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Are cardioprotective effects of NO-releasing drug molsidomine translatable to chronic anthracycline cardiotoxicity settings?

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ABSTRACT

Chronic anthracycline (ANT) cardiotoxicity is a serious complication of cancer chemotherapy. Molsidomine, a NO-releasing drug, has been found cardioprotective in different models of I/R injury and recently in acute high-dose ANT cardiotoxicity. Hence, we examined whether its cardioprotective effects are translatable to chronic ANT cardiotoxicity settings without induction of nitrosative stress and interference with antiproliferative action of ANTs. The effects of molsidomine (0.025 and 0.5 mg/kg, *i.v.*) were studied on the well-established model of chronic ANT cardiotoxicity in rabbits (daunorubicin/DAU/ 3 mg/kg/week for 10 weeks). Molsidomine was unable to significantly attenuate mortality, development of heart failure and morphological damage induced by DAU. Molsidomine did not alter DAU-induced myocardial lipoperoxidation, MnSOD down-regulation, up-regulation of HO-1, IL-6, and molecular markers of cardiac remodeling. Although molsidomine increased 3-nitrotyrosine in the myocardium, this event had no impact on cardiotoxicity development. Using H9c2 myoblasts and isolated cardiomyocytes, it was found that SIN-1 (an active metabolite of molsidomine) induces significant protection against ANT toxicity, but only at high concentrations. In leukemic HL-60 cells, SIN-1 initially augmented ANT cytotoxicity (in low and clinically achievable concentrations), but it protected these cells against ANT in the high concentrations. UHPLC–MS/MS investigation demonstrated that the loss of ANT cytotoxicity after co-incubation of the cells *in vitro* with high concentrations of SIN-1 is caused by unexpected chemical depletion of DAU molecule. The present study demonstrates that cardioprotective effects of molsidomine are not translatable to clinically relevant chronic form of ANT cardiotoxicity. The augmentation of antineoplastic effects of ANT in low (nM) concentrations may deserve further study.

1. Introduction

Anthracyclines (ANTs, *e.g.*, doxorubicin or daunorubicin/DAU/) are effective anticancer drugs which are often used to treat different types of malignant neoplasms (Ewer and Ewer, 2015a). Cardiotoxicity, which is a class effect typical of all of ANT derivatives, ranks among the most serious drawbacks of their clinical use (Ewer and Ewer, 2015a; Menna et al., 2012). Two major forms of ANT cardiotoxicity are distinguished in clinical practice with respect to the onset, manifestation and clinical significance (Menna et al., 2007). Acute forms are mostly represented by subclinical changes in cardiovascular functions that occur soon after drug administration and they are rarely a significant clinical problem (Suter and Ewer, 2013; Harake et al., 2012). Most of the concerns are related to the chronic forms that are manifested

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weeks/months and even years after the chemotherapy (termed early and late-onset type, respectively) (Ewer and Ewer, 2015a; Suter and Ewer, 2013). Chronic ANT cardiotoxicity usually results into irreversible cardiomyopathy and heart failure. Many mechanisms responsible for development of chronic forms have been proposed (Salvatorelli et al., 2012; Sterba et al., 2013), but direct ANT-induced oxidative (and nitrosative (Fogli et al., 2004)) damage to the myocardium received the highest appreciation. However, recent data suggest that oxidative stress may be a downstream event resulting from interaction of ANTs with TOP2b in cardiomyocytes (Zhang et al., 2012; Vejpongsa and Yeh, 2014).

Many different agents including numerous antioxidants have been tested with an intention to protect the heart from ANT-induced damage (Sterba et al., 2013). While the majority of them has showed promising outcomes in acute high-dose cardiotoxicity models, they failed when were studied using clinically relevant models of chronic ANT cardiotoxicity (Sterba et al., 2013). Similarly, those few agents tested in clinical trials yielded negative outcomes (Sterba et al., 2013; van Dalen et al., 2011). To date, the only agent with well-established cardioprotective efficacy against chronic ANT cardiotoxicity both in animal models and clinical trials is dexrazoxane (Menna et al., 2012; Sterba et al., 2013; Zerra et al., 2013). It shows significant cardioprotective effects irrespectively of ANT derivative employed. Unfortunately, dexrazoxane is rarely used in current clinical medicine due to the concerns related its adverse effects (Vejpongsa and Yeh, 2014; Zerra et al., 2013). Majority of patients currently receive ANT-containing chemotherapy without cardioprotective intervention, although it is recognized that each ANT cycle may represent certain damage to the heart (Aapro et al., 2011; Ewer and Ewer, 2015b). Thus, an intensive search for safe and effective cardioprotectant continues.

Nitric oxide (NO)-mediated cardioprotection has been suggested as promising intervention against myocardial I/R injury (Jones and Bolli, 2006). Inorganic nitrites/nitrates, which act from a significant part *via* NO release in the tissue (Omar and Webb, 2014), have been shown promising in different models of myocardial I/R injury (Omar and Webb, 2014; Calvert and Lefer, 2009). They were also reported effective against acute high-dose ANT cardiotoxicity (Zhu et al., 2011). Using well-established model of chronic ANT cardiotoxicity, we have recently demonstrated that high-dose parenteral nitrite can positively modulate certain pathways leading to cardiotoxicity, but its overall efficacy was limited – especially when compared to outstanding outcomes of dexrazoxane (Lencova-Popelova et al., 2016). The latter may be related to poor bio-activation of nitrite to NO in cardiotoxicity settings. This reaction is promoted by hypoxia and acidosis accompanying I/R injury (Omar and Webb, 2014; Calvert and Lefer, 2009), but the heart repeatedly exposed to ANTs is lacking these conditions. Hence, it can be hypothesized that direct NO-donors could overcome this limitation and achieve a higher cardioprotective efficacy.

Among clinically used NO donors, molsidomine is unique in its mechanism of NO release, because it does not require enzymatic reduction of organic nitrate to NO in the targeted tissue. Molsidomine is rapidly metabolized by liver esterases to morpholinosydnonimine (SIN-1) which releases NO in the tissue through its spontaneous chemical degradation (Rosenkranz et al., 1996). Molsidomine/SIN-1 has been shown cardioprotective against I/R *in vivo* and H/R injury *in vitro* using various experimental models (Fiedler et al., 1985; Fung et al., 1994; Vértesi et al., 1991; Siegfried et al., 1992; Mehta et al., 2002; Siegmund et al., 1998; Schluter et al., 1996). Moreover, recent study has demonstrated that molsidomine may protect the heart against acute ANT cardiotoxicity (Disli et al., 2013). Unfortunately, nothing is known about translatability of these findings to chronic cardiotoxicity induced by repeated administration of individual therapeutic doses over several weeks or months. Furthermore, it remains to be determined whether molsidomine/SIN-1 could act as a double-edge sword in ANT cardiotoxicity settings because under some circumstances it may also produce peroxynitrite (Singh et al., 1999) which is a toxic radical implicated in the pathogenesis of chronic ANT cardiotoxicity (Fogli et al., 2004).

Hence, the aim of this study was to evaluate a cardioprotective potential of molsidomine against chronic ANT cardiotoxicity and impact of the drug on antiproliferative action of ANTs.

2. Materials and methods

2.1. Animals and experimental design

Adult male New Zealand rabbits (n = 40, 3.0–3.5 kg) were obtained from VELAZ (Prague, Czech Republic). Rabbits were kept under standard conditions with free access to standard pellet diet and drinking water. Chronic ANT cardiotoxicity was induced by DAU in a well-established schedule (Daunoblastina, Pfizer, Rome, Italy, 3 mg/kg *i.v.*, weekly for ten weeks, n = 10) (Popelova et al., 2009; Simunek et al., 2004). Daunorubicin was administered into the ear vein through flexible intravenous cannula (Neoflon 24GA, BD, Franklin Lakes, NJ, USA). Molsidomine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline and administered intravenously one hour before each DAU injection in two doses (0.025 and 0.5 mg/kg, n = 8 in each group, respectively). The lower dose corresponds with the typical individual dose (2 mg) used in adult patients with angina pectoris, while 20-fold higher dose releases sufficient amount of NO to affect hemodynamics as it was described in previous animal experiments (Fiedler et al., 1985). Another group of rabbits (n = 6) received molsidomine-alone (0.5 mg/kg *i.v.*) and the control group received saline (1 mL/kg *i.v.*, n = 8). The protocol was approved by the internal Animal Welfare Body of the Faculty of Medicine in Hradec Králové, Charles University.

All non-invasive procedures were performed under anesthesia containing ketamine (25 mg/kg *i.m.*, Narketan; Vétoquinol, Ittigen, Switzerland) and midazolam (1.25 mg/kg *i.m.*, Midazolam Torrex, Chiesi Pharmaceuticals, Vienna, Austria), while freshly prepared pentobarbital solution (4% [w/w], Sigma-Aldrich, St. Louis, MO, USA) was used for intravenous surgical anesthesia during final invasive hemodynamic measurement and for animal overdose at the end of the experiment.

After animal sacrifice the heart was excised, washed, and transverse sections of ventricles were taken for histological examinations. The rest of the LV wall was pulverized in liquid nitrogen and stored at −80 °C.

2.2. Determination of cardiac troponin T in plasma

Plasma concentrations of cardiac troponin T were determined in samples obtained at the end of the study before the surgical anesthesia using Elecsys Tropo T hs STAT (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's recommendations. A limit of detection was 0.003 μg/L. Data are expressed as last measured value.

2.3. Examination of left ventricular function

The LV systolic function was examined by catheterization of the left ventricular chamber performed using a Mikro-Tip pressure catheter (2.3F, Millar Instruments, Houston, TX, USA) and the Chart 5.4.2 software (ADInstruments, Bella Vista NSW, Australia) was used for data analysis and calculation of index of systolic function (dP/dt_{max}).