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Ameliorative Effect of Captopril Against 5-Fluorouracil-Induced Cardiotoxicity in Rats: A Study with the Light and Electron Microscopes

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ABSTRACT

The chemotherapeutic agent 5-fluorouracil (5-FU) is a widely accepted part of many cancer treatment protocols. Its cardiotoxic potential is known, but considered uncommon and usually not life-threatening, although some cases of severe cardiotoxicity related to 5-FU have been reported. The objective of this study was to determine the effectiveness of captopril, an angiotensin-converting enzyme (ACE) inhibitor containing sulphhydril (-SH) group, in alleviating 5-FU-induced cardiotoxicity in male rats. Forty adult male rats were assigned to four equal groups: Group I, rats received normal saline solution (control group); Group II, rats received 5-FU 20 mg /kg/day, intraperitoneally for 5 days (5-FU alone group); Group III, rats received captopril 20 mg /kg/day, orally for 14 days (captopril alone group); Group IV, rats received 5-FU 20 mg /kg/day, intraperitoneally for 5 days + captopril 20 mg /kg/day, orally for 14 days (5-FU + captopril group). Animals of all groups were sacrificed and tissue samples from the cardiac muscle were taken and processed for both light and electron microscopical examination. Light microscopic observations revealed that administration of 5-FU caused variable signs of cardiotoxicity which are represented by focal atrophy, vacuolar degeneration, coagulative necrosis as well as cytolysis of myocytes. Interstitial oedema together with inflammatory cell infiltration in between the damaged myocardiocytes was also observed. Ultrastructural examination of these specimens confirmed the light microscopic findings and demonstrated abnormal-shaped mitochondria, dilated sarcoplasmic reticulum, interrupted Z lines and disorganization of the ordered parallel myofibrillar array. Pretreatment with captopril and its concomitant administration with 5-FU for 14 days attenuated 5-FU-induced myocardial damage and effectively reverted the abnormal structural changes to near normalcy. In conclusion, these results suggest that captopril has a protective potential in ameliorating 5-FU-induced cardiotoxicity.

Key words: 5-Fluorouracil; Captopril; Cardiotoxicity; Histology; Ultrastructure; Rats

Introduction

Chemotherapy in case of tumour involves the use of chemical agents to stop the cancer growth and eliminate cancer cells even those lying at distant sites from the origin of primary tumour. However, it does not distinguish between the cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including those of the hair and blood. More than half of all people diagnosed with cancer receive chemotherapy regimen that usually includes drugs to treat cancer as well as to help support the completion of the cancer treatment at the full dose on schedule (El-Sayyad et al., 2009). 5-Fluorouracil (5-FU) is an antimitabolite that acts during the S phase of the cell cycle. 5-FU is activated by thymidine phosphorylase into fluorodeoxyuridylate (5 fluoro 2'deoxyuridine 5'monophosphate, 5-FdUMP) that inhibits thymidylate synthase, thus preventing DNA synthesis; that leads to imbalanced cell growth and ultimately cell death (Shoemaker et al., 2004). Concentration of thymidine phosphorylase is 3-10 times higher in tumour cells compared to healthy tissues. This can enable selective drug activation of 5-FU at the tumour site and limits systemic toxicity (Saif et al., 2007). 5-FU is also converted to 5-fluorouridine monophosphate (5-FUMP) and can be incorporated into RNA and interfered with RNA processing and function. 5-FU is widely used alone or in combined protocols in the treatment of various malignancies including gastrointestinal, breast and head and neck cancer (Lemaire et al., 1992). It is metabolized through the liver and has a half-life of approximately 10 minutes (Sobrero et al., 1997). The common clinical side-effects of 5-FU include myelosuppression, diarrhoea, vomiting and mucositis. However, over the last decade, the number of reports of cardiotoxicity and neurotoxicity attributed to 5-FU has rapidly increased, probably because of using higher doses in continuous perfusion (Robben et al., 1993; Anand, 1994). The pathophysiology of 5-FU-induced cardiotoxicity is controversial and the conclusions are based on clinical studies and case reports more than on solid experimental evidence. 5-FU cardiotoxicity is suspected to be mediated by coronary vaso...
(explaining the occurrence of angina and myocardial infarction and rapid normalization of the ST segment) and free radical damage to the myocardium (Ensley et al., 1989). Other histomorphological and biochemical studies indicate a more direct drug-mediated cytotoxic action. Additional study support the hypothesis that 5-FU has direct endothelial toxicity resulting in thrombogenic effect and release of vasoactive substances (Singh et al., 2004).

Angiotensin-converting enzyme (ACE) inhibitors are considered to be effective clinical therapy for hypertension and heart failure. It has become obvious that ACE inhibitors reduce ischemic myocardial injury (Grover et al., 1991). Prolonged myocardial ischemia is accompanied by a time-dependent loss of the viability of myocardial cells in the jeopardized region of the heart. The protective effect of ACE inhibitors is considered to be mainly the result of reduction in myocardial oxygen demand and an increase in myocardial blood flow by inhibition of angiotensin II formation and bradykinin breakdown (Takeda et al., 1997).

Captopril is an angiotensin-converting enzyme (ACE) inhibitor that contains a sulphydryl (-SH) group. This sulphydryl group may have the ability to scavenge cytotoxic oxygen-derived free radicals, which play an important role in postischemic contractile dysfunction (Takeda et al., 1997). Previous evidence suggested that captopril can also improve the severity of inflammation through the following pathways. First, it inhibits endothelial derived growth factor (EDGF)/nitric oxide (NO) degradation through eliminating oxygen free radicals, and reinforced EDGF/NO functions (Kanno et al., 2001). Secondly, it improves microcirculatory disturbance through promoting the synthesis of vascular endothelial cell and the release of prostacyclin (Pawlak et al., 2000). Thirdly, it reduces endothelin release to attenuate tissue injury (Plusczcyk et al., 1999). Finally, it reduces intracellular Ca\(^{2+}\) levels by decreasing Ca\(^{2+}\) passage and inhibiting the activity of Ca\(^{2+}\) in passing through cell membrane to avoid intracellular Ca\(^{2+}\) overloading, thus attenuating cell injury (Krizanova et al., 1997).

This study was designed to investigate the possible ameliorative effect of an ACE inhibitor, captopril, on 5-FU-induced cardiac injury in rats.

Materials And Methods

Chemicals:

Captopril was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. 5-FU was donated from Choongawa Pharma Corporation, Seoul, Korea. 5-FU was given intraperitoneally at a dose of 20 mg/kg. This dose was guided by previous studies (Bozdag et al., 2001; Nayci et al., 2003; El-Sayyad et al., 2009). Captopril was administered by oral gavage at a dose of 20 mg/kg according to Shin et al. (2009).

Experimental animals:

Forty adult male Sprague–Dawely rats, each weighing 140 ± 10 g, were obtained from the Breading Unit of the Egyptian Organization for Biological and Vaccine Production, A.R.E. The rats were housed in stainless steel cages under conventional and controlled laboratory conditions of relative humidity (55 ± 5%), temperature (25 ± 2 °C) and light/dark cycle of 12 hr each. Standard laboratory rodent chow and tap water were provided ad libitum. The animals were acclimatized for a period of one week prior to the commencement of the experiment.

Experimental protocol:

The animals were divided into four groups each of ten rats as follows:

**Group I (Control):** The animals were given normal saline (2 ml/kg) b.w./day, parallel to the drug treated groups, throughout the course of the study of 14 days.

**Group II (5-FU alone group):** The animals first received normal saline (2 ml/kg b.w./day) by oral intubation for 9 days, and subsequently received 5-FU (20 mg in 2ml normal saline per kg b.w.) once daily by intraperitoneal injection in association with normal saline for additional 5 days.

**Group III (Captopril alone group):** The animals first received captopril alone (20 mg in 2 ml normal saline per kg b.w.) once daily by oral intubation for 9 days and subsequently received normal saline once daily by intraperitoneal injection in association with captopril for additional 5 days.

**Group IV (5-FU + captopril group):** The animals first received captopril alone at a dose of (20 mg/kg b.w./day) by oral intubation for 9 days, and subsequently received 5-FU (20 mg/kg b.w./day) by intraperitoneal injection in association with captopril for additional 5 days.
At the end of the experimental period, the tested animals were sacrificed 24 hr after the last dose of different administrations, the chest of the rat was opened and the heart specimens were rapidly removed and immediately processed for histopathological and ultrastructural studies.

**Light microscopic (L/M) study:**

For preparation and qualitative analysis of histological slides, halves of the left ventricles from both control and treated groups were taken and fixed in 10% neutral buffered formalin, dehydrated in ascending grades of ethyl alcohol to remove water content. After dehydration, the tissues were cleared in xylol, impregnated in paraffin wax and sectioned at 5-7 µm thickness using rotary microtome. The deparaffined sections were stained routinely with Harris’ haematoxylin & eosin (Bancroft and Stevens, 1977) and examined for any structural changes under a light microscope.

**Transmission electron microscopic (TEM) study:**

For ultrastructural examination, the other halves of the ventricular samples were cut into small pieces of about 1-mm³ in size and immediately fixed in 2.5% glutaraldehyde for 24-48 hr. The specimens were then washed in phosphate buffer (pH 7.2–7.4) 3-4 times for 20 min. every times and post-fixed in a buffered solution of 1% osmium tetroxide at 4 ºC for 2 hr., after that washed in the same buffer 4 times for 20 min. each. Fixed specimens were dehydrated in ascending grades of ethyl alcohol (30%, 50%, 70%, 90%, and 100%), cleared in two changes of propylene oxide, and embedded in Epon resin. Semithin sections (∼1 µm thick) stained with 1% toluidine blue were examined on a light microscope. The resin blocks were retrimmed to get rid of the undesired tissue. Ultrathin sections (60–90 nm thick) were cut with a diamond knife using an ultramicrotome (MT6000 – XL RMC, Inc.), mounted on copper grids and double stained with uranyl acetate and lead citrate (Weakly, 1981). Grids were viewed and photographed using a transmission electron microscope (JEOL JEM-1200 EX II, Japan) operated at 60-70 kV, Faculty of Science, Ain Shams University.

**Results**

**Light microscopic (L/M) results:**

At the light microscope level, H&E-stained sections and toluidine blue semithin sections of the cardiac muscle fibres of the left ventricle of the control group showed normal histological structure. It is formed of branching and anastomosing muscle fibres with central oval nuclei and granular acidophilic sarcoplasm. Blood vasculature appeared in between the muscle fibres. The intercalated discs could be detected in the longitudinally cut fibres (Figure 1a, 1b and 1c).

![Fig. 1: Light micrographs of the left ventricular myocardium of the control group showing: (a) normal appearance of longitudinally-oriented cardiac muscle fibres (H & E x100); (b) higher magnification of Figure 1a to identify myocardial bundles and central oval nuclei (N). Note the intercalated disc (arrows) (H & E x400); (c) normal appearance of transversely cut fibres. Notice the central vesicular nucleus (N) and the blood vasculature in between the muscle fibres (arrows) (Toluidine blue x400).](image-url)
H & E-stained sections of the left ventricle of rats treated with 5-FU showed atrophy, splitting, disruption and hyalinization of cardiomyocytes in focal manner (Figure 2a, 2b and 2c). Dilated blood vessel, severe interstitial oedema and inflammatory cell infiltration in between the damaged cardiomyocytes were also observed (Figure 2a and 2b). Semithin sections stained with toluidine blue revealed prominent cytopathic changes in the form of vacuolar degeneration, coagulative necrosis as well as cytolyis of myocytes (Figure 2d, 2e and 2f). Figure (2e) shows extravasation of the blood in between the muscle fibres. In addition to this, swelling of the perinuclear area and perivascular and interstitial oedema were also detected (Figure 2d and 2f).

In rats treated with 5-FU + captopril, H&E-stained sections of the left ventricle showed morphology nearly similar to that of control; however, there was focal haemorrhage in between the myocardial bundles (Figure 3a). Semithin sections revealed mild degree of swelling around the nuclei and congestion of the blood capillaries (Figure 3b).

Transmission electron microscopic (TEM) results:

Ultrastructural examination of the heart tissue of the control group revealed normal structure of cardiac muscle fibers which are connected end-to-end and side-to-side to one another by the intercalated discs. These fibres are composed of smaller myofibrils which contain repeating sections of sarcomeres. The mitochondria are arranged parallel to and in between the cardiac myofibrils and are found around the euchromatic nucleus. The Z line marks the boundary between two sarcomeres; it appears as a series of dark lines. Surrounding the Z line is the region of the light I band which contains actin thin filaments. Following the light I band is the dark A band.
which contains myosin thick filaments. Within the A band is a paler region called H zone which contains M line. The M line is at the center of the sarcomere and is the site at which the thick filaments are linked with each other. The cardiac muscle fibers exhibited central oval elongated nuclei. The nuclei are enclosed by nuclear envelope and displayed normal chromatin distribution (Figure 4a, 4b and 4c).

![Fig. 3](image1.png)

**Fig. 3:** Light micrographs of the left ventricular myocardium of a 5-FU + captopril group showing: (a) focal haemorrhage in between the myocardial bundles (arrows) (H&E x100); (b) mild degree of swelling around the nucleus (arrow) and congestion of the blood capillary (double arrows) (Toluidine blue x1000).

![Fig. 4](image2.png)

**Fig. 4:** Transmission electron micrograph of longitudinal muscle fibres of the left ventricular myocardium of the control group showing: (a) vesicular large nucleus (N), parallel arrays of myofilaments (arrow) and rows of mitochondria (M) are arranged in between the myofilaments and in the perinuclear space (x 2500); (b) striation of myofibrils with demarcated evident Z lines (ZL) and M line (ML) bisect the H zone (HZ). Note the light I band (IB), dark A band (AB) and the normal structure of mitochondria (M) with regular cristae (x 6000); (c) intercalated disc between the two adjacent cardiac muscle fibers (C1 & C2) (x 4000).

Ultrastructural examination of the left ventricular myocardium of FU-treated rats confirmed the light microscopic findings and demonstrated irregular nuclear envelope, swelling of the perinuclear area, dilatation of sarcoplasmic reticulum and interruption of Z line in focal areas (Figure 5a, 5b, 5c and 5d). Disorganized, electron-dense and bizarre shaped mitochondria and congestion of the blood capillaries were also observed (Figure 5c, 5d, 5e and 5f). Figure (5d and 5f) shows presence of large vacuoles in the sarcoplasm of the myocytes. Disruption, separation and lysis of myofibrils results in disorganization of the ordered parallel array (Figure 5c, 5d and 5f).
Fig. 5: Transmission electron micrographs of the left ventricular myocardium of 5-FU group showing: (a & b) irregular nuclear envelope (arrow), swelling of the perinuclear area (star), myofibrillar damage (double arrows) and dilatation of sarcoplasmic reticulum (curved arrows) (a: x2500; b: x3000); (c & d) bizarre-shaped mitochondria (M), interruption of Z line (ZL) in focal areas, separation and lysis of myofibrils (arrows) and presence of large vacuoles (V) in the sarcoplasm of the myocytes (c: x4000 x; d: x2000); (e) congestion of the blood capillaries (BC), fragmentation of myofibrils (arrows) and electron-dense mitochondria (M). RBC, red blood cell; E, endothelial cell (x1200); (f) disrupted myofibrils (arrows) and large membrane-limited vacuole (V) circumscribed by disorganized mitochondria (M). (x 4000).

In the left ventricle of rats treated with 5-FU + captopril, the myofilaments and nuclei appear somewhat normal. However, mild sarcoplasmic vacuolation and little myofibrillar damage are illustrated (Figure 6a and 6b).

Fig. 6: Transmission electron micrographs of the left ventricular myocardium of 5-FU + captopril group showing: (a & b) nearly normal appearance of the nucleus (N) and restoration of the regular arrangement of the myofibrils; however, small degree of myofibrillar damage (arrows) and mild sarcoplasmic vacuolation (V) are observed (a: x3000; b: x2500).
Discussion:

5-Fluorouracil cardiotoxicity is a serious phenomenon that requires a high index of suspicion whenever the decision is made to use this drug as a treatment modality. It is associated with a broad spectrum of cardiovascular symptoms, such as arrhythmias, with and without cardiopulmonary symptoms. Adverse effects could even progress to acute myocardial infarction (MI) symptoms, such as chest pain and shortness of breath with injury changes suggested on an electrocardiogram (ECG) study, ventricular dysfunction, cardiogenic shock, cardiac arrest, and sudden death (Millward et al., 1988; Ensley et al., 1989).

In the present investigation the most common histopathological abnormalities in the myocardium following 5-FU administration including vascular degeneration, coagulative necrosis, inflammatory cell infiltration and extravasation of the blood in between the damaged muscle fibres. In addition to this, the electron micrographs showed dilatation of sarcoplasmic reticulum, bizarre-shaped mitochondria, separation and lysis of myofibrils, interruption of Z lines and disorganization of the ordered parallel myofibrillar array. These results are consistent with previous studies reported by some investigators that during 5-FU treatment with serious consequences, in a previously healthy 23-year-old patient with squamous cell carcinoma of the tongue, the endomyocardial biopsy showed proliferation of the sarcoplasmic reticulum with marked vacuolization, similar to that found with doxorubicin cardiotoxicity (Kuropkat et al., 1999). Also, in a study of rabbits, one group was exposed to a single dose of 5-FU 50 mg/kg and the other group received repeated infusions of 15 mg/kg. The rabbits’ hearts were removed and analyzed shortly after death. The first group developed a massive haemorrhagic MI with spasms of the proximal coronary arteries and the second group developed left ventricular hypertrophy, foci of myocardial necrosis, thickening of intra-myocardial arterioles, and disseminated apoptosis in myocardial cells of the epicardium, as well as endothelial cells of the distal coronary arteries (Tsibirihi et al., 2006). Furthermore, a histopathological study of 5-FU induced cardiotoxicity was performed on 50 Swiss albino rats. Multiple interstitial myocardial haemorrhages, multifocal myofibre necrosis, inflammatory reaction, vascular changes, valvulitis and pericarditis have been noted especially in the left heart ventricles of rats under study. A probable reason has been proposed; it explains the endothelial damage leading to extravasation of the drug containing blood into myocardium resulting in myofibre necrosis and inflammatory reaction (Kumar et al., 1995).

The mechanism of 5-FU associated cardiotoxicity is controversial, but the prevalent hypothesis suggests ischemia to the myocardium. Ischemia could be due to a direct toxic effect on the vascular endothelium involving NO synthase, which leads to coronary vasospasms. The other mechanism of vasospasm endothelial is via protein kinase C (PKC)-mediated vasoconstriction (Alter et al., 2006). An alternative suggested hypothesis is that 5-FU toxicity is due to free radical damage to the myocardium (Ensley et al., 1989). Moreover, Spasojević et al. (2005) and Spasojević et al. (2008) have performed an ex vivo and in vivo study of the effects of 5-FU on erythrocytes, using a variety of biophysical techniques. Their research showed that 5-FU induced rapid increase in O2 consumption, which led to drastic changes in the metabolism of phosphate compounds in erythrocytes. Decrease in PO2 (Partial Pressure of Oxygen) provoked increase in production of 2, 3 bisphosphoglycerate (2, 3-BPG) and subsequent deoxygenation of oxyHb to deoxyHb. However, the most important effect of 5-FU on erythrocytes is severe decrease in the level of ATP. This could lead to a number of irreversible changes in erythrocyte structure and functioning, such as echinocytosis, increase in membrane fluidity, and non-functioning of membrane ion pumps. All these changes affect normal functioning of erythrocytes, leading to difficulties in oxygen transport and leaving the heart with an insufficient supply of oxygen, thus leading to cardiotoxicity.

In the present study, treatment with captopril could obviously mitigate the marked cardiotoxicity induced by 5-FU, resulting in morphology, to some extent, similar to that of the control group. This observation is similar to the results of van Gilst et al. (1984) who reported that captopril decreased ventricular fibrillation and the loss of high energy phosphate nucleotides after ischemia and reperfusion in isolated rat hearts. Also, Al-Shabanah et al. (1998) reported that captopril ameliorates doxorubicin-induced cardio-and haematotoxicity in normal rats. In addition, Okada et al. (2008) showed that captopril inhibits monocrotaline-induced hypertrophy and fibrosis in the right ventricles of rats. Moreover, Ibrahim et al. (2009) demonstrated that co-administration of captopril with doxorubicin to rats was able to attenuate doxorubicin-induced myocardial fibrosis and renal tubular damage.

The mechanism by which captopril is prescribed for the treatment of hypertension and congestive heart failure are not fully understood. Some evidence support the protective role of captopril as an ACE inhibitor on the cardiotoxicity. Yoshimura et al. (1989) postulated that since captopril inhibits the conversion of angiotensin I to II in not only serum but also in myocardium, this drug prevents the occurrence of necrotic foci in rat myocardium probably mediated by attenuation of accumulation of intracellular angiotensin II. Also, Sacco et al. (2009) suggested that inhibition of cardiac ACE by zofenopril and additional cardioprotective mechanism(s) may have a role in its ability to prevent myocardial damages in the rat subjected to chronic anthracycline treatment.
The potentiation of a free radical scavenging action by ACE inhibitors has also been postulated (Chopra et al., 1992). Sulphydryl groups on proteins and unsaturated fatty acids esterified into lipids are molecules susceptible to oxidation by oxygen attack (Guarnieri et al., 1980). Any molecule that reacts with a free radical is generally termed a free radical scavenger (Freeman and Crapo, 1982). Captopril has a sulphydryl group and can be converted into disulphides through the interaction with free radicals instead of cellular sulphydryl-containing proteins and enzymes (Pi and Chen, 1989). Similarly, Westlin and Mullane (1988) reported that captopril alleviated the postischemic contractile derangements of the left ventricle in the open chest dog; but enalaprilat, which lacks the sulphydryl group, did not improve it. Also, captopril has been suggested to have cardioprotective action because of its ability to act as an antioxidant; it reacts rapidly with hydroxyl radicals and is a powerful scavenger of hypochlorous acid (Aruoma et al., 1991). In addition, De-Cavanagh et al. (1995) suggested that ACE inhibitors may protect cell components from oxidative damage by increasing the enzymatic antioxidant defences in several mouse tissues. More recent, Mansour et al. (1999) reported that 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. The same authors - Mansour et al. (1999) - showed that captopril totally abolished leukotriene B4 (LTB4) formation from calcium ionophore-stimulated neutrophil. LTB4 is a highly superoxide anion generator (Crooks and Stockley, 1998). 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.

On the other hand, Deng et al., (2001) reported that captopril attenuates oxidative stress, reactive oxygen species-nitrict oxide (ROS-NO) interaction and generation of highly reactive and cytotoxic byproducts such as peroxynitrite - which can attack DNA, lipids, and proteins - by blockade of angiotensin II that upregulates nicotinamide-adenine dinucleotide phosphate oxidase which is thought to be a major source of ROS (Jones et al., 1996).

In conclusion, the findings of the present study clearly indicate that 5-FU induced histopathological and ultrastructural changes in the cardiomycocytes of rats and that pre-and co-administration of captopril with 5-FU lessened this toxicity. It is recommended that further studies must be carried out to corroborate these findings in clinical and experimental models.

References


