Role of Nicotinic and Muscarinic Cholinoreceptors in the Realization of the Cholinergic Anti-Inflammatory Pathway during the Early Phase of Sepsis

P. F. Zabrodskii, V. G. Lim, M. S. Shekhter, and A. V. Kuzmin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 656-659, May, 2012
Original article submitted February 7, 2011

Stimulation of nicotinic and muscarinic cholinoreceptors (nAChR, mAChR) in outbred albino mice with nicotine and aceclidine, respectively, in single equilethal doses 0.5 DL_{50} 6 h before sepsis induction significantly reduced animal mortality due to a decrease in blood concentrations of proinflammatory cytokines IL-1β, IL-6, and MIP-2. Stimulation of mAChR (injection of aceclidine) stimulated the neutrophilic phagocytic and metabolic activity. Realization of the cholinergic anti-inflammatory pathway (stimulation of the peripheral nicotinic cholinoreceptors (α7nAChR) and central muscarinic cholinoreceptors (mAChR) was modulated by stimulation of the muscarinic cholinoreceptors of the phagocytic monocytic system cells.

Key Words: *cholinergic anti-inflammatory pathway; sepsis; nicotinic and muscarinic cholinoreceptors; phagocytic monocytic system; cytokines*

Cholinergic stimulation (subcutaneous injection of armin, an irreversible cholinesterase inhibitor, to mice in doses of 0.12, 0.25, and 0.5 DL_{50}) significantly reduced mortality of albino mice from sepsis caused by intraperitoneal injection of *E. coli* [1]. The efficiency of cholinergic receptor agonists for urgent stimulation of nonspecific antibacterial resistance in infectious processes has been proven [2]. The parasympathetic pathway has been described in 2000; the vagus nerve plays the key role in it: stimulation of its afferent fibrils as a result of systemic inflammatory reactions to endotoxin eventually stimulates the anti-inflammatory reactions [5]. Stimulation of efferent fibers of the vagus nerve leads to reduction of proinflammatory cytokine production by liver macrophages (acetylcholine effect) [5]. Numerous recent studies [3,7,10,12] have confirmed the contribution of the cholinergic system stimulation to reduction of animal mortality from sepsis [1,2] caused by various infectious processes.

V. I. Razumovskii State Medical University, Saratov, Russia. ***Address for correspondence:*** pfzabrodsky@gmail.com. P. F. Zabrodsky

Chronic treatment with organophosphorus compounds can lead to realization of the cholinergic anti-inflammatory pathway [9,11,14], including stimulation of the brain muscarinic cholinoreceptors (mAChR), modulating the immunoregulatory function of the vagus nerve; stimulation of *n. vagus* efferent fibrils; acetylcholine effect on the receptors; stimulation of nicotonic cholinoreceptors (specifically, α7nAChR) by the phagocytic monocytic system (PMS) cells. The emergence of anti-inflammatory effect in the PMS cells is supported by JAK2 kinase, STAT3 transcription factor, and NF-kB transcription factor. Triggering of these biochemical mechanisms under conditions of cholinergic stimulation inhibits their production of TNF-α, B1 protein (HMGB1), MIP-2 macrophage inflammatory protein-2, IL-1β, and IL-6 [6].

Cholinergic stimulation causes acetylcholine stimulation of PMS cells (macrophages, monocytes, neutrophils) α7nAChR, this leading to reduction of mortality from experimental infection (during the early phase of sepsis) [1,8,12] as a result of low production of proinflammatory cytokines by PMS cells and lymphoid dendritic cells [9,10,11]. There are good grounds

to suggest a similar effect for muscarinic receptor agonists modulating mAChR in *n. vagus* nucleus in the medulla oblongata and mAChR of PMS [2].

We studied the relationship between mAChR and nAChR stimulation and mouse mortality from sepsis induced by experimental peritonitis and evaluated the phagocytic macrophage activity of neutrophils and plasma levels of proinflammatory cytokines IL-1β, IL-6, and MIP-2.

MATERIALS AND METHODS

Experiments were carried out on outbred albino mice of both genders (18-22 g). Aceclidine (muscarinic receptor agonist) penetrating through the blood-brain barrier) and nicotine (nicotinic receptor agonist) were injected subcutaneously in a single dose of 0.5 DL_{50} (DL_{50} for these drugs for mice being 4.12±0.22 and 35±4 mg/kg, respectively). Six hours after injections of cholinergic drugs, sepsis was induced in mice by intraperitoneal injection of 24-h culture of *E. coli* (2.5×10^9 microbial bodies) [1,5]. Mortality of mice receiving no cholinergic receptor agonist (control group 1) and treated with nicotine (group 2) or aceclidine (group 3) was evaluated 10 and 25 h after sepsis induction. These periods for mortality evaluation were chosen because of rather high mortality after 10 h, while after 25 h the mortality of animals from sepsis reached the peak and was virtually over [1,4]. Plasma concentrations of IL-1β, IL-6, and MIP-2 were measured in intact mice (control group 2), mice surviving 10 and 25 h after intraperitoneal injection of *E. coli* without cholinergic receptor agonists (control group 1), and mice injected with nicotine (group 2) and aceclidine (group 3). The measurements were carried out by ELISA with commercial kits. The phagocytic and metabolic activities of neutrophils under the effects of cholinergic receptor agonists (phagocytic index, phagocytic number, neutrophil activity index in spontaneous and induced NBT tests) were evaluated by the common methods [4]. Blood for analyses was collected from the retro-orbital venous sinus. The data were statistically processed by Student's *t* test.

TABLE 1. Effects of Nicotine and Aceclidine on Mouse Mortality after Sepsis Induction (*M*±*m*, *n*=36)

Experimental series	Period of mortality evaluation after *E. coli* injection, h	
	10	25
Sepsis (control group 1)	58.3±8.2	88.9±5.2
Nicotine+sepsis (group 2)	25.0±7.2*	52.8±8.3*
Aceclidine+sepsis (group 3)	33.3±7.8*	69.4±7.7*

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

RESULTS

Nicotine and aceclidine reduced (p<0.05) the mortality from sepsis in comparison with control group 1 (sepsis) by 2.33 and 1.75 times, respectively (by 33.3 and 25.0%), 10 h after *E. coli* injection and by 1.68 and 1.28 times (by 33.3 and 19.5%; p<0.05), respectively, after 25 h. This indicated that stimulation of mAChR and nAChR significantly improved the survival of animals during the early phase of sepsis (Table 1). Nicotine effect was slightly higher than that of aceclidine.

Plasma concentrations of cytokines IL-1β, IL-6 and MIP-2 increased by 13.29, 17.53, and 6.07 times,

TABLE 2. Effects of Nicotine and Aceclidine on Blood Concentrations (pg/ml) of Proinflammatory Cytokines in Mice after 10 and 25 h of Sepsis

Experimental series	IL-1β		IL-6		MIP-2	
	10 h	25 h	10 h	25 h	10 h	25 h
Intact mice (control group 2)	21±3 (*n*=7)	26±4 (*n*=7)	30±5 (*n*=7)	35±6 (*n*=7)	15±3 (*n*=7)	18±3 (*n*=7)
Sepsis (control group 1)	279±32* (*n*=7)	94±17*^{o} (*n*=4)	526±57* (*n*=7)	332±60*^{o} (*n*=4)	91±10* (*n*=7)	68±12*^{o} (*n*=4)
Nicotine+sepsis (group 2)	112±15*$^{+}$ (*n*=7)	51±6*$^{+o}$ (*n*=7)	282±30*$^{+}$ (*n*=7)	157±17*$^{+o}$ (*n*=7)	45±6*$^{+}$ (*n*=7)	31±4*$^{+o}$ (*n*=7)
Aceclidine+sepsis (group 3)	185±17*$^{+x}$ (*n*=7)	48±6*$^{+o}$ (*n*=7)	370±38*$^{+}$ (*n*=7)	178±18*$^{+o}$ (*n*=7)	67±6*^{ox} (*n*=7)	46±5*^{ox} (*n*=7)

Note. p<0.05 in comparison with: *control, $^{+}$sepsis, ^{o}after 10 h, ^{x}under the effect of nicotine.

TABLE 3. Effects of Nicotine and Aceclidine (0.5 DL_{50}) on Phagocytic and Metabolic Activity of Mouse Blood Neutrophils and Serum Lysozyme Concentrations after 25 h of Experimental Sepsis (*M*±*m*, *n*=14)

Parameter		Control group 2	Nicotine	Aceclidine
PI, %		26.1±2.2	34.2±3.4	49.0±4.0*
PN		1.75±0.18	1.97±0.24	2.94±0.31*
NAI	NBT spontaneous	0.28±0.03	0.34±0.04	0.51±0.05*
	NBT induced	0.50±0.06	0.65±0.08	0.82±0.09*
Lysozyme, mg/liter		6.5±0.6	8.0±0.7	9.9±0.8*

Note. PI, PN: phagocytic index and phagocytic number; NAI: neutrophil activity index. *$p<0.05$ in comparison with the control.

respectively ($p<0.05$), 10 h after sepsis induction in comparison with control group 2 (Table 2). Nicotine injection before *E. coli* resulted in a reduction of the cytokine concentrations after 10 h of sepsis in comparison with the values in sepsis without nicotine preinjection (control group 1) by 2.49, 1.87, and 2.02 times, respectively ($p<0.05$), the values being significantly ($p<0.05$) higher than in control group 2.

Aceclidine injection 6 h before sepsis induction reduced plasma concentrations of IL-1β, IL-6, and MIP-2 10 h after *E. coli* injection by 1.51, 1.42 ($p<0.05$), and 1.34 times ($p>0.05$), respectively, in comparison with the values in sepsis without preinjection of cholinergic receptor agonists (control group 1).

Similar, but less pronounced changes in blood levels of proinflammatory cytokines were detected 25 h after sepsis induction without preinjection of cholinergic receptor agonists (control group 1). Blood levels of IL-1β, IL-6, and MIP-2 cytokines were significantly ($p<0.05$) lower 25 h after sepsis induction in all experimental series in comparison with the concentrations recorded 10 h after *E. coli* injection.

Plasma concentrations of proinflammatory cytokines were significantly lower after 10 and 25 h of experimental peritonitis induced after preinjection of nicotine than after aceclidine preinjection, except IL-1β concentration after 25 h of peritonitis; the most significant ($p<0.05$) reduction was recorded for IL-1β after 10 h of sepsis and for MIP-2. The levels of the studied cytokines were significantly ($p<0.05$) higher than in the control (control group 2).

The data indicate that not only nAChR stimulation, but also exposure of mAChR (presumably, of the dorsal autonomic nucleus of *n. vagus* of the medulla oblongata) lead to reduction of animal mortality from sepsis as a result of reduction of blood concentrations of proinflammatory cytokines. Mortality reduction could also be caused by stimulation of mAChR of neutrophils and other PMS cells.

Analysis of nicotine and aceclidine effects on the phagocytic and metabolic activities showed (Table 3) that aceclidine increased the phagocytic index, phagocytic number, and neutrophil activity index in spontaneous and induced NBT test ($p<0.05$). Nicotine slightly increased all the studied parameters, presumably, as a result of stimulation of nAChR in the sympathetic ganglia and renal medulla and the subsequent norepinephrine and epinephrine effects on the PMS cell β-adrenoreceptors [4] against the background of the suppressive effect of nicotine. This hypothesis is supported by the previous study [13] proving that stimulation of the PMS cells and lymphocyte nAChR and mAChR leads to opposite effects (stimulation of nAChR reduces the functions of leukocytes, macrophages, and monocytes). Aceclidine treatment led to a significant increase of the phagocytic index, phagocytic number, and neutrophil activity index in spontaneous and induced NBT test – 1.88, 1.51, 1.82, and 1.64 times, respectively ($p<0.05$) in comparison with the control (control group 2). In addition, aceclidine stimulation of mAChR led to an increase of serum lysozyme concentration in mice (1.52 times; $p<0.05$).

These changes in the blood levels of proinflammatory cytokines in mice indicate that the cholinergic anti-inflammatory pathway [1-3,9,11], reducing animal mortality from sepsis, is realized not only as a result of monocyte, macrophage, and neutrophil α7nAChR stimulation [8], but also as a result of acetylcholine effect on the medulla oblongata *n. vagus* dorsal autonomic nucleus mAChR. In addition, the animal survival in sepsis may be due to stimulation of the PMS cell mAChR in the liver, gastrointestinal tract, and spleen, leading to increase of the phagocytic and metabolic activity.

Hence, stimulation of muscarinic and nicotinic cholinergic receptors by nicotine and aceclidine, injected in single doses equivalent to 0.5 DL_{50} 6 h before sepsis induction, caused a significant reduction of mouse mortality from experimental infectious process as a result of reduction of blood concentrations of proinflammatory cytokines IL-1β, IL-6, and MIP-2 and increase of the neutrophil phagocytic and metabolic

activity. Realization of the cholinergic anti-inflammatory pathway (stimulation of the peripheral α7nAChR and central mAChR) was modulated by stimulation of the PMS cell mAChR.

REFERENCES

1. P. F. Zabrodsky, *Byull. Eksp. Biol. Med.*, **119**, No. 8, 164-167 (1995).
2. P. F. Zabrodsky, *Ibid.*, **150**, No. 9, 309-311 (2010).
3. P. F. Zabrodsky, *Farmakol. Toksikol.*, **49**, No. 2, 57-60 (1987).
4. P. F. Zabrodsky and V. G. Mandych, *Xenobiotics Immunotoxicology* [in Russian], Saratov (2007).
5. L. V. Borovikova, S. Ivanova, M. Zhang, *et al.*, *Nature*, **405**, 458-462 (2000).
6. M. Gallowitsch-Puerta and V. A. Pavlov, *Life Sci.*, **80**, Nos. 24-25, 2325-2329 (2007).
7. I. A. Giebeleh, M. Leendertse, S. Florquin, and T. van der Poll, *Eur. Respir. J.*, **33**, No. 2, 375-381 (2009).
8. H. P. Hauber and P. Zabel, *Internist* (Berl.), **50**, No. 7, 779-780, 782-784, 786 (2009).
9. W. Kessler, T. Traeqer, A. Westerholt, *et al.*, *Langenbecks Arch. Surg.*, **391**, No. 2, 83-87 (2006).
10. C. Liu, F. M. Shen, and Y. Y. Le, *Crit. Care Med.*, **37**, No. 2, 634-641 (2009).
11. S. L. Oke and K. J. Tracey, *J. Leukoc. Biol.*, **83**, No. 3, 512-517 (2008).
12. V. A. Pavlov, *Int. J. Clin. Med.*, **1**, No. 3, 203-212 (2008).
13. S. Razani-Boroujerdi, M. Behl, F. F. Hahn, *et al.*, *J. Neuroimmunol.*, **194**, Nos. 1-2, 83-88 (2008).
14. M. Rosas-Ballina and K. J. Tracey, *J. Intern. Med.*, **265**, No. 6, 663-679 (2009).
15. D. J. Song, X. Y. Huang, L. C. Ren, *et al.*, *Zhonghua Shao Shang Za Zhi*, **25**, No. 1, 36-41 (2009).