Effects of Reversible Inhibition of Cholinesterase and Nicotine on Mouse Mortality and Blood Levels of Proinflammatory Cytokines during the Early Phase of Sepsis

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Experiments on outbred albino mice have shown that proserine (reversible cholinesterase inhibitor) and nicotine (nicotinic receptor agonist) in a equivalent dose of 0.2 DL_{50} injected 2 h before sepsis induction significantly reduced animal mortality from experimental infection due to reduction of blood concentrations of proinflammatory cytokines TNF-α, IL-1β, and IL-6.

Key Words: *sepsis; cholinergic anti-inflammatory mechanism; cholinesterase inhibition; nicotine; cytokines*

Cholinergic stimulation significantly reduces mortality of albino mice from sepsis induced by intraperitoneal or intrapulmonary injections of *E. coli* [1-4] and *Proteus vulgaris* [5]. The efficiency of cholinergic receptor agonists for urgent stimulation of nonspecific antibacterial resistance in various infectious processes has been proven in 1995 [4]. Numerous subsequent studies [7,10-12] have confirmed the role of activation of the cholinergic system in reduction of animal mortality from sepsis induced by various infections.

Improvement of animal survival after cholinergic stimulation (effects of cholinergic receptor agonists) [1,2,4] can be explained by realization of the cholinergic anti-inflammatory pathway [9,11,13].

Cholinergic stimulation leads to acetylcholine (AC) stimulation of α7-nicotinic cholinoreceptors (α7nAChR) on the monocyte/macrophage system (MMS) cells (monocytes, macrophages, neutrophils). It results in reduction of mortality from experimental infection [7,8,11,12] due to lesser production of proinflammatory cytokines (TNF-α, IL-1β, IL-6) [9,10, 11,14,15] by MMS cells [10,11] and lymphoid dendritic cells [6]. Presumably, nicotine causes a similar effect on α7nAChR.

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Here we studied the effects of reversible inhibition of acetylcholinesterase and nicotine on mouse mortality during the early phase of sepsis and on plasma levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6.

MATERIALS AND METHODS

Experiments were carried out on male and female outbred albino mice (18-22 g). Proserine (reversible cholinesterase inhibitor) and nicotine (nicotinic receptor agonist) were injected subcutaneously in a single dose of 0.2 DL_{50} (DL_{50} for these agents for mice were 0.55±0.10 and 35±4 mg/kg, respectively). Sepsis was induced 2 h after injection of cholinergic agents by intraperitoneal injection of 2.5×10^9 24-h culture of *E. coli* [4,14]. The mortality was registered for groups without cholinergic agents (control 1, group 1), for proserine (group 2), and nicotine (group 3) 10 and 25 h after sepsis induction. These periods for mortality evaluation have been chosen because an appreciable part of animals died after 10 h, while after 25 h the mortality from sepsis reached the peak and was virtually over [4,5]. Plasma concentrations of cytokines TNF-α, IL-1β, and IL-6 were measured in intact mice (control 2) and mice surviving 10 and 25 h after intraperitoneal *E. coli* without pre-injection of cholinergic

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agents (control group) and after pre-injection of proserine (group 2) or nicotine (group 3) by ELISA with the BioSource Int. kits. Blood for analysis was collected into tubes from the retro-orbital venous sinus. The data were statistically processed using Student's *t* test.

RESULTS

Study of the mortality of albino mice with experimental sepsis induced after preinjection of cholinergic agents and without them showed (Table 1) that proserine and nicotine significantly reduced ($p<0.05$) this parameter in comparison with the control group (sepsis) by 30.0 and 36.7% 10 h and by 23.3 and 30.0% ($p<0.05$), respectively, 25 h after intraperitoneal injection of *E. coli*. These results suggest that reduction of mouse mortality from experimental sepsis after injection of reversible cholinesterase inhibitor proserine and nicotinic receptor agonist nicotine was due to stimulation of AC and nicotine stimulation of MMS cell α7nAChR [7].

Plasma concentrations of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in sepsis (control group) increased significantly ($p<0.05$) in comparison with the intact group 10 h after sepsis induction (4.9, 14.0, and 21.7 times, respectively; Table 2).

The concentrations of TNF-α, IL-1β, and IL-6 in the blood 10 h after proserine injection (group 2) followed by sepsis simulation decreased by 1.6, 2.1, and 2.4 times, respectively ($p<0.05$) in comparison with the control (sepsis without proserine preinjection). The levels of proinflammatory cytokines were significantly higher ($p<0.05$) than in the intact group.

Injection of nicotine before sepsis induction (group 3) led to reduction of plasma concentrations of TNF-α, IL-1β, and IL-6 10 h after intraperitoneal injection of *E. coli* in comparison with the control (sepsis without proserine preinjection) by 1.8, 2.4, and 2.7 times, respectively ($p<0.05$). On the whole, nicotine caused a more pronounced reduction of the studied values, though the parameters in sepsis induced after preinjection of proserine (group 2) and nicotine (group 3) were virtually the same. The levels of cytokines were significantly ($p<0.05$) higher than in both control groups.

A less pronounced decrease in the cytokine concentrations in comparison with the intact group was recorded in sepsis induced without preinjection of cholinomimetics (control group) 25 h after intraperitoneal injection of *E. coli*.

Blood levels of TNF-α, IL-1β, and IL-6 25 h after sepsis induction significantly decreased ($p<0.05$) in all series of experiments (control group, groups 2 and 3) in comparison with cytokine concentrations 10 h after injection of *E. coli*. Blood levels of proinflammatory cytokines remained higher than in the intact group ($p<0.05$) after 25 h, except TNF-α level which was virtually the same as in the intact group. In sepsis modeled after preinjection of proserine (group 2) and nicotine (group 3), the concentration of TNF-α was lower in comparison with its level in sepsis (control group) by 1.3 and 1.4 times ($p<0.05$), respectively. Cytokine concentrations in the blood in sepsis after preinjections of proserine and nicotine were virtually the same.

These shifts in plasma concentrations of proinflammatory cytokines indicate that preinjection of proserine (reversible cholinesterase inhibitor) leads to stimulation of the monocyte, macrophage, and neutrophil α7nAchR [8,9] (and presumably the receptors on natural killers [1]) by AC. This leads to realization of the "cholinergic anti-inflammatory pathway" [1-5,15] by reducing the levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in the blood and organs with MMS (liver, gastrointestinal tract, spleen). These cytokines cause various pathological reactions leading to lethal outcome in sepsis and other infectious diseases [11,12]. Nicotine stimulates α7nAChR of MMS cell and induces a slightly higher effect than proserine in the equivalent dose.

Reduction of animal mortality 1-2 days after preinjection of reversible and irreversible inhibitors of cholinesterase (including the organophosphorus compounds), AC, and aceclidine (muscarinic receptor agonist) [1-4] is caused by AC reactions with the macrophage, monocyte, and neutrophil α7nAChR [11] and by stimulation of the hypothalamic—pituitary—adrenal system followed by elevation of blood concentrations of corticosteroids [5]. In addition, the increase of nonspecific antibacterial resistance can be due to activation of muscarinic receptors of MMS cell by AC and muscarinic receptor agonist, leading to an increase in the neutrophil phagocytic metabolic activity and to an increase in serum bactericidal activity, production

TABLE 1. Effects of Proserine and Nicotine on the Mortality of Mice with Experimental Sepsis ($M\pm m$, n=30)

Experimental series	Period of mortality evaluation after injection of *E. coli*, h	
	10	25
Sepsis (control group)	66.7±8.6	83.3±6.9
Proserine+sepsis (group 2)	36.7±8.8*	60.0±8.9*
Nicotine+sepsis (group 3)	30.0±8.4*	53.3±9.1*

Note. *$p<0.05$ in comparison with the control.

TABLE 2. Effects of Proserine and Nicotine on Blood Concentrations of Proinflammatory Cytokines 10 and 25 h after Sepsis Induction (pg/ml; *M*±*m*)

Experimental series	TNF-α		IL-1β		IL-6	
	10 h	25 h	10 h	25 h	10 h	25 h
Intact group	39±6 (7)	42±4 (7)	29±3 (7)	24±3 (7)	35±4 (7)	30±4 (7)
Sepsis (control group)	190±20* (10)	63±16*° (5)	405±42*	117±27*° (5)	760±80* (10)	332±83*° (5)
Proserine+sepsis (group 2)	119±12*+ (9)	49±5° (9)	191±20*+ (9)	56±6*+° (9)	313±34*+ (9)	150±17*+° (9)
Nicotine+sepsis (group 3)	105±11*+ (10)	45±5° (9)	167±17*+ (10)	51±6*+° (9)	282±30*+ (10)	167±18*+° (9)

Note. The number of animals in experiment is shown in parentheses; $p<0.05$ in comparison with: *intact group, +control group, °after 10 h.

of lysozyme and platelet cationic protein by the blood cells [1,5].

It is noteworthy that the studies of the early phase of sepsis characterize the effects of cholinergic agents in experimental sepsis not on the humoral and cellular immunity reactions, but on the anti-infection nonspecific resistance [1-4]. The synthesis of IL-6 by Th2 lymphocytes (and Th0 cells) in response to *E. coli* injection starts during the formation of immune response only 5-7 days after injection of this antigen [6].

Hence, proserine (cholinesterase reversible inhibitor) and nicotine (nicotinic receptor agonist) in a single equivalent dose of 0.2 DL_{50} injected 2 h before sepsis induction cause a significant reduction of mouse mortality from experimental infection due to reduction of the blood concentrations of proinflammatory cytokines TNF-α, IL-1β, and IL-6.

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