Metaiodobenzylguanidine: Evaluation of Its Potential as a Tracer for Monitoring Doxorubicin Cardiomyopathy

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We evaluated alterations in cardiac adrenergic neuron activity and progression of left ventricular dysfunction in comparison with the severity of structural changes using a rat model of adriamycin cardiomyopathy. Rats were treated with adriamycin (2 mg/kg s.c. once a week) for 6, 7, 8 and 9 wk. Accumulation of ^{125}I-metaiodobenzylguanidine (MIBG) 4 hr after intravenous administration was determined and left ventricular ejection fraction (LVEF) was calculated from gated blood-pool images. H & E and Masson-Trichrome stained specimens of the myocardium were examined by light microscopy. Histopathologic examination demonstrated dose-dependent myocyte damage, although there were no differences between the 8-wk and 9-wk groups. LVEF did not differ between controls and the 6-wk group (81.3% ± 5.5% versus 82.1% ± 4.8%, p = ns). LVEF began to decrease slightly in the 7-wk group (75.0% ± 5.7%, $p < 0.05$) and showed a remarkable decrease in the 8-wk group (53.7% ± 2.6%, $p < 0.001$). In the 9-wk group, LVEF diminished to 47.9% ± 3.1% ($p < 0.001$), accompanied by massive pleural effusions and ascites. MIBG accumulation in the heart (%ID/heart) significantly and progressively diminished; 1.42% ± 0.15% in the 6-wk group, 1.06% ± 0.16% in the 7-wk group, 0.77% ± 0.13% in the 8-wk group and 0.34% ± 0.11% in the 9-wk group, respectively $p < 0.001$, compared to controls (1.99% ± 0.30%). These results demonstrate that MIBG accumulation in the heart showed a greater and more linear dose-dependent decrease than LVEF. Furthermore, MIBG uptake was significantly reduced in the 6-wk group where only mild myocyte damage (isolated vacuolation or myofibrillar loss) was observed. Thus, MIBG may be a sensitive biochemical marker of adriamycin cardiomyopathy.

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Adriamycin is one of the most important cancer chemotherapeutic agents. Its spectrum of antitumor activity extends from hematologic malignancies to breast and thyroid carcinomas as well as bone and soft-tissue sarcomas. Unfortunately, repetitive administration of adriamycin and other anthracycline antibiotics is associated with significant chronic cardiotoxicity. This cardiotoxicity is dose-dependent myocardial cell damage that may culminate in congestive heart failure (*1*). The incidence of congestive heart failure is 3.5% at 400 mg/m^2, 7% at 550 mg/m^2 and 18% at 700 mg/m^2 (*2*). However, there is considerable variation in individual susceptibility to the cardiotoxic effects of adriamycin (*3*) and tumoricidal efficacy is similarly effected by dose level, especially in lymphoma and breast carcinoma (*4,5*). In clinical practice, it may be difficult to arbitrarily limit the cumulative dose of adriamycin.

Although a number of invasive and noninvasive techniques have been used to monitor the cardiotoxicity of adriamycin, only endomyocardial biopsy and radionuclide angiography have proven clinically useful (*6–9*). Serial endomyocardial biopsies are associated with considerable risk and a retrospective necropsy study of patients treated with anthracyclines has indicated the potential limitations of relying on histologic changes at the light microscopic level for predicting significant cardiotoxicity (*10*).

Radionuclide angiography is a noninvasive technique which can be performed repeatedly and serial examinations have become an effective clinical method for predicting adriamycin-induced congestive heart failure (*9*). However, in some instances, rest radionuclide angiography has been shown to be relatively insensitive; some patients with normal resting left ventricular ejection fraction (LVEF) show an abnormal response to exercise (*11*). Clinically evident congestive heart failure is a late manifestation of progressive subclinical myocardial damage and the dose-response relationship in adriamycin cardiomyopathy is frequently nonlinear (*6*). A certain amount of morphologic damage must occur before cardiac function begins to decrease, but once this critical degree of damage is reached, deterioration proceeds rapidly (*6*). Therefore, a better in-

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TABLE 1
Necropsy Findings

	No. of rats	Body weight (g)	Heart (g)	Pleural effusion	Ascites
Control	12	339 ± 29	0.88 ± 0.08	0/12	0/12
Adriamycin					
6-wk dose	6	289 ± 12*	0.83 ± 0.09	0/6	0/6
7-wk dose	5	285 ± 13*	0.77 ± 0.15	0/5	0/5
8-wk dose	8	247 ± 10†	0.66 ± 0.08†	4/8	2/8
9-wk dose	5	291 ± 35	0.62 ± 0.10†	5/5†	5/5†

*$p < 0.01$; †$p < 0.001$, compared to control.

dicator for the detection of adriamycin cardiomyopathy would be desirable.

The sympathetic nervous system is activated in chronic heart failure which may contribute to vasoconstriction and progressive impairment of left ventricular function (*12*). In numerous previous studies, it has been demonstrated that myocardial concentrations of catecholamines are significantly reduced in the failing heart (*13,14*). Recently, radiolabeled metaiodobenzylguanidine (MIBG), an analog of norepinephrine, was developed (*15*). The kinetics of MIBG accumulation in the myocardium can serve as an indicator of cardiac adrenergic neuron injury and function (*16*). In a previous study, we reported that a marked adriamycin dose-dependent reduction of myocardial MIBG accumulation, may reflect impaired cardiac adrenergic activity (*17*).

In the present study, we evaluated alterations in cardiac adrenergic neuron activity and progression of left ventricular dysfunction in comparison with the severity of structural changes using a rat model of adriamycin cardiomyopathy.

MATERIALS AND METHODS

Adriamycin was supplied from Kyowa Hakko Kogyo Co., Ltd (Japan). Iodine-125-MIBG (specific activity: 100 mCi/mg) was purchased from Daiichi Radioisotope Laboratory (Tokyo, Japan). Radiochemical purity was >99% as determined by silica gel TLC (ethyl acetate: ethanol (1:1), R_f = 0.30) and reverse-phase TLC (80% methanol R_f = 0.05).

Male Wistar rats, weighing 250 g ± 20 g (Charles River Breeding Laboratories, MA) were treated with adriamycin (2 mg/kg, sc) once a week for 6, 7, 8 and 9 wk. Control rats were injected with saline. At 2 wk after the final treatment in each group, radionuclide angiography with ^{99m}Tc-IgG (15 mCi) was performed under ketamine anesthesia. Three days later, the animals were injected with ^{125}I-MIBG (20 μCi, 740 KBq) and killed 4 hr later.

For gated blood-pool imaging, the rats were positioned supine and imaged approximately 1 cm from the 3-mm pinhole insert of a large field-of-view gamma camera interfaced to a dedicated computer (Technicare model 438/Technicare model 560, Solon, OH). This configuration results in visualization of only the chest of the rat (approximately 12× magnification). Thirty-two gated images were acquired in the frame mode using a 64 × 64 matrix over 10–12 min and LVEF was calculated as previously described (*18*).

Iodine-125-MIBG accumulation (%kg ID/g and %ID/organ) in the heart, lung, liver, spleen, kidney, adrenal gland and blood was measured with an automatic well-type gamma counter. Two-millimeter thick transverse sections perpendicular to the long-axis of the heart at the mid-ventricular level were prepared and regional accumulation of MIBG was determined in the right ventricular wall, septum and left ventricular free wall.

H & E and Masson-Trichrome-stained specimens of the myocardium were examined histopathologically by light microscopy. Mean numbers of damaged myocytes with vacuolation or myofibrillar loss were assessed from five fields (250×) in each myocardial sample. Blood samples were analyzed for serum BUN, creatinine, albumin, triglycerides and cholesterol.

Statistical Analysis

All results were expressed as mean ± s.d. The experimental groups were compared by unpaired t-tests with corrections for multiple comparisons. A p value less than 0.05 was used to define statistical significance.

RESULTS

No mortality occurred in the controls, 6-wk, 7-wk or 8-wk groups, but 50% of the rats in the 9-wk group died during the 2 wk following final adriamycin treatment. Body weights in the 6-wk, 7-wk and 8-wk groups decreased (Table 1). Heart weights in the 8-wk and 9-wk groups decreased relative to controls. Pleural effusions and ascites began to be appear in the 8-wk group and massive pleural effusions and ascites were observed in all rats of the 9-wk group. The blood chemistry data demonstrated marked hypoalbuminemia, hypertriglycemia and hypercholesteremia in the adriamycin treatment groups and BUN was increased in the 9-wk group (Table 2).

Histopathology

Histopathologic examination demonstrated dose-dependent adriamycin-induced myocyte damage, although there was no difference between the 8-wk and 9-wk groups (Fig. 1). In the 6-wk group, isolated vacuolation or myofibrillar loss was observed and there were 3–4 affected cells/field (250×). In the 7-wk group, there were 10–11 affected cells/field and some lesions showed clusters of 2–3 affected cells. In the 8-wk and 9-wk group, there were 19–20 affected cells/field and some lesions showed clusters of 3–10 affected cells (Fig. 2). Definite myocyte necrosis was not observed even in the 9-wk group.

TABLE 2
Blood Chemistry

	BUN (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control	16 ± 2	0.4 ± 0.1	1.4 ± 0.1	59 ± 15	86 ± 9
Adriamycin					
6-wk dose	15 ± 3	0.5 ± 0.1	0.6 ± 0.3**	461 ± 290**	372 ± 128**
7-wk dose	17 ± 5	0.5 ± 0.1	0.2 ± 0.1**	631 ± 233**	559 ± 105**
8-wk dose	25 ± 4**	0.6 ± 0.2	0.1 ± 0.1**	770 ± 302**	728 ± 111**
9-wk dose	29 ± 11**	0.7 ± 0.3*	0.1 ± 0.1**	846 ± 542**	654 ± 186**

*$p < 0.01$; **$p < 0.001$, compared to control.

Radionuclide Angiography

There was no difference in left ventricular global wall motion between controls and the 6-wk group (Fig. 3). Left ventricular wall motion was slightly reduced in the 7-wk group. In the 8-wk and 9-wk groups, left ventricular wall motion was markedly reduced. LVEF did not differ between controls and the 6-wk group (81.3% ± 5.6% versus 82.1% ± 4.8%, p = ns). LVEF began to decrease slightly in the 7-wk group (75.0% ± 5.7%, $p < 0.05$) and showed an abrupt decrease at 8 wk (53.7% ± 2.6%, $p < 0.001$). LVEF diminished to 47.9% ± 3.1%, $p < 0.001$) at 9 wk.

Iodine-125-MIBG Accumulation

MIBG accumulation (%kg ID/g) in the hearts of the adriamycin treated animals diminished progressively (Fig. 4); 0.47% ± 0.07% in the 6-wk group, 0.37% ± 0.09% in the 7-wk group, 0.31% ± 0.06% in the 8-wk group and 0.15% ± 0.05% in the 9-wk group. The change was significant in all groups ($p < 0.001$) when compared to controls (0.74% ± 0.09%). MIBG accumulation in the lung, spleen and adrenal gland also decreased, but the effect was less pronounced. Blood concentration of MIBG increased significantly ($p < 0.001$) in the 7-wk, 8-wk and 9-wk groups compared to controls. MIBG concentration in the kidney increased ($p < 0.001$) in the 7-wk, 8-wk and 9-wk groups. There was no change in MIBG accumulation in the liver.

When the data were expressed as %ID/organ, a similar dose-dependent decrease in myocardial accumulation of MIBG was observed; 1.42% ± 0.15% in the 6-wk group, 1.06% ± 16% in the 7-wk group, 0.77% ± 0.13% in the 8-wk group and 0.34% ± 0.11% in the 9-wk group ($p < 0.001$ for all groups when compared to controls (1.99% ± 0.30%)). Also, there was a tendency for a dose-dependent decrease in MIBG accumulation in the lung and spleen.

Regional myocardial accumulation of MIBG also showed a dose-dependent decrease (Fig. 5). MIBG concentration in the right ventricular wall was higher compared to the septum and left ventricular wall in controls and all adriamycin treatment groups.

Marked reduction in MIBG accumulation was accompanied by abnormal LVEFs; when LVEF was plotted against MIBG concentration (%ID/heart) an R^2 of 0.58 ($p < 0.001$) was obtained. However, significant reductions in MIBG accumulation were also observed in rats with almost normal LVEFs. MIBG accumulation in the heart showed a greater and more linear dose-dependent decrease than LVEF (Fig. 6).

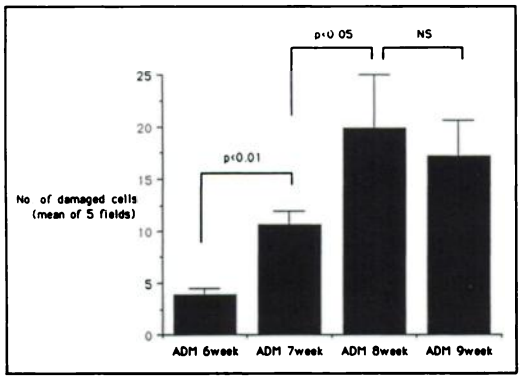

FIGURE 1. Number of damaged myocytes with vacuolation or myofibrillar loss. Adriamycin dose-dependent damage of myocytes was demonstrated, although there was no difference between the 8-wk and 9-wk groups. In the 6-wk group, only isolated vacuolation or myofibrillar loss was observed.

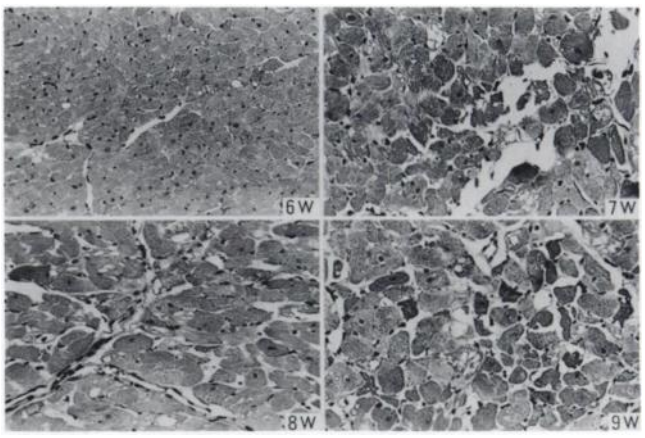

FIGURE 2. Histologic sections (H & E - 250×) of cardiac tissue from adriamycin treated rats. In the 6-wk group (6W), isolated vacuolation was observed. In the 7-wk group (7W), some lesions showed clusters of 2–3 affected cells. In the 8-wk group (8W) and 9-wk group (9W), some lesions showed clusters of 3–10 affected cells. Definite myocyte necrosis was not observed even in the 9-wk group.

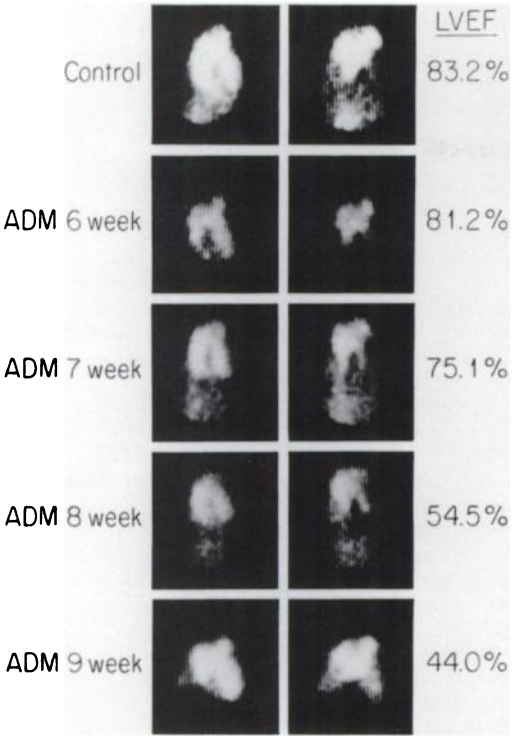

FIGURE 3. Representative gated blood pool images (end diastolic image on the left and end systolic image on the right) from each group. Left ventricular global wall motion did not differ between controls and the 6-wk group. In the 7-wk group, wall motion was slightly reduced. In the 8-wk and 9-wk groups, wall motion was markedly reduced.

DISCUSSION

Myocardial MIBG accumulation, determined 4 hr after injection, can be considered to reflect cardiac adrenergic neuron activity (*17*). The present study demonstrates that impaired cardiac adrenergic neuron function as expressed by reduced MIBG accumulation is a more sensitive indicator than LVEF for predicting congestive heart failure due to adriamycin cardiomyopathy in the rat model. Although the baseline ejection fractions were considerably higher than the values in humans, they are similar to previously reported LVEFs in rats (*18*). In the present study, LVEF did not differ between controls and animals treated with adriamycin for 6 wk. Beginning at 7 wk, LVEF began to decrease slightly and at 8 wk, it decreased abruptly. In the 9-wk group, LVEF decreased markedly and was accompanied by massive pleural effusions and ascites. Although these findings could also be related to the nephrotic syndrome (associated with hypoalbuminemia and hyperlipidemia) induced by adriamycin nephrotoxicity, they are similar to the clinical observation that myocardial function is preserved until a critical adriamycin dose is reached,

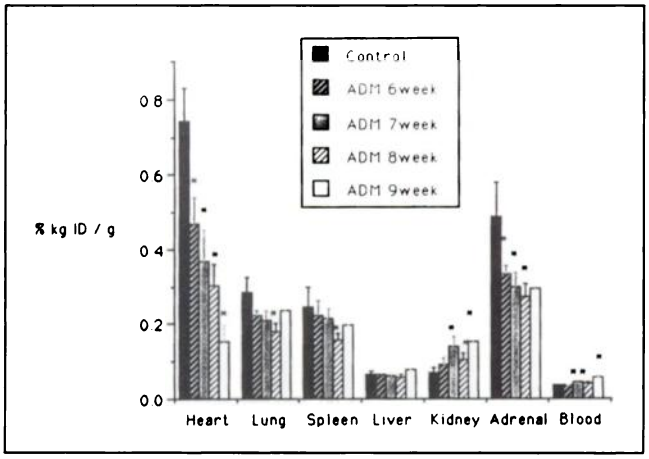

FIGURE 4. MIBG accumulation (%kg ID/g). MIBG accumulation in the hearts of adriamycin treated rats significantly and progressively diminished. MIBG accumulation in the lung, spleen and adrenal gland also decreased, but the effect was less marked. Blood and kidney concentrations increased in the 7-wk, 8-wk and 9-wk groups. There were no changes in MIBG accumulation in the liver (*p < 0.001, compared to controls).

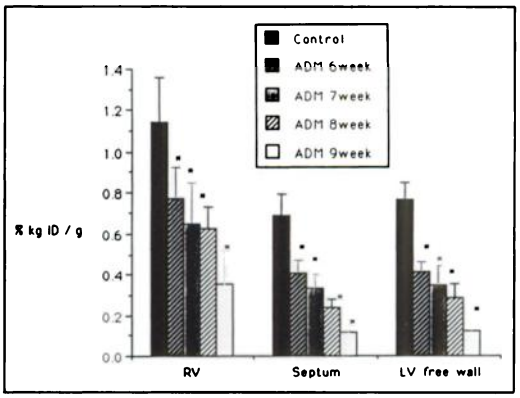

FIGURE 5. Regional myocardial MIBG accumulation. Dose-dependent decreases in MIBG accumulation were detected in the right ventricular wall (RV), septum and left ventricular wall. MIBG concentrations in RV were higher compared to the septum and left ventricular wall in both the controls and the adriamycin treatment groups (*p < 0.001, compared to controls).

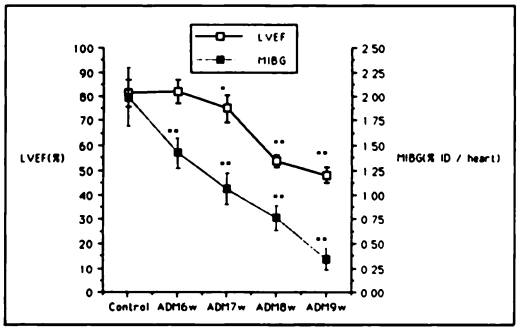

FIGURE 6. Progression of left ventricular dysfunction and decrease in MIBG accumulation. LVEF began to decrease slightly in the 7-wk group and markedly decreased in the 8-wk group. MIBG accumulation in the heart significantly diminished in the 6-wk group and showed a greater and more linear dose-dependent decrease than LVEF (*p < 0.05; **p < 0.001, compared to controls).

after which myocardial performance deteriorates rapidly (*6*).

MIBG accumulation in the heart (both %ID/heart and %kg ID/g) decreased significantly even in the 6-wk group. This dose-dependent decrease was greater and more linear than the decrease in LVEF. The finding of slightly higher MIBG concentrations in the right ventricle is consistent with previous studies of the myocardial distribution of norepinephrin in dogs (*19*), nonhuman primates (*20,21*) and humans (*22*). A possible explanation for this phenomenon is the simple dilution of sympathetic nerve endings in the left ventricle (*22*). Interestingly, there was also a tendency for a dose-dependent decrease in MIBG accumulation in the lung and spleen which are adrenergic-rich organs similar to the heart. These observations suggest that impaired adrenergic neuron activity is selectively induced in the heart in adriamycin cardiomyopathy and may be detectable by imaging.

Furthermore, by histopathological examination, we observed only mild myocyte damage (isolated vacuolation or myofibrillar loss) in the 6-wk group, and there was no difference in the severity of myocyte damage between the 8-wk and 9-wk groups. Thus, MIBG scintigraphy appears to be a sensitive and reliable method for monitoring adriamycin cardiotoxicity.

It has recently been reported that ^{111}In-antimyosin scans provide evidence of myocyte damage before LVEF deteriorates (*23*). However, the results of the present study suggest that the primary pathological features of adriamycin cardiomyopathy are vacuolar degeneration and myofibrillar loss, not myocyte necrosis which may appear after progression of myocyte damage (*24,25*). Definite evidence of myocyte necrosis was not observed even in the 9-wk group in which LVEF was markedly decreased with the appearance of massive pleural effusion and ascites. Histopathological confirmation is therefore required to validate the results of studies with antimyosin.

The following three possible mechanisms for the observed reduction of myocardial MIBG accumulation in adriamycin cardiomyopathy might be suggested: (1) acceleration of exocytotic MIBG release due to enhanced activity of the whole adrenergic nervous system; (2) energy depletion in cardiac adrenergic neurons; and (3) neuronal destruction (irreversible damage). Additional studies are needed to differentiate these possibilities.

In conclusion, myocardial MIBG accumulation in a rat model of adriamycin cardiomyopathy showed a greater and more linear dose-dependent decrease than alterations in left ventricular function and morphological changes in myocytes. Although these data suggest that scintigraphy with MIBG may be superior to serial measurements of LVEF for monitoring adriamycin cardiomyopathy, further studies, particularly in humans, are required to validate the clinical application of this radiopharmaceutical.

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