Hemin Treatment Ameliorates Doxorubicin-Induced Cardiotoxicity in Rats

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Abstract: The possible protective effect of hemin, the heme oxygenase-1 inducer, against acute doxorubicin cardiotoxicity was investigated in rats. Cardiac toxicity was induced by a single injection of doxorubicin (20 mg/kg, i.p.). Hemin treatment (40 µmol/kg/day, s.c.) was started 7 days before doxorubicin administration and continued for 10 consecutive days. Hemin significantly reduced the elevated serum creatine kinase level resulted from doxorubicin administration. Also, hemin significantly reduced the elevations of malondialdehyde, nitric oxide, tumor necrosis factor-α, caspase-3, and Bax/Bcl-2 ratio, and significantly prevented the decrease of total antioxidant status in doxorubicin-challenged rats. It was concluded that hemin, through its antioxidant, anti-inflammatory, and anti-apoptotic activities, protected against doxorubicin cardiotoxicity, which is a major dose-limiting clinical problem.
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1. Introduction

Doxorubicin is an anthracycline anticancer antibiotic commonly used for treatment of hematological malignancies and solid tumors. Despite the broad therapeutic effectiveness, the clinical use of doxorubicin is often limited because of its dose-dependent cardiotoxic adverse effects which frequently lead to congestive heart failure (Singal and Iliskovic, 1998; Minotti et al., 2004). It is recognized that oxidative stress with increased generation of reactive oxygen species plays an important role in the pathogenesis of doxorubicin-induced cardiotoxicity. Also, several antioxidants have proved effective in protecting against cardiac tissue damage induced by doxorubicin (Andreadou et al., 2007; Jiang et al., 2008; Singh et al., 2008).

Heme oxygenase-1 (HO-1), the rate-limiting enzyme in heme catabolism, is important for regulating the adaptive protection of tissues against oxidative stress and inflammation (Hangaishi et al., 2000; Morio et al., 2006). Hemin, the HO-1 inducer, was proved effective in protecting against oxidative and inflammatory tissue injuries in various experimental models (Wen et al., 2007; Chen et al., 2008). Recently, pharmacological induction of HO-1 by hemin protected rat testes against ischemia/reperfusion injury resulted from testicular torsion and detorsion (Yang et al., 2007). However, to the best of our knowledge, the effect of hemin against doxorubicin-induced cardiac damage was not yet studied. Therefore, the present study was conducted to evaluate the cardioprotective potential of hemin against acute injury induced by doxorubicin in rats. Also, the possible mechanisms underlying this effect were investigated.

2. Material and Methods

Drugs and chemicals

Hemin (Sigma-aldrich, USA) was dissolved in physiological saline. Doxorubicin hydrochloride (Sigma-aldrich, USA) was dissolved in physiological saline. The doses of the drugs used in the present study were selected based on previous investigations.

Animals

Thirty-two male Sprague-Dawley rats, weighing 200 ± 10 g were obtained from the Animal House, College of Medicine, Al-Ahsa, King Faisal University. The animals were housed at 24 ± 1°C, 45 ± 5% humidity, and 12 h light/dark cycle. They were supplied with standard laboratory chow and water *ad libitum*, and acclimated for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Experimental design

The rats were randomly divided into four groups (n = 8, each) as follows: First group (control) received a single intraperitoneal injection of physiological saline (vehicle of doxorubicin). Second group received a single intraperitoneal injection of doxorubicin at a dose of 20 mg/kg (Andreadou et al., 2007), and a daily subcutaneous injection of physiological saline (vehicle of hemin) for 10 days starting 7 days before doxorubicin administration. Third group received a single intraperitoneal injection of doxorubicin at a dose of 20 mg/kg (Andreadou et al., 2007), and a daily subcutaneous injection of hemin at a dose of 40 µmol/kg/day (Nagababu et al.,

1995) for 10 days starting 7 days before doxorubicin administration.

Fourth group received hemin only at a dose of 40 µmol/kg/day (Nagababu et al., 1995) for 10 days.

Sampling and biochemical analysis

Rats were euthanized by thiopental (70 mg/kg, i.p.) at the end of the experiments. Blood was collected via left ventricular puncture, and serum creatine kinase as a biomarker of myocardial injury was measured using colorimetric assay kit following the instructions of the manufacturer (Stanbio Laboratory).

The heart was removed from each animal and its fresh weight was recorded. The isolated hearts were kept at −80°C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was used to assess malondialdehyde (MDA), total antioxidant status (TAS), and nitric oxide (NO) by colorimetric kits (Biovision, USA). ELISA kits were also used to measure tumor necrosis factor-α (TNF-α) (R & D Systems, USA), and Bax, and Bcl-2 (LifeSpan Biosciences, USA) in cardiac tissue homogenates.

In addition, a colorimetric kit (R & D Systems, USA) was used to determine caspase-3 activity. Degradation of a specific enzyme substrate releases p-nitroaniline (pNA). Absorbance of pNA of different groups was measured by spectrophotometry at 405 nm, and compared to the control.

Statistics

GraphPad Prism software program (version 5) was used for analysis of the results (mean ± S.E.M.) by applying one-way ANOVA test followed by Tukey test for *post hoc* comparisons using, and significance was at $p < 0.05$.

3. Results

Doxorubicin administration in a single dose (20 mg/kg, i.p.) resulted in significant elevations ($p < 0.05$) of serum creatine kinase as compared to the corresponding control value. Hemin treatment, at a daily dose of 40 µmol/kg/day, s.c., for 10 days, starting 7 days before doxorubicin injection, significantly reduced ($p < 0.05$) serum creatine kinase in rats challenged with doxorubicin (Figure 1).

In addition doxorubicin caused significant increases ($p < 0.05$) in cardiac tissue MDA, and NO, and a significant decrease in cardiac TAS as compared to the corresponding control values. On the other hand, hemin significantly decreased ($p < 0.05$) MDA, and NO, and significantly decreased TAS in cardiac tissue of rats received doxorubicin (Figure 2).

Moreover, rats received a single doxorubicin administration showed significant increases ($p < 0.05$) of TNF-α and caspase-3 in cardiac tissue homogenates, as compared to the control rats. On the contrary, rats received hemin treatment showed significant reductions ($p < 0.05$) in cardiac tissue levels of TNF-α and caspase-3 ratio as compared to rats challenged with doxorubicin and non-treated with hemin (Figure 3 and Figure 4).

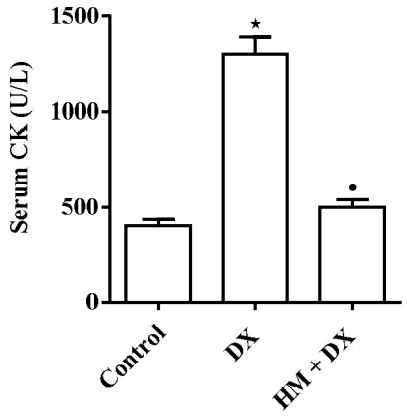

Figure 1. Effects of hemin (HM) on serum creatine kinase (CK) in rats received doxorubicin (DX). Results are mean ± S.E.M., $^{*}p < 0.05$ vs. control, $^{\bullet}p < 0.05$ vs. DX.

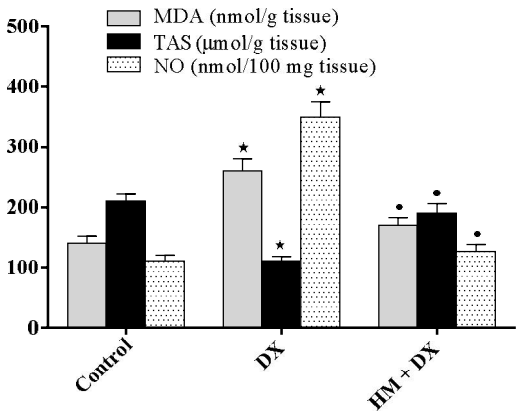

Figure 2. Effects of hemin (HM) on cardiac malondialdehyde (MDA), nitric oxide (NO), and total antioxidant status (TAS) in rats received doxorubicin (DX). Results are mean ± S.E.M., $^{*}p < 0.05$ vs. control, $^{\bullet}p < 0.05$ vs. DX.

Besides, doxorubicin administration lead to a significant increase ($p < 0.05$) of cardiac tissue Bax/Bcl-2 ratio in comparison with the control group. However, hemin treatment caused a significant decrease ($p < 0.05$) of Bax/Bcl-2 ratio in cardiac tissue in doxorubicin-challenged rats (Figure 5).

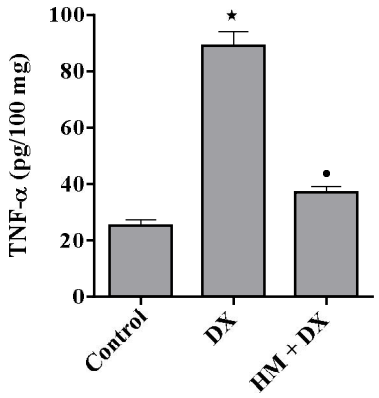

Figure 3. Effects of hemin (HM) on tumor necrosis factor-α (TNF-α) in cardiac tissues of rats received doxorubicin (DX). Results are mean ± S.E.M., $^{*}p < 0.05$ vs. control, $^{\bullet}p < 0.05$ vs. DX.

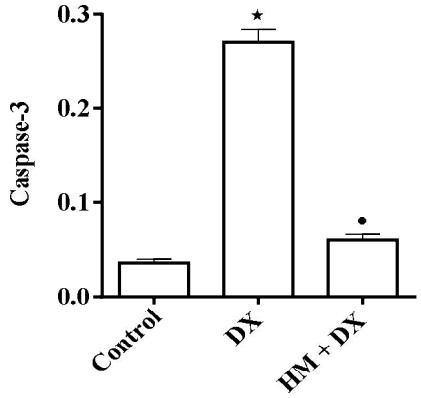

Figure 4. Effects of hemin (HM) on caspase-3 in cardiac tissues of rats received doxorubicin (DX). Results are mean ± S.E.M., $^{*}p < 0.05$ vs. control, $^{\bullet}p < 0.05$ vs. DX.

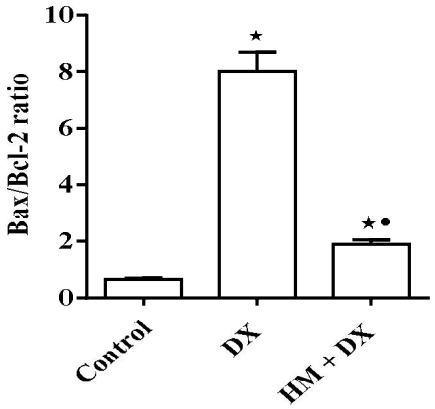

Figure 5. Effects of hemin (HM) on Bax/Bcl-2 in cardiac tissues of rats received doxorubicin (DX). Results are mean ± S.E.M., $^{*}p < 0.05$ vs. control, $^{\bullet}p < 0.05$ vs. DX.

4. Discussions

The present study revealed that hemin treatment provided a significant protective effect against doxorubicin-induced cardiotoxicity in rats.

The present results, in accordance with previous studies, showed that oxidative stress, depletion of antioxidant defenses, increased production of inflammatory mediators, and apoptosis are implicated in the pathogenesis of doxorubicin cardiotoxicity (Andreadou et al., 2007; Jiang et al., 2008; Ammar et al., 2011; Xin et al., 2011). In addition, it was reported that doxorubicin caused a significant elevation of cardiac NO levels due to increased expression of inducible nitric oxide synthase in the cardiac tissue (Sayed-Ahmed et al., 2001; Andreadou et al., 2007). This may be due to the ability of TNF-α to up-regulate the inducible nitric oxide synthase gene (Morris and Billiar, 1994). Excess NO reacts with superoxide anion to produce peroxynitrite radical which causes further cardiac tissue damage by oxidizing and nitrating cellular macromolecules. Also, excess NO depletes intracellular GSH increasing the susceptibility to oxidative stress (Clancy and Abramson, 1995).

In the present study, figure 1 showed that hemin treatment significantly reduced serum level of creatine kinase, the biomarker of myocardial tissue injury, in rats received doxorubicin. This indicates that hemin preserved myocardial integrity and function in doxorubicin-challenged rats. In addition, figure 2 demonstrated that doxorubicin significantly increased MDA, the oxidative biomarker, and NO, the nitrosative stress biomarker, and significantly reduced TAS, the indicator of endogenous antioxidant defenses, in cardiac tissue of rats. Figure 2 also showed that hemin provided a significant antioxidant, and antinitrosative effects as indicating by reduced cardiac MDA and NO, and increased cardiac TAS in rats challenged with doxorubicin. Besides, figure 3 displayed that hemin treatment significantly reduced TNF-α, the main pro-inflammatory cytokine, in cardiac tissues of rats received doxorubicin indicating the anti-inflammatory properties of hemin.

Hemin is an inducer of heme oxygenase-1 (HO-1), which is the rate-limiting enzyme in heme catabolism. HO-1 plays an important role in protecting against tissue injury through its antioxidant and anti-inflammatory properties (Bulger et al., 2003; Wen et al., 2007). The enzyme is induced during oxidative and inflammatory tissue injuries. Up-regulation of HO-1 following hemin administration catalyzes oxidative degradation of heme into carbon monoxide, biliverdin and ferrous iron. Carbon monoxide induces the nuclear-factor erithroid 2-related factor 2 (Nrf2) activating the antioxidant response elements which regulate genes for many

endogenous antioxidant enzymes (Otterbein and Choi, 2000; Chan et al., 2001). In addition, biliverdin is converted by biliverdin reductase into bilirubin which has a higher free radical-scavenging activity than other physiological antioxidants as vitamin C and α-tocopherol (Stocker et al., 1987). Administration of bilirubin or biliverdin prevents gluthatione depletion and lipid peroxidation, and increases superoxide dismutase activity (Chiu et al., 2002). This antioxidant activity by the fact that bilirubin oxidized into biliverdin is recycled back by biliverdin reductase deriving a powerful redox cycle that allows for continuous cytoprotection against oxidative stress (Florczyk et al., 2008).

Along the same line of the current work, previous investigations revealed that the mitochondrial-dependent apoptotic pathway is involved in doxorubicin cardiotoxicity (Jing et al., 2018). This pathway is controlled by the Bcl-2 family proteins, mainly Bcl-2, the anti-apoptotic protein, and Bax, the pro-apoptotic protein. Increasing Bax and decreasing Bcl-2 disrupts the integrity of mitochondrial membranes leading to mitochondrial release of cytochrome C into the cytosol, and activation of caspase-3, the main executioner of cell apoptosis (Anghel et al., 2018). In the present work, figure 4 and figure 5 showed that hemin treatment significantly reduced cardiac Bax/Bcl-2 ratio and caspase-3 activity in rats challenged with doxorubicin. Therefore, it can be concluded that hemin significantly inhibited the mitochondrial apoptotic pathway in cardiac tissue of doxorubicin-challenged rats.

The results of the present work indicated that hemin, the HO-1 inducer, significantly protected against doxorubicin cardiotoxicity in rats. This is most probably mediated by inhibiting oxidative/nitrative stress, inflammation, and apoptosis. Therefore, it can be assumed that hemin may be a feasible candidate to protect against cardiac toxicity and dysfunction in cancer patients treated with doxorubicin. However, this needs to be clarified by further investigations.

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Conflicts of interest
The author declare that he has no conflict of interest.

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