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Research Article

The Combined Effect of Organophosphorus Compounds and Its Antidote Atropine on Implementation of Cholinergic Anti-Inflammatory Pathway

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Abstract

Experiments on random-bred albino mice showed that the acute Intoxication of organophosphorus compounds (DDVP, 0.75 LD_{50}) is implemented the cholinergic anti-inflammatory pathway (reduced mortality of mice from sepsis and the blood concentration of proinflammatory cytokines TNF-α, IL-1β, IL-6). Sepsis caused by the intraperitoneal administration of saline diurnal culture of *E. coli* O157:H7 (2.5×10^9 CFUs). The use of the atropine sulfate (10 mg/kg) antidote of organophosphorus compounds after acute intoxication of DDVP does not affect the implementation of the cholinergic anti-inflammatory pathway.

Keywords: Anti-Inflammatory Pathway; Sepsis; *E. coli;* Organophosphorus Compounds; Atropine Sulfate

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* [3,4,5,8]. 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 α7n-acetylcholine receptors (α7nAChRs) 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 [5,9-18]. Reduced production of TNF-α, IL-1β, IL-6 (anti-inflammatory effect occurrence) for cholinergic anti-inflammatory pathway is provided kinase JAK2, transcription factor STAT3, NF-κB transcription factor) [10,16-18].

The aim of the study was to evaluate the effect of acute intoxication of organophosphate compounds in combination with its antidote atropine sulfate on the mortality of mice from sepsis caused by experimental peritonitis (*E. coli*), and the concentration of pro-inflammatory cytokines TNFα, IL-1β 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 = 45) was injected subcutaneously once with saline (0.5 ml), after 2 h mice received 2.5×10^9 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 = 30) were injected with OPC – DDVP (O, O-dimethyl-O-2,2-dichlorovinyl phosphate) (Sigma-Aldrich) intramuscularly once at a dose of 0.75 LD_{50} 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 LD_50 DDVP was 52.5 ± 3.3 mg/kg. The fourth group of mice (n = 30) received atropine sulfate (Sigma-Aldrich) at a dose of 10 mg/kg in 0.5 ml saline (single subcutaneous injection). The fifth group of mice (n = 35) received DDVP intramuscularly once at a dose of 0.75 LD_{50} and atropine sulfate

(10 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 1-8) 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 LD_{50}), combined effect of OPC and atropine sulfate (10 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 3.17 and 2.45 times ($p < 0.05$) (by 28.9 and 25.1%), and after 24 h - in 1.73 and 1.67 times (by 36.7 and 35.3%) ($p < 0.05$), respectively. The administration of atropine sulfate (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 atropine sulfate after acute intoxication of OPC does not affect 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 *E. coli*, h	
	4	24
Sepsis (control group 2, n = 45	42,2 ± 7,0	86,7 ± 5,0
DDVP + sepsis (group 3, n = 30)	13.3 ± 6.1*	50.0 ± 9,1*
Atropine sulfate + sepsis (group 4, n = 30)	30.0 ± 8,4	73.3 ± 8,1
DDVP + atropine sulfate + sepsis (group 5, n = 35)	17.1 ± 6.3*	51,4 ± 8,4*

Table 1: Combined effect of OPC (DDVP, 0.75 LD_{50}) and atropine sulfate (10 mg/kg) on the mortality of mice from sepsis (**M** ± m).

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

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	ФНОα		ИЛ1β		ИЛ-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]
Atropine sulfate + sepsis (group 4)	503 ± 70[a]	42 ± 7[c]	412 ± 65[a]	88 ± 11[ac]	1790 ± 235[a]	185 ± 30[ac]
DDVP + atropine sulfate + sepsis (group 5)	180 ± 33[ab]	37 ± 6[c]	226 ± 28[ab]	65 ± 9[abc]	300 ± 43[ab]	87 ± 9[abc]

Table 2: Combined effect of OPC (DDVP, 0.75 LD_{50}) and atropine sulfate (10 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.

Acute intoxication of OPC (DDVP) decreased the TNF-α, IL-1β 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 3.2; 2.3 and 6.6 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-1β and IL-6 24 h after sepsis modeling decreased in comparison with these parameters after 4 h, remaining below group 2 values in 1.2 ($p > 0.05$), 2.4 and 2.6 times ($p < 0.05$), respectively.

The parameters of TNF-α, IL-1β and IL-6 after administration of atropine sulfate 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-1β and IL-6, when using OPC (DDVP, 0.75 LD50) and its antidote atropine sulfate (combined effect) decreased 4 h after the sepsis modeling (group 5) compared to the parameters of the control group 2, respectively, in 3.8; 2.6 and 7.3 times ($p < 0.05$). A 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-1β and IL-6 remained below the values of group 2, respectively, in 1.4 ($p > 0,05$); 1.8 and 2.8 times ($p < 0.05$).

The IL-1β 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-1β and IL-6 in blood after sepsis modeling with acute intoxication of OPC (DDVP, 0.75 LD_{50}) and with combined effect of OPC and atropine sulfate (10 mg/kg) were not significantly different.

Discussion

4 and 24 h compared with the control group 2 (sepsis). Acute intoxication of OPC decreased the TNF-α, IL-1β and IL-6 blood concentrations after sepsis modeling (group 3) compared to the control group 2 (sepsis without the use of drugs). Numerous studies have shown that the established effects are associated with acetylcholine m1AChRs activation of the brain [18,21], α7nAChRs of MMS cells, nAChRs of adrenal medulla and other mechanisms [9-11,14,16,17,19]. The implementation of the reduction of proinflammatory cytokines TNF-α, IL-1β, IL-6, and others (the oc currence of anti-inflammatory effect) is provided by JAK2 kinas STAT3 transcription factor, transcription factor NF-κB) [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 atropine sulfate resulted in insignificant decrease (by 13.4% 24 h after sepsis modeling) of mouse mortality, as well as in reducing the concentrations of cytokines TNF-α, IL-1β and IL-6 after sepsis modeling. On average, atropine sulfate reduced the level of pro-inflammatory cytokines 4 h after modeling sepsis in 1.33 times. This is probably due to the activation of the sympathetic nervous system, which occurs when cholinolytics (atropine and others) are blocked by mAChRs of various organs and systems [5,8]. There is reason to believe that along with «cholinergic anti-inflammatory pathway» exist adrenergic anti-inflammatory mechanism associated with sepsis, inflammatory bowel disease and other infectious processes with the activation of n-cholinergic receptors of the adrenal medulla and sympathetic ganglia, which results in adrenaline and noradrenaline production, which are probably exciting adrenergic receptors of cells of MMS system (direct action) [17,22], α2-adrenoceptors (β2ARs) splenic T-lymphocytes (indirect action) [11,17], causing the same effect as the activation α7n-acetylcholine receptors (α7nAChRs), resulting in reduction of the synthesis of pro-inflammatory cytokines by cells of the MMS [17,18].

The mortality of mice and values of TNF-α, IL-1β, IL-6 in blood after sepsis modeling with acute intoxication of OPC and with combined effect of OPC and atropine sulfate were not significantly different. This suggests that with combined effect of OPC and atropine sulfate implemented a mechanism associated with the activation of the OPC cholinergic system, as well as to certain extent the adrenergic anti-inflammatory mechanism (activation of α7nAChRs of MMS cells, nAChRs of adrenal medulla and other) [16-19]. Atropine sulfate has no effect on the implementation of this mechanism (effect of OPC).

Conclusion

1. The acute Intoxication of organophosphorus compounds (DDVP, 0.75 LD_{50}) is implemented the cholinergic anti-inflammatory pathway (reduced mortality of mice from sepsis and the blood concentration of proinflammatory cytokines TNF-α, IL-1β, IL-6).

2. The use of the atropine sulfate (10 mg/kg) antidote of organophosphorus compounds after acute intoxication of OPC (DDVP, 0.75 LD_{50}) does not affect the implementation of the cholinergic anti-inflammatory pathway.

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