EFFECT OF CAPTOPRIL ON DOXORUBICIN-INDUCED NEPHROTOXICITY IN NORMAL RATS

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Biochemical evaluations of the effects of the sulfhydryl-containing angiotensin-converting enzyme inhibitor (captopril) on the nephrotoxicity induced by doxorubicin in normal rats were carried out. A single dose of doxorubicin (15 mg kg\(^{-1}\)) which caused nephrotoxicity was manifested biochemically by the elevation of serum urea after 24 and 48 h of administration. Also a severe decrease in total proteins and albumin after 4, 24 and 48 h was observed. Moreover, a decrease of non-protein sulfhydryl (–SH) concentrations in the kidney tissues after 24 h and an increase in the lipid peroxidation was observed after 4 h administration of doxorubicin. Captopril (60 mg kg\(^{-1}\) i.p.) injection did not induce any change in the biochemical parameters measured, however, captopril administered 1 h before doxorubicin ameliorated the biochemical toxicity induced by doxorubicin. This was evidenced by a significant reduction in serum urea and the lipid peroxidation after 4 and 24 h and a significant reduction in creatinine after 48 h. Also, the captopril amelioration was evidenced by an increase in total proteins and albumin after 4 and 24 h of doxorubicin administration. Captopril did not change non-protein sulfhydryl (–SH) concentrations or protein content in the kidney tissues. These results suggest that captopril may be beneficial as a protective agent against nephrotoxicity induced by doxorubicin.

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KEY WORDS: captopril, doxorubicin, nephrotoxicity, rats.

INTRODUCTION

Doxorubicin, an anthracyclin antibiotic represents a class of anticancer agents. It shows broad spectrum antitumour activities in certain human cancers including breast cancer, small cell carcinoma of the lung and acute leukaemia [1]. The optimal use of doxorubicin is limited by a number of side-effects; the most important are cardiotoxicity, haematotoxicity [2–4] and a dose-limiting nephrotoxicity [5]. The exact mechanism of doxorubicin-induce nephrotoxicity is not yet known. However, it has been suggested by many investigators that cellular damage induced by doxorubicin is mediated by the formation of an iron anthracyclin free radical [6, 7] which in turn causes severe damage to the plasma membrane [8].

Angiotensin-converting enzyme (ACE) activity plays a major role in arterial hypertension and nephrotoxicity and hence ACE inhibitors such as captopril are effectively used as antihypertensive agents [9], and suggested to ameliorate nephrotoxicity [10]. Recently, Al-Shabanah et al. [11] have reported that captopril ameliorates doxorubicin-induced cardio- and haematotoxicity in normal rats. The involvement of ACE inhibitors in amelioration of nephrotoxicity induced by doxorubicin is controversial and depends on the type and mode of action [12]. Some investigators [13, 14] reported that ACE inhibitors induced functional renal insufficiency, while others [15] declared that captopril partially inhibits the development of the functional and morphological damage induced by doxorubicin.

In view of the existing controversies on the role of ACE inhibitors (captopril) on the nephrotoxicity, it is worthwhile to gain further insight into the role of captopril as a free radical scavenger on the nephrotoxicity induced by doxorubicin.


MATERIALS AND METHODS

Chemicals
Captopril was obtained from Sigma Chemical Company, St Louis, MO, USA. Doxorubicin was purchased from Farmitalia, Carlo Erba, Milan, Italy. Drugs were dissolved in distilled water just before use.

Animals
Adult albino rats of both sex, weighing 250–300 g, were obtained from the Experimental Animal Care Center of King Saud University, Riyadh, KSA. Animals were maintained under standard conditions of humidity with regular light/dark cycles and free access to food (Purina chow) and water.

Experimental protocol
Eighty rats were divided into four groups, each consisting of 20 rats. The first, control group, received normal saline. The second, doxorubicin group, consisting of 20 rats. The first, control group, received 15 mg kg⁻¹ doxorubicin i.p. The third, captopril group, received 60 mg kg⁻¹ captopril i.p. and the fourth, doxorubicin–captopril group, received 60 mg kg⁻¹ captopril i.p. and 1 h later 15 mg kg⁻¹ doxorubicin i.p. The rats in each group (20 rats) were then divided into four subgroups (five rats per subgroup) to be used for serum analysis after drug administration at intervals of 4, 24, 48 and 72 h.

At 4-, 24-, 48- and 72-h intervals, after the administration of the drugs, five rats from each group were anaesthetised with ether and blood samples were collected from each rat by intracardiac puncture. Serum was separated for the measurement of kidney functions, plasma proteins, albumin and electrolytes. Kidneys were removed from the animal, washed with saline, weighed and homogenised in ice-cold bidistilled water in a Branson sonifier (250, VWR Scientific, Danbury, CT, USA).

Serum urea, creatinine and albumin were measured according to the methods of Halet and Cook [16], Bonsnes and Taussky [17] and Wrenn and Flichtmeir [18], respectively, while total proteins were measured according to the method of Lowry et al. [19]. Serum calcium, sodium and potassium were determined using atomic absorption spectrophotometer (AA 180, Varian, Sunnyvale, CA, USA).

Lipid peroxidation (malondialdehyde production) and total non-protein sulfhydryl groups in the kidney tissues were determined according to the methods of Ohkawa et al. [20] and Ellman [21], respectively, while tissue proteins were determined according to the method of Lowry et al. [19] using turbidity blank for each sample, consisting of kidney homogenate added to the reagent blank.

Statistical analysis
Data are expressed as (means ± SEM). Statistical comparison between different groups were done by using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. Significance was accepted at \( P < 0.05 \).

RESULTS

Serum level of urea and creatinine
Treatment with captopril failed to alter the serum urea and creatinine concentrations, however, doxorubicin administration significantly increased urea concentrations after 24 and 48 h (\( P < 0.05 \)) whereas, creatinine concentrations tended to increase at 24 and 48 h compared to the values in the normal group.

The doxorubicin-induced increase in the renal function tests was reduced after treatment with captopril. Elevated concentrations of serum urea and creatinine were decreased after captopril administration, however, captopril failed to decrease the serum urea after 48 h. These changes were statistically significant as compared to the values obtained after treatment with doxorubicin alone (Table I).

Total proteins and albumin
The plasma proteins and albumin were not affected after captopril treatment (Table I) however, doxorubicin therapy was shown to decrease total proteins and albumin significantly after 4, 24 and 48 h as compared to the control values. Pretreatment with captopril significantly increased the total proteins and albumin concentrations after 4 and 24 h. However, the concentrations of plasma proteins are still significantly lower than the control after 24 and 48 h.

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>Treatment</th>
<th>Urea (mg%)</th>
<th>Creatinine (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>Control</td>
<td>31.6 ± 2.1</td>
<td>0.4 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>29.6 ± 0.28</td>
<td>0.3 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>48.7 ± 5.5</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>23.6 ± 9.6</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>24 h</td>
<td>Control</td>
<td>36 ± 3.9</td>
<td>0.55 ± 0.022</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>38.5 ± 2.8</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>57.1 ± 4.7</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>37.1 ± 4.2</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>48 h</td>
<td>Control</td>
<td>31 ± 6.2</td>
<td>0.546 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>33.8 ± 4.3</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>62.8 ± 8.7</td>
<td>0.68 ± 0.035</td>
</tr>
</tbody>
</table>
|                      | Cap + Dox.| 53.4 ± 3.4| 0.34 ± 0.08**†

Data are expressed as mean ± se (n = 5).

*Significant difference from the control \( P < 0.05 \).
†Significantly different from doxorubicin group \( P < 0.05 \).
Table II
Effect of captopril on doxorubicin-induced decrease in serum albumin and total proteins

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>Treatment</th>
<th>Serum Total proteins (g %)</th>
<th>Albumin (g %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>Control</td>
<td>6.1 ± 0.28</td>
<td>3.8 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>5.9 ± 0.09</td>
<td>3.4 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>4.4 ± 0.33*</td>
<td>2.2 ± 0.11*</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>5.7 ± 0.05†</td>
<td>3.3 ± 0.05†</td>
</tr>
<tr>
<td>24 h</td>
<td>Control</td>
<td>7.4 ± 0.09</td>
<td>3.4 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>7.8 ± 0.31</td>
<td>3.46 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>4.2 ± 0.12*</td>
<td>2.26 ± 0.16*</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>5.6 ± 0.15*†</td>
<td>3.15 ± 0.07†</td>
</tr>
<tr>
<td>48 h</td>
<td>Control</td>
<td>7.9 ± 0.55</td>
<td>3.4 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>7.66 ± 0.4</td>
<td>3.46 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>4.7 ± 0.08*</td>
<td>2.6 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>5.8 ± 0.173*</td>
<td>3.14 ± 0.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± se (n = 5).
*Significantly different from the control P < 0.05.
†Significantly different from the doxorubicin group P < 0.05.

Tissue proteins, lipid peroxides and non-protein sulfhydryl (–SH)
Doxorubicin treatment was found to increase lipid peroxides (MDA) significantly after 4 h. In the presence of captopril the malondialdehyde was significantly decreased (Table II). A single i.p. injection of doxorubicin resulted in a decrease in the total non-protein sulphydryl groups (glutathione) content in the kidney tissues after 24 h. Pretreatment with captopril did not increase the level of total non-protein sulphydryl groups. There was no significant difference in the levels of tissue proteins among different groups.

Serum levels of calcium, sodium and potassium
There was no significant differences in the levels of calcium, sodium and potassium between different groups tested after captopril treatment. Also a single i.p. injection of doxorubicin did not induce any change in serum electrolyte levels.

DISCUSSION

In the present study, a single dose of doxorubicin (15 mg kg⁻¹) induced nephrotoxicity manifested biochemically by a significant increase in serum urea and by a significant decrease in total proteins and albumin after 4, 24 and 48 h. Furthermore, our results are supported by a significant increase in malondialdehyde and a significant decrease in glutathione concentrations in the renal cells after treatment with doxorubicin. These results are consistent with previous studies reported by other investigators [5, 15, 22, 23] that doxorubicin induced nephrotoxicity in normal rats. A single dose of captopril (60 mg kg⁻¹) given 1 h before doxorubicin, protected the kidney from damage induced by doxorubicin. This protection was clearly reflected by a decrease of serum creatinine, urea and by an increase in total proteins and albumin. Although captopril can not increase the glutathione content in the kidney tissues, it decreased lipid peroxidation induced by doxorubicin, thus captopril may protect kidneys by preventing lipid peroxidation.

The results of the present study that captopril did not elevate the glutathione content and decreases serum creatinine are not in agreement with the finding of Al-Harbi [24] who claimed that captopril decreases the glutathione level in the renal cells and did not decrease the higher creatinine concentrations after doxorubicin administration. However, captopril pre-treatment has been shown to be protective against nephrotoxicity induced by cisplatin. The basis of the controversies is due to the difference in the doses used and in the experimental

Table 3
Effect of captopril on doxorubicin-induced increase in lipid peroxide and decrease in glutathione content

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>Treatment</th>
<th>Kidney tissues MDA (nmol g⁻¹ kidney %)</th>
<th>Sulphydryl content (µmol g⁻¹ kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>Control</td>
<td>408 ± 12.6</td>
<td>3.3 ± 0.144</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>372 ± 8.6</td>
<td>2.86 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>519 ± 41.3*</td>
<td>2.77 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>411 ± 18†</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>24 h</td>
<td>Control</td>
<td>414 ± 9.4</td>
<td>3.2 ± 0.022</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>360 ± 14.1</td>
<td>3.0 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>471 ± 23</td>
<td>1.27 ± 0.075*</td>
</tr>
<tr>
<td></td>
<td>Cap + Dox.</td>
<td>377 ± 28†</td>
<td>1.5 ± 0.122*</td>
</tr>
</tbody>
</table>

*Significant difference from the control P < 0.05.
†Significantly different from doxorubicin group P < 0.05.
design. Reduced glutathione was reported to protect the cells from cytotoxic damage induced by many compounds [25] and it is generally known as a potent factor in the control of lipid peroxidation [26]. Recently Al-Shabanah et al. [11] declared that captopril ameliorates cardio- and haematotoxicity induced by doxorubicin.

Several proposals for the mechanisms of doxorubicin-induced nephrotoxicity have been put forward, involving the role of free radicals which are increasingly produced by doxorubicin. The findings of the present study that doxorubicin administration decreased the glutathione content in rat kidney after 24 h and increased lipid peroxidation after 4 and 24 h, are consistent with the previous report [27] that cisplatin administration to rats resulted in depletion of glutathione in the kidney cortical slices and that a relation exists between the cisplatin-induced nephrotoxicity and the depletion of glutathione content of kidney tissues [28]. It seems that lipid peroxidation is the main cause of doxorubicin-induced nephrotoxicity and that doxorubicin-induced lipid peroxidation is probably due to depletion of nonprotein sulfhydryl containing compounds.

Several natural and synthetic antioxidants have been implicated to minimise toxicity induced by free radical generation of doxorubicin, among them thymoquinone (TQ), a product derived from Nigella sativa seeds [29]. It has been reported that TQ inhibits membrane lipid peroxidation and eicosanoids generation by stimulated leucocytes [30]. The possibility that captopril protects the renal tissues against the doxorubicin-induced toxicity by scavenging the superoxide anion radicals is uncertain, since we recently reported that captopril at micromolar concentrations was not able to scavenge superoxide radicals in pure chemical systems [31]. However, an ongoing investigation in our laboratory shows that captopril totally abolished leukotriene B4 formation from calcium ionophore-stimulated neutrophil. Leukotriene B4 is a highly superoxide anion generator [32]. Therefore, it is possible that captopril may protect the renal tissues against doxorubicin-induced renal toxicity by indirectly inhibiting the generation of superoxide anion via inhibition of LTB4 formation.

Bolli et al. [33] reported that thiols can react with superoxide anion radicals, but the rate constant for these reactions is very low, usually $< 10^{-3} \text{ M}^{-1} \text{s}^{-1}$. Therefore, other free radicals, such as hydroxyl radical and hypochlorous acid may be involved in mediating the toxicity of doxorubicin. Captopril has been reported to react rapidly with hydroxyl radicals (rate constant $> 10^{-3} \text{ M}^{-1} \text{s}^{-1}$) and hypochlorous acid at micromolar concentrations [34]. Moreover, captopril was found to enhance the enzymatic activity of superoxide dismutase and selenium-dependent glutathione-peroxidase [35]. Thus, the possible hydroxyl radical and hypochlorous acid scavenging ability, together with the interference of captopril with the binding of doxorubicin to DNA may explain at least in part, the observed protective effect of captopril against undesirable side-effects of doxorubicin.

In conclusion, lipid peroxidation and depletion of glutathione content are the main causes of doxorubicin-induced nephrotoxicity. Captopril which was useful for amelioration of cardio- and haematotoxicity induced by doxorubicin, can also protect the kidney against nephrotoxicity induced by doxorubicin. Further investigations are needed to explore the possible mechanism of the protective actions of captopril.

REFERENCES


