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Short-term and long-term models of doxorubicin-induced cardiomyopathy in rats: A comparison of functional and histopathological changes

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ABSTRACT

Objectives: Doxorubicin (DXR), an anthracyclic antineoplastic agent, is one of the most commonly drug utilized to induce dilated cardiomyopathy (DCM) and heart failure (HF), but the well optimized protocol for cardiomyopathy induction leading to development of cardiac systolic dysfunction is unclear. This study aims to critically compare short-term and long-term DXR injection protocols for the induction of DCM in rats.

Methods: Animals were allocated into 3 experimental groups: a ST (short-term DXR injection) group, in which animals received 6 intraperitoneal (i.p.) injections of DXR (2.5 mg/kg per dose) over a period of 2 weeks (cumulative dose of 15 mg/kg); a LT (long-term DXR injection) group in which animals received weekly i.p. injections of DXR (2 mg/kg per dose) over a period of 9 weeks (cumulative dose of 18 mg/kg); and a control group in which animals received an appropriate volume of 0.9% saline i.p. All animals were submitted to echocardiography analysis at baseline and after completion treatment. Afterwards, the hearts were collected for conventional light microscopy and collagen quantification.

Results: Morphological myocardial analysis of both DXR-treated groups showed an identical pattern of swollen and vacuolated cardiomyocytes and disorganization of myofibrils. There was pronounced interstitial fibrosis in both groups of DXR-treated hearts as compared to controls, as assessed by the interstitial collagen volume fraction. There was no difference in interstitial fibrosis between the ST and LT groups. The echocardiography analysis of the LT group showed structural and functional findings compatible with DCM, including increased left ventricular systolic (5.02 ± 0.96 mm) and diastolic (7.68 ± 0.96 mm) dimensions and reduction of ejection fraction ($69.40 \pm 8.51\%$) as compared to the ST group (4.10 ± 0.89 mm, 7.32 ± 0.84, and $79.68 \pm 7.23\%$, respectively) and control group (4.07 ± 0.72 mm, 7.17 ± 0.68 mm and $80.08 \pm 4.71\%$, respectively), ANOVA $p < 0.01$.

Conclusions: These results indicate that LT injection of DXR is more effective than ST injection in inducing left ventricular dysfunction and structural cardiac changes resembling those found in dilated cardiomyopathy.

Abbreviations: DCM, dilated cardiomyopathy; DXR, doxorubicin; HF, heart failure, ST group short-term doxorubicin injection; LT group, long-term doxorubicin injection; LV, left ventricle, LVEF left ventricle ejection function; LVDd, LV end-diastolic dimension; LVDs, end-systolic dimension.

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1. Introduction

Animal models of cardiovascular disease have proved critically important for the investigation of pathophysiological mechanisms and for the testing of new therapies (Cohn et al., 1997; Schwarz et al., 1998). Based on the high prevalence and clinical importance of cardiovascular disease, investigators have developed numerous small animal models over the past fifty years to study the very complex syndrome of chronic heart failure (Cohn et al., 1997; Monnet and Chachques, 2005; Smith and Nuttall, 1985). Ideally, chronic animal models of heart failure should mimic the pathophysiology seen in humans, including not only myocardial histopathologic changes but also cardiac remodeling with impaired left ventricular systolic function. Several methods have been used to create this experimental condition including volume and pressure overload, myocardial infarction, rapid pacing, and drug-induced cardiomyopathies (Monnet and Chachques, 2005). Doxorubicin (DXR), an anthracyclic antineoplastic agent widely used to treat a number of malignancies, is one of the most commonly utilized experimental models to induce dilated cardiomyopathy (DCM) and heart failure (HF) (Monnet and Chachques, 2005; Smith and Nuttall, 1985).

The reference model of chronic anthracycline-induced DCM, initially described in rabbits, was comprised of a long-term injection of daunorubicin over a period of 8–12 weeks (Jaenke, 1974; Maral et al., 1967). This long-term injection model has also been utilized in rats and was found to be successful in inducing not only morphological changes, but also cardiac remodeling and left ventricular systolic dysfunction (Mettler et al., 1977; Schwarz et al., 1998; Wakasugi et al., 1993a, 1993b).

An alternative model of inducing cardiotoxicity consisting in the injection of a large dose of anthracyclines during a shorter period of 2 weeks has also been largely used (Kawasaki et al., 1996; Pouna et al., 1995; Richard et al., 2008; Robert, 2007; Teraoka et al., 2000; Tokudome et al., 2000). This short-term model holds the promise of reducing the duration of the experiment to 2 weeks with lower associated costs. In these studies, the derangement of cardiac function was documented using the Langendorff preparation, a highly sensitive method for the assessment of myocardial contractility. However, the efficiency of the short-term model in producing functional and structural cardiac changes consistent with those of dilated cardiomyopathy has not been confirmed in the studies using *in vivo* imaging techniques to assess the cardiac function and remodeling. Thus, this study aims to perform a head-to-head comparison of short-term and long-term DXR injection protocols for the induction of left ventricular systolic dysfunction and cardiac remodeling in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, weighing an average of 250 g, were obtained from the breeding colony of the Faculty of Medicine of Ribeirão Preto. They were housed under controlled temperature conditions (22–24 °C). They consumed tap water and standard commercial rat chow *ad libitum* on a 12:12 h light:dark cycle. All animals were weighted once a week, early in the morning, using an appropriate digital scale. All of the experimental procedures were approved by the Animal Care Committee of the Faculty of Medicine of Ribeirão Preto.

2.2. Chemical products

Adriablastina® RD (Doxorubicin chloride) was purchased from *Pfizer (Pharmacia, Milan, Italy),* dissolved in saline (10 mg/100 ml) and administered intraperitoneally to induce cardiomyopathy. Intramuscular injection of Ketamine cloridrate (*Vetbrands, Jacarei, SP, Brazil*) and Xylazine (Calier, *Les Franqueses del Vallés, Barcelona, Spain*) were used for anesthesia.

2.3. Experimental protocol

Animals were allocated into three experimental groups. The ***ST group*** (short-term DXR injection group, n = 50) consisted of animals that received 6 intraperitoneal injections of DXR (2.5 mg/kg per dose) over a period of 2 weeks (cumulative dose of 15 mg/kg of body weight). The ***LT group*** (long-term DXR injection group, n = 51) received weekly intraperitoneal injections of DXR (2 mg/kg/dose) over a period of 9 weeks (cumulative dose of 18 mg/kg). A ***control group*** (n = 14) was composed of animals of matched ages that received an appropriate volume of 0.9% saline intraperitoneally during 9 weeks.

2.4. Transthoracic 2D echocardiography

Cardiac function was evaluated at baseline and after DXR treatment by using 2D echocardiography performed as previously described (Romano et al., 2012). After DXR injection, the surviving animals were re-evaluated for left ventricular (LV) function at time points defined according to prior published studies using short-term (Teraoka et al., 2000) and long-term (Wakasugi et al., 1993b) models. In the ST group, animals surviving the DXR injections (n = 26) were submitted to echocardiographic study 4 weeks after the last dose of DXR. In the LT group, rats surviving the DXR injections (n = 26) underwent echocardiographic study 2 weeks after the last DXR dose or saline injection. In the LT group one animal was excluded of the final analysis due to artifacts in the echocardiographic images, resulting in a final sample size of the LT group = 25. Control animals were evaluated at the same time point as those used for the ST group.

Two-dimensional short-axis imaging using M-mode echocardiography of the left ventricle (LV) at the level of the papillary muscle was used to determine the internal diameters of the left ventricles during systole and diastole, to calculate the fractional shortening (%FS), which was used as an index of systolic function, and to calculate the left ventricular ejection fraction (LVEF) by using the Teichholz method (Teichholz et al., 1976). In addition, LV posterior and septal wall thickness were measured and used to calculate the LV mass. A single investigator performed all measurements. A second investigator, who was also experienced in echocardiographic analysis of rodent hearts, reviewed all measurements. The LV end-diastolic dimension (LVDd) was measured at maximal diastolic dimension, and the LV end-systolic dimension (LVDs) was measured at maximal anterior motion of the posterior wall. Measurements represented the mean of five consecutive cardiac cycles using the same transducer position and angle in the same stop image frame. The time interval between two consecutive cardiac cycles was measured for heart rate calculation.

2.5. Histopathology

2.5.1. Harvesting and preparation of hearts

Six animals from each group, randomly chosen, were used for histopathological analysis. The hearts were rapidly removed, rinsed in ice-cold 0.9% saline solution, and fixed as a whole by immersion in phosphate-buffered 10% formalin for 24 h at 4 °C for histological study. Both ventricles from each heart were isolated and cut into two fragments by a midventricular coronal section. Each block was serially cut in the same direction at thickness of 4–7 μm being appropriate for each stain, and sections were stained