The Influence of Cholinergic and Adrenergic Drugs on Mortality of Mice and the Concentration of Proinflammatory Cytokines in Blood at Sepsis

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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 α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adenomimetic (β2ARs) agonist (hexaprenaline 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

Introduction

From all the lethal outcomes associated with diseases and their complications, mortality from sepsis, including that caused by opportunistic Gram-negative microorganisms (E. 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” after the study of its realization at the organismal, cellular and subcellular levels [5,6,7]. 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-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].

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 adrenoreceptors 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].

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-1β, 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 0.5ml of isotonic sodium chloride solution (saline) 10-30 minutes later in 2.0ml of saline. The second group of mice (control group 2) was injected i.p., once with 0.5-1.0ml of saline. Fifteen to 60 minutes after the administration of saline, mice received 2.5×10⁹ CFUs in 2.0ml of saline diurnal culture of E. coli O157:H7 (modeling of sepsis) [3,4,5,15]. All the drugs (groups of mice 3-9) were administered (i.p., a single) in 0.5-1.0ml of saline.
M-cholinomimetic aceclydine (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 drags were for mice, respectively, 3.8±0.2, 30.0±2.5, 0.45±0.10mg/kg). The sixth group of mice received the n-cholinomimetic α7nAChRs agonist GTS-21 [3-(2,4-dimethoxybenzyldiene)-anabaseine dihydrochloride] (Sigma-Aldrich) at a single dose of 5mg/kg [16]. Epinephrine hydrochloride (Sigma-Aldrich) 0.5mg/kg (group 7) and selective β2ARs 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, 1.0-2.0 h 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 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

#### Table 1: Effect of cholinergic and adrenergic drugs on mortality of mice with E. coli-induced sepsis (M±m).

<table>
<thead>
<tr>
<th>Series of experiments</th>
<th>Term study of mortality after the introduction of E. coli, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Sepsis (control group 2, n = 85)</td>
<td>36.4±5.2</td>
</tr>
<tr>
<td>Acetycline + sepsis (group 3, n = 36)</td>
<td>27.7±7.5</td>
</tr>
<tr>
<td>Nicotine + sepsis (group 4, n = 36)</td>
<td>19.4±6.5*</td>
</tr>
<tr>
<td>Neostigmine methyl sulfate + sepsis (group 5, n = 37)</td>
<td>18.9±6.6*</td>
</tr>
<tr>
<td>α7nAChRs agonist (GTS-21) + sepsis (group 6, n = 37)</td>
<td>13.5±5.6*</td>
</tr>
<tr>
<td>Epinephrine + sepsis (group 7, n = 35)</td>
<td>14.3±5.9*</td>
</tr>
<tr>
<td>β2ARs agonist (hexaprenaline sulfate) + sepsis (group 8, n = 32)</td>
<td>15.6±6.6*</td>
</tr>
</tbody>
</table>

#### Table 2: Effect of cholinergic and adrenergic drugs on the concentration of pro inflammatory 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>ФНОα</th>
<th>ИЛ1β</th>
<th>ИЛ-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>Acetycline + sepsis (group 3)</td>
<td>248±26ib</td>
<td>48±6c</td>
<td>357±40in</td>
</tr>
<tr>
<td>Nicotine + sepsis (group 4)</td>
<td>177±19ibd</td>
<td>50±6c</td>
<td>205±28ibed</td>
</tr>
<tr>
<td>Neostigmine methyl sulfate + sepsis (group 5)</td>
<td>155±20ibd</td>
<td>57±7c</td>
<td>199±22ibed</td>
</tr>
<tr>
<td>α7nAChRs agonist (GTS-21) + sepsis (group 6)</td>
<td>134±21ibd</td>
<td>36±5c</td>
<td>174±18ibd</td>
</tr>
<tr>
<td>Epinephrine + sepsis (group 7)</td>
<td>170±23ibd</td>
<td>47±6c</td>
<td>21±27ibd</td>
</tr>
<tr>
<td>β2ARs agonist (hexaprenaline sulfate) + sepsis (group 8)</td>
<td>151±24ibd</td>
<td>40±7c</td>
<td>195±19ibd</td>
</tr>
</tbody>
</table>

The use of m-cholinomimetic acetycline, 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 24hours - by 20.0, 36.6, 30.0, 40.8, 26.8 and 39.4 (p<0.05) (Table 1,2). The effect of acetycline is less pronounced than that of other drugs (p> 0.05).
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 of the mortality of mice in the modeling of sepsis with administration of *E. coli* (i.p.) after the use of a reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate) and n-cholinomimetic (nicotine) is due to activation of acetylcholine (action of neostigmine methyl sulfate), central m-cholinergic receptors by acetylcholine, and activation of α7nAChRs and β2ARs cells monocyte-macrophage system [3, 5-18, 20]. It should be noted that in large doses acetylcholine 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 acetylcholine, 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 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, 63.3, 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 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 h 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]. Reducing the production of TNFα, IL1β and IL-6 under the influence of epinephrine, adrenomimetic agonist β2ARs (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].

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.

**Conclusions**

1. The use of m-holinomimetic (acetylcholine), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-cholinomimetic α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β2ARs 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 acetylcholine, nicotine, neostigmine methyl sulfate, α7nAChR agonist (GTS-21), epinephrine hydrochloride, adrenomimetic β2ARs agonist (hexaprenaline sulfate) practically simultaneously with modeling of sepsis, decreased blood concentrations of TNFα, IL-1β and IL-6 compared with parameters at sepsis without the use of drugs.

3. M-cholinomimetic acetylcholine 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.

**References**


