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Iron chelation-afforded cardioprotection against chronic anthracycline cardiotoxicity: A study of salicylaldehyde isonicotinoyl hydrazone (SIH)

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Abstract

Pyridoxal-derived aroylhydrazone iron chelators have been previously shown as effective cardioprotectants against chronic anthracycline cardiotoxicity. In this study we focused on a novel salicylaldehyde analogue (salicylaldehyde isonicotinoyl hydrazone, SIH), which has been recently demonstrated to possess marked and dose-dependent protective effects against oxidative injury of cardiomyocytes. Therefore, in the present study the cardioprotective potential of SIH against daunorubicin (DAU) cardiotoxicity was assessed *in vitro* (isolated rat ventricular cardiomyocytes; DAU 10 μM, 48 h exposure) as well as *in vivo* (chronic DAU-induced cardiomyopathy in rabbits; DAU 3 mg/kg, i.v. weekly, 10 weeks). *In vitro*, SIH (3–100 μM) was able to partially, but significantly decrease the LDH leakage from cardiomyocytes. *In vivo*, SIH co-administration was capable to reduce (SIH dose of 0.5 mg/kg, i.v.) or even to completely prevent (1.0 mg/kg, i.v.) the DAU-induced mortality. Moreover, the latter dose of the chelator significantly improved the left ventricular function (LV $dP/dt_{max} = 1185 \pm 80$ kPa/s versus 783 ± 53 kPa/s in the DAU group; $P < 0.05$) and decreased the severity of the myocardial morphological changes as well as the plasma levels of cardiac troponin T. Unfortunately, further escalation of the SIH dose (to 2.5 mg/kg) resulted in a nearly complete reversal of the protective effects as judged by the overall mortality, functional, morphological as well as biochemical examinations. Hence, this study points out that aroylhydrazone iron chelators can induce a significant cardioprotection against anthracycline cardiotoxicity; however, they share the curious dose–response relationship which is unrelated to the chemical structure or the route of the administration of the chelator.

Keywords: Cardioprotection; Iron chelator; Cardiotoxicity; Anthracyclines; Salicylaldehyde isonicotinoyl hydrazone; SIH

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1. Introduction

Cardiotoxicity ranks among the most serious and hardly manageable toxic effects of anticancer chemotherapy. It is particularly dangerous due to the significant vulnerability of the cardiomyocytes to the oxidative damage, a very limited regenerative potential of the myocardial tissue, and the cardinal physiological importance of the heart. Besides some novel anticancer drugs, which have been only recently recognized as potentially cardiotoxic (e.g., imatinib, trastuzumab) (Ewer et al., 2005; Kerkela et al., 2006), anthracyclines (e.g., doxorubicin and daunorubicin) have remained for more than 40 years the main threat regarding the cardiac safety of the patients undergoing cancer treatment (Minotti et al., 2004; Jones et al., 2006).

From the clinical point of view, it is the chronic type of anthracycline cardiotoxicity which represents the major problem. Its risk is known to be closely related to the cumulative dose of anthracyclines (Von Hoff et al., 1979). The anthracycline-induced myocardial injury is typically characterized by a progressive myofibrillar loss and degeneration of the left ventricular (LV) cardiomyocytes ultimately resulting in dilated cardiomyopathy and congestive heart failure (Herman et al., 1997). In addition, the cardiac injury may manifest itself even after a considerable delay (4–20 years) and therefore it can compromise the cardiac function of survivors of childhood cancers even during their productive adulthood (Lipshultz et al., 1991).

Despite extensive scientific efforts, the molecular basis of anthracycline cardiotoxicity remains enigmatic, which significantly hampers the rational development of both less cardiotoxic anthracycline derivates as well as effective pharmacological cardioprotectants. Albeit many theories of anthracycline cardiotoxicity has been proposed (Minotti et al., 2004), the most widely accepted is the "ROS and iron hypothesis" (Keizer et al., 1990). The anthracyclines are known to generate ROS by a redox-cycling of the anthracycline aglycon as well as through their complexes with iron (Keizer et al., 1990; Minotti et al., 2004). The quinone part of the anthracycline aglycon can be converted into a semiquinone form by the one electron acquisition. In the further step of cyclic reaction, the superoxide anion radicals are formed by the interaction of semiquinone with molecular oxygen. Superoxide can further dismutate, either spontaneously or enzymatically (by superoxide dismutase) forming the hydrogen peroxide which is in turn converted in to the extremely reactive hydroxyl radicals through iron-catalyzed Haber–Weiss reaction. Furthermore, iron is involved in the anthracycline-induced ROS generation as part of Fe^{3+}-anthracycline complex. In the presence of the reducing systems (e.g., NADH cytochrom P-450 reductase or glutathione), Fe^{3+}-anthracycline complexes can be reduced and the products (Fe^{2+}-anthracycline complexes) are promptly oxidized back via the interaction with molecular oxygen yielding again superoxide radicals. In addition, hydrogen peroxide can also participate in the latter reaction which results into the direct formation of hydroxyl radicals (Keizer et al., 1990). Alternatively, an internal electron shift within the iron–anthracycline complex may give reduction of iron, which subsequently delivers this electron to molecular oxygen, yielding further superoxide anions. In the excess of NO, the superoxide radicals can also react with NO and thus nitrogen reactive species (e.g., peroxynitrite) are formed (Weinstein et al., 2000; Fogli et al., 2004; Pacher et al., 2007).

The only cardioprotective drug with well-evidenced efficacy in both experimental and clinical settings is dexrazoxane – ICRF-187 (Cvetkovic and Scott, 2005). Today it is generally believed that dexrazoxane acts as a pro-drug which is bioactivated to the metal chelating compound ADR-925. The latter agent has been shown to chelate free or loosely bound iron within the cardiac cells and/or displace iron from its complexes with anthracyclines and thus it prevents the formation of reactive oxygen species, particularly the extremely toxic hydroxyl radicals (Hasinoff et al., 1998; Kwok and Richardson, 2000). Unfortunately, the clinical effectiveness of dexrazoxane is partially counterbalanced by its myelotoxicity which may aggravate the myelosuppression induced by anthracyclines and by its higher costs (Cvetkovic and Scott, 2005). Furthermore, there was a suspicion of decreased tumor response in dexrazoxane co-treated patients in one arm of a single clinical study (Swain et al., 1997), although other studies did not confirm this (Swain and Vici, 2004). All the arguments given above may be responsible for the recent estimation that in Europe cardioprotection with dexrazoxane is employed only in 6–7% of anthracycline-treated patients (Swain and Vici, 2004).

Although iron chelation seems to be a plausible mechanism of cardioprotective intervention against anthracycline cardiotoxicity, the potential of this approach was not appropriately appraised yet. So far, only several iron chelating agents have been examined (Link et al., 1996; Saad et al., 2001; Barnabe et al., 2002; Hasinoff et al., 2003; Xu et al., 2006) and, importantly, only very few *in vivo* studies on the relevant chronic mod-