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Full Length Article

Investigation of novel dexrazoxane analogue JR-311 shows significant cardioprotective effects through topoisomerase IIbeta but not its iron chelating metabolite

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ABSTRACT

Novel dexrazoxane derivative JR-311 was prepared to investigate structure-activity relationships and mechanism(s) of protection against anthracycline cardiotoxicity. Its cardioprotective, antiproliferative, iron (Fe) chelation and inhibitory and/or depletory activities on topoisomerase IIbeta (TOP2B) were examined and compared with dexrazoxane. While in standard assay, JR-311 failed in both cardioprotection and depletion of TOP2B, its repeated administration to cell culture media led to depletion of TOP2B and significant protection of isolated rat neonatal ventricular cardiomyocytes from daunorubicin-induced damage. This effect was explained by a focused analytical investigation that revealed rapid JR-311 decomposition, resulting in negligible intracellular concentrations of the parent compound but high exposure of cells to the decomposition products, including Fe-chelating JR-H2. Although chemical instability is an obstacle for the development of JR-311, this study identified a novel dexrazoxane analogue with preserved pharmacodynamic properties, contributed to the investigation of structure-activity relationships and suggested that the cardioprotection of bis-dioxopiperazines is likely attributed to TOP2B activity of the parent compound rather than Fe chelation of their hydrolytic metabolites/degradation products. Moreover, this study highlights the importance of early stability testing during future development of novel dexrazoxane analogues.

1. Introduction

Anthracyclines (ANTs), such as doxorubicin or daunorubicin (DAU), belong to the most effective anticancer drugs in current clinical practice. However, the risk of cardiotoxicity may significantly affect the morbidity, mortality and quality of life of cancer survivors. This cardiotoxicity can develop months or years after the completion of treatment and manifests as dilated cardiomyopathy and congestive heart failure (Sterba et al., 2013). A number of hypotheses have been proposed to explain the mechanism(s) of ANT-induced cardiotoxicity. While the traditional theory highlights the role of Fe-mediated production of reactive oxygen species (ROS), recent data imply the key role of an interaction of the drug with topoisomerase IIβ (TOP2B) in cardiomyocytes (Deweese and Osheroff, 2009).

Dexrazoxane (DEX, ICRF-187), a bisdioxopiperazine TOP2 inhibitor, is to date the only approved drug capable of preventing ANT-induced cardiotoxicity both in experimental models and clinical practice (Shaikh et al., 2016). Only several closely related compounds have been reported to alleviate ANT-related cardiotoxicity (Sterba et al., 2013), but most structural modifications of DEX have led to a loss of the cardioprotection and/or increase of toxicity (Jirkovska-Vavrova et al., 2015).

Despite the long history of use of DEX in clinical practice, the mechanism of its cardioprotective effects still remain unclear. The prevailing hypothesis attributes the cardioprotection to a DEX metabolite ADR-925 that is formed *via* enzymatic hydrolysis of the piperazine rings (Fig. 1A).

This analogue of metal chelator EDTA is hypothesized to bind intracellular Fe (Hasinoff et al., 2003b) and thus it could prevent ROS formation induced by either ANT-Fe complexes or the Fenton reaction (Simunek et al., 2009). However, several recent studies have challenged this theory, arguing that 1) similar to EDTA, ADR-925 complexes with

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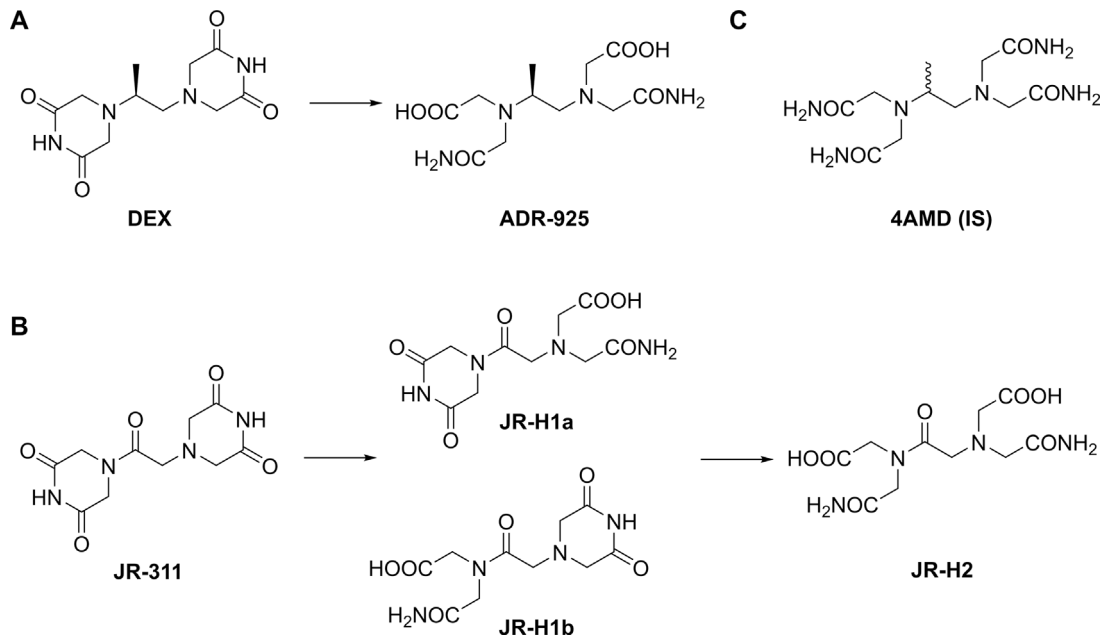

Fig. 1. Chemical structures of dexrazoxane (DEX) and its metabolite/degradation product ADR-925 (**A**), proposed degradation pathway of JR-311 to the metabolites/degradation products JR-H1a, JR-H1b and JR-H2 (**B**) and structure of internal standard 4AMD used for LC–MS/MS assay (**C**).

Fe are still redox active (Malisza and Hasinoff 1995; Potuckova et al., 2014) and therefore cannot effectively prevent ROS damage, 2) antioxidants and ROS scavengers (such as tocopherol or *N*-acetylcysteine) have failed to prevent the ANT-induced cardiotoxicity in relevant experimental models and in clinical trials (Sterba et al., 2013), 3) stronger and more selective Fe chelators were unable to provide a comparable degree of cardioprotection against ANT-toxicity as DEX (Hasinoff et al., 2003a; Sterba et al., 2013). Hence, it seems that the mechanisms of DEX-induced cardioprotection are more complex and go beyond simple Fe chelation. This idea is further supported by a pivotal study demonstrating that TOP2B is a primary target for ANT in cardiomyocytes and that oxidative stress is rather a downstream consequence of this interaction (Zhang et al., 2012). It is noteworthy that DEX was originally developed as a powerful inhibitor of TOP2 and as an anticancer drug (Hasinoff et al., 1995). Furthermore, it has also been shown to induce TOP2B depletion in proliferating H9c2 myoblasts (Lyu et al., 2007) and we have confirmed that the same occurs in neonatal cardiomyocytes and rabbit hearts under the conditions where it is cardioprotective against ANTs (Lencova-Popelova et al., 2016). Thus, we and others (Deng et al., 2014) have hypothesized that TOP2B may be responsible for the cardioprotective effects of DEX. Indeed, full understanding of the underlying mechanism(s) of the effects of DEX requires further research, which is a prerequisite for the rational development of more effective cardioprotective agents.

In the present study, we prepared a novel structural analogue of DEX, namely, 4,4′-(1-oxoethane-1,2-diyl)bis(piperazine-2,6-dione) (JR-311; Fig. 1B), which can be (by analogy to DEX) bioactivated by hydrolytic cleavage of the piperazine rings (Fig. 1B). Furthermore, this compound was picked in our *in vitro* screening to inhibit catalytic activity of TOP2 (Fig. S1). Hence, JR-311 was a perfect candidate for investigation of its cardioprotective effects to advance our understanding of the structure-activity relationship of DEX and its derivatives. Furthermore, novel LC–MS/MS assay was developed and utilized to investigate the fate of JR-311 in a cell culture medium and cardiac cells to provide explanations for some unexpected pharmacodynamic observations. Finally, the experimental schedule of the cardioprotective assay was modified to confirm the hypothesis.

2. Material and methods

2.1. Materials and chemicals

Dulbecco's Modified Eagle's Medium (DMEM), DMEM with Ham's F-12 nutrient mixture (DMEM/F12), horse serum (HS), fetal bovine serum (FBS), penicillin/streptomycin solution (5000 U/mL; P/S) and sodium pyruvate solution (100 mM; PYR) were purchased from Lonza (Basel, Switzerland). The sera were heat-inactivated prior to use. DEX was obtained from Huaren Chemicals (Changzhou, Jiangsu, China). DAU was purchased as Daunoblastina inj. from Pfizer (Rome, Italy). Salicylaldehyde isonicotinoyl hydrazone (SIH) was prepared and characterized as described previously (Edward et al., 1988). RPMI-1640 medium with L-glutamine and $NaHCO_3$, ferric ammonium citrate, lactic acid, nicotinamide adenine dinucleotide (NAD^+), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); methanol, acetonitrile, ammonium formate, formic acid (all MS grade); EDTA potassium salt, DMSO as well as other chemicals (*e.g.*, the constituents of various buffers) were purchased from Sigma-Aldrich (St. Louis, Montana, U.S.A.) or Penta (Prague, Czech Republic) and were of the highest available pharmaceutical or analytical grade. Milli-Q water was prepared using Millipore purification system (Merck–Millipore, Darmstadt, Germany).

JR-311 was synthesized and characterized as described previously (Roh et al., 2016). The degradation products JR-H1–mixture of *N*-(carbamoylmethyl)-*N*-(2-(3,5-dioxopiperazin-1-yl)-2-oxoethyl)glycine (JR-H1a) and *N*-(carbamoylmethyl)-*N*-(2-(3,5-dioxopiperazin-1-yl) acetyl)glycine (JR-H1b), JR-H2–*N,N*′-bis(carbamoylmethyl)glycinamide-*N,N*′-diacetic acid and internal standard (1,2-diaminopropane-*N,N,N*′,*N*′-tetraacetamide − 4AMD, IS) were prepared as described in the Supplementary material section.

2.2. Cellular proliferation assessments

The effects on leukemia cell proliferation of various agents and their combinations were examined using the HL-60 human acute promyelocytic leukemia cell line (Gallagher et al., 1979). The HL-60 cells were purchased from the American Type Culture Collection (Manassas, Virginia, U.S.A.) and cultured in RPMI-1640 supplemented with 10% FBS and 1% P/S in 75 cm^2 tissue culture flasks at 37 °C in a humidified