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EFFECT OF MESITYLENE ON ETHANOL METABOLISM IN RAT LIVER MICROSONMES

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Received 9 July 1992, Revised 5 November, 1992

Increased catalase activity was observed in the liver microsomal fraction of ethanol-treated rats (10% v/v aqueous ethanol solution per os for 5 weeks). In contrast, cytochrome P-450 concentration and specific activity of NADPH-cytochrome c reductase remained at the same level as in the liver of control rats (drinking water). The ratio of microsomal H2O2-generation to catalase activity was lower in the "ethanol" group than in the control one. This phenomenon seems to be related to the increased contribution of the "peroxidatic" reaction (increased rate of ethanol oxidation). Administration of mesitylene (1,3,5-trimethylbenzene) by gastric tube for 3 days (5 mmoles per kg daily) increased cytochrome P-450 concentration, specific activity of NADPH-cytochrome c reductase and ethanol metabolism.

Ethanol is oxidised in the liver by at least three enzyme systems, mainly (80%) by alcohol dehydrogenase (ADH\(^1\)) in the cytosol [1], but also partly by the microsomal ethanol oxidizing system (MEOS) which involves cytochrome P-450 [2, 3] and catalase [4, 5, 6]. Studies with the use of

\(^1\)Abbreviations: ADH, alcohol dehydrogenase; MEOS, microsomal ethanol oxidizing system
catalase inhibitors indicate that this reaction is probably responsible for not more than 10% of ethanol oxidation [7].

Chronic ethanol consumption enhances the activity of the microsomal ethanol oxidizing system (MEOS) [2, 3]. Ethanol induces the molecular form of cytochrome P-450 (CYP II E1) [8, 9] which shows high activity towards ethanol and aniline as substrates [10, 11]. The specific concentration of alcohol-oxidizing cytochrome P-450, in liver microsomes, was increased 2 to 4 fold by administration of the following agents: isoniazid, trichloroethylene, pyrazole, imidazole and acetone [12, 13].

The oxidation of ethanol via catalase was described by Keilin & Hartree [14]. In the liver cells, catalase is mainly located in peroxisomes, but it has also been identified in the endoplasmic reticulum and cytoplasm [15, 16, 17]. Ethanol oxidation by rat liver catalase depends on the ratio of the H2O2-generation rate to catalase concentration [18].

Mesitylene (1,3,5-trimethylbenzene) is well known as a cytochrome P-450 inducer [19]; it increases the amount of the three previously described cytochrome P-450 isoforms in rat liver [19].

In the present study, we investigated the ability of mesitylene to induce cytochrome P-450-dependent enzyme activities and oxidation of ethanol. The results obtained with a mesitylene-treated group were compared with those for the ethanol-treated group.

MATERIALS AND METHODS

Rat liver microsomes were prepared by the method of Teschke et al. [20]. The washed microsomes were suspended in 0.15 M KCl. White male Wistar rats (200 - 250 g-weight) were used for all experiments. The animals were kept on a standard diet. Group I (control group) received drinking water for 5 weeks, group II – 10% (v/v) aqueous ethanol solution for the same time (ad libitum), group III drinking water and mesitylene dissolved in soya oil administered by gastric tube for 3 days (5 mmol × kg\(^{-1}\) per day) before being killed, and group IV received both ethanol for 5 weeks and mesitylene for 3 days.

Oxidation of ethanol by microsomes was monitored by measuring the production of acetaldehyde by the methods described previously [21]. The reaction mixture was prepared as described by Lieber & De Carli [3].
Cytochrome P-450 content in liver microsomes was assayed by the method of Omura & Sato [22] and cytochrome b5 content according to Johnson et al. [23]. The absorption coefficients of 91 mM$^{-1}$cm$^{-1}$ (450 nm - 490 nm) for cytochrome P-450 and 185 mM$^{-1}$cm$^{-1}$ (424 nm - 409 nm) for cytochrome b5 were used [22].

The activity of NADPH-cytochrome c reductase was assayed by following the reduction of cytochrome c at 550 nm [24].

The aminopyrine N-demethylase activity was determined by measuring formaldehyde production as described by Nash [25] and the aniline hydroxylase activity by formation of p-aminophenol [26]. The reaction mixture was prepared as described by Pyykkö [19].

Catalase activity was assayed by the Beers & Sizer method [27].

The rate of microsomal H$_2$O$_2$ generation was measured according to the procedure of Cederbaum et al. [28].

Protein determination was carried out using the biuret method [29].

Mesitylene (1,3,5-trimethylbenzene) (purity, 99%) was obtained from BDH Chemicals Ltd. (Poole, Dorset, England). All other reagents were of analytical grade.

RESULTS

As compared with the control rats, the mesitylene-treated group showed a significant increase in NADP-cytochrome c reductase activity, cytochrome P-450 concentration and ethanol metabolism (Table 1).

In contrast, chronic ethanol treatment (for 5 weeks) increased the rate of ethanol oxidation and cytochrome b5 contents by more than 100% but had no effect on cytochrome P-450 content and the activity of NADPH-cytochrome c reductase.

Administration of ethanol for 5 weeks and mesitylene for 3 days had an additive effect on ethanol metabolism. In comparison with the mesitylene treated rats, combined ethanol and mesitylene treatment did not increase the cytochrome P-450 content or NADPH-cytochrome c reductase activity.

In the ethanol-treated group, the aniline hydroxylase activity was increased by 92%. The animals treated with mesitylene for 3 days showed a 56% increase in aniline hydroxylation (Table 1). A similar rise in aminopyrine N-demethylase activity was observed in the liver microsomal
Statistics difference against the control group calculated by the Student's-t test was p<0.01; other values were not statistically different by this ct.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethanol</th>
<th>Mesylamine</th>
<th>Ethanol</th>
<th>Mesylamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1.87 ± 0.15</td>
<td>1.83 ± 0.16</td>
<td>1.82 ± 0.13</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.75 ± 0.03</td>
<td>0.69 ± 0.07</td>
<td>0.61 ± 0.04</td>
<td>0.41 ± 0.07</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.78 ± 0.03</td>
<td>0.72 ± 0.04</td>
<td>0.42 ± 0.03</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>Alcohol</td>
<td>2.81 ± 1.29</td>
<td>1.48 ± 1.4</td>
<td>1.48 ± 1.4</td>
<td>1.48 ± 1.4</td>
</tr>
<tr>
<td>Alcohol</td>
<td>2.61 ± 2.0</td>
<td>4.8 ± 0.8</td>
<td>9.8 ± 0.8</td>
<td>9.8 ± 0.8</td>
</tr>
<tr>
<td>Alcohol</td>
<td>6.96 ± 0.67</td>
<td>7.7 ± 1.3</td>
<td>7.7 ± 1.3</td>
<td>7.7 ± 1.3</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the percentage of the control value. Values are the means from 7 experiments ± SD and are all expressed per mg protein.

**Effect of mesylamine and ethanol on microsomal enzymes in rat liver**

*Table I*
fraction of rats treated with ethanol or mesitylene separately, or ethanol and mesitylene given simultaneously (Table 1).

Ethanol and/or mesitylene treatment did not change the microsomal protein content. These data obtained were in agreement with the findings of Pyykkö [19] and Howell et al. [30].

A 75% increase of catalase activity was noted in the ethanol-treated group. On the other hand, the rate of H₂O₂ formation decreased by 30% (Table 2). In contrast, the mesitylene-treated group showed a slight decrease in catalase activity and a 40% increase in the rate of H₂O₂-generation. Administration of mesitylene enhanced the ratio of the H₂O₂-generation rate to catalase activity, whereas in ethanol-treated group this ratio was decreased.

DISCUSSION

The results presented in this paper indicate that administration of mesitylene enhances cytochrome P-450 concentration and ethanol metabolism in rat liver. Increased concentration of cytochrome P-450 and ethanol oxidation were accompanied by enhanced activity of other P-450 mediated microsomal reactions. Mesitylene administration increased aniline hydroxylase activity too, but had little effect on the aminopyrine N-demethylase activity. It should be noted that the liver microsomal cytochrome b₅ concentration in mesitylene-treated rats remained at the control level.

Consequently, it seems probable that cytochrome b₅ does not limit hydroxylation reactions after mesitylene treatment. This confirms the findings of other investigators [31, 32, 33].

The induction of a mixed type caused by mesitylene, increased the concentration of the three isoforms of cytochrome P-450: CYP 2A1, CYP 2B1 and CYP 1A1 [9, 19]. Different substrate specificity of these enzymes was reported. Cytochrome CYP 2B1 preferentially demethylates aminopyrine. Aniline is hydroxylated by the three isoforms of cytochrome P-450: CYP 2A1, CYP 2B1 and CYP 1A1 [19]. In consequence, stimulation of aniline hydroxylation was higher than that of aminopyrine demethylase (Table 1).

These results indicate that in the mesitylene-treated group ethanol is oxidized, in addition to cytosol ADH, by the microsomal cytochrome P-450-dependent monooxygenase system.
By contrast, enhanced catalase activity was noted in response to chronic ethanol treatment (Table 2). No changes were observed in the cytochrome P-450 content in the ethanol-treated group (Table 1). These results are not consistent with those reported in earlier papers [2, 3]. Chronic ethanol consumption by rats resulted in an increase of NADPH-cytochrome P-450 reductase activity and in induction of a specific cytochrome P-450 isoforms [10, 11, 12, 34]. According to those paper, the induction of cytochrome P-450 isoform (CYP 2E1) by ethanol is associated with an increase in hepatic microsomal aniline and ethanol oxidation. However, treatment of the animals with different agents also results in an increase of cytochrome CYP 2E1 content (imidazole and acetone are more effective inducers than ethanol) [12].

Table 1 illustrates, the 92% and 104% elevation of the aniline hydroxylase activity and ethanol metabolism, respectively, as a result of ethanol consumption. The cytochrome b5 content was also increased more than two-fold after ethanol treatment (Table 1). This suggests that cytochrome b5 may participate in the transfer of the second electron to cytochrome P-450, and confirms the findings of other investigators [35, 36, 37].

On the other hand, catalase activity was increased by treatment with ethanol. However, decreased generation of H2O2 in the microsomal fraction of the same animals was found despite increased catalase activity (Table 2).

### Table 2

**Effects of ethanol and mesitylene on catalase activity and H2O2 - generation rate**

Values are the means from 7 experiments ± SD and are all expressed per mg protein. Numbers in parentheses indicate the percentage of the control value.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Catalase activity (ΔA240nm/min)</th>
<th>H2O2-generation rate (nm/min)</th>
<th>Ratio of H2O2-generation rate to catalase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.45 ± 0.03</td>
<td>1.37 ± 0.15</td>
<td>3.04</td>
</tr>
<tr>
<td>Mesitylene</td>
<td>0.40 ± 0.01 (89%)</td>
<td>1.88 ± 0.16 (137%)</td>
<td>4.70</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.79 ± 0.04 (175%)</td>
<td>0.97 ± 0.07 (71%)</td>
<td>1.23</td>
</tr>
</tbody>
</table>

All values were statistically different with respect to the control assuming $p \leq 0.01$.
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The nature of this phenomenon is unknown. Oshino et al. [18] have found that ethanol oxidation by rat liver catalase depends on the ratio of the \( \text{H}_2\text{O}_2 \)-generation rate to catalase concentration. A decrease of this ratio in microsomes of the ethanol-treated group caused an increase in the contribution of the "peroxidatic" reaction and, consequently, an increase in the apparent rate of ethanol oxidation (assuming that higher activity of catalase observed in the experiments reflects higher concentration of this enzyme). Consequently, in the mesitylene-treated group, an increase in the ratio of \( \text{H}_2\text{O}_2 \)-generation rate to catalase activity enhances the "catalatic" reaction (Table 2).

From our studies we conclude that chronic alcohol ingestion shifts ethanol metabolism in rat liver microsomes from the cytochrome P-450-dependent to the catalase-mediated pathway.

REFERENCES


