



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

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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- α , IL-1 β 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- α , IL-1 β , 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- κB (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- α , IL-1 β , 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 2.5×10^9 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) (3rd group of mice) penetrating the blood-brain barrier, n-cholinomimetic nicotine (Sigma-Aldrich) (4th group), reversible acetylcholine esterase inhibitor - neostigmine methyl sulfate (Sigma-Aldrich) (5th group) was administered at dose of 0.3 LD₅₀ (LD₅₀ data drugs were for mice, respectively, 3.8 ± 0.2 , 30.0 ± 2.5 , 0.45 ± 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 (8th 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 MyBioSoure) in accordance with the manufacturer's instructions. Monoclonal antibodies MyBioSoure (TNF-α, IL1β, IL-6 - #MBS494184, #MBS494492, #MBS335516) were used to determine the concentration of pro-inflammatory cytokines. Blood for research was taken from the retro-orbital 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, adrenomimetic 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, adrenomimetic ago-

nist β 2ARs (hexaprenaline sulfate) occurs as result of direct and indirect (through β 2ARs T spleen T cells) activation of monocyte-macrophage system cells [3,9]. It is known that monocytes and macrophages have β ARs, and their activation leads to an anti-inflammatory effect [14] due to inhibition of the nuclear transcription factor NF- κ 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 α		IL1 β		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 ^a	55 \pm 8 ^e	528 \pm 63 ^a	135 \pm 17 ^{ac}	1975 \pm 243 ^a	213 \pm 24 ^{ac}
Aceclidine + sepsis (group 3)	248 \pm 26 ^{ab}	48 \pm 6 ^e	357 \pm 40 ^{ab}	92 \pm 10 ^{abc}	435 \pm 45 ^{ab}	115 \pm 12 ^{abc}
Nicotine + sepsis (group 4)	177 \pm 19 ^{abd}	50 \pm 6 ^e	205 \pm 28 ^{abd}	64 \pm 8 ^{abcd}	293 \pm 32 ^{abd}	72 \pm 9 ^{abcd}
Neostigmine methyl sulfate + sepsis (group 5)	155 \pm 20 ^{abd}	57 \pm 7 ^e	199 \pm 22 ^{abd}	57 \pm 6 ^{abc}	268 \pm 31 ^{abd}	80 \pm 10 ^{abcd}
α 7nAChRs agonist (GTS-21) + sepsis (group 6)	134 \pm 21 ^{abd}	36 \pm 5 ^e	174 \pm 18 ^{abd}	39 \pm 6 ^{abc}	200 \pm 27 ^{abd}	59 \pm 8 ^{abcd}
Epinephrine + sepsis (group 7)	170 \pm 23 ^{abd}	47 \pm 6 ^e	211 \pm 27 ^{abd}	62 \pm 8 ^{abc}	232 \pm 28 ^{abd}	73 \pm 10 ^{abcd}
β 2ARs agonist (hexaprenaline sulfate) + sepsis (group 8)	151 \pm 24 ^{abd}	40 \pm 7 ^e	195 \pm 19 ^{abd}	48 \pm 7 ^{abc}	220 \pm 30 ^{abd}	65 \pm 7 ^{abcd}

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 α 7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β 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, α 7nAChR agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β 2ARs agonist (hexaprenaline sulfate) practically simultaneously with modeling of sepsis, decreased blood concentrations of TNF- α , IL-1 β 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 α 7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β 2ARs agonist (hexaprenaline sulfate) are more pronounced.

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