Influence of Starvation and Chronic Malnutrition on Lipid Peroxidation in Rats Treated with Ethanol

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Summary The effect of starvation and chronic food restriction on lipid peroxidation during treatment with ethanol was studied. The experiment was carried out on 64 female Wistar rats. The formation of malondialdehyde was determined in postmitochondrial liver fraction (basal level and after stimulation with ascorbate). The concentration of reduced glutathione was measured in the same fraction.

In rats fed *ad libitum*, the consumption of ethanol, given with the drinking water for either 3 or 28 days, promoted a slight, but statistically nonsignificant, activation of lipid peroxidation. In animals not given ethanol, a 3-day fast increased the formation of lipid peroxides after stimulation with ascorbate. However, some tendency toward inhibition of lipid peroxidation was established after consumption of a restricted diet (6–8 g standard pellet food daily) for 28 days. The treatments with alcohol for 3 and 28 days were combined respectively with a 3-day fast and a 28-day food restriction. In both a statistically significant increase in lipid peroxides was observed in comparison with the controls, which were fed *ad libitum* and were not given ethanol.

The concentration of reduced glutathione was decreased in all experimental groups, but only with the 3-day treatments did the extent of its depletion correspond to the activation of lipid peroxidation. The results show that both starvation and chronic malnutrition potentiate lipid peroxide formation in rats treated with ethanol.

Key Words: starvation, malnutrition, alcohol, lipid peroxidation, glutathione

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Alcoholism is often attended by malnutrition [1]. It is due both to the intake of "empty" calories from ethanol and to secondary factors such as anorexia, impaired absorption, and metabolic disturbances. Besides, in many cases socioeconomic factors and behavioral deviations contribute additionally to the malnutrition. Many authors consider lipid peroxidation (LPO) to be a basic mechanism leading to denaturation of the hepatic membrane structure in alcohol intoxication [2-6]. There are some observations that malnutrition inhibits LPO [7-9]. However, other data suggest that with reduced food intake the detoxification mechanisms against free radicals could be impaired, which would stimulate LPO [10-14].

The present study was aimed at establishing the influence of starvation and prolonged food restriction on LPO processes in rats treated with alcohol. The results were compared with the changes in the concentration of the hepatic reduced glutathione (GSH).

MATERIALS AND METHODS

Female Wistar albino rats with an initial body mass of 190–210 g were used. Sixteen animals were fed ad libitum and served as controls. The others were divided into the following 6 groups, each containing 8 animals:

- Three-day treatment with ethanol given with the drinking water (1st day, 6 vol.%; 2nd day, 9 vol.%; 3rd day, 12 vol.%) with free access to standard pellet food.
- Total food deprivation for 3 days with free access to drinking water.
- Combined 3-day treatment with ethanol and starvation (as above).
- Twenty-eight-day treatment with ethanol given with drinking water (1st week, 6 vol.%; 2nd week, 9 vol.%; 3rd and 4th week, 12 vol.%) with free access to standard pellet food.
- Restricted diet (6-8 g pellet food daily) for 28 days.
- Combined 28-day treatment with restricted diet and ethanol (as above).

The rats were killed at the end of the treatment by decapitation. Blood was collected in tubes containing 0.05 ml 0.1 M EDTA. Na₂, and plasma was obtained by centrifugation. Lipid peroxides in plasma were determined according to the method of Yagi [15]. The liver was removed, perfused via the portal vein with chilled saline solution and homogenized in 1.15 M KCl, containing 0.1 M Tris, pH 7.4 (dilution 1 : 4, w/v). Then the 9,000×g (30 min) supernatant fraction was used for determination of lipid peroxides. Their concentration was measured as a basal level according to Buege and Aust [16] and after stimulation with ascorbate [17]. The results were presented as the quantity of malondialdehyde (MDA), an end product of LPO, and were expressed per mg of protein, which was determined by the method of Schacterle and Pollack [18]. GSH in the same supernatant fraction was also determined [19]. The results were statistically processed by means of variation analysis at a significance level of p<0.05.

RESULTS

The changes in the body mass are presented in Table 1. As the intake of liquid in the animals treated for 3 days with alcohol + starvation was smaller than that in the rats receiving alcohol for 3 days but having free access to food, the amounts of ingested ethanol were unequal (an average of 77 mmol vs. 233 mmol per animal). The rats in both groups treated with ethanol for 28 days (with and without diet restriction) consumed practically the same volume of ethanol, an average of 36 mmol daily during the 1st week, 60 mmol during the 2nd, and 83 mmol during the 3rd and 4th weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body mass change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (after 28 days)</td>
<td>+18±2.3</td>
</tr>
</tbody>
</table>

3-day treatments
- Alcohol: +0.4±3.3**
- Starvation: -17.1±1.7**
- Alcohol+starvation: -16.2±1.4**

28-day treatments
- Alcohol: -1.5±7.5**
- Malnutrition: -23.8±4.8**
- Alcohol+malnutrition: -8.5±6.7**

The results are expressed as means±SD. Each experimental group contained 8 animals; and the control group, 16. Comparison with the control: **p<0.01.

Table 2. Lipid peroxides in plasma and postmitochondrial liver fraction and GSH in the same fraction.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma (nmol MDA/ml)</th>
<th>Basic level in postmitochondrial liver fraction</th>
<th>Level in postmitochondrial liver fraction after stimulation with ascorbate</th>
<th>GSH in postmitochondrial liver fraction (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.11±0.42</td>
<td>0.686±0.189</td>
<td>4.42±1.11</td>
<td>6.9±2.0</td>
</tr>
</tbody>
</table>

3-day treatments
- Alcohol: 3.93±0.54** 0.837±0.193 4.88±0.58 6.0±1.2
- Starvation: 3.20±0.38 0.681±0.210 6.15±1.44** 5.3±1.0*
- Alcohol+starvation: 3.80±0.31*** 0.883±0.133** 6.07±1.39** 4.8±1.6*

28-day treatments
- Alcohol: 3.17±0.39 0.839±0.321 5.22±0.84 5.1±0.8*
- Malnutrition: 2.70±0.20** 0.543±0.183 3.64±1.11 4.3±0.9**
- Alcohol+malnutrition: 3.56±0.45* 1.080±0.235*** 6.10±0.96** 5.0±0.7*

The results are expressed as means±SD. Each experimental group contained 7-8 animals; and the control group, 16. Comparison with the control: *p<0.05; **p<0.01; ***p<0.001.
Ethanol consumption for either 3 or 28 days led to a slight activation of LPO (Table 2). The 3-day fast increased the formation of lipid peroxides in the postmitochondrial liver fraction after stimulation with ascorbate. Malnutrition for 28 days revealed some tendency to decrease LPO, and this decrease was statistically significant in plasma. The combination of alcohol with fast or chronically restricted diet augmented the formation of lipid peroxides (both of their basal level and level after stimulation with ascorbate).

GSH was decreased in all experimental groups. Its reduction was most evident in the animals with alcohol + fast and chronic malnutrition without ethanol.

DISCUSSION

The 3-day treatment with ethanol increased the concentration of lipid peroxides in plasma only, which is an integral parameter reflecting the extent of LPO in the various tissues and organs [15]. A similar trend, although nonsignificant, could be seen in the hepatic postmitochondrial fraction both after 3- or 28-day ethanol treatment. This supports the opinion of the important role of LPO in alcoholism [4, 14].

The augmented lipid peroxide formation (after stimulation with ascorbate) in the 3-day fast could be related to the increased output of catecholamines during stress, which activates specific phospholipases. They release polyunsaturated fatty acids from the phospholipid molecules. During starvation an increased H$_2$O$_2$ formation in the liver also takes place, because of the stimulated $\beta$-oxidation [20]. The observed tendency toward decreased LPO in animals with chronically reduced food intake has been reported by other authors as well [21]. The elevated activity of some antioxidative enzymes [22-24] and the increased metabolism of MDA could be possible reasons for this reduction [21].

When ethanol intoxication was combined with fasting a statistically significant increase in lipid peroxides was established both in plasma and in liver (basal level and level after stimulation with ascorbate). Bearing in mind the considerably smaller ethanol intake in these animals (as compared with the group on alcohol only, in which the values of lipid peroxides were lower), one can assume that total starvation potentiates LPO in alcohol consumption.

Although the 28-day treatment with the restricted diet only decreased slightly lipid peroxide formation, the addition of reduced food intake to chronic alcohol consumption intensified the LPO processes (like starvation in the 3-day ethanol treatment). It is possible that acetaldehyde formed during the catabolism of alcohol could compete with MDA for NAD-dependent aldehyde dehydrogenase [21], which would decrease MDA metabolism.

The changes in GSH concentration in the 3-day treatments are in good accordance with the data on LPO. The increased formation of lipid peroxides in the postmitochondrial liver fraction after the combined 3-day treatment was clearly demonstrated, which corresponds to the low GSH value. The depletion of

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GSH favors LPO [11, 12]. One could suppose that fasting potentiates LPO by decreasing GSH. Such interrelation between the concentration of GSH and the extent of LPO were not established in the 28-day treatments. In spite of the comparatively low degree of LPO in the group with chronic malnutrition, the GSH concentration was also found to be rather low. In contrast, in the animals treated with both restricted diet and ethanol, by which LPO activation was obtained, GSH was less decreased. Obviously, LPO processes can not be simply explained by GSH depletion only.

Although the exact mechanisms of LPO require further elucidation, the present study shows that the interrupted or restricted food intake could potentiate LPO in alcohol consumption.

REFERENCES

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