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ORIGINAL ARTICLE

Effects of ∝-ketoglutarate on antioxidants and lipid peroxidation products in rats treated with sodium valproate

Murugesan Vidya, Perumal Subramanian

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar – 608 002, Tamil Nadu, India

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Summary
Oxidative stress may cause free radical reactions to produce deleterious modifications in membranes, proteins, enzymes and DNA. Valproic acid is a major anti-epileptic drug with a broad spectrum of anti-epileptic activity. Chronic treatment with valproic acid can lead to elevated serum ammonia levels and specific oxidative metabolites of valproic acid have been associated with the drug's toxicity. The influence of sodium valproate treatment on lipid peroxidation and lipid profiles and the detoxifying effects of α-ketoglutarate on sodium valproate induced toxicity were studied in rats. The levels of thiobarbituric acid reactive substances, hydroperoxides and lipid profile variables (cholesterol, phospholipids, triglycerides and free fatty acids) were significantly increased in sodium valproate treated rats. Further, non-enzymic antioxidants (reduced glutathione) and the activities of the enzymic (superoxide dismutase, catalase, glutathione peroxidase) antioxidants were significantly decreased in sodium valproate treated rats. The levels were observed to be normal in α-KG + sodium valproate treated rats. These biochemical alterations during α-KG treatment could be due to (i) its ubiquitous collection of amino groups in body tissues, (ii) the participation of α-KG in non-enzymatic oxidative decarboxylation of the hydrogen peroxide decomposition process and (iii) its role in the metabolism of fats which could suppress oxygen radical generation and thus prevent lipid peroxidative damage.

Key words: α-ketoglutarate – sodium valproate – antioxidants – lipid peroxidation

INTRODUCTION

Valproate is often prescribed as a long-term therapeutic mood-stabilizing agent for individuals with bipolar disorder (Wang et al. 2003). Valproate (Valproic acid – VPA) is a major anti-epileptic drug with a broad spectrum of anti-epileptic activity. It has been the drug of choice in the treatment of most forms of primary generalized epilepsies and is also efficient against partial seizures (Rowan 1997). Valproic acid affects hepatocellular defence mechanisms and suggests that a predisposition of hepatocytes to oxidative stress may play a role in the fatal hepatotoxicity of valproic acid in epileptic patients (Klee et al. 2000). Specific oxidative metabolites of valproic acid have been associated with the clinically defined toxicity of the drug (Graf et al. 1998). Hyperammonemia is a documented side effect of valproate (VPA)

✉ P. Subramanian, Reader, Department of Biochemistry, aculty of Science, Annamalai University, Annamalainagar – 608 002, Tamil Nadu, India
☎ +91-04144-238343 extn: 218.
🖷 +91-04144-238080.
💻 psub@rediffmail.com

treatment (Stephens and Levy 1994). Lipid peroxidation may be involved as an additional mechanism of valproic acid induced liver damage in rats. Alterations in mental status may develop in patients receiving sodium valproate through a direct hepatotoxic effect of the agent or its metabolites (Zaret et al. 1982).

α-ketoglutarate (α-KG) is an intermediate of the citric acid cycle and is the natural ubiquitous collector of amino groups in body tissues (Lehninger et al. 2000). Because of its chemical structure, α-KG is a potent natural detoxifying agent (Velvizhi et al. 2002a, Dakshayani et al. 2002) and is used as an antidote to cyanide poisoning where it reacts with the cyanide molecule to form cyanohydrin as a reaction product (Dunaley et al. 1999). α-KG also improves myocardial protection in patients undergoing coronary operations (Kjellman et al. 1997). Further, α-KG also decreases muscle protein catabolism (Wernerman et al. 1990) and improves recovery after trauma. Another important function of α-KG consists in the formation of carnitine (Copper and Kristal 1997). Carnitine is a molecule that acts as a carrier of fatty acids into cell mitochondria so that proper metabolism of fats can proceed (Roe et al. 2000).

The detoxifying characteristics of α-KG were analysed in the present study by estimating the levels of thiobarbituric acid reactive substances (TBARS), hydroperoxides (the products of lipid peroxidation), non-enzymic antioxidant (reduced glutathione), activities of enzymic antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and the lipid profile variables (cholesterol, triglycerides, phospholipids and free fatty acids) in blood and tissues (liver and brain) so as to assess its role in suppressing the side effects induced by sodium valproate.

MATERIALS AND METHODS

Animals

Adult Wistar rats (180-220g) obtained from Central Animal House, Faculty of Medicine, Annamalai University, were kept at room temperature (32±2°C). All animal experiments were approved by the ethical committee, Annamalai University and were in accordance with the National Institutes of Health: Guide for the Care and Use of laboratory animals (NIH 1985). Animals were fed with the standard pellet diet (Agro Corporation Private Limited, Bangalore, India) which, as with water was available to the animals *ad libitum*.

α-ketoglutarate (disodium salt) was purchased from SRL Private Limited, Mumbai, India. Sodium valproate and all other chemicals used in this study were of analytical grade. The animals were randomized and divided into four groups (n=6 in each group). Group I animals served as controls. Group II animals were treated with sodium valproate (300 mg/kg body weight) every day orally for 8 weeks. Group III animals received sodium valproate at the same dose as in Group II along with α-KG (2 g/kg body weight) and Group IV rats received α-KG at the same dose as Group III animals for 8 weeks.

At the end of the experimental period, animals were sacrificed by cervical dislocation. Liver and brain tissues were excised for the determinations of biochemical parameters. The levels of TBARS (Nichans and Samuelson 1968), hydroperoxides (Jiang et al. 1992), GSH (Ellman 1959), cholesterol (Zlatkis et al. 1953), phospholipids (Zilversmit and Davis 1950), triglycerides (Foster and Dunn 1973), free fatty acids (Falholt et al. 1973) and the activities of superoxide dismutase (Kakkar et al. 1984), catalase (Sinha 1972), glutathione peroxidase (Rotruk et al. 1973) were analyzed in the liver and brain tissues.

Statistical analysis

The Mean±SD of the measured variables in each group was calculated. The Analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test was carried out to detect significant differences between the control and the experimental groups.

RESULTS

The levels of TBARS, hydroperoxides, lipid profiles such as cholesterol, phospholipids, triglycerides, free fatty acids and the antioxidant status of SOD, catalase, GPx, GSH in the liver and kidney tissues are shown in Table 1 and 2. A decrease in the status of antioxidants such as catalase, SOD, GPx and GSH with a concomitant increase in the rate of TBARS and hydroperoxides were observed in the sodium valproate (Group II) treated rats when compared with the control (Group I) rats. On treatment with α-KG (Group III) there was a significant increase in the activities of antioxidant enzymes and glutathione with a corresponding decrease in TBARS and hydroperoxides when compared to the sodium valproate (Group II) treated rats. The α-KG treated group (Group IV) alone did not show any significant change.

The levels of cholesterol, phospholipids, triglycerides and free fatty acids were increased significantly in the sodium valproate treated rats (Group II). The sodium valproate and α-KG treated (Group III) rats showed significantly lower levels when compared with the sodium valproate treated rats. The levels of these parameters were found to be normal in the α-KG (Group IV) treated rats.

Table 1. **Effect of α-ketoglutarate on liver antioxidant and lipid profile status against sodium valproate induced toxicity in rats**

Parameters	Control (Group I)	NaVPA (Group II)	NaVPA + α-KG (Group III)	α-KG (Group IV)
TBARS (mM/100g tissue)	0.90 ± 0.26	2.03 ± 0.19*	1.65 ± 0.16^{*+}	0.80 ± 0.15^{ns}
Hydroperoxides (mM/100g tissue)	75.46 ± 6.51	110.06 ± 9.14*	92.38 ± 3.64^{*+}	69.63 ± 1.95^{ns}
Superoxide dismutase (50% inhibition of NBT reaction/min/mg protein)	5.81 ± 0.59	3.44 ± 0.31*	4.74 ± 0.30^{*+}	6.66 ± 0.62^{c}
Catalase (µmoles/min/mg protein)	71.95 ± 1.60	45.14 ± 2.33*	61.41 ± 3.12^{*+}	71.37 ± 0.59^{ns}
Glutathione peroxidase (µg of GSH consumed/min/mg protein)	6.50 ± 0.11	4.72 ± 0.12*	5.20 ± 0.35^{*+c}	6.85 ± 0.54^{ns}
Reduced glutathione (mg/g tissue)	45.33 ± 4.13	22.53 ± 2.34*	37.46 ± 3.94^{*+}	46.66 ± 4.13^{ns}
Cholesterol (mg/100g tissue)	330.66 ± 16.52	581.33 ± 37.40*	405.33 ± 16.52^{*+}	336.00 ± 17.52^{ns}
Phospholipids (mg/100g tissue)	930.00 ± 37.41	1680.00 ± 74.56*	1310.00 ± 64.49^{*+}	920.00 ± 33.46^{ns}
Triglycerides (mg/100g tissue)	334.56 ± 10.97	660.37 ± 19.75*	408.88 ± 17.41^{*+}	328.00 ± 11.73^{ns}
Free fatty acids (mg/100g tissue)	664.88 ± 18.72	942.92 ± 39.72*	726.61 ± 47.68^{*+}	658.84 ± 48.20^{ns}

§ mean ± S.D; n=6
NaVPA – sodium valproate
α-KG - α-ketoglutarate
ANOVA followed by Least Significant Difference (LSD).
Group I is compared with groups II, III and IV (*statistically significant at the level 2α=0.05)
Group II is compared with group III ($^{+}$ statistically significant at the level 2α=0.05, ns-not significant)

DISCUSSION

The cytotoxic activity of valproate is the result of the generation of hydrogen peroxide and the production of highly reactive hydroxyl radicals (Tabatabaei and Abbott 1999). This could lead to the increased levels of TBARS and hydroperoxides and decreased levels of enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH) antioxidants in the Group II rats. Further sodium valproate treatment through a process of free radical damage causes functional damage in the liver (Siddique et al. 1999). The SOD catalyzes the dismutation of superoxide anion to H_2O_2. The latter can be converted to the more harmful hydroxyl radicals (OH). Subsequently, H_2O_2 is reduced to H_2O and O_2 by peroxidases (e.g., GPx) or CAT. GPx scavenges H_2O_2 in the presence of reduced glutathione (GSH) to form H_2O and oxidized glutathione (Michiels et al. 1994). Earlier reports showed that α-KG offers protection against oxidative damage by participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process (Sokolowska et al. 2000). Reports have also shown that α-KG is a glutamine precursor from which GSH is formed (Cynober 1999) which is essential for the activity of GPx. It is known that the ammonia-induced inhibition of antioxidant enzymes is mediated by the activation of NMDA receptors of nitric oxide synthase and the formation of nitric oxide, which inhibits the activities of antioxidant enzymes (Kosenko et al. 2000).

Table 2. **Effect of α-ketoglutarate on brain antioxidant and lipid profile status against sodium valproate induced toxicity in rats**

Parameters	Control (Group I)	NaVPA (Group II)	NaVPA + α-KG (Group III)	α-KG (Group IV)
TBARS (mM/100g tissue)	1.05 ± 0.16	2.35 ± 0.22*	1.70 ± 0.15^{*+}	1.00 ± 0.15^{ns}
Hydroperoxides (mM/100g tissue)	112.65 ± 3.40	148.76± 7.04*	127.31± 10.02^{*+}	110.05 ± 10.67^{ns}
Superoxide dismutase (50% inhibition of NBT reaction/min/mg protein)	8.50 ± 0.41	5.53 ± 0.31*	7.44 ± 0.17^{*+}	8.83 ± 0.79^{ns}
Catalase (µmoles/min/mg protein)	3.12 ± 0.41	1.14 ± 0.27*	2.74 ± 0.16$^{ns,+}$	3.35 ± 0.39^{ns}
Glutathione peroxidase (µg of GSH consumed/min/mg protein)	3.13 ± 0.26	1.10 ± 0.11*	2.75 ± 0.31^{*+}	3.25 ± 0.29^{ns}
Reduced glutathione (mg/g tissue)	32.66 ± 3.01	17.53 ± 1.52*	28.53 ± 2.66^{*+}	36.13 ± 3.44*
Cholesterol (mg/100g tissue)	1146.66 ± 66.83	2003.33 ± 70.89*	1246.66 ± 60.55^{*+}	1138.33 ± 47.08^{ns}
Phospholipids (mg/100g tissue)	731.66 ± 77.04	995.00 ± 65.34*	848.33 ± 85.65^{*+}	720.00 ± 40.00^{ns}
Triglycerides (mg/100g tissue)	332.37 ± 13.55	424.20 ± 6.78*	376.11 ± 25.79^{*+}	326.14 ± 19.90^{ns}
Free fatty acids (mg/100g tissue)	24.17 ± 2.96	40.49 ± 3.56*	28.88 ± 1.97$^{ns,+}$	22.96 ± 1.87^{ns}

Symbols as in Table 1

In the present study, the administration of sodium valproate caused a significant increase in the levels of lipid profile variables (cholesterol, triglycerides, phospholipids and free fatty acids). It has been reported that sodium valproate could deplete the levels of α-KG (Kifune et al. 2000) and this could elevate the levels of acetyl CoA. This acetyl CoA may be used for the synthesis of fatty acids and cholesterol, since fatty acids of different sources are used as substrates for synthesizing triglycerides and phospholipids. Further treatment with valproate causes a kind of drug induced mitochondrial cytopathy with microvesicular lipid deposition. The lipid deposits are likely to be a result of the inhibited mitochondrial fatty acid oxidation (Melegh and Trombitas 1997).

The enhanced level of lipid profile variables could be due to the carnitine transport defect associated with the treatment of sodium valproate. Our results are in accordance with the report of Heldenberg et al (1983) who found an increase in cholesterol levels in epileptic children treated with VPA. Increased levels of free fatty acids in the sodium valproate treated rats may be due to the decrease in the α-KG levels (Velvizhi et al. 2002b) which lead to the accumulation of free fatty acids which might be reversed during the treatment by α-KG as reflected in group III and group IV. Exogenous administration of α-KG could lead to the normalization of fat metabolism and could increase the oxidation of fats (Bellei et al. 1989) offering protection against lipid peroxidation and oxidative stress (Dakshayani et al. 2002, Velvizhi et al. 2002b).

In conclusion, exogenous administration of α-KG could cause the biochemical alterations by (i) participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process and (ii) enhancing the proper metabolism of fats, which could suppress oxygen radical generation and prevent lipid peroxidative damage in rats.

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