PRETREATMENT WITH PARACETAMOL INHIBITS METABOLISM OF ENFLURANE IN RATS

A. N. HANNA, J. S. MCDONALD, C. H. MILLER JR AND D. COURI

Enflurane is a widely used anaesthetic agent generally believed to be safe [1]. It is a substrate of the mixed function oxidase system in the liver [2–8] and has a degree of metabolism of approximately 2% [9]. Fluoride concentrations in serum, liver and urine are used as indicators of its biotransformation [1].

Paracetamol is a commonly used antipyretic analgesic drug that is generally safe in therapeutic doses [10]. It is eliminated mainly by conjugation to the metabolites paracetamol glucuronide and sulphate. A small portion is metabolized by the liver microsomal enzyme system to form reactive metabolites that are inactivated by conjugation with reduced glutathione. If the amount of reduced glutathione is depleted, these reactive metabolites bind covalently to the macromolecules of the liver cell, thereby producing liver cell damage and necrosis [11–13].

This study has investigated the effect of pretreatment with paracetamol (acetaminophen U.S.P.) on biotransformation of enflurane.

MATERIALS AND METHODS

Treatment groups

Sixteen male Sprague–Dawley rats (300 (SEM 50) g) were allocated to four equal groups. The first group of rats were given paracetamol 7.5 mg/100 g body weight per day by mouth for five consecutive days. The second group received this regimen plus, 15 min after the last dose, exposure to 1% enflurane for 2 h. The third group was only exposed to 1% enflurane for 2 h. The fourth group consisted of untreated controls. The animals were placed in metabolic cages for 6 h for collection of urine, after which time they were placed in a chamber containing carbon dioxide to produce anaesthesia. After removal from the chamber for blood sampling by intracardiac

SUMMARY

We studied the interaction between paracetamol (acetaminophen U.S.P.) and enflurane. Sixteen rats were assigned to four groups (n = 4) to receive: paracetamol 7.5 mg/100 g body weight; paracetamol plus 1% enflurane; 1% enflurane alone, or no treatment (controls). Animals were killed 6 h later. A second series of 16 were treated identically, but were killed after 24 h. Measurements were made of fluoride concentrations in serum, liver and urine (indicators of biotransformation of enflurane), paracetamol concentrations in urine, pathological changes in liver samples, and concentrations of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum. Pretreatment with paracetamol significantly decreased urinary fluoride at 6 and 24 h after exposure to enflurane, but decreased fluoride concentrations in serum and liver only at 6 h after exposure to enflurane. Paracetamol concentrations in urine did not change after exposure to enflurane. Exposure to paracetamol alone increased AST and ALT. At 24 h after exposure to enflurane, serum concentrations of enzymes in rats pretreated with paracetamol were similar to those of control rats. Pretreatment with paracetamol may therefore inhibit metabolism of enflurane. Although no hepatic damage was observed, the increased in AST and ALT suggested subclinical liver damage in rats given only paracetamol.

A. N. HANNA, M.D. (Departments of Anesthesiology and Pharmacology); J. S. MCDONALD, M.D. (Department of Anesthesiology); C. H. MILLER JR, PH.D., D. COURI, PH.D. (Department of Pharmacology, Toxicology Division); The Ohio State University, 410 West Tenth Avenue, Columbus, Ohio 43210–1228, U.S.A. Accepted for Publication: May 30, 1988.

Correspondence to J. S. McD.
puncture, they were returned to the chamber to be killed [14, 15]. The same procedure was repeated on 16 other rats, except that these animals were kept in metabolic cages for 24 h instead of 6 h. Samples of urine, blood and liver tissue were taken for analysis and examination.

Administration of drugs

A solution was prepared by dissolving 600 mg of paracetamol in 30 ml of distilled water containing 1% ethanol. Paracetamol 7.5 mg/100 g body weight was given via a No. 18 feeding needle. Groups not treated with paracetamol were given distilled water by the same method.

Rats treated with enflurane were exposed to the anaesthetic in a 420-litre chamber at 23 (SEM 1) °C. To achieve a concentration of 1% enflurane, enflurane 31.0 g was vaporized inside the chamber. Chamber samples were taken at 15-min intervals, to confirm the concentration of enflurane by gas–liquid chromatography. Control animals were placed in similar chambers without enflurane.

Analyses

Serum concentrations of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the University Hospital Clinical Pathology Laboratory [16, 17].

Serum fluoride concentration was determined at 6 or 24 h after exposure to enflurane. One millilitre of serum was added to 1 ml of total ionic strength adjusting buffer. Fluoride ion was determined using an ion selective electrode (the Orion Combination Electrode). A standard curve was prepared from samples containing sodium fluoride 0.01–1.0 µg ml−1 with zero adjustment set at 0.01 µg ml−1 [18]. The fluoride standard curve was linear when plotted on a semilogarithmic scale, fitting with a coefficient of correlation of 0.99.

Urinary fluoride concentration was determined using an Orion Combination Electrode. One millilitre of urine was added to 1 ml of total ionic strength adjusting buffer. A standard curve was prepared from samples containing sodium fluoride 1–100 µg ml−1, with zero adjustment set at 1.0 µg ml−1 [19]. A standard curve was linear when plotted on a semilogarithmic scale fitting, with a coefficient of correlation of 0.99.

Urine concentrations of paracetamol were measured as described elsewhere [20, 21]. A standard curve was linear at 0–500 µg ml−1, with a coefficient of correlation of 0.99.

Histopathology

Liver samples were examined histologically by the University Clinical Pathology Laboratory.

Data are presented as mean values (SEM). Data were analysed by one-way analysis using a log transformation of data. Turkey’s studentized range test and Scheffes’s test were used for multiple comparison of each pair of treatment groups. P < 0.05 was regarded as significant [22, 23].

RESULTS

The concentration of enflurane in the chambers remained at 1.02 (0.01) %. At 6 h after exposure to enflurane, fluoride concentrations in serum and liver samples were significantly greater in animals exposed to only enflurane than in animals pretreated with paracetamol before exposure to enflurane (figs 1, 2). Also, the concentration and total amount of fluoride in urine and in the whole body were significantly greater in the animals exposed only to enflurane than in animals pretreated with paracetamol and exposed to enflurane (table I). By 24 h after exposure to enflurane, fluoride concentrations in serum and liver had returned to control values (serum 40 (10) ng ml−1; liver 38 (13) ng ml−1). However, the concentration and total amount of fluoride in urine and in

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**Fig. 1.** Fluoride concentrations in serum 6 h after 16 rats were given paracetamol 7.5 mg/100 g body weight only (P); pretreatment with paracetamol and exposure to 1% enflurane (P+E); 1% enflurane only (E) or no treatment (controls) (C). Significant differences (P < 0.05): * compared with the two groups not given enflurane; † compared with group pretreated with paracetamol and exposed to enflurane.
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**TABLE I.** Mean (SD) values for fluoride in urine 6 h after exposure of rats to enflurane. Significant differences (P < 0.01): **compared with two groups not given enflurane; †† compared with group given paracetamol and enflurane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume of urine (ml)</th>
<th>Concentration of fluoride (µg ml⁻¹)</th>
<th>Total fluoride (µg)</th>
<th>Fluoride per body wt (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>2.3 (0.7)</td>
<td>4.4 (0.4)</td>
<td>12.4 (2.7)</td>
<td>36 (5)</td>
</tr>
<tr>
<td>Paracetamol + enflurane</td>
<td>3.0 (0.2)</td>
<td>20.6 (1.9)**</td>
<td>61.7 (5.0)**</td>
<td>175 (12)**</td>
</tr>
<tr>
<td>Enflurane</td>
<td>2.8 (0.2)</td>
<td>33.0 (0.8)††</td>
<td>90.8 (7.6)**††</td>
<td>290 (32)**</td>
</tr>
<tr>
<td>None (controls)</td>
<td>3.2 (1.3)</td>
<td>4.0 (0.7)</td>
<td>12.1 (3.0)</td>
<td>43 (13)</td>
</tr>
</tbody>
</table>

**TABLE II.** Mean (SD) values for fluoride in urine 24 h after exposure of rats to enflurane. Significant differences: † P < 0.05 compared with group given paracetamol and enflurane; **P < 0.01 compared with two groups not given enflurane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume of urine (ml)</th>
<th>Concentration of fluoride (µg ml⁻¹)</th>
<th>Total fluoride (µg)</th>
<th>Fluoride per body wt (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>8.2 (0.7)</td>
<td>4.5 (1.8)</td>
<td>39.4 (17.3)</td>
<td>143 (54)</td>
</tr>
<tr>
<td>Paracetamol + enflurane</td>
<td>8.7 (3.0)</td>
<td>9.5 (3.7)**</td>
<td>79.0 (28.0)**</td>
<td>306 (66)**</td>
</tr>
<tr>
<td>Enflurane</td>
<td>8.4 (2.2)</td>
<td>17.0 (7.4)††</td>
<td>133.1 (30.2)**†</td>
<td>470 (84)**†</td>
</tr>
<tr>
<td>None (controls)</td>
<td>8.1 (0.8)</td>
<td>4.3 (1.5)</td>
<td>40.3 (16.2)</td>
<td>139 (30)</td>
</tr>
</tbody>
</table>

the whole body were significantly greater in animals exposed to enflurane alone than in pretreated animals exposed to enflurane (table II).

The volume of urine did not differ for all four groups at 6 h (2.8 (0.6) ml) or 24 h (8.4 (2.0) ml).

At 24 h, the concentration of paracetamol and its metabolites (sulphate and glucuronide) in urine were similar for the group given paracetamol alone (17.5 (2.1) µg) and the group given enflurane in addition (16.9 (3.6) µg). These values represent 85% recovery of the doses given.

Paracetamol alone increased both AST (from 165 to 475 units ml⁻¹) and ALT (from 60 to 185

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**Fig. 2.** Fluoride concentrations in liver samples 6 h after 16 rats were given paracetamol 7.5 mg/100 g body weight only (P); pretreatment with paracetamol and exposure to 1% enflurane (P + E); 1% enflurane only (E); or no treatment (controls) (C). Significant differences (P < 0.05): * compared with the two groups not given enflurane; † compared with animals pretreated with paracetamol and exposed to enflurane.

**Fig. 3.** Serum concentrations of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) after 16 rats were given paracetamol 7.5 mg/100 g body weight only (P); pretreatment with paracetamol and exposure to 1% enflurane (P + E); 1% enflurane only (E); or no treatment (controls) (C). ** Significant difference (P < 0.01) from all the other groups.
units ml⁻¹). The other two treatment groups and the control group had similar values for these two enzymes (fig. 3).

There were no pathological changes evident on histological examination of any liver sample.

**DISCUSSION**

Pretreatment with paracetamol appears to inhibit biotransformation of enflurane, as indicated by decreased concentrations of fluoride ion in serum, liver and urine. Although paracetamol is eliminated mainly by formation of the glucuronide and sulphate, a small and important portion is metabolized by the liver microsomal enzyme system, mainly cytochrome P₄₅₀, to form reactive metabolites [12]. Enflurane also is a substrate of the microsomal enzyme system of the liver. Inhibition of the biotransformation of enflurane by paracetamol may occur because of competition between the drugs for the microsomal enzyme system of the liver (possibly the enzyme system cytochrome P₄₅₀), or because of a toxic effect on the mixed function oxidase system.

Dehalogenation of enflurane was probably inhibited by paracetamol or its metabolites, as no changes occurred in urinary volume or in the concentration of paracetamol and its conjugates in urine, whereas fluoride output in urine was decreased by paracetamol at both 6 and 24 h, and serum fluoride decreased at 6 h, but not 24 h, after exposure of rats to enflurane. The decrease in serum and liver concentrations of fluoride after pretreatment with paracetamol further suggests that the difference in urinary concentrations of fluoride between treatment groups was a result, not of an effect on fluoride excretion, but of differences in the concentration of fluoride ion in serum; that is, enflurane underwent less metabolism in the liver when rats were pretreated with paracetamol.

For both groups given paracetamol there was no difference in its concentration in urine after incubation of urine samples with β-glucuronidase and sulphatase. This implies that enflurane had no effect on conjugation of the drug with glucurionate or sulphate.

The rats given paracetamol alone exhibited the greatest concentrations of AST and ALT. The group given the drug with enflurane exhibited AST and ALT concentrations similar to those of the control and enflurane-only groups. Enflurane may decrease reactive metabolities by altering the metabolic pathway of paracetamol or by creating competition between paracetamol and enflurane for the microsomal enzyme system of the liver.

Although no pathological changes were observed in any liver sample, the increases in the concentrations of AST and ALT in the group given paracetamol alone suggests subclinical liver damage.

**REFERENCES**


