



The Cholinergic Anti-Inflammatory Pathway. Combined Effect of Anticholinesterase Compounds and its Antidote Cholinesterase Reactivator Carboxim

Pavel Franzevich Zabrodskii*

Saratov Medical University "REAVIZ", Saratov, Russia

*Corresponding Author: Pavel Franzevich Zabrodskii, Saratov Medical University "REAVIZ", Saratov, Russia.

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Abstract

Experiments on random-bred albino mice showed that the acute intoxication of anticholinesterase compounds (DDVP, 0.75 LD₅₀) is implemented the cholinergic anti-inflammatory pathway (reduced mortality of mice from sepsis and the blood concentration of proinflammatory cytokines TNF- α , IL-1 β , IL-6). Sepsis caused by the intraperitoneal administration of saline diurnal culture of *E. coli* O157:H7 (2.5 \times 10⁹ CFUs). The use of the cholinesterase reactivator carboxim (15 mg/kg) - antidote of organophosphorus compounds - after acute intoxication of DDVP diminished affect the implementation of the cholinergic anti-inflammatory pathway.

Keywords: Anti-Inflammatory Pathway; Sepsis; *E. Coli*; Anticholinesterase Compounds; Cholinesterase Reactivator Carboxim

Introduction

Anticholinesterase compounds (organophosphate compounds – OPC, anticholinesterase drugs) are widely used in agriculture, various industries and households, in medicine. OPC can cause environmental pollution, as well as acute and chronic intoxications [1-7]. Cholinergic stimulation, as we established in 1987 [2] and in subsequent studies, significantly reduces the mortality of white mice from sepsis caused by intraperitoneal or intrapulmonary administration, respectively of *E. coli* and *P. vulgaris* [3-5,8]. Thus, the cholinergic anti-inflammatory mechanism has been discovered in 1987 [2], named «cholinergic anti-inflammatory pathway» in 2002 [9] after the research its implementation at the organismal, cellular and subcellular levels [3,4,9,10]. It should be noted that in 1995 it was proved the possibility of cholinomimetics for emergency activation of antimicrobial resistance of the organism in sepsis [3,4]. In the future, the study of the cholinergic anti-inflammatory pathway caused by the action of acetylcholine on α 7n-acetylcholine receptors (α 7nAChRs) cells of the monocyte-macrophage system (MMC), followed by inhibition of the production by the cells of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and reduced mortality from sepsis were devoted hundreds

of articles various authors [5-18]. Reduced production of TNF- α , IL-1 β , IL-6 (anti-inflammatory effect occurrence) for cholinergic anti-inflammatory pathway is provided kinase JAK2, transcription factor STAT3, NF- κ B transcription factor) [10,16,17,18].

Aim of the study

The aim of the study was to evaluate the effect of acute intoxication of anticholinesterase compounds (OPC, anticholinesterase drugs) in combination with its antidote cholinesterase reactivator carboxim on the mortality of mice from sepsis caused by experimental peritonitis (*E. coli*), and the concentration of pro-inflammatory cytokines TNF α , IL-1 β and IL-6 in blood.

Materials and Methods

Experiments were performed on random-bred albino mice of both sexes weighing 18-22 g. Control group of mice (control group 1, n = 8) received i.p. 2.0 ml isotonic sodium chloride solution (saline) 2 h after subcutaneous administration of 0.5 ml saline. The second group of mice (control group 2, n = 50) was injected subcutaneously once with saline (0.5 ml), after 2 h mice received 2.5 \times 10⁹ CFUs in 2.0 ml of saline diurnal culture of *E. coli* O157:H7 (sepsis modeling) [2-4 9,16-18,20]. Mice of the third group (n = 40)

were injected with OPC – DDVP (O, O-dimethyl-O-2,2-dichlorovinyl phosphate) (Sigma-Aldrich) intramuscularly once at a dose of 0.75 LD₅₀ in 0.5 ml of a 0.25% solution of dimethyl sulfoxide (DMSO). DDVP was dissolved in DMSO, 0.25% aqueous solution containing a toxicant was prepared. The LD₅₀ DDVP was 52.5±3.3 mg/kg. The fourth group of mice (n = 25) received cholinesterase reactivator OPC of antidote carboxim (5-[[[2-[Benzyl(dimethyl)ammonio]ethyl]amino]carbonyl]-2-[(hydroxyimino)methyl]-1-methylpyridinium ichloride) (Pharmzaschita Research and Production Center “Pharmzaschita”, Russia) at a dose of 15 mg/kg in 0.5 ml saline (single subcutaneous injection). The fifth group of mice (n=45) received DDVP intramuscularly once at a dose of 0.75 LD₅₀ and carboxim (15 mg/kg, single subcutaneous injection) 5-10 minutes after the administration of OPC (DDVP).

The concentration of TNF-α, IL1β and IL-6 was studied in blood plasma of all groups of mice (groups 1-5) 4 and 24 h 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 proinflammatory 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

Acute intoxication of OPC (DDVP, 0.75 LD50), combined effect of OPC and carboxim (15 mg/kg) 2 h before the sepsis modeling caused a significant decrease in mortality of mice after 4 h compared with the control group 2 (sepsis), respectively, in 4.80 and 1.80 times (p < 0.05) (by 38.0 and 21.3%), and after 24 h - in 1.68 and 1.33 times (by 32.5 and 20.0%) (p < 0.05), respectively.

The administration of carboxim (2 h before the sepsis modeling) did not significantly (p > 0.05) reduce the mortality of mice after 4 and 24 h (after the administration of *E. coli*). The obtained research results indicate that the administration of carboxim after acute intoxication of OPC (before sepsis modeling) diminished the effect of OPC, which causes a reduction in the mortality of mice after sepsis modeling (Table 1).

Series of experiments	Term study of mortality after the administration of <i>E. coli</i> , h	
	4	24
Sepsis (control group 2, n = 50)	48,0 ± 7,0	80,0 ± 5,7
DDVP + sepsis (group 3, n = 40)	10.0 ± 4.7*	47.5 ± 7,9*
Carboxim + sepsis (group 4, n = 25)	36.0 ± 9,6	68.0 ± 9,3
DDVP + carboxim + sepsis (group 5, n = 45)	26.7 ± 6.6**	60,0 ± 7,3*

Table 1: Combined effect of OPC (DDVP, 0.75 LD50) and carboxim (15 mg/kg) on the mortality of mice from sepsis (M±m).

* -p <0,05 as compared to control (group 2). ** -p <0,05 as compared to group 3.

The concentrations of cytokines TNF-α, IL-1β and IL-6 after sepsis modeling (control group 2) significantly increased in the blood of mice after 4 h compared to control group 1 (intact animals), respectively, at 17.0; 19.9 and 53.7 times (p <0.05), the concentrations of TNF-α, IL-1β and IL-6 after 24 h compared with their level after 4 h significantly decreased, exceeding the parameters of intact animals (group 1) in 1.3 (p>0.05), 4.7 and 6.5 times (p <0.05), respectively (Table 2).

Series of experiments	TNFα		IL1β		IL-6	
	4	24	4	24	4	24
Control group 1	40 ± 6	42 ± 7	29 ± 5	25 ± 5	41 ± 6	37 ± 6
Sepsis (control group 2)	680 ± 87 ^a	53 ± 8 ^c	577 ± 72 ^a	118 ± 24 ^{ac}	2200 ± 250 ^a	242 ± 36 ^{ac}
DDVP + sepsis (group 3)	215 ± 26 ^{ab}	46 ± 7 ^c	252 ± 35 ^{ab}	59 ± 8 ^{abc}	331 ± 42 ^{ab}	94 ± 11 ^{abc}
Carboxim + sepsis (group 4)	520 ± 65 ^a	38 ± 7 ^c	378 ± 63 ^a	72 ± 12 ^{ac}	1600 ± 210 ^a	161 ± 29 ^{ac}
DDVP + carboxim + sepsis (group 5)	325 ± 30 ^{abd}	35 ± 5 ^c	355 ± 33 ^{abd}	63 ± 7 ^{abc}	605 ± 64 ^{abd}	105 ± 12 ^{abc}

Table 2: Combined effect of OPC (DDVP, 0.75 LD50) and carboxim (15 mg/kg) on concentrations of proinflammatory cytokines in blood of mice after sepsis modeling, pg/ml (M ± m; n = 6-8).

Note. 4 and 24 - after sepsis modeling, h; a-p <0,05 as compared to control (group 1); b-p <0,05 as compared to the corresponding parameter in sepsis (control group 2); c-p <0,05 as compared to the 4 h; d-p <0,05 as compared to the corresponding parameter in group 3.

Acute intoxication of OPC (DDVP) decreased the TNF- α , IL-1 β and IL-6 blood concentrations 4 h after sepsis modeling (group 3) compared to the control group 2 (sepsis without the use of drugs), respectively, by 3.16; 2.29 and 6.65 times ($p < 0.05$). At the same time, the concentrations of proinflammatory cytokines in the blood significantly ($p < 0.05$) exceeded the corresponding parameters of the control group 1. The concentrations of TNF- α , IL-1 β and IL-6 24 h after sepsis modeling decreased in comparison with these parameters after 4 h, remaining below group 2 values in 1.15 ($p > 0.05$), 2.00 and 2.57 times ($p < 0.05$), respectively.

The parameters of TNF- α , IL-1 β and IL-6 after administration of carboxim in 4 h and 24 h after modeling sepsis (group 4) were not significantly reduced ($p > 0.05$) compared with the parameters of the control group 2.

The values of TNF- α , IL-1 β and IL-6, when using OPC (DDVP, 0.75 LD₅₀) and its antidote carboxim (combined effect) decreased 4 h after the sepsis modeling (group 5) compared to the parameters of the control group 2, respectively, in 2.09; 1.63 and 3.64 times ($p < 0.05$). The reduction of content of proinflammatory cytokines in the blood was established 24 h after the sepsis modeling compared with the corresponding values after 4 h, while the concentrations of TNF- α , IL-1 β and IL-6 remained higher than the values of group 1, respectively, in 1.26 ($p > 0.05$); 4.72 and 6.54 times ($p < 0.05$).

The IL-1 β and IL-6 blood concentrations in groups 3, 4 and 5 were significantly higher ($p < 0.05$) than the corresponding values of the control group 1. The TNF- α blood concentrations in these groups were significantly higher ($p < 0.05$) only 4 h after the sepsis modeling.

The values of TNF- α , IL-1 β , and IL-6 in blood 4 h after sepsis modeling and combined action of OPC and carboxim (group 5) compared to acute OPC intoxication (DDVP, after sepsis modeling, group 3) were significantly higher ($p < 0.05$), but these values were significantly lower ($p < 0.05$) in group 2 (sepsis modeling).

Discussion

After acute OPC intoxication 2 h after sepsis modeling (groups 3) mouse mortality is significantly reduced after 4 and 24 h compared with the control group 2 (sepsis). Acute intoxication of OPC decreased the TNF- α , IL-1 β and IL-6 blood concentrations after sepsis modeling (group 3) compared to the control group 2 (sep-

sis without the use of drugs). Numerous studies have shown that the established effects are associated with acetylcholine m1AChRs activation of the brain [18,21], α 7nAChRs of MMS cells, nAChRs of adrenal medulla and other mechanisms [9-11,14,16,17,19]. The implementation of the reduction of pro-inflammatory cytokines TNF- α , IL-1 β , IL-6, and others (the occurrence of anti-inflammatory effect) is provided by JAK2 kinase, STAT3 transcription factor; transcription factor NF- κ B) [5,10,15-19]. In addition, the decrease in mortality from sepsis after acute intoxication of OPC due to the suppression of the synthesis of proinflammatory cytokines is also associated with the effect of corticosteroids (activation of the hypothalamic-pituitary-adrenal system) [5,8,11]. The administration of carboxim (15 mg/kg) practically did not reduce the mortality of mice, as well as the concentrations of cytokines TNF- α , IL-1 β and IL-6 after sepsis modeling.

The decrease of the implementation of cholinergic anti-inflammatory pathway under the combined action of DDVP (anticholinesterase compounds, OPC, anticholinesterase drugs) and cholinesterase reactivator OPC antidote carboxim is caused by acetylcholinesterase reactivation (associated with OPC), decrease in concentration in blood and synapses of parasympathetic nervous system of acetylcholine [5,8-11,14,16,17,19]. As a result, the effect of acetylcholine on brain m1AChRs, α 7nAChRs of MMS cells, nAChRs of adrenal medulla and the effect of cholinergic anti-inflammatory pathway decreases. This is supported by the data we have provided on the administration of carboxim after acute intoxication of OPC (before sepsis modeling) diminished the effect of OPC, which causes a reduction in the mortality of mice after sepsis modeling, as well as by the results of our studies, which show that the values of TNF- α , IL-1 β , and IL-6 in blood 4 h after sepsis modeling and combined action of OPC and carboxim (group 5) compared to acute OPC intoxication (DDVP, after sepsis modeling, group 3) were significantly higher ($p < 0.05$).

Conclusion

The anticholinesterase compounds acute intoxication is implemented the cholinergic anti-inflammatory pathway (reduced mortality of mice from sepsis and the blood concentration of proinflammatory cytokines TNF- α , IL-1 β , IL-6). The use of cholinesterase reactivator anticholinesterase compounds (OPC) of antidote carboxim (15 mg/kg) after acute intoxication of OPC (DDVP, 0.75 LD₅₀) before the sepsis modeling diminished affect the implementation of the cholinergic anti-inflammatory pathway.

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