Sex differences in the metabolism of ethanol and acetaldehyde in normal subjects

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(Received 18 November 1983; 6 March 1984; accepted 28 March 1984)

Summary

1. Blood ethanol and acetaldehyde concentrations were compared in normal young male and female subjects after intravenous infusion of 0.5 g of ethanol/kg body weight.

2. After the infusion was completed, females had significantly higher mean concentrations of blood ethanol than males, but a significantly lower apparent volume of distribution ($V_d$) of ethanol ($0.56 \pm 0.06$ l/kg vs $0.68 \pm 0.17$ l/kg, $P < 0.05$). There were no differences in ethanol elimination rate (EER) (females $1.78 \pm 0.3$ mmol h$^{-1}$ kg$^{-1}$; males $1.87 \pm 0.41$ mmol h$^{-1}$ kg$^{-1}$). The mean value of the areas under the acetaldehyde/time curves (AUC) were significantly greater for males ($88.5 \pm 26.4$ μmol/l h) than for females ($58.6 \pm 31.5$ μmol/l h, $P < 0.05$).

3. Since the ethanol elimination rate was similar in both sexes, the observed differences in AUC for acetaldehyde may reflect the sex differences in metabolism of this substrate by the liver.

Key words: acetaldehyde, ethanol, liver diseases.

Abbreviations: AUC, area under acetaldehyde/time curve; EER, ethanol elimination rate.

Introduction

Alcohol consumption in Great Britain has been increasing in the past 20 years, but proportionately the rise has been most marked in women. Consequently, alcohol-related disorders, particularly liver disease, are becoming increasingly common in the female population [1]. Furthermore, clinical and epidemiological studies have suggested that women develop alcoholic liver disease at a lower daily alcohol intake than men and after a shorter period of excessive drinking [2–7]. Although sex differences in body weight and composition have been implicated, the exact reason for susceptibility to alcoholic liver disease in women is not known.

Recent interest in the pathogenesis of liver damage in alcoholic liver disease has centred on the role of acetaldehyde and the enzyme acetaldehyde dehydrogenase. Acetaldehyde metabolism is disturbed in patients with alcoholic liver disease, and this is due to depressed activity of the cytosolic fraction of acetaldehyde dehydrogenase [8–9]. It is not clear whether this is a primary abnormality in alcoholics or secondary to the liver disease [9–10].

We have investigated the hypothesis that the observed sex difference in susceptibility to alcoholic liver disease may be related to sex differences in the metabolism of ethanol and/or acetaldehyde. This study compares blood ethanol and acetaldehyde concentrations after a standard alcohol load in normal male and female volunteers.

Methods

Subject selection

Twenty normal healthy volunteer subjects were studied: 10 males (mean age 21.9 years, range 21–24 years) and 10 females (mean age 22 years, range 20–26 years). All subjects had a regular intake of less than 80 g of ethanol/week and all had normal liver function tests (including serum
bilirubin, aspartate and alanine aminotransferase and \( \gamma \)-glutamyltransferase). None was taking medications known to affect hepatic enzyme activity, including the oral contraceptive pill. Subjects in each group were matched for alcohol intake (\( \pm 30 \) g of ethanol/week). Subjects gave informed consent and the study was approved by the local Ethical Committee.

**Study protocol**

Studies commenced at 09.00 hours, each subject having fasted for 12 h and consumed no ethanol for 48 h before the study. An indwelling Teflon catheter was inserted into a forearm vein in each arm, one for ethanol administration and the other for blood sampling. Subjects were fasted throughout the study and remained at rest.

Absolute ethanol (0.5 g/kg body weight) was diluted to a total volume of 250 ml with sodium chloride solution (154 mmol/l) and administered intravenously over 75 min. Samples (2 ml each) of venous blood were taken immediately before the ethanol infusion and then every 20 min for 4 h.

**Sample handling**

Each 2 ml sample of blood was divided into 1 ml aliquots for duplicate analysis, and then placed into a 20 ml polycarbonate tube to which was added 0.1 ml of thiourea (1 mol/l), 0.25 ml of propan-1-ol (50 mmol/l, for use as an internal standard) and 8.65 ml of perchloric acid (0.36 mol/l). All solutions and containers were maintained at 0-4°C on ice. After thorough mixing, each sample was then stored on ice for up to 4 h and subsequently centrifuged at 2000 g for 10 min at 4°C. The supernatant was stored at -20°C for a maximum of 3 days.

**Blood ethanol and acetaldehyde assay**

Blood ethanol and acetaldehyde concentrations were determined by gas-liquid chromatography (GLC), by the head-space technique of Brien & Loomis [11].

**Standard curves**

Standard curves were prepared daily, using aqueous standards in the concentration ranges of 20–26 mmol/l for ethanol and 0–260 \( \mu \)mol/l for acetaldehyde. For each blood sample the concentrations of ethanol and acetaldehyde were determined by calculation of the peak height (expressed as a ratio to the peak height of the internal standard, propan-1-ol) and interpolation on the standard curve, using the slope and intercept of the line of best fit.

**Precision of assay**

Within-run precision of the assay was determined by analysing several samples of blood containing the same known concentrations of ethanol and acetaldehyde on one day. The results were similar to those reported by Brien & Loomis [11].

**Calculations and statistical analysis**

Ethanol concentration-time curves were produced for each subject and analysed using zero order kinetic analysis. The rate of ethanol elimination from blood \( (V_{\text{max}}) \) was obtained by linear least squares regression analysis of the decline in blood ethanol concentration. The derived ethanol concentration at the start of the ethanol infusion \( (C_0) \) was determined from the \( y \) intercept of the regression line; the apparent volume of distribution \( (V_d) \) of ethanol by dividing the total dose of ethanol by \( C_0 \) and then expressed per kg body weight. The total body ethanol elimination rate \( (\text{EER}) \) was obtained from the product of \( V_{\text{max}} \) and \( V_d \). The area under the ethanol concentration-time curve was calculated using the trapezoidal method, measuring from the moment of ethanol administration to 4 h later. Blood acetaldehyde/time curves were analysed for area under curve only.

Statistical comparisons between male and female groups were made, with Student's \( t \)-test being used for unpaired data.

**Results**

**Blood ethanol concentrations**

There were no significant differences in mean blood ethanol concentration between males and females during infusion of ethanol, and peak blood ethanol concentrations were similar. During the elimination phase, however, females had higher mean concentrations of blood ethanol and these were statistically significant at several time-points (Fig. 1). Females had a significantly lower \( V_d \) for ethanol (0.56 ± 0.06 \( \text{litre/kg} \)) than males (0.69 ± 0.17 \( \text{litre/kg}; P < 0.05 \)), but there were no sex differences in \( V_{\text{max}} \), EER or AUC for ethanol (see Table 1).

**Blood acetaldehyde concentrations**

There were no statistically significant differences between males and females for mean blood
Sex differences in ethanol metabolism

**Fig. 1.** Mean blood ethanol concentration/time curves for males (○—○) and females (△—△) ± SD (n = 10). *P < 0.05; **P < 0.01.

**Fig. 2.** Mean blood acetaldehyde concentration/time curves for males (○—○) and females (△—△) ± SD (n = 10).

![Blood ethanol concentration](image1)

![Blood acetaldehyde concentration](image2)

**Table 1.** Ethanol pharmacokinetics in males and females after intravenous infusion of ethanol

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>t-test</th>
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<tbody>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (mmol h&lt;sup&gt;-1&lt;/sup&gt; l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.21 ± 0.78</td>
<td>2.95 ± 1.15</td>
<td>N.S.</td>
</tr>
<tr>
<td>EER (mmol h&lt;sup&gt;-1&lt;/sup&gt; kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.78 ± 0.3</td>
<td>1.87 ± 0.41</td>
<td>N.S.</td>
</tr>
<tr>
<td>AUC (mmol/l. h)</td>
<td>36.25 ± 5.86</td>
<td>32.34 ± 6.29</td>
<td>N.S.</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt; (l/kg)</td>
<td>0.56 ± 0.06</td>
<td>0.69 ± 0.17</td>
<td>P &lt; 0.05</td>
</tr>
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</table>

Acetaldehyde concentrations at any time-point in the study (Fig. 2). However, when the AUC for acetaldehyde was calculated for each subject, the mean AUC for females (58.6 ± 31.5 μmol l<sup>-1</sup> h<sup>-1</sup>) was significantly lower than the mean AUC for males (88.5 ± 26.4 μmol l<sup>-1</sup> h<sup>-1</sup>, P < 0.05).

**Discussion**

After infusion of ethanol (0.5 g/kg body weight) intravenously in normal, healthy, young volunteers, we have demonstrated higher blood concentrations of ethanol in females than in males. One possible explanation would be a sex difference in the metabolism or excretion of ethanol. However, this study clearly demonstrates that there is no sex difference in the rate of clearance of ethanol from peripheral blood (V<sub>max</sub>) or in total body ethanol elimination rate (EER). Furthermore, other workers have been unable to demonstrate a sex difference in the pathways or rate of metabolism of ethanol [12-15]. An alternative explanation for the higher concentrations of blood ethanol found in females is the difference in the apparent volume of distribution of ethanol (V<sub>d</sub>) observed in this study. Females had a lower V<sub>d</sub> for ethanol than males, an equal dose per kg body weight therefore leading to higher peripheral blood concentrations of ethanol. Marshall et al. [15] have previously demonstrated a sex difference in V<sub>d</sub> for ethanol and shown a strong correlation with total body water (r = 0.99, P < 0.001) measured by a <sup>3</sup>H<sub>2</sub>O dilution method. Women have less body water and more body fat per kg body weight than men [16], thus explaining the difference in volume of distribution of ethanol between the two sexes. These differences may account for the increased susceptibility of women to ethanol-induced liver disease, but this has not been conclusively established.

Recently, there has been much interest in the role of acetaldehyde in the pathogenesis of ethanol-induced liver injury. Acetaldehyde is formed after oxidation of ethanol by alcohol dehydrogenase, catalase or the microsomal enzyme oxidizing system in the liver [17]. Acetaldehyde, a highly reactive toxic compound, is rapidly metabolized in the liver, by acetaldehyde dehydrogenase, to acetate [17] and only small concentrations enter the blood [18, 19]. After administration of ethanol to alcoholic subjects, higher blood levels of acetaldehyde have been reported than in controls [8, 19, 20]. Furthermore, after ethanol ingestion, relatives of alcoholic subjects develop higher concentrations of blood acetaldehyde than controls, suggesting a primary defect in acetaldehyde metabolism predisposing to alcoholism [21].
Studies of hepatic acetaldehyde dehydrogenase activity have shown a reduction in the activity of this enzyme in subjects with alcoholic liver disease compared with normal controls and subjects with other liver diseases [8, 9, 22, 23]. After abstention from ethanol, both a persistent reduction [9] and a return to normal activity [10] of this enzyme have been reported. It is therefore not clear whether the reduction in hepatic acetaldehyde dehydrogenase activity and consequent elevated levels of blood acetaldehyde reported in alcoholics is a primary abnormality or secondary to the effects of ethanol on the liver.

If acetaldehyde is important in the pathogenesis of ethanol-induced liver injury, a sex difference in the metabolism of acetaldehyde might contribute to the apparent susceptibility of women to such injury. We have compared blood acetaldehyde concentrations in normal male and female subjects after ethanol infusion. The technique used [11] for determination of acetaldehyde in blood may be subject to error due to non-enzymatic formation of acetaldehyde from ethanol during deproteinization. Thiourea is included to reduce this, but inhibition is not complete [24, 25]. The values reported here for blood acetaldehyde concentration may therefore be an overestimate of their true value. Nevertheless, within this study valid comparisons can be made between male and female groups. The mean area under the acetaldehyde/time curve (AUC), which reflects the total release of acetaldehyde into the peripheral blood, was significantly smaller in females than in males. There are several possible explanations for this finding. Firstly, higher blood concentrations of ethanol in females may have led to an increased excretion via the breath or urine and thus decreased presentation of substrate to the liver for oxidation to acetaldehyde. However, the sex difference in AUC for acetaldehyde is proportionately greater than the differences observed in either blood ethanol concentrations or $V_d$. Other factors must therefore be involved. Another theoretical possibility is that extrahepatic metabolism or excretion of acetaldehyde may differ between the sexes. We cannot comment on the contribution of such factors from the data in this study. However, more than 80% of ethanol elimination is due to hepatic metabolism [17] to acetaldehyde, the latter being rapidly metabolized by the liver as discussed previously. Therefore the most likely explanation of the observed difference in AUC for acetaldehyde is a sex difference in the rate of hepatic metabolism of this substrate, with a faster rate in females than males.

Similar results for acetaldehyde excretion in breath have been reported in mice after ethanol administration, with greater levels reported in males than females [26]. It was of interest that castration reduced the response of male mice to the female range but ovariectomy had no effect. Sex hormone influence on the activity of acetaldehyde dehydrogenase is a possible explanation of our findings in humans, and this area warrants further investigation.

A direct hepatotoxic action of acetaldehyde has been postulated in the pathogenesis of ethanol-induced liver injury [27]. As women are more susceptible than men to such injury, we might have expected to find higher acetaldehyde levels and AUC (acetaldehyde/time) in women, whereas our results demonstrate opposite findings. This suggests that a direct hepatotoxic action of acetaldehyde may not be the explanation for increased susceptibility of women to ethanol-induced liver injury.

Acetaldehyde may act as a substrate for the enzyme xanthine oxidase. During this reaction, oxygen-derived free radicals, such as superoxide, are produced and this pathway of acetaldehyde metabolism has been suggested as a route by which hepatocyte injury may occur [28]. Although speculative, subsequent metabolism of acetaldehyde leading to hepatocyte injury is an hypothesis which could link the results of this study with the increased susceptibility of women to alcohol-induced liver injury.

Acknowledgments

We thank Mrs Isabella Strachan for preparing this manuscript, and David Corina for his expert technical advice.

References

Sex differences in ethanol metabolism


