Urinary coproporphyrin in lead intoxication: a study in the rabbit

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(Received 10 August 1982/24 March 1983; accepted 22 May 1983)

Summary

1. Lead acetate was administered to adult New Zealand White rabbits in their drinking water. Their mean blood lead level rose to 4.5 μmol/l within a week and then remained relatively constant.

2. The rabbits developed a marked coproporphyrinuria. Plasma levels of coproporphyrin increased but not in proportion to the urine excretion. Thus the renal clearance of coproporphyrin rose from a mean of 1.8 ml/min to 32.2 ml/min whilst creatinine clearance remained constant.

3. The concentration of coproporphyrin in renal venous blood from control rabbits was found to be slightly lower than that in arterial blood. In the lead intoxicated rabbits the concentration of coproporphyrin in renal venous blood was approximately three times higher than the arterial concentration.

4. Significantly higher levels of lead, porphobilinogen, uroporphyrin, coproporphyrin and protoporphyrin were found in renal tissue than in brain, heart or liver.

5. Renal tissue homogenates from control rabbits were able to synthesize porphobilinogen, uroporphyrin, coproporphyrin and protoporphyrin when incubated with 5-aminolaevulinic acid. Renal tissue from lead intoxicated rabbits was also able to synthesize these haem precursors although at a reduced rate.

6. Three enzymes from the haem biosynthetic pathway were assayed in renal mitochondria. Compared with those from controls, mitochondria from lead intoxicated rabbits showed no significant difference in ferrochelatase activities, but the activities of coproporphyrinogen oxidase were decreased, and those of 5-aminolaevulinate synthase were increased.

7. It was concluded that a large portion of the excess coproporphyrin excreted by the lead intoxicated rabbits was of renal origin.

Key words: coproporphyrin, kidney, lead.

Introduction

Lead intoxication in man and animals results in an increased urinary excretion of porphyrins [1, 2]. Coproporphyrin is the major porphyrin excreted, being 80–85% of the total [3]. The porphyrinuria has been assumed to result from the inhibition of haem synthetic enzymes, predominantly in erythropoietic tissue [4]. There is no doubt that lead affects erythropoietic tissue; some of the earliest signs of lead intoxication are observed in circulating erythrocytes and a hypochromic, macrocytic anaemia can be induced by chronic lead intoxication. However, lead is also nephrotoxic and proximal renal tubular function is particularly vulnerable [5, 6]. The possibility that a renal mechanism contributes to the coproporphyrinuria of lead intoxication was investigated in an animal model.

Methods

Materials

Young adult (2.5–3.0 kg) female New Zealand White rabbits were kept in metabolic cages. Twelve rabbits were given lead acetate solution (13.2 mmol/l) to drink ad libitum in place of the normal
drinking water given to the ten control rabbits. Of the lead intoxicated rabbits, seven were used for the serial estimation of the variables described along with five controls, and five were killed for tissue enzyme studies together with five controls.

Porphyrin standards

Coproporphyrin in pre-weighed 5 µg vials was obtained from Sigma Chemical Co. Protoporphyrin and uroporphyrin were purchased as the methyl esters (Sigma), and free porphyrin standards were prepared by the method of Chisholm & Brown [7].

Analyses

In the porphyrin methods described porphyrinogens are all oxidized to porphyrins [8]. The methods therefore estimate the total of porphyrin and porphyrinogen. Plasma coproporphyrin was measured by the fluorimetric method of Moore et al. [9], using excitation and emission wavelengths of 399 nm and 592 nm respectively. Urine coproporphyrin was measured by the method of Rimington [10] and quantification was by fluorimetry as above.

Tissue porphyrin and enzyme studies were performed on 10% (w/v) homogenates in ice cold triethanolamine citrate buffer (0.3 mol/l, pH 7.5).

Portions (5.0 ml) of renal tissue homogenates were incubated with 5-aminolaevulinic acid (2.0 mmol/l) and glucose (10 mmol/l) for 1 h at 37°C. Porphobilinogen, uroporphyrin, coproporphyrin and protoporphyrin were then assayed in these homogenates.

The extraction of coproporphyrin and protoporphyrin was based on the method of Rimington [10]. Uroporphyrin was recovered from the residual aqueous washings and extracts by adsorption on to alumina columns [11]. The porphyrins were measured by fluorimetry at the following excitation and emission wavelengths: coproporphyrin 399 nm and 592 nm; protoporphyrin 399 nm and 602 nm; uroporphyrin 399 nm and 597 nm. The method of porphobilinogen measurement was based on that used in the assay of 5-aminolaevulinate dehydratase [12].

After a 9 week period of lead intoxication, each rabbit was anaesthetized with 2-3 ml of sodium pentobarbitone solution (30 ng/ml)/kg. The abdomen was opened and arterial and renal venous blood collected.

Urine samples were tested semi-quantitatively for the presence of glucose by using Clinistix (Ames Co.).

Blood lead levels were estimated by use of the Delves' cup system and an IL353 atomic absorption spectrophotometer.

Details of other methods used can be found by reference to the following: urine lead [13], faecal and tissue lead [14], creatinine [15], phosphate [15], protein [16], preparation of mitochondria [17], 5-aminolaevulinic synthase [18], coproporphyrinogen oxidase [19] and ferrochelatase [20].

Data are presented as the means ± 1 SD of the results from either the experimental or the control group of rabbits.

Results

Chronic intoxication studies

The lead intoxicated rabbits showed no overt signs or symptoms of toxicity. They had a healthy general appearance with normal posture and mobility.

Balance studies, over 6 days, showed that the mean lead intake was 2.85 ± 0.52 mmol/24 h, the mean faecal excretion was 2.61 ± 0.43 mmol/24 h and the mean urinary excretion was 0.82 ± 0.48...
Urine coproporphyrin in lead intoxication

Thus the rabbits had a mean daily retention of 0.24 mmol of lead, 9% of their intake.

The mean blood lead concentration of the experimental rabbits rose sharply from 0.5 μmol/l to 4.6 μmol/l during the first week of lead ingestion. It then increased more slowly for the next 8 weeks to 5.7 μmol/l.

The urinary excretion of coproporphyrin rose from 12 ± 3 nmol/24 h to 443 ± 156 nmol/24 h (Fig. 1). Plasma levels of coproporphyrin increased, but only by a factor of 3; thus the renal clearance rose from an initial 1.8 ± 0.7 ml/min to 23.2 ± 8.5 ml/min.

The porphyrins present in the urine were separated and examined by the thin-layer chromatographic procedure of Doss [21]. Coproporphyrin was clearly predominant with small amounts of hexa- and penta-porphyrin. Very little uroporphyrin was present.

Some aspects of renal function were assessed: glomerular function by calculating creatinine clearance; tubular reabsorptive function by measuring phosphate clearance and by testing the urine for glucose. Creatinine clearance was not significantly affected by the lead intoxication, being 6.0 ± 1.3 ml/min before and 5.7 ± 1.3 ml/min after the 9 weeks of lead ingestion. Phosphate clearances were similarly unaffected, with an initial mean of 1.5 ± 0.5 ml/min and a final mean of 1.3 ± 0.5 ml/min. There was no evidence of glycosuria. Urine pH was found to be consistently high, ranging from 6.5 to 8.5.

Coproporphyrin concentration in arterial and renal venous plasma

In the control rabbits renal venous plasma was found to have a slightly lower coproporphyrin concentration than the arterial plasma. However, in the lead intoxicated rabbits the renal venous plasma was found to have a far higher coproporphyrin concentration than arterial plasma (Fig. 2).

Rabbit tissue studies

Analysis of the tissues of lead intoxicated rabbits revealed that the kidneys contained higher

Table 1. Concentrations of lead and haem precursors in kidney, liver, heart and brain from the control and lead intoxicated animals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Lead intoxicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (μg/g of protein)</td>
<td>&lt;0.2</td>
<td>41 ± 12</td>
</tr>
<tr>
<td>Porphobilinogen (nmol/mg of protein)</td>
<td>2.0 ± 1.6</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Uroporphyrin (pmol/mg of protein)</td>
<td>23 ± 12</td>
<td>654 ± 100</td>
</tr>
<tr>
<td>Coproporphyrin (pmol/mg of protein)</td>
<td>32 ± 2</td>
<td>233 ± 24</td>
</tr>
<tr>
<td>Protoporphyrin (pmol/mg of protein)</td>
<td>78 ± 20</td>
<td>456 ± 80</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (μg/g of protein)</td>
<td>&lt;0.2</td>
<td>9.3 ± 3.2</td>
</tr>
<tr>
<td>Porphobilinogen (nmol/mg of protein)</td>
<td>2.3 ± 2.2</td>
<td>6.3 ± 2.7</td>
</tr>
<tr>
<td>Uroporphyrin (pmol/mg of protein)</td>
<td>22 ± 5</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>Coproporphyrin (pmol/mg of protein)</td>
<td>34 ± 8</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>Protoporphyrin (pmol/mg of protein)</td>
<td>92 ± 13</td>
<td>210 ± 52</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (μg/g of protein)</td>
<td>&lt;0.02</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Porphobilinogen (nmol/mg of protein)</td>
<td>0.6 ± 0.5</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Uroporphyrin (pmol/mg of protein)</td>
<td>12 ± 5</td>
<td>29 ± 10</td>
</tr>
<tr>
<td>Coproporphyrin (pmol/mg of protein)</td>
<td>16 ± 3</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>Protoporphyrin (pmol/mg of protein)</td>
<td>69 ± 1</td>
<td>86 ± 20</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (μg/g of protein)</td>
<td>&lt;0.2</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>Porphobilinogen (nmol/mg of protein)</td>
<td>0.5 ± 0.3</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>Uroporphyrin (pmol/mg of protein)</td>
<td>21 ± 11</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>Coproporphyrin (pmol/mg of protein)</td>
<td>20 ± 2</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>Protoporphyrin (pmol/mg of protein)</td>
<td>60 ± 6</td>
<td>87 ± 28</td>
</tr>
</tbody>
</table>
TABLE 2. Accumulation of haem precursors by kidney homogenates during 2 h incubation with 5-aminolaevulinic acid (2.0 mmol/l) and glucose (10 mmol/l)

Results are expressed as the means ± 1 SD. N.S., Not significant.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lead intoxicated</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphobilinogen (nmol h⁻¹ mg⁻¹ of protein)</td>
<td>272 ± 38</td>
<td>69 ± 25</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Uroporphyrin (pmol h⁻¹ mg⁻¹ of protein)</td>
<td>554 ± 100</td>
<td>645 ± 100</td>
<td>N.S.</td>
</tr>
<tr>
<td>Coproporphyrin (pmol h⁻¹ mg⁻¹ of protein)</td>
<td>122 ± 21</td>
<td>53 ± 19</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Protoporphyrin (pmol h⁻¹ mg⁻¹ of protein)</td>
<td>165 ± 39</td>
<td>144 ± 52</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

TABLE 3. Activities of three enzymes from the haem biosynthetic pathway, assayed in isolated renal mitochondria

Results are expressed as the means ± SD with the level of significance indicated. N.S., Not significant.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Lead intoxicated</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Aminolaevulinate synthetase (nmol of ALA synthesized h⁻¹ mg⁻¹ of protein)</td>
<td>0.20 ± 0.05</td>
<td>0.32 ± 0.09</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Coproporphyrinogen oxidase (nmol of PROTO synthesized h⁻¹ mg⁻¹ of protein)</td>
<td>0.13 ± 0.05</td>
<td>0.04 ± 0.02</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Ferrochelatase (nmol of mesohaem synthesized h⁻¹ mg⁻¹ of protein)</td>
<td>0.71 ± 0.23</td>
<td>0.52 ± 0.12</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

levels of lead than liver, brain or heart (Table 1). The kidneys were also found to have higher levels of the haem precursors. Kidney homogenates from the lead intoxicated animals, incubated with 5-aminolaevulinic acid, synthesized haem precursors but at a slower rate than the controls (Table 2).

Activities of some of the mitochondrial enzymes of haem synthesis are shown in Table 3.

Discussion

An animal model has been used to examine the possibility of a renal contribution to the coproporphyrinuria associated with lead intoxication. Lead treated rabbits developed marked biochemical abnormalities without overt clinical signs or symptoms. Renal function, as assessed by creatinine and phosphate clearances and qualitative urine testing for glucose, was unaffected by the lead intoxication. Urinary excretion of coproporphyrin rose from a mean of 12 nmol/24 h to 443 nmol/24 h. A coproporphyrinuria of this magnitude is consistent with observations made by other workers [2, 3, 22]. One interesting feature of the rabbit's urine was its persistent alkalinity. This may have contributed to the magnitude of the coproporphyrin excretion since it is known to be pH dependent, increasing as urine pH rises [23].

Although the urinary excretion of coproporphyrin increased nearly 40-fold, the plasma levels rose only threefold. Thus the renal clearance of coproporphyrin, which initially was of a similar order to that of phosphate, increased dramatically and exceeded that of creatinine. This suggested two possibilities: either that the kidneys were actively secreting coproporphyrin, or that the urinary coproporphyrin largely originated from renal tissue.

In an attempt to resolve this question coproporphyrin was measured in plasma from arterial and renal venous blood. The observation of higher levels of coproporphyrin in the renal venous blood of the lead intoxicated animals led us to conclude that these kidneys were synthesizing and releasing large amounts of coproporphyrin.

The increased vulnerability of the kidneys to the toxic effects of lead was emphasized by the finding that lead had accumulated to a greater degree in renal tissue than in liver, brain or heart, as previously recorded [2, 14]. The kidneys were also found to contain the highest levels of haem precursors. Whether this accumulation was due to the effects of lead on renal metabolism per se, or was the result of renal uptake from the plasma, is not known. The ability of renal homogenates to synthesize haem precursors from 5-aminolaevulinic
Acid was demonstrated and this persisted in the lead intoxicated tissue despite the partial inhibition of 5-aminolaevulinate dehydratase activity. However, there is evidence against the renal uptake of 5-aminolaevulinic acid and its subsequent conversion into porphyrins. Large intravenous infusions of 5-aminolaevulinic acid produced little increase in the urinary excretion of coproporphyrin. When 5-[14C]aminolaevulinic acid was administered to human subjects only a small percentage of 5-aminolaevulinate dehydratase activity. How- ever, there had been an accumulation of protoporphyrin in the liver. Recovered in the form of urinary coproporphyrin, the vast majority being incorporated into haem pigments in the bone marrow and liver [25].

One apparent anomaly was that higher tissue levels of uroporphyrin than of coproporphyrin were found and yet very little uroporphyrin was excreted in the urine. Quantitative analysis by San Martin de Vialle et al. [3] of the porphyrin excreted by lead poisoned rabbits had shown that uroporphyrin constituted only 1–2% of the total. The explanation for the findings is not known but it may be that uroporphyrin cannot leave the renal tubular cells as readily as coproporphyrin.

When the mitochondrial enzymes of haem synthesis were examined no significant difference was found in the levels of ferrochelatase between controls and experimental rabbits (Table 3). However, there had been an accumulation of protoporphyrin in renal tissue, indicating inhibition in vivo. The inhibition of ferrochelatase by lead has been well documented [4, 26], but it has also been shown that it may be difficult to demonstrate this inhibition in vitro without adding lead to the assay incubation mixture [2]. The increased activity of 5-aminolaevulinate synthase was probably due to derepression [26], since the enzyme is normally under negative feedback control by haem. The observed inhibition of coproporphyrinogen oxidase would have provided a metabolic block, which, together with the elevated activity of 5-aminolaevulinate synthase, could explain the accumulation of coproporphyrinogen.

Our observations suggest that the marked increase in the urinary excretion of coproporphyrin by the lead intoxicated rabbits was mainly due to an increased concentration of coproporphyrinogen in the renal tissues, which in turn was a consequence of deranged renal metabolism. Recent evidence by Day & Eales [27, 28] supports the proposition that renal tissue may be the major site of urinary coproporphyrin, both in porphyric patients and in patients with no known porphyria disorder. Those findings, together with the observations presented in this paper, challenge the conventional view that the excess excretion of coproporphyrin relates to the disturbances in the major haem synthetic organs of bone marrow and liver.

Acknowledgments
We thank the Employment Medical Advisory Service (EMAS) for the financial support of this work.

References


