A small animal model of non-ischemic cardiomyopathy and its evaluation by transthoracic echocardiography

Ernst R. Schwarz, Charles Pollick, Joan Dow, Mike Patterson, Yochai Birnbaum, Robert A. Kloner*

The Heart Institute, Good Samaritan Hospital and Division of Cardiology, University of Southern California, Los Angeles, CA, USA

Received 15 September 1997; accepted 3 December 1997

Abstract

Background: Costs for large animal studies have escalated. Therefore there is a need to develop small animal models of non-ischemic cardiac failure and accurate non-invasive techniques that will allow serial quantitation of left ventricular function. Objectives: The purpose of our study was to determine the efficacy and reliability of adriamycin for inducing cardiomyopathy in rats. We hypothesized that high frequency transthoracic 2-dimensional and M-mode echocardiography would allow for serial testing of cardiac function in this small animal model. Methods: Adriamycin was administered at a dose of 2.5 mg/kg intravenously once a week for 10 weeks in 54 rats. Transthoracic echocardiography by use of a 7.5 MHz transducer was performed in 19 rats at baseline and additionally at 12 weeks after beginning of adriamycin therapy to measure left ventricular dimensions and calculate fractional shortening. Results: The mortality rate during the treatment period was 11%, but increased to 52% at 13 weeks. Transthoracic echocardiography provided adequate visualization of left ventricular dimensions and cardiac function in a parasternal short axis view. In follow-up echocardiography, pericardial effusion was detected in 8/19 rats (42%). Compared to baseline, end-diastolic diameters increased from 0.56±0.06 to 0.64±0.08 mm (p<0.001), end-systolic diameters increased from 0.27±0.03 to 0.42±0.08 mm (p<0.001), and fractional shortening decreased from 52.8±4.0 to 34.3±7.1% (p<0.001) at 12 weeks. Electron microscopy in a subset of rats revealed cardiomyocyte degeneration, mitochondrial and sarcoplasmatic reticular edema, numerous intracellular vacuoles and 'onion-ring' shaped mitochondrial cristae, characteristic for adriamycin cardiotoxicity in human patients. Conclusion: Adriamycin at an intravenous dose of 2.5 mg/kg over 10 weeks can be used to create a reliable model of non-ischemic dilated cardiomyopathy with a high success rate. For in-vivo diagnostic purposes, transthoracic echocardiography provides a reliable technique to non-invasively assess cardiac function quantitatively and qualitatively in follow-up studies in rat cardiomyopathy. This small animal model can easily be used for testing new therapeutic strategies in cardiac failure. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Cardiomyopathy; Adriamycin; Rats; Echocardiography

1. Introduction

Chronic heart failure in man, which can be caused by ischemic or non-ischemic cardiomyopathy, remains a major cause of morbidity and mortality. Chronic animal models are needed to mimic the pathophysiological condition of heart failure including severely impaired hemodynamic function, clinical signs of heart failure, and cellular, subcellular, and metabolic alterations in the myocardium. To study the effects of new drugs or therapeutic concepts several animal models for congestive heart failure are available, i.e. cardiac failure caused by experimental volume and pressure overload, myocardial ischemia or infarction, rapid pacing, or drug-induced cardiomyopathies [1]. It is, however, difficult to perform survival studies in heart failure models using large animals because of increasing costs. Based on financial restraints, technical equipment needs, skills and availability, small animal models such as rats or mice represent the future targets in cardiovascular research, including use of transgenic animals. Even though models of chronic heart failure

*Corresponding author. Address for correspondence: Heart Institute Research, Good Samaritan Hospital, 1225 Wilshire Boulevard, Los Angeles, CA 90017, USA. Tel.: +1-213-977-4050; Fax: +1-213-977-4107.

Time for primary review 24 days.
exist, imaging techniques that can be used to assess left ventricular function in these small animals with cardiomyopathy, have not been widely established, yet.

Adriamycin, an anthracycline antibiotic and antineoplastic agent, is used in chemotherapy for the treatment of several cancers in humans. One well known side-effect of prolonged therapy is the development of congestive heart failure due to a proposed calcium overload and its direct cardiotoxicity. It is hypothesized that lipid peroxidation and generation of oxygen-derived free radicals [2,3] disrupt cellular membranes finally leading to degenerative dilated cardiomyopathy, which is relatively refractory to conventional treatment strategies [4–7].

In human patients, echocardiography is used as a non-invasive diagnostic technique to visualize morphologic or functional changes in adriamycin-induced cardiomyopathy [8–10]. Models using adriamycin-induced cardiomyopathy have mainly been reported in rabbits [11–13], but also in dogs [14], mice [15], and rats [16]. To our knowledge, functional non-invasive follow-up studies in the rat model have not been described, yet. Therefore, the purpose of our study was to (1) determine whether a reliable small animal model of dilated cardiomyopathy could be developed in the rat and (2) to evaluate whether transthoracic echocardiography represents a reliable technique to image the heart in this small animal model.

2. Methods

A total of sixty female Sprague-Dawley rats, weighing between 164 and 253 g were used. Fifty-four rats were used for later treatment with adriamycin. Histologic analysis including electron microscopy of the left ventricle was performed in a subset of five adriamycin-treated rats. All experiments were conducted in accordance with the institutional guidelines for use and care of laboratory animals which conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.1. A model of dilated cardiomyopathy

Fifty-four rats received 2.5 mg/kg adriamycin as an intravenous tail vein infusion once a week for a total of 10 weeks. Six rats served as controls and received the same amount of saline as an intravenous tail vein infusion once a week for a total of 10 weeks. For the intravenous injection, the rats were restrained. Transthoracic echocardiography was performed in all rats at baseline and a subgroup of 22 rats was chosen for follow-up echocardiography (19 adriamycin-treated rats and 3 controls) after 12 weeks (2 weeks after cessation of adriamycin treatment). To compare baseline and post-treatment echocardiography in the adriamycin-treated group, data from these 19 rats were used for further analysis.

2.2. Echocardiography

For echocardiographic studies, inhalative anesthesia was induced and maintained by the use of methoxyflurane (closed container technique). The chest was shaved, and the animals were positioned on their left side. A 7.5 MHz commercially available standard pediatric transducer which used phased array technology for beam formation (64 elements) and a lateral and axial resolution between 0.2 and 0.5 mm was connected to an echocardiographic computer console (Hewlett Packard 1500, Andover, MA). The interrogation depth was set at 4 cm.

A parasternal long axis view was followed by a parasternal short axis view. Left ventricular end-systolic and end-diastolic diameters were measured at the level of the papillary muscles using two-dimensional guided M-mode imaging. Fractional shortening was calculated. Measurements of left ventricular dimensions were performed online from the screen and repeated later. To avoid individual variations due to potential failures of measurements caused by difficulties in detection of endocardial border zones in some cases and to ensure reproducibility of the measurements beat-to-beat and intraobserver variability were analyzed. For all rat hearts, between three and six beats were measured using the same transducer position and angle in the same stop image frame. The mean values of all measurements were used for further analysis. All measurements were conducted by the same investigator (E.R.S.), but were reviewed by two other investigators (C.P. and R.A.K.), who had experience in echocardiographic analysis of rodent hearts [17,18]. The intra- and interobserver variability, which has been described before from our group [19], was analyzed in a subgroup of 10 rat hearts for intra- and 30 rat hearts for inter-observer variability. For measurements of left ventricular dimensions, the recommendations of the American Society of Echocardiography were used [20]. The total examination time was less than 10 min; thereafter rats were allowed to recover.

3. Results

3.1. Follow-up

None of the control rats — which were healthy and only treated with saline — died during the 10-week saline injection period. One rat in the treated group died during the first injection of adriamycin; additionally two rats died within the first week. Three rats died during the ninth and tenth week of therapy, presumably due to severe cardiac failure. Therefore, the total mortality rate at follow-up (10 weeks after start of the 10-week adriamycin therapy) was 11%. However, after completion of the adriamycin treatment period, cumulative mortality was 37% within the following 2 weeks (total of 12 weeks) and increased to
parasternal site, which enabled a short axis view. The circumference of the left ventricle in its middle portion and at the level of the mitral valve could clearly be detected. Epicardial and endocardial borders could be visualized. However, in some animals, the anterior wall and the interventricular septum could not clearly be distinguished in a short axis view. In these animals, a parasternal long axis view and substernal four chamber view were performed additionally to measure left ventricular dimensions. Left ventricular diameters were $0.56 \pm 0.06$ cm at end-diastole and $0.27 \pm 0.03$ cm at end-systole. Fractional shortening was $52.8 \pm 4.0\%$ (Table 1). Follow-up echocardiography in three control rats with saline injections for 10 weeks revealed no reduction in fractional shortening if compared to baseline echocardiography.

3.2. Transthoracic echocardiography

To analyze beat-to-beat variability the highest and the lowest of the three to six measurements were taken. The beat-to-beat correlation coefficient for the absolute end-diastolic diameter was $r=0.91$ with a standard error of the estimate (SEE) of $0.04$ cm and $r=0.98$ (SEE=0.018) for the absolute end-systolic diameter. Intraobserver variability was measured by analyzing the heart beats in the same echocardiographic image frames at two separate occasions in 10 randomly selected hearts. The correlation for end-diastolic diameters was $r=0.973$ (SEE=0.013) and for end-systolic diameters $r=0.975$ (SEE=0.009). Interobserver variability between two investigators was analyzed in 30 measurements and revealed a correlation of $r=0.956$ (SEE=0.044) for end-systolic and end-diastolic diameters.

3.2.1. Baseline echocardiography

At baseline, the left ventricle was well visualized in all 19 rats and measurements of left ventricular dimensions were performed. The transducer was positioned on the left

<table>
<thead>
<tr>
<th>Transthoracic echocardiography</th>
<th>End-diastolic diameter (mm)</th>
<th>End-systolic diameter (mm)</th>
<th>Fractional shortening (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline ($n=19$) weight: 226±13 g</td>
<td>0.56±0.06</td>
<td>0.27±0.03</td>
<td>52.8±4.0</td>
</tr>
<tr>
<td>Follow-up ($n=19$) weight: 229±31 g</td>
<td>0.64±0.08*</td>
<td>0.42±0.08*</td>
<td>34.3±7.1*</td>
</tr>
</tbody>
</table>

* = $p<0.001$ versus baseline.

3.2.2. Cardiomyopathy

In the 19 rats subjected to adriamycin therapy and follow-up echocardiography, baseline echocardiographic dimensions were not different compared to the control population. Physical examination at 10 weeks revealed ascites in 13 animals (68%) but in none of the control rats. Initially, body weight increased in the adriamycin-treated animals (from $225.9\pm12.9$ g to $242.6\pm29.8$ g at 10 weeks, $p<0.05$), but then decreased by 12 weeks (to $228.9\pm30.5$ g, not significant versus baseline), presumably due to worsening of the clinical condition. In contrast, body weight in the six control rats increased from $186.0\pm11.3$ g to $335.0\pm20.0$ g at 12 weeks (2 weeks after cessation of saline injection, $p<0.001$ versus baseline and versus the adriamycin-treated rats). Follow-up echocardiography showed pericardial effusions in eight adriamycin-treated animals (42%), and increased left ventricular diameters. In control rats, no pericardial effusions were detectable. The methods and duration of anesthesia using methoxyflurane were identical for both the baseline and the follow-up echocardiographic examinations. At follow-up, fractional shortening was reduced in all adriamycin-treated rats (Table 1 and Figs. 2–6) but not in controls.

In 5/19 rats, end-diastolic diameters did not increase, but end-systolic diameter did and thus, fractional shortening was reduced. Two of these rats died between the 13th and 14th week after start of adriamycin treatment. The three others survived the observation period, but died at the 17th week.
3.3. Electron microscopy

Electron microscopic analysis was performed in five adriamycin-treated rats and revealed partially degenerated cells, numerous intracellular vacuoles, swollen mitochondria with ‘onion ring’ shaped cristae, and swollen sarcoplasmatic reticulum (Figs. 7–9). Two of these hearts were among those in which end-diastolic diameters did not increase at echocardiographic follow-up. The histologic pattern of adriamycin-induced cardiotoxicity was similar to those with enlarged end-diastolic diameters (Fig. 9).

4. Discussion

The major findings of our study are: (1) adriamycin reliably caused dilated cardiomyopathy in the rat model resulting in severe heart failure; (2) electron microscopic analysis demonstrated cellular degeneration and ultrastructural changes including myocyte vacuolation and interstitial edema, characteristic for adriamycin-induced cardiotoxicity [4,6,16]; (3) transthoracic echocardiography represents a useful technique for imaging the development of dilated cardiomyopathy in this small animal model with accurate assessment of left ventricular dimensions with a low variability of measurements, and demonstrated a significant increase in end-diastolic and end-systolic diameters, and a decrease in fractional shortening.

4.1. Adriamycin-induced cardiomyopathy in the rat

Adriamycin-induced cardiomyopathy has been described before in the rat model [16,21]. However, the definite dosage of adriamycin, i.e. from 1 mg/kg body weight to 2.5 mg/kg, the frequency and the route of administration, i.e. once a week or once every 3 weeks, subcutaneously, intraperitoneally or intravenously, and the total duration of treatment, i.e. from 6 to 20 weeks, varied. Using our techniques, i.e. 2.5 mg/kg adriamycin, administered intravenously once a week for 10 weeks, all animals developed cardiac failure. This was shown by the clinical condition of the animals including a high incidence of ascites, initial increase but later decrease of body weight after the 10-week treatment period, and echocardiographic evidence of reduced global cardiac function. Moreover, in contrast to Wakasugi et al., who found a mortality rate of 82% within the treatment period in rats treated with 2 mg/kg adriamycin, administered subcutaneously for 10 weeks [21], our mortality rate during the treatment period was less than 12% (only three rats died during the first week and three treated rats died in the ninth or tenth week), although it increased thereafter. The reason for this difference is not clear, but the subcutaneous route of adriamycin administration might have caused severe local and systemic infections, which may contribute to the high...
Fig. 5. Short axis view of the left ventricle of a rat at baseline (upper panels) and 2 weeks after completion of a 10-week adriamycin treatment period (lower panels). To the left, the left ventricle is shown at end-diastole, to the right, the left ventricle is shown at end-systole. At baseline, the changes of the left ventricular dimensions are easily detectable. In contrast, adriamycin-induced cardiomyopathy shows almost no difference in left ventricular dimensions between systole and diastole with very low fractional shortening (LV=end ventricular cavity, ed=end-diastole, es=end-systole).

Fig. 6. M-mode echocardiographic images of a rat heart at baseline (upper panel) and at 2 weeks after completion of a 10-week adriamycin treatment period (lower panel). In adriamycin-induced cardiomyopathy, there is global left ventricular hypokinesis and an enlarged left ventricular cavity (end-systolic diameter 0.322 cm, end-diastolic diameter 0.525 cm), compared to the same heart at baseline (end-systolic diameter 0.203 cm, end-diastolic diameter 0.458 cm). Calculated fractional shortening is 56% at baseline and 39% after adriamycin-induced cardiomyopathy had been developed (LV=end ventricular cavity, SEPT=septal wall, PW=posterior wall).
shortening (Fig. 4). In contrast, in the rabbit model Pye et al. found a reduction in ejection fraction only in 7/16 animals, indicating 56% of unsuccessfully treated animals [22]. Therefore, the model described here using the rat represents a cost-effective, reproducible and accurate model with a high incidence of cardiomyopathy and a low mortality during the treatment phase but increased mortality due to severe cardiac failure during the observation period of 4 weeks thereafter.

4.2. Transthoracic echocardiography

Qualitative and mainly quantitative 2-dimensional echocardiography provides in vivo dynamic assessment of ventricular function, cardiac motion and mechanical abnormalities in human patients as well as in experimental animals. In research studies, echocardiography is used in different species up to the size of the transducer itself, i.e. in mice we recently demonstrated that 2-D echocardio-

Fig. 7. Electron micrograph of left ventricular tissue from a rat subjected to 10 weeks of adriamycin therapy. Typically, numerous vacuoles in between the myofilaments are present. Magnification ×2900.

Fig. 8. Micrograph showing swollen, enlarged mitochondria, characteristic for adriamycin cardiotoxicity. Magnification ×58 000.
raphy provides accurate estimation of left ventricular dimensions and function [17]. In the rat heart, 2-D echocardiography is an established procedure for assessment of ventricular geometry, global left ventricular function, stroke volume, and cardiac index in normal, hypertrophied, congenitally malformed, and ischemic or necrotic myocardium [23–29]. However, most of these studies had no follow-up and non-ischemic rat models of cardiomyopathy are rare. Since echocardiography is frequently used in human patients treated with adriamycin for detection of functional changes [6,8–10,30], there is need for an adequate comparable animal model.

Evaluation of cardiac function by echocardiography in dogs with adriamycin-induced cardiomyopathy has been reported recently [14], demonstrating gradually reduced fractional shortening over an observation period of 21 weeks. In this study, however, no increase in left ventricular dimensions has been shown. This is in contrast to our findings in the rat model, since we found a significant increase of end-systolic as well as end-diastolic diameters, which easily could be visualized by two-dimensional and M-mode echocardiography at 12 weeks. In rabbits, transthoracic echocardiography revealed a reduction in ejection fraction and an increase in diastolic left ventricular dimensions at 20 weeks after start of an 8-week adriamycin treatment period, compared to control animals [22]. In this study, however, no intra-individual follow-up echocardiography had been performed.

To our knowledge, we present the first study demonstrating the feasibility of follow-up echocardiography to detect a change in left ventricular dimensions and a reduction of contractile cardiac function in adriamycin-induced cardiomyopathy in rats. This technique might represent a promising non-invasive, cost-effective diagnostic tool which reliably can be performed to study different treatment strategies.

4.3. Histological findings

As previously reported by use of a histological scoring system in rabbits, a negative correlation was found between ejection fraction and the amount of myocardial degeneration [22]. Since the findings of cellular degeneration, swollen mitochondria and vacuolation were typical for severe adriamycin cardiotoxicity in human patients [4–7], our findings indicate that the functional changes detected by echocardiography are caused by adriamycin cardiotoxicity. Thus, in addition to the availability of a model of non-ischemic cardiomyopathy and a diagnostic non-invasive technique for follow-up studies, this rat model may be used to study (1) the cellular and metabolic effects of adriamycin cardiotoxicity which are similar to findings in human patients, and (2) the avoidance of these morphologic and functional alterations by use of different protective treatment strategies.

One limitation of this study is the absence of invasively evaluated hemodynamic data. However, invasive surgical procedures may limit a follow-up and survival studies and might even alter the hemodynamic condition (i.e. a chronically occluded carotid artery after invasively measured blood pressure) in rats. On the other hand, alterations of hemodynamics including cardiac output in adriamycin-treated rats have been reported before [31–34]. Therefore, inclusion of invasive hemodynamic measurements was not part of our study design. It is also unlikely that humans
with adriamycin-induced cardiomyopathy would be subjected to invasive hemodynamic testing.

4.4 Conclusion

The rat model described here represents an adequate, cost-effective, and reproducible model for adriamycin-induced cardiomyopathy, resembling morphologic and functional changes in human patients treated with adriamycin. Transthoracic echocardiography by use of two-dimensional and M-mode imaging represents an accurate diagnostic technique for non-invasive follow-up studies in the cardiomyopathy rat model. This small animal model and transthoracic echocardiography can be used for testing new therapeutic strategies and drugs in chronic heart failure.

References