Effect of Acetylcholine on Mortality of Mice from Sepsis and Proinflammatory Cytokine Production

P. F. Zabrodskii

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Experiments on outbred mice showed that acetylcholine chloride in a dose of 20 mg/kg 6 h after subcutaneous injection significantly reduces mortality of mice from sepsis induced by intraperitoneal injection of 2×10^9 *E. coli* bacterial bodies and the blood levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6.

Key Words: *sepsis; acetylcholine; proinflammatory cytokines*

The mortality from sepsis is responsible for one third of all lethal outcomes of diseases and their complications [5]. Cholinergic stimulation reduces significantly mortality of albino mice from sepsis induced by experimental infections [1]. The possibility of using cholinomimetics, including acetylcholine (AC), for urgent stimulation of antibacterial resistance in infectious processes was hypothesized [2]. Several thousands of reports have been published in recent years, discussing therapy of septic conditions (infectious processes) by stimulation of the cholinergic system, specifically, of AC α7n cholinoreceptor (α7nAChR) [6,7,10,11] and the role of cytokines (mainly TNF-α, IL-1β, IL-6, and IL-10) in the pathogenesis of sepsis and cholinergic system regulation of the production of these cytokines by monocytes, macrophages, neutrophils, and lymphoid dendritic cells [7,8,13,14].

The "cholinergic anti-inflammatory mechanism" phenomenon [8,10,12] in the early phase of sepsis [7], formulated recently, confirms the results of previous studies [1,2] and indicates a close relationship between the nervous and immune systems [3-5]. It is now essential to evaluate the effects of AC and its agonists on the mortality from sepsis caused by infections and evaluate blood levels of proinflammatory cytokines determining the course and outcome of sepsis [10-12].

Saratov Military Institute of Biological and Chemical Safety, Russia.
Address for correspondence: pz@renet.com.ru. P. F. Zabrodskii

We evaluated the effects of AC on mortality of mice from sepsis caused by experimental peritonitis and on sepsis-associated changes in blood 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 weighing 18-22 g. The animals were divided into 3 groups. Group 1 mice (control) received intraperitoneal and subcutaneous injections of isotonic saline (2 and 0.5 ml, respectively). Group 2 mice (sepsis) were intraperitoneally injected with suspension (2×10^9 bacterial bodies) of 24-h *E. coli* culture in 2 ml isotonic NaCl and subcutaneously injected with 0.5 ml isotonic NaCl. Subcutaneous injection of 0.5 ml isotonic NaCl in groups 1 and 2 was carried out 6 h before intraperitoneal injection of 2 ml isotonic saline and 2 ml suspension (2×10^9) of 24-h *E. coli* culture, respectively. In group 3 mice (AC+sepsis), sepsis was induced by intraperitoneal injection of 24-h *E. coli* culture (2×10^9) under conditions of complete absence of clinical signs of cholinergic stimulation 6 h after AC injection [3]. AC was injected subcutaneously (common administration route for AC [3]) in a single dose of 20 mg/kg, inducing pronounced cholinergic stimulation [2,3] and increase of mouse survival in experimental infection [2]. The method of administration of bacterial cells and their dose were substantiated in our previous studies [1-3] and published reports [13].

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The mortality of mice in groups 2 and 3 was evaluated 10 and 25 h after septic process induction. These periods are determined by the fact that an appreciable part of animals die 10 h after sepsis induction, while after 25 h mortality from sepsis reached the peak and was virtually over [1,3]. Plasma concentrations of cytokines TNF-α, IL-1β, and IL-6 in group 1 animals and survivors in groups 2 and 3 after intraperitoneal injection of *E. coli* (10 and 25 h postinfection) were measured by ELISA (BioSource Int. kits were used). Blood for analysis was collected from the caudal vein. The data were statistically processed using Student's *t* test.

RESULTS

Studies of albino mouse mortality after experimental sepsis with and without AC injection (Table 1) showed that AC reduced animal mortality by 36% ($p<0.05$) 10 h after *E. coli* injection and by 24% ($p<0.05$) after 25 h. More marked reduction of mortality from sepsis under the effect of AC during the earlier period (up to 10 h) after bacterial infection is worthy of note.

Measurements of plasma cytokine concentrations (Table 2) showed that the levels of TNF-α, IL-1β, and IL-6 10 h after infection increased by 4.9, 13.8, and 18.3 times, respectively ($p<0.05$), in sepsis in comparison with the control.

Acetylcholine treatment significantly reduced plasma levels of proinflammatory cytokines in animals. After 10 h, blood levels of TNF-α, IL-1β, and IL-6 decreased by 2.3, 2.5, and 2.6 times, respectively ($p<0.05$), in comparison with the cytokine concentrations in sepsis without AC injection. Cytokine levels were significantly ($p<0.05$) higher than in the control. After 25 h, the concentrations of blood cytokines (TNF-α, IL-1β, IL-6) decreased significantly ($p<0.05$) in comparison with the previous term (10 h postinfection), but remained above the control ($p<0.05$), except TNF-α, which was just slightly elevated (by 1.3 times; $p>0.05$).

It is noteworthy that the decrease in animal mortality from sepsis after AC injection was paralleled by reduction of blood concentrations of TNF-α, IL-1β, and IL-6. In addition, AC-induced reduction of mortality from sepsis, more pronounced before 10 h than 25 postinfection (Table 1), was paralleled by a greater AC inhibition of TNF-α production during the earlier period (10 h) of sepsis induction than later (25 h postinfection). The level of TNF-α under conditions of AC treatment decreased by 2.29 times 10 h and 1.35 times 25 h after infection.

Published data suggest that realization of the cholinergic anti-inflammatory mechanism [1,2,12] is mediated by interaction of AC with α7nAChR on monocytes, macrophages, and neutrophils [7,10] (and presumably natural killer cells [2]), which decreases the levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in the blood and organs of the monocytic and macrophage systems (including the spleen, liver, and gastrointestinal tract) and induces pathological reactions leading to lethal outcomes in sepsis and other infections [10,11].

TABLE 1. Effects of AC on Mouse Mortality (%) after Sepsis Induction (*M*±*m*; *n*=25)

Group	Time after *E. coli* injection, h	
	10	25
2 (sepsis)	56.0±9.9	84.0±7.3
3 (AC+sepsis)	20.0±8.0*	60.0±9.8*

Note. *$p<0.05$ compared to the control (group 1).

TABLE 2. Effects of AC on Plasma Concentrations of Proinflammatory Cytokines in Mice 10 and 25 h after Injection of *E. coli* (pg/ml; *M*±*m*)

Group	TNF-α		IL-1β		IL-6	
	10 h	25 h	10 h	25 h	10 h	25 h
1 (control)	34±5 (*n*=6)	37±4 (*n*=6)	25±3 (*n*=6)	20±3 (*n*=6)	30±4 (*n*=6)	35±4 (*n*=6)
2 (sepsis)	167±18* (*n*=6)	65±21^{o} (*n*=4)	346±38*^{o} (*n*=6)	135±45*^{o} (*n*=4)	578±60*^{o} (*n*=6)	375±73*^{o} (*n*=4)
3 (AC+sepsis)	73±8^{+} (*n*=6)	48±5^{o} (*n*=6)	138±15^{+o} (*n*=6)	44±6^{+o} (*n*=6)	221±22^{+o} (*n*=6)	133±16^{+o} (*n*=6)

Note. $p<0.05$ compared to: *control, $^{+}$control and the parameter in sepsis, ^{o}the corresponding parameter after 10 h.

The increase in blood levels of TNF-α, IL-1β, and IL-6 characterizes reduction of their synthesis by macrophages, monocytes, natural killer cells (TNF-α), and lymphoid dendritic cells, resultant from AC effect on their cholinergic receptors. Production of IL-6 by Th2 (and Th0) lymphocytes in response to foreign protein antigens presumably starts during the formation of immune response only on days 5-7 after the antigen (*E. coli* in our experiments) challenge [4].

Previously [1-3] we revealed a "paradoxical" reduction of animal mortality 1-2 days after acute intoxication with highly toxic cholinergic receptor agonists (organophosphorus zarine, VX, insecticides), reversible and irreversible cholinesterase inhibitors used in medicine. It was caused by not only AC effect [1,2] (for example, on macrophage and monocyte α7nAChR [10]), but also by stimulation of the hypothalamic-pituitary-adrenal system with subsequent elevation of blood corticosteroid level [3].

Hence, AC (20 mg/kg) injected 6 h before induction of sepsis (experimental infectious process) significantly reduced animal mortality and blood concentrations of proinflammatory cytokines TNF-α, IL-1β, and IL-6.

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