Toxic Effects of Low Level Lead on the Blood-Brain Barrier in Rats

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Abstract: Toxic Effects of Low Level Lead on the Blood-Brain Barrier in Rats: Su-yun Ruan, et al. Shanghai Institute of Labour Hygiene and Occupational Diseases—This study was designed to investigate damage to the permeability and ultrastructure of the blood-brain barrier caused by low level lead by using Lanthanum nitrate tracing and to explore the blood and the brain lead threshold level which induced such damage. The results showed that there was no obvious damage to the blood-brain barrier in the 10 mg/L lead exposure group when the blood lead level reached 1.67 times as high as that in the control group, and the brain lead level was not much higher than that in the control group. But in the 30 mg/L lead exposure group, when the blood and brain lead levels reached 2.46 and 1.34 times that in the control group, respectively, lanthanum granules seeped into the base membrane and pericytes through the space between neighbouring endotheliocytes. The results suggested that the damage in close junctions between endotheliocytes was an early and easily observable brain marker of exposure to low level lead. In animal experimentals the lanthanum nitrate tracing technique with cardiac flush fixation is a sensitive and effective method for revealing changes in the permeability of the blood-brain barrier. It can be used in experiments on lead or other toxic environmental pollutants affecting the blood-brain barrier, and also in observing the effects of new preventive and therapeutic medicaments.

Materials and Methods

1. Animal model

The 36 rats just after weaning used in this study were Spraque-Dawley (18 rats of each sex). Their average body weight was 46.5 ± 4.38 g. They were equally divided into three groups: control group, low lead dose group and high lead dose group. The rats in the control group drank non-ion water, and the rats in the lead dose groups were given Pb at 10 and 30 mg lead/L in their drinking water for 3 months (corresponding to lead acetate at 18 and 54 mg/L). At the end of the treatment, from each group 6 rats were killed to take blood and brain for lead level analysis, and 6 rats for La-tracing.

2. Blood and brain lead level analysis

The animals were anaesthetized and the abdominal aorta was cut off to take the blood and then the skull was opened to take the brain and weighed. The blood was pretreated with diluted nitrate and the brain pretreated with perchloric acid, and then both the blood and brain samples were transferred into a graphite furnace to analyze the lead by atomic absorption spectrometry.

3. La-tracing by cardiac flush fixation

The animals were anaesthetized and the chest was cut off to intubate and flush from the left ventricle to the artery, first with 250 µ/ml heparin for 30 sec, then with the fixative of one part 4% lanthanum nitrate and two parts 6% glutaraldehyde-0.1 M sodium cacodylate (pH 7.40–7.50) for 2 h, then washed with water containing 1% lanthanum nitrate-0.1 M sodium cacodylate for 15 min, and then again with a fixative of 1% osmium oxide-
1% lanthanum nitrate for 2 h and in the washing water for 15 min. After the rats' skulls were opened to remove brain tissues from parietal lobus of the brain, the tissues were dehydrated and embedded according to the routine method, stained with uranium acetate and lead nitrate, and examined under an H-800 model electronmicroscope.

Result

1. Blood and brain lead level

The average blood lead level in the low and high dose groups was 1.65 times and 2.45 times, respectively, as high as in the control group. The average brain lead level in the low dose group was not significantly different from that in the control group, but in the high dose group it was 1.34 times as high as in the control group and this difference was significant (Table 1).

<table>
<thead>
<tr>
<th>Pb (mg/L)</th>
<th>n</th>
<th>Blood Lead (µg/L)</th>
<th>Brain Lead (µg/G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>38.57 ± 1.03</td>
<td>0.099 ± 0.012</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>64.47 ± 4.96*</td>
<td>0.100 ± 0.013</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>94.67 ± 12.87*</td>
<td>0.133 ± 0.016*</td>
</tr>
</tbody>
</table>

*p<0.01, compared with control group at a dose of 0 mg/L.

Table 1. Lead levels in blood and whole brain of rats fed Pb in drinking water for 3 months (mean SD)

2. Observation of permeability of the blood-brain barrier by La-tracing of cardiac flush fixation

In the control group: The capillaries had three typical structures, i.e., endotheliocytes, base membrane and peripheral cells. The endotheliocytes were closely junceted each other to form consecutive layer. Some capillaries with relatively small cavities were covered by rather thick endotheliocyte plasma (Fig. 1). Some capillaries with relatively large cavities were covered by rather thin endotheliocyte plasma (Fig. 2) and some other capillaries with large cavities were covered by a little endotheliocyte plasma (Fig. 3), but the lanthanum granules were all located at the cavities in all capillaries and closely aligned along the plasmalemma of the endotheliocyte endosurface. In the base membrane no lanthanum granules were found and the neuromechanisms on the periphery of capillaries were clear and close (Fig. 1, 2 and 3).

The low dose group gave results similar to those of the control group, no lanthanum granules seeped to the base membrane in any of the capillaries. In the high dose group the capillaries of small cavities covered by relatively thick endotheliocyte plasma were similar to those of the controls. In the capillaries with large cavities covered by relatively thin endotheliocyte plasma, the lanthanum granules were found to seep to base membrane and pericytes through the space between endotheliocytes (Figs. 4 and 5). The capillaries with large cavities covered by a little endotheliocyte plasma gave the results similar to those covered by relatively thin...
endotheliocyte plasma but the parts in the former into which lanthanum granules seeped were more numerous than those in the latter, and the glial base membrane was swollen (Fig. 6).

**Discussion**

The La-tracing is a relatively new technique used to research cell junction and permeability of plasma membrane⁴. In the present study adding a little lanthanum nitrate to the fixative not only can avoid having the tracing substance itself damage the blood-brain barrier⁴ ⁵, but also provide an opportunity to directly observe the fine structure and permeability of the blood-brain barrier with the electronmicroscope. In this study, in the control and low dose groups, the lanthanum granules were not found to seep into the base membrane in any of the capillaries, but, in the high dose group, it was found the lanthanum granules seeped into the base membrane through the space between the neighbouring endotheliocytes of the capillaries with large cavities. This is probably because the close junctions between neighbour endotheliocytes were damaged. In the capillaries with large cavities the junctions between neighbouring endotheliocytes were more numerous than those in the capillaries with relatively small cavities because in the former the quantity of endotheliocytes covering the cavity was greater than in
the latter, so the chance that the damaged junction could be seen, is greater in the former.

Furthermore, in this study when the blood lead level was 1.65 times that in the control group the permeability of the blood-brain barrier was not changed, but when the blood level was 2.45 times that in the control group and the brain lead level significantly increased compared with the control group the lanthanum granules were found to seep into the base membrane and the peripheral cells through the space between endotheliocytes. The results indicated that the seepage of lanthanum granules caused by damage to close junctions between endotheliocytes was a relatively early and easily observable marker of brain damage caused by low level lead exposure. In animal experiments the La-tracing technique with cardiac flush fixation is a sensitive and effective method for revealing changes in the permeability of the blood-brain barrier. The technique can be used to research the toxic effects of lead and other environmental pollutants on the blood-brain barrier and to observe the effects of preventive and therapeutic medicaments.

References