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ETHANOL-INDUCED CHANGES OF THE LIPID PROFILE IN RAT KIDNEY

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A well known biochemical consequence of alcohol abuse is the fat accumulation in the liver. Taking into account that the kidney also contributes to the metabolism of ethanol, we have investigated the effect of ethanol consumption on the lipid profile and lipid peroxidation in the rat kidney. Thirty-two Wistar rats were randomly divided into two equal groups and were treated as follows: the control group and the ethanol-fed group (2 g/kg body weight/24 hours). The animals were sacrificed after 10 weeks, and respectively 30 weeks of ethanol feeding, and the renal tissue was isolated and analysed. Ethanol administration caused the significant increase in the triglycerides and cholesterol levels, toghether with the reduction of the phospholipids content, the most noticeable changes being registered after 30 weeks of ethanol ingestion. No significant modification regarding the lipid peroxidation process was reported, but a significant enhancement of glutathione peroxidase activity was noticed. This study suggests that chronic ethanol consumption alters the lipid in profile in the rat kidney, with possible consequences in the composition, structure and stability of the biomembranes, and disturbance of the renal function.

INTRODUCTION

Ethanol is oxidized primarily in the liver and the main pathway involves alcohol dehydrogenase (ADH). The metabolism of ethanol by ADH, couples the oxidation of ethanol to acetaldehyde with the reduction of nicotinamide adenine dinucleotide (NAD^+). The large amount of reducing equivalents generated by the ethanol metabolism via ADH distorts the redox homeostasis, and, consequently, serious metabolic disorders associated with different tissues and organs may occurre.[1] Various studies have demonstrated that the ethanol administration induces changes of the various lipid constituents in the liver of both animals and humans.[2,3] The lipid abnormalities detected after alcohol consumption include the alteration in the level of cholesterol, triglyceride, fatty acid and, particularly, fatty acid composition of membrane phospholipids.[4-6] Although hepatic effects of alcohol abuse on lipid metabolism have been extensively investigated, the changes of the lipid content in the kidney were subject to a lesser number of studies. Thus, the aim of the present study was to evaluate the effects of chronic ethanol administration, for 10 and 30 weeks, on the levels and the profile of the lipids in rat kidney.

RESULTS

Table 1 shows the lipid content in the kidneys of rats after chronic ethanol ingestion (ethanol-fed animals and control group). Significant increases in the lipid content were observed after 10 weeks

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of ethanol administration (the triglicerydes level rised by 29.3%, and the cholesterol by 21.2%, whereas the phospholipids level decreased by 13%). The modification of the lipid profile was even more evident after the administration of ethanol for a longer period of time. Thus, after 30 weeks of ethanol feeding, the level of triglycerides increased by 62.3%, the cholesterol level rised by 32.2%, while the phospholipids content decreased by 27.5% as compared as the control group.

Figure 1 shows the effects of chronic ethanol administration on the malondialdehyde (MDA) level (a measure of lipid peroxidation process) in rat kidney. No important change in the MDA level was recorded in the kidney of rats exposed to ethanol, for 10 and 30 weeks, respectively, in comparison to the control groups ($p > 0.05$).

Table 1

Effects of ethanol ingestion for 10 and 30 weeks on the content of lipids in rat kidney.
Values are means (n=8) ±SEM. * $p<0.05$ vs. control

	10 weeks experiment		30 weeks experiment	
	Control	Ethanol	Control	Ethanol
Triglycerides (μg/mg protein)	38.2 ± 2.5	49.4 ± 1.8*	40.1 ± 3.5	65.1 ± 4.8*
Cholesterol (μg/mg protein)	32.5 ± 1.5	39.4 ± 2.7*	36.3 ± 2.9	48.0 ± 3.9*
Phospholipids (μg/mg protein)	129.9 ± 10.5	113.1 ± 11.9*	135.8 ± 9.5	98.5 ± 15.8*

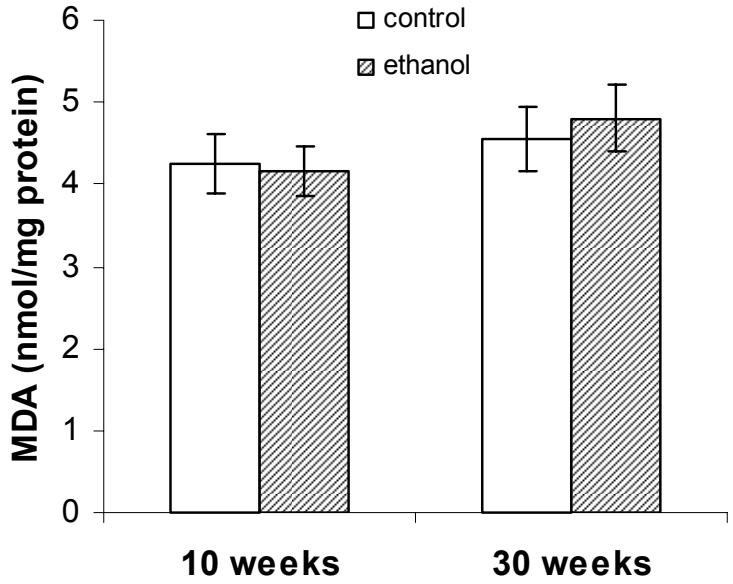

Fig. 1 – Effects of chronic ethanol consumption on the malondialdehyde level in rat kidney.
Values are means (n=8) ± SEM.

The effect of chronic ethanol feeding on Se-glutathione peroxidase (Se-GPx) activity is shown in Figure 2. The GPx activity increassed by 21.2% and 26.4% after 10 and 30 weeks of treatment, respectively.

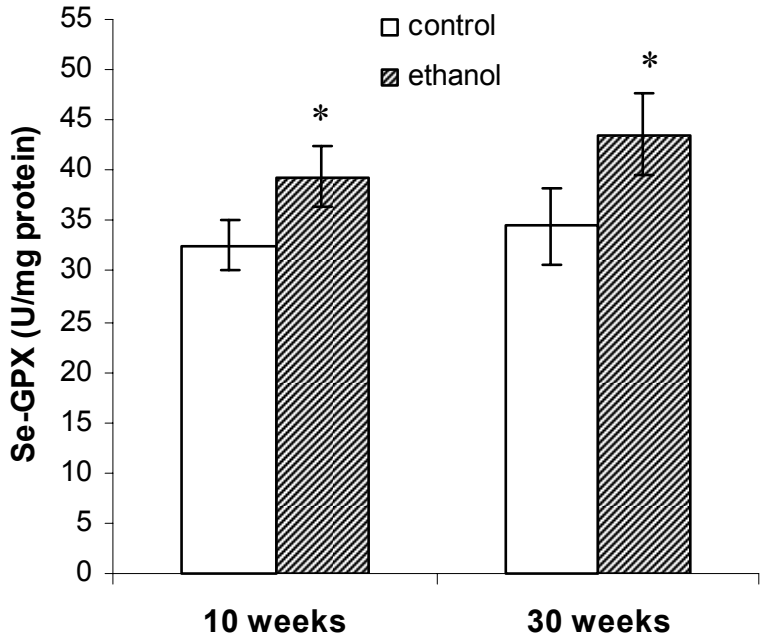

Fig. 2 – Effects of chronic ethanol consumption on the Se-GPx activity in rat kidney.
Values are means (n=8) ± SEM. * $p<0.05$ vs. control.

DISCUSSION

The oxidation of ethanol via the alcohol dehydrogenase (ADH) pathway results in the production of acetaldehyde and the reduced form of nicotinamide adenine dinucleotide (NADH). A major contribution to the adverse effects of ethanol arises from the changes in the redox status, due to the increase in NADH and decrease in nicotinamide adenine dinucleotide (NAD^+) concentration. The generation of a large amount of reducing equivalents in liver, via ADH pathway, has been correlated with a number of metabolic disorders, including hyperlipidemia.[4] Our previous studies have shown that a significant consequence of chronic ethanol administration was the increase of ADH activity in the rat kidney, and the subsequent decrease of NAD^+/NADH.[7] Thus, the capacity of the kidney to promote hyperlipidemia and fat accumulation after ethanol administration was of great interest. In order to clarify the changes of the lipid profile, the level of triglycerides, cholesterol and phospholipids were investigated.

Our results show changes in the level of triglyceride and cholesterol in the rat kidney, a more significant increase being registered after 30 weeks of ethanol administration. Alcohol feeding is known to increase the biosynthesis and to decrease the catabolism of fatty acid and cholestrol, resulting in hypertriglyceridemia and hypercholesterolemia.[8-10] The high levels of triglyceride after ethanol administration may be due to a number of factors such as the increased availability of the fatty acids for esterification.[11] Furthemore, our results show an increase of the cholesterol level in rat kidney, registered for both periods of ethanol administration. This finding may be related with an increase in 3-hydroxy-3-methyl-glutaryl CoA reductase activity by ethanol, taking into account that this enzyme catalyze is the rate limiting step in cholesterol biosynthesis.[12] Phospholipids, the major component of biomembranes, are the primary targets of peroxidation process, and they can be altered by ethanol consumption.[13] In the present study, we reported that phospholipids level was decreased in kidney tissue of ethanol-fed rats, mainly after the administration of ethanol for 30 weeks. The decrease in the phospholipids concentration may be due to an accelerated degradation, and can result in the modification of composition, structure and stability of biomembranes thus leading to cellular dysfunction. Earlier reports also have shown that the administration of ethanol has resulted in the decrease of the phospholipid content in both kidney and liver of rats.[11,14]

Multiple functional abnormality of kidney may be associated with ethanol-induced changes in membrane composition and lipid peroxidation.[15] Despite the high content in long-chain polyunsaturated fatty acids, we have noticed a low susceptibility of the kidney to the peroxidative

damage under ethanol exposure. This finding reveals either an enhanced protection against free radical attack, or an augmentation of fatty acid turnover, or perhaps both. In our study, an increase of GPx activity was reported, which might have contributed significantly to the removal of organic hydroperoxides. GPx is an antioxidant enzyme that reduces cellular hydroperoxides to alcohols, by performing an enzymatic protection against the detrimental action of the lipid peroxidation. In comparison to the kidney, a number of studies have shown that ethanol administration enhances the lipid peroxidation in the liver, and the peroxidation of liver lipids is a factor in the pathogenesis of ethanol-induced liver injury.[4,16,17] We assume that the kidney is less susceptible to the peroxidative damage than the liver because of its specific metabolism and physiology, and also because the peroxidative process is facilitated by the accumulation of iron in the liver after chronic ethanol ingestion.[18]

EXPERIMENTAL

Animals and tissue processing. Thirty-two healthy male Wistar rats weighing 140-160 g were housed two per cage under controlled conditions of a 12 hours light/dark cycle, 50% humidity and 24°C. Before the experiments, rats were monitored daily and had free access to water and to a standard pellet diet (10 g/100 g body weight/day). After one week of adaptation, the animals were randomly divided into two groups of sixteen each. Group 1, the control group, continued to receive water for fluid. Group 2, the ethanol-fed group, was treated daily with 1.0 mL of 35% (w/w) ethanol, equivalent to 2 g/kg body weight, as an aqueous solution, using an intragastric tube. After 10 weeks, eight rats of each group were killed by cervical decapitation under light ether anesthesia. The remained rats of each group were sacrificed in the same conditions after 30 weeks.
Both kidneys of each rat were quickly excised, cleared of the adhering fat, rinsed with a cold 0.9% sodium chloride solution, and weighted. One kidney of each rat was used for lipid peroxidation assay and the other one was used for lipids determination.

Lipid peroxidation. The kidney was homogenized in cold 0.15 M NaCl using a Potter-Elvehjem homogenizer. Homogenates were centrifuged at 10,000*g* for 15 min at 4°C. The levels of lipid peroxidation were measured via the thiobarbituric acid color reaction (TBARS assay) according to the method of Ohkawa *et al*.[19] A malondialdehyde (MDA) solution fredhly made by the hydrolysis of 1,1,3,3-tetramethoxy propane was used as a standard. The results were expressed as nmol of MDA per mg protein.

Glutathione peroxidase activity. The Se-glutathione peroxidase (Se-GPx) activity was assayed using a Sigma assay Kit (Sigma, Saint Louis, Missouri, USA), based on the principle that the oxidized glutathione (GSSG) produced upon reduction of tert-butyl hydroperoxide by GPX, is immediately recycled to its reduced form (GSH) with concomitant oxidation of NADPH to $NADP^+$. The oxidation of NADPH was monitored as a decrease in absorbance at 340 nm. One GPx-340 unit (U) is defined as 1 nmol of NADH oxidized per minute under the assay conditions. The enzymatic activity was expressed as U per mg protein.

Lipid profile analysis. In order to prepare the lipid extracts, each gram (wet weigh) of rat renal tissue was homogenized with 20 mL of chloroform/methanol (2/1, by volume), and lipids were extracted according to Folch *et al*.[20] The extracts were evaporated on a rotary evaporator and the dried lipid residues were dissolved in a small amount of chloroform. The kidney triglyceride concentration was measured enzymatically using the peroxidase-coupled method of McGrowan *et al*.[21] The cholesterol content was estimated by the method of Allain *et al*.,[22] while the phospholipids level was analyzed by the method of Zilversmit and Davis.[23] The concentrations were expressed as mg/mg protein.

Protein concentration. The protein concentration, expressed as mg/mL, was determined by the method of Lowry,[24] using bovine serum albumine as a standard.

Statistical analysis. All values were expressed as means ± SEM. The differences between control and ethanol-treated experimental groups were compared by Student's t test using standard social science statistical packages. The results were considered significant only if the p value was less than 0.05.

CONCLUSIONS

Our results suggest that alcohol consumption causes a significant change in the lipid profile of the rat kidney, as well as in an enhancement of the enzymatic protection against lipid peroxidation. The alteration in the lipid profile may be associated with some pathological changes observed in the kidney after ethanol consumption.

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