



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



Zabrodskii PF*

Saratov Medical University "REAVIZ", Saratov, Russian Federation

*Corresponding author: PF Zabrodskii, Saratov Medical University "REAVIZ", Saratov, Russian Federation, Russia

Submission: 📅 March 22, 2018; Published: 📅 May 18, 2018

Abstract

Experiments on albino mice showed that administration m-cholinomimetic (aceclidine), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-cholinomimetic $\alpha 7$ nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic $\beta 2$ ARs 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-1 β 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 $\alpha 7$ n-acetylcholinoreceptors ($\alpha 7$ nAChRs) of cells of the monocyte-macrophage system, followed by inhibition of the production of these cells by pro-inflammatory cytokines TNF- α , IL-1 β , 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].

The cholinergic anti-inflammatory pathway is realized due to the activation of acetylcholine m-acetylcholine receptors type 1 (m1AChR) of the brain, modulating the immunoregulatory function of the vagus nerve; excitation of efferent fibers n. vagus; the action of acetylcholine on $\alpha 7$ nAChRs cells of monocyte-macrophage system [3,6,7,12,13]. In cells of the monocyte-macrophage system, the anti-inflammatory effect is provided by the kinase JAK2, the transcription factor STAT3, the transcription factor NF- κ B (nuclear factor kappa B, NF-kappa B) [3,6].

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], $\beta 2$ -adrenoreceptors ($\beta 2$ ARs) of spleen T-lymphocytes [3,9], cause the same effect as the activation of $\alpha 7$ nAChRs, 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-1 β , 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 2.5×10^9 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 aceclidine (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 drugs 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 $\alpha 7$ nAChRs agonist GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride] (Sigma-Aldrich) at a single dose of 5mg/kg [16]. Epinephrine hydrochloride (Sigma-Aldrich) 0.5mg/kg (group 7) and selective $\beta 2$ ARs agonist (8th group) dexmedetomidine hydrochloride (Orion Pharma) were used as adrenergic drugs, which was administered at a single dose of 25 μ g/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 , IL1 β 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 , IL1 β , 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 \pm m).

Series of experiments	Term study of mortality after the introduction of <i>E. coli</i> , h	
	6	24
Sepsis (control group 2, n = 85)	36.4 \pm 5.2	89.4 \pm 3.4*
Aceclidine + sepsis (group 3, n = 36)	27.7 \pm 7.5	69.4 \pm 7.7*
Nicotine + sepsis (group 4, n = 36)	19.4 \pm 6.5*	52.8 \pm 8.3*
Neostigmine methyl sulfate + sepsis (group 5, n = 37)	18.9 \pm 6.6*	59.4 \pm 8.1*
$\alpha 7$ nAChRs agonist (GTS-21) + sepsis (group 6, n = 37)	13.5 \pm 5.6*	48.6 \pm 8.2*
Epinephrine + sepsis (group 7, n = 35)	14.3 \pm 5.9*	62.6 \pm 8.2*
$\beta 2$ ARs agonist (hexaprenaline sulfate) + sepsis (group 8, n = 32)	15.6 \pm 6.6*	50.0 \pm 9.0*

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 \pm m; n = 7-9).

Series of experiments	ФНО α		ИЛ1 β		ИЛ-6	
	6	24	6	24	6	24
Control group 1	50 \pm 7	38 \pm 6	26 \pm 4	28 \pm 5	73 \pm 10	65 \pm 9
Sepsis (control group 2)	606 \pm 84 ^a	55 \pm 8 ^c	528 \pm 63 ^a	135 \pm 1 ^{ac}	1975 \pm 243 ^a	213 \pm 24 ^{ac}
Aceclidine + sepsis (group 3)	248 \pm 26 ^{ab}	48 \pm 6 ^c	357 \pm 40 ^{ab}	92 \pm 10 ^{abc}	435 \pm 45 ^{ab}	115 \pm 12 ^{abc}
Nicotine + sepsis (group 4)	177 \pm 19 ^{abd}	50 \pm 6 ^c	205 \pm 28 ^{abd}	64 \pm 8 ^{abcd}	293 \pm 32 ^{abd}	72 \pm 9 ^{abcd}
Neostigmine methyl sulfate + sepsis (group 5)	155 \pm 20 ^{abd}	57 \pm 7 ^c	199 \pm 22 ^{abd}	57 \pm 6 ^{abc}	268 \pm 31 ^{abd}	80 \pm 10 ^{abcd}
$\alpha 7$ nAChRs agonist (GTS-21) + sepsis (group 6)	134 \pm 21 ^{abd}	36 \pm 5 ^c	174 \pm 18 ^{abd}	39 \pm 6 ^{abc}	200 \pm 27 ^{abd}	59 \pm 8 ^{abcd}
Epinephrine + sepsis (group 7)	170 \pm 23 ^{abd}	47 \pm 6 ^c	211 \pm 27 ^{abd}	62 \pm 8 ^{abc}	232 \pm 28 ^{abd}	73 \pm 10 ^{abcd}
$\beta 2$ ARs agonist (hexaprenaline sulfate) + sepsis (group 8)	151 \pm 24 ^{abd}	40 \pm 7 ^c	195 \pm 19 ^{abd}	48 \pm 7 ^{abc}	220 \pm 30 ^{abd}	65 \pm 7 ^{abcd}

The use of m-cholinomimetic aceclidine, n-cholinomimetic nicotine, reversible inhibitor of acetylcholine esterase (neostigmine methyl sulfate), n-cholinomimetic agonist $\alpha 7$ nAChRs (GTS-21), epinephrine, adrenergic agonist $\beta 2$ ARs (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 aceclidine 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 $\alpha 7$ nAChRs agonist (GTS-21), minimal effect - when using m-cholinomimetic aceclidine. 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 aceclidine, and activation of $\alpha 7$ nAChRs and $\beta 2$ ARs cells monocyte-macrophage system [3-5,18-20]. It should be noted that in large doses aceclidine activates probably not only the central m1AChRs [6,14], but and the $\alpha 7$ nAChRs.

The concentration in the blood of TNF α , IL1 β 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 TNF α content was significantly reduced ($p < 0.05$), reaching almost the control level, and the concentration of IL1 β and IL-6 exceeded the control parameters by 4.8 and 8.5 times, respectively ($p < 0.05$).

After the use of aceclidine, nicotine, neostigmine methyl sulfate, n-cholinomimetic agonist $\alpha 7$ nAChRs (GTS-21), epinephrine, adrenomimetic $\beta 2$ ARs agonist (group 2-8), followed by modeling of sepsis (administration of *E. coli*) concentration of TNF- α 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% ($p < 0.05$); IL1 β 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 TNF α 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), IL1 β concentration with m-cholinomimetic aceclidine 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 aceclidine, and by 67.2% on other drugs ($p < 0.05$).

It should be noted that, on average, the reduction of the concentrations of TNF α , IL1 β and IL-6 6 hours after the administration of *E. coli* with the use of aceclidine (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 TNF α , IL1 β and IL-6 after administration of aceclidine 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 $\alpha 7$ nAChRs

agonist (GTS-21) are associated with the activation of $\alpha 7$ nAChRs cells of the monocyte-macrophage system of liver, gastrointestinal, and spleen nAChRs [20,21]. Reduction of production of TNF α , IL1 β and IL-6 under the influence of epinephrine, adrenomimetic agonist $\beta 2$ ARs (hexaprenaline sulfate) occurs as result of direct and indirect (through $\beta 2$ ARs T spleen T cells) activation of monocyte-macrophage system cells [3,9]. It is known that monocytes and macrophages have β ARs, and their activation leads to an anti-inflammatory effect [14] due to inhibition of the nuclear transcription factor NF- κ B [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 (aceclidine), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-cholinomimetic $\alpha 7$ nAChRs agonist (GTS-21), epinephrine hydrochloride, adenomimetic $\beta 2$ ARs 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 aceclidine, nicotine, neostigmine methyl sulfate, $\alpha 7$ nAChR agonist (GTS-21), epinephrine hydrochloride, adrenomimetic $\beta 2$ ARs agonist (hexaprenaline sulfate) practically simultaneously with modeling of sepsis, decreased blood concentrations of TNF- α , IL-1 β and IL-6 compared with parameters at sepsis without the use of drugs.
3. M-cholinomimetic aceclidine compared with the effects of nicotine, the reversible cholinesterase inhibitor neostigmine methyl sulfate, the $\alpha 7$ nAChRs agonist (GTS-21), epinephrine hydrochloride, adrenomimetic $\beta 2$ ARs agonist (hexaprenaline sulfate) are more pronounced.

References

1. Link A, Selejan S, Maack C, Lenz M, Böhm M (2008) Phosphodiesterase 4 inhibition but not beta-adrenergic stimulation suppresses tumor necrosis factor-alpha release in peripheral blood mononuclear cells in septic shock. *Crit Care* 12(6): R159.
2. Martin GS (2012) Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. *Expert Rev Anti Infect Ther* 10(6): 701-706.
3. Zabrodskii PF, Gromov MS, Maslyakov VV (2016) Role of $\beta 2$ -adrenoreceptors in adrenergic anti-inflammatory mechanism in sepsis. *Bull Exp Biol Med* 162(12): 718-721.
4. Zabrodskii PF (1987) Effect of armin on nonspecific resistance factors of the body and on the primary humoral immune response. *Farmakol Toksikol* 50(1): 57-60.
5. Zabrodskii PF (1995) Change in the non-specific anti-infection resistance of the body exposed to cholinergic stimulation. *Bull Exp Biol Med* 120(8): 164-166.

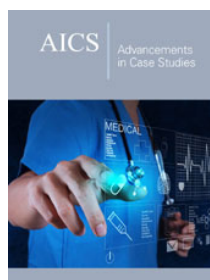
6. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina G, et al. (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405(6785): 458-462.
7. Bernik TR, Friedman SG, Ochani M, Di Raimo R, Ulloa L, et al. (2002) Pharmacological stimulation of the cholinergic anti-inflammatory pathway. *J Exp Med* (195) 6: 781-788.
8. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, et al. (2003) Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421(6921): 384-388.
9. Bonaz BL, Bernstein CN (2013) Brain-gut interactions in inflammatory bowel disease. *Gastroenterology* 144(1): 36-49.
10. Fernandez R, Nardocci G, Navarro C, Reyes EP, Acuña-Castillo C, et al. (2014) Neural reflex regulation of systemic inflammation: potential new targets for sepsis therapy. *Front Physiol* 15(5): 489.
11. Reardon C (2016) Neuro-immune interactions in the cholinergic anti-inflammatory reflex. *Immunol Lett* 178: 92-96.
12. Kessler W, Traequer T, Westerholt A, Neher F, Mikulcak M, et al. (2006) The vagal nerve as a link between the nervous and immune system in the instance of polymicrobial sepsis. *Langenbecks Arch Surg* 391(2): 83-87.
13. Eftekhari G, Hajiasgharzadeh K, Ahmadi-Soleimani SM, Dehpour AR, Semnani S, et al. Activation of central muscarinic receptor type 1 prevents development of endotoxin tolerance in rat liver. *Eur J Pharmacol* 740: 736-741.
14. Scanzano A, Cosentino M (2015) Adrenergic regulation of innate immunity: a review. *Front Pharmacol* 13(6): 171.
15. Song DJ, Huanq XY, Ren LC, Yang XH, Xiao MZ, et al. (2009) Effect of lentiviral vector encoding on triggering receptor expressed on myeloid cells 1 on expression of inflammatory cytokine in septic mice infected by *Bacteroides fragilis*. *Zhonghua Shao Shang Za Zhi* 25(1): 36-41.
16. Sitapara RA, Antoine DJ, Sharma L, Patel VS, Ashby CR Jr, Gorasiya S, et al. (2014) The $\alpha 7$ nicotinic acetylcholine receptor agonist GTS-21 improves bacterial clearance in mice by restoring hyperoxia-compromised macrophage function. *Mol Med* 20: 238-247.
17. Ning Q, Liu Z, Wang X, Zhang R, Zhang J, et al. (2017) Neurodegenerative changes and neuroapoptosis induced by systemic lipopolysaccharide administration are reversed by dexmedetomidine treatment in mice. *Neurol Res* 39(4): 357-366.
18. Giebeleh IA, Leendertse M, Florquin S, van der Poll T (2009) Stimulation of acetylcholine receptors impairs host defence during pneumococcal pneumonia. *Eur Respir J* 33(2): 375-381.
19. Lin JN, Tsai YS, Lai CH, Chen YH, Tsai SS, et al. (2009) Risk factors for mortality of bacteremic patients in the emergency department. *Acad Emerg Med* 16: 749-755.
20. Kox M, Pickkers P (2015) Modulation of the innate immune response through the vagus nerve. *Nephron*. 131(2): 79-84.
21. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ (2003) The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol Med* 9(5-8): 125-134.
22. Tan KS, Nackle AG, Satterfield K, Maixner W, Diatchenko L, et al. (2007) Beta2 adrenergic receptor activation stimulates pro-inflammatory cytokine production in macrophages via PKA- and NF-kappaB-independent mechanisms. *Cell Signal* 19(2): 251-260.



Creative Commons Attribution 4.0
International License

For possible submissions Click Here

[Submit Article](#)



Advancements in Case Studies

Benefits of Publishing with us

- High-level peer review and editorial services
- Freely accessible online immediately upon publication
- Authors retain the copyright to their work
- Licensing it under a Creative Commons license
- Visibility through different online platforms