α-TOCOPHEROL REDUCES DOXORUBICIN-INDUCED TOXICITY IN RATS - HISTOLOGICAL AND BIOCHEMICAL EVIDENCES

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Abstract: The beneficial effect of α-tocopherol on doxorubicin-induced toxicity was studied in rats. α-Tocopherol (400 mg/kg/day) was administered orally, daily for a period of 2 months along with/without doxorubicin (2.5 mg/kg, i.v. weekly once for 8 weeks). Histology showed liver necrosis, heart myocyte degeneration, glomerular and tubular degeneration, cellular infiltration and desquamation of intestinal mucosa in doxorubicin treated animals. There was a significant increase in lipid peroxide levels measured in terms of "TBA reactants" in all these organs. These changes were associated with elevated levels of serum enzymes such as transaminases, creatine kinase and lactate dehydrogenase.

The pathological observations were minimal in animals receiving both doxorubicin and α-tocopherol. The lipid peroxide levels were low with concomitant normal levels of serum and intestinal enzymes in those animals.

Key words: doxorubicin-lipid peroxidation-tissue necrosis α-tocopherol-antioxidant

INTRODUCTION

The anthracycline antibiotic doxorubicin is one of the potent drugs in the field of cancer chemotherapy (1). However its repetitive administration in patients and in experimental animals has been associated with the development of cardiotoxicity (2) and other drastic side effects (3).

Doxorubicin is known to generate superoxide radical ions either enzymatically (4) or non-enzymatically (5) and to stimulate lipid peroxidation (6). The formation of free radicals as well as accumulation of lipid peroxides in doxorubicin treatment has been well documented, and this is recognised as one of the possible biochemical mechanisms for the doxorubicin associated side effects (6).

Yamanaka et al (7) observed the beneficial effects of antioxidants for protection against doxorubicin-toxicity. Infact, α-tocopherol was shown to prevent cardiotoxicity effectively, presumably by inhibiting lipid peroxidation.

Since lipid peroxidation has been reported to be associated with various deleterious effects including tissue damage and necrosis, direct evidence like histopathology of a particular organ may throw more light on the effect of α-tocopherol on the doxorubicin-induced lipid peroxidation.

Hence in the present investigation histochimical observations were made on liver, heart, kidney and intestine of doxorubicin treated animals and compared with those coadministered with α-tocopherol. Levels of some clinically important enzymes in serum and in intestinal mucosa were determined and compared.

METHODS

Doxorubicin hydrochloride (Sigma Chemical Company, USA) was dissolved in the vials with sterile saline and used within 48 hr. The solution was kept in ice in a dark atmosphere until use.

Adult male Wistar rats weighing 150-160 g
were used for the study. The rats were fed with commercial pelleted rat chow and water given ad libitum. The rats were divided into 4 groups. Groups 1 served as control. Group 2 rats were injected doxorubicin 2.5 mg/kg, weekly once for a period of 8 weeks (8). Group 3 rats were fed orally, α-tocopherol, 400 mg/kg, daily for 2 months. Group 4 animals received both doxorubicin and α-tocopherol at the above mentioned dosages.

After the experimental period, the rats were killed by cervical decapitation. Blood was collected and the serum separated was used for the assay of transaminases (9) lactate dehydrogenase (9) and creatine kinase (10).

Immediately after the sacrifice, liver, kidney, heart and intestine were removed and washed in ice-cold saline. A portion of the tissues were fixed in 10% formalin-saline and stained with hematoxylin and eosin for histological examinations. Another portion of the tissues were homogenised in 0.1 M phosphate buffer pH 7.4 and used for the estimation of lipid peroxides in terms of “TBA reactants” (11). 1,1‘3,3‘ tetramethoxy propane was used as the standard.

After washing the food contaminants in the intestine the mucosal scrappings were collected, weighed and homogenised. The homogenate was used for the assay of Na+, K+ - ATPase (12) Ca2+ ATPase (13) and alkaline phosphatase (14) activities. Protein was determined by the method of Lowry et al (15).

RESULTS

Figs. 1 and 2 show the tissue architecture of liver and heart (a, b and c denote the groups 1, 2 and 4, respectively). Hyperaemia, mild foci of centrilobular necrosis, irregular nuclear pyknosis, diffuse cloudy swelling, mild hydropic changes and focal fatty changes are common in doxorubicin treated rat liver. Fig. 1b shows the region of extensive necrosis. These changes are minimum in group 4 rats. Other changes like cellular infiltration and zones of necrosis observed in doxorubicin treatment are also absent.

Heart sections show focal myocyte degeneration, cloudy swelling, loss of striations and multifocal hemorrhage in doxorubicin treated rats. Proliferation of Anitskor cells and edema were also present. Neither necrosis nor myocyte degeneration are seen in group 4 rats. But hyperaemia and mild hemorrhage are noted.

Glomerular hyperaemia, atrophy of glomerulus with tubular necrosis are frequent in sections of kidney from doxorubicin treated animals (Fig.3)

Intestinal sections (Fig 4) from group 2 rats show necrosis and desquamation of the mucosa and infiltration of mononuclear cells. The goblet cell activity is much reduced. These changes are minimum in group 4 rats.

In all these sections control animals show normal architecture and there were no significant

<p>| TABLE I : Levels of lipid peroxides in liver, heart, kidney and intestinal mucosa of control and experimental rats. Values are expressed as mean ± SD for 6 rats in each group. |
|----------------------------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Rats treated with</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Intestinal mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>240.5±12.9</td>
<td>160.2±10.9</td>
<td>262.5±13.6</td>
<td>179.5±11.7</td>
</tr>
<tr>
<td>2</td>
<td>Doxorubicin</td>
<td>320.1±19.1**</td>
<td>241.0±11.6**</td>
<td>310.2±11.0**</td>
<td>249.5±20.3**</td>
</tr>
<tr>
<td>3</td>
<td>α-tocopherol</td>
<td>230.2±11.6m</td>
<td>153.0±11.5m</td>
<td>250.0±20.2m</td>
<td>170.8±10.8m</td>
</tr>
<tr>
<td>4</td>
<td>Doxorubicin+ α-tocopherol</td>
<td>260.3±10.6m</td>
<td>17120±11.3m</td>
<td>276.0±12.5m</td>
<td>190.6±18.5m</td>
</tr>
</tbody>
</table>

Lipid peroxide levels of expressed as n moles malonaldehyde/gm tissue. For statistical analysis Groups 2, 3 and 4 are compared with group

** P < 0.001  ** P < 0.01  NS - Non significant
Histology of liver (Fig. 1) and heart (Fig. 2) of rats (Hematoxylin-Eosin x 200).

(a) untreated, control animals; (b) animals treated with doxorubicin and (c) animals treated with doxorubicin + α-tocopherol
(Treatment as given in 'Materials and Methods')

Note: Necrosis (1b) and only occasional pyknosis (1c); degeneration of myocytes and marked hemorrhages (2b) and only mild hyperaemia and hemorrhage (2c).
Fig. 3 and 4:
Histology of kidney (Fig.3) and Intestine (Fig.4) of rats (Hematoxylin-Eosin x 200).
(a) untreated, control animals; (b) animals treated with doxorubicin and (c) animals treated with doxorubicin + α-tocopherol.

Note: glomerular necrosis, hemorrhage and tubular degeneration (3b) and only mild hyperaemia (3c); necrosis; cellular infiltration, desquamation of mucosa (4b) and only mild infiltration of cells (4c).
alterations in group 3 animals which shows the non toxic nature of α-tocopherol at this dosage.

Levels of "TBA-reactants" in liver, kidney, heart and intestinal mucosa are given in Table I. There is a significant elevation in the levels of TBA reactants in all these organs in the order of Heart < intestine < liver < kidney. The formation of "TBA reactants" is significantly controlled in group 4 animals. Group 3 animals show significantly low levels of "TBA reactants" when compared to that of group 1 animals.

**TABLE II:** Activities of transaminases, lactate dehydrogenase and creatine kinase in the serum of control and experimental rats. Values are expressed as mean ± SD for 6 rats in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats treated with</th>
<th>Glutamate oxaloacetate transaminase</th>
<th>Glutamate pyruvate transaminase</th>
<th>Lactate dehydrogenase</th>
<th>Creatine kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>27.3 ± 2.4</td>
<td>10.3 ± 2.7</td>
<td>82.6 ± 4.3</td>
<td>301.5 ± 5.2</td>
</tr>
<tr>
<td>2</td>
<td>Doxorubicin</td>
<td>37.4 ± 2.1**</td>
<td>14.4 ± 0.8**</td>
<td>103.0 ± 7.3*</td>
<td>362.0 ± 11.2**</td>
</tr>
<tr>
<td>3</td>
<td>α-tocopherol</td>
<td>25.1 ± 1.9**</td>
<td>11.2 ± 0.9**</td>
<td>77.5 ± 5.6**</td>
<td>312.2 ± 9.6**</td>
</tr>
<tr>
<td>4</td>
<td>Doxorubicin+ α-tocopherol</td>
<td>29.3 ± 1.5**</td>
<td>9.25 ± 0.75**</td>
<td>90.0 ± 7.2**</td>
<td>290.0 ± 11.2*</td>
</tr>
</tbody>
</table>

The enzyme activities are expressed as I.U/litre, Statistically significant variations are derived by comparing group 2, 3 and 4 with group 1.

**P < 0.001** **P < 0.01** NS - Non significant

Table II shows the activities of transaminases creatine kinase and lactate dehydrogenase in the serum of control and experimental animals. Activities of these enzymes are elevated in doxorubicin treated rats (Group 2). But the elevation is significantly minimum in group 4 rats. Group 3 rats didnot show any significant changes when compared to control rats.

**DISCUSSION**

Doxorubicin has been shown to be a potential source of free radicals (16). Among the organs studied, heart is found to be more susceptible (Table I). Superoxide radicals and hydrogen peroxide radicals have been reported to be formed during quinone-semiquinone reactions involved
during doxorubicin metabolism (17). The necrotic changes observed here could have been attributed to the lipid peroxidative nature of doxorubicin.

Certain chemicals such as carbon tetrachloride, chloroform and ethanol have been shown to induce necrosis in liver through lipid peroxidation (18,19). Intermediates such as conjugated dienes and hydroperoxides have been reported to be the primary causes.

Levels of transaminases, creatine kinase and lactate dehydrogenase are the diagnostic indicators of hepatic and myocardial functions (20). Results from Table II clearly show the cardiotoxic and hepatotoxic nature of doxorubicin which have been demonstrated by various workers (2, 3).

As noted from the histological reports the hepatic necrosis and heart myocyte degeneration could have resulted in the leakage of enzymes into the blood stream. Generally, the quantity of enzyme released from the damaged tissue is a measure of number of necrotic cells. α-tocopherol co-administered rats show low levels of malonaldehyde in liver and heart and no necrosis. This is also reflected in the normal serum enzyme levels in group 4 animals. α-tocopherol is known to act as peroxyl radical-trapping chain breaking antioxidant and also act as a scavenger of free radicals (21, 22).

Nephrotoxicity has been reported in doxorubicin therapy (23). Histopathology of kidney from group 2 rats show glomerular necrosis and hemorrhage into the Bowman’s capsule and tubular degeneration. α-tocopherol significantly minimize these changes.

The elevated lipid peroxidation in the intestinal mucosa would have been responsible for the observed decrease in the activities of membrane bound ATPases and alkaline phosphatase because biomembranes are more susceptible for free radical attack. Ca²⁺ and Na⁺, K⁺ ATPases have been shown to be -SH group containing enzymes (24) and in many cases the inhibition of enzymes by quinone anticancer drugs was considered to be due to the attack on the -SH groups essential for the catalytic activity (25). Nicotera et al (26) have associated the loss of critical protein -SH groups with inactivation of Ca²⁺ dependent ATPase. It has been demonstrated that the prevention of doxorubicin toxicity by α-tocopherol is due to maintenance of protein thiols (27). Alkaline phosphatase was reported to be a glycoprotein which were shown to be a preferential target for the active oxygen free radicals (28).

As is well known α-tocopherol is capable of protecting membrane lipids from lipid peroxidation in vivo (29) Diplock and Lucy (20) have demonstrated the interaction between phytyl side chain of α-tocopherol with membrane phospholipid arachidonic acid which is the major site of lipid peroxidation.

From this investigation it could be supported that α-tocopherol prevents the peroxidative tissue damage induced by doxorubicin probably through its antioxidant capacity.

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REFERENCES


