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Slow-Release Doxorubicin Pellets Generate Myocardial Cardiotoxic Changes in Mice Without Significant Systemic Toxicity

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Abstract

An increasing volume of pre-clinical and clinical-translational research is attempting to identify novel biomarkers for improved diagnosis and risk-stratification of chemotherapy-induced cardiotoxicity. Most published animal models have employed weekly intraperitoneal injections of doxorubicin to reach a desired cumulative dose. This approach can be associated with severe systemic toxicity which limits the animal model usefulness, particularly for advanced imaging. In the current study, slow-release subcutaneous doxorubicin pellets demonstrated histopathologic evidence of cardiotoxicity at doses similar to standard human dose-equivalents without limiting animal survival or ability to participate in advanced imaging studies. This approach may provide a more robust cardiotoxicity animal model.

Keywords Cardio-oncology · Animal model · Cardiotoxicity · Chemotherapy

Abbreviations

CMR	Cardiac magnetic resonance imaging
PET	Positron emission tomography
IP	Intraperitoneal
TUNEL	Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling

Introduction

Cancer treatment with anthracyclines is associated with an incidence of overt cardiotoxicity of 6% and subclinical cardiotoxicity in as much as 18% of patients. [1] With doxorubicin, doses of 400–450 mg/m^2 (~ 8–12 mg/kg) have been identified as high risk for the development of chronic cardiotoxicity, and prescribed doses are generally lower than these levels [2]. Non-invasive imaging with echocardiography, cardiac MRI (CMR), and/or positron emission tomography (PET) have been recognized as potentially useful screening tools to identify myocardial damage prior to deterioration of cardiac function [3]. If myocardial damage is identified at an early stage, treatment regimens could be modified to mitigate the potential for chronic cardiotoxicity. In mice, peak doses of approximately 25 mg/kg of doxorubicin have been shown to result in chronic cardiotoxicity [4]. Most published models have employed intraperitoneal (IP) injections of approximately 5–8 mg/kg spread over 4–5 weeks to reach desired cumulative dose, however these models have generally not been used in advanced imaging studies which require frequent intravenous contrast injections and multiple rounds of prolonged anesthesia. Our goal in this study was to develop a mouse model of doxorubicin cardiotoxicity for the purpose of advanced, multimodality cardiac imaging with CMR and PET.

Methods and Results

Initially, we sought to replicate the standard IP approach. Note that all animal studies were approved by the local Institutional Animal Care and Use Committee (IACUC) and conform to National Institutes of Health guidelines on the protection of animals used for scientific purposes. With IP injections, we found mice consistently died before reaching peak dose or became too ill to tolerate imaging, likely from systemic toxicity. In our initial experiment, a total of 11, 10–12 week old C57/BL6 female mice were divided into treatment ($n = 8$) or control ($n = 3$) groups. The treatment group was dosed at 8 mg/kg in 100 µL saline IP of

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doxorubicin over 3 weeks for a cumulative dose of 24 mg/kg. The control group was given 100 µL IP saline injections. All mice were scheduled to undergo MRI at baseline, and at weeks 2 and 4 following treatment initiation. In the treatment group, only three mice were able to complete the imaging protocol (three deaths and two mice too ill to undergo final imaging). The surviving treatment group lost 1.8 ± 2.0 g (10% body weight) and the non-surviving treatment mice lost 4.3 ± 2.1 g (25% body weight), while the control group gained 2.3 ± 1.5 g.

We therefore sought to develop a more robust cardiotoxicity mouse model utilizing a subcutaneous slow release doxorubicin pellet with a 5 mg/kg total cumulative dose which is released over 21 days (~ 0.25 mg/kg/day) (Innovative Research of America, Sarasota, FL). In $n = 7$ 8-week-old C57/BL6 male mice, pellets were implanted in the subcutaneous nape/shoulder each week for five weeks resulting in a total cumulative does of 25 mg/kg. The 1/8 inch length pellets contain a matrix consisting of cholesterol, cellulose, lactose, phosphates, and stearates fused with doxorubicin. The doxorubicin is released through a process of matrix erosion and doxorubicin diffusion which occurs at a predictable rate. Doxorubicin release begins immediately at implantation. Mice were anesthetized with inhaled 0.5–2% isoflurane mixed with oxygen during implantation. Following surgical skin prep of the site, an ~ 1 mm incision and pocket were made with micro hemostat. The pellet was deposited and skin closure was performed with surgical wound clips. One mouse was euthanized each week beginning on day 21 after treatment initiation (cumulative treatment dose 5.3 mg/kg) for histologic and electron micrograph assessment. The final mouse was euthanized on day 70 after treatment initiation (14 days after reaching total cumulative dose of 25 mg/kg). For euthanasia, mice were anesthetized using inhalation of 0.5–2.0% isoflurane mixed with oxygen, and a ketamine/xylazine mixture (50–100 mg/kg (K), 5–10 mg/kg (X) was injected IP for cardiac harvest. In this experiment, no mice died prematurely, and there was no evidence of distress documented during in-cage observation. Mice gained on average 2.3 ± 1.3 g, and no mouse lost weight prior to sacrifice. On electron microscopy, myocardial damage consistent with cardiotoxicity including vacuolization, mitochondrial enlargement, and thickened cristae were present at a cumulative dose of 15 mg/kg (day 28 after treatment initiation) (Fig. 1). Advanced changes including complete sarcomere disarray, fibrosis, and progressive mitochondrial enlargement were seen at doses of 25 mg/kg (day 49 after treatment initiation), with one of three mice who reached this dose exhibiting overt apoptosis on terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay.

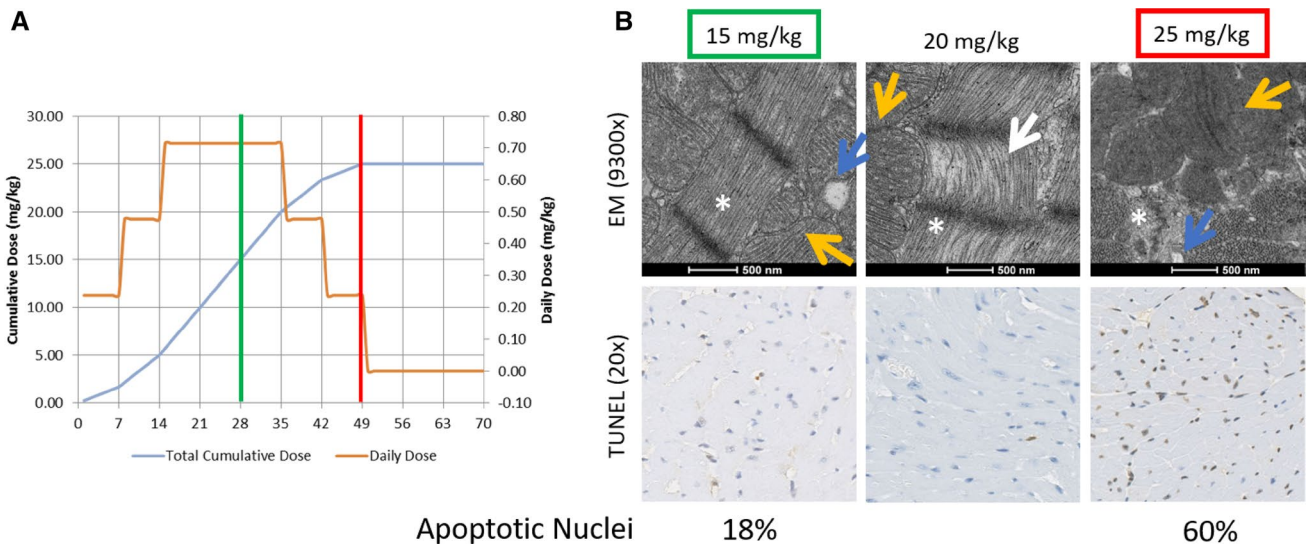

Fig. 1 Dosing profile of 5 mg/kg subcutaneous doxorubicin pellets when delivered as one pellet per week for 5 weeks. The maximum cumulative dosage is 25 mg/kg in seen in panel A. Panel B demonstrates representative images of terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay histopathologic slides (20x) and electron micrographs (grayscale, 9300x). TUNEL assay indicates apoptosis when nuclei are stained brown, which is seen prominently at the 25 mg/kg dose. Apoptotic nuclei percentages were calculated using the average number of apoptotic nuclei divided the average number of total nuclei multiplied by 100 when counting ten 40× regions on TUNEL slides. Early changes associated with cardiotoxicity are seen in the 15 mg/kg (day 28, green line in A) specimen with enlarged mitochondria, thickened cristae (orange arrow), and vacuolization (blue arrow). The sarcomeres (white *) are preserved. These changes progress with increasing doses characterized by myofilament disruption at 20 mg/kg (day 35) (white arrow), and complete disarray at 25 mg/kg (day 49, red line in A)

Discussion

Our findings suggest that doxorubicin cardiotoxicity can be induced in mice with subcutaneous, slow release pellets at doses as low as 15 mg/kg, which is lower than commonly reported in mice and is more in line with typical human dose ranges. While echocardiographic studies are relatively short and minimally invasive, CMR studies in mice utilizing advanced imaging protocols can require mice to be under anesthesia for nearly 2 h and tolerate gadolinium contrast injection. Dynamic PET studies which may hold promise for identifying early changes in cardiac myocyte energy utilizing requires mice to be anesthetized for > 1 h. Systemic toxicity from chemotherapy resulting in severe illness makes the performance of these advanced imaging techniques tenuous, especially when performed over short intervals.

In general, we found the pellet delivery method to be straightforward and no additional technologist training was required. This technique is a more reliable delivery approach compared to our experience with intravenous drug delivery and is at least as straightforward as IP injections which can damage bowel or visceral organs if not performed correctly. We chose to implant pellets using a small incision, but trochar delivery devices are also available and may further ease the delivery process. Finally, we noted no local skin damage or difficulties with wound healing at the site of insertion, while IP injection can lead to peritonitis or other local inflammatory responses which may adversely impact animal survival.

This work is subject to several limitations. First, we relied on vendor dose release rates and kinetics to estimate the daily and weekly total accumulation of doxycycline and did not conduct a systematic dose response and pharmacokinetic evaluation. Without quantitative knowledge of actual blood and end organ clearance information, no rigorous pharmacological comparison can be drawn between subcutaneous doxorubicin pellets and standard IP injection. Also, additional dose and timeline strategies were not explored in this study. Finally, only male mice were used for this systematic assessment of the subcutaneous pellets. Males were chosen in attempt to mitigate hormonal effects on cardiac function that would be difficult to control for in this small group [5]. Future work will evaluate how mouse gender and modulating hormonal levels impact histopathologic and imaging findings related to cardiotoxicity.

In conclusion, the mouse model we have described may reduce systemic toxicity of doxorubicin to better allow assessment of advanced imaging approaches for cardiotoxicity diagnosis.

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Compliance with Ethical Standards

Conflicts of interest JCC has a research relationship with Siemens, and reports travel/accommodations expenses reimbursed by Siemens. All additional authors have no disclosures.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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