Spectrofotometrical Determination of Lipid Peroxidation in Brain of Cadmium, Nickel and Zinc Treated Mice

D. Juciūtė*, R. Bernotienė

Neuroscience Institute and Department of Biochemistry, Medical Academy, Lithuanian University of Health Sciences, Lithuania

*E-mail: juciute.d@gmail.com

Introduction. After industrial revolution the use and contamination of heavy metals rapidly increased. One of the main reasons of contamination is the low re-cycle rate [1].

Heavy metals – cadmium (Cd) and nickel (Ni) are acknowledged as human carcinogens [1, 2]. Due to prolonged biological half-time in humans body which is 10-30 years, Cd induce oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues [3]. Also this metal generate reactive oxygen species (ROS) which causes lipid peroxidation, DNA and proteins damage [4].

Ni is a known haematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic and carcinogenic agent [2]. Ni as well as Cd induces ROS production and provoke lipid peroxidation. The end products of lipid peroxidation can cause protein damage in all organisms due to their distribution. One of end products – malondialdehyde (MDA) is used as biomarker for lipid peroxidation [5].

Zinc (Zn) is a fundamental element for sub-cellular metabolism and is an essential component of catalytic sites of at least one enzyme in every enzyme classification [6]. It is an essential microelement for organism and it can protect against oxidative damage. Zn deficiency increases the level of cellular ROS, also has been linked to Alzheimer’s and Parkinson’s disease in humans [7].

However, there is little information about intracellular mechanisms implicated in Zn-dependent protection of an organism against deleterious effects of Cd or Ni. The present study was conducted to evaluate the effects of Cd, Ni and Zn ions on lipid peroxidation in mice brain and a possible Zn antioxidant effect against Cd and Ni toxicity choosing 14 days intraperitoneal (i.p.) injections.

Materials and methods. Experiments were done on 4-6-week-old outbreed white laboratory mice weighing 20-25 g. All experiments performed according to the Republic of Lithuania Law on the Care, Keeping and Use of Animals (License of State Veterinary Service for Working with Laboratory Animals No 0136).

We have chosen the model of sub-acute prolonged 14 days intoxication with the metal salts as indicated in the Table 1. Mice were randomly assigned into six groups: five experimental and one control group. The number of mice in each group was from 8 to 15.
Table 1. Experimental groups and the concentrations of used solutions

<table>
<thead>
<tr>
<th>Group No</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>A single daily injection of CdCl$_2$ (14 μmol Cd/kg body weight)</td>
</tr>
<tr>
<td>2</td>
<td>A single daily injection of NiCl$_2$ (19 μmol Ni/kg body weight)</td>
</tr>
<tr>
<td>3</td>
<td>A single daily injection of ZnSO$_4$ (24 μmol Zn/kg body weight)</td>
</tr>
<tr>
<td>4$^a$</td>
<td>A single daily injection of ZnSO$_4$ and CdCl$_2$ in aforementioned doses</td>
</tr>
<tr>
<td>5$^a$</td>
<td>A single daily injection of ZnSO$_4$ and NiCl$_2$ in aforementioned doses</td>
</tr>
</tbody>
</table>

$^a$ – For 14 days, mice of 4$^{th}$ and 5$^{th}$ experimental groups were daily pre-treated by i.p. injections of ZnSO$_4$ and after 20 min they received i.p. injections of CdCl$_2$ or NiCl$_2$ solutions, respectively. Mice were terminated after 24 h following the last CdCl$_2$ or NiCl$_2$ i.p. injections.

Control group animals received daily i.p. injections of the same volume of physiological solution for 14 days.

**Determination of malondialdehyde in mice brain**

Lipid peroxides were estimated by measuring thiobarbituric-acid-reactive substances [8]. The brain was removed and homogenized with 9 volumes (weight:volume) of cold 1.15% KCl to make 10% homogenate. 3 ml of 1% H$_3$PO$_4$ and 1 ml of 0.6% thiobarbituric acid aqueous solutions were added to 0.5 ml of this homogenate. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation (8000 x g for 10 minutes) and supernatant absorbance was determined at 535 and 520 nm using Perkin Elmer UV/VIS spectrometer Lambda 25 model. The content of MDA was calculated from the calibration curve (1.1.3.3-tetrahydroxypropane was used as a standard) and expressed as nmol/g of wet brain weight.

**Statistical analysis**

The data are expressed as the mean ± SEM. Differences among means were analyzed by one-way ANOVA. $p<0.05$ value was considered statistically significant (SPSS version 19.0, SPSS).

**Results.** Lipid peroxidation is a naturally occurring process in cell, but under stressful conditions it rapidly increases. This process includes oxidation of cell membrane polyunsaturated fatty acids, resulting formation of various final products, including epoxy-fatty acids, aldehydes (e.g. MDA), alkenes and alkanes. As mentioned, MDA is one of the most frequently used indicators of lipid peroxidation [5].

The effects of Cd, Ni and Zn ions on the content of MDA in mice brain after 14 days of i.p. injections of CdCl$_2$, NiCl$_2$ or/and ZnSO$_4$ solutions are shown in Fig. 1.
Fig. 1. The content of MDA in mice brain after 14 days of exposure to Cd, Ni and/or Zn ions and control mice group.

The number of mice in each group was from 8 to 15.* = p < 0.05 as compared to the control mice; # = p < 0.05 as compared to the group of Cd, Ni - treated mice.

In our experiments we determined that after 14 days of CdCl$_2$ or ZnSO$_4$ injections, the content of MDA in mice brain was significantly increased by 16% and 73%, respectively, as compared to control mice group. Meanwhile, in Ni treated mice group MDA content was decreased by 19% as compared to control mice group.

Also, our results indicated that in Zn+Cd treated mice group the content of MDA in mice brain was decreased by 27%, but in Zn+Ni treated group this parameter increased by 38% as compared to control mice group.

Our results did not confirm our hypothesis that 14 days of exposure to Ni, can cause oxidative stress in mice brain and Zn can protect against this toxicity. In our experiments we determined, that Zn ions increased lipid peroxidation and Ni ions decreased this process in mice brains. Also we did not found the proof that Zn has antioxidant effect in mice brain after 14 days of exposure.

**References**


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The present investigation was undertaken to evaluate the influence of Cd, Ni and Zn ions on the content of MDA (marker of lipid peroxidation) in mice brain. Experiments were done on outbred white laboratory mice using intraperitoneal injections of CdCl₂, NiCl₂ and/or ZnSO₄ solutions. The exposure time was 14 days. Our results showed that after 14 days the content of MDA was increased by 16% after exposure to Cd, by 73% after exposure to Zn and by 38% in Zn+Ni treated mice group. Meanwhile, in Zn+Cd and Ni treated mice groups, MDA content in mice brain was decreased by 27% and 19%, respectively, as compared to control mice group.