THE INFLUENCE OF LOW-LEVEL SARIN INHALATION EXPOSURE ON THE HOST RESISTANCE AND IMMUNE REACTION OF INBRED BALB/C MICE AFTER THEIR INFECTION WITH *FRANCISELLA TULARENSIS LVS*

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SUMMARY

To study the influence of low-level sarin inhalation exposure on immune functions, inbred BALB/c mice were exposed to two low concentrations of sarin for 60 minutes in the inhalation chamber and then infected with Francisella tularensis LVS on the 7th day following the exposure to sarin. 24 hours after infection, the level of some isotypes of antibodies (IgM, IgA) against tularaemia was significantly decreased regardless of the sarin concentration used while the lymphoproliferation was significantly increased regardless of the mitogen and sarin concentration used. Later, the level of some isotypes of antibodies (IgM, IgA) against tularaemia and the vitality of Francisella tularensis LVS was significantly increased in the case of exposure of mice to clinically symptomatic concentration of sarin (7 days after infection) while the lymphoproliferation was significantly decreased regardless of the concentration of sarin when specific tularaemic antigen Ag4 was used as a mitogen (3 weeks after infection). Thus, the results indicate that not only symptomatic but also asymptomatic dose of sarin is able to alter the host resistance and reaction of immune system, especially at 24 hours and 7 days following infection with Francisella tularensis LVS. Nevertheless, the alteration of immune functions following the inhalation exposure to a symptomatic concentration of sarin seems to be more pronounced.

Key words: sarin, low-level inhalation exposure, immunotoxicity, *Francisella tularensis* LVS, BALB/c mice

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INTRODUCTION

Nerve agents, highly toxic organophosphorus compounds (OPs) represent potential threats to both military and civilian population, as evidenced in recent terroristic attacks in Japan. The irreversible binding to and subsequent inactivation of acetylcholinesterase (AChE, EC 3.1.1.7) leading to the accumulation of acetylcholine in the cholinergic synapses is generally believed to be the major mechanism of OP poisoning. In addition, OPs have many other effects. They are called as non-specific or non-cholinergic effects and involve mutagenic, stressogenic, immunotoxic, hepatotoxic, membrane and haematotoxic effects (1).

Several studies on the immunotoxic effects of OP compounds in experimental animals have demonstrated the laboratory signs of suppression of cell-mediated as well as humoral immune functions as a supression of the primary IgM and IgG response to sheep erythrocytes in inbred mice (2) or inhibition of mitogen-induced lymphocyte proliferation (3) following the exposure to OPs, especially to organophosphorus insecticides (OPI) at relatively high toxic doses. The immunotoxic effects of OPI have been also

shown in humans but the evaluation of human immunotoxicity of OP compounds is limited to few studies (4, 5). Most above-mentioned studies described the results of OPI exposure but there are also studies demonstrating the immunotoxic effects of nerve agents (6).

Much is known about the acute effects of high level exposure to nerve agents and OPI but there have been very few studies about harmful effects of OPs following low level exposures (7). Therefore, the research dealing with the evaluation of the influence of low-level exposure to OP compounds on various physiological functions including the immune functions in OP-exposed organisms is needed. The purpose of this study is to find out whether chosen nerve agent (sarin) at low doses is able to produce the alteration of the host resistance and immune reaction to the infection with *Francisella tularensis* LVS in inbred mice.

MATERIAL AND METHODS

Specific-pathogen-free, female BALB/c mice weighing 15-18 g were purchased from Konárovice (Czech Republic). They were kept in an air-conditioned room and allowed to access to standard food and tap water ad libitum. Food as well as water were sterilized before their use. The mice were divided into groups of ten animals. Handling of the experimental animals was done under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy in Hradec Králové (Czech Republic).

Sarin of 98% purity was obtained from Military Technical Institute in Zemianske Kostolany (Slovak Republic). Its purity was assayed by acidimetric titration. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Mice were exposed to low concentrations of sarin in the inhalation chamber for 60 minutes. Two low concentrations of sarin were used for the inhalation exposure of mice:

• concentration resulting in no clinical signs or symptoms and erythrocyte AChE inhibition of < 20 % following 60 minute inhalation exposure (0.5 μg/l) - LEVEL 1.

• concentration resulting in mild clinical signs such as salivation without convulsions and in an inhibition of erythrocyte AChE of 40-50% following 60 min inhalation exposure (1.6 μg/l) – LEVEL 2.

The total specific antibodies against tularaemia and their isotypes (IgA, IgG1, IgG2a, IgG2b, IgE, IgM) were evaluated in serum with the help of ELISA method (8). Non-specific mitogens (concanavalin A, lipolysaccharides) or specific mitogen (tularaemic antigen Ag4)-induced spleen cell proliferation was evaluated through synthesis of nucleic acids with the help of the measurement of incorporated [^{3}H] thymidine (9). The vitality of *Francisella tularensis* bacteria was quantified by the measurement of the number of cultivated bacteria of *Francisella tularensis* LVS in spleen homogenates of infected mice with the help of colony forming units (CFU) by plating on McLeod culture agars and cultivation for 72 hours at 37 °C.

The experimental data were compared with the control values obtained from the infected mice exposed to pure air instead of sarin. The statistical significance was determined by Student`s t-test that was used to evaluate a statistical significance between experimental and control data. The differences were considered significant when $p < 0.05$.

RESULTS

The results of the study related to the evaluation of sarin-induced alteration of host resistance and immune reaction following low-level sarin inhalation exposure of mice are summarized in Tables 1 and 2. While the serum level of total antibodies did not show any changes, the level of some isotypes of antibodies (IgM, IgA) against tularaemia was significantly decreased ($p < 0.01$) regardless of the sarin concentration used at 24 hours after infection. On the other hand, serum level of both isotypes of antibodies against tularaemia was significantly increased ($p < 0.01$) in mice exposed to LEVEL 2 of sarin at 7 days after infection (Table 1).

Concanavalin A and tularaemic antigen Ag4-induced lymphoproliferation was significantly increased ($p < 0.01$) regardless of sarin concentration used at 24 hours after infection. On the other hand, it was significantly decreased ($p < 0.05$) regardless of the LEVEL of sarin three weeks after infection when specific tularaemic antigen Ag4 was used as a mitogen (Table 2).

Table 1. *The changes in the chosen immune parameters one day, seven days and twenty one days following sarin inhalation exposure*

Level of sarin exposure		Control	Level 1	Level 2
Serum IgM antibodies level measured by index	1 day	1.45 ± 0.184	**0.926 ± 0.157****	**1.064 ± 0.194****
	7 days	1.273 ± 0.122	1.349 ± 0.314	**1.619 ± 0.084****
	21 days	1.59 ± 0.204	1.78 ± 0.017	1.61 ± 0.063
Serum IgA antibodies level measured by index	1 day	1.188 ± 0.025	**0.42 ± 0.014*****	**0.607 ± 0.009****
	7 days	0.482 ± 0.01	0.378 ± 0.015	**1.54 ± 0.021*****
	21 days	0.471 ± 0.018	0.506 ± 0.015	0.357 ± 0.017
Number of colony forming units in spleen cells	1 day	5.0 ± 5.0	40.0 ± 76.4	38.6 ± 43.4
	7 days	6,580 ± 9,710	2,580 ± 3,420	**81,100 ± 66,500***
	21 days	3.33 ± 7.41	5.0 ± 7.64	2.0 ± 2.0

Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2. *The changes in the chosen immune parameters one day and twenty one days following the inhalation exposure*

Level of sarin exposure			Control	Level 1	Level 2
Spleen cell lymphoproliferation stimulated by mitogens measured by index	**Concanavalin A**	1 day	31.8 ± 23.4	**211.26 ± 130.8****	**261.99 ± 192****
		21 days	77.42 ± 54	101.6 ± 61	88.4 ± 60
	Lipopolysaccharides	1 day	7.5 ± 1.98	16.2 ± 6.55	11 ± 8.4
		21 days	15.11 ± 13	9.7 ± 5.2	12.3 ± 6.5
	Tularemic antigen Ag4	1 day	1.0 ± 0.22	**2.8 ± 0.66****	**2.7 ± 1.64****
		21 days	40.46 ± 38.0	**16.7 ± 10.9****	**20.6 ± 7.25***

Statistical significance: * $p < 0.05$; ** $p < 0.01$

The number of CFU in spleen homogenates shows that the viability of *Francisella tularensis* LVS was significantly increased ($p < 0.05$) in the case of exposure of mice to LEVEL 2 of sarin at 7 days after infection (Table 1). Some observed alterations of immune reaction markers of sarin-exposed mice on infection with *Francisella tularensis* LVS were found to be dose-dependent.

DISCUSSION

Generally, organophosphate-induced immunosuppression is associated with severe cholinergic stimulation. The immunosuppression may results from direct action of acetylcholine (ACh) upon immune system or it may be secondary to the toxic chemical stress associated with cholinergic poisoning (2). In addition, the OP-induced alteration of immune functions can be caused through the direct effects of accumulated ACh on lymphocytes because immune functions are, at least in part, under control of an independent non-neuronal lymphocytic cholinergic system (10).

Our results confirm that not only symptomatic but also asymptomatic dose of nerve agent sarin is able to modify the resistance and immune reaction of sarin-exposed mice after the infection with *Francisella tularensis* LVS. The observed data show the decrease in the resistance of sarin-exposed host (the decrease in the level of some isotypes of antibodies against tularaemia and lymphoproliferation stimulated with tularaemic antigen, the increase in the viability of *Francisella tularensis* LVS) compared to the control animals exposed to the pure air instead of sarin. Some immune reactions of sarin-exposed mice to *Francisella tularensis* LVS were found to be increased in comparison with control mice. The increase in some immune functions following the inhalation exposure to low-level sarin and infection with *Francisella tularensis* LVS appears to be the result of compensatory reactions of immune functions rather than the result of direct effects of inhalation exposure to low-level sarin. The explanation of complex effects of low-level sarin inhalation exposure on immune reaction of sarin-exposed mice on infection could be found in very complicated communication between immune and neuroendocrine systems (11).

In conclusion, nerve agents such as sarin seem to be able to alter some immune functions in exposed mice not only at non-convulsive symptomatic dose but also at low, asymptomatic dose (7). Although these findings are difficult to extrapolate directly to human low-level exposures to nerve agents, they indicate that subtle alteration of immune system could also occur in humans at exposures which do not cause any clinical manifestation.

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