Reduction of δ-aminolevulinate dehydratase concentration by bromobenzene in rats

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ABSTRACT The effects of bromobenzene on δ-aminolevulinate (ALA) dehydratase (EC 4.2.1.24) were examined in rats. The enzyme in the bone marrow was irreversibly inhibited after 12 hours of treatment with bromobenzene (intraperitoneally) without any change in the enzyme concentration. When bromobenzene treatment was prolonged to 72 hours, the concentrations of the enzyme in the bone marrow and in the liver were reduced proportionally to the decrease in the enzyme activity. Neither the activity nor the concentration of ALA dehydratase in the peripheral erythrocytes were reduced even after 72 hours treatment with bromobenzene. These findings indicate that bromobenzene decreases ALA dehydratase activity in a biphasic manner; firstly, through an irreversible inhibition probably due to the formation of mercaptide with the essential SH groups and, secondly, through a reduced synthesis of the enzyme.

The activity of δ-aminolevulinate (ALA) dehydratase (EC 4.2.1.24) is well known to be inhibited by exposure to lead and the degree of the inhibition of the enzyme activity is recognised as an indicator of lead uptake. The inhibition by lead is due to the formation of mercaptide between the essential SH groups of the enzyme molecule and lead. Therefore, substances able to bind to essential SH groups might also have an inhibitory effect on ALA dehydratase activity. Recently, several organic solvents such as trichloroethylene, styrene, and bromobenzene have been reported significantly to reduce ALA dehydratase activity. These solvents are used widely in industry and studies of their biological effects should provide important information for evaluating the health status of workers exposed to them. Trichloroethylene is metabolised by cytochrome P-450 dependent mixed function oxidase to form reactive metabolites, which are considered to inhibit ALA dehydratase activity irreversibly by binding to the essential SH groups. Styrene, however, decreases the enzyme concentration by reducing enzyme synthesis at transcriptional and post-translational levels and this reduction in enzyme concentration results in reduced ALA dehydratase activity. These reports indicated at least two different ways to decrease ALA dehydratase activity, though both trichloroethylene and styrene are oxidised by the cytochrome P-450 system.

Bromobenzene is also metabolised by the cytochrome P-450 system to form highly reactive intermediates (bromobenzene-3,4-oxide and -2,3-oxide), which possibly decrease the dehydratase activity in two different ways. One is through the inhibition of the enzyme activity due to the formation of mercaptide with the essential SH groups as in the case of lead and trichloroethylene. The other is by the reduced synthesis of the enzyme protein as in the case of styrene. The first mechanism has been reported in vitro, but the second has not previously been examined.

Materials and methods

Animals and treatment with bromobenzene
Male Wistar rats, 7 weeks old and weighing 200–300 g, were divided into a control (n = 8) and a bromobenzene treated group (n = 8). Bromobenzene was administered intraperitoneally once a day in a dose of 4 mmole bromobenzene (dissolved in 0.5 ml of soybean oil)/kg body weight. Soybean oil (0.5 ml/kg body weight) was administered to the controls once a day. Four rats from each group were killed at 12 hours and 72 hours after the first administration of bromobenzene and blood, liver, and bone marrow from tibias and femurs were collected. Samples were assayed for packed cell volume (blood), protein concentration (liver and bone marrow), and activity and
concentration of ALA dehydratase (blood, liver, and bone marrow). Rats were fed with a certified chow (Funahashi Nojo, Hamamatsu, Japan) and distilled water ad libitum. They were housed four to a wire bottom cage in a room designed to control temperature (24–26°C), relative humidity (50–70%), and photocycle (12 hours). In all the experiments the rats were killed or treated between 0900 and 1000.

**PURIFICATION OF ALA DEHYDRATASE**

ALA dehydratase was purified from rat erythrocytes as described previously. The purified enzyme preparation was homogenous on gel filtration chromatography and in analytic polyacrylamide elec-
trophoresis both in the absence and presence of sodium dodecyl sulphate. The enzyme was labelled with 125-I (Amersham International Ltd, Bucking-
hamshire, UK) as reported previously. The specific radio activity of 125-I ALA dehydratase was 6-0 μCi/μg of enzyme protein.

**PREPARATION OF ANTIBODY AGAINST ALA DEHYDRATASE**

The antibody was prepared by the method described in an earlier report using the ALA dehydratase purified from rat erythrocytes as described above. At a dilution of 1:15 000 the IgG preparation could bind approximately 50% of the labelled antigen (= labelled ALA dehydratase).

**PREPARATION OF MICROSONES**

Liver microsomes from rats treated with phenobarbi-
tal were prepared as reported previously.

**ASSAY OF ALA DEHYDRATASE ACTIVITY**

ALA dehydratase activity was assayed both in the absence and presence of 10 mM dithiothreitol and 0.1 mM zinc acetate. Unless otherwise stated, the enzyme activity in the text represents the activity after the reactivation with dithiothreitol and zinc. One unit of the dehydratase activity was defined as 1 μmole of porphobilinogen formed at 37°C per hour. Radioim-

**REAGENTS**

ALA hydrochloride was purchased from Sigma Chemicals Co (St Louis). Other chemicals used were all of analytical grade.

**STATISTICS**

Student’s t test (two tailed) was applied for the examination of significant differences between the control groups and groups treated with bromobenzene.

**RESULTS**

The activity of ALA dehydratase decreased signifi-
cantly after 12 hours of bromobenzene adminis-
tration only in the bone marrow (table 1). It was not accompanied with a decrease in the enzyme concentra-
tion. Treatment with dithiothreitol and zinc could not restore the decreased activity of ALA dehydratase, indicating an irreversible inhibition of ALA dehydratase by bromobenzene. By contrast, ALA dehydratase activity in the erythrocytes and in the liver was unaltered after 12 hours of treatment with bromobenzene. Table 1 indicates the significant decrease of ALA dehydratase concentration and of

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**Table 1** Effect of treatment with bromobenzene on ALA dehydratase

<table>
<thead>
<tr>
<th>ALA dehydratase (No of rats)</th>
<th>Duration of bromobenzene treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 hours</td>
</tr>
<tr>
<td>Bone marrow (n = 4)</td>
<td>Activity</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
</tr>
<tr>
<td>Erythrocytes (n = 4)</td>
<td>Activity</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
</tr>
<tr>
<td>Liver (n = 4)</td>
<td>Activity</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
</tr>
</tbody>
</table>

To compare the enzyme activity with its concentration, both values were described as relative values (%) in comparison with the means of concurrent controls.

Means and standard deviations (in parentheses) of ALA dehydratase activities and concentrations in the pooled control—that is, the control at 12 hours and 72 hours, eight animals in total—were 17.9 (2.15) × 10⁶ unit/mg protein and 854 (108.0) ng/mg protein in the bone marrow, 194 (27.6) × 10⁴ unit/ml of packed cells and 7.73 (2.43) μg/ml of packed cells in erythrocytes, and 6.19 (1.38) × 10⁶ unit/mg protein and 615 (99.8) ng/mg protein in liver.

*Difference from the control is significant at p < 0.01(**) or < 0.05(*).
ALA dehydratase activity in the bone marrow and liver but not in erythrocytes 72 hours after the first administration of bromobenzene. Results of radioimmunoassay show that the reduced enzyme concentration was proportional to the reduced enzyme activity in the bone marrow and in the liver (to 47% and 67% of the control, respectively).

Difference was observed between rats treated with bromobenzene for 12 hours and those treated for 72 hours (Table 1) when the activity/mass ratio (= activity of ALA dehydratase/concentration of ALA dehydratase) was examined in the bone marrow (Table 2). The activity/mass ratio of the control rats was 21.2 units/mg of enzyme, which is almost compatible with the specific activity of purified ALA dehydratase (rat, 23.8–27.0 units/mg of enzyme). This observation shows the accuracy of our purification and of the determination of the enzyme. As shown in Table 2, a significant decrease of the activity/mass ratio in the bone marrow was observed after only 12 hours of the first treatment with bromobenzene. This shows that the reduced enzyme activity in the early stage of intoxication should be an inhibition of enzyme activity. The activity/mass ratio in the rats treated for 72 hours, however, was almost identical with that in the control rats (20.1 vs 21.2) indicating that the reduced enzyme activity in rats treated for 72 hours is due to a reduced enzyme concentration.

To elucidate the site of the reduced enzyme concentration by bromobenzene, the following experiments were carried out. ALA dehydratase activity was decreased to 61% of the control when the enzyme was incubated in vitro with the mixed function oxidase system and bromobenzene (Table 3). The enzyme concentration determined by radioimmunoassay, however, did not decrease after in vitro incubation. It is, therefore, unlikely that the enzyme concentration was depressed by post-translational degradation.

**Discussion**

Lead inhibits the activity of ALA dehydratase by the formation of mercaptide with the essential SH groups of the enzyme molecule and the inhibition of ALA dehydratase is one of the most sensitive indicators of lead exposure. ALA dehydratase activity in the peripheral blood is routinely determined in the medical examination of lead workers. Recently, clear evidence of the increased synthesis of ALA dehydratase by lead was reported in rats and in man. Therefore, attention should be given to enzyme synthesis as well as to enzyme activity in the study of effects of chemicals on ALA dehydratase.

Several organic solvents such as trichloroethylene, styrene, and bromobenzene have been reported to decrease ALA dehydratase activity in vivo. In general, two mechanisms should be considered when we examine mechanisms by which enzyme activity to reduced in vivo. One is the inhibition of the enzyme activity due to an interaction between the enzyme molecules and the chemicals or the metabolite(s), or both. In the case of trichloroethylene, ALA dehydratase is inhibited by the chemical after bio-activation with mixed function oxidases and the

### Table 2  Time course of the decrease of ALA dehydratase in the bone marrow. (Figures are means and standard deviations in parentheses)  

<table>
<thead>
<tr>
<th>Rats</th>
<th>Activity (× 10^2 unit/mg protein)</th>
<th>Concentration (ng/mg protein)</th>
<th>Activity/mass ratio (units/mg enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.9 (2.15)</td>
<td>854 (108)</td>
<td>21.2 (1.40)</td>
</tr>
<tr>
<td>12 hours treated</td>
<td>14.1 (1.54)†</td>
<td>869 (43)</td>
<td>16.8 (1.34)‡</td>
</tr>
<tr>
<td>72 hours treated</td>
<td>8.1 (2.47)‡</td>
<td>403 (92)</td>
<td>20.1 (4.76)</td>
</tr>
</tbody>
</table>

Activity/mass ratio was calculated as activity of ALA dehydratase/concentration of ALA dehydratase. Other conditions as in legend to table 1 are in the text.

†Difference from the control is significant at p < 0.01(**) or p < 0.05(*).

### Table 3  Effects of bromobenzene on ALA dehydratase in the presence of the mixed function oxidase system in vitro. Figures in the table are means (n × 3) and standard deviations (in parentheses)  

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>ALA dehydratase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes NADPH</td>
<td>Activity (%)</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>100 (5)†</td>
</tr>
<tr>
<td>+</td>
<td>102 (3)</td>
</tr>
<tr>
<td>+</td>
<td>61 (4)*</td>
</tr>
</tbody>
</table>

Purified ALA dehydratase (7 μg protein/ml) was incubated with the mixed function oxidase system and bromobenzene as described previously.  

*Significantly decreased when compared with the control (p < 0.01).  
†Taken as control.
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The enzyme in animals treated with bromobenzene.

The idea that bromobenzene reduces ALA dehydratase synthesis may explain the time lag between the decrease of non-protein SH (reduced form) content in the liver and the reduction of ALA dehydratase activity observed in the liver. The amount of non-protein SH in the liver reached a minimum after 24 hours of bromobenzene administration and, thereafter, the concentration was gradually restored. Non-protein SH group is considered to play a part in the protection against the toxicity of the reactive intermediates of bromobenzene and trichloroethylene on macromolecules of animals. The addition of dithiothreitol prevents the inhibition of ALA dehydratase by intermediates of trichloroethylene in vitro. Thus the time course of the decrease in ALA dehydratase activity should be synchronised with the fluctuations in non-protein SH contents, since the interaction between intermediates and the enzyme should correlate inversely with the concentration of non-protein SH. In fact, this was not the case. Our results show that the activity of ALA dehydratase decreased progressively in the liver even after 72 hours, notwithstanding the partial recovery of non-protein SH content (48 hours in (8)). This may be due to the prolonged reduction in the synthesis of ALA dehydratase by the interaction between nucleic acids and the reactive intermediate(s) of bromobenzene.

All correspondence to Dr N Ishihara.

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