Detection of Abnormal Cardiac Adrenergic Neuron Activity in Adriamycin-Induced Cardiomyopathy with Iodine-125-Metaiodobenzylguanidine

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Radiolabeled metaiodobenzylguanidine (MIBG), an analog of norepinephrine (NE), serves as an index of adrenergic neuron integrity and function. Using a rat model of adriamycin-induced cardiomyopathy, we tested the hypothesis that abnormal cardiac adrenergic neuron activity may appear and be exacerbated dose-dependently in adriamycin cardiomyopathy. The degree of vacuolar degeneration of myocardial cells was analyzed in relation to the duration of adriamycin treatment (2 mg/kg, once a week). There were no abnormalities or only isolated degeneration in the 1- or 2-wk treatment groups, isolated or scattered degeneration in half of the 3-wk group, frequent scattered degeneration in the 4-wk group, scattered or focal degeneration in the 5-wk group, and extensive degeneration in the 8-wk group. Myocardial accumulation of [^{125}I]MIBG 4 hr after intravenous injection did not differ between the controls and the groups treated 3 wk or less. However, the 4-wk group had a slightly lower accumulation in the right ventricular wall (82% of the control) and significantly lower accumulation in the left ventricular wall (about 66% of the control: $p < 0.05$). In the 5-wk group, MIBG accumulation in the right and left ventricular wall was 35% and 27% of that in controls, respectively ($p < 0.001$). In the 8-wk group, MIBG accumulation in the right and left ventricular wall was 18% and 14% of that in controls, respectively ($p < 0.001$). Thus, MIBG accumulation in the myocardium decreased in an adriamycin dose-dependent manner. The appearance of impaired cardiac adrenergic neuron activity in the presence of slight myocardial impairment (scattered or focal vacuolar degeneration) indicates that MIBG scintigraphy may be a useful method for detection of adriamycin-induced cardiomyopathy.

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Adriamycin is a potent chemotherapeutic agent useful for a wide variety of tumors. However, its dose-dependent cardiotoxicity can irreversibly injure myocardial cells, causing congestive heart failure which is frequently fatal (*1,2*).

Electrocardiography and echocardiography have been found to be nonspecific predictors of early or impending left ventricular (LV) failure (*3*). Radionuclide angiography is currently a valuable noninvasive method for identifying patients likely to develop cardiomyopathy (*4–8*). However, radionuclide determined left ventricular ejection fraction (LVEF) at rest is frequently insensitive, and LVEF during exercise is nonspecific because it also detects minor and nonspecific abnormalities in LV performance not associated with adriamycin-caused cardiotoxicity (*5*). LVEF does not decrease linearly with the adriamycin dose level. LV function is preserved until a critical dose or degree of damage is reached, after which myocardial performance deteriorates rapidly (*9*). It is possible that fatal heart failure can suddenly develop at some total dose levels. Furthermore, because myocardial cells have already been injured irreversibly when LVEF shows a significant reduction, discontinuation of adriamycin treatment upon appearance of significant LVEF reduction probably cannot prevent persistent LV dysfunction, although it may prevent fatal aggravation of the impairment.

At present, endomyocardial biopsy is the most reliable method for monitoring cardiac damage due to adriamycin (*10*). This technique, however, is problematic in that an invasive procedure has to be repeatedly performed. It has recently been reported that ^{111}In-antimyosin scans provide evidence of myocyte damage before LVEF deteriorates (*11*). However, because this finding has not been confirmed by histopathological examination, it is unknown how well antimyosin uptake in the myocardium reflects early myocyte damage (vacuolar degeneration or myofibrillar loss). Considering the finding from histopathological examination of experimentally-induced adriamycin cardiomyopathy that myocyte necrosis appeared after progression of myocyte damage (*12*), it seems unlikely that antimyosin uptake occurs in the early stage of myocyte damage.

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Vacuolar destruction of cardiac neurons is reported in a rat model of adriamycin-induced cardiotoxicity (*13*). Damage to intrinsic cardiac neurons, induced by daunorubicin (a drug closely related to adriamycin), in patients with cancer is also reported (*14*). Adriamycin is an anthracycline antibiotic that stops RNA synthesis and also inhibits its DNA synthesis. Neurons are rich in RNA and are dependent on it for normal functioning. It is possible that cessation of RNA synthesis in neurons results in their damage. Recently, radiolabeled metaiodobenzylguanidine (MIBG), an analog of the adrenergic neurotransmitter norepinephrine (NE), was developed. Changes in myocardial MIBG accumulations are reported to reflect cardiac adrenergic neuron integrity and function (*15–17*). Therefore, we speculated that dose-dependent impairment of cardiac adrenergic neuron activity occurs in adriamycin-induced cardiomyopathy and may be a new indicator for the detection of adriamycin cardiomyopathy. To test this hypothesis, we assessed alterations in myocardial MIBG accumulations in a rat model of adriamycin-induced cardiomyopathy.

MATERIALS AND METHODS

Adriamycin powder was supplied from Kyowa Hakko Kogyo Co., Ltd. Male Wistar rats, weighing 250 ± 20 g, were injected with adriamycin (2 mg/kg, once a week via the tail vein for 1, 2, 3, 4, 5 and 8 wk. The number of animals allocated to each treatment group was 4, 4, 7, 3, 16 and 2 for 1-wk, 2-wk, 3-wk, 4-wk, 5-wk and 8-wk treatment groups, respectively (Fig. 1). The controls consisted of 25 rats injected with distilled water (1 ml/kg) via the tail vein once a week. In each group, [^{125}I]MIBG (20 μCi, 740 KBq) was injected via the femoral vein under ether anesthesia 2 wk after the final treatment with adriamycin or distilled water.

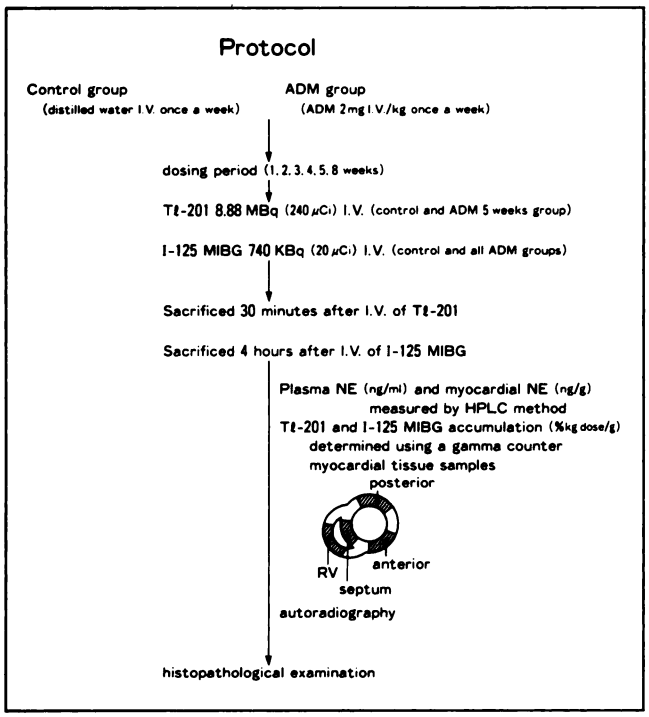

FIGURE 1. Study protocol. ADM = adriamycin and NE = norepinephrine.

Iodine-125-MIBG (Daiichi Radioisotope Laboratory) had a specific activity of 100 mCi/mg and a chemical purity of 100%. Total MIBG dose was 0.53-1.29 μg/kg.

Four hours after MIBG injection the animals were killed under ether anesthesia and autopsied. First, the animals were examined for pleural effusion and ascites. Then, blood was sampled by cardiac puncture, followed by removal of the heart, lungs, liver and kidneys.

In the control group and the 5-wk treatment group, five animals were examined for serum potassium, creatinine, BUN, triglyceride, cholesterol and albumin level and plasma-renin activity (PRA). The plasma-norepinephrine (NE) level was examined by high-performance liquid chromatography (HPLC).

Blood [^{125}I]MIBG level (%kg dose/ml) and cardiac [^{125}I]MIBG accumulation (%kg dose/g) were determined with an auto-well gamma counter.

Myocardial [^{125}I]MIBG accumulation was determined in the right ventricle (RV), septum, LV anterior and posterior walls by preparing a 2-mm thick transverse section perpendicular to the long-axis of the heart in the mid-ventricular region. The NE content in the remaining ventricular myocardium was measured by HPLC.

HE-stained specimens of myocardial regions, liver, lung and kidney were histopathologically examined by light microscopy. For myocardial tissue specimens, the degree of vacuolar degeneration of myocytes, a characteristic of adriamycin-induced cardiomyopathy, was graded on a six-grade scale (Table 1).

Iodine-125-MIBG (3700 KBq) was injected via the tail vein to one animal each from the control group and the 5-wk treatment group. Four hours later, the heart was removed from these animals for semi-microautoradiography.

The autoradiograph was analyzed using a computer-assisted image-processing system (Fujix bioimage analyzer BAS 2000). Thallium-201 (240 μCi, 8.85 MBq) was injected via the tail vein in five animals each from the control group and the 5-wk treatment group. Thirty minutes later, they were killed for determination of blood ^{201}Tl level (% kg dose/ml) and myocardial ^{201}Tl accumulation (% kg dose/g) with an auto-well gamma counter.

Statistical Analysis

Results were expressed as mean ± s.d. Rat groups were compared with unpaired t-tests. A p value less than 0.05 defined statistical significance.

RESULTS

The control group (n = 25) showed satisfactory weight gain from the pre-dosing level of 270 ± 14 g to the post-

TABLE 1
Morphologic Evaluation of Myocardial Damage

Degrees	Definition
0	Normal
0.5	Isolated minimally affected cells
1	Scattered affected cells
2	Focal groups of affected cells
3	Confluent groups of affected cells
4	Most of the cells damaged

dosing level of 375 ± 36 g (2 wk after the end of the dosing period) (Table 2). Pre-dosing body weight for adriamycin-treated animals was 266 ± 35 g (n = 36). Post-dosing body weight (determined 2 wk after the final treatment) was 334 ± 13 g for the 1-wk treatment group (n = 4), 330 ± 14 g for the 2-wk group (n = 4), 328 ± 22 g for the 3-wk group (n = 7), 283 ± 32 g for the 4-wk group (n = 3), 236 ± 31 g for the 5-wk group (n = 16) and 155 ± 7 g for the 8-wk group (n = 2). Thus, the post-dosing body weight in treated animals was lower than that in the controls, and the difference became more marked as the total dose (dosing period) increased. Pleural effusion and ascites were absent in the 3 wk or less treatment groups, while they were observed in 33%, 31%, and 50% of animals in the 4-wk, 5-wk and 8-wk treatment groups, respectively. The ratio of heart weight-to-body weight in the 5-wk treatment group (0.0034 ± 0.0002, $p < 0.001$) and the 8-wk treatment group (0.0041 ± 0.0003, $p < 0.001$) was significantly higher than that in the controls (0.0025 ± 0.0001).

Compared to the controls, the 5-wk treatment group showed significant reduction in PRA (0.63 ± 0.13 versus controls 29.1 ± 11.0, $p < 0.01$) and serum albumin (3.15 ± 0.53 versus controls 3.86 ± 0.24, $p < 0.01$) as well as significant increase in cholesterol (554 ± 174 versus 53 ± 6, $p < 0.001$) and triglyceride (2097 ± 824 versus 169 ± 69, $p < 0.001$). Between the control and 5-wk treatment groups, no significant difference was observed in creatinine (0.47 ± 0.15 versus 0.42 ± 0.04), BUN (21.7 ± 7.5 versus 14.4 ± 2.4) and potassium (5.7 ± 0.8 versus 5.9 ± 0.7).

Histopathology

The groups treated for 4 wk or less showed no signs of congestion of the lung and liver. In the 5-wk treatment group, slight congestion of the lung and liver was observed. Congestion of these organs became more marked in the 8-wk treatment group, although it was not severe and not accompanied by centrilobular hepatic necrosis. Renal changes such as congestion, dilated tubules and vacuolated glomeruli began to be seen in the 5-wk treatment group and were enhanced in the 8-wk treatment group.

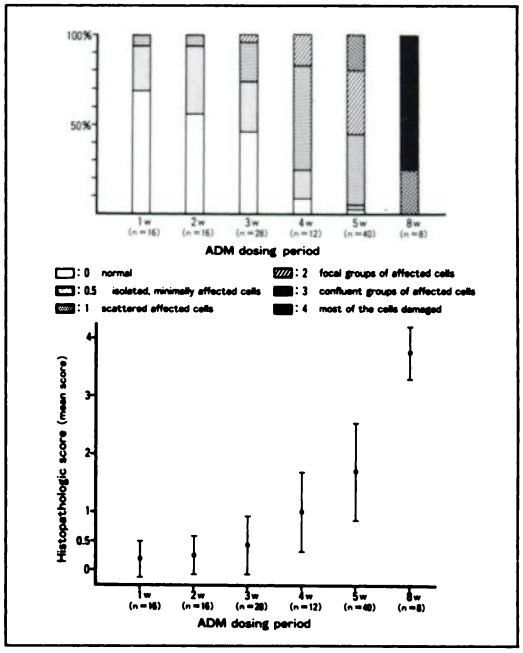

FIGURE 2. Myocardial damage dependent on adriamycin dosing period. The upper row shows the incidence of each grade of lesions at each adriamycin treatment period. Mean scores for each treatment group are shown in the lower row. Animals treated with adriamycin for 3 wk or less showed no abnormalities or only isolated myocyte degeneration. In the 4-wk treatment group, on the other hand, scattered myocyte degeneration was observed. In the 5-wk treatment group, scattered or focal myocardial degeneration was apparent. After 8 wk of adriamycin treatment, most myocardial cells showed a high degree of degeneration. ADM = adriamycin, W = week, and N = number of tissue samples.

When vacuolar degeneration was examined in myocardial specimens from different regions in relation to the duration of adriamycin treatment, groups treated for 1 wk or 2 wk showed no abnormalities or only isolated vacuolar degeneration. The 3-wk group showed no abnormalities in half of the cases but did show isolated or scattered degeneration in half of the cases. In the 4-wk treatment group, however, normal findings markedly decreased, and scattered vacuolar degeneration tended to be frequently observed. In the 5-wk treatment group, about half of the animals showed scattered vacuolar degeneration, and focal vacuolar degeneration was also frequently observed. In the 8-wk treatment group, severe myocardial damage, represented by extensive vacuolar degeneration, was noted (Figs. 2 and 3).

Thallium-201 Accumulation

Thallium-201 accumulation (%kg dose/g) in each region of the myocardium 30 min after injection did not significantly differ between the control and 5-wk treatment groups (RV, 0.60 ± 0.13 versus 0.73 ± 0.25; septum, 0.52 ± 0.08 versus 0.59 ± 0.16; anterior wall, 0.54 ± 0.06 versus

TABLE 2
Necropsy Findings

	No. of rats	Body weight (mean ± s.d. (g))	Ascites	Pleural effusion	Heart/Body weight ratio (mean ± s.d.)
Control	25	375 ± 36	0	0	0.0025 ± 0.0001
Adriamycin:					
1-wk dose	4	334 ± 13	0	0	0.0025 ± 0.0002
2-wk dose	4	330 ± 14	0	0	0.0026 ± 0.0002
3-wk dose	7	328 ± 22	0	0	0.0028 ± 0.0003
4-wk dose	3	283 ± 32†	1	1	0.0030 ± 0.0004
5-wk dose	16	236 ± 31†	5*	4*	0.0034 ± 0.0002†
8-wk dose	2	155 ± 7†	1	1	0.0034 ± 0.0003†

* $p < 0.01$ compared to controls.
† $p < 0.001$ compared to controls.

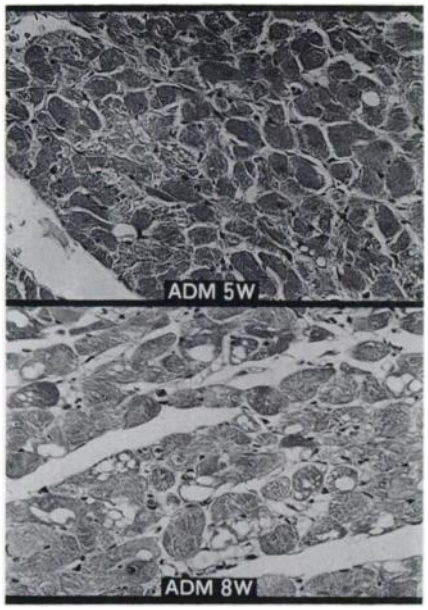

FIGURE 3. The upper row shows the left ventricular myocardium from a rat after a 5-wk intravenous adriamycin treatment (2 mg/kg/wk). Note focal groups of affected cells. The lower row shows the left ventricular myocardium from a rat after an 8-wk intravenous adriamycin treatment (2 mg/kg/wk). Note damage to most cells. H & E × 330. ADM = adriamycin and W = week.

0.53 ± 0.16; posterior wall, 0.56 ± 0.07 versus 0.61 ± 0.16). The blood ^{201}Tl level (%kg dose/ml) was significantly higher in the 5-wk treatment group (0.020 ± 0.006) than in the controls (0.014 ± 0.002) ($p < 0.05$).

Myocardial NE Content and Plasma NE Concentration

The myocardial NE content (Fig. 4) did not significantly differ between the groups treated for 3 wk or less and the controls. In comparison to the controls (799 ± 144 ng/g), this parameter was significantly lower in the 4-wk treatment group (438 ± 168, $p < 0.01$), the 5-wk treatment group (488 ± 114, $p < 0.001$) and the 8-wk treatment group (421 ± 110, $p < 0.001$). However, the parameter did not significantly differ between any two of the 4-, 5- and 8-wk treatment groups. Thus, the decrease of this parameter was not dose-dependent.

Plasma NE concentration also did not significantly differ between the 3-wk or less treatment groups and the controls. In comparison to the controls (5.8 ± 3.9 ng/ml), the parameter was significantly higher in the 4-wk treatment group (17.7 ± 15.37, $p < 0.05$), the 5-wk group (17.7 ± 12.7, $p < 0.01$) and the 8-wk group (17.7 ± 4.5, $p < 0.001$), although the increase in this parameter was not dose-dependent.

Iodine-125-MIBG Accumulation in the Heart

In the control group, [^{125}I]MIBG accumulation (Fig. 5) 4 hr after injection was significantly higher in the right ventricle (0.89 ± 0.14 %kg dose/g) than in the septum (0.64 ± 0.08), the anterior wall (0.64 ± 0.09) and the posterior wall (0.66 ± 0.10) ($p < 0.01$). This higher accumulation in the RV than in the other myocardial areas was also observed in each adriamycin treatment group ($p < 0.01$).

Accumulation in any myocardial region did not significantly differ between the groups treated for 3 wk or less

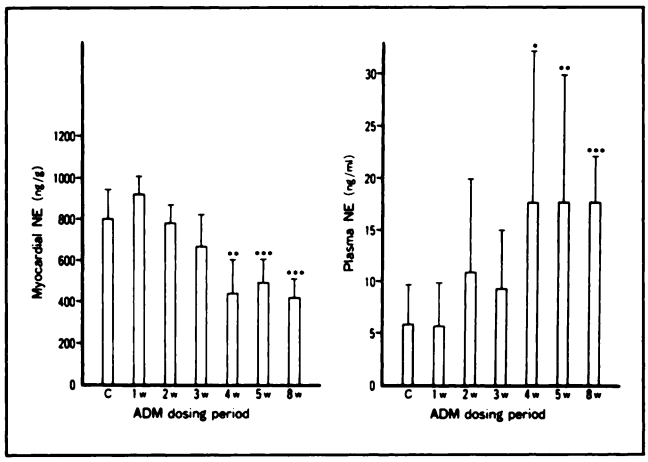

FIGURE 4. Myocardial NE content and plasma NE concentration related to adriamycin dosing period. Adriamycin treatment for 3 wk resulted in no significant change in myocardial NE content as compared to the controls. Myocardial NE content significantly decreased in the 4-, 5-, and 8-wk treatment groups as compared to the controls, although the parameter did not significantly differ between any two of the 4-, 5- and 8-wk treatment groups (i.e., no dose-dependent decrease). An opposite tendency of change was observed also for the plasma NE concentration. ADM = adriamycin, C = control, W = duration of dosing in weeks, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (versus controls).

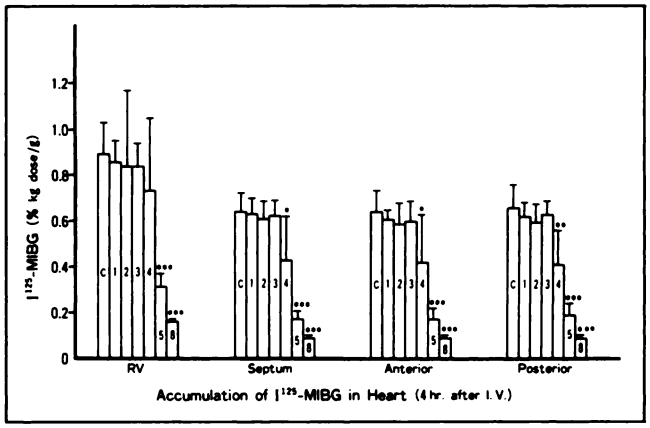

FIGURE 5. Iodine-125-MIBG accumulation in the heart (4 hr after injection). Accumulation was higher in the right ventricular wall than in other areas of the myocardium in the control group and all adriamycin-treated groups. Iodine-125-MIBG accumulation in the 3-wk or less treatment groups did not significantly differ from that in controls. In the 4-wk treatment group, accumulation in the right ventricular wall was about 82% of that in controls (not significant) and accumulation in the left ventricular wall was about 66% of the controls ($p < 0.05$). In the 5-wk treatment group, accumulation in the right and left ventricular wall was about 35% ($p < 0.001$) and 27% ($p < 0.001$) of the controls. In the 8-wk group, it was about 18% ($p < 0.001$) and 14% ($p < 0.001$) of the controls, respectively. Thus, [^{125}I]MIBG accumulation in the heart decreased in an adriamycin dose-dependent manner. C = control (n = 8). 1: 1-wk adriamycin treatment (n = 4); 2: 2-wk adriamycin treatment (n = 4); 3: 3-wk adriamycin treatment (n = 6); 4: 4-wk adriamycin treatment (n = 3); 5: 5-wk adriamycin treatment (n = 7); 8: 8-wk adriamycin treatment (n = 2); and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (versus controls).

and the controls. In the 4-wk treatment group, accumulation in all regions but the RV was significantly lower than that in the controls (RV, 0.73 ± 0.32 versus 0.89 ± 0.14 %kg dose/g, p = ns; septum, 0.43 ± 0.19 versus 0.64 ± 0.08, $p < 0.05$; anterior wall, 0.42 ± 0.21 versus 0.64 ± 0.09, $p < 0.05$; posterior wall, 0.41 ± 0.15 versus 0.66 ± 0.10, $p < 0.01$). In the 5-wk treatment group, accumulation in all regions was significantly lower than in the controls (RV, 0.31 ± 0.06, $p < 0.001$; septum, 0.17 ± 0.04, $p < 0.001$; anterior wall, 0.17 ± 0.05, $p < 0.001$; posterior wall, 0.19 ± 0.05, $p < 0.001$). In the 8-wk treatment group, a larger, significant decrease of accumulation was observed for each myocardial region as compared to the controls (RV, 0.16 ± 0.007, $p < 0.001$; septum, 0.085 ± 0.007, $p < 0.001$; anterior wall, 0.085 ± 0.007, $p < 0.001$; posterior wall, 0.085 ± 0.007, $p < 0.001$). Thus, myocardial [^{125}I]MIBG accumulation showed a marked, dose-dependent decrease in adriamycin-treated animals.

The blood [^{125}I]MIBG level did not significantly differ between the groups treated for 3 wk or less and the controls. In comparison to the controls (0.04 ± 0.01 %kg dose/ml), the parameter was significantly higher in the 4-wk group (0.06 ± 0.01, $p < 0.01$), the 5-wk group (0.07 ± 0.01, $p < 0.001$) and the 8-wk group (0.07 ± 0.01, $p < 0.001$).

Iodine-125-MIBG Semi-Microautoradiography

Figure 6 shows the myocardial accumulation of [^{125}I] MIBG obtained 4 hr after injection to one animal each from the control group and the 5-wk treatment group. In the control animal, only the apical region showed reduced accumulation and high accumulation was observed in the right and left ventricular myocardium. In the 5-wk treatment animal, markedly reduced accumulation was observed in the right and left ventricular myocardium, as well as in an extensive area of the apical region.

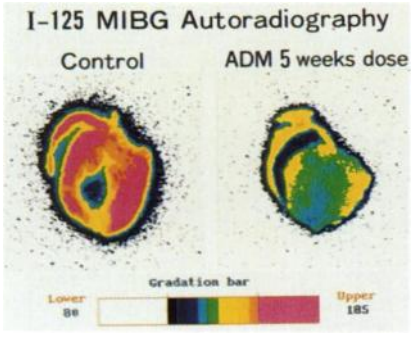

FIGURE 6. Iodine-125-MIBG semi-microautoradiography from single animals each from the control group and the 5-wk treatment group. The control animals showed reduced accumulation only in the apical region. The 5-wk treatment animals showed an intense, extensive reduction of accumulation in the apical region as well as a markedly reduced accumulation in the right and left ventricular wall. ADM = adriamycin.

Relationship Between the Degree of Vacuolar Degeneration and [^{125}I]MIBG Accumulation

Figure 7 shows the relationship between the degree of myocyte vacuolar degeneration and [^{125}I]MIBG accumulation for both RV and LV myocardium (including the septum). In both adriamycin-treated animals and untreated controls, accumulation was lower in the left ventricle than in the right ventricle. Figure 7 also shows some correlation between the histopathologic score and MIBG accumulation. MIBG accumulation was markedly reduced not only in specimens showing marked vacuolar degeneration but also in specimens showing slight or no degeneration.

DISCUSSION

Although not a perfect analog of [^{3}H]NE, [^{125}I]MIBG appears to enter and leave the heart in patterns similar to those of [^{3}H]NE (*15*). Therefore, kinetics of [^{125}I]MIBG in the myocardium can serve as an indicator of cardiac adrenergic neuron injury and function. When the time course of MIBG accumulation in the vesicles at the adrenergic neuron terminal was examined to clarify the time when myocardial MIBG accumulation best reflects cardiac adrenergic activity, extra-vesicular accumulation rapidly decreased after MIBG injection while intra-vesicular accumulation was relatively constant after MIBG injection to reach a plateau level (about 50% of the myocardial level) 4 hr later (*18*). However, myocardial MIBG accumulation is influenced by specific activity, and it markedly decreases following a high MIBG dose (*19*). A more recent rat experiment using MIBG with a higher specific radioactivity revealed accumulation of 80%–90% of MIBG in cardiac neurons 3 hr after injection (dose: 0.2-1.0 μg/kg) and storage of 70%–80% in adrenergic vesicles (Inoue M, unpublished data). In this study, MIBG with high specific activity (100 mCi/mg) was used at a low dose (0.53–1.29 μg/kg). Therefore, myocardial MIBG accumulation, determined 4 hr after injection in the present study, can be regarded as reflection of cardiac adrenergic neuron activity.

In the control group and in each adriamycin-treated group, [^{125}I]MIBG accumulation in RV myocardium 4 hr after injection was higher than that in the ventricular septum and LV anterior and posterior walls, supporting previous findings that adrenergic nerve control of the right ventricle was predominant over that of the left ventricle (*20*). Myocardial [^{125}I]MIBG accumulation did not significantly differ between the groups treated with adriamycin for 3 wk or less and the control group. In the 5-wk treatment group, however, [^{125}I]accumulation in all myocardial regions but the right ventricle was lower than the control values (by about 35%). In the 5-wk treatment group, accumulation in each myocardial region was lower than that in the controls (by about 73% in the left ventricle). In the 8-wk group, accumulation further decreased (about 86% lower than the control value in the left ventricle). Thus, a dose-dependent decrease in myocardial ^{125}I

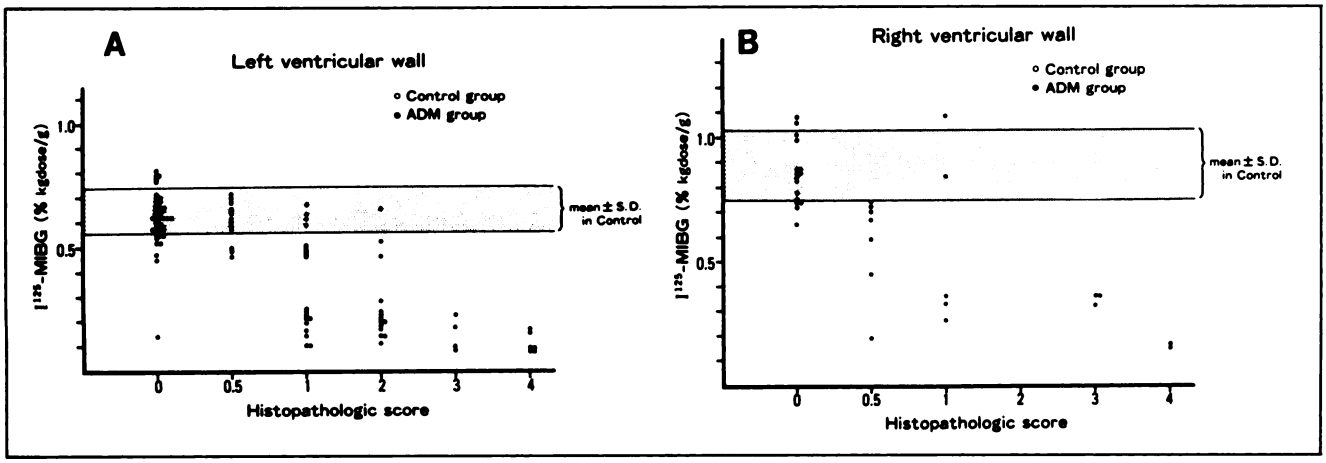

FIGURE 7. Relationship between the degree of myocardial tissue damage (expressed as scores) and [^{125}I]MIBG accumulation. In myocardial tissue that had suffered a high degree of cellular damage, MIBG accumulation was often markedly decreased even in tissues that showed only slight cellular damage. ADM = adriamycin.

accumulation was observed in adriamycin-treated animals, suggesting that adriamycin impairs cardiac adrenergic neuron activity. Myocardial NE content was also significantly lower in the 4-wk, 5-wk and 8-wk treatment groups than in the control group. However, the degree of its reduction was lower (40%–47% reduction from the controls), and the decrease was not dose-dependent.

It has been reported that [^{3}H]NE uptake is more sensitive than myocardial NE content as an indicator of adrenergic reinnervation of surgically impaired canine heart (*21*). In the present study, myocardial abnormalities were reflected more closely by myocardial accumulation of [^{125}I] MIBG (an NE analog) than myocardial NE content, suggesting that myocardial MIBG accumulation may be a more sensitive indicator of cardiac adrenergic neuron integrity and function. The reduction in myocardial [^{125}I] MIBG accumulation was linearly related to the adriamycin dose level even at the following stages: (1) a stage where vacuolar degeneration shows a scattered or focal distribution and slight morphological abnormalities are observed under a light microscope; and (2) a stage where myocardial ^{201}Tl accumulation [an indicator of myocardial blood flow that may decrease related to a decrease in myocardial cells per unit mass or injured but viable myocardial cells that might require less oxygen and, accordingly, less flow (*22*)] does not differ between the control and adriamycin-treated groups. This finding suggests that impairment of cardiac adrenergic neuron integrity and function (reflected into reduced myocardial MIBG accumulation) is a new and useful indicator for clinical detection of adriamycin cardiomyopathy.

We did not perform radionuclide angiography. Therefore, it is not clear that assessment of cardiac adrenergic neuron activity will be useful beyond the conventional radionuclide determined LVEF. Two possible mechanisms for the decrease of myocardial MIBG accumulation are: (1) adriamycin myocardial toxicity causes a cardiomyopathic change, which leads to congestive heart failure and systemic adrenergic hyperactivity, resulting in enhanced NE release from cardiac adrenergic neurons, i.e., enhanced washout of [^{125}I]MIBG from the heart; and (2) disruption of cardiac adrenergic neuron integrity and function due to adriamycin cardiac neurotoxicity.

In the present study, the groups treated with adriamycin for 4 wk or more, which showed reduced myocardial MIBG accumulation, had an increased plasma NE and a reduced myocardial NE level, reflecting systemic hyperactivity of the adrenergic nervous system. In the 5-wk treatment group, however, myocardial MIBG accumulation was markedly reduced, although congestion of the lung, liver and kidney was very slight. Considering a previous report that 10-wk treatment of rats with 2 mg/kg adriamycin (intravenously once a week) resulted in apparent congestive heart failure accompanied by cardiomegaly and centrilobular hepatic necrosis (*13*), the adriamycin treatment period in the present study seems to be relatively short. We cannot rule out that pleural effusion and ascites observed in the present study are associated with nephrotic syndrome due to adriamycin nephrotoxicity (low plasma renin and albumin levels as well as hyperlipidemia), because there was only mild histopathologic evidence of congestive heart failure.

The plasma NE level, which has been regarded as an indicator of systemic adrenergic hyperactivity, showed a significant increase but no adriamycin dose-dependent increase, contrary to myocardial MIBG accumulation which showed a decrease dependent on adriamycin doses. The reduction in myocardial accumulation of MIBG was greater in LV myocardium than in RV myocardium where the adrenergic nervous system is predominant. When these results are taken together with the histopathological evidence of slight congestive heart failure, it is difficult to explain the reduced myocardial MIBG accumulation based on systemic adrenergic hyperactivity alone.

In addition to the established myocardial toxicity, an animal study suggests that adriamycin has a neurotoxic

effect on the peripheral nervous system, resulting in neuronal nuclear abnormalities and neuronal necrosis (*23*). Damage to the intrinsic cardiac neurons by adriamycin or daunorubicin (a drug closely related to adriamycin) was seen in a rat model of adriamycin-induced cardiotoxicity (*13*) and in a postmortem study of clinical cases (*14*).

A number of laboratories have failed to confirm neurotoxicity in the heart, and the effect of adriamycin on adrenergic neuron in the heart is not well established. However, these past reports are from a histopathological study on neuronal damage and not from a study of adrenergic neuron activity such as performed in this present study. Because reduced myocardial [^{125}I]MIBG accumulation in this study is difficult to explain only by systemic adrenergic hyperactivity due to congestive heart failure, adriamycin-induced cardiac neurotoxicity and disruption of cardiac adrenergic neuron integrity are strongly suggested.

In summary, a marked reduction of myocardial [^{125}I] MIBG accumulation, which reflects impaired cardiac adrenergic activity, was observed in the presence of slight morphological abnormalities of myocytes (i.e., in the presence of scattered or focal vacuolar degeneration of myocytes). This finding indicates that evaluation of cardiac adrenergic activity provides a new and useful approach for detection of adriamycin-induced cardiomyopathy which causes fatal congestive heart failure.

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