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Content of Liver and Brain Ubiquinol-9 and Ubiquinol-10 after Chronic Ethanol Intake in Rats Subjected to Two Levels of Dietary α-Tocopherol

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To assess the effect of chronic ethanol ingestion in the content of the reduced forms of coenzymes Q_9 (ubiquinol-9) and Q_{10} (ubiquinol-10) as a factor contributing to oxidative stress in liver and brain, male Wistar rats were fed *ad libitum* a basal diet containing either 10 or 2.5 mg α-tocopherol/100 g diet (controls), or the same basal diet plus a 32% ethanol–25% sucrose solution. After three months treatment, ethanol chronically-treated rats showed identical growth rates to the isocalorically pair-fed controls, irrespectively of α-tocopherol dietary level. Lowering dietary α-tocopherol led to a decreased content of this vitamin in the liver and brain of control rats, without changes in that of ubiquinol-9, and increased levels of hepatic ubiquinol-10 and total glutathione (tGSH), accompanied by a decrease in brain tGSH. At the two levels of dietary α-tocopherol, ethanol treatment significantly decreased the content of hepatic α-tocopherol and ubiquinols 9 and 10. This effect was significantly greater at 10 mg α-tocopherol/100 g diet than at 2.5, whereas those of tGSH were significantly elevated by 43% and 9%, respectively. Chronic ethanol intake did not alter the content of brain α-tocopherol and tGSH, whereas those of ubiquinol-9 were significantly lowered by 20% and 14% in rats subjected to 10 and 2.5 mg α-tocopherol/100 g diet, respectively. It is concluded that chronic ethanol intake at two levels of dietary α-tocopherol induces a depletion of hepatic α-tocopherol and ubiquinols 9 and 10, thus contributing to ethanol-induced oxidative stress in the liver tissue. This effect of ethanol is dependent upon the dietary level of α-tocopherol, involves a compensatory enhancement in hepatic tGSH availability, and is not observed in the brain tissue, probably due to its limited capacity for ethanol biotransformation and glutathione synthesis.

Keywords: Chronic ethanol ingestion, ubiquinols, α-tocopherol, total glutathione, liver, brain

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INTRODUCTION

Oxidative stress has been suggested to contribute to the toxic effects of ethanol consumption produced in the liver of experimental animals and in extrahepatic tissues including gastric mucosa, heart, testes, and central nervous system.[1–3] This condition is established by an increased generation of oxygen- and ethanol-derived free radicals at the microsomal level, as well as oxyradical production by cytosolic xanthine oxidase and the mitochondrial respiratory chain, coupled to a derangement in antioxidant defense systems.[3] Although clinical data support this contention in alcoholics, the role of oxidative stress in the pathogenesis of ethanol-induced cellular injury has not been clearly established. However, both experimental and clinical studies suggest that dietary regimens diminishing or preventing ethanol-induced free radical-mediated reactions may ameliorate the incidence of ethanol toxicity in humans.[3,4] This suggestion is based on the nutritional depletion of antioxidant components reported in alcoholics, represented by depressed serum levels of ascorbic acid,[5,6] selenium,[6–10] zinc,[10,11] and α-tocopherol.[6,9,10,12–14] In addition, diminished content of hepatic α-tocopherol has been reported in patients with alcoholic liver disease[15,16] and in experimental animals subjected to different models of prolonged ethanol ingestion,[17–20] an effect that does not seem to be due to a decreased absorption of the vitamin.[21] The effects of chronic ethanol ingestion on rat liver α-tocopherol levels, however, appear to depend upon the ethanol intake and on the vitamin E content of the diet.[18,22] Antioxidants incorporated into biological membranes control the deleterious effects of free radicals and electronically excited states by preventing the initiation and propagation reactions of lipid peroxidation. This is accomplished by a free-radical scavenging action or by singlet molecular oxygen quenching, as shown for α-tocopherol, carotenoids, and oxycarotenoids.[23] Besides these compounds, coenzyme Q has been proposed to act as an antioxidant protecting a number of cellular membranes from free radical damage, in addition to its main function as an electron shuttle in the mitochondrial electron transport system.[24] Structurally, coenzyme Q contains a quinone ring and a long-chain isoprenoid moiety that provides the molecule with its hydrophobic character. A one-electron reduction of the ring structure results in the formation of a relatively stable semiquinone that defines the antioxidant properties of coenzyme Q, which has been supported by numerous studies in subcellular and cellular systems, intact animals, and human subjects in the clinical setting.[24] Contrarily to α-tocopherol and carotenoids, coenzyme Q is synthesized in the endoplasmic reticulum–Golgi system of the liver and is subsequently distributed to various cellular membranes.[25] In view of these considerations, the present work evaluates the influence of prolonged ethanol intake on the content of the reduced form of the coenzymes Q_9 (ubiquinol-9) and Q_{10} (ubiquinol-10), as a possible factor contributing to oxidative stress in rat liver and brain. Studies were carried out at two different levels of dietary α-tocopherol and results obtained were correlated with changes in the content of total glutathione (tGSH), the reduced component of which is the main cellular water soluble antioxidant.

MATERIALS AND METHODS

Animal Treatment

Twenty-seven male Wistar rats maintained from weaning on a commercial diet (Purina Ind., Brasil) were individually housed in stainless steel cages in a room with controlled temperature (22–23°C) and photoperiod (12 h/day). They were randomly divided into two groups receiving purified basal diets containing either 10 mg α-tocopherol/100 g (diet I) or 2.5 mg α-tocopherol/100 g (diet II). The composition of the basal diet (g/100 g) was casein, 18; sucrose, 42.5; dextrin (white technical grade), 10; corn oil, 20; cellufil (non-nutritive

bulk), 3; vitamin mix, 2; mineral mix, 4; and L-cysteine, 0.5. Mineral mix and vitamin mix were prepared according to the National Research Council requirements for laboratory animals.[26] Half of the animals in each group were given an aqueous solution containing 32% ethanol and 25% sucrose (w/v)[27] by means of Richter drinking tubes for three months. Control animals were pair-fed isocalorically with ethanol-treated rats, being ethanol calories replaced by isocaloric amounts of sucrose. Experiments were performed under fed conditions both in control and ethanol-treated groups. In the latter case, the sucrose–ethanol solution was replaced by water 48 h before sacrifice, in order to avoid the effects of any residual ethanol. All animal care procedures were consistent with those outlined in the Guide for the Use and Care of Laboratory Animals.[28]

Biochemical Parameters

Animals were sacrificed by cervical dislocation, the abdominal cavity was opened, and ice-cold 0.9% w/v NaCl was infused through the inferior vena cava to eliminate blood. The livers and brains were removed, weighed, and were homogenized in 0.5 M $HClO_4$ for the determination of tGSH.[29]

For the determination of α-tocopherol, lipid extracts were prepared by homogenizing approximately 100 mg of tissue in 1 mL of water containing 0.05 mL of 10 mg/mL butylated hydroxytoluene to prevent lipid autoxidation. After the addition of 1 mL of 0.1 M SDS, the mixture was vortexed for 30 s, supplemented with 4 mL of hexane, vigorously vortexed for 1 min, and centrifuged for 5 min at $1000\times g$ to separate the organic layer. A 3 mL aliquot of the hexane layer was dried under N_2, and the residue was redissolved in 0.5 mL of methanol/ethanol (1/1, v/v) and filtered through a 0.22 μm-pore membrane. To determine ubiquinols, the samples were chemically reduced as follows. Aliquots of 0.4 mL of extract were mixed with 1 mL of methanol, 0.5 mL of distilled water, and 20 mg of sodium borohydride. This mixture was vortexed and incubated at room temperature for 30 min in the dark, and then extracted with 4 mL hexane. After centrifugation for 5 min at $1000\times g$, the hexane layer was removed, dried under N_2, and resuspended in 0.5 mL of methanol/ethanol (1/1, v/v) for HPLC analysis. Quantitative measurements of α-tocopherol and ubiquinols were made by HPLC[30] of lipid extracts using a Bioanalytical System electrochemical detector (LC-4C) coupled to isocratic delivery system. Samples were injected through a Reodhyne system (loop: 20 μL) to a C8 Nova-Pak column, 4 μm, with dimensions of 3.9×150 mm. Separation was done using a flow rate of 1 mL/min of the mobile phase (20 mM lithium perchlorate in methanol/water 98/2 [v/v]). Electrochemical detection was done using an applied oxidation potential of 0.6 V. Standard solutions of α-tocopherol, ubiquinol-9, and ubiquinol-10 were prepared by dissolving the pure compounds in 200 μL of ethanol : methanol (1 : 1).[31]

Reagents

Analytical reagents were obtained from Sigma Chemical Co. (St. Louis, MO), except for HPLC-reagents (Riedel-de Haen AG, Seelze, Germany). Ubiquinols (9 and 10) standards were obtained by reduction of the respective ubiquinones using sodium borohydride.[32]

Statistical Analysis

Results are expressed as means ± SEM for the indicated number of separate determinations. Statistical comparisons were performed by two-way ANOVA and the Tukey's test for multiple comparisons (GraphPad Prism™ Version 2.0, GraphPad Software, San Diego, CA).

RESULTS

Ethanol administration to rats fed basal diets containing 2.5 or 10 mg α-tocopherol/100 g diet

for three months resulted in a final caloric regimen characterized by relatively high percentages of ethanol, together with a decrease in the caloric percentages of protein (56% in both diets) and fat (53% in both diets) (Table I). Carbohydrate intake in all experimental groups was similar, as control animals subjected to diets I and II were isocalorically supplemented with sucrose (added to the basal diet) to compensate the calories given as ethanol (Table I). In these conditions, rats chronically treated with ethanol exhibited comparable growth rates to those observed in isocalorically pair-fed control animals, irrespectively to the dietary level of α-tocopherol (Table I).

Reduction in the dietary supply of α-tocopherol from 10 to 2.5 mg/100 g diet resulted in a significant 40% decrease in the content of hepatic α-tocopherol in control rats, without changes in that of ubiquinol-9, and increases in both ubiquinol-10 (46%; $P < 0.05$) and tGSH (52%; $P < 0.05$) levels (Table II). At the two levels of dietary α-tocopherol, chronic ethanol ingestion

TABLE I Body weight, growth rate, and diet, caloric and ethanol intakes in control (C) and chronically ethanol-treated (E) rats subjected to two levels of dietary α-tocopherol*

	Diet I (10 mg α-tocopherol/100 g diet)		Diet II (2.5 mg α-tocopherol/100 g diet)	
	C	E	C	E
Initial body weight (g)	103 ± 2	102 ± 2	102 ± 2	101 ± 1
Final body weight (g)	271 ± 2	273 ± 3	270 ± 3	261 ± 2
Growth rate (g/day)	1.83 ± 0.02	1.86 ± 0.04	1.84 ± 0.01	1.74 ± 0.02
Diet intake (g/day)	6.70 ± 0.01	6.70 ± 0.03	6.60 ± 0.01	6.60 ± 0.03
Protein intake (% of total calories)	16	7	16	7
Fat intake (% of total calories)	38	18	38	18
Carbohydrate intake (% of total calories)	46	41	46	41
Ethanol intake (% of total calories)	—	34	—	34

*Values shown are means ± SEM, for 5 animals per experimental group.

TABLE II Content of α-tocopherol, ubiquinol-9, ubiquinol-10, and total glutathione (tGSH) in the liver and brain of control (C) and chronically ethanol-treated (E) rats subjected to two levels of dietary α-tocopherol*

	Diet I (10 mg α-tocopherol/100 g diet)		Diet II (2.5 mg α-tocopherol/100 g diet)	
	C	E	C	E
Liver				
α-tocopherol (nmol/g liver)	60.4 ± 1.6	34.4 ± 0.9[a]	35.9 ± 0.5[b]	23.0 ± 0.6[a]
Ethanol effect (nmol/g liver)	−26.0 ± 1.9 (−43%)[†]		−12.9 ± 0.6 (−36%)[c]	
Ubiquinol-9 (nmol/g liver)	62.4 ± 2.1	26.7 ± 1.3[a]	58.6 ± 3.8	29.5 ± 3.2[a]
Ethanol effect (nmol/g liver)	−35.7 ± 3.1 (−57%)		−29.1 ± 5.8 (−50%)	
Ubiquinol-10 (nmol/g liver)	12.5 ± 0.5	5.0 ± 0.4[a]	18.3 ± 0.4[b]	13.1 ± 0.5[a]
Ethanol effect (nmol/g liver)	−7.5 ± 0.7 (−60%)		−5.2 ± 0.5 (−28%)[c]	
tGSH (μmol/g liver)	5.90 ± 0.07	8.46 ± 0.18[a]	8.96 ± 0.14[b]	9.74 ± 0.41[a]
Ethanol effect (μmol/g liver)	2.56 ± 0.16 (43%)		0.78 ± 0.48 (9%)[c]	
Brain				
α-Tocopherol (nmol/g brain)	20.5 ± 0.7	19.1 ± 0.9	8.2 ± 0.5[a]	7.0 ± 0.3
Ubiquinol-9 (nmol/g brain)	27.9 ± 0.8	22.3 ± 0.08[b]	26.9 ± 3.8	23.2 ± 1.0[b]
Ethanol effect (nmol/g brain)	5.6 ± 0.9 (−20%)		3.7 ± 0.9 (−14%)[c]	
tGSH (μmol/g brain)	2.01 ± 0.03	2.04 ± 0.03	1.47 ± 0.05[a]	1.55 ± 0.03

*Values shown are means ± SEM for 5 animals per experimental group. Significance studies were carried out by two-way ANOVA followed by Tukey's test for multiple comparisons: [a]$P < 0.05$ compared to the respective control group; [b]$P < 0.05$ compared to the control group subjected to diet I; [c]$P < 0.05$ compared to diet I.
[†]Numbers in parentheses indicate the ethanol effect expressed as percentage of the respective control values.

induced significant decreases in the liver content of α-tocopherol, ubiquinol-9, and ubiquinol-10 as compared to the respective control animals. The ethanol effect was more pronounced ($P < 0.05$) for α-tocopherol and ubiquinol-10 in rats subjected to 10 mg α-tocopherol/100 g diet than in those given 2.5 mg α-tocopherol/100 g diet (Table II). Contrarily to the above changes, chronic ethanol treatment enhanced liver tGSH content by 43% ($P < 0.05$) in conditions of higher dietary α-tocopherol supply and by 9% ($P < 0.05$) in animals subjected to the lower level α-tocopherol diet (Table II).

Reduction in the dietary supply of α-tocopherol from 10 to 2.5 mg/100 g of diet led to a decrease in the content of brain α-tocopherol (60%; $P < 0.05$) and tGSH (27%; $P < 0.05$) in control animals, without a significant alteration in that of ubiquinol-9 (Table II). No measurable ubiquinol-10 was detected in rat brain under the conditions studied. In the two α-tocopherol regimens, chronic ethanol administration did not significantly alter the content of brain α-tocopherol and tGSH, whereas that of ubiquinol-9 was reduced by 20% ($P < 0.05$) and 14% ($P < 0.05$) in animals given 10 and 2.5 mg α-tocopherol/100 g diet, respectively, compared to the respective control groups (Table II).

DISCUSSION

Data presented indicate that chronic ethanol ingestion leads to differential changes in the content of liver and brain antioxidants in animals concomitantly subjected to two levels of dietary α-tocopherol for three months. At the level of 10 mg of α-tocopherol/100 g diet, chronic ethanol treatment significantly decreases the hepatic content of α-tocopherol and ubiquinols 9 and 10, in addition to a compensatory enhancement in liver tGSH levels, suggesting the development of a higher oxidative stress status in this tissue. Most long-term studies in rats fed liquid diets containing ethanol have reported increased liver reduced glutathione (GSH) levels associated with enhanced hepatic turnover and synthesis of GSH, that seem to be triggered by the elevated loss of the tripeptide into the sinusoidal space[32] secondary to oxidant damage to the plasma membrane.[1,33] Reduction in the dietary supply of α-tocopherol from 10 to 2.5 mg/100 g diet effectively diminishes the content of the vitamin in the liver, and elicits a significant increase in liver tGSH. At this low level of dietary intake of α-tocopherol, chronic ethanol administration also decreases the content of hepatic α-tocopherol and ubiquinols 9 and 10 as found for the high level of vitamin exposure, however, the observed changes are of a smaller magnitude. The latter finding may be related to the observation that reduction in the dietary supply of α-tocopherol, which did not alter the hepatic levels of ubiquinol-9, significantly enhanced those of ubiquinol-10 and GSH. This effect may involve synthesis *de novo* of the biomolecules as an adaptive response to sustain a suitable antioxidant potential in the liver tissue against the prooxidant actions underlying ethanol biotransformation.[1,3,20,33,34]

At the two levels of dietary α-tocopherol, the basal levels of brain α-tocopherol, ubiquinol-9, and tGSH were substantially lower than those in the liver, supporting the contention that the brain tissue is less well protected against the action of free radicals and other reactive species.[35] As found in the liver, reduction in the dietary supply of α-tocopherol led to a 60% diminution in the content of brain α-tocopherol, however, this change is accompanied by a 26% decrease in brain tGSH levels as compared to the 52% enhancement observed in the liver. Furthermore, chronic ethanol intake did not alter the content of brain α-tocopherol and tGSH at the two levels of dietary α-tocopherol, whereas a marginal decrease in ubiquinol-9 was attained. These findings are probably related to differences in the metabolic characteristics of the liver compared with the brain tissue. In fact, the liver is the major organ involved in the biotransformation of xenobiotics, it has the greatest content of tGSH, and plays a key role in the interorgan GSH homeostasis.[36]

Thus, lack of major changes in brain antioxidants by chronic ethanol ingestion may be related to its rather low capacity for ethanol biotransformation, a process that predominates in the liver associated with the development of severe oxidative stress.[1,3,33] It is concluded that chronic ethanol feeding at two levels of dietary α-tocopherol significantly depletes the cellular stores of the hepatic membrane-bound antioxidants α-tocopherol and ubiquinols 9 and 10, thus contributing to ethanol-induced oxidative stress in the liver tissue. These effects of ethanol seem to be dependent upon the dietary level of α-tocopherol, are partially compensated by an enhancement in hepatic tGSH availability, and are not observed in the brain tissue, probably due to its limited metabolic capacity for ethanol biotransformation and GSH synthesis.

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