EFFECT OF NALOXONE ON ALCOHOL-INDUCED HEPATOTOXICITY IN THE RAT

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SYNOPSIS

Histochemical and biochemical studies indicate that rats treated for 30 days with alcohol (10 g/kg; 40% solution) plus naloxone (i.p., 4 mg/kg), showed more prominent hepatocellular necrosis, leukocytic infiltration, and zonal distribution of the lesion than with alcohol alone. SGOT and SGPT activities, in vivo microsomal lipid peroxidation, and GSH level exhibited a trend of greater hepatic injury in the alcohol-plus-naloxone-treated animals than those treated with alcohol alone, whereas naloxone alone had no deteriorative effect on hepatic functions under the same conditions. The present study indicates that alcohol-induced hepatotoxicity is potentiated further by an anti-opiate mechanism, implicating the involvement of an opiate system in the alcohol-induced hepatotoxicity.

INTRODUCTION

Alcohol abuse has become the most important drug dependence problem in present societies all over the world. It is well-known that ethanol is a potent hepatotoxic agent and causes injury both at the level of cellular and subcellular membrane (Cederbaum & Rubin, 1975; Hawkins & Kalan, 1972; Lieber & Davidson, 1962). Although several recent studies (Catley et al., 1981; Jeffocate et al., 1979; Jeffocate et al., 1981; Mattila et al., 1981) have been directed towards understanding the sensitivity of alcohol dependence to naloxone they have resulted in several contradictory reports or observations, and the role of endogenous opiate system in alcohol intoxication still remains to be elucidated appropriately. In view of these facts, the present investigation was undertaken to determine whether chronic ethanol consumption which caused biochemical as well as physiological alterations initiated at the level of cellular and subcellular membrane can be counteracted by

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anti-opiate treatment, in order to reveal the plausible involvement of endogenous opiate system in alcohol toxicity.

METHODS

Materials

The male adult rats (100-150 g body wt.), used in these experiments, were of Charles-Foster Strain. Rats were kept at 22°C on a 12 hr. light - 12 hr. dark cycle. Unless otherwise stated, animals were given stock laboratory diet ad lib.

Rats were divided into 4 groups. Rats in Group I served as control group. Group II rats were treated orally with 40% alcohol daily at a dose of 10 g/kg through the 30th experimental day. Group III rats were treated daily with naloxone (4 m/kg, i.p.), half an hour before the treatment of alcohol. Group IV rats were treated with naloxone only.

Drugs and Reagents

Absolute alcohol was purchased from the Bengal Chemical, Calcutta. Naloxone was kindly supplied by Endo Laboratories, U.S.A. All other reagents were of analytical grades and purchased from Sigma Chemicals, U.S.A.

Measurement of enzyme activities

Animals were killed by decapitation 24 hours after the last treatment. Blood samples were collected, sera prepared, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were measured by the method of Reitman and Franknel (1957). Microsomal lipid peroxidation was measured according to the method of Klaassen and Plaa (1969). Glutathione content (GSH) was measured by using Ellman's reagent (1959) with the modifications of Jollow et al., (1974). Histological studies were carried out according to the method of Harris (1900) and Brown (1978) using haematoxylin-eosin stain. Statistical analysis was performed by Student's t-test.

DISCUSSION

The results of the present study, like those of (Cederbaum & Rubin, 1975) and of other investigators (Hawkins & Kalant, 1972; Lieber & Davidson, 1962) indicate that high doses of alcohol can produce fatty liver and elevated levels of serum transaminases in rats. They also clearly indicated that lipid peroxidation increases
significantly whereas reduced glutathione content, a good marker of determining hepatotoxicity (Deiss et al., 1970; Hochstein et al., 1978), undergoes considerable decrease leading to the conclusion that hepatocytes are severely damaged. Histological changes (see Figures 1 and 2) generally parallel the increase in serum transaminase levels, degree of lipid peroxidation as well as decrease in GSH levels (Table 1).

Table - 1

Naloxone Effects on Ethanol-Induced Changes in SGOT, SGPT Values, Lipid Peroxidation Rate and GSH Content of Rat Liver (means ± S.E.M.'s)

<table>
<thead>
<tr>
<th>Parameter Studied</th>
<th>Control</th>
<th>Alcohol (10 g/kg; 40% solution x 30 days)</th>
<th>Naloxone (4 mg/kg x 30 days)</th>
<th>Alcohol + Naloxone (4 mg/kg x 30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (uM Pyruvate/ mg protein/hr.)</td>
<td>6.35±0.07</td>
<td>8.95±0.04 *</td>
<td>6.40±0.15</td>
<td>9.58±0.05 *</td>
</tr>
<tr>
<td>SGPT (uM Pyruvate/ mg protein/hr.)</td>
<td>1.65±0.02</td>
<td>2.85±0.01 *</td>
<td>1.61±0.07</td>
<td>3.18±0.09 *</td>
</tr>
<tr>
<td>Lipid Peroxidation (OD x 10^-3 /mg protein)</td>
<td>10.16±1.15</td>
<td>19.71±1.03 *</td>
<td>10.25±2.13</td>
<td>23.36±1.06 *</td>
</tr>
<tr>
<td>GSH content (uM/g wet wt.)</td>
<td>3.52±0.17</td>
<td>0.49±0.19 *</td>
<td>3.94±0.27</td>
<td>0.07±0.01 *</td>
</tr>
</tbody>
</table>

* Rats were decapitated 24 hours after the last feeding of the alcohol on the 30th experimental day. Each group consisted 6 rats. Significance of difference from control was determined using Student's t-test.

Our study also reveals that naloxone alone does not produce any significant damage in the hepatocytes, as evident from the histological picture (see Figure 3). SGOT, SGPT, lipid peroxidation, and GSH level in naloxone-treated rats exhibited no significant alteration as compared to control indicating thereby that naloxone causes no
hepatotoxic injury. However, in animals treated simultaneously with alcohol and naloxone, prominent fatty changes and cellular necrosis were observed (see Figure 4). The advanced cellular necrosis formation in this case has also been supported by the fact that serum transaminases as well as in vivo lipid peroxidation levels were boosted to a great extent. Hepatocytes were also severely damaged as the GSH level went well below the normal level.

This study indicates that some endogenous opiate system, which may attenuate the alcohol-induced hepatotoxicity, is somehow antagonized by anti-opiate naloxone. Involvement of an endogenous opiate system, which is not induced by alcohol treatment alone, to prevent the alcohol-induced hepatotoxicity is obvious from the present study, although exactly which naloxone-sensitive endogenous system is responsible for this phenomenon is yet to be elucidated.

CONCLUSION

Based on the above study, we conclude that naloxone, instead of attenuating alcohol intoxication, potentiates alcohol-induced hepatotoxicity to a great extent. Further, we suggest involvement of an endogenous opiate system in preventing the alcohol induced hepatotoxicity.

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REFERENCES


Figure 1. Histology of the liver: normal.

Figure 2. Histology of the liver: following high doses of alcohol.
Figure 3. Histology of the liver: following naloxone.

Figure 4. Histology of the liver: following alcohol plus naloxone.