BIOLOGIJA. 2006. Nr. 3. P. 92–95

Influence of long-term cadmium and selenite exposure on resistance to *Listeria monocytogenes* during acute and chronic infection in mice

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The aim of the present study was to evaluate the effect of long-term exposure to cadmium and selenite ions on the BALB/c mice resistance to experimental *Listeria monocytogenes* infection. A low-dose six-week exposure to cadmium ions (10 mg/l in drinking water) decreased the clearance of listeria from mice liver and spleen at 24 h in the early phase of infection. Long-term treatment of mouse with selenite ions (0.15 mg/l) did not activate the elimination of bacteria from the organs. Eight weeks of poisoning with high doses (100 mg/l) of cadmium during chronic infection affected the growth rate and survival of mice while selenite ions increased these parameters. Long-term Cd^{2+} and SeO_3^{2-} exposure increased ($p < 0.05$) the number of bacteria carriers in all the experimental groups of mice. Higher doses of Cd^{2+} increased listeria persistence in liver as compared to lower Cd^{2+} doses, but the difference was not significant. The titer of anti-listerial antibodies in blood serum in the group treated with SeO_3^{2-} was lower ($p < 0.05$) than in the control group, but higher than in the mice intoxicated with Cd^{2+}. In conclusion, mice intoxicated with high doses of cadmium were more susceptible to *L. monocytogenes* infection than non-intoxicated mice or intoxicated with a small concentration of cadmium ions. Selenite ions did not reduce the negative effect of cadmium on the resistance of mice to bacterial infection, although increased the production of antibodies to *L. monocytogenes*.

Key words: cadmium, selenite, experimental mice, infection, *Listeria monocytogenes*

INTRODUCTION

Cadmium is one of the most toxic heavy metals and has the toxic biological effects at concentrations smaller than almost any commonly found mineral. Its toxicity has been widely studied and reported [1, 2]. This metal is a serious environmental and occupational contaminant and may represent a serious health hazard to man and animals [3–5]. The basis of Cd toxicity is its negative influence on the enzymatic systems of cells, resulting from substitution of other metal ions (mainly Zn^{2+}, Cu^{2+} and Ca^{2+}) in metalloenzymes and its very strong affinity to biological structures containing –SH groups, such as proteins, enzymes and nucleic acids [6, 7]. Many effects of Cd action result from interactions with necessary micro- and macroelements, especially Ca, Zn, Cu, Fe and Se [8, 9].

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Selenium has many biological functions mediated through an array of selenoproteins. These proteins can influence a range of biochemical systems in the body including those involved in antioxidant mechanisms, thyroid hormone metabolism and redox control [10]. All these processes can impinge on elements of the immune system, and it is therefore not surprising that Se can influence both the humoral and cell mediated immune responses in animals and human [11]. A deficiency of Se impairs the body's immune system and ability to fight infections [12, 13].

It is known that a long-term exposure to heavy metals may cause changes in the immune response of the organism [14–17]. At present, the direct immunotoxicity of heavy metals is the subject of extensive studies, especially in *in vitro* models. Heavy metals may regulate the immune response of the body at its different stages, modifying early and late inflammatory reactions, among others through changing the number of circulating B and T lymphocytes, NK cells and immunological memory cells [18].

Heavy metal environmental pollutants increase the susceptibility of affected individuals to bacterial and viral infections, but the mechanisms responsible for this effect are not known [19, 20]. Despite the well-known facts about the diminishing effect of Se on Cd toxicity to the immune system, little is known how a combination of these two factors may affect the process of infection.

To understand this mechanism, we chose an experimental murine model of listerial infection [21]. The aim of this work was to study the effect of long-term exposure to cadmium and selenium on the resistance of mice to experimental *L. monocytogenes* infection.

MATERIALS AND METHODS

Experiments were done on BALB/c mice weighing 10–20 g. The experiments were performed according to requirements of the European Ethics Committee for Laboratory Animal Sciences with the permission of the Lithuanian LAS Ethical Committee No. 0028, 04.02.2001). Experimental infection was induced by a single injection of virulent 1860 1/2a serotype *L. monocytogenes* mutant [21] resistant to streptomycin. In the experiments we used 2.5×10^3 colony-forming units (CFU) per 1 g of body weight, which equals to 0.05 LD_{50}.

In the first experiment we formed three groups of mice and started the following oral treatment: control group (n = 20) – deionized drinking water, Cd group (n = 30) – water containing $CdCl_2$ 10 mg/l, and Cd+Se group (n = 30) – water containing $CdCl_2$ 10 mg/l and Na_2SeO_3 0.15 mg/l. After six weeks the mice were injected intravenously with *L. monocytogenes* suspension. The presence of the bacteria in the organs of mice was determined 15 min, 3 and 24 hours after infection. The spleen and liver were removed and homogenized with sterile sand. Next we sowed 0.1 ml of the diluted homogenate of the organs on agar medium (Columbia-Agar (Basis), Diagnostica Merck) with 0.25 mg/ml of streptomycin. The quantity of listerial CFU was calculated after incubation at 37 ˚C for 24 h.

Investigating the long-term infection and exposure to heavy metals in the second experiment, we evaluated the frequency of *L. monocytogenes* persistence in mouse organs. On the first day of experiment we infected the animals and started the same oral treatment, but here we had two Cd-groups: the Cd^{I} group (n = 30) got water containing $CdCl_2$ (10 mg/l) and the Cd^{II} group (n = 30) received water containing $CdCl_2$ (100 mg/l). All groups were sacrificed after eight weeks. About the influence of infection we judged by changes of weight, mortality of mice and results of bacteriological examination. The survival of *L. monocytogenes* in the liver of experimental animals was evaluated by the presence of bacteria CFU on the agar medium after 30 days of keeping the organs' homogenate in broth (CASO-broth, Merck) at 4 ˚C and 24 h incubation at 37 ˚C. Results obtained by this method were combined with the results of the procedure described by Gray [21].

The accumulation of antibodies against *L. monocytogenes* antigens in the blood serum of mice was estimated using a direct agglutination test. Blood serum samples were prepared by double-dilutions (1:20→1:320) and incubated at 37 ˚C for 24 h with *L. monocytogenes* culture inactivated with 1% of formaldehyde. The agglutination was evaluated with a spectrophotometer (Specord UV-VIS, Germany) at 410 nm.

The results were evaluated statistically and expressed as mean ± S.E.M. Differences were considered significant at the p value below 0.05.

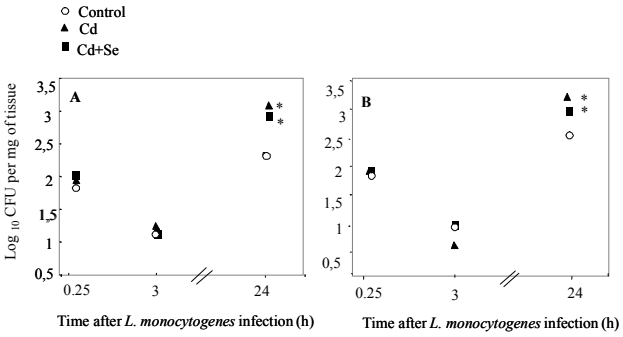

Fig. 1. Bacterial growth during the early phase of *L. monocytogenes* infection in liver (A) and spleen (B) in cadmium- and cadmium/selenite-exposed mice

* Significantly differ from control value ($p < 0.05$).

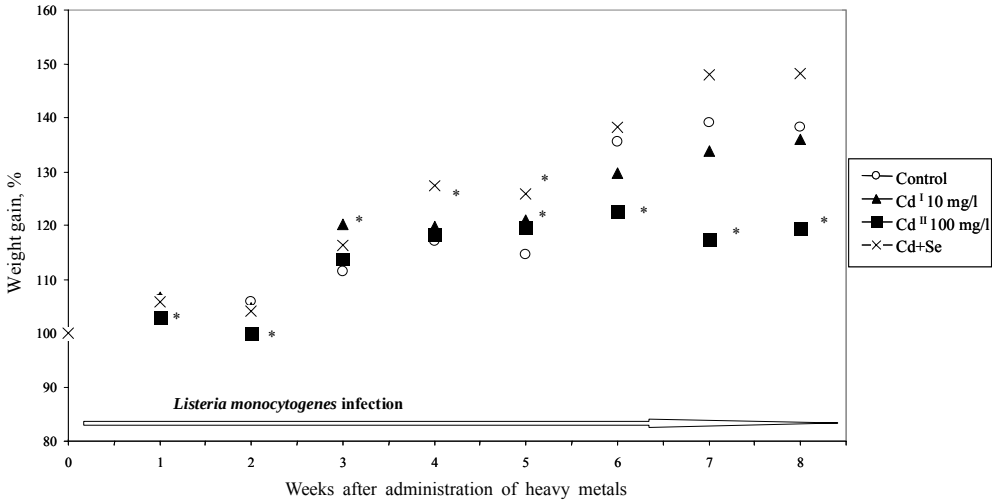

Fig. 2. Body weight gain in cadmium- and cadmium/selenite-treated and *L. monocytogenes* infected mice

* Significantly differ from control value ($p < 0.05$).

RESULTS

To evaluate the effect of Cd^{2+} on the state of non-specific immunity which reacts to the infection at early stages, we determined the number of infectious agents in the liver and spleen of experimental animals within 24 h of infection. After chronic $CdCl_2$ or $CdCl_2$ and Na_2SeO_3 treatment, already 3 h after *L. monocytogenes* injection, the first clearance of listeria in the liver of mice and spleen was the same (Fig. 1). After 24 h there was a significant increase in CFU in the liver and spleen of Cd- and Cd+Se-treated groups of mice as compared to control.

Infected mice during the 8-week period of exposure to metals presented some clinical symptoms: loss of weight, coordination and mortality. Evaluating a long-term exposure to $CdCl_2$, we used two concentrations of Cd^{2+} (Cd^{I} 10 mg/l ir Cd^{II} 100 mg/l). As is shown in Fig. 2, mice treated with a higher concentration of cadmium ions demonstrated a rather similar manner in body weight changes as compared to other groups of mice, however, from the sixth week this parameter decreased. The biggest increase of body weight was found in the Cd+Se group.

The results of mortality and bacteriological studies, which were obtained after a long-term $CdCl_2$ or $CdCl_2$ and Na_2SeO_3 exposure and *L. monocytogenes* infection, are shown in Table 1. The mortality of mice and the growth of bacteria in the control group were lower than in all experimental groups, though insignificantly. A higher Cd dose receiving group showed a higher mortality than the other groups of mice. We have found that selenite ions do not play a protective role in murine resistance to bacterial pathogen, because at the end of the experiment the organs of Cd+Se-treated mice were colonized by bacteria.

At the end of the experiment (after 8 weeks) we investigated the effect of a long-term exposure to $CdCl_2$ or $CdCl_2$ and Na_2SeO_3 on the colonization of mouse liver by *L. monocytogenes*. Data presented in Table 2 indicate that a long-term exposure increased ($p < 0.05$) the number of bacteria carriers in all the experimental groups. A higher dose of Cd^{2+} increased the listerial persistence in the liver as compared to the lower Cd^{2+} dose, but the difference was not significant. SeO_3^{2-} very slightly decreased this parameter as compared with the two Cd-receiving groups of mice.

After a long-term exposure to heavy metals there was a significant increase of spleen and liver weight indexes in the entire Cd groups and a decrease in the Cd+Se-groups as compared to controls (Table 2). We can see that SeO_3^{2-} decreased the weight index of mice organs.

Listeria belongs to the type of bacteria that are independent of antibodies; nevertheless, antibodies against intracellular *L. monocytogenes* are produced in infected animals. As is seen in Table 2, the humoral immunity in groups of mice treated with two different doses of Cd significantly decreased as compared to control group. The antibody titer in Cd+Se-group was lower ($p < 0.05$) than in control group, but higher than in Cd-treated mouse groups.

DISCUSSION

The present work demonstrates that mice chronically intoxicated with cadmium ions display an extensive impairment of resistance to an intracellular pathogen. This conclusion is based on high mortality rates observed in the second experiment in two Cd-treated mouse groups (Table 1). The results of the first experiment show that Cd^{2+} decrease the non-specific resistance of mice too,

Table 1. **Effect of cadmium and selenite ions on mouse mortality and bacteriological examination of liver and spleen**

Mouse groups	Weeks								Mouse mortality, %
	1	2	3	4	5	6	7	8	
Control (n = 20)	-	-	-	1*/1**	-	1/0	2/0	-	20
Cd ^{I} (n = 30)	1/1	-	2/2	1/1	-	-	2/1	2/2	26.6
Cd ^{II} (n = 30)	-	2/2	3/3	-	-	1/1	1/1	3/3	33.3
Cd+Se (n = 30)	-	2/2	-	2/2	-	-	1/1	2/2	23.3

*/** The number of dead mice / the number of bacteria carriers in dead mice.

Table 2. **Effect of long-term cadmium and selenite exposure on mouse liver colonization by *L. monocytogenes*, organ weight index and accumulation of antibodies against listeria in blood serum of mice**

Parameter	Control	Cd^{I} (10 mg/l)	Cd^{II} (100 mg/l)	Cd+Se
Percentage of mice carrying bacteria in liver	36.8	82.1*	89.6*	77.7*
Liver index of mice (mg liver weight/g body weight): mean ± SD	54.00 ± 1.1	62.9 ± 1.6*	65.8 ± 0.86*	53.9 ± 1
Spleen index of mice (mg spleen weight/g body weight): mean ± SD	6.6 ± 0.6	9.16 ± 0.85*	9.4 ± 0.86*	6.42 ± 1
Maximal dilution of serum which agglutinated the bacterial antigen	237 ± 49	97 ± 38*	69 ± 16*	207 ± 30

*Significantly differ from control value ($p < 0.05$).

because at 24 h after infection there was an intensive bacterial colonization of mouse spleen and liver (Fig. 1).

Cadmium accumulation in the liver and kidney was associated with degeneration and inflammatory changes in these organs (unpublished observations). This might explain a significant increase of spleen and liver weight indexes in all Cd-treated groups of mice (Table 2). We conclude that cadmium causes a significant suppression of cell-mediated immunity in mice, which could be explained by its cytotoxic action on liver, kidney and immune cells.

It is known that selenium also supports immune functions and neutralizes certain poisonous substances such as cadmium, mercury and other heavy metals [11, 12]. Our bacteriological studies show that the carriers' stage was less in the liver of the Se-treated than in both Cd-treated groups of mice, though insignificantly (Table 2). Comparing it with our previous results [22], we see that selenium does not play a protective role in cadmium neutralization like zinc and does not increase the resistance to bacterial intracellular persistence. Maybe this is related to the property of selenium to antagonize much stronger with Hg but not with Cd [9]. We have noted above that listeria belong to the type of bacteria that are independent of antibodies; nevertheless, we see that the antibody titer in Cd+Se-group was higher in Cd-treated mice group and reached the normal level (Table 2). Our data show that selenium exerts a positive effect on the humoral system of mice and the production of anti-listerial antibodies.

Studies on the immunotoxic effects of metals such as cadmium, lead, zinc and selenium are often conducted at higher concentrations than those found in the environment or in the human blood. Moreover, most of these studies examine the effects of one metal at the time on human or animal cells. However, humans are often exposed to mixtures of toxic metals rather than to one single metal. Therefore, the potential effects of mixed exposures to these contaminants require evaluation. Understanding the effects of mercury, cadmium, lead, zinc and selenium on human immune functions could be helpful in determining the risk associated with human exposure to these metals at physiologically relevant concentrations. This would help in designing the strategies of preventing chronic toxicity to heavy metals.

Received 14 February 2006
Accepted 3 June 2006

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LĖTINIS KADMIO IR SELENITO JONŲ POVEIKIS PELIŲ ATSPARUMUI *LISTERIA MONOCYTOGENES* ESANT ŪMIAI IR LĖTINEI INFEKCIJAI

S a n t r a u k a

Šio tyrimo tikslas – nustatyti, kaip kadmio ir selenito jonai paveikė BALB/c pelių atsparumą eksperimento metu sukeltai *Listeria monocytogenes* infekcijai. Girdymas kadmio jonais (10 mg/l) 6 savaites slopino listerijų eliminavimą iš pelių kepenų ir blužnies po infekavimo praėjus 24 val., o girdant kartu ir selenito jonus (0,15 mg/l) bakterijų pašalinimas iš šių organų nepagerėjo. Po ilgalaikio girdymo kadmio jonais (100 mg/l), esant lėtinei *L. monocytogenes* infekcijai, sumažėjo pelių kūno masės prieaugis ir padažnėjo žūties atvejai. Pridėjus į kadmio tirpalą selenito, šie parametrai normalizavosi. Ilgalaikis girdymas kadmio, taip pat kadmio ir selenito jonais didino bakterijų nešiotojų skaičių visose eksperimentinėse pelių grupėse ($p < 0,05$). Didesnė kadmio jonų dozė nedaug padidino listerijų persistavimą kepenyse, lyginant su mažesne doze. Nors specifinių antikūnų prieš listerijas titras selenitu paveiktoje pelių grupėje buvo didesnis nei vien kadmiu paveiktų pelių kraujo serume ($p < 0,05$), jis nesiekė kontrolinės grupės pelių antikūnų titro. Gauti rezultatai rodo, kad didesne kadmio doze paveiktos pelės yra jautresnės *L. monocytogenes* infekcijai, nei nuodytos maža doze. Selenito jonai nepašalina neigiamo kadmio poveikio pelių atsparumui *L. monocytogenes* infekcijai, nors ir padidina specifinių antikūnų sintezę.