



J. Egypt. Soc. Toxicol.
(Vol. 35: 123-129 July 2006)
WWW.estoxicology.org

EVALUATION OF THE POTENTIAL PROTECTIVE EFFECT OF CAPTOPRIL AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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ABSTRACT

The purpose of this study was to investigate the possible protective effect of Cap, an angiotensin-converting enzyme inhibitor with an antioxidant property, against DOX-induced cardiotoxicity. Results of the present study showed that DOX decreased significantly plasma activities of CK, CK-MB and LDH, while it produced a significant increase in serum NO level, cardiac MDA level and CAT activity. Significant reductions in cardiac GSH level and GPx activity were also produced. Administration of Cap one hour before DOX treatment attenuated the reduction in CK-MB activity. The combined treated group showed significant decrease in cardiac MDA level. Also, DOX concentration was markedly decreased in both serum and cardiac tissue. Data of the present study showed that Cap had limited protective effect on cardiotoxicity produced by DOX treatment. The mechanism by which Cap exerts its protective effect may involve inhibition of lipid peroxidation..

Key words: Captopril – Doxorubicin – Cardiac tissue.

INTRODUCTION

Doxorubicin (DOX) is one of the most widely prescribed antineoplastic agents having broad spectrum of activity. It is effective against various localized and disseminated neoplasms; lymphoblastic and myeloblastic leukaemia, neuroblastomas, bone marrow sarcomas and carcinomas of breast, bladder, and thyroid (Wallace 2003). However, the clinical use of DOX is limited by its cumulative dose-related cardiotoxicity which may ultimately lead to severe and irreversible forms of cardiomyopathy (Hrdina *et al.*, 2000). Several mechanistic pathways have been suggested to explain such toxicity. These include; disturbance of calcium homeostasis (Zucchi and Ronca-Testoni, 1997), interference with membrane phospholipids (Goormaghtigh *et al.*, 1980), metabolite toxicity (Licata *et al.*, 2000), mitochondrial dysfunction (Chacon and Acosta, 1991), imbalance in nitric oxide production (Pacher *et al.*, 2003) and liberation of reactive free radicals (Venkatesan, 1998). However, the primary cause of DOX- cardiotoxicity was focused on the induction of oxidative stress and lipid peroxidation (Venkatesan, 1998; Quiles *et al.*, 2002).

Captopril (Cap) is one of the thiol-containing angiotensin converting enzyme inhibitors (ACEIs) that has achieved wide spread usage in treatment of hypertension and congestive heart failure (Okumura *et al.*, 2002). Its sulfhydryl group binds tightly to the zinc ion at active site of angiotensin converting enzyme with an affinity that is 30,000 times greater than that of angiotensin I (Cushman and Ondetti 1980). Cap has been shown to be a potent

scavenger of free radicals (De cavanagh *et al.*, 2000). Although some studies showed that both sulfhydryl and non- sulfhydryl containing ACEIs act as effective free radical scavengers (Mehta *et al.*, 1990; Mira *et al.*, 1994), others suggested that sulfhydryl-containing inhibitors have other additional cardioprotective effects (Liu and Xu, 1993; De Nigris *et al.*, 2001). It has been reported that Cap is very effective in scavenging free radicals in a manner similar to glutathione (GSH) and N-acetylcysteine (Goldshmidt and Tallarida, 1991). It has been shown to scavenge efficiently hydroxyl radical and hypochlorous acid (Arouma *et al.*, 1991) and enhance the antioxidant enzymatic activity of superoxide dismutase and selenium-dependent glutathione-peroxidase (GPx) in mouse liver (Elena *et al.*, 2000). Moreover, Cap has been shown to have cardioprotective effects against DOX toxicity in different animal models (Alshabanah *et al.*, 1998; Dogan *et al.*, 1998; Abdelaziz *et al.*, 2001). However, these primary data need further substantiation. Therefore, the present study was designed to investigate the possible protective effects of Cap against DOX-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Drugs and chemicals

DOX was obtained as a solution of doxorubicin hydrochloride (2 mg/ ml) for infusion. The drug was supplied from EBewe, Austria. Cap powder was obtained from Spimaco, Alqaseim Pharmaceutical Plant, Saudi

Arabia. It was dissolved in normal saline. Thiobarbituric acid, 1,1,3,3-tetraethoxypropane, N-ethylmaleimide, O-phthalaldehyde, reduced nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH), oxidized glutathione (GSSG) and oxidised glutathione reductase were obtained from Sigma-Aldrich Chemical Co. MO, USA. All other used chemicals were of high analytical grade and all the solutions were prepared in bidistilled deionized water.

Animals and animal treatment

Adult male albino Wistar rats weighing 170-200 g were used. The rats were obtained from the animal facility of King Fahad Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia and were kept for two weeks before starting the experiment under controlled conditions of temperature, humidity and light. The rats were allowed free access to food and water *ad libitum*. They were classified into four groups (each ten rats) as follows: Group 1: rats served as a control group and injected (I.P) with normal saline twice weekly for two consecutive weeks. Group 2: rats were injected (I.P.) with DOX at a dose of 4.5 mg/ kg twice weekly for two consecutive weeks in a total cumulative dose of 18 mg/ kg (Tubaro *et al.*, 1985). Group 3: rats were treated (P.O.) with Cap at a dose of 60 mg/ kg twice weekly for two consecutive weeks (Al-shabanah *et al.*, 1998; Javanovic *et al.*, 2005). Group 4: rats were treated with the same schedules and doses of treatments as groups 2 and 3 but DOX was injected 1 hour post-each Cap treatment.

Sample preparation

Forty eight hours after the last treatment, the rats were anaesthetized with ether and blood samples were withdrawn by eye puncture in plain and heparin containing tubes. The blood was centrifuged at 600 g (Hermle centrifuge, Germany) for 20 minutes to separate serum and plasma. Serum was used for determination of nitric oxide (NO) concentration and DOX level while plasma was used for assessment of total creatine kinase (CK), creatine kinase isoform (CK-MB) and lactic dehydrogenase (LDH) activities. The rats were killed by cervical dislocation, abdomens were opened and hearts were rapidly removed, rinsed from excess blood using ice cold saline, blotted dry with filter paper. The hearts were homogenized in ice-cold 0.1 M phosphate buffer pH 7.4 using Krien-Lerzern homogenizer (Switzerland) to prepare 10 % w/v homogenate. The resultant homogenate was centrifuged at 600 g in cold centrifuge (Sigma, USA) at 4 °C for 10 minutes. The supernatant was kept at -60 °C for the assessment of the biochemical parameters. The level of lipid peroxides (LPO) and the contents of GSH and GSSG were determined. The activities of glutathione peroxidase (GPx) and catalase (CAT) were investigated. In addition, protein content and the levels of DOX and NO were also assessed.

Biochemical analyses

The activities of total CK and CK-MB were determined adapting the methods of Szasz *et al.* (1978) and Wurzburg

et al. (1976) respectively. Lactic dehydrogenase (LDH) activity was determined spectrophotometrically as reported by Witt and Trendelenburg (1982). The level of lipid peroxide as maleondialdehyde (MDA) was determined spectrophotometrically (Yoshioka *et al.*, 1978). The contents of GSH and GSSG were assessed following Hissin and Hifi (1976). The activity of GPx was measured spectrophotometrically following the procedure of Lawrence and Burk (1976) while the activity of CAT was investigated adapting the method of Aedi (1984). Protein content was determined colourimetrically according to Lowry *et al.* (1951). Nitric oxide (NO) concentration was determined using assay-Design kit (USA) following the method of Green *et al.*, (1982) while, DOX level was measured spectrofluorometrically at excitation and emission wave lengths of 470 nm and 485 nm respectively (Bachar *et al.*, 1970).

Statistical analysis

Statistical analysis was performed using software SPSS® version 12.0 for windows. Data were expressed as mean ± standard error of mean (SEM). Comparison between groups was carried out using one way analysis of variance (ANOVA) followed by Tukey-kramer test for multiple comparisons. Comparison between two groups was carried out using Student's *t*-test. Statistical significance was accepted at $P \leq 0.05$.

RESULTS

DOX- alone treatment significantly decreased the normal activities of plasma CK, CK-MB and LDH by 30.25%, 31.23% and 52.72% respectively. Also, Cap-alone treated group showed marked inhibition in the normal LDH activity (64.35%). In addition, administration of Cap one hour before DOX injection, failed to ameliorate DOX-induced decreases in the assessed parameters; it produced significant decrease in the normal activities of CK and LDH by 26.51%, and 57.25% respectively (Table 1).

Table (2) shows that DOX treatment exhibited significant increase in the normal level of MDA (154.62%) associated with marked inhibition in the normal GSH content (75.98%). Moreover, Cap-alone treatment significantly decreased the normal level of GSH by 22.53%. The combined treatment (DOX+Cap) showed marked decrease in the level of MDA (72.86 ± 13.7 nmol/g tissue) when compared to DOX alone-treated group (163.39 ± 15.52 nmol/g tissue). However the combined regimen failed to preserve the normal GSH level (71.71%). Assessment of GSSG content did not exhibit any significant change.

Table (3) shows that the normal enzymatic activity of GPx was significantly decreased by 73.9% while CAT activity was significantly increased by 37.65% after DOX treatment. Also, Cap treatment caused significant decrease in GPx activity amounted to 36.89% of the control group. Furthermore, DOX+Cap treated group showed marked inhibition in GPx activity (1.95 ± 0.28 μ mol/ g protein) and elevation in CAT activity (4.2 ± 0.23 μ mol/ mg protein).

Table 1: Effects of DOX, Cap and their combination on plasma CK, CK-MB and LDH activities in rats

	CK (U/L)	CK-MB (U/L)	LDH (U/L)
Control	113.49 ± 9.64	15.56 ± 1.65	245.36 ± 12.70
DOX	79.15 ^a ± 1.00	10.70 ^a ± 1.12	116.00 ^a ± 19.06
Cap	93.97 ± 3.38	13.10 ± 1.00	157.89 ^a ± 22.87
DOX + Cap	83.40 ^a ± 2.21	11.98 ± 1.03	104.88 ^a ± 13.34

- DOX was injected (I.P) at a cumulative dose of 18 mg/kg divided into four injections over a period of two consecutive weeks.
- Cap was administered orally at a dose of 60 mg/kg twice weekly for two consecutive weeks.
- In the combined treatment group, the drugs were taken by the same doses and schedules of treatment and Cap was administered one hour before each DOX injection.
- Data are expressed as mean ± SEM of 10 rats.
- a indicates significant difference from corresponding control at $p \leq 0.05$.

Table 2: Effects of DOX, Cap and their combination on the levels of LPO, GSH and GSSG in cardiac tissues of rats

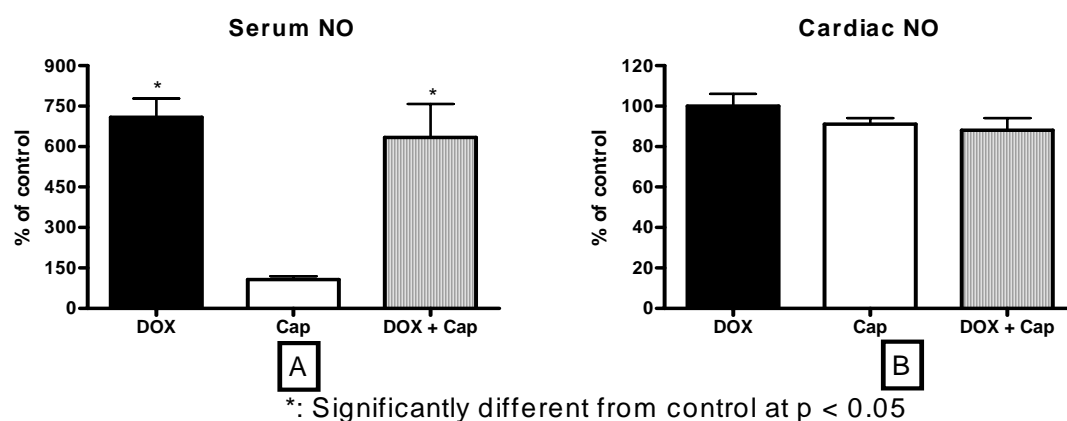
	MDA (nmol/g tissue)	GSH (µg/g tissue)	GSSG (µg/g tissue)
Control	105.67 ± 13.66	522.83 ± 28.07	12.77 ± 0.58
DOX	163.39 ^a ± 15.52	397.29 ^a ± 31.00	10.76 ± 0.82
Cap	91.00 ± 8.65	405.03 ^a ± 16.93	13.66 ± 1.03
DOX + Cap	72.86 ^b ± 13.70	374.95 ^a ± 23.28	11.37 ± 0.86

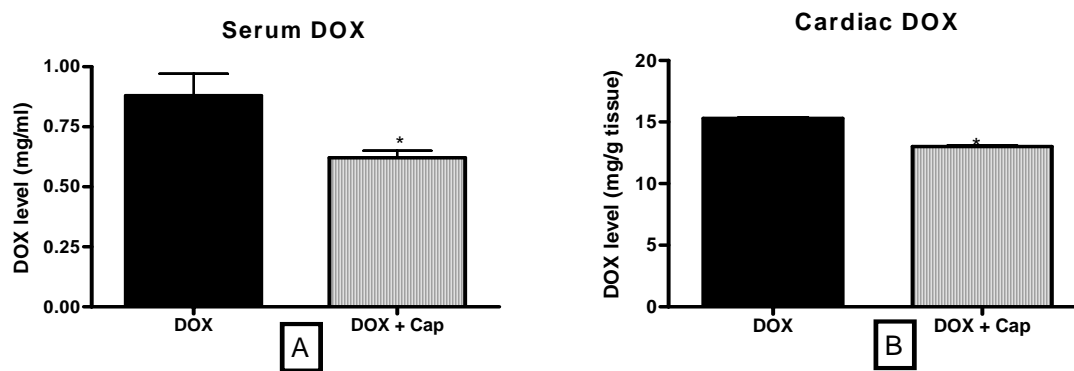
- Details of legend are the same as in Table 1.
- a indicates significant difference from corresponding control at $p \leq 0.05$.
- b indicates significant difference from corresponding DOX at $p \leq 0.05$.

Table 3: Effects of DOX, Cap and their combination on the activities of GPx, and CAT in cardiac tissues of rats

	GPx (µmol/mg protein)	CAT (µmol/mg protein)
Control	6.18 ± 1.13	3.32 ± 0.16
DOX	1.61 ^a ± 0.13	4.57 ^a ± 0.21
Cap	2.28 ^a ± 0.34	3.99 ± 0.19
DOX + Cap	1.95 ^a ± 0.28	4.20 ^a ± 0.23

- Details of legend are the same as in Table 1.
- a indicates significant difference from corresponding control at $p \leq 0.05$.

**Fig. (1): Effects of DOX, Cap and their combination on the level of NO in serum (A) and cardiac tissues (B) of rats**



*: Significantly different from DOX-treated group at $p < 0.05$

Fig. (2): The level of DOX (injected alone or in combination with Cap) in serum (A) and cardiac tissues (B) of rats

Administration of the used drugs failed to change NO level in the cardiac tissue. However, DOX treatment significantly increased the serum normal NO level when injected alone (7 fold) or in combination with Cap (6 fold) (Figure 1 A & B). Treatment with Cap in combination with DOX resulted in significant decrease in the levels of DOX and / or its metabolites in both serum (0.88 vs. 0.62 $\mu\text{g/ml}$) and cardiac tissue (15.29 vs. 13.33 $\mu\text{g/g tissue}$) (Figure 2 A & B).

DISCUSSION

In the present study, DOX in a total cumulative dose of 18 mg/ kg produced a significant decrease in plasma CK, CK-MB and LDH. Our results gain support by the work of Robison *et al.* (1989). The inhibition of CK activity and its isoform CK-MB can be attributed to either depletion of ATP levels (Robison *et al.*, 1989) or /and oxidative inactivation of the enzyme SH group caused by reductive free radicals or oxidative activation of DOX (Muraoka and Miura, 2004). On the other hand, several investigators reported that DOX induced a significant increase in serum CK and LDH (Mostafa *et al.*, 1999; Abdelgawad and El-sawalhi, 2003). However, Herman *et al.* (1988) reported that DOX administered once a week for 12 weeks failed to induce significant changes in the serum activities of enzymes of rats. This discrepancy may be attributed to the differences in the doses, schedules of DOX administration and sampling time after treatment.

Cap alone produced a significant decrease in LDH activity. The suggested mechanism for Cap induced reduction in LDH may be due to inhibition of anaerobic oxidation of glucose. Swislocki *et al.* (1999) reported enhanced capacity of Cap for lipid oxidation and decreased handling of glucose for energy metabolism in rats. Our data indicate that coadministration of Cap with DOX abolished the decrease in CK-MB without affecting the activity of CK

and LDH. This can be explained on the basis of the reported selective cardioprotective properties of Cap (Liu and Xu 1993; De Nigris *et al.*, 2001). This effect could be reflected specifically on the enzyme activity of CK-MB; which is a sensitive marker of cardiac integrity (Abdelaziz *et al.*, 2001).

In our study, DOX-treated rats showed significant increase in lipid peroxides level associated with significant depletion of GSH content with no change in GSSG level in the cardiac tissues. Several investigators reported marked increases in MDA levels in cardiac tissues after DOX treatment (Dziegiel *et al.*, 2003; Mohan *et al.*, 2006). Concerning the depletion of GSH, there is a body of in vitro and in vivo studies which are in parallel to our findings (Paranka and Dorr, 1994; Zhou *et al.*, 2001; Al-majed *et al.*, 2002). Lipid peroxidation of cell membrane and cell organelles membrane especially the mitochondria leads to damage of cardiomyocytes (Tong *et al.*, 1991). The increase in lipid peroxides level is related to DOX metabolism in heart muscle that generates large numbers of oxygen-based radicals (Seifert *et al.*, 1994). The quinone ring of DOX in the presence of flavoenzymes is reduced to its respective semiquinone free radical (Venkatesan, 1998). In addition the interaction of DOX with iron can form DOX-iron III complex which in presence of oxygen, yielding superoxide radicals (Esterbauer, 1993). These in turn initiate free radical mediated chain reactions which result in conversion of the membrane unsaturated fatty acid to lipid peroxides (Doroshov *et al.*, 1990). It is anticipated that redox cycling and subsequent formation of reactive intermediates play a role in the DOX-induced GSH depletion. The observed insignificant change in GSSG may be due to the conversion of GSH to other derivatives such as mixed disulfides (Wefers and Sies, 1983).

Our data showed that DOX treatment significantly decreased GPx activity associated with marked increase in CAT activity in the cardiac tissue. The decrease in GPx

activity is in accordance with a bundle of in-vitro (Paranka and Dorr, 1994) and in-vivo (Li and Singal 2000; Mohan *et al.*, 2006) studies. The inhibition in the enzyme activity may be due to the parallel decrease in the enzyme protein or due to the inhibition of the enzyme activity caused by lipid peroxides liberation (Choi and Tappel, 1969). The elevation of CAT activity is also in agreement with different previous investigations (Dziegiel *et al.*, 2003; Yilmaz *et al.*, 2006). The increase in CAT activity is an evidence of adaptive response to the massive production of hydrogen peroxide in the myocardium (Li and Singal, 2000).

In our study, Cap abolished the increase in MDA level produced by DOX. This was accompanied by non significant changes in the levels of GSH and GSSG as well as the activities of GPx and CAT. Our results are in agreement with that of Abdelaziz *et al.* (2001), who showed that treatment of Cap before DOX administration significantly reduced the heart level of MDA with no changes in either GSH or GSSG levels. These findings are supported by the work of Dogan *et al.* (1998) who showed that Cap treatment markedly inhibited myocardial MDA level during ischemia-reperfusion injury.

In our study, treatment with DOX resulted in a significant increase in serum NO level. The increase in serum NO level is in accordance with pervious studies carried out on rats (Lind *et al.*, 1997). The increase in serum NO is a pivotal indication of cardiotoxicity (Guerra *et al.*, 2005). Induction of inducible nitric oxide synthase (Pacher *et al.*, 2003) and endothelial nitric oxide synthase (Kalivendi *et al.*, 2001) have also been observed after DOX treatment. Administration of Cap in the present study failed to cause any change in either serum or cardiac NO level. A finding, which is in consistence with studies carried out on human subjects (Doger *et al.*, 1996) and rats (Bernatova, 2000). This result can indicate that the beneficial effect of Cap is not mediated through modulation of NO production. Administration of Cap before DOX produced insignificant change in serum and cardiac NO levels. The combined administration of DOX + Cap significantly decreased the concentration of DOX in both serum and cardiac tissues. This may be attributed to the ability of Cap to affect DOX kinetics under our experimental conditions. In conclusion, Cap exhibited limited protective effects against DOX-induced cardiotoxicity via inhibiting lipid peroxidation.

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