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Doxorubicin: Comparison between 3-h continuous and bolus intravenous administration paradigms on cardio-renal axis, mitochondrial sphingolipids and pathology

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ABSTRACT

Doxorubicin (DOX) is a potent and effective broad-spectrum anthracycline antitumor agent, but its clinical usefulness is restricted by cardiotoxicity. This study compared pharmacokinetic, functional, structural and biochemical effects of single dose DOX bolus or 3-h continuous iv infusion (3-h iv) in the Han–Wistar rat to characterize possible treatment-related differences in drug safety over a 72 h observation period.
Both DOX dosing paradigms significantly altered blood pressure, core body temperature and QA interval (indirect measure of cardiac contractility); however, there was no recovery observed in the bolus iv treatment group. Following the 3-h iv treatment, blood pressures and QA interval normalized by 36 h then rose above baseline levels over 72 h. Both treatments induced biphasic changes in heart rate with initial increases followed by sustained decreases. Cardiac injury biomarkers in plasma were elevated only in the bolus iv treatment group. Tissue cardiac injury biomarkers, cardiac mitochondrial complexes I, III and V and cardiac mitochondrial sphingolipids were decreased only in the bolus iv treatment group. Results indicate that each DOX dosing paradigm deregulates sinus rhythm. However, slowing the rate of infusion allows for functional compensation of blood pressure and may decrease the likelihood of cardiac myocyte necrosis via a mechanism associated with reduced mitochondrial damage.

1. Introduction

Doxorubicin (DOX) is a potent and effective anthracycline antibiotic used for the treatment of multiple cancers, including soft tissue sarcomas and myelomas (Mishra et al., 2013; Walczak and Irwin, 2013). Doxorubicin exerts its therapeutic effects by stabilizing topoisomerase II enzyme (Momparler et al., 1976; Pigram et al., 1972). However, the therapeutic potential of DOX is limited by its action to induce life-threatening cardiotoxicity (Green et al., 2001; Lefrak et al., 1973). Approximately 10% of patients treated with DOX or its derivatives will develop cardio-complications up to 10 years after the cessation of chemotherapy (Octavia et al., 2012). Cardiomyopathy caused by DOX may develop in an acute or chronic clinical setting. In the acute situation, reversible, abnormal electrocardiographic changes can occur with ST-T wave alterations and arrhythmias (Octavia et al., 2012). In the chronic setting, cardiomyopathy is characterized by cumulative dose-related toxicity, which manifests as congestive heart failure, with a 50% mortality rate ("Doxorubicin", 2015).

The mechanism of DOX-mediated toxicity is not completely understood. However, the cationic form of DOX generates reactive oxygen species (ROS), which are associated with mitochondrial damage in cardiac myocytes in vitro (Berthiaume and Wallace, 2007), and in vivo in mice (Rosenoff et al., 1975), rats (Gandhi et al., 2013), dogs (Henderson et al., 1982) and man (Rinehart et al., 1974). Reactive oxygen species damage nucleic acids causing single and double strand

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breaks. They also increase cell membrane permeability and inactivate membrane receptors and other enzymes, possibly by inducing lipid peroxidation (Keizer et al., 1990). Structural phospholipids and sphingolipids located on the inner membrane of the mitochondria are likely targets for doxorubicin-induced ROS. Little is known about the role of membrane lipids in cardiotoxicity (Marcillat et al., 1989). Sphingolipids and their metabolites are major components of biological membranes; they also serve as key cellular signaling molecules. Their functional diversity is closely related to their structural diversity in which sphingolipids encompass a large group of lipid classes, including sphingosine (So), ceramide (Cer), sphingomyelin (SM), and glycosylated derivatives (Fig. 1). Mass spectrometry (MS)-based lipidomics is well-suited for detailed lipid structural and content analysis to further elucidate the role of lipids in biology and disease.

Normally, DOX is dosed iv in the clinic as a single, rapid intravenous infusion, which is repeated every 21 days ("Doxorubicin", 2015). To limit cardiac toxicity, different dosing formulations, dosing rates and methods of administering doxorubicin have been developed (Anderson and Lokich, 1994). However, there is limited evidence regarding the comparative success of the various dosing regimens. For example, although continuous infusion of DOX has been reported by some to reduce cardiotoxicity (Brown et al., 2013), others indicate no clinical benefit (Lipshultz et al., 2012). Detailed characterization of DOX effects in animal models of bolus versus continuous infusions are not available, but such comparisons may provide support for clinical application. This study compared functional, structural and biochemical differences between a 5-min bolus iv versus 3-h continuous (i.e., 3-h) iv DOX administration. Pharmacokinetic properties of DOX were evaluated in tandem with multiple, clinically-relevant parameters. To assess cardiac functional integrity, electrocardiography (ECG), blood pressure (BP), heart rate (HR), contractility (QA interval; an indirect measure of cardiac contractility), and core body temperature (CBT) were measured. Cardiotoxicity was assessed using plasma and tissue-based biomarkers of cardiac injury, histopathological examination and semi-quantification of changes in mitochondrial complexes. In addition, metabolomic analyses of mitochondrial lipid profiles were used to explore the impact of DOX treatment on cardiac mitochondrial sphingolipids. Because DOX is known to adversely affect renal structure (Herman et al., 1988), urinary biomarkers of kidney injury, as well as functional measures of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were evaluated. Summary data of salient treatment-related changes in hematology, clinical chemistry, renal functional measures, urine chemistry and acute kidney injury biomarkers are reported separately (see Tables S1, S2, S3 and S4, respectively, in Kamendi, et al. 2015, in press).

2. Materials and methods

All animal procedures were performed with the approval of the internal AstraZeneca Institutional Animal Care and Use Committee (IACUC), in accordance with recommendations of the panel on euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication: "The Guide for the Care and Use of Laboratory Animals" (ILAR, 1996). Animals identified as moribund or distressed prior to study start were not included in the study.

2.1. Surgical implantation of radio telemetry and catheters and study design

Han Wistar rats were surgically implanted with PhysioTel® multiplus radio transmitters (model TL11M2-C50-PXT) by Charles River laboratories (Raleigh, NC) for measurement of BP, CBT and electrocardiogram (ECG), as per recommendation of the manufacturer and with a partially-modified protocol for ECG biopotential placement (Sgoifo et al., 1996). The ability to record parameters was confirmed using Dataquest A.R.T.™ Version 4.2 (DSI, St. Paul, MN) before completion of surgery by the vendor. Following two weeks of recovery, the rats were outfitted with in-dwelling jugular and femoral vein catheters (Chen et al., 2013) under isoflurane anesthesia for continuous infusion of test articles and blood sampling, respectively. Cannula patency was maintained with 1:1 heparin–glycerol when rats were not on study. The animals were shipped to AstraZeneca (Waltham, MA) four days later. Eighteen rats arrived at AstraZeneca seven days before the study and were individually housed for the duration of study. Animal identification and conditions involving housing, acclimatization, environment, diet and water were in accordance with facility standard operating procedures. On study day −2, rats were moved from home cages and placed into the Automated Blood Sampling and Telemetry System (ABST), an integrated pharmacology testing platform (Chen et al., 2013; Kamendi et al., 2010; Litwin et al., 2011) for the duration of study.

Using simple randomization, animals were assigned to one of the three study groups: vehicle, bolus iv DOX or 3-h continuous iv DOX with 6 animals per group. This group size provides adequate power (80%) to assess hemodynamic changes (Kamendi et al., 2010).

On day 1 at t = 0, rats (n = 6/group) were dosed with either vehicle (saline), DOX (Sigma, St. Louis, MO) 30 mg/kg bolus iv or DOX 30 mg/kg 3-h iv, as described in Table 1. Automated blood sampling of 100 μl whole blood occurred at 0, 0.08, 0.17, 0.3, 0.5, 1, 2, 3, 4, 8, 16, 24, 48 and 72 h for the vehicle and bolus iv groups and at 0, 1, 2, 3, 4, 8, 16, 24, 48 and 72 h for the 3-h iv group. For each sample, the blood volume removed was replaced immediately with an equal volume of isotonic saline. Total daily blood volume sampled from each animal was within

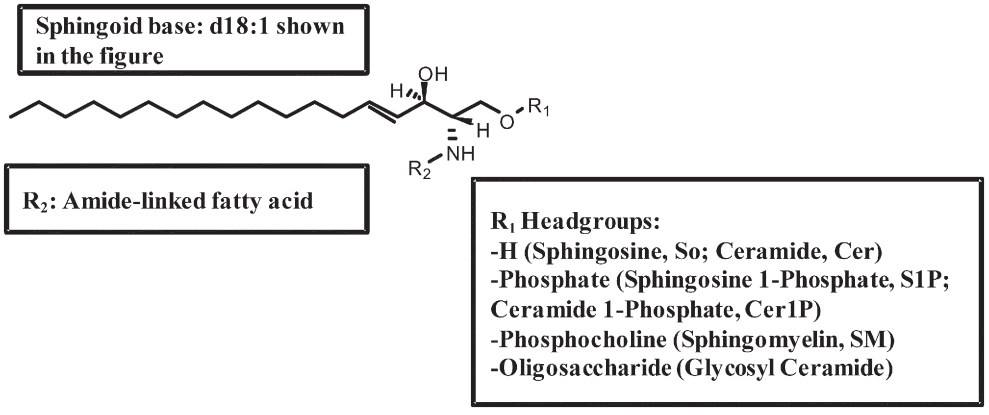

Fig. 1. General sphingolipid structure. All sphingolipids contain a sphingoid, long-chain base (e.g. sphingosine), which is linked to a fatty acid molecule through an amide bond, thereby forming the ceramide unit. The headgroup on the primary hydroxyl group varies from being as simple as a hydrogen to complex structures, such as oligosaccharides.