ORIGINAL ARTICLES

An Experimental Study on Nerve Conduction Velocities and Biochemical Parameters in Lead-Administered Rats

Yingfa PANG*, Koichi HARADA, Takashi MIYAKITA, Junichi MISUMI, and Hajime MIURA

Department of Hygiene, Kumamoto University Medical School, Honjo-2-2-1, Kumamoto 860 Japan

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Abstract: An experimental study was carried out to clarify the relationships between lead exposure and the changes in nerve conduction velocities or in porphyrin metabolism in rats. The rats in Groups A, B and C received intraperitoneal lead injections in respective doses of 0, 0.2 and 2.0 mg/kg of lead, once a week for 22 weeks. During the experiment, all rats grew normally, and there was no significant difference in the group mean body weights. Lead concentrations in the blood and organs increased in these groups as the lead dose increased. Significant changes in nerve function and in biochemical indices relating to porphyrin metabolism were observed only in Group C treated with 2.0 mg/kg of lead, and not in the other groups. In Group C, urinary coproporphyrin and free erythrocyte protoporphyrin increased from an early stage, 4-6th week, of the lead administration, and urinary δ-aminolevulinic acid increased from the 8th week. Slight but significant slowing of conduction velocity in motor nerves appeared from the 10th week, when the rats had received lead injection 9 times in total. The change in distal motor latency occurred from the 16th week. No functional change in sensory nerves was observed in this study.

Key words: Lead—Nerve conduction velocity—Porphyrin—FEP—ALA-U—CP-U

INTRODUCTION

Interest has recently intensified in the slowing of the conduction velocities in peripheral nerves as an indicator of lead poisoning. However, the minimum Pb-B level at which a measurable impairment of peripheral nerve function will occur has not been settled. Furthermore, the relationship in lead poisoning

Reprint requests to: Dr. H. Miura at the above address.

* Present address: Dr. Y. Pang, Institute of Health, China National Center for Preventive Medicine, Nan Wei Road 29, Beijing 100050, China
between the changes in nerve conduction velocity and porphyrin metabolism also remains unsolved.

Extensive studies\textsuperscript{3,9} have been made on the clinical changes in neuropathy due to lead, especially under excessive exposure. Several experimental studies\textsuperscript{10,12} on peripheral neuropathy were reported in animals exposed to lead, and some results\textsuperscript{10,11} demonstrated that lead exposure induces impairment of peripheral nerve function in the experimental animals.

In the present study, maximum motor conduction velocity (MCV), maximum sensory conduction velocity (SCV) and parameters relation to porphyrin metabolism were measured in rats to clarify the effect of the degree of lead administration on electrophysiological responses and changes in porphyrin metabolism, under low levels of lead exposure.

**Materials and Methods**

Forty-nine male rats (Donryu strain) aged 8 weeks, weighing 240 to 260 g, were used for the experiment. After being prefed for 2 weeks, the rats were divided into 3 groups, the A group (16 rats) received injections of 5%-glucose solution intraperitoneally as a control, the B and C groups (16 and 17 rats) were treated respectively with 0.037% or 0.37% lead acetate in 5%-glucose solution. The treatment schedule, starting after the first week, consisted of weekly intraperitoneal injections of lead at a dose of 0 (A), 0.2 (B) and 2.0 (C) mg/kg for 22 weeks. The lead injections were performed on every Wednesday after checking the body weight and clinical symptoms including neurological signs. Each group was further divided into 2 subgroups. Once every 2 or 3 weeks, one subgroup served for the electrophysiological measurements, and the other was used to determine the biochemical parameters. The measurement of conduction velocity in nerves and the collection of urine and blood samples were performed on Wednesdays before the lead injection. On the first day of the 16th and 22nd week in the experiment, 5 or 6 and 10 or 11 rats in each group were killed, respectively, without the lead injection for that week. Blood and organ samples were stored at \(-20^\circ\text{C}\) until the time of lead analysis.

All rats were fed diet pellets (CE-2, CLEA JAPAN Co.) using a paired feeding method. Water was given \textit{ad libitum}. The feeding room temperature was controlled at about \(24^\circ\text{C}\) during the experimental period.

**Biochemical measurements**

The specific gravity of urine was measured with a refractometer (Uricon-S, Atago Co.). Then the hematocrit (Ht) was measured immediately by the capillary method. All biomaterials were stored in a freezer at \(-20^\circ\text{C}\) until analyzed.

Coproporphyrin in urine (CP-U) was determined by the method of Sano & Granick.\textsuperscript{13} The details of this method were described by Ohmori \textit{et al.}\textsuperscript{14}
Free erythrocyte protoporphyrin (FEP), which is defined as all of the protoporphyrin extractable from whole blood with acidic solvent, was determined by Harada’s microfluorometric method within the sampling day.

Urinary δ-Aminolevulinic acid and porphobilinogen (ALA-U, PBG-U) were measured by the Mauzerall and Granick method using ion-exchange resins. Lead in the blood and organs were determined as follows: Samples were digested by wet ashing. The diluted samples were injected into a Zeeman-effect atomic absorption spectrophotometer (Model. 170-70s Hitachi Co.), and lead levels were determined by the calibration method.

**Electrophysiological examinations**

Maximum motor and sensory nerve conduction velocity (MCV and SCV) and distal motor latency (D.L.) were measured in the tail nerves in the rats.

The electrodiagnostic technique for detection of peripheral nerve dysfunction has been described in detail in the reports of Misumi. The proximal, middle and distal sections along the rat’s tail were named respectively A, B and C. The MCV was tested in section A-B, and the SCV in sections A-B (proximal part), B-C (distal part), and A-C (whole length). The nerves were supramaximally stimulated with a single pulse of 0.3 msec duration at a frequency of 1 Hz, delivered by an electric stimulator (MNS-1101, Nihon Kohden Co.). Action potentials were amplified with a time constant of 0.01 sec, and displayed on the screen of an addscope (ATAC-250, Nihon Kohden Co.) and recorded on graph paper with an X-Y recorder (3086-22, Yokogawa Denki Co.). The skin temperature

![Graph showing changes in body weight during the experiment.](image)

**Fig. 1. Changes in body weight during the experiment.**

Note. The number of animals before and after the 16th week: Group A, n=16 & 10; Group B, n=16 & 10; Group C, n=17 & 11.
of the tail was checked before the measurement. It ranged from 33.0 to 36.0°C (A, B and C points). The room temperature was controlled at 28 to 30°C.

Student's t-test was used for the statistical analyses. The Welch's correction

Fig. 2. Changes in CP-U and FEP levels during the experiment.

Note. Mark and bar: Mean ± SD.
The number of animals before and after the 16th week: Group A, n=8 & 5; Group B: n=8 & 5; Group C, n=9 & 6.
Significance levels: * p<0.05, ** p<0.01.
method was used when a difference between variances was significant.

**RESULTS**

There were no significant differences in the body weight between the exposed and control groups, although a slight inhibition of weight gain in the C group occurred in the latter stages of exposure (Fig. 1). No clinical symptoms or neurological signs were observed in any rats during the whole period of the experiment.

**Hematological and biochemical measurements**

The means±SD of the hematocrit (Ht) in the A and C groups were 51.1±2.3% (n=8), 47.4±2.6% (n=9), respectively, at the 6th week of the experiment, and the difference between those means was significant (p<0.01). Although the significant differences (p<0.01 or 0.05) between the groups continued until the end of the experiment, the values remained within the normal range. The mean Ht in the B group decreased temporarily at the 10th and 13th week, but recovered in the following weeks.

In the C group treated with 2.0 mg/kg of lead, the mean CP-U and FEP increased significantly from the 4th to the 6th week after the treatment started, compared with the control group, and these increases continued until the end of the experiment (Fig. 2). However, the CP-U and FEP in the B group did not increase during the whole period of the experiment, but maintained almost the same levels as those in the control group. Ranges of weekly average of CP-U and FEP in the control group were 50–103 μg/l, 55–107 μg/dl.pcv, respectively.

The mean ALA-U at the 8th week in the control and C groups were 0.56±0.17 mg/l (n=8) and 2.57±2.02 mg/l (n=9), respectively. The latter is significantly

<table>
<thead>
<tr>
<th>Table 1. Lead concentrations in blood and tissues</th>
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<td><strong>Group</strong></td>
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*: p<0.05 vs. the control, **: p<0.01 vs. the control.

s) The data of the 16th and 22nd were pooled, because there was no significant difference between the weeks in any item.
higher (p<0.05, with Welch's correction) than the former. This significant increase continued until the end of the experiment. The mean ALA-U in the B group was not significantly different from the control group, except at the 2nd week. ALA-U levels in the control group ranged from 0.60 to 1.20 mg/l. There were no significant differences in PBG-U between the poisoned (B, C) and control groups during the whole experimental period.

The mean Pb-B in the control group remained at a level of 5.0±4.5 µg/dl. As the lead doses increased, the lead levels in the blood and organs increased in the B and C groups, and they were significantly higher than those in the control group (Table 1). The mean Pb-B levels in the C group at the 16th and 22nd week were 18.8 and 31.6 µg/dl, respectively, while those in the B group remained at 10±11 µg/dl. The decreasing order of lead concentrations in organs in both

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**Fig. 3. Changes in conduction velocity of sensory nerve fibers (SCV) and motor nerve fibers (MCV) in the rat tail.**

Note. Mark and bar: Mean±SD.

The number of rats in the each group: n=8 before the 16th week and n=5 thereafter.

Significance levels: * p<0.05, ** p<0.01.
of the poisoned groups was bone > kidney > spleen > liver.

**Electrophysiological examinations**

In the C group, there was no significant reduction of the SCV in the whole length of the tail, except for a slowing tendency at the 19th and 22nd week (Fig. 3). Also there was no significant difference in the SCV between the control and B groups, except for a temporary slowing in the proximal part of tail at the 16th week. In the distal part of the tail, there was no significant difference in the SCV among the three groups during the whole experimental period.

The mean MCV measured after the 10th week decreased significantly in the C group compared with the control group (Fig. 3). In the B group there was no significant difference from the control throughout the experiment.

In all three groups, the D.L. tended to become gradually reduced as the experiment progressed. The mean D.L. in the control group was 2.83±0.44 msec (n=8) at the beginning and 1.57±0.07 msec (n=5) at the end of the experiment. At the 16th week, the mean D.L. was 1.67±0.08 msec (n=8) in the A group and 1.84±0.07 msec (n=8) in the C group. There was a significant difference (p<0.01) in the D.L. between the A and C groups from the 16th week to the end of the experiment. In the B group, however, there was no significant difference from the control, except at the 16th week.

**DISCUSSION**

The lead levels in the blood and organs were elevated with increasing lead doses (Table 1). The order of lead levels in organs of the poisoned groups showed a typical distribution of lead in the body, and was in good agreement with the results of the study reported by Mouw et al.\(^\text{18}\)

None of the rats treated with lead for 22 weeks had any apparent clinical symptoms or signs of lead poisoning, except for a slight inhibition of weight gain.

<table>
<thead>
<tr>
<th>Stage of dosing period (week)</th>
<th>4th</th>
<th>6th</th>
<th>8th</th>
<th>10th</th>
<th>13th</th>
<th>16th</th>
<th>19th</th>
<th>22nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dose (mg/kg)</td>
<td>6.0</td>
<td>10.0</td>
<td>14.0</td>
<td>18.0</td>
<td>24.0</td>
<td>30.0</td>
<td>36.0</td>
<td>42.0</td>
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<tr>
<td>Pb-B (µg/dl)</td>
<td>18.8</td>
<td>31.6</td>
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<td>CP-U</td>
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<td>ALA-U</td>
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<td>D.L.</td>
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+: p<0.05 vs. the control; #: p<0.01 vs. the control.
in the C group during the latter stage of the experiment. In the C group, there were changes in several electrophysiological and biochemical parameters in the peripheral nerves and heme biosynthesis after the lead administration. The times of onset of significant changes in the parameters are summarized in Table 2. In the B group treated with the smaller dose of lead, only temporary changes occurred in some parameters, as in SCV, D.L., ALA-U and Ht, and they recovered in the subsequent week. Subclinical dysfunction in peripheral nerves accompanying metabolic changes in the porphyrin biosynthesis was clearly detected in rats treated with 2.0 mg/kg of lead.

The electrophysiological findings suggesting subclinical peripheral neuropathy in this experiment were in good agreement with the results reported by Jo et al. and Fullerton using rats and guinea pigs. In contrast to those findings, Hopkins failed to produce any apparent lesions in the peripheral nerves in 15 baboons, although a very high level of Pb-B (100 µg/dl or more) was maintained for one year. These facts suggest a difference in susceptibility to lead among species.

It is important to estimate the critical Pb-B level at which detectable functional impairment will occur in the peripheral nerve. All of the available information on this has been obtained from workers who have been chronically exposed to lead. Araki and Homma reported that a significant reduction in MCV of the median nerve was observed in the workers having 29–77 µg/dl of Pb-B. And they suggest that a no-response level may be in the range of 31 to 50 µg/dl of Pb-B. Seppäläinen et al. also attempted to define the no-effect level of Pb-B for impairment of nervous function. They used data from 78 lead workers and 34 non-exposed reference workers. The conduction velocity of slower motor fibers (CVSF) of the ulnar nerve was the most sensitive indicator. The CVSF in the group with maximal Pb-B levels between 40 and 49 µg/dl differed significantly from that of the referents. So the authors suggest that nerve conduction impairment is induced in some subjects at Pb-B levels of 40 to 50 µg/dl. However, because of the rather small sample, a definite conclusion cannot yet be drawn.

The highest mean Pb-B observed in the present experiment was 31.6 µg/dl in the C group at the final week. This level seems to be rather low. However, the Pb-B levels obtained in this study indicated the lowest level during the week, because blood samples for lead analysis were collected just prior to the subsequent lead administration at the beginning of the 16th and 22nd week. With the intravenous lead injections the Pb-B level became elevated soon after the injection, and it decreased rapidly within 2–4 hrs to maintain a fairly stable level. Although we did not check an actual variation of the Pb-B level after the intraperitoneal lead administration, variations in Pb-B levels may be smaller and slower with such injections than with intravenous injection. If so, slight dysfunction in peripheral nerve will be induced at a lower Pb-B level in rats than that estimated in humans.
As shown in Fig. 3, the mean MCVs in the C and control groups were about 36 m/sec and 42 m/sec, respectively, after the 10th week of the experiment. On the other hand, as described in the results, no significant reduction of SCV occurred in either the B or C groups during the whole experimental period. This indicates that lead mainly attacks the motor nerve, as described in the classical clinical observations.

The lack of correlation between the changes of nerve function and biochemical indices in lead workers has been reported by many investigators. For instance, Ashby found no evidence of a relationship between neurophysiological and either FEP levels or Hb concentrations. On the other hand, there are some reports in which a relationship between biochemical and neurophysiological changes has been observed after exposure to lead. Sessa et al. observed that a decrease in ulnar MCV was paralleled by an increase in FEP. Catton et al. noted an association between nerve dysfunction and anaemia. Under the conditions of exposure in our present study, as shown in Table 2, a decrease in motor conduction velocity occurred from the 10th week, after metabolic changes in porphyrin had already developed. From this the question arises whether accumulated protoporphyrin interferes directly with neurological function in vivo. An accumulation of protoporphyrin in the nervous system as a result of lead intoxication has been demonstrated by Whetsell et al., who used cultured neural tissue. The accumulation of excess protoporphyrin was most prominent in the glial cells, which were damaged most in childhood lead neuropathy. However, the direct effect of protoporphyrin on peripheral nerve still remains unknown.

In conclusion, the lead levels of blood and organs increased with an increase of the lead dose. The decreasing order of lead levels in organs in poisoned rats was bone > kidney > spleen > liver. Furthermore, the present study indicates that the disorders in porphyrin metabolism appear at first in the early stage of the lead exposure, and then, significant but slight changes in nerve function occur several weeks later as the lead exposure progresses.

**REFERENCES**

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