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Potential Protective Role of Angiotensin-converting Enzyme Inhibitors Captopril and Enalapril against Adriamycin-induced Acute Cardiac and Hepatic Toxicity in Rats

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Captopril and enalapril—angiotensin-converting enzyme (ACE) inhibitors—were evaluated for their antioxidative protective action against adriamycin-induced cardiac and hepatic toxicity. Rats were treated with either captopril (10 mg kg^{-1}) or enalapril (2 mg kg^{-1}) intragastrically (i.g.) daily for 7 days before single intraperitoneal (i.p.) injection with adriamycin (15 mg kg^{-1}). The animals were killed 30 h after adriamycin administration. Adriamycin produced significant elevation in thiobarbituric acid reactive substances (TBARS), which is an indicator of lipid peroxidation, and significantly inhibited the activity of superoxide dismutase (SOD) in heart and liver tissues, with a significant rise in the serum levels of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), creatine kinase isoenzyme (CK-MB) and lactic dehydrogenase (LDH), indicating acute cardiac toxicity. A single injection of adriamycin did not affect the cardiac or hepatic glutathione (GSH) content or cardiac catalase (CAT) activity, but hepatic CAT activity was elevated. Pretreatment with ACE inhibitors significantly reduced the TBARS concentration in both heart and liver and ameliorated the inhibition of cardiac and hepatic SOD activity. In addition, the ACE inhibitors significantly improved the serum levels of GOT, GPT, CK-MB and LDH in adriamycin-treated rats. Thus, these results suggest that captopril and enalapril possess antioxidative potential that may protect the heart against adriamycin-induced acute oxidative toxicity. This protective effect might be mediated, at least in part, by the limitation of culprit free radicals and the amelioration of oxidative stress.

INTRODUCTION

Adriamycin—a quinone-containing anthracycline antibiotic—is an important anticancer drug used in treating a wide spectrum of human neoplasms. Nevertheless, cardiac toxicity is a major factor that limits its use in cancer chemotherapy.[1,2] The exact mechanism of adriamycin-induced toxicity remains unclear. However, several different pieces of evidence have indicated that free radicals are involved.[3]

The chemical structure of adriamycin is prone to the generation of free radicals and the induction of oxidative stress that correlates with cellular injury.[4] In addition, adriamycin administration is associated with a decrease in the endogenous antioxidants responsible for the scavenging of free radicals.[5] A decrease in antioxidants and an increase in oxidants resulted in increased oxidative stress, which is followed by the development of cardiotoxicity and hepatotoxicity.[6,7] During recent years, considerable efforts have been focused on the use of antioxidants to prevent adriamycin-induced toxicity. Strategies have been used in an attempt to prevent adriamycin-induced cardiac toxicity, without affecting its anti-tumour activity, include administration of adriamycin in combination with cardioprotective agents and prevention of oxidative stress-induced injury by adjunctive therapy with antioxidants.[8]

Angiotensin-converting enzyme (ACE) inhibitors are commonly used as cardioprotective drugs.[9,10] In addition, ACE inhibitors have been shown to be potent scavengers of free radicals.[11,12] Therefore, the aim of the present study was to investigate the possible protective effects of the ACE inhibitors captopril and enalapril against adriamycin-induced cardiac and hepatic toxicity in rats. In addition, the study was designed to examine the involvement of an anti-free-radical mechanism in this protection.

MATERIALS AND METHODS

Adriamycin was obtained from Pharmacia & Upjohn, Italy. Captopril was obtained from Bristol-Myers Squibb Company, Egypt. Enalapril was obtained from Merk & Co. Inc., USA. All other chemicals were purchased from Sigma Chemical (St Louis, MO).

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Adult male rats weighing 150–180 g, bred at the university experimental animal care centre, were used in the study. They were maintained under standard conditions and fed a standard chow and water *ad libitum*. All experiments were carried out in accordance with protocols approved by the local experimental animal ethics committee. Rats were divided randomly into six groups of eight animals each, as follows.

Group 1 received 0.5 ml of water intragastrically (i.g.) daily for 7 days and served as the control group. Group 2 received 10 mg captopril kg^{-1} body weight dissolved in distilled water daily i.g. for 7 days.[13] Group 3 received 2 mg kg^{-1} body weight of enalapril dissolved in distilled water daily i.g. for 7 days.[14] Group 4 received a single intraperitoneal (i.p.) injection of adriamycin (15 mg kg^{-1}).[13] Group 5 received captopril and adriamycin at the same doses and schedule as groups 2 and 4. Group 6 received enalapril and adriamycin at the same doses and schedule as groups 3 and 4.

The rats were sacrificed by decapitation 30 h after adriamycin administration. Blood was collected from carotid arteries and the sera were separated by centrifugation at 3000 rpm for 10 min and frozen at $-20\,^{\circ}C$ for estimation of serum activities of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT),[15] creatine kinase isoenzyme (CK-MB)[16] and lactic dehydrogenase.[17] The heart and liver were isolated, weighed, cooled and homogenized (100 mg 2 ml^{-1} in ice-cool phosphate buffered saline, pH 7.4, for 30 s) for biochemical determinations of the product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS),[18] the activity of superoxide dismutase (SOD),[19] catalase (CAT) activity[20] and glutathione (GSH) content.[21] For SOD and CAT the homogenates were sonicated for 2 min.

Statistical analysis

Statistical comparison was made using analysis of variance (ANOVA) followed by Newman–Keuels' *post hoc* test.

RESULTS

The results of the present study as shown in Tables 1–3 revealed that the administration of either captopril (10 mg kg^{-1}) or enalapril (2 mg kg^{-1}) induced insignificant changes in the tested parameters compared with the control.

Table 1 shows the effect of ACE inhibitor captopril (10 mg kg^{-1}) or enalapril (2 mg kg^{-1}) on adriamycin (15 mg kg^{-1}) adriamycin-induced biochemical changes in the serum of the treated rats. Animals that received adriamycin (15 mg kg^{-1}) had significantly higher activities of GOT, GPT, CK-MB and LDH when compared with control values. Administration of captopril or enalapril daily for 7 days before adriamycin treatment prevented the increase in the activities of these enzymes in the serum

Table 1—Effect of captopril (10 mg kg^{-1}) and enalapril (2 mg kg^{-1}) on adriamycin (15 mg kg^{-1})-induced biochemical changes in serum of rats

Treatment	Serum enzyme levels (U l^{-1})			
	GOT	GPT	CPK-MB	LDH
Control	79.0 ± 3.5	14.6 ± 1.9	10.5 ± 1.3	260 ± 19.3
Captopril	81.3 ± 5.5	15.7 ± 1.7	13.0 ± 1.1	278 ± 20.2
Enalapril	83.5 ± 6.3	15.7 ± 1.4	13.1 ± 1.1	281 ± 20.7
Adriamycin	$105.4 \pm 7.4^{**}$	$37.2 \pm 2.3^{**}$	$36.6 \pm 1.8^{**}$	$377 \pm 30.1^{**}$
Captopril + adriamycin	$88.8 \pm 7.6^{\dagger\dagger}$	$16.5 \pm 1.5^{\dagger\dagger}$	$18.3 \pm 2.9^{**,\dagger\dagger}$	$288 \pm 29.9^{\dagger\dagger}$
Enalapril + adriamycin	$89.9 \pm 8.1^{\dagger\dagger}$	$17.2 \pm 1.3^{\dagger\dagger}$	$19.1 \pm 1.1^{**,\dagger\dagger}$	$301 \pm 28.8^{**,\dagger\dagger}$

Values are means ± SEM of eight animals;
** significant compared with control group, $P < 0.01$;
†† significant compared with adriamycin-treated group, $P < 0.01$.

Table 2—Effect of captopril (10 mg kg^{-1}), enalapril (2 mg kg^{-1}) and adriamycin (15 mg kg^{-1}) on lipid peroxidation (nmol TBARS g^{-1} fresh tissue), superoxide dismutase (SOD, U g^{-1} fresh tissue), catalase (CAT, U g^{-1} fresh tissue) and glutathione (GSH, mg g^{-1} fresh tissue) in rat heart

Treatment	Parameter levels			
	TBARS	SOD	CAT	GSH
Control	94 ± 9.5	1328 ± 98	0.109 ± 0.03	0.510 ± 0.06
Captopril	110 ± 11.1	1368 ± 39	0.110 ± 0.01	0.496 ± 0.08
Enalapril	105 ± 10.9	1329 ± 80	0.112 ± 0.03	0.480 ± 0.06
Adriamycin	$355 \pm 40^{**}$	$625 \pm 40^{**}$	0.115 ± 0.03	0.512 ± 0.05
Captopril + adriamycin	$170 \pm 21^{**,\dagger\dagger}$	$1080 \pm 85^{**,\dagger\dagger}$	0.114 ± 0.03	0.518 ± 0.06
Enalapril + adriamycin	$160 \pm 15.2^{**,\dagger\dagger}$	$1139 \pm 34^{**,\dagger\dagger}$	0.107 ± 0.01	0.485 ± 0.07

Values are means ± SEM of eight animals;
** significant compared with control group, $P < 0.05$;
†† significant compared with adriamycin-treated group, $P < 0.05$.

Table 3—Effect of captopril (10 mg kg^{-1}), enalapril (2 mg kg^{-1}) and adriamycin (15 mg kg^{-1}) on lipid peroxidation (nmol TBARS g^{-1} fresh tissue), superoxide dismutase, (SOD, U g^{-1} fresh tissue), catalase (CAT, U g^{-1} fresh tissue) and glutathione (GSH, mg g^{-1} fresh tissue) in rat liver

Treatment	Parameter levels			
	TBARS	SOD	CAT	GSH
Control	85.2 ± 7.3	1128 ± 74	0.100 ± 0.01	3.58 ± 0.25
Captopril	95 ± 8.5	1284 ± 70	0.105 ± 0.02	3.25 ± 0.10
Enalapril	82.5 ± 6.0	1207 ± 68	0.106 ± 0.03	3.67 ± 0.18
Adriamycin	$390 \pm 35.5^{**}$	$990 \pm 60^{**}$	$0.138 \pm 0.03^{**}$	3.08 ± 0.35
Captopril + adriamycin	$290 \pm 30.2^{**,\dagger\dagger}$	$1280 \pm 90^{\dagger\dagger}$	$0.124 \pm 0.03^{**}$	2.91 ± 0.31
Enalapril + adriamycin	$247 \pm 22.0^{**,\dagger\dagger}$	$1292 \pm 97^{\dagger\dagger}$	$0.128 \pm 0.03^{**}$	3.37 ± 0.3

Values are means ± SEM of eight animals.
** significant compared with control group, $P < 0.05$;
†† significant compared with adriamycin-treated group, $P < 0.05$.

and displayed insignificant changes when compared with the control.

In addition, a significant increase in lipid peroxidation (LPO) was induced in the heart and liver, as indicated from the rise of TBARS concentration in both tissues 30 h after ADR treatment (Tables 2 and 3). The present results also revealed a good amelioration of LPO in both tissues against an adriamycin-induced rise in TBARS level by oral administration of either captopril or enalapril daily for 7 days before adriamycin treatment.

The activity of SOD in heart and liver was inhibited significantly after 30 h of adriamycin treatment (Tables 2 and 3). This effect was prevented significantly in both tissues by i.g. pre-administration of either captopril or enalapril before adriamycin treatment. The activity of CAT did not changed in the heart after 30 h of adriamycin treatment (Table 2). On the other hand, CAT activity in the liver was increased significantly in the liver of the same adriamycin-treated rats. This effect was modulated significantly in the liver by oral administration of either captopril or enalapril before adriamycin treatment but still showed higher activity than the control value (Table 3). The non-enzymatic antioxidant GSH did not changed in the heart or liver in the same adriamycin-treated rats 30 h after treatment (Tables 2 and 3).

DISCUSSION

Adriamycin is an anthracycline derivative and is one of the most frequently used antineoplastic agents.[22] However, its clinical effectiveness is restricted due to toxic effects such as cardiac, hepatic and myelo-toxicity.[7,23] The mechanism of adriamycin-induced toxicity is not fully understood. One hypothesis proposed for this mechanism is the involvement of free radicals.[24,25] Adriamycin has been demonstrated to be a potent generator of free radicals.[26,27]

Administration of adriamycin in combination with agents that would block its free-radical-mediated toxicity without affecting its anti-tumour activity, and prevents its oxidative stress and tissue injury, might serve as a novel combination. Angiotensin-converting enzyme inhibitors are reported to provide protection against free-radical-mediated damage.[28,29] However, their effects on adriamycin-induced organ toxicity also are unknown.

The purpose of this study was to investigate the potential protective effect of ACE inhibitors captopril and enalapril on adriamycin-induced cardiac and hepatic toxicity in rats and their possible mechanism of protection. The results of this study showed an obvious cardiac and hepatic protection by prophylactic administration of captopril (10 mg kg^{-1}) or enalapril (2 mg kg^{-1}) for 7 days before adriamycin treatment (15 mg kg^{-1}), as evidenced by biochemical and histopathological changes in the heart and liver. The functional parameters, namely GOT, GPT, CK-MB and LDH, were greatly reduced. At the same time, the activity of the antioxidant enzyme SOD in heart and liver was increased significantly after its suppression by adriamycin.

Adriamycin caused a significant increase in TBARS concentration in heart and liver, indicating increased LPO, which is a basic deteriorative process in the cell. This is in agreement with several reports in heart,[30,31] liver[7] and the nervous system.[32] One of the most prevailing hypotheses of cardiac and hepatic damage resulting from adriamycin administration is the ability of the drug to produce free radicals and reduce the antioxidant defense mechanism.[24,33,34] Free radicals are known to damage several macromolecular and cellular components.[35] However, up-regulation of antioxidant gene expression occurred in response to adriamycin in mouse heart, although the antioxidant activities were not all increased.[30] The CAT activity was unaffected in rat heart but significantly elevated in liver 30 h after adriamycin treatment (Tables 2 and 3). Yin *et al.*[30] and Venkatesan[31] demonstrated increased CAT activity in the heart after 4 and 2 days, respectively. Thus, the effect of adriamycin on the CAT activity might be time dependent. In addition, the difference in the effect of adriamycin on CAT activity in heart and liver after 30 h of adriamycin administration might, explain the higher resistance of liver compared with heart with respect to the adriamycin-induced toxicity recorded in the present study.

Pretreatment with either captopril or enalapril produced significant reduction in TBARS formation and maintained the antioxidant levels in ACE inhibitors combined with adriamycin-treated rats. These findings confirm the previous reports on the association of ACE inhibitors and their antioxidant action.[12,29] Moreover, the present data support the hypothesis that the generation of free radicals might play an important role in adriamycin-induced toxicity.[25,36] Consistent with this notion, several authors have shown TBARS as a marker of free radicals to be increased in adriamycin-treated cells.[37–39] Adriamycin-induced free

radicals may attack the heart or liver cell membranes and cause protein and lipid peroxidation, which would affect the cellular integrity and potentially account for the increased serum enzymes (GOT, GPT, CK-MB and LDH), as well as contributing to the pathological changes recorded in the heart.[8]

Several groups of investigators have investigated some aspects of possible free radical scavenging by ACE inhibitors.[40] Studies by Westlin and Mullane[41] have demonstrated, both *in vitro* and *in vivo*, that captopril scavenges superoxide radicals and improves myocardial dysfunction. Al-Harbi[13] stated that the free radical scavenging action of captopril was found to be equivalent to the combined effects of SOD, CAT and allopurinol. In addition, the ACE inhibitors captopril, Epicaptopril, Zofenopril and enalapril significantly scavenged free radicals and enhanced glutathione-dependent antioxidant defenses.[29,42]

It is demonstrated that the sulfhydryl compound GSH (glutathione) inhibits adriamycin-induced cardiotoxicity in rat.[43] Although some studies have suggested that the sulfhydryl portion of the ACE inhibitors is required for the optimal free radical scavenging effect,[12,41,42] other studies[10,44,45] have found that ACE inhibitors both with and without the sulfhydryl moiety act as effective free radical scavengers. In the present study captopril contains a sulfhydryl group, whereas enalapril does not; both drugs produced a similar protection against adriamycin-induced cardiac and liver toxicity.

Recently, it is demonstrated that both captopril and enalpril increased SOD activity in kidney medulla, heart and erythrocytes, whereas CAT activity was unaffected by either treatment.[46,47] These results were supported by the present observations that neither captopril nor enalapril treatment had an effect on CAT activity.

The prophylactic impact of captopril or enalapril against LPO supports the foregoing proposal and could be explained by a mechanism involving the stimulation of radical scavenger and sustained antioxidant capacity in cells. Tables 2 and 3 showed that the SOD activity was significantly higher in ACE-inhibitor-protected rats than in adriamycin-treated rats. The increased SOD activity is important and is correlated with increased resistance towards free radicals and exerts a radical-scavenging impact. In conclusion, these results show that the generation of oxygen free radicals may be a possible primary event in the development of adriamycin-induced acute cardiac and hepatic toxicity. Secondly, ACE inhibitors have a protective antioxidant effect on adriamycin-induced toxicity and they might serve as a novel combination with adriamycin to limit its free-radical-mediated organ injury.

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