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Effect of leptin administration on membrane-bound adenosine triphosphatase activity in ethanol-induced experimental liver toxicity

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Abstract

Hepatic injury elicits intracellular stress that leads to peroxidation of membrane lipids accompanied by alteration of structural and functional characteristics of the membrane, which affects the activity of membrane-bound ATPases. We have explored the effect of leptin on hepatic marker enzyme and membrane-bound adenosine triphosphatases in ethanol-induced liver toxicity in mice. The experimental groups were control, leptin (230 μg kg^{-1}, i.p. every alternate day for last 15 days), alcohol (6.32 g kg^{-1}, by intragastric intubation for 45 days), and alcohol plus leptin. Ethanol feeding to mice significantly ($P < 0.05$) elevated the plasma leptin, alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT) and hepatic lipid hydroperoxides (LOOH), and plasma and hepatic total ATPases, Na^+, K^+-ATPase and Mg^{2+}-ATPase. There was a significant decrease in Ca^{2+}-ATPase and reduced glutathione (GSH). Leptin injections to ethanol-fed animals further elevated the levels of hepatic LOOH, plasma and hepatic total ATPases, Na^+, K^+-ATPase and Mg^{2+}-ATPase, while the Ca^{2+}-ATPase and GSH were decreased significantly. In addition, leptin administration was found to increase the plasma levels of leptin, ALT, ALP, GGT, Na^+ and inorganic phosphorous, and decrease the levels of K^+ and Ca^{2+} in ethanol-fed mice. These findings were consistent with our histological observations, confirming that leptin enhanced liver ailments in ethanol-supplemented mice.

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Introduction

Ethanol manifests its harmful effects either through direct generation of reactive metabolites, including free radical species that react with most of the cell components, changing their structures and functions, or by contributing to other mechanisms that finally promote enhanced oxidative damage (Nordmann 1994). The liver is the major target of ethanol toxicity, and the role of oxidative stress in the pathogenesis of alcohol-related disease, particularly in the liver, has been repeatedly confirmed (Lieber 1997). Peroxidation of membrane phospholipids not only alters the lipid milieu and structural as well as functional integrity of cell membranes, but also affects the activity of various membrane-bound enzymes including total ATPases, Mg^{2+}-ATPase, Ca^{2+}-ATPase and Na^+, K^+-ATPase (Rauchcova et al 1995). As membrane integrity is important for cell viability, this study was undertaken to evaluate the effect of leptin on membrane-bound phosphatases and inorganic cation transport in the liver of ethanol-treated mice.

Leptin, a 16-kDa peptide product of the obese (*ob*) gene (Zhang et al 1994), is a potent adipocyte-derived hormone that plays a key role in the control of energy balance and food intake (Pelleymounter et al 1995). Leptin receptors, initially found primarily in central nervous tissues such as the hypothalamus (Tartaglia et al 1995), have also been localized to other tissues, including the liver (Wang et al 1997). Interestingly, isolated hepatic stellate cells (HSCs) have been shown to produce leptin during the in-vitro transactivation process (Potter et al 1998). Further, it has been reported that serum leptin levels are increased in patients with alcohol-induced

cirrhosis (Henriksen et al 1999). These observations suggested a possible involvement of leptin in the pathogenesis of liver disease.

The aim of this study was to explore the effect of administering exogenous leptin on membrane bound adenosine triphosphatases such as total ATPases, Na^+, K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase, and inorganic cation transport in ethanol-mediated experimental hepatic toxicity in mice.

Materials and Methods

Animals

Sixty adult (4-week-old) male Swiss albino mice, bred and maintained in the local animal house, were used in this study. They were housed three animals per plastic cage ($47 \times 34 \times 18$ cm) lined with husk that was renewed every 24 h. The mice had free access to drinking water and standard pellet diet (Agro Corporation Private Limited, Bangalore, India). The animals were kept at room temperature ($26 \pm 2°C$) under semi-natural light–dark conditions (12-h light/dark). The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and the study was approved by the Institutional Animal Ethics Committee, Annamalai University.

Reagents

Mouse recombinant leptin (purity > 97% as determined by SDS-PAGE and HPLC) was purchased from Sigma Chemical Co. (St Louis, MO). Leptin was reconstituted by adding 0.5 mL 0.2 μm-filtered 15 mM HCl. After dissolving the protein, 0.3 mL 7.5 mM 0.2 μm-filtered NaCl was added. The dissolved protein was then stored in a refrigerator. The hormone was diluted with phosphate-buffered saline (pH 7.4) just before use. Ethanol was obtained from Cuddalore District, South India. All other chemicals used were of analytical grade and the organic solvents were distilled before use.

Experimental design

Animals were divided into four groups of 15 mice each. Groups 1 and 2 received a normal diet of standard pellets and isocaloric glucose from a 40% glucose solution. This was to ensure that the caloric intake by all the animals in the different groups was the same. Liver cell damage was induced in the mice of groups 3 and 4 by administering 16% ethanol, 1 mL each ($6.32\,g\,kg^{-1}$) as an aqueous solution using an intragastric tube daily for 30 days (Balasubramaniyan et al 2003). At the end of this period, the animals were treated as follows for the next 15 days. Group 1 animals continued to receive standard pellet diet and isocaloric glucose from a 40% glucose solution orally daily and served as control. Group 2 animals continued to receive standard pellet diet, isocaloric glucose from a 40% glucose solution orally and were administered exogenous leptin ($230\,\mu g\,kg^{-1}$, i.p.) every alternate day. Group 3 animals continued to receive standard pellet diet and 16% ethanol orally. Group 4 animals continued to receive standard pellet diet, 16% ethanol orally + leptin ($230\,\mu g\,kg^{-1}$, i.p.) every alternate day.

The total experimental duration was 45 days. At the end of this time, the animals were fasted overnight, anaesthetized with an intramuscular injection of ketamine hydrochloride ($30\,mg\,kg^{-1}$) and killed by cervical dislocation. Blood was collected in heparinized tubes and plasma was separated by centrifugation at $2000\,rev\,min^{-1}$. Plasma leptin levels were measured using a radioimmunoassay kit (Linco Research, INC). The assays were performed according to the manufacturer's instructions. Assay of alanine transaminase (ALT, EC 2.6.1.2) was performed by the method of Reitman & Frankel (1974), plasma alkaline phosphatase (ALP, EC 3.1.3.1) by the method of King & Armstrong (1988). The activity of plasma γ-glutamyl transferase (GGT; EC 2.3.2.2) was assayed by the method of Fiala et al (1972). Plasma inorganic phosphorous was measured by the colorimetric method using a Sigma-Aldrich kit (St Louis, MO). Plasma sodium, potassium and calcium were measured by flame photometry. Liver tissue was removed, cleared of blood and collected in ice-cold containers containing 0.9% NaCl for the assay of hepatic lipid peroxidation products, antioxidants and ATPases. The concentration of lipid hydroperoxides (LOOH) was estimated by the method of Jiang et al (1992) and the activity of reduced glutathione (GSH) was assayed by the method of Ellman (1959). The amount of phosphorus liberated by the enzymes were assessed to quantify the activity of total ATPases (Hokins et al 1973), Na^+, K^+-ATPase EC 3.6.3.9 (Jorgensen 1988), Ca^{2+}-ATPase EC 3.6.3.8 (Hjerten & Pan 1983), and Mg^{2+}-ATPase EC 3.6.3.2 (Ohinishi et al 1982). The levels of total protein in liver homogenate and plasma were quantified by the procedure of Lowry et al (1951).

Histological investigations

Liver slices fixed for 48 h in 10% formalin were processed for paraffin embedding following the standard microtechnique (Galighar & Kozloff 1971). Sections (5 μm) of liver were stained with haematoxylin and eosin (H&E) and mounted in neutral disterene dibutyl phthalate xylene medium (DPX) under a light microscope and examined.

Statistical analysis

Results were statistically evaluated using one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT). All the grouped data were statistically determined with SPSS/10 software. The differences were considered significant at $P < 0.05$.

Results

Effect of leptin and alcohol on histopathological changes in the liver

Figure 1a–d shows respectively the histopathological changes in the liver of untreated control, leptin-treated, alcohol-treated, and alcohol plus leptin-treated groups in mice.

Liver of control animals showed hepatocytes arranged around the central vein resembling the spokes of a wheel (Figure 1A). The liver sections of animals administered 16% ethanol revealed fatty degeneration predominantly of the macrovesicular type. However, very few uninvolved hepatocytes were seen in the top right corner (Figure 1C). Leptin + ethanol-treated liver (Figure 1D) showed fatty change (macrovesicular) involving all the hepatocytes. Leptin injections given to control mice showed dilated sinusoids with some areas showing degenerated hepatocytes, but the hepatocytes close to the central vein appeared normal (Figure 1B). These findings clearly indicated that exogenous leptin administration enhanced ethanol-induced hepatic injury.

Effect of leptin and alcohol on the plasma leptin and hepatic marker-enzymes

Table 1 shows the levels of plasma leptin and hepatic marker enzymes such as ALT, ALP and GGT in the control and experimental animals. Plasma leptin, ALT, ALP and GGT were significantly higher in ethanol-treated mice (group 3) as compared with those of the control mice (group 1). These levels were markedly elevated on administering leptin to ethanol-fed mice (group 4). Leptin itself (group 2) did not significantly alter plasma ALT, ALP and GGT levels but plasma leptin levels were significantly increased as compared with those of the untreated normal control (group 1) and ethanol-treated animals (group 3), respectively.

Effect of leptin and alcohol on hepatic LOOH and GSH

The levels of hepatic LOOH and the activity of GSH in control and experimental animals are summarized in

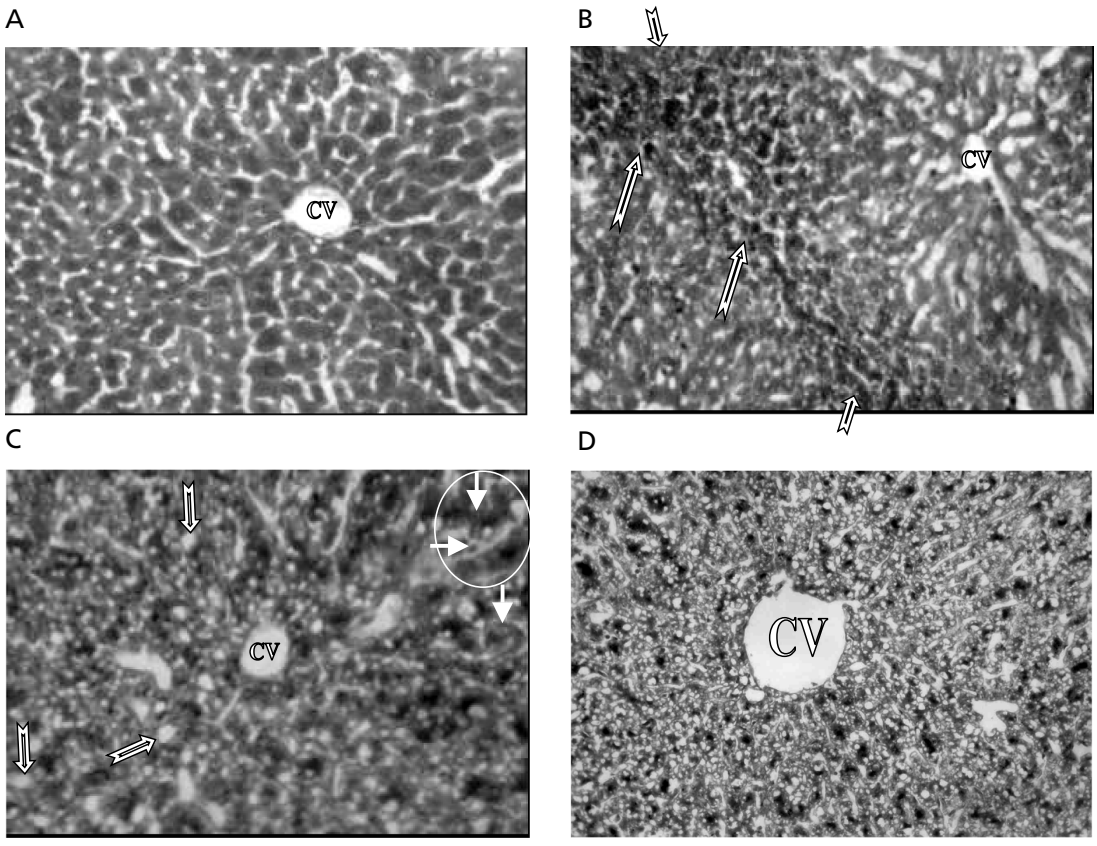

Figure 1 Representative photomicrographs of histopathological changes in the liver of mice after administering ethanol and/or leptin. A. Normal control mouse liver shows central vein (CV) surrounded by normal hepatocytes which are distributed in single plates in the form of spokes of a wheel. B. (⇛) Leptin-treated mouse liver shows dilated sinusoids with some areas of degenerated hepatocytes. The hepatocytes close to the central vein appeared normal. C. (⟶) Ethanol-treated mouse hepatocyte shows fatty degeneration predominantly of macrovesicular type (⇛). Very few uninvolved hepatocytes are seen in top right corner. D. All the hepatocytes around the central vein are involved and they show predominantly macrovesicular type of fatty degeneration. Photomicrographs were taken using a Sanyo (Aver Version 300) digital camera. Haematoxylin and eosin × 20. × – depicts the power of the objective.

Table 1 Effect of administering leptin and alcohol on plasma leptin and hepatic marker enzymes

Groups	Leptin (ng mL^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})	GGT (IU L^{-1})
Control	2.4 ± 0.4^{a}	28 ± 8.9^{ae}	59 ± 9.3^{ae}	4 ± 0.5^{ae}
Control + leptin	4.4 ± 0.4^{b}	29 ± 8.7^{be}	57 ± 12.7^{be}	5 ± 0.8^{be}
Alcohol	8.7 ± 1.6^{c}	118 ± 12.3^{c}	136 ± 12.9^{c}	17 ± 1.4^{c}
Alcohol + leptin	11.4 ± 2.3^{d}	152 ± 13.7^{d}	157 ± 13.3^{d}	27 ± 1.2^{d}

ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase. Values are means $\pm$ s.d. for six mice in each group. a–e: values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 2. Lipid peroxidation indicated by lipid hydroperoxides was significantly higher in the liver of alcohol-treated animals, whereas the activity of GSH was significantly lowered, as compared with the control mice (group 1). On administering leptin to alcohol-treated animals (group 4), further significantly elevated LOOH and lowered GSH levels were observed as compared with those of the untreated normal control mice (group 1) and alcohol-treated (group 3) mice, respectively.

Effect of leptin and alcohol on plasma electrolytes – Na^+, K^+ and Ca^{2+}

Table 3 shows the concentrations of plasma electrolytes and inorganic phosphorous of control and experimental animals. The plasma concentrations of Na^+ and inorganic phosphorous were significantly increased whereas, the plasma concentrations of K^+ and Ca^{2+} were significantly decreased in ethanol-treated mice (group 3) as compared with the normal control mice (group 1). Leptin administration to ethanol-treated mice (group 4) further elevated the levels of Na^+ and inorganic phosphorous and significantly reduced the levels of K^+ and Ca^{2+} in the plasma as compared with control (group 1) and ethanol-fed (group 3) mice. Leptin itself did not alter the levels of Na^+, K^+, and inorganic phosphorous significantly, whereas Ca^{2+} ion concentration was found to be decreased significantly as compared with those of the control (group 1) mice.

Table 2 Effect of administering leptin and alcohol on hepatic lipid hydroperoxides (LOOH) and glutathione (GSH)

Groups	LOOH (mmol (g tissue)$^{-1}$)	GSH (mmol (g tissue)$^{-1}$)
Control	64.7 ± 6.0^{ae}	0.43 ± 0.03^{ae}
Control + leptin	63.0 ± 6.2^{be}	0.41 ± 0.02^{be}
Alcohol	87.2 ± 7.0^{c}	0.29 ± 0.02^{c}
Alcohol + leptin	96.9 ± 9.2^{d}	0.23 ± 0.03^{d}

Values are means $\pm$ s.d. for six mice in each group. a–e: values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 3 Effect of administering leptin and alcohol on plasma electrolytes and inorganic phosphorous (iP)

Groups	Na^+ (mEq L^{-1})	K^+ (mEq L^{-1})	Ca^{2+} (mg dL^{-1})	iP (mg dL^{-1})
Control	148 ± 8^{ae}	5.4 ± 0.7^{ae}	9 ± 0.6^{ae}	8 ± 0.9^{ae}
Control + leptin	148 ± 7^{be}	5.5 ± 0.5^{be}	8 ± 0.5^{be}	7 ± 0.9^{be}
Alcohol	191 ± 7^{c}	3.3 ± 0.6^{c}	5 ± 0.5^{c}	16 ± 0.8^{c}
Alcohol + leptin	218 ± 7^{d}	2.3 ± 0.3^{d}	3 ± 0.4^{d}	19 ± 1.0^{d}

Values are means $\pm$ s.d. for six mice in each group. a–e: values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Effect of leptin and alcohol on the activity of membrane bound enzymes

Tables 4 and 5 show the activity of total ATPases, Na^+, K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in the plasma membrane and liver of control and experimental animals. The activity of total ATPases, Na^+, K^+-ATPase and Mg^{2+}-ATPase were found to be increased significantly, whereas Ca^{2+}-ATPase activity was significantly decreased in ethanol intoxicated mice (group 3) as compared with the normal control mice (group 1). Administration of exogenous leptin to alcohol-treated mice (group 4) further elevated the activity of total ATPases, Na^+, K^+-ATPase and Mg^{2+}-ATPase, and the activity of Ca^{2+}-ATPase was found to be lowered as compared with those of untreated control (group 1) and alcohol fed mice (group 3), respectively. Leptin itself (group 2) induced significant changes in hepatic total ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase, whereas Na^+, K^+-ATPase activity was not significantly altered as compared with the normal control mice.

Discussion

Damage to the liver after ethanol ingestion is a well-known phenomenon and the obvious sign of tissue injury is the leakage of cellular enzymes into plasma. The extent of hepatic damage is assessed by the level of released cytoplasmic ALT, ALP and GGT in circulation (Sallie et al 1991). Our results showed increased activity of

Table 4 Effect of administering leptin and alcohol on plasma ATPases

Groups	Total ATPase*	Na^+, K^+-ATPase*	Ca^{2+}-ATPase*	Mg^{2+}-ATPase*
Control	0.97 ± 0.14^{ae}	0.60 ± 0.1^{ae}	0.77 ± 0.1^{ae}	0.35 ± 0.1^{ae}
Control + leptin	1.07 ± 0.16^{be}	0.61 ± 0.1^{be}	0.74 ± 0.16^{be}	0.42 ± 0.1^{be}
Alcohol	1.96 ± 0.24^{c}	1.10 ± 0.1^{c}	0.49 ± 0.09^{c}	0.85 ± 0.1^{c}
Alcohol + leptin	2.43 ± 0.10^{d}	1.34 ± 0.1^{d}	0.31 ± 0.02^{d}	1.2 ± 0.2^{d}

*μmol inorganic phosphorous formed min^{-1} (mL plasma)$^{-1}$. Values are means $\pm$ s.d. for six mice in each group. a–e: values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 5 Effect of administering leptin and alcohol on hepatic ATPases

Groups	Total ATPase*	Na^+, K^+-ATPase*	Ca^{2+}-ATPase*	Mg^{2+}-ATPase*
Control	3.53 ± 0.35^{a}	1.86 ± 0.27^{ae}	6.76 ± 1.02^{a}	2.39 ± 0.3^{a}
Control + leptin	4.28 ± 0.23^{b}	2.48 ± 0.34^{be}	5.1 ± 0.33^{b}	3.07 ± 0.2^{b}
Alcohol	6.44 ± 0.38^{c}	4.55 ± 0.48^{c}	2.56 ± 1.05^{ce}	6.03 ± 0.3^{c}
Alcohol + leptin	7.50 ± 0.4^{d}	6.10 ± 0.23^{d}	2.42 ± 1.21^{de}	7.26 ± 0.2^{d}

*μmol inorganic phosphorous formed min^{-1} (mg protein)$^{-1}$. Values are means $\pm$ s.d. for six mice in each group. a–e: values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

ALT, ALP and GGT in the plasma of ethanol-treated mice, which correlated with a study by Senthil Kumar et al (2002). Exogenous leptin injections to ethanol-fed mice significantly elevated the plasma levels of ALT, ALP and GGT, demonstrating augmented hepatic damage caused by administration of leptin.

Chronic ethanol intake may alter serum leptin levels by different mechanisms. Chronic alcoholics, frequently undernourished, have reduced fat mass and are more susceptible to infections (Nicolas et al 2001). In this context Mantzoros et al (1998) reported that obesity and alcohol intake are positively related to serum leptin levels, whereas De Silva et al (1998) and Lagiou et al (1999) failed to find a relationship between ethanol intake and leptin. In alcoholic liver cirrhosis, raised serum leptin levels have been reported by Henriksen et al (1999). Finally, Nicolas et al (2001) found high serum leptin levels in both cirrhotic and non-cirrhotic alcoholic out-patients assisted for alcohol dependence. Our results were in agreement with the observations of the above researchers, as we had also observed significantly increased plasma levels of leptin in ethanol-treated mice.

Determination of membrane associated enzyme activity such as ATPases indicates membrane changes under pathological conditions (Suhail & Rizvi 1989). ATPases are lipid-dependent enzymes involved in the active transport process and have been implicated in the pathogenesis of liver cell injury (Rahman et al 2000). Enhanced susceptibility of membranes to oxidative stress can lead to loss of protein thiol, thereby changing membrane functions (Adhirai & Selvam 1997). Further, toxic insult to the liver can promote a variety of chemical reactions including depletion of GSH, which affects membrane-bound ATPases as they require SH groups to maintain their structure and function (Kaplowitz 2002; Marinou et al 2005). In this study, we observed increased activity of LOOH, total ATPases, Na^+, K^+-ATPase, Mg^{2+}-ATPase and decreased activity of Ca^{2+}-ATPase and GSH levels in the liver of ethanol-treated mice, and exogenous leptin administration further aggravated these changes.

Membrane-bound Na^+, K^+-ATPase is concerned with the maintenance of a low intracellular concentration of Na^+. Decreased activity of Na^+, K^+-ATPase can lead to a decrease in sodium efflux and thereby altered membrane permeability (Kako et al 1988). Acute or chronic ingestion of ethanol causes considerable metabolic derangement as well as changes in the structure and function of hepatocellular organelles (Lieber 2000). We have observed increased hepatic and plasma Na^+, K^+-ATPase activity in ethanol-supplemented mice. The exact mechanisms by which ethanol enhances Na^+, K^+-ATPase activity are unknown. It was originally postulated that the increase in Na^+, K^+-ATPase in the liver after ethanol administration was due to increased oxidative phosphorylation and oxygen consumption to supply ATP for ATPase activity (Rodrigo et al 1998). Thus our results were in agreement with those of Johnson & Crider (1989) who observed increased Na^+, K^+-ATPase in whole liver homogenates, but contrasted with the findings of Ricci et al (1981). The explanation for these different findings may be in the

different amounts of ethanol administered. Ricci et al (1981) administered 6 g kg^{-1} ethanol for five days, whereas we gave 6.32 g kg^{-1} ethanol.

Ethanol intoxication (group 3) caused a significant overall increase in the levels of total ATPases and Mg^{2+}-ATPase. Mg^{2+}-ATPase is involved in the energy requiring processes of the cell. The significant increase in the activity of Mg^{2+}ATPase on ethanol administration may have been due to the loss of protein-SH, a consequence of lipid peroxidative damage that is observed on ethanol intoxication. In this context our result agreed with that of Rodrigo et al (1998), who showed that chronic ethanol administration to rats resulted in elevated Mg^{2+}-ATPase activity. Administration of exogenous leptin further elevated the levels of hepatic lipid peroxidations indicated by increased LOOH and hepatic activity of total ATPases, Na^+, K^+-ATPase and Mg^{2+}-ATPase in ethanol-treated mice. Leptin administration to ethanol-fed mice results in hyperleptinaemia, which can lead to the interaction between superoxide anion (O^{2-}) and the free radical nitric oxide ($NO^{\bullet}$) to produce peroxy nitrite ($ONOO^{\bullet}$) (Fruhbeck 1999). Subsequently, nitric oxide may rapidly and spontaneously react with molecular oxygen to yield a variety of nitrogen oxides such as nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) (Grisham 1994). NO_2 and N_2O_3 are potent oxidizing and N-nitrosating agents, which in turn increase lipid peroxidation. This may be the cause for the significantly elevated levels of total ATPases, Na^+, K^+-ATPase and Mg^{2+}-ATPase observed in the plasma and liver of alcohol-treated mice administered leptin.

Transport of Ca^{2+} ions across plasma membranes is an important process for regulating the cellular concentration of free Ca^{2+}. In addition to Ca^{2+} influx and mobilization of intracellular Ca^{2+}, the maintenance of cytosolic Ca^{2+} by plasma membrane Ca^{2+}-ATPase is equally important (Pereira et al 1996). Perturbation of cell calcium homeostasis has been reported to occur during hepatocyte injury induced by a number of toxic compounds, including ethanol (Sepulveda & Mata 2004). It has been postulated, in this connection, that an irreversible rise in cytosolic calcium may be a relevant mechanism leading to cell death in toxic conditions (Parola et al 1990). Studies have shown alterations in almost all membrane-dependent biochemical processes to various extents. The ability of alcohol to inhibit Ca^{2+} transport has been postulated by Sepulveda & Mata (2004) and it has been attributed to their membrane partitioning property. Since active transport of calcium is accomplished by Ca^{2+}-ATPase located in plasma membrane, the depressant action of ethanol on the nervous system has focused the attention of researchers mainly on the brain membrane systems. However, it is equally important to study its action in the liver, as liver is the primary site for ethanol metabolism. The rapid oxidation of ethanol in liver causes marked disruption of normal metabolic processes. As shown in Tables 3 and 4, feeding ethanol for 45 days decreased the activity of Ca^{2+}-ATPase in the plasma and liver. This inhibition of Ca^{2+}-ATPase may account for the accumulation of intracellular calcium observed in tissues of ethanol-fed rats (Gandhi & Ross 1989). Moreover, administering exogenous leptin further lowered the hepatic GSH and Ca^{2+}-ATPase activity in the plasma and liver of mice treated with ethanol. Lowered GSH levels may be due to its increased utilization to scavenge the significantly elevated levels of ROS formed on administering ethanol and leptin.

Alcohol appears to directly influence the kidney's handling of sodium and other electrolytes, potentially resulting in hypernatraemia. In general, neither acute nor chronic alcohol consumption directly causes significant changes in serum sodium concentrations, although impaired sodium excretion is a frequent complication of advanced liver disease (Carini et al 2000). In our study, the concentration of plasma ionic Na^+ was marginally elevated whereas ionic K^+ and Ca^{2+} levels were significantly decreased on chronic alcohol exposure. Administering leptin to ethanol-fed mice elevated the levels of Na^+ and decreased the levels of K^+ and Ca^{2+}, which showed that leptin promoted the active transport of inorganic cations.

Histopathology of the liver sections of ethanol-treated mice showed fatty degeneration predominantly of the macrovesicular type, however very few uninvolved hepatocytes were seen in the top right corner (Figure 1). Control mice treated with leptin showed dilated sinusoids with some areas showing degenerated hepatocytes, but the hepatocytes close to the central vein appeared normal. Administering leptin to ethanol-supplemented mice significantly intensified the fatty degeneration and macrovesicular type of fatty change involving all the hepatocytes, which proved that elevated systemic leptin levels could augment ethanol-induced hepatotoxicity.

Conclusion

The results suggested that administration of exogenous recombinant leptin augmented ethanol-induced membrane damage. The aggravating effect of leptin may have been due to its ability to increase free radical production in the liver during ethanol metabolism.

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