EFFECTIVENESS OF ALPHA LIPOIC ACID AS HEPATOPROTECTIVE AND ANTIOXIDANT

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ABSTRACT
The present study investigates the hepatoprotective and antioxidant activity of alpha lipoic acid (ALA) on carbamazepine (CBZ) administered rats. 30 animals were divided into 5 groups. Animals in group 1 received drinking water orally and served as control, group 2 received CBZ 50 mg/Kg dissolved in water daily by oral gavage, group 3, 4 and 5 received (50, 100 and 200 mg/Kg) of ALA in 0.2% carboxy methyl cellulose respectively 1 hr prior to administration of 50 mg/Kg CBZ for a duration of 45 days. The levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, bilirubin, albumin and total protein were estimated to determine the hepatoprotective activity. The levels of superoxide dismutase (SOD), glutathione (GSH), catalase and lipid peroxidation levels were estimated to determine the antioxidant activity. Histopathological examination was also done. CBZ increased the level of SGOT, GSH, catalase and increased lipid peroxidation. Administration of ALA reversed the CBZ induced hepatotoxicity. Histopathological examination revealed preservation of liver integrity in ALA administered rats compared to CBZ alone treated rats. The hepatoprotective effect of ALA was due to its antioxidant activity by scavenging free radicals.

Keywords: Antioxidant, hepatoprotective, Alpha lipoic acid, Free radicals.

INTRODUCTION
Carbamazepine (CBZ) is used for the treatment of seizure, as well as affective and behavioral disorders. The common adverse effects of CBZ include hepatitis and hepatotoxicity1,2. CBZ produce serious hepatotoxicity and manifest as acute granulomatous hepatitis which is self-limiting if the drug is withdrawn. Administration of CBZ has produced primary biliary cirrhosis, primary sclerosing cholangitis, heavy portal liver fibrosis (precirrhotic process), acute or chronic hepatotoxicity, cholestatic3, hepatocellular injury4 and even granuloma formation5 in the liver. Oxidative stress occurs due to an imbalance in the oxidants and antioxidants system causing potential cellular damage6. Oxidative stress plays an vital role in the pathogenesis of liver injuries, hepatotoxicity7 and liver diseases8. The clinical utilization of aromatic antiepileptic drug is limited by its diverse adverse effects and hepatotoxicity9. The aromatic antiepileptic drug provoked hepatotoxicity is attributed due to defective detoxification by the epoxide hydrolase and accumulation of arene oxides10. There is a growing body of evidence suggesting that idiosyncratic drug-induced hepatotoxicity may be mediated by oxidative stress, characterized by enhanced levels of ROS such as hydroxyl radical, superoxide anion and
hydrogen peroxide, due to reduced elimination and increased production of ROS\textsuperscript{11}. It has been recently demonstrated that aromatic antiepileptic drug mediated hepatotoxicity is associated with mitochondrial dysfunction and genetic or acquired mitochondrial defects\textsuperscript{8}. In the present study, we have addressed the oxidative stress as a potential mechanism responsible for CBZ-induced hepatotoxicity.

**MATERIALS AND METHODS**

**Animals**

The pathogen free adult male albino rats weighing 150-250 g were used. The rats were housed in polypropylene cages at room temperature (25 ± 3°C) with 12/12 hours light and dark cycle and the animals were fed with a balanced diet and tap water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee of M.S. Ramaiah College of Pharmacy, Bangalore, Karnataka (Ref. No. 220/abc/CPCSEA).

**Study Protocol**

The rats were divided into five groups with six animals in each group. First group served as control and received drinking water orally daily by gavage for 45 days. Second group received 50 mg/Kg CBZ dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12.00 hrs. Third, fourth and fifth group received 50, 100 and 200 mg/Kg (p.o) of ALA respectively 1 hr prior to administration of 50 mg/Kg CBZ for 45 days between 11.00 and 12.00 hrs. On 45\textsuperscript{th} day of drug administration, the animals were anaesthetized under light ether anaesthesia and the blood samples were collected from retro orbital plexus for estimation of biochemical parameters such as SGOT, SGPT, ALP, total bilirubin, total protein and albumin. Serum was separated by centrifuging blood at 2500 rpm for 10 minutes and the levels of SGOT, SGPT, ALP, bilirubin, albumin and total protein were analyzed by using a commercially available enzymatic kit (AGAPPE, India) and an autoanalyser (Chemistry Analyser (CA 2005), B4B Diagnostic Division, China). The animals were then sacrificed, liver tissues were isolated and rinsed with cold phosphate buffer (PB, 100 mM, pH 7.4), weighed, sliced for histopathological studies and stored at -40°C. The stored tissues were homogenized and the homogenate was centrifuged at 10,000 x g for 10 minutes at 4°C. The supernatant was stored at -40°C for further biochemical estimations of endogenous activities of antioxidants such as SOD, GSH\textsuperscript{12}, catalase and lipid peroxidation\textsuperscript{13}.

**Histopathological Studies**

The histopathological study in liver tissue was conducted according to Li et al\textsuperscript{14}. Rats were anesthetized under ether anaesthesia and sacrificed. The liver was fixed in 4% paraformaldehyde overnight. A block was prepared in block preparation unit (Shandon Histocenter-2) and coronal sections (10 µm) were cut with the help of a microtome (Leica RM 2255, Lab India) and picked up on poly-l-lysine coated slides and were stained with haematoxylin and eosin (HE).

**Statistical analysis**

The results are expressed as mean±SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) with Tukey’s post hoc statistical tests. P < 0.001 was considered significant.

**RESULTS**

The CBZ treated group significantly elevated the levels of SGOT, SGPT, ALP and total bilirubin, whereas reduced the levels of total protein and albumin (P<0.001) as compared to the control group. Administration of CBZ along with ALA showed significant reduction in the levels of SGOT, SGPT, ALP and total bilirubin and increased the levels of total protein and albumin (P < 0.001) (Table 1). The carbamazepine treated group significantly increased the liver lipid peroxidation as compared to the control group. CBZ plus ALA 50, 100 and 200 mg/kg showed dose dependent reduction (P < 0.001) in the levels of CBZ induced lipid peroxidation (Table 1).

Table 2 shows the effect of chronic treatment of CBZ and CBZ + ALA on SOD, GSH and catalase. Chronic CBZ treatment significantly decreased the reduced SOD, GSH and catalase levels when compared to control animals. ALA at the dose of 50, 100 and 200 mg/Kg significantly increased the SOD, GSH and catalase when compared to CBZ treated animals (Table 2).

At the end of 45\textsuperscript{th} day of treatment with CBZ, there was a statistically significant decrease in bodyweight and an increase in the absolute and relative liver weights when compared to the control group (P < 0.001). ALA at the dose of 50, 100 and 200 mg/kg showed increase in body weight and decrease in absolute and relative liver weights compared with CBZ group (P < 0.001) (Table 3).

**Histopathological Studies**

Figure 1: Histopathology of liver (A) Control liver reveals normal hepatocytes. In (B) CBZ treated group some of the hepatocytes confirmed haemorrhage, centrilobular and
sinusoidal congestion with hepatic damage. (C) CBZ + ALA at a dose of 50 mg/kg showed fatty degeneration and hepatic necrosis. (D) CBZ + 100 mg/kg ALA showed hepatic necrosis. (E) CBZ + 200 mg/kg ALA revealed normal hepatocytes with central vein and appeared similar to the control liver.

DISCUSSION

The imbalance in the serum oxidant/antioxidant status of epileptic children under antiepileptic monotherapy with CBZ was demonstrated and it was suggested that the hepatotoxic effects might be associated with the side effects of these drugs. Our study suggests that the toxicity of CBZ might be secondary to the oxidative stress induced by their metabolites, probably the intermediate arene oxides, formed after biotransformation of aromatic antiepileptic drug and due to disturbance in the GSH metabolism. In the present study, we observed the depletion of GSH in liver. Oxidative stress might have occurred as a consequence of disruption of thiol redox circuits, which are controlled by GSH.

Liver is the main detoxifying organ for many toxic agents and drugs that contribute to oxidative stress. The increased ROS production due to depletion of GSH might lead to oxidative damage and a critical role in the development of hepatic damage. ALA is effective in preventing the development of hepatic damage. ALA is found to be a potential therapeutic agent in the treatment and prevention of different pathologies that are related to an imbalance of the oxidoreductive cellular status, which occurs in the case of hepatic disorder status. In addition, several researchers have recently reported the protective effects of ALA on the liver which is induced by oxidative agents.

CONCLUSION

In the present study, supplementation with ALA decreased the markers of hepatotoxicity such as SGOT, SGPT, bilirubin and increased the levels of albumin and total protein. ALA (50 mg/Kg) showed mild hepatoprotective activity, fatty degeneration and hepatic necrosis. But, ALA (200 mg/Kg) improved the histopathological changes and hepatic damage induced by CBZ. ALA exerts significant protection against CBZ induced toxicity by its ability to decrease the lipid peroxidation and thus oxidative stress through its free radical scavenging activity, which improved the levels of antioxidant defense system.

ACKNOWLEDGEMENT

We wish to thank Dr. V. Madhavan, Principal, M.S. Ramaiah College of Pharmacy for encouraging us to successfully carryout this work.

Table 1: Effect of chronic treatment of carbamazepine and carbamazepine + ALA on liver enzymes, bilirubin, albumin, total protein and liver lipid peroxidation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Carbamazepine</th>
<th>Carbamazepine + ALA 50mg/kg</th>
<th>Carbamazepine + ALA 100mg/kg</th>
<th>Carbamazepine + ALA 200mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT</td>
<td>260.5±4.8***</td>
<td>378.6±3.59***</td>
<td>318.6±1.201***</td>
<td>295.8±5.61***</td>
<td>266±1.46***</td>
</tr>
<tr>
<td>SGPT</td>
<td>67.05±1.42***</td>
<td>91.47±1.11***</td>
<td>75.01±0.669***</td>
<td>72±0.84***</td>
<td>68.5±0.61***</td>
</tr>
<tr>
<td>ALP</td>
<td>149.5±3.35***</td>
<td>252.6±3.44***</td>
<td>215.6±2.39***</td>
<td>180.16±6.00***</td>
<td>157.6±2.04***</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1.22±0.00***</td>
<td>2.63±0.06***</td>
<td>1.98±0.118***</td>
<td>1.72±0.05***</td>
<td>1.405±0.06***</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.6±0.13***</td>
<td>3.02±0.05***</td>
<td>3.785±0.14***</td>
<td>3.98±0.15***</td>
<td>4.36±0.14***</td>
</tr>
<tr>
<td>Total Protein</td>
<td>7.965±0.22***</td>
<td>6.15±0.17***</td>
<td>6.35±0.22***</td>
<td>6.785±0.058***</td>
<td>7.5±0.0568***</td>
</tr>
<tr>
<td>LLP</td>
<td>33.19±0.61***</td>
<td>120.42±0.57***</td>
<td>91.13±0.77***</td>
<td>74.97±1.43***</td>
<td>52.59±1.26***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. *** (p<0.001), ***(p<0.01), *(p<0.05) Vs Control group. +++*(p<0.001), ++*(p<0.01), +(p<0.05) Vs CBZ group.

SGOT: Serum Glutamate Oxaloacetate Transaminase
SGPT: Serum Glutamate Pyruvate Transaminase
ALP: Alkaline Phosphatase
LLP: Liver Lipid Peroxidation
Table 2: Effect of CBZ and CBZ + NAC on Superoxide dismutase, catalase and glutathione

<table>
<thead>
<tr>
<th>Groups</th>
<th>Superoxide Dismutase (superoxide anion reduced/mg protein/min)</th>
<th>Catalase (µmolH₂O₂ degraded/ mg protein/min)</th>
<th>Glutathione (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.35±0.2455***</td>
<td>60.03±0.88***</td>
<td>17.43±0.61***</td>
</tr>
<tr>
<td>Carbamazepine (50 mg/Kg)</td>
<td>2.023±0.197***</td>
<td>37.47±0.58***</td>
<td>10.58±0.42***</td>
</tr>
<tr>
<td>Carbamazepine + ALA 50 mg/Kg</td>
<td>3.88±0.168****</td>
<td>42.6±0.35****</td>
<td>12.69±0.20****</td>
</tr>
<tr>
<td>Carbamazepine + ALA 100mg/Kg</td>
<td>4.6±0.202****</td>
<td>52.83±1.09****</td>
<td>13.67±0.32****</td>
</tr>
<tr>
<td>Carbamazepine + ALA 200 mg/Kg</td>
<td>5.58±0.096****</td>
<td>58.24±0.702****</td>
<td>14.64±0.25****</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals. ***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group. +++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Carbamazepine group.

Table 3: Effect of CBZ and CBZ + Alpha Lipoic Acid on body weight, absolute and relative liver weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight in gram</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% Change</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>268.3±2.1</td>
<td>19.2±0.93***</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>221.16±2.3</td>
<td>201.6±1.0</td>
<td>18.7±1.03***</td>
</tr>
<tr>
<td>Carbamazepine + ALA 50mg/Kg</td>
<td>226.6±1.0</td>
<td>215±1.52</td>
<td>14.73±0.72***</td>
</tr>
<tr>
<td>Carbamazepine + ALA 100mg/Kg</td>
<td>209.16±4.3</td>
<td>206.6±4.0</td>
<td>11.16±0.52***</td>
</tr>
<tr>
<td>Carbamazepine + ALA 200mg/Kg</td>
<td>225±2.5</td>
<td>221±3.3</td>
<td>11.5±0.37***</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals. ***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group. +++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Carbamazepine group.

Normal hepatic architecture

Fig. a): control
Effect of ALA on CBZ induced alterations in liver histopathology

Fig. b): Carbamazepine (50 mg/Kg)

Fig. c): Carbamazepine + ALA (50 mg/Kg)

Fig. d): Carbamazepine + ALA (100 mg/Kg)
Fig. 1: Micrograph showing effect of CBZ on hepatocytes.
(A) Control liver showed normal hepatic architecture.
(B) Carbamazepine treated group showed haemorrhage, centrilobular and sinusoidal congestion.
(C) Carbamazepine + ALA at a dose of 50 mg/kg showed fatty degeneration and hepatic necrosis.
(D) Carbamazepine + 100 mg/kg ALA showed hepatic necrosis.
(E) Carbamazepine + 200 mg/kg ALA showed normal hepatocytes with central vein.

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