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Abstract

Chronic Ethanol Feeding Causes Oxidative Stress in Rat Liver Mitochondria. Prevention by S-Adenosyl Methionine

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INTRODUCTION

Much experimental evidence shows that oxidative stress is involved in the pathogenesis and progression of alcoholic hepatopathy.[1,2] Thus, chronic alcoholism is associated with decreased hepatic lipid reduced glutathione (GSH) levels and increased hepatic lipid peroxide levels.[2,3] Chronic ethanol feeding increases lipid peroxides and glutathione turnover in rat liver.[4] In addition, changes in mitochondrial morphology and function are also common in alcoholic hepatopathy.[5] Ethanol causes an increase in oxygen consumption[5] which in turn may increase the mitochondrial generation of reactive oxygen species. Oxygen free radicals are also generated in mitochondria through acetaldehyde oxidation by aldehyde oxidase.[6] Chronic alcoholism causes glutathione depletion in rat liver mitochondria;[7] therefore, liver mitochondria would likely be more susceptible to oxidative stress.

The aims of our study were: (1) to determine whether chronic alcoholism causes oxidative stress in rat liver mitochondria; (2) to determine whether a glutathione precursor, such as S-adenosyl methionine, prevents this oxidative stress.

MATERIALS AND METHODS

Animals Male Wistar rats of 4–6 months of age were used. They were divided into three groups.

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A group fed ethanol for 6 weeks, a control group, and a group fed ethanol for 6 weeks but treated with S-adenosyl methionine. Animals were pair-fed a liquid diet[8] containing 36% of calories as ethanol or a isocaloric mixture with maltose instead of ethanol. The amount of ethanol consumed daily by the ethanol-fed group was 13 g of ethanol/kg of body weight. S-adenosyl methionine was administered s.c. for 6 weeks at a daily dose of 12 mg/kg body weight.

Assays GSH was measured as in Garcia de la Asunción *et al.*;[9] Oxidized glutathione (GSSG) levels and malondialdehyde levels were measured by hplc as in García de la Asunción *et al.*[9] and Young *et al.*,[10] respectively.

RESULTS AND DISCUSSION

GSH/GSSG ratio and malondialdehyde (MDA) levels were measured in liver mitochondria as indices of oxidative stress. Our results show that chronic ethanol feeding causes a significant increase in mitochondrial GSSG levels, which causes a decrease in mitochondrial GSH/GSSG ratio (see Table I). However, GSH levels were not significantly lower in mitochondria from ethanol-fed rats than in pair-fed controls. Mitochondrial MDA levels were significantly higher in ethanol-fed rats than in controls (see Table I). Therefore, our results demonstrate that chronic ethanol feeding causes oxidative stress in rat liver mitochondria.

Administration of S-adenosyl methionine (SAM) prevented the increase in GSSG levels which occurs in liver mitochondria from ethanol-fed rats and, hence, it prevented the decrease in mitochondrial GSH/GSSG ratio (see Table I). Treatment with SAM also prevented the increase in MDA levels found in liver mitochondria from ethanol-fed rats (see Table I). Therefore, administration of SAM prevented oxidative stress in liver mitochondria from chronic ethanol-fed rats.

The occurrence of oxidative stress in liver mitochondria from ethanol-fed rats may be due to an impairment in antioxidant defence and/or an increased generation of reactive oxygen species. Fernandez-Checa *et al.*[7] previously found that ethanol-fed rats exhibit decreased mitochondrial GSH levels due to an impaired entry of cytosol GSH into mitochondria. This could render liver mitochondria more susceptible to oxidative stress. Nevertheless, in the present work we have not found a significant decrease in mitochondrial GSH levels in liver of ethanol-fed rats. Thus, an increased generation of reactive oxygen species by mitochondria – without GSH depletion – should not be ruled out as an important contributor to oxidative stress in liver from ethanol-fed rats.

Recently, we have demonstrated that mitochondrial glutathione oxidation correlates with oxidative damage to mitochondrial DNA.[9] Thus, the glutathione oxidation which occurs in liver mitochondria from ethanol-fed rats indicates that oxidative damage to mitochondrial DNA is likely associated to chronic alcoholism.

In conclusion, our results support the role of mitochondrial oxidative damage in the pathogenesis of alcoholic liver disease and point out

TABLE I Effect of chronic ethanol feeding on GSH, GSSG and malondialdehyde levels in liver mitochondria

	GSH (μmol/mg prot.)	GSSG (nmol/mg prot.)	GSH/GSSG	MDA (nmol/mg prot.)
Control	9.8 ± 2.8	89 ± 28	141 ± 71	7.5 ± 0.9
Ethanol-fed	8.6 ± 1.7	120 ± 24*	73 ± 20*	11.6 ± 1.9*
Ethanol-fed + SAM	9.9 ± 2.9	70 ± 40**	136 ± 50**	8.1 ± 2.9

Results are expressed as mean ± S.D. for 4–12 experiments. Statistically significant differences are indicated as follows:
*$P < 0.05$ vs the control group;
**$P < 0.05$ vs the ethanol-fed group.

the therapeutical usefulness of agents, such as S-adenosyl methionine, capable of preventing the oxidative stress associated with chronic alcoholism.

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