CRIMSON PUBLISHERS
Wings to the Research

Advancements in Case Studies

ISSN 2639-0531

Case Report

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 (aceclydine), 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].

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 α7nAChRs 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-κB (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], 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 drags (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-dimethoxybenzylidene)-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).

Series of experiments	Term study of mortality after the introduction of *E. coli*, h	
	6	24
Sepsis (control group 2, n = 85)	36.4±5.2	89.4±3.4*
Aceclydine + sepsis (group 3, n = 36)	27.7±7.5	69.4±7.7*
Nicotine + sepsis (group 4, n = 36)	19.4±6.5*	52.8±8.3*
Neostigmine methyl sulfate + sepsis (group 5, n = 37)	18.9±6.6*	59.4±8.1*
α7nAChRs agonist (GTS-21) + sepsis (group 6, n = 37)	13.5±5.6*	48.6±8.2*
Epinephrine + sepsis (group 7, n = 35)	14.3±5.9*	62.6±8.2*
β2ARs agonist (hexaprenaline sulfate) + sepsis (group 8, n = 32)	15.6±6.6*	50.0±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 ± m; n = 7-9).

Series of experiments	ФНОα		ИЛ1β		ИЛ-6	
	6	24	6	24	6	24
Control group 1	50±7	38±6	26±4	28±5	73±10	65±9
Sepsis (control group 2)	606±84^{a}	55±8^{c}	528±63^{a}	135±1^{ac}	1975±243^{a}	213±24^{ac}
Aceclydine + sepsis (group 3)	248±26^{ab}	48±6^{c}	357±40^{ab}	92±10^{abc}	435±45^{ab}	115±12^{abc}
Nicotine + sepsis (group 4)	177±19^{abd}	50±6^{c}	205±28^{abd}	64±8^{abcd}	293±32 ^{abd}	72±9^{abcd}
Neostigmine methyl sulfate + sepsis (group 5)	155±20^{abd}	57±7^{c}	199±22^{abd}	57±6^{abc}	268±31^{abd}	80±10^{abcd}
α7nAChRs agonist (GTS-21) + sepsis (group 6)	134±21^{abd}	36±5^{c}	174±18^{abd}	39±6^{abc}	200±27^{abd}	59±8^{abcd}
Epinephrine + sepsis (group 7)	170±23^{abd}	47±6^{c}	211±27^{abd}	62±8^{abc}	232±28^{abd}	73±10^{abcd}
β2ARs agonist (hexaprenaline sulfate) + sepsis (group 8)	151±24^{abd}	40±7^{c}	195±19^{abd}	48±7^{abc}	220±30^{abd}	65±7^{abcd}

The use of m-cholinomimetic aceclydine, 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 aceclydine is less pronounced than that of other drugs ($p> 0.05$)

How to cite this article: Zabrodskii P. The Influence of Cholinergic and Adrenergic Drugs on Mortality of Mice and the Concentration of Proinflammatory Cytokines in Blood at Sepsis . Adv Case Stud. 1(3). AICS.000512.2018. DOI: 10.31031/AICS.2018.01.000512

The results indicate that the applied drags, 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 aceclydine. 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 aceclylidine, and activation of α7nAChRs and β2ARs cells monocyte-macrophage system [3-5,18-20]. It should be noted that in large doses aceclydine 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 aceclydine, 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 TNF-α in the blood of mice decreased in 6 hours compared to parameters for sepsis without the use of drags (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 drags in sepsis did not practically differ from the parameters of control group 1 (intact mice) and group 2 (sepsis without drags), IL1β concentration with m-cholinomimetic aceclydine 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 aceclydine, 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 aceclydine (59.7%) compared to other drags (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 aceclydine 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]. Reduction of 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 (aceclydine), n-cholinomimetic (nicotine), reversible inhibitor of acetylcholinesterase (neostigmine methyl sulfate), n-cholinomimetic α7nAChRs agonist (GTS-21), epinephrine hydrochloride, adenomimetic 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 aceclydine, 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 TNF-α, IL-1β and IL-6 compared with parameters at sepsis without the use of drugs.

3. M-cholinomimetic acetylidine 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

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 β2-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, Traeqer 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, Semnanian 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 α7 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, Nackley 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.