Adriamycin-induced Cardiotoxicity (Cardiomyopathy and Congestive Heart Failure) in Rats

F. P. Mettler, D. M. Young, and J. M. Ward

Comparative Pathology Section, Laboratory of Toxicology, Experimental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland

SUMMARY

The recent development of numerous analogs of the anthracycline class of oncolytic agents has resulted in an urgent need for a standardized, accurate, reproducible, and cost-effective system for cardiotoxicity testing. The present studies were designed to determine the feasibility of using the rat as a model for induction of the chronic type of cardiotoxicity (i.e., cardiomyopathy and congestive heart failure). Adriamycin (ADR) was administered to rats at doses of 1 to 2 mg/kg/week for 10 to 14 weeks. The majority of ADR-treated rats developed cardiomyopathy from 3 to 23 weeks after the last injection. Forty to 70% of those rats with cardiomyopathy had gross evidence of congestive heart failure (pleural effusions, ascites, hepatomegaly, cardiomegaly). Histological myocardial changes consisted of myocyte vacuolization and degeneration, interstitial edema, and mild fibroplasia. In addition, damage to atrial ganglion cells was evident in several rats. Ultrastructural alterations involved sarcoplasmic disruption with distentions of subcellular organelles and loss of myofilaments. Extracardiac toxic effects of ADR (nephrotoxicity, myelosuppression, enteropathy, arrested osteogenesis) were minor and were observed more frequently at higher cumulative doses.

The results of the present study suggest that the rat model is an accurate, reproducible, and cost-effective system for large-scale cardiotoxicity testing of analogs of ADR and daunorubicin.

INTRODUCTION

Cardiotoxicity is a well-characterized, major dose-limiting complication of ADR therapy (6, 17). Although numerous studies have described the effects of this broad-spectrum antineoplastic agent on cellular structure and function, the pathogenesis of the cardiomyopathy remains an enigma (15, 17, 43). Current investigations appear to be directed at the identification of noncardiotoxic analogs of ADR and DNR and the characterization of potential antidotal procedures that prevent or reverse the development of cardiomyopathy. More than 300 analogs of the anthracycline class of oncolytic agents have been defined chemically. Therefore, there is an urgent need for a standardized quantitative system to screen, assay, or test analogs for cardiotoxic activity in comparison to the parent compounds. Of the number of animal models described as exhibiting ADR-induced cardiotoxicity (43), the rabbit appears to be the most widely used by various laboratories. Although the rabbit model provides a highly reproducible incidence of cardiotoxicity, it is costly to use for large-scale testing of analogs. Therefore, the current studies were designed to determine the feasibility of using the rat as a model for induction of the chronic type of cardiotoxicity (i.e., cardiomyopathy and CHF).

MATERIALS AND METHODS

A total of 70 male 4-week-old CDF (Fischer) rats (Charles River Breeding Laboratory, Wilmington, Mass.) were used in this study. Rats were maintained in filter-topped polycarbonate cages on NIH Open Formula Rat and Mouse Ration 5018 (Ralston Purina Co., St. Louis, Mo.) and water ad libitum throughout the experiment. At 6 weeks of age, the rats were divided into 5 groups for either s.c. or i.v. (tail vein) injection. ADR (kindly supplied by Dr. Harry Wood, Drug Synthesis and Chemistry Branch, National Cancer Institute, NIH, Bethesda, Md.) was administered i.v. in 0.9% NaCl solution at a dose schedule of 1 mg/kg/week (Group 1) and 2 mg/kg/week (Group 2) and s.c. at 2 mg/kg/week (Group 3). Control rats received equivalent volumes of 0.9% NaCl solution i.v. (Group 4) and s.c. (Group 5) once a week. Rats were lightly anesthetized with ether prior to i.v. injections with 27-gauge needles. With careful technique it was possible to avoid perivenous infiltration, sclerosis, and necrosis of the tails. In order to avoid development of skin ulcerations, s.c. injections were performed at alternate sites each week. Body weights were recorded weekly. Samples of blood were collected from the tail veins 24 hr after injection on a bimonthly basis for determination of packed cell volume (microhematocrit). Injections of ADR were discontinued after 10 weeks for rats in Group 2, between 11 and 14 weeks for rats in Group 1, and at 13 weeks for rats in Group 3. Rats either died of toxicity or were sacrificed in poor condition by an overdose of ether. Terminal blood samples from rats being sacrificed were collected from the abdominal aorta, and sera were analyzed for calcium (Technicon Auto Analyzer), phosphorus (Hycl phosphorus), sodium, potassium (Technicon STaT-ion), blood urea nitrogen (19, 37), creatine phosphokinase (30, 33), cholesterol (14), and...
triglycerides (3). Necropsies were performed with collection of the following tissues in Telly's fixative (36) for histopathological evaluation: heart, lung, liver, kidney, spleen, intestines, diaphragm, and proximal tibia. Sections were cut at 6 μm and stained with hematoxylin and eosin and Masson's trichrome. Samples of ventricular myocardium from 8 ADR-treated rats were minced into small cubes (0.5 mm), fixed in 1.5% glutaraldehyde, postfixed in 1% osmium tetroxide (10), dehydrated in graded ethanol, and embedded in Epon-Araldite (22). The blocked samples were cut at 250 to 500 Å with a Reichert Om-U-2 ultramicrotome, stained with uranyl acetate and lead citrate (31), and examined with a JEOLCO 100B electron microscope. Samples from 3 control hearts were processed and evaluated in a similar manner.

Blood and tissue samples from appropriately matched controls were utilized in these studies, and data were analyzed statistically using Student's t test (39).

RESULTS

All rats maintained similar growth rates throughout the 1st 4 weeks of injections (Chart 1). Thereafter, rats in Group 2 became inactive and failed to maintain weight gains as observed in other treated and control rats. Rats given ADR, 2 mg/kg s.c., gained weight at a rate similar to that of rats receiving ADR, 1 mg/kg i.v. Blood collected from rats in Group 2 revealed that the packed cell volume had decreased from a base-line range of 39 to 46%, after 10 weeks of treatment, to 15 to 25%. Microhematocrit values from rats in Group 1 were only slightly decreased (range, 35 to 40%) compared to controls (range, 40 to 48%). One rat in Group 2 died of toxicity after 9 weekly i.v. injections. The remaining ADR-treated rats survived the full course of the experiment except for 1 rat in Group 1 and 3 rats in Group 2, which died as a result of inadvertent ether overdose prior to weekly i.v. injections. Nine rats in Group 1 died from 1 to 23 weeks after the last ADR injection (Table 1). The remaining 10 rats in Group 1 were sacrificed at various times ranging from 1 to 32 weeks after the final ADR injection.

In Group 2, 8 of the 17 rats died from 1 week before to 5 weeks after the last injection of ADR (Table 1). The remaining 9 rats were sacrificed from 1 to 12 weeks after the last ADR injection. Analysis of terminal serum samples from rats in Group 2 revealed (mean ± S.E.) hypocalcemia (8.9 ± 1.8 mg/100 ml), hyperkalemia (6.9 ± 2.3 mEq/liter), hyperphosphatemia (9.3 ± 2.8 mg/100 ml), elevated blood urea nitrogen levels (73 ± 23 mg/100 ml), and hypercholesterolemia (396 ± 128 mg/100 ml). These values were significantly different (p < 0.05) from corresponding control means of calcium (10.6 ± 0.2 mg/100 ml), potassium (4.8 ± 0.3 mEq/liter), phosphorus (5.4 ± 1.5 mg/100 ml), blood urea nitrogen (22 ± 1 mg/100 ml), and cholesterol (110 ± 24 mg/100 ml). Changes in serum parameters of terminal samples from other ADR-treated groups did not differ significantly from control values.

CHF was evident grossly at necropsy (pleural effusions, ascites, cardiomegaly) in 14 of the ADR-treated rats (Table 1). One rat in Group 1 died 23 weeks after the last of 14 weekly ADR injections (84 mg/sq m total dose) with fulminating CHF.

Due to severe postmortem autolysis, heart samples were not collected for light microscopy from 8 rats in Group 1 and 4 rats in Group 2. Histopathological evaluation of hearts from the majority of the remaining ADR rats revealed varying degrees of myocardial alterations (Table 1). The most consistent and predominant finding was marked vacuolization of myocytes, especially in subepicardial and subendocardial regions in addition to perivascular locations (Figs. 1, 3, and 4). Although vacuolar lesions were observed frequently in ventricles and septa, several rats had severe vacuolation of atrial myocytes (Fig. 2). In addition, vacuolar degeneration of ganglion cells was observed in 2 of 3 rats where atrial sections were found to include clusters of cardiac neurons (Fig. 5). Three rats in Group 3 had focal atrial thromboses evident histopathologically. Myocyte lysis, interstitial edema, and mild fibrosis were evident in occasional areas of more severely affected rat hearts. The incidence and severity of the cardiomyopathy appeared to be dose dependent (Table 1).

Ultrastructural evaluation of samples of myocardium from ADR-treated rats revealed varying degrees of sarcoplasmic vacuolation (Fig. 6). The vacuoles occurred predominantly in a perinuclear position and consisted of distentions of the sarcoplasmic reticulum, the T-tubule system, and Golgi vesicles. In myocytes where vacuolation was severe, there was a concomitant reduction in the quantity of myofilaments. A few scattered myocytes were found to contain fragmented sarcromeres with disruption of the characteristic parallel arrays of myofilaments. In general, mitochondria did not appear to be altered markedly, although several were swollen with disruption of cristae. Formation of myelin figures was not a predominant feature of mitochondrial alterations.

Histopathological evaluation of other tissues from ADR rats revealed lesions in kidneys, liver, small intestine,
Table 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of rats</th>
<th>Route of administration</th>
<th>Treatment schedule</th>
<th>Mean total ADR dose (mg/sq m)</th>
<th>Mean wk survival</th>
<th>Died/sacrificed</th>
<th>Cardiomyopathy*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>i.v.</td>
<td>ADR, 1 mg/kg/wk for 11–14 wk</td>
<td>75 (31) 12–44*</td>
<td>9/10</td>
<td>2 (9) 4 (36)*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>i.v.</td>
<td>ADR, 2 mg/kg/wk for 10 wk</td>
<td>120 (13) 9–22</td>
<td>8/9</td>
<td>8 (12) 12 (92)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>s.c.</td>
<td>ADR, 2 mg/kg/wk for 13 wk</td>
<td>156 (16) 14–20</td>
<td>5/5</td>
<td>4 (10) 10 (100)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>i.v.</td>
<td>0.9% NaCl solution, 1 ml/kg/wk for 14 wk</td>
<td>0</td>
<td>0/10</td>
<td>0 (100) 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>s.c.</td>
<td>0.9% NaCl solution, 1 ml/kg/wk for 13 wk</td>
<td>0</td>
<td>0/10</td>
<td>0 (100) 0</td>
<td></td>
</tr>
</tbody>
</table>

* Determined by histopathological evaluation.

Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>In vivo/in vitro Parameters and end-point evaluations</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick embryo</td>
<td>ECG evaluation, microscopic pathological evaluation</td>
<td>26, 27</td>
</tr>
<tr>
<td>Mouse</td>
<td>Survival time, accumulative dose, microscopic and ultrastructural pathological evaluation</td>
<td>18, 35</td>
</tr>
<tr>
<td></td>
<td>Survival time, heart wt, myocardial malondialdehyde content</td>
<td>21, 23</td>
</tr>
<tr>
<td></td>
<td>+ Myocardial cell culture, beating frequency, automaticity, and rhythmic contractile activity</td>
<td>9, 24</td>
</tr>
<tr>
<td>Rat</td>
<td>+ Altered myocardial DNA synthesis</td>
<td>34</td>
</tr>
<tr>
<td>Rabbit</td>
<td>+ ECG evaluation, accumulative dose</td>
<td>1, 5, 46</td>
</tr>
<tr>
<td></td>
<td>Survival time; accumulative dose; gross, microscopic, and ultrastructural pathological evaluation</td>
<td>15, 16, 43, 45</td>
</tr>
<tr>
<td></td>
<td>+ Myocardial calcium accumulation</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>+ + Inhibition of sodium-potassium-ATPase (heart, kidney)</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>+ Inhibition of mitochondrial respiratory enzymes</td>
<td>12</td>
</tr>
<tr>
<td>Monkey</td>
<td>+ Survival time, accumulative dose, gross and microscopic pathological evaluation</td>
<td>11*</td>
</tr>
</tbody>
</table>


cecum, and bone. Renal changes were characterized by dilated tubules, vacuolated glomeruli, and thickening of Bowman's capsule (Fig. 7). One rat in Group 1 died with histopathological evidence of chronic renal failure and secondary hyperparathyroidism 17 weeks after the last ADR injection. Rats with CHF had various degrees of centrolobular hepatic necrosis. Midsagittal sections of proximal tibiae from the majority of ADR rats contained lesions in the epiphyseal-metaphyseal area consisting of a deficiency of trabeculae, osteoblasts, and osteoclasts (Fig. 8). These changes were interpreted as consistent with an arrest of chondroosteogenesis. Lesions observed in the small intestine and cecum consisted of varying degrees of epithelial regeneration. One rat in Group 1 had a squamous cell carcinoma of Zymbal's gland at 22 weeks after the last ADR injection. No other neoplastic processes were observed grossly or histopathologically in either ADR-treated or control rats.

DISCUSSION

The need for a model system to test analogs of ADR and DNR for cardiotoxicity has resulted in numerous investigations and several proposed experimental systems (Table 2). These systems vary considerably between species with re-
spect to suggested markers or indices of cardiotoxicity. None of the recommended procedures has been tested rigorously on a large-scale basis to determine the accuracy and reproducibility of its predictiveness. Any biological system used to assay for anthracycline-induced cardiotoxicity should be: (a) cost-effective with respect to large-scale screening of ADR-DNR analogs; (b) accurate and reproducible for predicting the development of cardiomyopathy and CHF; and (c) relatively simple to develop and perform in order to be used readily in various laboratories without special facilities, equipment, or highly trained personnel.

The results of the present study indicate that the rat model may be the closest to an "ideal animal model" for cardiotoxicity testing (43). Large numbers of animals can be placed on test and maintained at a reasonable cost. The injection procedures and evaluation of end-point parameters are simple and routinely used in a number of laboratories. The induction of the cardiomyopathy occurs with a high reproducible incidence and appears to be time and dose related. The myocardial lesion has histological characteristics similar to those reported in humans and rabbits (i.e., myocyte vacuolation and degeneration, edema, mild fibrosis) (4, 15-17, 43, 45). In addition, the cardiotoxicity is delayed, progressive, and frequently manifested by development of CHF with measurable quantities of fluid retained in body cavities. The rat model could be utilized in dual experimentation to determine oncolytic and cardiotoxic activity of analogs. ADR and DNR have been found to be moderately active against s.c.-implanted Walker 256 carcinosarcoma in rats (J. Venditti, personal communication). One limitation of the rat model as utilized in our studies is the time required for induction of the cardiomyopathy (10 to 20 weeks). Studies are being designed to determine whether a characteristic subchronic ECG change correlates consistently and reproducibly with eventual cardiomyopathy and CHF in the rat model. Zbinden et al. (1, 5, 46) have proposed the use of ECG evaluation in rats as an experimental system to detect anthracycline-induced cardiotoxicity. They administered analogs and parent agents i.p. on a daily basis for 5 to 25 injections. ECG's were recorded in unanesthetized rats 1 to 4 times weekly, and the rats were kept alive for 8.5 weeks. The cumulative dose of ADR (120 mg/sq m) administered is comparable to that for rats in our study (Table 1). Widening of the QRS complex and mean QRS duration plus S-wave trough were used as end points in evaluation of ECG's. In this respect, Zbinden et al. (46) calculated the "minimal cumulative cardiotoxic dose" for ADR as 68 mg/sq m. No significant histopathological changes were evident in myocardia of test rats, and minor alterations ("slight focal fiber degeneration or atrophy with modest proliferation of interstitial connective tissue") were not correlated with ECG findings (46). Perhaps test rats were sacrificed too early for manifestations of the characteristic cardiomyopathy and fulminating CHF. The cumulative cardiotoxic doses for ADR in our studies ranged from 75 to 156 mg/sq m, which is slightly higher than Zbinden's minimal cumulative cardiotoxic dose. The major difference between these 2 rat studies is the time of evaluation of end-point parameters (i.e., 8.5 versus 9 to 44 weeks). Further studies are needed to determine if there is a significant correlation between Zbinden's acute-subchronic rat model and our proposed chronic rat model. A comprehensive comparative study may lead to the identification of a sub-chronic parameter alteration which could serve as a precise index or marker of ADR-induced cardiotoxicity in rats.

Chalcroft et al. (7) did not observe myocardial changes by light microscopy in rats receiving single i.v. doses of DNR, 25 mg/kg. Myocardial tissues were collected from test rats 1 to 5 days after injection and prepared for light and electron microscopic evaluation. Ultrastructural changes in myocytes consisted of mitochondrial alterations with swelling, loss of cristae, degeneration, and formation of myelin figures. Mitochondrial changes were not predominant features of myocardial lesions in rats from our study. This discrepancy is most probably related to the differences in experimental design (i.e., acute, single high dose versus chronic, multiple low dose). Studies by Philips et al. (29) revealed the absence of histopathological changes in myocardia 1 to 15 weeks after single high i.v. dosing of ADR (10 and 20 mg/kg) in rats. They also reported the effects of supralethal i.v. doses (10 and 20 mg/kg) in rabbits. Although myocardial lesions were evident 5 to 24 hr after ADR administration, the histopathological characteristics were dissimilar from those reported after chronic, multiple, low-dose treatment (15, 43). These results appear to underscore again the lack of similarity of findings between acute and chronic studies of anthracycline-induced cardiotoxicity. It is well known in toxicology investigations that acute studies are the least reliable, whereas chronic, repeated dose studies are the most reliable in evaluating the side effects of drugs (28).

The observation of lesions in atrial ganglia of rats from our study was a serendipitous finding. The predominant histopathological alteration was cytoplasmic vacuolation. Occasional ganglia cells appeared swollen, with sparse granular cytoplasm. The adverse effects of anthracyclines on nerve cells and ganglia have been a subject of considerable debate. Smith (38) reported on damage to intrinsic cardiac neurons induced by DNR in patients with cancer. The alterations were evident at the light microscopic level and consisted of unusual tinctorial properties, degeneration and death of neurons, proliferation of amphotyes, neuronophagia, and swollen and misshapen processes. Studies by other investigators evaluating animal and human myocardia after treatment with DNR or ADR have failed to confirm the finding of neuronal lesions (4, 15, 43). Histo-fluorometric studies by Mazzanti et al. (20) suggest that DNR is rapidly taken up by nerve cell cytoplasm in the Auerbach plexuses of rats and guinea pigs receiving i.p. injections. Other animal studies indicate that histopathological degeneration of spinal root ganglia occurs regularly after ADR administration (E-S. Cho, personal communication). Further studies are required to characterize and elucidate the pathogenesis of anthracycline-induced changes in nerve cells.

The extracardiac toxic effects of ADR in the rat model (i.e., nephrotoxicity, hematopoietic suppression, arrested osteogenesis) are minor and occur more frequently with higher doses. The nephrotoxic effects of ADR and DNR have been well characterized in rats (29, 40) and rabbits.
(43). The chondroosseous toxic effects of ADR in growing rabbits have been reported in detail previously (44). Therefore, the observed extracardiac toxic effects of ADR in rats were not an unexpected finding.

The selection of an animal model for cardiotoxicity testing should take into account that the response of the myocardium to toxic agents appears to vary considerably among species. Hearse et al. (13) found major species differences in development of myocardial damage as measured by biochemical and ultrastructural evaluation. Rat and mouse hearts were resistant whereas guinea pig and rabbit hearts were susceptible to early onset of damage. These findings correlate well with the observation that rabbit myocardium is uniquely sensitive to the cardiomyopathic effects of ADR (15, 16, 43, 45). Nutritional and physiological peculiarities between species should also be examined. Rabbits have a rather unusual calcium homeostatic mechanism and an unusual, yet undefined, nutritional interaction of selenium and vitamin E (8). Perhaps the normally high serum calcium levels play a role in the sensitivity of this species to ADR. These species differences should be considered in attempts to identify markers or indices of ADR-induced cardiotoxicity. Biochemical or ultrastructural alterations of injured myocardia may not be readily extrapolated across species lines. In addition, some species may be more prone to develop spontaneous myocardial lesions (2, 42).

The results of this study suggest that the rat model is an efficient, reproducible, cost-effective system for large-scale testing of ADR and DNA analogs. Rats develop cardiomyopathy at considerably lower total doses of ADR compared to rabbits (15, 16, 43, 45). For convenience and ease of standardization, it is recommended that rats receive analogs s.c. weekly for 13 consecutive weeks. Initial test doses should be one-tenth to one-fifth of the 50% lethal dose administered s.c. Rats should be observed and weighed regularly. Six weeks after the last dose all rats should be sacrificed. Necropsies should be performed on all rats with special notation for evidence of CHF. The heart should be removed, trimmed, and weighed. Tissues should be collected for histopathological evaluation, with the heart (including atria) sectioned transversely at 3 to 5 equidistant levels. The following qualitative changes should be recorded: vacuolation of myocytes, interstitial edema, myocyte degeneration, and fibroplasia. The lesions can be assigned a semiquantitative score (16) or a more refined measurement using morphometric analysis (32). Measurement of the aforementioned parameters would allow for an accurate, reproducible, and precise evaluation of the cardiotoxic activity of ADR and DNA analogs.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Myra Grabin and Bernard Taylor for animal care and administration of injections, Reginald Reagan for electron microscopy services, and Jo F. Pelham and Cindy Bunnell for technical and editorial assistance.

REFERENCES

ADR-induced Cardiotoxicity in Rats
Adriamycin-induced Cardiotoxicity (Cardiomyopathy and Congestive Heart Failure) in Rats

F. P. Mettler, D. M. Young and J. M. Ward


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/8_Part_1/2705

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.