

ENHANCEMENT OF CIRCULATORY ANTIOXIDANTS BY α -KETOGLUTARATE DURING SODIUM VALPROATE TREATMENT IN WISTAR RATS

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The effects of α -ketoglutarate (α -KG) on sodium valproate-induced hyperammonemia and hepatotoxicity were studied in biochemical experiments in rats. The levels of ammonia, urea, serum transaminases, hydroperoxides and thiobarbituric acid reactive substances were significantly increased in sodium valproate-treated rats. These levels were significantly decreased in α -KG- and sodium valproate-treated rats. Further, non-enzymatic (vitamins C and E) and enzymatic (superoxide dismutase and catalase) antioxidants were significantly decreased in sodium valproate-treated rats and were increased in α -KG- and sodium valproate-treated rats. These biochemical alterations during α -KG treatment could be due to (i) its ability to act as an ubiquitous collector of amino groups in body tissues, (ii) the participation of α -KG in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process and (iii) enhancing the proper metabolism of fats which could suppress oxygen radical generation and, thus, prevent the lipid peroxidative damages in rats.

Key words: *α -ketoglutarate, sodium valproate, hyperammonemia, antioxidants, lipid peroxidation*

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INTRODUCTION

Hyperammonemia is a documented side effect of valproate (VPA) treatment [35]. Valproate is one of the most frequently used antiepileptic drugs [15]. VPA-associated hepatotoxicity includes hepatocellular damage, including microvesicular steatosis, cellular ballooning, intrahepatic cholestasis, proliferation of bile ducts and hepatic necrosis [20]. Valproic acid and its unsaturated metabolites, 2-ene VPA and (E)-2, (Z)-3'-diene VPA, demonstrated dose-dependent cytotoxicity in primary cultures of rat hepatocytes [16]. Sodium valproate treatment, through a process of free radical-induced damage, causes functional changes in the liver [33].

α -Ketoglutarate (α -KG) is a Krebs cycle intermediate, which is the natural, ubiquitous collector of amino groups in body tissues [23]. α -KG acts as a detoxifying agent in hyperammonemia, hyperaminoacidurias, or exposures to toxic nitrogen chemicals such as cyanide, ammonia, ammonium compounds, amines and hydrazines [28, 40]. α -KG also improves myocardial protection in the patients undergoing coronary operations [18]. Further, α -KG also decreases the muscle protein catabolism [41] and improves recovery after trauma. Another important function of α -KG consists in the formation of carnitine [7]. Carnitine is a molecule that acts as a carrier of fatty acids into cell mitochondria so that proper metabolism of fats can proceed [32].

The capacity of α -KG to elevate the levels of antioxidants was systematically analyzed in the present study by estimating the levels of ammonia, urea, transaminases, thiobarbituric acid reactive substances (TBARS), and hydroperoxides, non-enzymatic antioxidants (vitamins C and E) and enzymatic antioxidants (superoxide dismutase and catalase).

MATERIALS and METHODS

Adult Wistar rats (weighing 180–220 g) obtained from Central Animal House, Faculty of Medicine, Annamalai University, were kept at room temperature ($32 \pm 2^\circ\text{C}$). All studies were conducted in accordance with the National Institutes of Health: Guide for the Care and Use of Laboratory Animals [24]. Animals were randomized and divided into four groups (Group I – control, Group II – sodium valproate-treated, Group III – sodium valproate- and α -KG-treated, Group IV – α -KG-treated; $n = 6$ in

each group). Animals were fed with the standard pellet diet (Agro Corporation Private Limited, Bangalore, India) and water which were available *ad libitum* to animals.

α -KG (disodium salt) was purchased from Sisco Research Laboratories Private Limited, Mumbai, India. Sodium valproate and all other chemicals used in this study were of analytical grade. Group I animals served as controls. Group II animals were administered with (300 mg/kg) sodium valproate orally every day for 8 weeks [36]. Group III animals were treated with sodium valproate as in group II along with α -KG as oral solution (2 g/kg) [29] and Group IV rats received α -KG orally (2 g/kg) for 8 weeks.

Biochemical determinations were performed after 8 weeks of sodium valproate and/or α -KG administration. Animals from all the groups were sacrificed by cervical dislocation. Blood samples were collected from each group of rats. Serum and plasma were separated. Various biochemical analyses were done using blood, serum hemolysate and plasma samples. The levels of ammonia [8], urea [38], TBARS [26], hydroperoxides [13], vitamin E [2] and catalase [34] were measured in plasma, vitamin C content was determined in blood [31], the levels of aspartate transaminase (AST) and alanine transaminase (ALT) [30] were analyzed in serum and the levels of superoxide dismutase [17] were evaluated in hemolysate.

Analysis of variance followed by Least Significant Difference (LSD) test were carried out to detect the significant differences between control and experimental groups.

RESULTS

Sodium valproate-treated rats showed significant increases in body weight compared to the control rats. Sodium valproate- and α -KG-treated group gained body and tissue weights nearly as control group. Body weight gain in group IV rats was slightly increased in comparison with the control rats (Tab. 1).

The concentrations of ammonia, urea, TBARS and hydroperoxides in plasma were increased significantly ($p < 0.001$) in sodium valproate-treated group (Tab. 2). Group III (sodium valproate- and α -KG-treated) showed significantly lower levels ($p < 0.001$) when compared to other corresponding sodium valproate-treated group. Ammonia, urea,

Table 1. Body weight changes in the rats treated with sodium valproate and/or α -ketoglutarate (α -KG)

Group	Body weight (g)		
	Initial body wt	Final body wt	Net gain
Normal	180 \pm 14	225 \pm 17	45.70 \pm 2.64
Sodium valproate	200 \pm 18	290 \pm 20	90.26 \pm 8.87***
Sodium valproate + α -KG	185 \pm 16	245 \pm 19	66.50 \pm 6.37***,a
α -KG	190 \pm 15	242 \pm 18	52.16 \pm 5.11 ^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD). Group I is compared with groups II, III and IV (*** $p < 0.001$). Group II is compared with group III (^a $p < 0.001$); ns – not significant

Table 2. Levels of ammonia, urea, TBARS and HP

Group	NH ₃ (μ mol/l)	Urea (mg/dl)	TBARS (ml/dl)	HP $\times 10^{-5}$ (mM/100 ml)
Normal	96.26 \pm 7.66	38.40 \pm 2.70	0.12 \pm 1.68	9.75 \pm 0.72
Sodium valproate	323.33 \pm 14.28***	85.71 \pm 5.51***	0.23 \pm 1.54 ***	16.29 \pm 0.67 ***
Sodium valproate + α -KG	165.73 \pm 14.28***,a	29.73 \pm 3.18***,a	0.18 \pm 1.98***,a	11.26 \pm 0.27***,a
α -KG	91.56 \pm 6.80 ^{ns}	32.28 \pm 3.92 ^{ns}	0.10 \pm 1.47 ^{ns}	9.58 \pm 0.47 ^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD). Group I is compared with groups II, III and IV (*** $p < 0.001$). Group II is compared with group III (^a $p < 0.001$); ns – not significant

Table 3. Levels of non-enzymatic and enzymatic antioxidants

Group	Vitamin C (mg/dl)	Vitamin E (mg/dl)	SOD (50% inhibition of NBT reaction/mg protein)	CAT (mmoles/dl)
Normal	1.85 \pm 0.16	1.43 \pm 0.18	2.50 \pm 0.24	53.51 \pm 11.54
Sodium valproate	0.85 \pm 5.47***	0.60 \pm 0.14***	1.13 \pm 0.18***	26.41 \pm 2.61***
Sodium valproate + α -KG	1.50 \pm 9.24 ^{ns,a}	1.24 \pm 0.11 ^{ns,a}	1.83 \pm 0.10***,a	49.67 \pm 3.29 ^{ns,a}
α -KG	1.76 \pm 0.10 ^{ns}	1.58 \pm 0.29 ^{ns}	2.51 \pm 0.17 ^{ns}	60.17 \pm 1.34 ^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD). Group I is compared with groups II, III and IV (*** $p < 0.001$). Group II is compared with group III (^a $p < 0.001$); ns – not significant

Table 4. Levels of AST and ALT

Group	AST (Iu/l)	ALT (Iu/l)
Normal	157.86 \pm 10.91	40.78 \pm 3.31
Sodium valproate	246.91 \pm 10.80***	66.13 \pm 5.68***
Sodium valproate + α -KG	176.61 \pm 11.65***,a	53.96 \pm 5.88***,a
α -KG	144.20 \pm 7.98 ^{ns}	37.40 \pm 3.81 ^{ns}

Statistical significance was evaluated using ANOVA followed by Least Significant Difference (LSD). Group I is compared with groups II, III and IV (*** $p < 0.001$) (** $p < 0.005$). Group II is compared with group III (^a $p < 0.001$); ns – not significant

TBARS and hydroperoxides were found to be normal in α -KG-treated rats. Administration of sodium valproate caused a significant decrease in the levels of vitamins C and E, when compared to the control rats (Tab. 3). Sodium valproate- and α -KG-treated groups showed significantly increased levels of vitamins C and E, when compared with corresponding sodium valproate-treated group. α -KG-treated rats showed near normal levels of antioxidant vitamins (C and E) when compared with the controls. Similar alterations were observed in the activities of enzymatic antioxidants (catalase and superoxide dismutase).

Sodium valproate-treated (group II) rats showed significant increase in the activities of ALT and AST (Tab. 4). The levels were almost normal in group III (sodium valproate- and α -KG-treated) animals. α -KG-treated animals (group IV) showed near normal levels of ALT and AST.

DISCUSSION

Body weight changes

Our results revealed that sodium valproate-treated rats showed significantly increased body weights in comparison with other rats. Experimental evidences suggest that VPA interferes with endogenous fatty acid metabolism [19]. VPA and one of its desaturated metabolites, 4-ene VPA, inhibit β -oxidation of fatty acids under *in vitro* and *in vivo* conditions in rats. Inhibition of fatty acid metabolism in rats led to the increased food intake [11] and feeding efficiency, which would lead to the increased weights. Further, carnitine stores can be depleted through chronic VPA therapy [4]. As a consequence of carnitine transport defects, the fatty acid oxidation might be inhibited leading to weight gain observed in sodium valproate-treated rats.

Groups III and IV rats gained body weight as control rats. This could be due to detoxifying effects of α -KG and also to the increased synthesis of proteins [39]. Furthermore, α -KG fulfills an important function in the formation of carnitine [7] which could maintain proper catabolism of lipids and prevent their accumulation.

Ammonia and urea

The elevated levels of ammonia and urea in the plasma of sodium valproate-treated rats could be

due to valproate-induced hyperammonemia which is probably due to depletion of mitochondrial acetyl CoA and decreased production of N-acetyl glutamate, the obligatory activator of the first enzyme of urea cycle, carbamyl phosphate synthetase I [5]. Stimulation of glutaminase by VPA in the kidney results in glutamine uptake and ammonia release [12]. The levels could be reversed during the α -KG treatment. It has already been reported that α -KG collects amino ($-\text{NH}_2$) groups and traps ammonia in blood and in body tissues forming glutamate, glutamine and other amino acids through deamination and transamination thus detoxifying ammonia [25].

Lipid peroxidation

Cytotoxic activity of valproate is the result of the generation of hydrogen peroxide and the production of highly reactive hydroxyl free radicals [37]. This could lead to the increased levels of TBARS and hydroperoxides and decreased levels of enzymatic and non-enzymatic antioxidants in group II rats. Earlier reports showed that the elevated levels of α -KG offer protection against oxidative damages, by participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process [22].

Exogenous administration of α -KG could lead to the normalization of fat metabolism and might increase the oxidation of fats [3] offering natural protection against lipid peroxidation and oxidative stress. VPA also decreased the levels of α -KG [9] and exogenous administration of α -KG prevented the rise in the TBARS and hydroperoxides levels in rats treated with sodium valproate [40].

Transaminases

Serum transaminases are sensitive indicators of liver cell injury [6]. Their elevated levels in sodium valproate-treated rats may be due to a transient hepatic dysfunction with increased liver enzyme activities or hepatomegaly. An abnormal VPA metabolite was also described [10].

Earlier studies indicated that AST activity was inhibited by elevated levels of α -KG [27]. Similarly α -KG was known to elevate vitamin C levels [1]. Our present findings corroborate the previous results. The increase in vitamin E levels could be due to the decreased levels of free radicals and TBARS in group III rats.

In our study, the decreased activities of antioxidant enzymes (SOD and CAT) in sodium valproate-treated rats may be due to the inhibition of these enzymes by nitric oxide. It is known that ammonia-induced inhibition of antioxidant enzymes is mediated by the activation of NMDA receptors, of nitric oxide synthase and formation of nitric oxide which inhibits the activities of antioxidant enzymes [21].

Valproate has been reported to inhibit the tricarboxylic acid cycle at the α -KG dehydrogenase step [14] and also the activity of TCA cycle which may lead to a decrease in α -KG levels. Therefore, exogenous administration of α -KG would restore the activity of TCA cycle and also reverse the toxic effect of valproate.

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, (ii) acting as a ubiquitous collector of amino groups in body tissues and (iii) enhancing the proper metabolism of fats which could suppress oxygen radical generation and prevent the lipid peroxidative damages in rats.

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