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 (aceclydine), *n*-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), *n*-cholinomimetic α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β2ARs 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-1β 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 organonal, 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 α7n-acetylcholinoreceptors (α7nAChRs) of cells of the monocyte-macrophage system, followed by inhibition of the production of these cells by pro-inflammatory cytokines TNF-α, IL-1β, IL-6, B1-HMG1 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 α7-acetylcholine receptors type 1 (α7nAChR) of the brain, modulating the immunoregulatory function of the vagus nerve; excitation of efferent fibers n. vagus; the action of acetylcholine on α7n-acetylcholinoreceptors (α7nAChRs) of cells of the monocyte-macrophage system, followed by inhibition of the production of these cells by pro-inflammatory cytokines TNF-α, IL-1β, IL-6, B1-HMG1 protein, macrophage-inflammatory protein-2-MIP-2 and decrease in mortality from sepsis was devoted to hundreds of articles by different authors [8-11].

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M-cholinomimetic accecdine (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 administrated at dose of 0.3 LD₅₀ (LD₅₀ data drags 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 α7nAChRs agonist GTS-21 [3- (2,4-dimethoxybenzylidene)-anabaseine dihydrochloride] (Sigma-Aldrich) at a single dose of 5 mg/kg [16].

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], β2-adrenoreceptors (β2ARs) of spleen T-lymphocytes [3,9], cause the same effect as the activation of α7nAChRs, leading to reduction of synthesis of proinflammatory cytokines by cells of the monocyte-macrophage system [3,7,10].

**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 × 10⁹ CFUs in 2.0 ml of saline diurnal culture of *E. coli*O157:H7 (modeling of sepsis) [3-5,15]. All the drags (groups of mice 3 - 9) were administrated (i.p., a single) in 0.5-1.0 ml of saline.  

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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, #MBS35516) were used to determine the concentration of proinflammatory 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 acetylcholine, n-cholinomimetic nicotine, reversible inhibitor of acetylcholine esterase (neostigmine methyl sulfate), n-cholinomimetic agonist α7nAChRs (GTS-21), epinephrine, adrenergic agonist β2ARs (hexoprenaline 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 aceclydine is less pronounced (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 aceclydine is less pronounced compared to with the control group 2 (sepsis without the use of drugs) 6 and 24 hours after the introduction of *E. coli*, h

<table>
<thead>
<tr>
<th>Series of experiments</th>
<th>Term study of mortality after the introduction of <em>E. coli</em>, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis (control group 2, n = 85)</td>
<td>36.4 ± 5.2, 89.4 ± 3.4*</td>
</tr>
<tr>
<td>Acetylcholine + sepsis (group 3, n = 36)</td>
<td>27.7 ± 7.5, 69.4 ± 7.7*</td>
</tr>
<tr>
<td>Nicotine + sepsis (group 4, n = 36)</td>
<td>19.4 ± 6.5*, 52.8 ± 8.3*</td>
</tr>
<tr>
<td>Neostigmine methyl sulfate + sepsis (group 5, n = 37)</td>
<td>18.9 ± 6.6*, 59.4 ± 8.1*</td>
</tr>
<tr>
<td>α7nAChRs agonist (GTS-21) + sepsis (group 6, n = 37)</td>
<td>13.5 ± 5.6*, 48.6 ± 8.2*</td>
</tr>
<tr>
<td>Epinephrine + sepsis (group 7, n = 35)</td>
<td>14.3 ± 5.9*, 62.6 ± 8.2*</td>
</tr>
<tr>
<td>β2ARs agonist (hexoprenaline sulfate) + sepsis (group 8, n = 32)</td>
<td>15.6 ± 6.6*, 50.0 ± 9.0*</td>
</tr>
</tbody>
</table>

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 acetylcholine. 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 aceclydine, and activation of α7nAChRs and β2ARs cells monocyte-macrophage system [3-5,18-20]. It should be noted that in large doses aceclydine 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 exceed the control parameters by 4.8 and 8.5 times, respectively (p < 0.05).

After the use of aceclydine, nicotine, neostigmine methyl sulfate, n-cholinomimetic agonist α7nAChRs (GTS-21), epinephrine, adrenergic β2ARs agonist (group 2-8), followed by modeling of sepsis (administration of *E. coli*) concentration of TNFα 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, 63.7, 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 acetylcholine 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 acetylcholine, 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 acetylcholine (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 acetylcholine 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-

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Conclusions

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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.

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 ± m; n = 7-9).

<table>
<thead>
<tr>
<th>Series of experiments</th>
<th>TNFα</th>
<th>IL-1β</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Control group 1</td>
<td>50 ± 7</td>
<td>38 ± 6</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Sepsis (control group 2)</td>
<td>606 ± 84*</td>
<td>55 ± 8*</td>
<td>528 ± 63*</td>
</tr>
<tr>
<td>Acedycline + sepsis (group 3)</td>
<td>248 ± 26*</td>
<td>48 ± 6*</td>
<td>357 ± 40*</td>
</tr>
<tr>
<td>Nicotine + sepsis (group 4)</td>
<td>177 ± 19*</td>
<td>50 ± 6*</td>
<td>205 ± 28*</td>
</tr>
<tr>
<td>Neostigmine methyl sulfate + sepsis (group 5)</td>
<td>155 ± 20*</td>
<td>57 ± 7*</td>
<td>199 ± 22*</td>
</tr>
<tr>
<td>α7nAChRs agonist (GTS-21) + sepsis (group 6)</td>
<td>134 ± 21*</td>
<td>36 ± 5*</td>
<td>174 ± 18*</td>
</tr>
<tr>
<td>Epinephrine + sepsis (group 7)</td>
<td>170 ± 23*</td>
<td>47 ± 6*</td>
<td>211 ± 27*</td>
</tr>
<tr>
<td>β2AR agonist (hexaprenaline sulfate) + sepsis (group 8)</td>
<td>151 ± 24*</td>
<td>40 ± 7*</td>
<td>195 ± 19*</td>
</tr>
</tbody>
</table>

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 (acecdyline), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-Cholinomimetic α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β2ARs agonist (hexaprenaline sulfate) occurs as result of direct and indirect (through β2ARs T spleen T cells) activation of monocyte-macrophage system cells [3,9]. It is known that monocytes and macrophages have βARs, and their activation leads to an anti-inflammatory effect [14] due to inhibition of the nuclear transcription factor NF-κB [22].

2. Administration to mice of m-cholinomimetic acecdyline, nicotine, neostigmine methyl sulfate, α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β2ARs agonist (hexaprenaline sulfate) practically simultaneously with modeling of sepsis, decreased blood concentrations of TNF-α, IL-1β and IL-6 compared with parameters at sepsis without the use of drugs.

3. M-Cholinomimetic acetyldyline compared with the effects of nicotine, the reversible cholinesterase inhibitor neostigmine methyl sulfate, the α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β2ARs agonist (hexaprenaline sulfate) are more pronounced.


Bibliography


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