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Early and delayed cardioprotective intervention with dexrazoxane each show different potential for prevention of chronic anthracycline cardiotoxicity in rabbits

Eduard Jirkovský[a], Olga Lenčová-Popelová[a], Miloš Hroch[a,b], Michaela Adamcová[c], Yvona Mazurová[d], Jaroslava Vávrová[e], Stanislav Mičuda[a], Tomáš Šimůnek[f], Vladimír Geršl[a], Martin Štěrba[a,*]

[a] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University in Prague, Šimkova 870, Hradec Králové 500 38, Czech Republic
[b] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University in Prague, Šimkova 870, Hradec Králové 500 38, Czech Republic
[c] Department of Physiology, Faculty of Medicine in Hradec Králové, Charles University in Prague, Šimkova 870, Hradec Králové 500 38, Czech Republic
[d] Department of Histology and Embryology, Faculty of Medicine in Hradec Králové, Charles University in Prague, Šimkova 870, Hradec Králové 500 38, Czech Republic
[e] Department of Clinical Biochemistry and Diagnosis, University Hospital Hradec Králové, Sokolská 581, Hradec Králové 500 05, Czech Republic
[f] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Heyrovského 1203, Hradec Králové 500 05, Czech Republic

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ABSTRACT

Despite incomplete understanding to its mechanism of action, dexrazoxane (DEX) is still the only clearly effective cardioprotectant against chronic anthracycline (ANT) cardiotoxicity. However, its clinical use is currently restricted to patients exceeding significant ANT cumulative dose (300 mg/m^2), although each ANT cycle may induce certain potentially irreversible myocardial damage. Therefore, the aim of this study was to compare early and delayed DEX intervention against chronic ANT cardiotoxicity and study the molecular events involved. The cardiotoxicity was induced in rabbits with daunorubicin (DAU; 3 mg/kg/week for 10 weeks); DEX (60 mg/kg) was administered either before the 1st or 7th DAU dose (i.e. after ≈300 mg/m^2 cumulative dose). While both DEX administration schedules prevented DAU-induced premature deaths and severe congestive heart failure, only the early intervention completely prevented the left ventricular dysfunction, myocardial morphological changes and mitochondrial damage. Further molecular analyses did not support the assumption that DEX cardioprotection is based and directly proportional to protection from DAU-induced oxidative damage and/or deletions in mtDNA. Nevertheless, DAU induced significant up-regulation of heme oxygenase 1 pathway while heme synthesis was inversely regulated and both changes were schedule-of-administration preventable by DEX. Early and delayed DEX interventions also differed in ability to prevent DAU-induced down-regulation of expression of mitochondrial proteins encoded by both nuclear and mitochondrial genome. Hence, the present functional, morphological as well as the molecular data highlights the enormous cardioprotective effects of DEX and provides novel insights into the molecular events involved. Furthermore, the data suggests that currently recommended delayed intervention may not be able to take advantage of the full cardioprotective potential of the drug.

Abbreviations: ALAS1, 5-aminolevulinate synthase 1; ANT, anthracycline; BVR A, biliverdin reductase A; COX1, COX4, mitochondrial and nuclear genome-encoded complex IV subunits, respectively; DAU, daunorubicin; DEX, dexrazoxane; FU, post-treatment follow up; GSH, reduced glutathione; GSSG, oxidized glutathione; HIF1α, hypoxia-inducible factor 1α; HO1, heme oxygenase 1; LV FS, left ventricular fractional shortening; MDA, malondialdehyde; MnSOD, mitochondrial superoxide dismutase; mtDNA, mitochondrial DNA; ND1, ND4 mitochondrial genome-encoded complex I subunits; nDNA, nuclear DNA; NDUFS2, nuclear genome-encoded complex I subunit; NOX2, NOX4, NADPH oxidases 2 and 4; NQO1, NAD(P)H dehydrogenase [quinone] 1; NRF1, nuclear respiratory factor 1; Nrf2, nuclear factor erythroid 2-related factor 2; PRDX3, peroxiredoxin 3; TFAM, mitochondrial transcription factor A.

* Corresponding author. Tel.: +420 495 816 312; fax: +420 495 513 597.
E-mail address: sterbam@lfhk.cuni.cz (M. Štěrba).

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1. Introduction

Anthracycline (ANT) antibiotics (e.g. doxorubicin, daunorubicin or epirubicin) continue to form a backbone of anticancer therapy in many hematological and solid malignancies. However, all ANTs are also known for their risk of chronic cardiotoxicity which may result in cardiomyopathy and heart failure with either early or late onset. Current clinical guidelines attempt to minimize the risks by limitation of the total cumulative dose of ANTs and by implementation of non-invasive monitoring of cardiac function (Aapro et al., 2011; Menna et al., 2012). Nevertheless, it is becoming clear that there is no completely safe cumulative dose of ANTs. Instead, it is now appreciated that each ANT cycle likely induces certain molecular and ultrastructural damage to the myocardium which is largely irreversible and manifests itself after accumulation of the damage from multiple cycles (Ewer and Benjamin, 2006). Hence, patients undergoing ANT treatment may carry clinical or more often subclinical cardiac burden to their post-cancer life (Aapro et al., 2011; Ewer and Benjamin, 2006; Menna et al., 2012).

The optimal approach to management of ANT cardiotoxicity risk is its effective prevention. Hence, the search for an effective pharmacological cardioprotectant began soon after the early clinical trials recognizing this complication. More than 40 years of intensive research yielded many disappointing outcomes which also concerned classic antioxidants including vitamin E or acetylcysteine (Dresdale et al., 1982; Legha et al., 1982; Myers et al., 1983; Sterba et al., 2013). However, major success has also been achieved in this field-development of dexrazoxane (DEX) which is still the only drug which has been convincingly demonstrated to provide effective cardioprotection in both clinical and experimental settings (Cvetkovic and Scott, 2005; van Dalen et al., 2011). Furthermore, DEX has been shown to induce effective protection from both ANT-induced degenerative changes and apoptotic death of cardiomyocytes (Popelova et al., 2009; Sawyer et al., 1999). Traditionally, DEX is believed to be a pro-drug of iron chelating metabolite (ADR-925) which prevents participation of iron in ANT redox cycling in the heart (Cvetkovic and Scott, 2005; Hasinoff and Herman, 2007). More recently, DEX has been proposed to specifically protect mitochondrial DNA (mtDNA) from oxidative stress-induced common deletions and impaired expression of mtDNA-encoded respiratory chain subunits (Lebrecht et al., 2007). However, stronger and more selective intracellular iron chelators failed to provide better or at least comparable cardioprotection as DEX in chronic ANT cardiotoxicity models (Sterba et al., 2013) which argues against this hypothesis and some investigators have questioned even the whole pro-drug concept (Hasinoff and Herman, 2007; Lyu et al., 2007; Zhang et al., 2012). Thus, the mechanisms responsible for effective cardioprotection provided by DEX are poorly understood.

Despite its well-evidenced cardioprotective effects, DEX is currently recommended to be only used when a critical cumulative ANT dose of 300 mg/m^2 is achieved (Hensley et al., 2009). This recommendation was originally driven by outcomes of a single clinical trial suggesting significant impact of DEX on objective response rate of breast cancer to the ANT-based chemotherapy (Swain et al., 1997b). Although the interpretation of this finding is far from being straightforward (Swain and Vici, 2004) and an independent meta-analysis of all randomized clinical trials has failed to find any evidence for this hypothesis (van Dalen et al., 2011), the guidelines for clinical use of DEX remain still the same. Indeed, even the recommended "delayed" administration of DEX showed a benefit in a clinical trial (Swain et al., 1997a), however, there is a considerable lack of understanding what happens to the myocardium in this settings as compared to the "early" intervention, where DEX is administered before each ANT cycle. Furthermore, such investigation could shed more light on molecular events important for effective cardioprotection.

Therefore, the aim of the present study was to evaluate cardioprotective effects of DEX administered either before each daunorubicin (DAU) dose or with significant delay (from cumulative DAU dose of 300 mg/m^2) on a well-established rabbit model of chronic ANT cardiotoxicity. Furthermore, functional, morphological as well as molecular aspects of both cardioprotective interventions were investigated.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich (MO, USA) unless stated otherwise.

2.1. Study design and animals treatment

The study used well-established model of chronic ANT cardiotoxicity (Gersl et al., 1999; Popelova et al., 2009; Simunek et al., 2004). All animal handling and procedures were approved and supervised by Ethical Committee of the Faculty of Medicine in Hradec Králové. Chinchilla male rabbits (n = 77, ~3.5 kg, ~0.2 m^2 body surface area) were randomized to treatments as follows: cardiotoxicity was induced with daunorubicin (3 mg/kg/week, i.v., for 10 weeks; the DAU group, n = 27). DEX (60 mg/kg, i.p. 30 min before DAU) was administered either from the 1st DAU dose (early DEX intervention, DD_1 group, n = 16) or from the 7th one (delayed DEX intervention, DD_7 group, n = 15), i.e. after exceeding cumulative DAU dose of 300 mg/m^2 as calculated according to Ward (2008). Controls received saline (1 mL/kg/week, i.v., for 10 weeks, CTR group n = 19). A week after the last administration, animals in each group were randomized for sacrifice or for a 10-week post-treatment follow up (FU; DAU, n = 11; CTR, n = 10; DD_1, n = 8; DD_7, n = 8). Mortality was determined during the treatment period, whereas during the FU period animals were sacrificed whenever weekly echocardiography examination showed left ventricular fractional shortening (LV FS) to be lower than 20%, which indicates decompensated cardiac failure, to avoid loss of myocardial samples because of sudden deaths.

All non-invasive procedures and blood sampling were performed under light anesthesia mixture of ketamine (30 mg/kg) and midazolam (2.5 mg/kg), while pentobarbital (30 mg/kg) was used for invasive hemodynamic measurements and animal sacrifice.

After animal overdose, a gross autopsy was performed and the heart was rapidly excised and retrogradely perfused with ice-cold saline. Transversal sections of whole heart were cut for histological analysis, while the rest of the left ventricle (LV) was frozen in liquid nitrogen, pulverized under liquid nitrogen and stored at −80 °C.

2.2. Cardiac function examinations

Echocardiography was used to examine LV function during the study (Vivid 4 equipped with 10 MHz probe, GE Healthcare, UK). Left parasternal approach was employed to perform guided M-mode examinations in long and short axis view. LV end-systolic (LVESD) and LV end-diastolic (LVEDD) diameters were determined and LV fractional shortening (LV FS) was calculated as follows: LV FS (%) = ((LVEDD-LVESD)/LVEDD) × 100.

At the end of the study, invasive LV hemodynamic measurements were performed via *A. carotis sinistra* using Micro-Tip pressure catheter (2.3F, Millar Instruments, TX, USA) connected to a data acquisition system (Powerlab, ADInstruments Pty., Australia). The first derivative of the LV pressure change in the isovolumic phase of the systole and diastole (index dP/dt_{max} and dP/dt_{min}, respectively) were calculated using Chart 5.4.2 software (ADInstruments Pty.). All measurements were performed after the equilibration interval (15 min) to stabilize the animals after the preparation.

2.3. Plasma troponin T determination

Blood samples were obtained before the 1st, 5th, 7th, 10th drug administration, at the end of the study and in the 13th, 15th, 17th and 21st week in the FU period. Concentrations of cardiac troponin T in plasma were determined using Elecsys Troponin T hs assay kit (Roche Diagnostics, Switzerland) with a limit of detection of 0.003 μg/L.

2.4. Histopathological examinations

During autopsy, the blocks of heart tissue (each approx. 3 mm thick) were transversely cut off at the level under the atrioventricular septum, post-fixed by immersion in 4% neutral formaldehyde for 3 days and embedded in paraffin. Serial sections (6 μm thick), were stained with hematoxylin and eosin (H&E) and Masson's blue trichrome. Photomicrographs were made using the microscope Olympus BX 51 equipped with the digital camera DP 70 (Olympus, Japan) and Quick Photo Camera 2.3 software (Promicra, Czech Republic).