Ornithine α-ketoglutarate modulates the levels of antioxidants and lipid peroxidation products in ammonium acetate treated rats

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The effects of ornithine α-ketoglutarate (OKG) on ammonium acetate induced hepatotoxicity were studied in experimental rats. The levels of urea, non-protein nitrogen and thio-barbituric acid reactive substances were significantly increased in ammonium acetate treated rats; but these levels were significantly decreased in ammonium acetate-OKG treated rats. Similar patterns were observed in the levels of free fatty acids, triglycerides and phospholipids. Furthermore, non-enzymatic (reduced glutathione) and enzymatic (glutathione peroxidase, superoxide dismutase and catalase) antioxidants were significantly decreased in ammonium acetate treated rats, when compared with control and were significantly increased in ammonium acetate-OKG treated rats compared to ammonium acetate treatment alone.

Ornithine α-ketoglutarate (OKG), a salt formed from one molecule of α-ketoglutarate and two of ornithine was described as an ‘ammonia scavenger’. Its perfusion to cirrhotic patients effectively lowered ammonia levels. Similar results were reported in experimental hyperammonemia in mammals. After administration, it dissociates into α-ketoglutarate and ornithine in mammals. In cirrhotic patients, improved nitrogen balance and normalized amino acid profile were obtained by the treatment of OKG. In clinical studies on septic trauma, surgical, cancer and burn patients, OKG showed beneficial effects at the whole body level, as shown by improved nitrogen balance, reduced hyperphenylalaninemia and urinary 3-methylhistidine excretion, all evidence of an OKG-mediated decrease in proteolysis, an increase in protein synthesis, or both.

Ammonia formed in mammals as a product of catabolism of proteins and other nitrogenous compounds is neurotoxic at high levels, leading to functional disturbances of central nervous system, that can lead to coma and death. Its intoxication reduces the α-ketoglutarate levels in Krebs cycle and impairs mitochondrial function and ATP synthesis and also to increased formation of free radicals. The sustained hyperammonemia in mice leads to increased lipid peroxidation in liver and brain reflecting an oxidative stress condition.

The present study deals with the systematic investigation on the levels of thio-barbituric acid reactive substances (TBARS – the products of lipid peroxidation) and the levels of reduced glutathione (non-enzymatic antioxidant) and glutathione peroxidase, superoxide dismutase and catalase (enzymatic antioxidants) during hyperammonemia and during the treatment of OKG. Further, the levels of urea, non-protein nitrogen and lipid constituents such as free fatty acids, triglycerides and phospholipids were also investigated.

Ornithine α-ketoglutarate (monohydrate) was provided as gift from Chiesi Company, Courbevoie, France. Ammonium acetate and other chemicals were of analytical grade. Adult Wistar rats (180-200 g), obtained from Central Animal House, Faculty of Medicine, Annamalai University were kept at experimental room temperature (32±3°C). Animals were randomly divided into four groups (Group I – control; Group II – ammonium acetate treated; Group III – ammonium acetate and α-ketoglutarate treated and Group IV – α-ketoglutarate treated; n=6 in each group). Food pellets (Agro Corporation Private Limited, Bangalore, India) and water were available ad libitum to animals. Groups I, III and IV were pair-fed with respect with Group II.

Animals were handled, ethically treated and sacrificed as per the rules and instructions of ethical committee of animal care of Annamalai University in accordance with the Indian National Law on animal care and use. Group I rats were injected with saline intraperitoneally throughout the experimental period (8 weeks). Group II rats were treated with ammonium acetate every day (100 mg/kg) intraperitoneally for 8 weeks. Group III animals were treated with ammonium acetate at the same dose as in group II animals and also with ornithine α-ketoglutarate solution (2 g/kg) every day orally for 8 weeks. Group IV ani-
mals received OKG alone at the same dose as in group III animals\(^{10}\).

At the end of the experimental period, animals were sacrificed (after overnight fasting) by cervical dislocation and blood was collected. Liver, kidney and brain tissues were removed for various analyses. Blood urea\(^{11}\) and non-protein nitrogen (NPN)\(^{12}\) were estimated. In tissues, thio-barbiturate acid reactive substances (TBARS)\(^{13}\), reduced glutathione (GSH)\(^{14}\), glutathione peroxidase (GPx)\(^{15}\), catalase (CAT)\(^{16}\), superoxide dismutase (SOD)\(^{17}\), triglycerides\(^{18}\), free fatty acids\(^{19}\) and phospholipids\(^{20}\) were estimated.

Mean±SD values of variables in different groups were calculated. Data were analysed statistically by analysis of variance (ANOVA), followed by least significant difference (LSD) method. There was no significant difference in food intake in all four groups of rats.

The concentration of blood urea and NPN were significantly increased in ammonium acetate treated rats when compared with control, but significantly decreased during ammonium acetate + OKG treatment. OKG-treated rats showed no significant differences in the concentration of urea and NPN (Table 1).

Concentration of TBARS in liver increased significantly in ammonium acetate treated group, but ammonium acetate-OKG treated group showed significantly low levels of TBARS, when compared to control (Table 2). Similar patterns were observed in kidney and brain tissues.

Administration of ammonium acetate caused a significant decrease in the levels of the antioxidants, reduced GSH, GPx and CAT in the liver, but ammonium acetate-OKG treated group showed significantly increased levels of these antioxidants. OKG-treated group showed significant increase in the levels of antioxidants when compared with control (Table 2). Similar patterns were noticed in kidney and brain tissues in all four groups.

The triglycerides, free fatty acids and phospholipids levels were increased significantly in ammonium

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### Table 1—Changes in blood urea\(^{11}\) and non-protein nitrogen (NPN)\(^{12}\) during hyperammonemia and treatment with ornithine \(\alpha\)-ketoglutarate in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dl)</th>
<th>NPN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>10.95±0.59</td>
<td>23.81±0.85</td>
</tr>
<tr>
<td>(NH_4) (II)</td>
<td>23.51±0.91*</td>
<td>52.16±1.47*</td>
</tr>
<tr>
<td>(NH_4)+OKG (III)</td>
<td>13.40±0.85</td>
<td>32.83±1.69*</td>
</tr>
<tr>
<td>OKG (IV)</td>
<td>11.06±0.55*</td>
<td>22.66±1.47</td>
</tr>
</tbody>
</table>

\(*, p<0.005\) when compared with control; \(^{1}\), \(p<0.005\) when compared with ammonium acetate (\(NH_4\)\(^+)\) treated group; ns, not significant when compared with control

### Table 2—Changes in lipid peroxidation, levels of triglycerides, free fatty acids and phospholipids in liver of rats during hyperammonemia and treatment with ornithine \(\alpha\)-ketoglutarate

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>(NH_4)(^+)</th>
<th>(NH_4)+OKG</th>
<th>OKG</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmole/mg protein)</td>
<td>1.83±0.06</td>
<td>3.91±0.24*</td>
<td>2.51±0.09*</td>
<td>1.74±0.14*</td>
</tr>
<tr>
<td>GSH (mg/g tissue)</td>
<td>21.46±1.37</td>
<td>10.53±0.60*</td>
<td>18.00±0.66*</td>
<td>24.80±1.43*</td>
</tr>
<tr>
<td>GPx (μg of GSH consumed/min/mg protein)</td>
<td>11.52±0.76</td>
<td>4.09±0.27*</td>
<td>8.30±0.87*</td>
<td>12.90±0.45*</td>
</tr>
<tr>
<td>CAT (molecules of (H_2O_2) consumed/min/mg protein)</td>
<td>70.98±4.43</td>
<td>36.11±2.50*</td>
<td>67.53±4.20*</td>
<td>81.42±3.99*</td>
</tr>
<tr>
<td>SOD (50% inhibition of NBT reaction/min/mg protein)</td>
<td>5.32±0.24</td>
<td>2.11±0.05*</td>
<td>4.61±0.28*</td>
<td>6.18±0.20*</td>
</tr>
<tr>
<td>Triglycerides (mg/100 g tissue)</td>
<td>332.37±15.88</td>
<td>656.00±23.46*</td>
<td>406.16±21.55*</td>
<td>328.00±14.37*</td>
</tr>
<tr>
<td>Free fatty acids (mg/100 g tissue)</td>
<td>668.50±52.38</td>
<td>942.52±51.28*</td>
<td>713.23±54.60*</td>
<td>652.79±15.87*</td>
</tr>
<tr>
<td>Phospholipids (mg/100 g tissue)</td>
<td>940.00±90.33</td>
<td>1691.66±58.79*</td>
<td>1320.00±75.89*</td>
<td>950.00±79.74*</td>
</tr>
</tbody>
</table>

\(*, p<0.005\) when compared with control; \(^{1}, p<0.005\) when compared with (\(NH_4\)\(^+)\) treated group; ns, not significant when compared with control
acetate-treated rats, but were decreased in ammonium acetate-OKG treated rats (Table 2). Similar trends of alterations were observed in kidney and brain tissues of four groups.

In the liver, ammonia is removed either in the form of urea in perportal hepatocytes and/or as glutamine in perivenous hepatocytes. Elevated levels of urea and NPN might indicate the elevated levels of ammonia in ammonium acetate treated rats in our study. OKG administration decreased plasma urea and NPN; the decrease in plasma urea could be due to the presence of α-ketoglutarate, which could accelerate ammonia removal by stimulating glutamine synthesis. Further, OKG is a precursor of glutamine and arginine which are known to be anti-ammoniagenic amino acids due to efficient recycling of ammonia into urea.

Ammonia intoxication enhances lipid peroxidation and generates free radicals. This could lead to increased levels of TBARS and decreased levels of non-enzymatic and enzymatic antioxidants in group II rats. Exogenous administration of α-ketoglutarate (in group III) could offer protection against oxidative damages by participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process. Further, arginine produced by ornithine would also promote scavenging of free radicals, as evidenced by the ability of ornithine α-ketoglutarate to enhance macrophage cytotoxicity in stress conditions.

Ammonium acetate could deplete the α-ketoglutarate and other Krebs cycle intermediates and thus elevate the levels of acetyl CoA which, in turn, could lead to increased levels of lipid profile (free fatty acids, triglycerides and phospholipids) as observed in our study.

Another important function of α-ketoglutarate occurs in the formation of carnitine, which acts as a carrier of fatty acids into cell mitochondria so that proper metabolism of fats can proceed. Probably, low levels of α-ketoglutarate in ammonium acetate-treated (group II) rats could lead to accumulation of fatty acids which might be reversed during the treatment of OKG.

Acknowledgement

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References