drugs on Mortality of Mice and the Concentration of Proinflammatory Cytokines in Blood at Sepsis Caused by *E. coli*

Pavel Franzevich Zabrodskii\*

Saratov Medical University "REAVIZ", Saratov, Russia

\*Corresponding Author: Pavel Franzevich Zabrodskii, Saratov Medical University "REAVIZ", Saratov, Russia.

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### Abstract

Experiments on albino mice showed that administration m-cholinomimetic (aceclidine), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-cholinomimetic  $\alpha 7nAChRs$  agonist (GTS-21), epinephrine hydrochloride, adrenomimetic  $\beta 2ARs$  agonist (hexoprenaline sulfate) causes decrease in mice mortality in sepsis caused by the administration (i.p.) of *E. coli* O157:H7 and the concentration of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 in the blood in comparison with parameters at sepsis without use of drugs.

**Keywords:** Sepsis; Cytokines; *E. coli*; Cholinergic Drugs; Adrenergic Drugs

From all the lethal outcomes associated with diseases and their complications, mortality from sepsis, including that caused by opportunistic Gram-negative microorganisms (*Escherichia coli*, etc.) varies from 12 to 60% depending on various factors [1] and the frequency of lethality from it increases [2,3]. In 1987, a cholinergic anti-inflammatory mechanism was discovered [4], named in 2002 "cholinergic anti-inflammatory pathway" [7] after the study of its realization at the organismal, cellular and subcellular levels [5-7]. It should be noted that in 1995 the possibility of using cholinomimetics for the immediate activation of antimicrobial resistance of the organism during sepsis was proved [5]. Further study of the cholinergic anti-inflammatory pathway caused by the action of acetylcholine on  $\alpha 7nAChRs$  of cells of the monocyte-macrophage system, followed by inhibition of the production of these cells by pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, B1-HMGB1 protein, macrophage-inflammatory protein-2-MIP-2 and decrease in mortality from sepsis were devoted to hundreds of articles by different authors [8-11].

The cholinergic anti-inflammatory pathway is realized due to the activation of acetylcholine m-acetylcholine receptors type 1 (m1AChR) of the brain, modulating the immunoregulatory function of the vagus nerve; excitation of efferent fibers n. vagus; the action of acetylcholine on  $\alpha 7nAChRs$  cells of monocyte-macrophage system [3,6,7,12,13]. In cells of the monocyte-macrophage system, the anti-inflammatory effect is provided by the kinase JAK2, the transcription factor STAT3, the transcription factor NF- $\kappa B$  (nuclear factor kappa B, NF-kappa B) [3,6].

Along with the cholinergic anti-inflammatory pathway, there is an adrenergic anti-inflammatory mechanism [3], associated with sepsis, inflammatory bowel diseases and other infectious processes involving the activation of adrenal medulla and sympathetic ganglia n-cholinergic receptors, which leads to epinephrine and norepinephrine production, which by exciting adrenoceptors of

cells of monocyte-macrophage system [3,14],  $\beta 2$ -adrenoceptors ( $\beta 2ARs$ ) of spleen T-lymphocytes [3,9], cause the same effect as the activation of  $\alpha 7nAChRs$ , leading to reduction of synthesis of proinflammatory cytokines by cells of the monocyte-macrophage system [3,7,10].

### Aim of the Study

The aim of the study was to study cholinergic and adrenergic drugs on mouse mortality in early phase of experimental sepsis caused by *E. coli* and the content of proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6 in the blood.

### Materials and Methods

The experiments were carried out on mongrel white mice of both sexes weighing 18 - 22g. The control group of mice (control group 1) received 0.5 ml of isotonic sodium chloride solution (saline) 10 - 30 minutes later in 2.0 ml of saline. The second group of mice (control group 2) was injected i.p., once with 0.5-1.0 ml of saline. Fifteen to 60 minutes after the administration of saline, mice received  $2.5 \times 10^9$  CFUs in 2.0 ml of saline diurnal culture of *E. coli* O157:H7 (modeling of sepsis) [3-5,15]. All the drugs (groups of mice 3 - 9) were administered (i.p., a single) in 0.5-1.0 ml of saline.

M-cholinomimetic aceclidine (Vector HRC of Virology and Biotechnology, Russia) (3<sup>rd</sup> group of mice) penetrating the blood-brain barrier; n-cholinomimetic nicotine (Sigma-Aldrich) (4<sup>th</sup> group), reversible acetylcholine esterase inhibitor - neostigmine methyl sulfate (Sigma-Aldrich) (5<sup>th</sup> group) was administered at dose of 0.3 LD<sub>50</sub> (LD<sub>50</sub> data drugs were for mice, respectively,  $3.8 \pm 0.2$ ,  $30.0 \pm 2.5$ ,  $0.45 \pm 0.10$  mg/kg). The sixth group of mice received the n-cholinomimetic  $\alpha 7nAChRs$  agonist GTS-21 [3- (2,4-dimethoxybenzylidene)-anabaseine dihydrochloride] (Sigma-Aldrich) at a single dose of 5 mg/kg [16]. Epinephrine hydrochloride (Sigma-Aldrich) 0.5 mg/kg (group 7) and selective  $\beta 2ARs$  agonist (8<sup>th</sup> group) dexmedetomidine hydrochloride (Orion

Pharma) were used as adrenergic drugs, which was administered at a single dose of 25 µg/kg [17]. In groups 3 and 8, 1.0 - 2.0h after the administration of the drugs, sepsis was modeled. The registration of the lethality of mice (groups 2 - 8) was performed 6 and 24 hours after the modeling of sepsis.

The concentration of TNF-α, IL1β and IL-6 was studied in blood plasma of all groups of mice (groups 1-8) 6 and 24 hours after the administration of *E. coli* (sepsis modeling) by enzyme immunosorbent assay (ELISA) using kits (ELISA Kits MyBioSource) in accordance with the manufacturer's instructions. Monoclonal antibodies MyBioSource (TNF-α, IL1β, IL-6 - #MBS494184, #MBS494492, #MBS335516) were used to determine the concentration of proinflammatory cytokines. 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 and Discussion

The use of m-cholinomimetic aceclidine, n-cholinomimetic nicotine, reversible inhibitor of acetylcholine esterase (neostigmine methyl sulfate), n-cholinomimetic agonist α7nAChRs (GTS-21), epinephrine, adrenergic agonist β2ARs (hexaprenaline sulfate) caused a decrease in mortality 6 hours after i.p. administration of *E. coli* compared to with the control group 2 (sepsis without the use of drugs), respectively, by 8.7 ( $p > 0.05$ ); 17.0, 17.5, 22.9, 22.1 and 20.8 ( $p < 0.05$ ), and after 24 hours - by 20.0, 36.6, 30.0, 40.8, 26.8 and 39.4 ( $p < 0.05$ ) (Table 1). The effect of aceclidine is less pronounced than that of other drugs ( $p > 0.05$ ).

Series of experiments	Term study of mortality after the introduction of <i>E. coli</i> , h	
	6	24
Sepsis (control group 2, n = 85)	36,4 ± 5,2	89,4 ± 3,4 *
Aceclidine + sepsis (group 3, n = 36)	27,7 ± 7,5	69,4 ± 7,7*
Nicotine + sepsis (group 4, n = 36)	19,4 ± 6,5*	52,8 ± 8,3*
Neostigmine methyl sulfate + sepsis (group 5, n = 37)	18,9 ± 6,6*	59,4 ± 8,1*
α7nAChRs agonist (GTS-21) + sepsis (group 6, n = 37)	13,5 ± 5,6*	48,6 ± 8,2*
Epinephrine + sepsis (group 7, n = 35)	14,3 ± 5,9*	62,6 ± 8,2*
β2ARs agonist (hexoprenaline sulfate) + sepsis (group 8, n = 32)	15,6 ± 6,6*	50,0 ± 9,0*

**Table 1:** Phytochemical screening of extracts from *A. vera*, *Aloe volkensii* and *Aloe secundiflora*

GRA: Green Rid Aqueous; LPA: Leaf Pulp Aqueous; +++: High; ++: Moderate; +: low; -: Absent

The results indicate that the applied drugs, both cholinomimetics, and agonists reduced the mortality of mice from sepsis (administration of *E. coli*) approximately equally, the maximum effect was observed when applying α7nAChRs agonist (GTS-21), minimal

effect - when using m-cholinomimetic aceclidine. The results obtained suggest that the decrease the mortality of mice in the modeling of sepsis with administration of *E. coli* (i.p.) after the use of a reversible inhibitor of acetylcholine esterase (neostigmine methyl sulfate) and n-cholinomimetic (nicotine) is due to activation of acetylcholine (action of neostigmine methyl sulfate), central m-cholinergic receptors by aceclidine, and activation of α7nAChRs and β2ARs cells monocyte-macrophage system [3-5,18-20]. It should be noted that in large doses aceclidine activates probably not only the central m1AChRs [6,14], but and the α7nAChRs.

The concentration in the blood of TNFα, IL1β and IL-6 mice after the modeling of sepsis (administration of *E. coli* i.p.) after 6 hours compared with the control (group 1) increased by 12.1, 20.3, 27.1 times ( $p < 0.05$ ). For 24 hours, the blood TNFα content was significantly reduced ( $p < 0.05$ ), reaching almost the control level, and the concentration of IL1β and IL-6 exceeded the control parameters by 4.8 and 8.5 times, respectively ( $p < 0.05$ ).

After the use of aceclidine, nicotine, neostigmine methyl sulfate, n-cholinomimetic agonist α7nAChRs (GTS-21), epinephrine, adrenergic agonist β2ARs agonist (group 2-8), followed by modeling of sepsis (administration of *E. coli*) concentration of TNF-α in the blood of mice decreased in 6 hours compared to parameters for sepsis without the use of drugs (control group 2), respectively, at 59.1, 70.8, 74.4, 77.9, 71.9 and 75.1% ( $p < 0.05$ ); IL1β concentration decreased by 42.6, 61.2, 63.3, 67.0, 60.0 and 63.1% ( $p < 0.05$ ), and the concentration of IL-6 - by 78.0, 85.2, 86.4, 89.9, 88.3 and 88.9% ( $p < 0.05$ ), respectively. 24 hours after the administration of *E. coli*, the content of TNFα in the blood after the application of cholinergic and adrenergic drugs in sepsis did not practically differ from the parameters of control group 1 (intact mice) and group 2 (sepsis without drugs), IL1β concentration with m-cholinomimetic aceclidine decreased by 31.9% ( $p < 0.05$ ) compared with the parameter in group 2 (sepsis), and in the case of other drugs - an average of 60.0% ( $p < 0.05$ ). The concentration of IL-6 in 24 after the administration of *E. coli* decreased by 46.0% ( $p < 0.05$ ) compared with the parameter in group 2 (sepsis) with the use of aceclidine, and by 67.2% on other drugs ( $p < 0.05$ ).

It should be noted that, on average, the reduction of the concentrations of TNFα, IL1β and IL-6 6 hours after the administration of *E. coli* with the use of aceclidine (59.7%) compared to other drugs (decrease of 74.8% on average) was 15.1% less is expressed ( $p < 0.05$ ).

The literature data suggest that the reduction of synthesis of pro-inflammatory cytokines TNFα, IL1β and IL-6 after administration of aceclidine in sepsis is due to its effect on m1AChR of the brain [6,9,21] and subsequent activation of the cholinergic anti-inflammatory pathway. Effects of nicotine, acetylcholine esterase inhibitor neostigmine methyl sulfate, n-cholinomimetic α7nAChRs agonist (GTS-21) are associated with the activation of α7nAChRs cells of the monocyte-macrophage system of liver, gastrointestinal, and spleen nAChRs [20,21]. Reduction of production of TNFα, IL1β and IL-6 under the influence of epinephrine, adrenergic ago-

nist  $\beta$ 2ARs (hexaprenaline sulfate) occurs as result of direct and indirect (through  $\beta$ 2ARs T spleen T cells) activation of monocyte-macrophage system cells [3,9]. It is known that monocytes and macrophages have  $\beta$ ARs, and their activation leads to an anti-inflammatory effect [14] due to inhibition of the nuclear transcription factor NF- $\kappa$ B [22].

Thus, n-cholinomimetics, reversible inhibitors of acetylcholinesterase, adrenomimetics and m-holinomimetics can be considered as promising drugs, along with other drugs, for the treatment of septic conditions, inflammatory bowel diseases and other infectious diseases caused by opportunistic Gram-negative microorganisms.

Series of experiments	TNF $\alpha$		IL1 $\beta$		IL-6	
	6	24	6	24	6	24
Control group 1	50 $\pm$ 7	38 $\pm$ 6	26 $\pm$ 4	28 $\pm$ 5	73 $\pm$ 10	65 $\pm$ 9
Sepsis (control group 2)	606 $\pm$ 84 <sup>a</sup>	55 $\pm$ 8 <sup>e</sup>	528 $\pm$ 63 <sup>a</sup>	135 $\pm$ 17 <sup>ac</sup>	1975 $\pm$ 243 <sup>a</sup>	213 $\pm$ 24 <sup>ac</sup>
Aceclidine + sepsis (group 3)	248 $\pm$ 26 <sup>ab</sup>	48 $\pm$ 6 <sup>e</sup>	357 $\pm$ 40 <sup>ab</sup>	92 $\pm$ 10 <sup>abc</sup>	435 $\pm$ 45 <sup>ab</sup>	115 $\pm$ 12 <sup>abc</sup>
Nicotine + sepsis (group 4)	177 $\pm$ 19 <sup>abd</sup>	50 $\pm$ 6 <sup>e</sup>	205 $\pm$ 28 <sup>abd</sup>	64 $\pm$ 8 <sup>abcd</sup>	293 $\pm$ 32 <sup>abd</sup>	72 $\pm$ 9 <sup>abcd</sup>
Neostigmine methyl sulfate + sepsis (group 5)	155 $\pm$ 20 <sup>abd</sup>	57 $\pm$ 7 <sup>e</sup>	199 $\pm$ 22 <sup>abd</sup>	57 $\pm$ 6 <sup>abc</sup>	268 $\pm$ 31 <sup>abd</sup>	80 $\pm$ 10 <sup>abcd</sup>
$\alpha$ 7nAChRs agonist (GTS-21) + sepsis (group 6)	134 $\pm$ 21 <sup>abd</sup>	36 $\pm$ 5 <sup>e</sup>	174 $\pm$ 18 <sup>abd</sup>	39 $\pm$ 6 <sup>abc</sup>	200 $\pm$ 27 <sup>abd</sup>	59 $\pm$ 8 <sup>abcd</sup>
Epinephrine + sepsis (group 7)	170 $\pm$ 23 <sup>abd</sup>	47 $\pm$ 6 <sup>e</sup>	211 $\pm$ 27 <sup>abd</sup>	62 $\pm$ 8 <sup>abc</sup>	232 $\pm$ 28 <sup>abd</sup>	73 $\pm$ 10 <sup>abcd</sup>
$\beta$ 2ARs agonist (hexaprenaline sulfate) + sepsis (group 8)	151 $\pm$ 24 <sup>abd</sup>	40 $\pm$ 7 <sup>e</sup>	195 $\pm$ 19 <sup>abd</sup>	48 $\pm$ 7 <sup>abc</sup>	220 $\pm$ 30 <sup>abd</sup>	65 $\pm$ 7 <sup>abcd</sup>

**Table 2:** Effect of cholinergic and adrenergic drugs on the concentration of proinflammatory cytokines in blood of mice with *E. coli* sepsis, pg/ml (M  $\pm$  m; n = 7-9).

Note: 6 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 at sepsis (control group 2); c: p < 0.05 compared with parameter after 4 h; d: p < 0,05 - compared with corresponding parameter of group 3.

## Conclusions

1. The use of m-Cholinomimetic (aceclidine), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-cholinomimetic  $\alpha$ 7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic  $\beta$ 2ARs agonist (hexaprenaline sulfate) causes a decrease in mice mortality in sepsis caused by the administration i.p. of *E. coli* O157:H7.
2. Administration to mice of m-cholinomimetic aceclidine, nicotine, neostigmine methyl sulfate,  $\alpha$ 7nAChR agonist (GTS-21), epinephrine hydrochloride, adrenomimetic  $\beta$ 2ARs agonist (hexaprenaline sulfate) practically simultaneously with modeling of sepsis, decreased blood concentrations of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 compared with parameters at sepsis without the use of drugs.
3. M-cholinomimetic acetylde compared with the effects of nicotine, the reversible cholinesterase inhibitor neostigmine methyl sulfate, the  $\alpha$ 7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic  $\beta$ 2ARs agonist (hexaprenaline sulfate) are more pronounced.

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