Protective effects of dexrazoxane against acute ischaemia/reperfusion injury of rat hearts

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Abstract: Dexrazoxane (DEX), an inhibitor of topoisomerase II and intracellular iron chelator, is believed to reduce the formation of reactive oxygen species (ROS) and protects the heart from the toxicity of anthracycline antineoplastics. As ROS also play a role in the pathogenesis of cardiac ischaemia/reperfusion (I/R) injury, the aim was to find out whether DEX can improve cardiac ischaemic tolerance. DEX in a dose of 50, 150, or 450 mg·(kg body mass)$^{-1}$ was administered intravenously to rats 60 min before ischaemia. Myocardial infarct size and ventricular arrhythmias were assessed in anaesthetized open-chest animals subjected to 20 min coronary artery occlusion and 3 h reperfusion. Arrhythmias induced by I/R were also assessed in isolated perfused hearts. Only the highest dose of DEX significantly reduced infarct size from 53.9% ± 4.7% of the area at risk in controls to 37.5% ± 4.3% without affecting the myocardial markers of oxidative stress. On the other hand, the significant protective effect against reperfusion arrhythmias occurred only in perfused hearts with the dose of DEX of 150 mg·kg^{-1}, which also tended to limit the incidence of ischaemic arrhythmias. It is concluded that DEX in a narrow dose range can suppress arrhythmias in isolated hearts subjected to I/R, while a higher dose is needed to limit myocardial infarct size in open-chest rats.

Key words: heart, dexrazoxane, ischaemia, reperfusion, infarct size, arrhythmias, cardioprotection, reactive oxygen species.

Résumé : Le dexrazoxane (DEX), un inhibiteur de topoisomérase II et un chélateur de fer intracellulaire, est présumé réduire la formation d'espèces réactives d'oxygène (ERO) et protéger le cœur de la toxicité d'antinéoplasiques de la famille des anthracyclines. Puisque les ERO jouent aussi un rôle dans la pathogenèse des dommages d'ischémie/reperfusion (I/R), notre but était de déterminer si le DEX pouvait améliorer la tolérance cardiaque à l'ischémie. Une dose de 50, 150 ou 450 mg·(kg de poids corporel)$^{-1}$ de DEX a été administrée à des rats par injection intraveineuse 60 minutes avant l'ischémie. La taille de l'infarctus du myocarde et l'arythmie ventriculaire ont été évaluées sur des animaux anesthésiés à thorax ouvert soumis à une occlusion de l'artère coronaire de 20 minutes et une reperfusion de 3 heures. L'arythmie induite par l'I/R a aussi été évaluée sur des cœurs perfusés isolés. Seule la dose la plus élevée de DEX réduisait significativement la taille de l'infarctus de 53,9 ± 4,7 % de la zone à risque des contrôles à 37,5 ± 4,3 % sans affecter les marqueurs myocardiques du stress oxydant. D'un autre côté, l'effet protecteur significatif contre l'arythmie de reperfusion survenait seulement sur les cœurs perfusés à une dose de 150 mg·kg^{-1} de DEX, qui tendait aussi à limiter l'incidence d'arythmie ischémique. Nous concluons que le DEX peut supprimer l'arythmie de cœurs isolés soumis à une I/R dans une fenêtre de concentrations étroite, alors qu'une dose plus élevée est requise pour limiter la taille de l'infarctus du myocarde des rats à thorax ouvert.

Mots-clés : cœur, dexrazoxane, ischémie, reperfusion, taille de l'infarctus, arythmie, cardioprotection, espèces réactives d'oxygène.

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Introduction

A bisdioxopiperazine derivative, dexrazoxane (DEX), which is a strong catalytic inhibitor of topoisomerase II (Hasinoff et al. 1995), is currently the only drug approved for the protection of myocardium from cardiotoxicity induced by anthracycline (ANT) chemotherapeutics. Potent cardioprotective effects of this drug have been demonstrated in various experimental models of anthracycline cardiotoxicity (e.g., Herman and Ferrans 1987; Cvetković and Scott 2005; Popelová et al. 2009) as well as in randomized clinical trials (Swain et al. 1997; Scully and Lipshultz 2007; van Dalen et al. 2011).

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This article is one of a number of papers published in the Special Issue entitled "Heart Health and Care," which focuses on new knowledge of the physiology of cardiovascular functions in health, and pathophysiology of cardiovascular dysfunctions.

Although the precise mechanisms of ANT cardiotoxicity remain unknown, it has been hypothesized that a major role may involve ANT-induced and iron-catalyzed production of reactive oxygen species (ROS) within the myocardium (Singal and Iliskovic 1998; Li and Singal 2000). In this regard, DEX has been characterized as a prodrug that is bioactivated in cardiomyocytes towards its metal chelating metabolite ADR-925 (Schroeder and Hasinoff 2002). The chelation of free (catalytically active) iron within the cells is believed to prevent both the redox cycling of ANT–Fe^{3+} complexes and the production of highly cytotoxic hydroxyl radicals through the Fe-catalyzed Haber–Weiss reaction (reviewed in Šimůnek et al. 2009).

It is now established that ROS play a role in the pathogenesis of acute cardiac ischaemia/reperfusion (I/R) injury. In particular, it has been well documented that ROS contribute to early reperfusion arrhythmias and that antioxidants can significantly attenuate their incidence and severity (reviewed in Li and Jackson 2002 and Wang et al. 2002). In contrast to the clearly protective effects of antioxidants against reperfusion-induced arrhythmias, their efficacy against other endpoints of I/R injury is rather controversial, and it is still debated whether ROS can significantly contribute to cell death. Although numerous experimental studies have demonstrated the beneficial effects of ROS dampening on ischaemic arrhythmias and infarct size, other reports showed only minor (if any) protection (Miura et al. 1988; Matejíková et al. 2009; Neckář et al. 2009; Imani et al. 2011). Nevertheless, prevention of the production of cytotoxic ROS might be a target for effective cardioprotection, especially during the reperfusion phase. Of note, the main theoretical advantage of DEX over traditional antioxidants could reside in its ability to prevent the cyclic production of most cytotoxic forms of ROS instead of the less effective attempts to eliminate them after their production.

Although the cardioprotective effects of DEX on ANT-induced cardiotoxicity have been well established, there are only a few studies addressing the potential protective effects of this agent against myocardial injury caused by acute oxygen deprivation. This is rather surprising, as DEX is a clinically approved drug that has passed all stages of preclinical and clinical development, and thus it could be readily available for use in other cardioprotective settings. Ten years ago, Hasinoff (2002) suggested that DEX can protect neonatal cardiac myocytes against hypoxia–reoxygenation damage, detected as lactate dehydrogenase release. Similarly, DEX has been reported to improve the postischaemic recovery of cardiac contractile functions after global I/R in isolated rat hearts (Ramu et al. 2006). Finally, the recent study of Zhou et al. (2011) showed that preischaemic administration of DEX reduced myocardial remodelling and improved indices of ventricular dysfunction in rats with permanent coronary artery occlusion. However, it is still unknown whether DEX can protect the heart against irreversible tissue injury and limit the incidence and severity of ventricular arrhythmias in a whole animal model of acute I/R. The aim of our study was, therefore, to find out whether DEX treatment reduces myocardial infarct size and arrhythmias induced by regional I/R in open-chest rats and in isolated perfused hearts, and to find out its most effective dose.

Materials and methods

Animals

Experiments were performed on adult male Wistar rats (body mass 270–320 g). All animals had free access to water and a standard laboratory diet. The animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (ILAR 1996). Experimental protocol was reviewed and approved by the Animal Care and Use Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

DEX (Cardioxane®; Novartis, Switzerland) was dissolved in saline and administered in single doses of 50, 150, or 450 mg·(kg body mass)$^{-1}$ to anaesthetized animals (pentobarbital sodium, 60 mg·kg^{-1}, intraperitoneal injection; Sigma–Aldrich, USA) via the cannulated right jugular vein always 60 min prior to the induction of myocardial ischaemia. The time interval for DEX administration was selected with respect to the known pharmacokinetic profile and metabolism of the drug (Schroeder and Hasinoff 2002; Cvetković and Scott 2005). Control rats received the same volume of the vehicle (~1 mL).

Infarct size and ventricular arrhythmias in open-chest rats

Anaesthetized rats were ventilated (rodent ventilator 7026; Ugo Basile, Italy) via tracheal cannula with room air at 68 strokes·min^{-1} (tidal volume of 1.2 mL·(100 g body mass)$^{-1}$). Both blood pressure in the carotid artery and a single-lead electrocardiogram (ECG) were continually recorded and subsequently analyzed by our custom-designed software. The rectal temperature was maintained between 36.5 and 37.5 °C by a heated table throughout the experiment. Left thoracotomy was performed and regional myocardial ischaemia was induced after 10 min stabilization by tightening a polyester ligature 6/0 (Ethicon, UK) placed around the left anterior descending (LAD) coronary artery about 1–2 mm distal to its origin. Characteristic changes in the configuration of the ECG and a transient decrease in blood pressure verified the coronary artery occlusion. After a 20 min occlusion period, the ligature was released and reperfusion of previously ischaemic tissue continued.

The incidence of ventricular arrhythmias during ischaemic insult and the first 5 min of reperfusion was assessed according to the Lambeth Conventions (Walker et al. 1988). Premature ventricular complexes (PVCs) occurring as singles, salvos, or tachycardia (a run of 4 or more consecutive PVCs) were counted separately. The incidence of ventricular tachycardia (VT) and fibrillation (VF) was also evaluated. VF lasting more than 2 min was considered as sustained. The severity of arrhythmias in each individual heart was evaluated by means of a 5-point arrhythmia score, as described by Asemu et al. (2000). Scores were used for group analysis of the severity of arrhythmias.

At the end of 3 h of reperfusion, the hearts were excised and washed with 20 mL saline through the aorta. The area at risk and the infarct size were determined as described by Neckář et al. (2002) by staining with 5% potassium permanganate (Pliva-Lachema, Czech Republic) and 1% 2,3,5-triphenyltetrazolium chloride (Sigma–Aldrich, USA), respectively. The hearts were cut perpendicularly to the left

ventricular (LV) long axis into slices 1 mm thick and stored overnight in 10% neutral formaldehyde solution. The next day, the right ventricular free wall was separated and both sides of the LV slices were photographed. The size of the infarct area, the size of the area at risk, and the size of the LV were determined by a computerized planimetric method using the software Ellipse (ViDiTo, Slovakia) with a grid of about 400 points per slice. The infarct area was normalized to the area at risk, and the area at risk was normalized to the LV.

Ventricular arrhythmias in isolated perfused hearts

Animals were anaesthetized as above. Hearts were rapidly excised and perfused at constant flow (~10 mL·min^{-1}·g^{-1}) and temperature (37 °C), according to the method of Langendorff, under nonrecirculating conditions, by a modified Krebs–Henseleit solution (in mmol·L^{-1}: NaCl, 118.0; KCl, 3.2; $CaCl_2$, 1.25; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; glucose, 7.0). The medium was gassed with 95% O_2 and 5% CO_2 (pH 7.4). The expected heart masses were calculated from regression equations established on the basis of our previous data for heart mass-to-body mass ratio (Asemu et al. 1999).

Epicardial electrograms were continuously recorded and subsequently analyzed. To investigate the effect of DEX on ischaemic arrhythmias, after a 20 min stabilization period the hearts were subjected to 30 min ischaemia. To investigate the effect of DEX on reperfusion arrhythmias, the hearts were subjected to 15 min ischaemia followed by reperfusion. Ventricular arrhythmias occurring during 30 min ischaemia and the first 5 min of reperfusion, respectively, were counted and evaluated as for the open-chest experiments.

Biochemical analyses

Biochemical markers were assessed in additional groups of DEX-treated (150 and 450 mg·kg^{-1}) and vehicle-treated control rats subjected to 20 min LAD occlusion and 60 min reperfusion. Samples (about 80 mg each) were taken from the LV free wall area supplied by the occluded artery and from the interventricular septum (non-ischaemic tissue), frozen in liquid nitrogen, and stored at –80 °C until use. Preliminary experiments confirmed that non-ischaemic levels of the analyzed markers did not differ between these 2 parts of ventricular myocardium.

The reduced (GSH) and oxidized (GSSG) glutathione concentrations were determined simultaneously using the method of Reed et al. (1980) adapted by Yoshida (1996), with slight modifications. Briefly, the tissue was homogenized in cold 5% metaphosphoric acid containing 10 mmol·L^{-1} EDTA (1 mL). The precipitated proteins were removed by centrifugation, and the supernatant (0.4 mL) was reacted with 0.4 mol·L^{-1} iodoacetic acid (100 μL) to block the thiol group of GSH and then with 1-fluoro-2,4-dinitrobenzene (100 μL) to derivatize amino groups of both GSH and GSSG. The excess reagent was removed by incubation with glycine. The solution was analyzed using an HPLC system 1100 (Agilent, USA; Zorbax NH_2 column; 4.6 mm × 150 mm; 5 μm). The mobile phase for gradient elution was methanol–water 4:1 (*v*/*v*; solution A) mixed with 2 mol·L^{-1} sodium acetate–water–methanol 3:1:2 (*v*/*v*/*v*; solution B). UV detection was set at 365 nm.

The samples for malondialdehyde (MDA; marker of lipid peroxidation) determination were pulverized into a powder under liquid nitrogen. After adding 500 μL of the homogenization buffer (25 mmol·L^{-1} Tris–HCl and 0.1% Triton X-100), the samples were homogenized and centrifuged (1000*g*, 10 min, 4 °C). Supernatant (100 μL) was taken for the determination of MDA concentration. After adding 20 μL of NaOH (6 mol·L^{-1}) and vortexing, the samples were kept at 60 °C for 30 min followed by 5 min cooling at –20 °C, deproteinized by 50 μL of $HClO_4$ (35% *v*/*v*) and centrifuged (10 000*g*, 5 min, 4 °C). Supernatant (100 μL) was mixed with 10 μL of 2,4-dinitrophenylhydrazine (5 mmol·L^{-1}), kept in the dark for 10 min, and analyzed by an HPLC system (Shimadzu, Japan; column EC Nucleosil 100–5 C18; 4.6 mm × 125 mm; flow 1.0 mL·min^{-1}; sampling volume 30–100 μL) with the UV detection set on 310 nm. Concentration of MDA was normalized to total proteins determined by the method of Bradford (1976).

Data analysis

Results are expressed as the mean ± SEM. Differences in the number of PVCs between the groups were compared by Mann–Whitney *U* test. More than 2 groups were compared by Kruskal–Wallis nonparametric test. The incidence of ventricular fibrillation was examined by Fisher's exact test. ANOVA and subsequent Student–Newman–Keuls test were used for comparison of differences in parametric variables among the groups. Differences were considered as statistically significant at $P < 0.05$.

Results

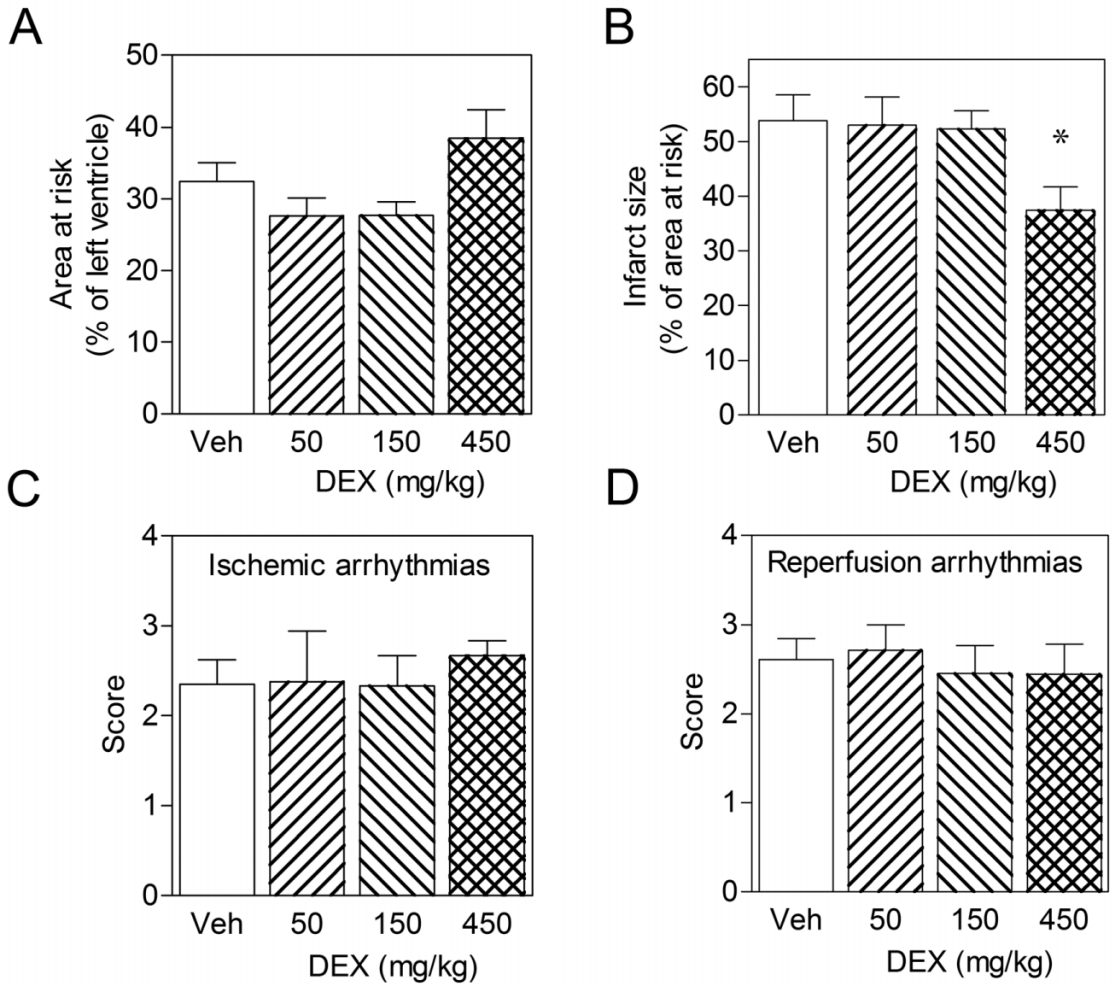
Myocardial infarct size

The normalized area at risk did not significantly differ among the groups except for the group treated with the highest dose of DEX, which exhibited a larger area at risk than the groups treated with lower doses (Fig. 1A). Nevertheless, only this high dose of DEX (450 mg·kg^{-1}) significantly reduced infarct size to 37.5% ± 4.3% of the area at risk, compared with the vehicle-treated controls (53.9% ± 4.7%), while the doses of 50 mg·kg^{-1} and 150 mg·kg^{-1} had no effect (Fig. 1B).

Ischaemic and reperfusion ventricular arrhythmias

None of the doses of DEX tested affected the values of ischaemic and reperfusion ventricular arrhythmia score (Figs. 1C and 1D, respectively) or the incidence of various types of arrhythmias (data not shown) in open-chest animals. Similarly, arrhythmia score did not differ between isolated perfused hearts from the control and DEX-treated rats (Fig. 2A) although the dose of 150 mg·kg^{-1} tended to decrease both the total number of PVCs (Fig. 2B) and the duration of tachyarrhythmias (VT + VF; Fig. 2C) without affecting temporal profile of arrhythmias (Fig. 2D) occurring during the 30 min ischaemic period. However, 150 mg·kg^{-1} DEX significantly reduced both arrhythmia score (Fig. 3A) and the incidence of VF (Fig. 3B) occurring during early reperfusion of isolated hearts; this effect was absent in the hearts of rats treated with the highest dose of DEX.

Fig. 1. (A) Myocardial area at risk, (B) infarct size, and (C) ventricular arrhythmia scores over 20 min ischaemia and (D) early reperfusion in open-chest control (Veh) and dexrazoxane (DEX)-treated rats. DEX was administered intravenously 60 min prior to the induction of ischaemia in doses of 50, 150, or 450 mg·(kg body mass)$^{-1}$. Values are the mean ± SEM from 7–12 rats in each group. *, $P < 0.05$ compared with Veh.



Biochemical markers

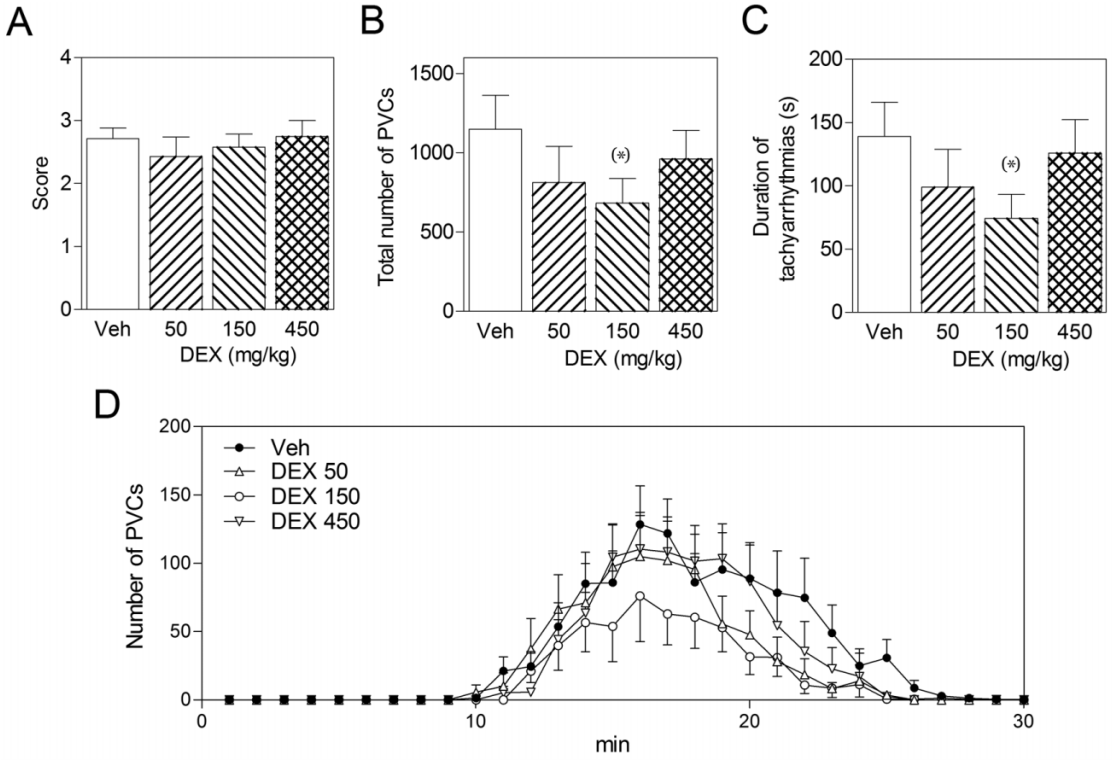
Figure 4 shows effects of I/R insult on myocardial ratio of GSH:GSSG (Fig. 4A) and concentration of MDA (Fig. 4B), expressed as a percentage of corresponding non-ischaemic values. In vehicle-treated controls, I/R decreased the GSH:GSSG ratio by 36% and increased the MDA concentration by 40%. DEX administered in the doses of 150 or 450 mg·kg^{-1} did not significantly affect these markers of oxidative stress.

Discussion

This study analyzed the cardioprotective potential of iron chelator DEX against acute I/R injury in rats (3 doses of DEX were used in both in-vitro and in-vivo settings). To the best of our knowledge, the infarct size and the susceptibility to ischaemic and reperfusion arrhythmias in DEX-treated animals were assessed for the first time. We have found a narrow dose range that can suppress ischaemic and, especially, reperfusion arrhythmias in isolated perfused hearts. On the other hand, only the highest dose of DEX (450 mg·kg^{-1}) significantly reduced the myocardial infarct size determined in open-chest rats without affecting various markers of oxidative stress associated with I/R.

Our observation of the infarct size-limiting effect of DEX is in line with results of Ramu et al. (2006), who showed improved postischaemic recovery of contractile function in isolated perfused rat hearts subjected to global I/R after DEX pretreatment, and with those of Hasinoff (2002), who detected lower lactate dehydrogenase release from DEX-treated neonatal cardiac myocytes exposed to anoxia–reoxygenation. The literature concerning the effects of other iron chelators on I/R-induced lethal myocardial injury is rather controversial and inconclusive. Several studies examined the influence of deferoxamine (DFO) (a prototype and most widely used iron chelator in the clinical practice) on infarct size in various animal species. They demonstrated smaller (Lesnefsky et al. 1990; Kobayashi et al. 1991; Chopra et al. 1992), unchanged (Maxwell et al. 1989; Reddy et al. 1991), or even increased (Watanabe et al. 1993) myocardial infarction in DFO-treated animals. Obviously, differences between species, I/R models, and experimental protocols including DFO dosage and timing could account for these discrepant results. Moreover, poor membrane permeability and a short plasma half-life of DFO can diminish the cardioprotective potential of iron chelation under in-vivo conditions.

Fig. 2. (A) Ventricular arrhythmia score, (B) total number of premature ventricular complexes (PVCs), (C) duration of tachyarrhythmias (ventricular tachycardia and fibrillation), and (D) temporal profile of PVCs over 30 min ischaemia of isolated perfused hearts of the control (Veh) and dexrazoxane (DEX)-treated rats. DEX was administered intravenously 60 min prior to the induction of ischaemia in doses of 50, 150, or 450 mg·(kg body mass)$^{-1}$. Values are the mean ± SEM from 7–12 hearts in each group. (*), $P < 0.1$ compared with Veh.



Based on current knowledge, both ANT-induced cardiotoxicity and I/R injury seem to share the involvement of ROS with a supposed catalytic role of free iron. Hence, intracellular iron chelation can be considered as a promising approach to cardioprotection. The main advantage of this strategy is that it is focused on prevention of the production of the most toxic forms of ROS instead of relatively difficult and poorly effective scavenging of ROS using antioxidants. In the present study, we determined 2 different markers of myocardial oxidative stress: ratio of GSH-to-GSSG characterizing one of the most important intracelullar antioxidant defence systems, and MDA as the main product of lipid peroxidation. The aim was to test the hypothesis that the protective effects of DEX were associated with decreased ROS formation during I/R insult. However, none of these conventional markers of oxidative stress showed any significant effect of DEX treatment, which strongly argues against its above-proposed and generally anticipated mechanisms of action. It should be noted that the active metabolite of DEX, which is effectively produced inside the cardiomyocytes, is not selective for iron. As an EDTA-like agent it can chelate and affect the biological function of other multivalent ions such as calcium. As a profound impairment of intracellular calcium handling plays a role in the pathogenesis of myocardial I/R injury, it is considered as an important target for cardioprotection. It should be also mentioned that DEX is a strong inhibitor of topoisomerase II (Hasinoff et al. 1995), which plays an important role in DNA transcription and replication. However, at least the latter effect seems unlikely to contribute to the cardioprotection observed in this study, because ventricular myocytes are terminally differentiated and myocardium contains only low levels of this enzyme (Hasinoff and Herman 2007).

Our study demonstrated that the dose of 150 mg·kg^{-1} DEX markedly reduced the incidence and severity of reperfusion arrhythmias and tended to diminish ischaemic arrhythmias in isolated perfused hearts. This finding corresponds with several earlier reports suggesting that hydroxyl radicals were likely involved in I/R-induced arrhythmogenesis and postischaemic stunning of rat hearts (Bernier et al. 1986; Bolli et al. 1987; Shuter et al. 1990; Chevion 1991; Appelbaum et al. 1990; Ferdinandy et al. 1998). In addition, many experimental studies demonstrated that the acute administration of ROS scavengers or antioxidants at the end of ischaemic period reduced reperfusion arrhythmias (reviewed in Wang et al. 2002). Nevertheless, no effect of DFO on reperfusion arrhythmias was demonstrated in 2 studies on dogs (da-Luz et al. 1993; Euler 1995) suggesting that species differences should be taken into account.

The reason for the loss of antiarrhythmic protection with increasing the dose of DEX in our experiments is unclear. This bell-shaped dose–response relationship of cardioprotective action has not been reported previously for DEX in any experimental setting. In anthracycline cardiotoxicity reports, the common dose–response has been described, while the studies dealing with I/R (Ramu et al. 2006) or permanent coronary artery occlusion (Zhou et al. 2011) used DEX in a single dose only. It might be hypothesized that formation of redox-inactive complexes of ADR-925 with iron requires a certain molar ratio between active metabolite and free iron within the cardiomyocytes. When a threshold ratio is ex-

Fig. 3. (A) Ventricular arrhythmia score and (B) the incidence of ventricular fibrillation during early (5 min) reperfusion of isolated perfused hearts of control (Veh) and dexrazoxane (DEX)-treated rats subjected to 15 min ischaemia. DEX was administered intravenously 60 min prior to the induction of ischaemia in doses of 50, 150, or 450 mg·(kg body mass)$^{-1}$. Values are the mean ± SEM from 12–18 hearts in each group. *, $P < 0.05$ compared with Veh.

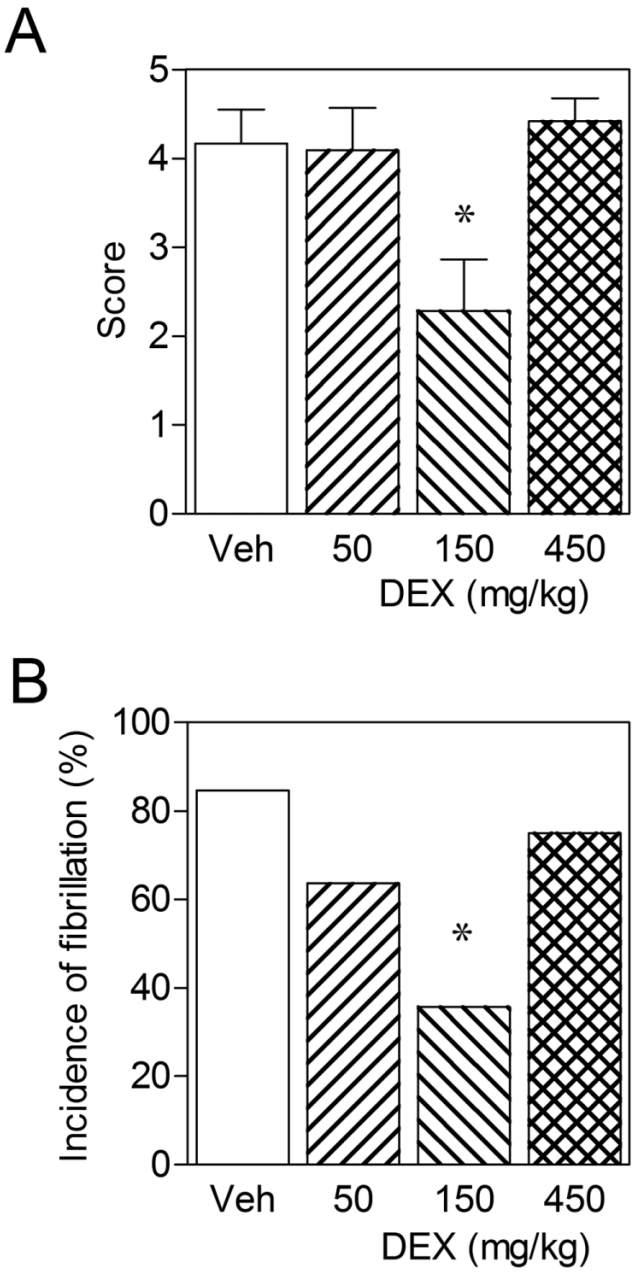
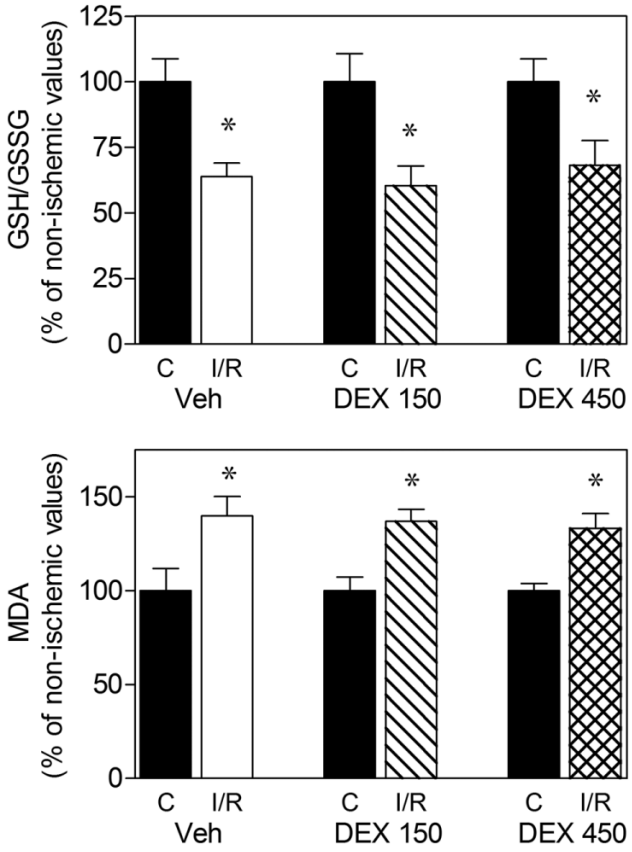


Fig. 4. (A) Myocardial ratio of reduced-to-oxidized glutathione (GSH:GSSG) and (B) concentration of malondialdehyde (MDA) in open-chest control (Veh) and dexrazoxane (DEX)-treated rats. Effects of 20 min ischaemia and 60 min reperfusion (I/R) are expressed as a percentage of corresponding non-ischaemic (C) values. DEX was administered intravenously 60 min prior to the induction of ischaemia in doses of 150 or 450 mg·(kg body mass)$^{-1}$. Values are the mean ± SEM from 6 hearts in each group. *, $P < 0.05$ compared with C.



ceeded, as with the highest DEX dose, different type of complexes can be formed that need not be effective in the prevention of ROS formation. This view is in accord with the presumed ROS-independent mechanism of infarct-size limitation in open-chest animals found in our study.

We have observed different effects of DEX on arrhythmias in open-chest rats and in isolated perfused hearts, despite the fact that both involved the same model of zero-flow regional ischaemia. Similarly, Asemu et al. (2000) reported distinct effects of chronic hypoxia on susceptibility of rat hearts to ischaemic arrythmogenesis assessed under in-vitro and in-vivo conditions. The reason for these differences is unknown at present. Apparently, the presence of blood components and neurohumoral control mechanism in open-chest animals can mask the antiarrhythmic potential of DEX.

The interval between DEX administration and the onset of ischaemia has been selected with regards to the pharmacokinetics and metabolism of DEX in rats (Schroeder and Hasinoff 2002). These authors have demonstrated that 60 min after intravenous administration of DEX, the distribution phase of the drug is largely completed. Furthermore, they have observed relatively rapid DEX metabolism. The supposed active metabolite ADR-925 was detectable in plasma as early as 5 min after DEX administration, and its concentration increased progressively to become comparable with that of the parent drug at 60 min. Hence, we believe that 60 min after drug administration, all pharmacokinetic considerations comply with the achievement of a cardioprotective response. Moreover, others have reported significant cardioprotective effects against I/R injury or anthracycline cardiotoxicity

when the drug has been administered even 30 min earlier (Ramu et al. 2006; Herman and Ferrans 1998).

In conclusion, DEX in the single dose of 150 mg·kg^{-1} effectively suppressed ventricular arrhythmias in isolated hearts subjected to acute I/R, but it was insufficient to reduce arrhythmias in open-chest animals, and only the highest dose of DEX (450 mg·kg^{-1}) decreased myocardial infarct size. This difference could be related to a more complex pathogenesis of I/R injury under in-vivo conditions. The protective effects of DEX were not accompanied by decreases of oxidative stress markers in the myocardium, suggesting that other protective mechanism(s) than limitation of ROS formation might play a role.

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