EFFECT OF LIV.100 AGAINST ANTITUBERCULAR DRUGS (ISONIAZID, RIFAMPICIN AND PYRAZINAMIDE) INDUCED HEPATOTOXICITY IN RATS.

S. D. SARASWATHY, V. SUJA, PREMA GURUMURTHY*, C. S. SHYAMALA DEVI

Department of Biochemistry, University of Madras Guindy Campus, Chennai - 600 025.
Department of Biochemistry, Tuberculosis Research Centre, Chetput, Chennai - 600 031.

Manuscript Received: 27-1-98 Revised: 14-3-98 Accepted: 8-5-98

SUMMARY

Objectives: To assess the protective effect of Liv.100 against antitubercular drugs (isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA)) induced hepatotoxicity in rats.

Methods: The simultaneous treatment of Liv.100 (400 mg/kg body weight) on antitubercular drugs (INH, RMP and PZA) induced lipid peroxidation in liver was studied in rats. Levels of marker enzymes such as lactate dehydrogenase, aspartate amino transferase, alanine amino transferase and alkaline phosphatase were assessed in liver and serum. The glutathione content and the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase were estimated in liver. The levels of lipid peroxides and the activities of Na +K+ ATPase, Ca++ ATPase and Mg++ ATPase were also assayed in the liver of experimental groups.

Results: In antitubercular drugs administered rats, a significant decrease was observed in the levels of marker enzymes in the liver with a corresponding increase in their levels in serum. The levels of lipid peroxides (in terms of YBA reactants) were increased significantly in their serum and liver. The activities of Na +K+ ATPase, Ca++ ATPase and Mg++ ATPase were decreased significantly in their liver. The glutathione content and the activities of antioxidant enzymes decreased significantly in the liver of antitubercular drugs administered rats when compared to normal control. Simultaneous administration of Liv. 100, showed near normal levels of marker enzymes, and the levels of lipid peroxides glutathione content on comparison with normal control.

Conclusion: Simultaneous treatment with Liv. 100 offers protection against hepatotoxicity induced by antitubercular drugs by reducing lipid peroxidation and restoring the antioxidant defense system.

KEY WORDS Hepatoprotection lipid peroxides antioxidant enzymes catalase glutathione

INTRODUCTION

Isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), used in short-course chemotherapy of tuberculosis are known to be potentially hepatotoxic1. Administration of anti-tubercular drugs (INH+ RMP + PZA) to white rats results in cytolytic liver injury. This manifests by hyperamino transferasemia, initiation of lipid peroxidation and suppression of the antioxidant system2. It has been established in experimental animals that anti-tubercular drugs administered in toxic doses affect the liver, its membranes and organelles. This is supported by release of aspartate and alanine aminotransferases and alkaline phosphatase in serum and by a decrease of the activity of Na +K+ ATPase, Ca++ ATPase and Mg++ ATPase in liver3.

Liv.100 (Himalaya Drug Co. Pvt. Ltd.) is a modified formulation of Liv.52, a popular hepatic stimulant4. Liv.100 is a scavenger of free radicals and it exhibits dose and time dependant protective response against hydrogen peroxide induced lipid peroxidation5. Since, free radical induced lipid peroxidation has been reported to be associated with various deleterious effects including tissue damage and necrosis, Liv.100 being a good scavenger of free radicals, has been chosen to study its effect on lipid peroxidation and activities of antioxidant enzymes during anti-tubercular drugs induced hepatotoxicity.

MATERIALS AND METHODS

Liv.100 an improvised and indigenous preparation of Liv.52 contains Cichorium intybus, Solanum...
Table 1. Serum and hepatic levels of aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in experimental groups.

<table>
<thead>
<tr>
<th>Treatment (Group)</th>
<th>Normal control (I)</th>
<th>INH+RMP+PZA (II)</th>
<th>Liv.100 (III)</th>
<th>Liv.100 +INH+RMP+PZA (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>A</td>
<td>1.35 ± 0.11</td>
<td>2.73 ± 0.19**</td>
<td>1.31 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.55 ± 0.04</td>
<td>0.8 ± 0.2**</td>
<td>1.53 ± 0.04</td>
</tr>
<tr>
<td>AST</td>
<td>A</td>
<td>0.38 ± 0.04</td>
<td>0.76 ± 0.04**</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.68 ± 0.05</td>
<td>0.49 ± 0.03**</td>
<td>0.71 ± 0.07</td>
</tr>
<tr>
<td>ALT</td>
<td>A</td>
<td>0.53 ± 0.04</td>
<td>0.89 ± 0.04***</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.93 ± 0.03</td>
<td>0.05 ± 0.02**</td>
<td>0.94 ± 0.03</td>
</tr>
<tr>
<td>ALP</td>
<td>A</td>
<td>0.69 ± 0.03</td>
<td>1.37 ± 0.04**</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.10 ± 0.03</td>
<td>0.81 ± 0.02**</td>
<td>1.07 ± 0.03</td>
</tr>
</tbody>
</table>


Values are expressed as mean ± S.D. for 6 animals in each group.

Students’ t test: Vs Gp I: ***P<0.001, *P<0.05.

The levels of LDH, AST, ALT and ALP in Serum are expressed as µkat/litre.

The levels of LDH, AST and ALT in liver are expressed as µ moles of pyruvate liberated/sec/g of protein. The level of ALP in liver is expressed as µ moles of phenol liberated/sec/g protein.

nigrum, Phyllanthus amarus, Piccorhiza kurroa and Embelica officinalis (5:3:75:2.62:2:1) and it was gifted by Himalaya Drug Company. Anti-tubercular drugs [viz, isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA)], epinephrine, NADPH, glutathione (reduced) and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO, USA). All the other chemicals used were of analytical grade.

Adult male albino rats of Wistar strain weighing 120-150 g were fed with commercial pelleted rat chow (M/s Hindustan Lever Limited, Bombay) and water ad libitum. They were maintained in clean, sterile, polypropylene cages. The rats were divided into 4 groups of 6 animals each.

Group I served as normal control. Group II rats were administered a combination of three anti-tubercular drugs (INH-7.5 mg/kg body wt., RMP-10 mg/kg body wt. and PZA-35 mg/kg body wt. for 45 days by intragastric administration) in sterile saline. Group III rats were given Liv.100 (400 mg/kg body wt., daily for a period of 45 days, orally). Group IV rats were administered both Liv.100 and the combination of three antitubercular drugs (INH+RMP+PZA) at the above-mentioned dosage for 45 days.

After the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected and the serum was used for the assay of marker enzymes, (lactate dehydrogenase (LDH), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP)). Immediately after the sacrifice, the liver was dissected out, washed in ice-cold saline, and a homogenate was prepared in 0.1 M Tris- HCl buffer (pH7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST), glutathione reductase (GRD), Na+K+ ATPase, Ca2+ ATPase and Mg2+ ATPase. Lipid peroxide levels were estimated in serum and liver in terms of TBA reactants using 1,
Table 2. The levels of lipid peroxides and the activities of Na\(^+\)K\(^+\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase in the liver of experimental groups.

<table>
<thead>
<tr>
<th>Treatment (Group)</th>
<th>Normal Control (I)</th>
<th>INH+RMP+PZA (II)</th>
<th>Liv. 100 (III)</th>
<th>Liv. 100+INH+RMP+PZA (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.69 ± 0.14</td>
<td>6.41 ± 0.10(^{**})</td>
<td>3.60 ± 0.16</td>
<td>3.55 ± 0.06</td>
</tr>
<tr>
<td>Serum</td>
<td>2.37 ± 0.12</td>
<td>5.67 ± 0.14(^{**})</td>
<td>2.26 ± 0.04</td>
<td>2.30 ± 0.14</td>
</tr>
<tr>
<td>Na(^+)K(^+) ATPase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.94 ± 0.04</td>
<td>0.53 ± 0.04(^{**})</td>
<td>0.97 ± 0.03</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>Ca(^{2+}) ATPase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.06 ± 0.04</td>
<td>0.34 ± 0.04(^{***})</td>
<td>0.64 9.0 3</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>Mg(^{2+}) ATPase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.73 ± 0.05</td>
<td>0.39 ± 0.03(^{***})</td>
<td>0.69 ± 0.01</td>
<td>0.70 ± 0.04</td>
</tr>
</tbody>
</table>

INH: Isoniazid; RMP: Rifampicin; PZA: Pyrazinamide. Values are expressed as mean ± S.D. for 6 animals in each group.

Students’ t test: Vs Gp I: ***P<0.001, **P<0.01, *P<0.05.

The level of lipid peroxide in liver is expressed as pmoles of TBA reactants/g protein whereas that in serum are expressed as pmoles of TBA reactants/litre.

The activities of Na\(^+\)K\(^+\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase are expressed as µmoles of phosphorus liberated/sec/g protein.

\(1;3,3’\) tetramethoxy propane as the standard. Protein was determined by the method of Lowry et al.

Student ‘t’ test was used for statistical analysis of data.

RESULTS

The rats which received anti-tubercular drugs showed a significant decrease in the levels of marker enzymes such as ALT, AST, LDH and ALP in liver accompanied by a corresponding significant increase in their levels in serum (Table 1). Rats administered with Liv.100 maintained the levels of marker enzymes in serum and liver. Group IV rats restored the levels of marker enzymes in the liver thereby preventing their increase in the serum.

The levels of lipid peroxides (as TBA reactive substances) and the activities of membrane bound Na\(^{+}\)K\(^{+}\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase in liver are presented in Table 2. Rats administered anti-tubercular drugs (INH+RMP+PZA) alone, showed a significant increase in lipid peroxide in serum (p<0.01) and liver (p<0.001) when compared to normal control. The rats from same group showed a significant decrease in the activities of Na\(^{+}\)K\(^{+}\) ATPase Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase. Group IV rats (Liv.100+INH+RMP+PZA) retained near normal lipid peroxide levels in serum and liver, and the activities of membrane bound enzymes in liver.

Table 3 presents the activities of antioxidant enzymes such as SOD, CAT, GPX, GST, GRD and glutathione in liver of experimental groups. Group II rats showed a significant decrease (p<0.001) in the activities of antioxidant enzymes when compared to normal control. Group IV rats restored the activities of antioxidant enzymes at near normal values. Rats administered Liv.100 alone, did not show any significant variations in the above-studied parameters when compared to normal control.

DISCUSSION

Recently, free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies. The serum levels of a number of hepatic enzymes are used as diagnostic indicators of hepatic injury. Increased levels of LDH, AST, ALT and ALP in serum of the anti-tubercular drugs treated animals certainly indicate liver damage. An increase in the levels of these marker enzymes in serum is due to the leakage of the enzymes from liver as a result of tissue damage. This is consistent
Table 3. The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and glutathione content in liver of experimental groups.

<table>
<thead>
<tr>
<th>Treatment (Group)</th>
<th>Normal Control (I)</th>
<th>INH+RMP+PZA (II)</th>
<th>Liv. 100 (III)</th>
<th>Liv.100+INH+RMP+PZA (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>61.79 ± 3.01</td>
<td>29.39 ± 0.17***</td>
<td>63.13 ± 2.51</td>
<td>57.45 ± 1.84**</td>
</tr>
<tr>
<td>Catalase</td>
<td>74.33 ± 3.33</td>
<td>56.50 ± 4.17***</td>
<td>73.50 ± 2.67</td>
<td>71.83 ± 3.0</td>
</tr>
<tr>
<td>Glutathione Peroxidase</td>
<td>184.42 ± 6.51</td>
<td>93.29 ± 5.42**</td>
<td>192.01 ± 10.31</td>
<td>189.30 ± 5.97</td>
</tr>
<tr>
<td>Glutathione transferase</td>
<td>160.54 ± 3.54</td>
<td>87.89 ± 2.99***</td>
<td>155.64 ± 4.08</td>
<td>156.46 ± 4.90</td>
</tr>
<tr>
<td>Glutathione-S-reductase</td>
<td>10.38 ± 0.24</td>
<td>5.77 ± 0.25***</td>
<td>10.56 ± 0.31</td>
<td>10.07 ± 0.29</td>
</tr>
<tr>
<td>Glutathione</td>
<td>4.58 ± 0.01</td>
<td>2.58 ± 0.05**</td>
<td>4.59 ± 0.09</td>
<td>4.53 ± 0.08</td>
</tr>
</tbody>
</table>

INH : Isoniazid; RMP : Rifampicin; PZA : Pyrazinamide. Values are expressed as mean ± S.D. for 6 animals in each group.

Students' t test: Vs Gp I: ***P<0.001, *P<0.05.

Level of superoxide dismutase is expressed in µkat/g protein. Level of catalase is expressed as nmoles of H₂O₂ decomposed/sec/g protein. Activity of glutathione peroxidase is expressed as nmoles of GSH utilised/sec/g protein. Level of glutathione reductase is expressed as nmoles of GSSG utilised/sec/g protein. Activity of glutathione-S-transferase is expressed as µmoles of CDNB conjugated/sec/g protein. Level of glutathione is expressed as nmoles of GSH/g tissues.

with the decrease in the levels of the marker enzymes in liver of anti-tubercular drugs treated animals.

On concurrent treatment with Liv.100, the serum marker enzyme levels in Group IV rats were near normal indicating protection against liver damage.

Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in the levels of lipid peroxides in serum and liver and decrease in activities of Na⁺K⁺ATPase, Ca²⁺ATPase and Mg²⁺ATPase in liver of rats administered anti-tubercular drugs as compared to normal control are in accordance with the observation of Skakun et al.²,³.

The increase in TBA reactive substances of liver indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals.¹⁸

Inactivation of Na⁺K⁺ATPase and Mg²⁺ATPase could be due to enhanced lipid peroxidation by free radicals on anti-tubercular drugs treatment since Na⁺K⁺ATPase is a ‘SH’ group containing enzyme and is lipid dependent.¹⁹

The rats given Liv.100 and anti-tubercular drugs concurrently retained the levels of TBA reactive substances to near normal in serum and liver when compared to normal control. This shows the protective action of Liv.100 on cellular membranes by lowering lipid peroxidation.²⁰ The activities of Na⁺K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase were also restored at near normal on administration of Liv.100 concurrently with anti-tubercular drugs. This could be due to the ability of Liv.100 to protect the ‘SH’ groups from oxidative damage through inhibition of peroxidation of membrane lipids.

GSH together with GSH dependant systems GPX, GST, GRD, and CAT-SOD couple, efficiently scavenge toxic free radicals.²¹ Decreased glutathione levels may be due to its increased utilisation in protecting ‘SH’ containing proteins from lipid peroxidation.
peroxides. The nonavailability of glutathione decreases the activity of glutathione peroxidase and transferase.

Isoniazid is known to induce the cytochrome P450 system resulting in increased metabolism, formation of toxic metabolites, depletion of glutathione stores and subsequent hepatocellular damage\(^1\). Inhibition of cellular glutathione biosynthesis by rifampicin in *M. smegmatis* has been reported\(^3\). Suppression of the antioxidant system in anti-tubercular drugs treated rats has also been reported\(^2\).

The glutathione levels and activities of glutathione dependent enzymes were restored to near normal levels in Group IV rats, which were treated simultaneously with Liv.100 and the combination of anti-tubercular drugs.

GSSG, the oxidised product of GSH has been reported to accumulate due to the inactivation of glutathione reductase. GSSG inactivates many enzymes containing the 'SH' group and inhibits protein synthesis\(^6\). On concurrent treatment with Liv.100 to the cellular thiol status is not significantly altered. Earlier reports with Liv.52 showed significant enhancement in the GSH level in animals treated with it and also normalize radiation-induced alterations in GSH levels\(^9\).

The decreased activities of SOD and CAT the primary antioxidant enzymes, observed in the anti-tubercular drugs treated rats may be due to the interaction of the accumulated free radicals with the associated metal ions or with the active amino acids of these enzymes\(^8\). During hepatotoxicity these enzymes are structurally and functionally impaired by free radicals resulting in liver damage. In Group IV rats the restoration of the activities of the antioxidant enzymes could be due to the ability of Liv.100 to scavenge reactive oxygen species within the lipid region of the membrane\(^5\).

The results indicate that simultaneous treatment with Liv.100 in animals offers protection to the liver against anti-tubercular drugs induced toxicity.

**ACKNOWLEDGEMENT**

The authors are extremely thankful to Dr. S. K. Mithra, Director, Himalaya Drug Company R&D, Makali, Bangalore for sending the drugs as gift.

**REFERENCES**


KETOROLAC 10mg IM ADEQUATE FOR RELIEF OF CANCER PAIN

IM ketorolac 10mg is adequate for the relief of cancer pain and has a similar effect to ketorolac 30mg and diclofenac 75mg. So say researchers in Italy who conducted a double-blind study involving patients with cancer pain who were randomised to receive a single IM injection of one of these 3 treatments (60 patients per study group). The patients had moderate-to-severe pain at baseline.

A visual analogue scale (VAS) and 4-point verbal scale were used to evaluate pain intensity after the injection. Pain relief was experienced soon after injection of ketorolac 10mg and lasted for up to 5 hours. There was no between-group difference in mean pain severity scores, as measured by VAS. Patient assessed pain relief was rated as ‘good’ or ‘fair’ in 75% of ketorolac 10mg recipients. The percentage of patients who terminated the study due to inadequate pain relief was 33,32 and 30% in the diclofenac, ketorolac 10 and 30mg groups, respectively.

(Pharmacotherapy 18:504-508, May-Jun 1998)