Interaction between vitamin E and glutathione in rat brain: Effect of chronic ethanol administration

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The protection against ethanol-induced lipid peroxidation is rendered by antioxidants such as vitamin E and glutathione (GSH) interacting with each other and also functioning independently. A study of the levels of GSH and activities of glutathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GST) in the cerebral cortex (CC), cerebellum (CB) and brain stem (BS) of vitamin E-supplemented and -deficient rats subjected to ethanol administration for 30 days was carried out. Chronic ethanol administration to vitamin E-supplemented rats elevated GP, GR and GST activities in the three regions and GSH levels in the CB. Chronic ethanol administration to vitamin E-deficient rats elevated GR activity in the three regions and GP activity in the CC and CB, decreased GST activity in the CC and CB, but did not alter GSH levels compared with normal rats subjected to chronic ethanol administration. The results indicate that vitamin E helps to maintain GSH levels to combat increased peroxidation while its absence has a deleterious effect.

Key words: vitamin E, glutathione, rat brain, ethanol, lipid peroxidation, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, malonaldehyde.

Introduction

Ingestion of large amounts of ethanol is known to have a depressant effect on the central nervous system (CNS) and over a prolonged period is associated with the reduction in CNS sensitivity to the effect of ethanol, leading to the development of tolerance and physical dependence. The neuronal plasma membrane has been shown to be the cellular target site for the action of ethanol in the brain. Apart from a direct physicochemical effect of ethanol on membranes,[1] increased formation of toxic peroxides consequent to its metabolism[2] is thought to play an important role in altered membrane functions in ethanol toxicity.

Vitamin E, a hydrophobic dietary antioxidant, is an integral component of biomembranes[3] and renders protection against free-radical-initiated injury,[4] both independently and through its interaction with the glutathione (GSH) system. The GSH system also helps in the elimination of the toxic metabolic products of ethanol, such as acetaldehyde.[5]

We have reported earlier that there is an increase in malonaldehyde ((MDA), as an index of lipid peroxidation) levels in different regions (e.g. cerebral cortex (CC), cerebellum (CB) and brain stem (BS)) of the rat brain after acute and long-term administration of ethanol.[6] The rise in MDA is dependent on the vitamin E status of the animal:[7] in vitamin E-supplemented rats (plus ethanol administration) the rise in MDA is significantly lower when compared with vitamin E-deficient rats after ethanol administration, indicating a protective antioxidant role for vitamin E.[6] We have also reported the effect of acute ethanol administration on the levels of GSH and activities of glutathione peroxidase (GP), glutathione reductase (GR) and glutathione-S-transferase (GST) in vitamin E-supplemented and -deficient rat brain regions (CC, CB and BS), and observed that the interaction between vitamin E and GSH is aimed at maintaining normal GSH levels to combat ethanol-induced peroxidative damage.[8] In this investigation, we present the levels of GSH and activities of GP, GR and GST in the three brain regions (CC, CB and BS) of vitamin E-supplemented and -deficient rats subjected to long-term ethanol administration in order to study the protective effects of vitamin E and the GSH system.

Materials and methods

Animals and diets

Adult albino rats of Wistar strain (body weight 150–200 g) were obtained from the College of Veterinary Sciences, Bangalore, India. They were maintained in a well-ventilated animal house with 12 h light and 12 h dark exposure. In each group six animals were used.

Vitamin E supplementation

One group of rats was fed orally DL-alpha-tocopherol at a daily dose of 10 mg/kg body weight in two divided doses for a period of 15 days.[9] The rats were fed standard rat pellet diet (Gold Mohur, Lipton India Ltd, Mumbai, India) *ad libitum*.

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Vitamin E deficiency

Vitamin E deficiency was induced in a second group of rats by feeding a vitamin E-deficient diet *ad libitum*, as suggested by Yang and Desai[10] with slight modifications, for a period of 2 months, as described by Marcus *et al.*[8] Coconut oil replaced vitamin E stripped corn oil and the salt and vitamin mixtures were prepared as described by Raghuramulu *et al.*[11] No significant differences in food consumption were observed during the period of study between the vitamin E-deficient and the control animals.[8] Random blood samples at the end of 2 months showed lowered serum vitamin E levels as assayed by the method of Kayden *et al.*[12] The vitamin E deficiency induced in this study was relatively mild and did not produce clinically manifest signs of Vitamin E deficiency as reported by us earlier.[8]

Ethanol administration

The two groups of animals were fed a mixture of 25% (w/v) sucrose and 32% (w/v) ethanol through a feeding bottle *ad libitum*, as described by Zucoloto *et al.*,[13] for a further period of 30 days and maintained on their respective diets.

Preparation of homogenates

The animals were killed by decapitation. The brains were dissected out and the cerebral cortex (CC), the cerebellum (CB) and the brain stem (BS) were separated out by the method of Sadasivudu and Lajtha.[14] Homogenates were prepared in appropriate media using a Potter–Elvehjem type homogeniser (Cole-Parmer Instruments International, Niles, IL, USA) with a Teflon pestle.

Glutathione assay

Glutathione levels were estimated in terms of non-protein sulfhydryl groups according to the method of Sedlak and Lindsay.[15]

Enzyme assays

Glutathione peroxidase (GP) was assayed according to the method of Paglia and Valentine.[16] The assay mixture, consisting of 2.6 mL of 0.05 mol/L phosphate buffer (pH 7) containing 1.125 mol/L sodium azide, 0.5 units of glutathione reductase, 0.1 mL of 0.15 mol/L GSH, 0.1 mL of 8.4 mmol/L NADPH and 0.1 mL of 2% homogenate of the tissue, was allowed to equilibrate for 10 min at 37 °C. The reaction was initiated by adding 0.1 mL of 2.2 mol/L H_2O_2. The increase in absorbance was measured at 340 nmol/L. The enzyme activity was calculated using a molar extinction coefficient of 6.1 $mmol^{-1} \times cm^{-1}$.

Glutathione reductase was determined by the method described by Carlberg and Mannervik.[17] The reaction mixture consisted of 2.5 mL of 0.1 mol/L Tris buffer (pH 8), 0.1 mL NADPH (4 mmol/L), 0.1 mL EDTA (0.015 mol/L), 0.1 mL GSSG (0.05 mol/L) and 0.1 mL 10% homogenate. The decrease in absorbance at 340 nm was measured and enzyme activity was calculated using a molar extinction coefficient of 6.1 $mmol^{-1} \times cm^{-1.}$

Glutathione-S-transferase activity was estimated by the method of Habig *et al.*[18] using 1-chloro 2,4-dinitrobenzene (CDNB) as substrate. The assay mixture consisted of 2 mL Tris buffer (0.15 mol/L, pH 6.5), 0.3 mL GSH (0.03 mol/L), 0.1 mL CDNB (0.03 mol/L), 0.5 mL water and 0.1 mL of the supernatant of the 10% homogenate of the brain tissue. The increase in absorbance was monitored at 340 nmol/L after the addition of the homogenate. The enzyme activity was determined using a molar extinction coefficient of 9.6 $mmol^{-1} \times cm^{-1}$.

Statistical analysis

Statistical analysis was carried out using Student's *t*-test. Comparisons were made between ethanol-treated normals[19] and vitamin E-supplemented plus ethanol, and ethanol-treated normals[19] and vitamin E-deficient plus ethanol-treated animals.

Results

In this study three groups of rats were used: one group fed a normal diet, a second group fed a vitamin E-supplemented diet and a third group fed a vitamin E-deficient diet. The choice of diet and response elicited have been described by Marcus *et al.*[8] Vitamin E supplementation brought about an increase in plasma vitamin E levels, and a mild vitamin E deficiency devoid of clinically manifest signs was observed by feeding the rats a vitamin E-deficient diet.

The GSH levels (μmol/g) and the activities of GP, GR and GST (μmol product formed/g/min) are given with respect to the three brain regions (CC, CB and BS) of vitamin E-supplemented and vitamin E-deficient rats subjected to chronic ethanol treatment in Table 1. The comparisons are made with respect to normal rats given ethanol for 30 days and reported by us earlier.[19]

Glutathione levels

There was no alteration in GSH levels in the three brain regions of vitamin E-deficient rats subjected to chronic ethanol treatment. However, in vitamin E-supplemented rats subjected to chronic ethanol administration, a significant increase in GSH levels was observed in the CB, while the level remained unchanged in the CC and BS.

Glutathione peroxidase activity

A significant increase in GP activity in the three brain regions of vitamin E-supplemented rats and in the CC and CB of vitamin E-deficient rats subjected to chronic ethanol administration was observed.

Glutathione reductase activity

Both vitamin E supplementation and deficiency significantly elevated the GR activity in all of the three brain regions of rats given ethanol for 30 days.

Glutathione-S- transferase

Vitamin E supplementation and chronic ethanol treatment increased the GST activity in the CC, CB and BS of rats, while vitamin E deficiency and ethanol treatment for 30 days depressed the GST activity in CC and CB.

Discussion

An important consequence of ethanol consumption is lipid peroxidation. The potential ill-effects of free-radicals are neutralized by the antioxidant defense systems. The primary defense systems include vitamins E, A and C, GSH, uric acid and enzymes such as superoxide dismutase, catalase and

Table 1. The levels of glutathione and activities of glutathione peroxidase, glutathione reductase and glutathione transferase in different brain regions of normal, vitamin E-supplemented and vitamin E-deficient rats subjected to chronic ethanol administration

	Normal with ethanol[a]	Vitamin E suplemented with ethanol	Vitamin E deficient with ethanol
Glutathione[b]			
Cerebral cortex	1.28 ± 0.28	1.40 ± 0.19	1.10 ± 0.19
Cerebellum	0.90 ± 0.23	1.20 ± 0.15*	1.10 ± 0.19
Brain stem	0.90 ± 0.26	1.00 ± 0.16	0.90 ± 0.18
Glutathione peroxidase[c]			
Cerebral cortex	25.10 ± 2.27	47.00 ± 5.48 ***	30.10 ± 3.43**
Cerebellum	27.70 ± 3.20	52.60 ± 6.87***	31.60 ± 2.15*
Brain stem	27.30 ± 1.08	53.70 ± 3.79***	30.50 ± 4.16
Glutathione reductase[c]			
Cerebral cortex	0.90 ± 0.15	2.30 ± 0.66***	1.69 ± 0.32***
Cerebellum	0.88 ± 0.22	1.60 ± 0.25***	1.80 ± 0.39***
Brain stem	0.75 ± 0.18	1.40 ± 0.25***	1.70 ± 0.31***
Glutathione-S-transferase[c]			
Cerebral cortex	1.99 ± 0.21	2.50 ± 0.32**	0.77 ± 0.15***
Cerebellum	1.60 ± 0.16	2.10 ± 0.27**	0.79 ± 0.23***
Brain stem	1.02 ± 0.23	2.30 ± 0.24***	0.81 ± 0.23

[a]Data from Marcus *et al.*[19] (reproduced with the kind permission of the publishers); [b] expressed as μmol/g tissue; [c] expressed as μmol product formed/g tissue/min. As compared with normals with ethanol treatment: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; mean ± SD; $n = 6$.

GP.[20] The cooperative interaction of the various antioxidants is responsible for protection against free-radical induced damage.

Vitamin E supplementation and chronic ethanol administration

In this study, chronic ethanol administration to vitamin E-supplemented rats elevated GP, GR and GST activities in CC, CB and BS, and GSH levels in CB only, when compared to normal rats subjected to chronic ethanol administration. Vitamin E is known to decrease lipid peroxidation and spare GP activity.[10] In our previous study, the administration of a single dose of ethanol did not alter GP activity in vitamin E-supplemented rat brain regions;[8] while in this study chronic ethanol administration showed a tendency to elevate the GP activity to that of control normal rats (vitamin E non-supplemented and minus ethanol administration). The increased GP activity helps to metabolise the increased peroxides consequent to ethanol administration and metabolism.

The elevated GR activity would satisfy the greater demand for GSH by both GP and GST activities. A similar increase in GR activity in vitamin E-supplemented rat brain regions, reported earlier by us,[8] was probably to protect the brain from any subsequent increase in peroxidations: a situation seen in the case of chronic ethanol administration.

Vitamin E deficiency and chronic ethanol administration

In vitamin E-deficient rat brain regions, chronic ethanol treatment brought about a significant increase in MDA levels,[6] in GP activity in CC and CB, and in GR activity in the three regions. There was a decrease in GST activity in CC and CB and the GSH levels remained unaltered as compared with normal rats subjected to chronic ethanol treatment. Chio and Tappel[21] have reported the inhibition of sulfhydryl enzymes such as GP by the products of lipid peroxidation, as also observed in the three brain regions of normal rats treated with ethanol for 30 days.[19] The comparatively low activities of GP (as compared with vitamin E non-supplemented and minus ethanol administration[8]) in the three regions of vitamin E-deficient, ethanol-treated rats is also suggestive of this type of inhibition. However, the slight elevation in GP activity in CC and CB of vitamin E-deficient, ethanol-treated rats and the concomitant decrease in GST is probably due to the reduction of hydroperoxides by GST.[22]

The GR activity maintains the GSH levels to combat lipid peroxidation and to conjugate with acetaldehyde formed from ethanol. The decreased GST activity in CC and CB of Vitamin E-deficient, ethanol-treated rats may lead to reduced removal of acetaldehyde by conjugation with GSH. However, the GSH levels remained unaltered in the three brain regions in spite of earlier reports of GSH exerting an antioxidant role even in vitamin E deficiency.[23]

The significant alterations in the activities of GSH-metabolising enzymes in vitamin E-supplemented rats are probably aimed at maintaining GSH levels in the brain and its effective utilization to ward off the peroxidative ill-effects of ethanol administration. Thus, the deleterious effects of ethanol through enhanced lipid peroxidation becomes more pronounced in vitamin E deficiency due to alterations observed in GSH-metabolising enzymes in spite of unaltered GSH levels.

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慢性酒精攝入影響的大白鼠腦組織中維生素E和谷胱甘肽的相互作用

摘要

維生素E和谷胱甘肽一類的抗氧化物可以防止酒精誘導的脂蛋白過氧化。這些抗氧化物即可相互合作又能獨立發揮其作用。本文報道了有關維生素E補充和缺乏對慢性酒精攝入（30天）影響的大白鼠大腦皮層，小腦和腦干中谷胱甘肽（GSH）水平，谷胱甘肽過氧化酶（GP），谷胱甘肽還原酶（GR）和谷胱甘肽轉化酶（GST）活性影響的研究。以正常大白鼠比較，慢性酒精攝入可以提高有維生素E補充的大白鼠大腦皮層，小腦和腦干中谷胱甘肽過氧化酶（GP），谷胱甘肽還原酶（GR）和谷胱甘肽轉化酶（GST）活性和小腦的中谷胱甘肽（GSH）水平。但是在維生素E缺乏的大白鼠，慢性酒精攝入僅能提高谷胱甘肽過氧化酶（GP）在大腦皮層，小腦和腦干中的活性以及谷胱甘肽過氧化酶（GP）在大腦和小腦中的活性，減低谷胱甘肽轉化酶（GST）在大腦和小腦中的活性，對谷胱甘肽（GSH）水平没有影響。該研究結果表明維生素E能幫助維持谷胱甘肽（GSH）水平從而抵抗過氧化作用的升高。維生素E的缺乏可導致有害的影響。

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