COMPARISON OF EFFECTS OF ACETAMINOPHEN ON LIVER MICROSOMAL DRUG METABOLISM AND LIPID PEROXIDATION IN RATS AND MICE

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Accepted January 20, 1978

Abstract—Studies were conducted to determine the in vivo effect of acetaminophen (AAP) on the lipid peroxidation, drug metabolizing enzyme activity and microsomal electron transfer system of rat and mouse liver. AAP was found to inhibit ethylmorphine N-demethylation activity in the presence of NADPH and this inhibition of the enzyme was due to decrease in cytochrome P-450 content, but not due to change in lipid peroxidation in liver microsomes. Kinetic data showed that AAP administration had no effect on Km values of ethylmorphine N-demethylase, however, a decrease in the Vmax values was seen in rats and mice. There was no significant effect of AAP on both NADPH-cytochrome c reductase and the content of cytochrome b5 3 hours after this administration to rats and mice. On the other hand, AAP induced a significant decrease in NADH-ferricyanide reductase in mice, but not in rats. The greatest decrease in cytochrome P-450 was observed among the components of the liver microsomal electron transfer system of rats and mice.

Acetaminophen (AAP) is widely used as an analgesic, and its covalent binding to macromolecules has been reported when a large oral dose only of AAP was given (1, 2), and such may be attributed to production of hepatic necrosis in man (3). The drug is, however, remarkably safe in therapeutic doses (4).

AAP is known to be metabolized by enzymes in liver microsomes and produces an active metabolite, N-hydroxy-acetaminophen (5, 6). Studies in experimental animals have shown that when higher doses are given, a reactive metabolite of the drug binds covalently with liver cell protein (5, 7, 8). Thorgeirsson et al. (9) demonstrated that changes in biochemical parameters were noted in both male mice and male hamsters after AAP administration.

Previous studies in this laboratory demonstrated that AAP has characteristics of type II substrate for microsomal mixed function oxygenase and inhibits ethylmorphine N-demethylation in the noncompetitive type and aniline hydroxylation in the competitive one, respectively (10). It has been reported that mice were susceptible to the hepatotoxic effects of AAP, while rats were resistant to the AAP poisoning (2).

The purpose of the present study was to investigate the comparison of the in vivo effects of AAP on drug metabolizing enzymes and lipid peroxidation in liver microsomes of rats and mice.
MATERIALS AND METHODS

Enzyme preparation

Male Wistar rats weighing 150-180 g and male ddy mice weighing about 20 g were used throughout. Following deprivation of all food, livers were removed and perfused with 1.15% KCl to remove the blood. These tissues were homogenized in 3 volumes of ice-cold 1.15% KCl in a Potter-Elvehjem type homogenizer with a Teflon pestle, and the homogenate was centrifuged at 9,000 g for 20 min. After recentrifugation of the supernatant of 10,5000 g for 1 hr, the microsomal pellet was resuspended in 1.15% KCl to contain about 2 mg protein per ml.

Enzyme assay

Ethylmorphine N-demethylase activity was measured by determination of formaldehyde formed according to the method of Nash (11). NADH-ferricyanide reductase and NADPH-cytochrome c reductase activities were assayed by the methods of Takesue and Omura (12) and Omura and Takesue (13), respectively.

Analytical methods

Cytochrome b$_5$ content was determined by the method of Omura and Takesue (13). Cytochrome P-450 content was determined from CO difference spectra of the dithionite-treated sample as described by Omura and Sato (14). Lipid peroxidation was measured by the method of Zalkin and Tappel (15).

Protein determination

The microsomal protein was determined according to the method of Lowry et al. (16). Statistical analysis of data was performed using Student’s t-test.

RESULTS

Effect of acetaminophen on ethylmorphine N-demethylase activity of liver microsomes in rats and mice

As shown in Table 1, a single administration of AAP (500 mg/kg, i.p.) to rats produced no significant change in ethylmorphine N-demethylase activity. However, treatment of rats with AAP for two successive days produced a decrease in the enzyme activity to about 60% that seen in control animals, when the incubation was conducted in the presence of EDTA, for the assay of the enzyme activity. EDTA is known to be an inhibitor of lipid peroxidation and stabilizes the activity of the drug metabolizing enzymes in liver microsomes (17). Ethylmorphine N-demethylase activity in mice after a single administration of AAP was significantly decreased in both the presence and absence of EDTA.

On the other hand, decrease in ethylmorphine N-demethylase of AAP-treated animals and controls occurred to the same extent in the presence and absence of EDTA. Therefore, it is likely that lipid peroxidation was not involved in the inhibition of ethylmorphine N-demethylase by AAP administration to rats and mice.

Effect of acetaminophen on the microsomal lipid peroxidation in rats and mice

Table 2 shows that no significant changes in lipid peroxidation occurred with liver
microsomes from rats and mice after AAP administration. This phenomenon supports
the finding shown in Table 1, that is, the ratios of the value with added EDTA to that without
added EDTA were not affected by AAP administration.

### Table 1. Effect of acetaminophen on ethylmorphine N-demethylase activities in liver
microsomes from rats and mice

| Species | Treatment | Dose (mg/kg) | Ethylmorphine N-demethylase
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDTA(+) (mg/mg protein)</td>
</tr>
<tr>
<td>Rat</td>
<td>Control</td>
<td>-</td>
<td>170.10 ± 22.11 (4)</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>500×1</td>
<td>173.89 ± 8.75** (4)</td>
</tr>
<tr>
<td>Rat</td>
<td>Control</td>
<td>-</td>
<td>132.93 ± 10.92 (5)</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>500×2</td>
<td>82.17 ± 9.33*** (5)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Control</td>
<td>-</td>
<td>94.24 ± 2.69 (13)</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>500×1</td>
<td>76.33 ± 2.58*** (5)</td>
</tr>
</tbody>
</table>

* In experiments with a single dosing, rats and mice were sacrificed 3 hr after i.p. administration of acetaminophen (500 mg/kg). Repeated administration to rats was carried out by giving the drug (500 mg/kg) i.p. twice at 12 hr-intervals, and the rats were sacrificed 24 hr after the second injection. Controls were given an injection of saline. The incubation mixture consisted of ethylmorphine (1 mM), 0.4 ml of microsomal suspension, NADPH-generating system (0.03 mM NADP, 8 mM glucose 6-phosphate, 15 μmoles MgCl₂ and 0.045 unit of glucose 6-phosphate dehydrogenase) and 0.3 ml of 0.2 M Na-K phosphate buffer (pH 7.4) in a final volume of 1 ml. The incubation mixture contained EDTA (0.1 mM), as required. Incubation was carried out at 37°C for 30 min. Enzyme activity represents nmoles formaldehyde formed/mg protein/30 min. Values are mean ± S.E. and the figures in parentheses represent number of animals used. **Not significantly different from corresponding values in the control group. ***Significantly different (p<0.05) from corresponding values in the control group. ****Significantly different (p<0.01) from corresponding values in the control group.

### Table 2. Effect of acetaminophen on liver microsomal lipid peroxidation in rats and mice

| Species | Treatment | Lipid peroxidation
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDTA(+)</td>
</tr>
<tr>
<td>Rat</td>
<td>Control</td>
<td>0.87±0.11</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>0.76±0.07*</td>
</tr>
<tr>
<td>Mouse</td>
<td>Control</td>
<td>0.97±0.13</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>0.94±0.09*</td>
</tr>
</tbody>
</table>

* Control groups were given saline injections while the acetaminophen administered groups were given the compound in a single administration of 500 mg/kg, i.p. (mice) or in repeated dosing in rats (500 mg/kg, two days) as described in the legend to Table 1. The incubation mixture consisted of the same components as described in the legend to Table 1 except that distilled water used instead of ethylmorphine was deproteinized with 1 ml of 10% trichloroacetic acid after incubation at 37°C for 30 min. After centrifugation, 3 ml of 0.67% thiobarbiturate dissolved in 0.5% sodium acetate solution was added to an aliquot (0.5 ml) of the resulting supernatant. The solution was mixed, boiled for 10 min, then the optical density of malondialdehyde formed was determined at 532 nm. Extinction coefficient of malondialdehyde was 156 × 10³ M⁻¹ cm⁻¹. Nmoles malondialdehyde formed/mg protein/30 min. Values are mean ± S.E. of 4 or 6 experiments. *Not significantly different from corresponding values in the control group.
Fig. 1. Effect of acetaminophen on Lineweaver-Burk plots of ethylmorphine N-demethylation in the presence and absence of EDTA in rats and mice. Liver microsomes of AAP-treated rats (500 mg/kg/day, 2 days, i.p.) and mice (500 mg/kg, i.p.) were prepared as described in MATERIALS AND METHODS. Km values for ethylmorphine N-demethylase of AAP treated mice (−−) and control (−−−) (A and B on the figure), and AAP-treated rats (−−−−) and controls (−−−−−−) (C and D on the figure) were calculated from Lineweaver-Burk plots. Experimental details on the incubation mixture were as described in Table 1 except that incubation was carried out at 37 °C for 20 min.

Effect of acetaminophen treatment on Km values for ethylmorphine N-demethylation in rats and mice

In vitro studies in our laboratory demonstrated that AAP was found to be a noncompetitive inhibitor for ethylmorphine N-demethylase. In the present study, in vivo effect of AAP on ethylmorphine N-demethylase activity was investigated. Fig. 1 shows a reciprocal plot of the mixed function oxygenase-dependent inhibition of ethylmorphine N-demethylation after AAP administration to rats and mice. As determined from a Lineweaver-Burk plot (18), Km values for control and AAP treated liver microsomes (mouse) were almost identical in the presence (0.83 mM) and absence (1.11 mM) of EDTA (0.1 mM) (Fig. 1A and 1B). Km values for rat liver microsomes were 0.25 mM in the presence of EDTA and 0.87 mM in the absence of the chemical (Fig. 1C and 1D).

Changes in microsomal electron transfer system after acetaminophen administration to rats and mice

To assess the functional state of liver microsomal drug metabolizing enzymes utilizing cytochrome P-450 as the terminal oxidase, an enzymatic reaction known to require cytochrome P-450 or to participate in the microsomal electron transfer system was measured. As shown in Table 3, AAP did not alter the specific activity of NADPH-cytochrome c reductase in both species after AAP treatment. The activity of NADH-ferricyanide reductase was slightly decreased, but such was not statistically significant, after AAP administration.
to rats. The enzyme activity in mice decreased to a greater degree (P<0.05) than that in rats. Table 3 also shows that AAP treatment produced a decrease in the amount of cytochrome P-450 in rats and mice, but there was no change in the amount of cytochrome b$_5$. 

### Table 3. Effect of acetaminophen on microsomal electron transfer system of rat and mouse liver

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Cytochrome P-450 Content</th>
<th>Cytochrome b$_5$ Content</th>
<th>NADPH-cytochrome c reductase</th>
<th>NADH-ferricyanide reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Control</td>
<td>0.964±0.079</td>
<td>0.696±0.083</td>
<td>0.064±0.005</td>
<td>2.002±0.145</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>0.652±0.058**</td>
<td>0.557±0.042*</td>
<td>0.055±0.006*</td>
<td>1.649±0.118*</td>
</tr>
<tr>
<td>Mouse</td>
<td>Control</td>
<td>0.615±0.034</td>
<td>0.415±0.054</td>
<td>0.065±0.003</td>
<td>1.623±0.054</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>0.394±0.042***</td>
<td>0.396±0.015*</td>
<td>0.055±0.004*</td>
<td>1.395±0.074**</td>
</tr>
</tbody>
</table>

Control groups were given saline injections while the acetaminophen administered groups were given the compound in a single administration of 500 mg/kg, i.p. (mice) or in repeated dosing in rats (500 mg/kg, two days) as described in the legend to Table 1. **mole/mg protein. ***mole/mg protein/min. *Not significantly different from corresponding values in the control group. **Significantly different (p<0.05) from corresponding values in the control group. ***Significantly different (p<0.01) from corresponding values in the control group. Values are mean±S.E. of 4 or 5 experiments.

### DISCUSSION

Our results herein show that there was no change in ethylmorphine N-demethylase activity in rats 3 hr after a single administration of AAP (500 mg/kg, i.p.), while significant decrease in the enzyme activity occurred in mice even after a single administration of the drug.

The decrease in ethylmorphine N-demethylase activity in AAP-treated mice and rats was not due to activation of liver microsomal lipid peroxidation, but to a significant depletion in the cytochrome P-450 content (Table 3). Kinetic data showed that administration of AAP to both rats and mice did not alter the Km values for ethylmorphine N-demethylase, however, Vmax values decreased in both species.

Since the activity of lipid peroxidation was greater in rats than in mice, the effect of EDTA on Km values for ethylmorphine N-demethylase was necessarily greater in rats than in mice. These findings were in accordance with our previous report (17).

The present work also demonstrated that AAP has no significant effect on the reductase activities associated with the microsomal electron transfer system, although the activity of NADH-ferricyanide reductase in mice was decreased. In contrast, cytochrome P-450 content was markedly decreased by AAP administration. Thorgeirsson et al. (9) demonstrated that incorporation of $^3$H-leucine into liver protein decreased even 3 hr after the administration of AAP, although this inhibition of protein synthesis may be the result of decrease in cytochrome P-450. The other components of drug metabolizing enzyme systems were not altered significantly except for the significant decrease in NADH-ferricyanide reductase in mice. Accordingly, this degradation of cytochrome P-450 was mediated through other mechanisms as described below.
Preliminary experiments in this laboratory have demonstrated that the in vitro addition of N-hydroxyaniline, analog of N-hydroxy-acetaminophen, to the cuvette containing liver microsomes caused a significant decrease in cytochrome P-450 CO-difference spectrum without appearance of cytochrome P-420. Moreover, addition of reduced glutathione prior to the addition of N-hydroxyaniline to the cuvette effectively blocked decrease in levels of cytochrome P-450 (data not shown).

From these results it can be speculated that liver cytochrome P-450 may be more susceptible to AAP metabolites than other constituents of the microsomal electron transfer system.

Finally, AAP is metabolized to an active metabolite, N-hydroxy-acetaminophen through liver microsomal drug metabolizing enzymes, and the labile metabolite formed from AAP by cytochrome P-450 reacts initially with cytochrome P-450 itself unless there is no scavenger(s) for the active metabolite existing in the microsomes. Therefore, severe destruction of liver function may be due to the active metabolite of AAP, but not to increase in lipid peroxidation.

Acknowledgements: This work was supported in part by a Grant in Aid from the Ministry of Education, Science and Culture, Japan. We are grateful for the excellent technical assistance of Misses Y. Sekiguchi and S. Ura.

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