Myocardial Uptake of $^{111}$In Monoclonal Antimyosin Fab in Detecting Doxorubicin Cardiotoxicity in Rats

Morphological and Hemodynamic Findings

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Background. The therapeutic value of doxorubicin (DOX) is limited by its cardiotoxicity, which is dose dependent. To improve the detection of such myocardial damage, this study was designed to determine whether the $^{111}$In antimyosin antibody Fab could serve as a marker for cardiotoxicity in treated versus control rats on the basis of comparative morphological and hemodynamic findings.

Methods and Results. DOX was administered by intravenous injection to rats at a dose of 3 mg·kg$^{-1}$·week$^{-1}$ for 5 weeks. Three weeks after the final injection, an intravenous dose of $^{111}$In antimyosin, 740 kBq (20 µCi), was administered, and tissue distribution of the radioisotope (percent kilogram dose per gram) was assessed in 48 hours. Myocardial uptake of radioactivity by both ventricles was more prominent in the DOX-treated rats than in control rats ($p<0.001)$. The heart-to-blood and heart-to-lung uptake ratios were markedly higher in the treated rats than in controls ($p<0.001)$. As the severity of the myocardial damage increased, there was a progressive increase in myocardial uptake. There was a strong correlation between the severity of myocardial damage and the ventricular end-diastolic pressure ($r=0.84$ and $r=0.83$ in the left and right ventricles, respectively). On microscopic immunoradiography of the DOX-treated heart, there was a specific immunolocalization of the radiotracer in the injured myocytes but no radioactivity in the control myocytes.

Conclusions. $^{111}$In antimyosin antibody appears to be a useful immunoradiotracer in detecting cardiac damage induced by DOX administration and in assessing the severity of cardiotoxicity. These data reinforce the clinical observation that myocardial imaging using $^{111}$In antimyosin Fab is able to provide information to guide the course of patients receiving DOX treatment. (Circulation 1992;86:1965–1972)

Key Words: Inium • antibodies • doxorubicin • cardiotoxicity • radiography

Although the anthracycline antibiotic doxorubicin is highly effective in treating certain hematologic malignancies as well as solid tumors, its administration is limited by a dose-dependent cardiotoxicity that may lead to congestive heart failure and even death. Various techniques have been used to quantify the severity of myocardial damage and cardiac dysfunction, among them cardiac catheterization, endomyocardial biopsy, radionuclide ventriculography, and echocardiography.

Experience is beginning to accumulate on myocardial imaging using radiopharmaceuticals to detect such drug-induced toxicity. Since the development of monoclonal antimyosin antibody Fab by Khaw et al.$^{7,8}$ $^{111}$In-labeled antimyosin has allowed the noninvasive detection of myocardial damage in acute myocardial infarction.$^{9,10}$ and myocarditis,$^{11–13}$ myocardial injury in cardiomyopathy,$^{14}$ and rejection after heart transplantation.$^{15–17}$ More recently, Estorch et al.$^{18}$ have investigated this method in evaluating the cardiotoxicity of doxorubicin in cancer patients.

The present investigation was designed to determine whether $^{111}$In antimyosin antibody Fab would be useful in evaluating the myocardial damage induced by doxorubicin in rats compared with results of morphological studies and hemodynamic observations.

Methods

Materials

Doxorubicin hydrochloride was generously donated by the Kyowa Hakko Kogyo Corp., Japan. The monoclonal antimyosin antibody Fab (R11D10, Centocor, Malvern, Pa.), 0.5 mg, conjugated to diethylenetriamine pentaacetic acid and labeled with $^{111}$In, was donated by the Daiichi Isotope Corp., Japan. The mouse monoclo-
nal anti–human IgG antibody Fab purchased from Oxoid Ltd., USA was used as an irrelevant antibody. Radioiodination of the anti-IgG antibody was by the Iodo-Gen method using [125I]sodium iodide (Amersham Japan). The radiolabeled anti-IgG antibody Fab was purified by exclusion chromatography on a Bio-Gel P-6 column (Bio-Rad Laboratory, Richmond, Calif.). The percentage of binding of isotope to the antibody was determined by thin-layer chromatography and exceeded 95%. The cross-reactivity of the anti–human IgG antibody Fab with rat IgG was examined by a method of enzyme immunoassay, showing little cross-reactivity of the antibody with rat IgG.

Animals and Experimental Protocol

Six-week-old male Wistar-Imamichi rats (weight, 154±15 g) were used in this study. They were allowed food and water ad libitum and were maintained under a constant room temperature and a 12-hour light–dark schedule. They were weighed each week, and their general condition was observed. The animals were divided into two groups. In the doxorubicin-treated group, the drug dissolved in 0.9% saline was administered intravenously to 20 rats at a dosage of 3 mg/kg given weekly for 5 weeks. That dosage was determined in a pilot study that showed that doxorubicin-related morphological damage and impairment of cardiac function would be induced in animals exposed to that dosage. The cumulative dose of 15 mg/kg in this study was considered to be equivalent to a dose of more 500 mg·kg⁻¹·m⁻² in a 50-kg human. The control group consisted of 11 rats that received an equal volume of 0.9% saline administered intravenously each week for the same period. Three weeks after the final injection of doxorubicin, In antimyosin was injected intravenously in control and doxorubicin-treated rats, and they were killed 48 hours later. In addition, a separate study was conducted with 125I-labeled monoclonal anti-IgG Fab antibody to determine whether the antimyosin antibody was specific to doxorubicin-induced damaged myocardium. Anti-IgG antibody was injected intravenously in 10 control and 10 doxorubicin-treated rats, and they were also treated in the same manner as described above.

Hemodynamic Measurements

At week 7 of the experiment, baseline hemodynamics were assessed after the administration of sodium pentobarbital (50 mg/kg i.p.). The right jugular vein was cannulated with a polyethylene catheter connected to a Statham transducer (P10EZ, Gould, Inc., Cleveland, Ohio). The catheter was advanced into the right ventricle. The external right carotid artery was exposed and cannulated with a micromanometer-tipped catheter (PR249, Millar Instruments, Houston, Tex.). Measurements were thus obtained of both the ventricular systolic and end-diastolic pressures and positive dP/dt of the left ventricle, aortic pressure, and heart rate by ECG monitoring.

Morphological Studies

After cardiac catheterization, the rats were killed by exsanguination. The heart and skeletal muscle were excised and fixed in 10% buffered formalin for histological study. Paraffin-embedded sections 4 μm thick were cut, stained with hematoxylin and eosin and Masson's trichrome stain, and mounted. For ultrastructural examination, samples were fixed in 3% glutaraldehyde, post fixed in a 2% solution of osmium tetroxide, and embedded in Epon. Sections were examined by electron microscopy. Morphological changes consistent with anthracycline damage based on a combination of light and electron microscopic findings were evaluated using Billingham et al’s grading system with ratings made on a scale of 0 to 3 as follows: 0, no change from normal; 1, scanty cells showing early myofibrillar loss and/or dis tended sarcoplasmic reticulum; 2, more widespread changes with marked myofibrillar loss and/or cytoplasmic vacuolization; and 3, diffuse myocyte damage with marked cellular changes and cell necrosis. Quantitative analysis of the myocardial damage was performed independently by two investigators who had no knowledge of the hemodynamic findings and myocardial uptake of tracer.

Tissue Distribution Studies

Each animal received a single intravenous injection of 740 kBq (20 μCi) of 111In antimyosin antibody or 125I-labeled anti-IgG antibody Fab, which was not an intact IgG, and 48 hours later each animal was killed by exsanguination via a cardiac puncture. Samples of the heart (right and left ventricles), lung, liver, skeletal muscle, sternum, and blood were obtained, weighed, and counted in an autogamma well counter with corrections made for background, radioactive decay, and count efficiency. Tissue uptake was expressed as the percent kilogram dose per gram (% kg dose/g=[μCi in organ/ g+μCi (dose)/kg body weight]×100). In addition, heart-to-blood ratios and heart-to-lung ratios of 111In antimyosin antibody were calculated from tissue uptake.

Immunoradiography of Heart

Each animal was injected intravenously with 3.7 MBq (100 μCi) of 111In antimyosin antibody and killed 48 hours later. Transverse sections of the excised heart filled with carboxymethyl cellulose gel were quickly frozen by immersion in acetone and dry ice at −80°C. The frozen heart sections were cut on a cryomicrotome (NA 200, Nakagawa, Tokyo) at −20°C into 20-μm-thick sections. These sections were placed onto an imaging plate (20×40 cm) with a resolution of 10 pixels/mm (Fujix Bio-imaging Analyzer BAS2000, Fuji Photo Film Co., Ltd., Tokyo) for 1 hour of exposure time. The dose of radioactivity in the heart sections was quantified promptly on the cathode ray tube screen and was printed in color. For a histological autoradiography, cardiac specimens were fixed in periodate-lysine-parafomaldehyde fixative (0.01 M sodium metaperiodic acid, 0.075 M lysine monohydrochloride, 2% paraformaldehyde, 0.0375 M phosphate buffer) for 6 hours at 4°C and quickly frozen in optimum cutting temperature compound (Miles Scientific, Elkhart, Ind.) cooled in liquid nitrogen. Cryostat sections at 2 μm were thaw-mounted on albumin-coated slides. The slides were dipped in Konika NR-M2 emulsion (Konika Corp., Tokyo) diluted 1:1.5 with water at 43°C, dried for 30 minutes, and stored in light-tight boxes at 4°C. After 6–8 weeks’ exposure, the slides were developed in Konidol X developer (Konika Corp., Tokyo), rinsed in 0.1 M acetic acid, fixed in Konifix (Konika Corp., Tokyo), and stained with hematoxylin-eosin. Sections
were examined and photographed in an Olympus AH2 microscope (Olympus Corp., Tokyo).

Statistical Analysis
The results are expressed as mean±SD. Comparisons between the two groups were performed by two-tailed, paired or unpaired Student's t-tests or Mann-Whitney test. One-way ANOVA and a Sheffe's method of comparison analysis were used to determine the statistical significance of the differences between the three groups. Linear correlation and least-squares linear regression also were performed. The accepted level of significance was p<0.05.

Results
Baseline Hemodynamics
Of the 20 rats given doxorubicin, six died during the experiment of congestive heart failure, and the remaining 14 developed pleural effusion, pericardial effusion, and ascites associated with heart failure. Significant weight loss was observed in the doxorubicin-treated rats compared with controls (Table 1). The ratio of heart to body weight was markedly increased in the drug-treated animals, suggesting myocardial hypertrophy. In the treated rats, both left and right ventricular end-diastolic pressures were significantly increased, whereas the systolic left ventricular and mean aortic pressures were decreased. A substantial decrease in positive dP/dt of the left ventricle was also evident in the treated rats, indicating a global impairment of left ventricular function.

Morphological Studies
On global examination of the heart, left and right ventricular dilatation was observed. Variable myocardial damage occurred with focal changes (Figure 1). That is, severely damaged cardiomyocytes were often observed adjacent to normal-appearing myocardial cells. The myocardium was characterized by degeneration of varying severity with cytoplasmic vacuolization, cellular hypertrophy, interstitial edema, and perivascular fibrosis. No cellular infiltration was observed in the myocardium. On electron microscopy, the damaged cardiomyocytes showed sarcoplasmic vacuolar degeneration, myofibrillar disorganization, mitochondrial swelling, disruption of the cristae, and the presence of myelin figures. Evaluation of severity of histopathological changes in the myocardial samples revealed mean scores of 2.2±0.7 and 1.5±0.7 in the left and right ventricles (p<0.01), respectively, being worse in the left ventricle than in the right in all treated animals. The effects of doxorubicin on skeletal muscle were very mild compared with the heart, with no areas of either myocyte damage or edema observed.

Tissue Distribution Studies
Table 2 summarizes the concentrations of 125I antimyosin in the blood, heart, lung, liver, skeletal muscle, and sternum of the treated versus control rats. The myocardial uptake of radioactivity was significantly increased in the drug-treated animals compared with the control group (p<0.001). The left ventricular uptake of the tracer was significantly higher than that of the right ventricle (p<0.001) in all doxorubicin-treated rats. In contrast, there was a significantly lower uptake of radioactivity in the blood, muscle, and sternum of the treated rats (p<0.001). No differences in the uptake of radioactivity in liver and lung were noted between the treated and control groups. A summary of the concentration ratios of 125I antimyosin for heart to blood and heart to lung appears in Table 3. The ratios of left and right ventricles to blood in the doxorubicin-treated rats were 15-fold and 10-fold, respectively, significantly higher than the control values (p<0.001). Similar results were observed with the heart-to-lung concentration ratios of the doxorubicin-treated rats (p<0.01). On the other hand, there was little tissue difference of 125I anti-IgG antibody uptake between the doxorubicin-treated and control rats in Table 4, suggesting that specificity is associated with the use of the antimyosin antibody and that the myocardial uptake of the radiolabeled antibody is not from permeability in the injured myocardium.

Myocardial Uptake Versus Morphological Changes
Figure 2 shows the mean ventricular uptake of 125I antimyosin. The left ventricular uptake (percent kilogram dose per gram) in the doxorubicin-treated rats was 0.180±0.020 (n=3), 0.231±0.021 (n=6), and 0.340±0.070 (n=5) for pathology scores 1, 2, and 3, respectively. In the right ventricle, the myocardial uptake (percent kilogram dose per gram) was 0.133±0.020 (n=9), 0.199±0.021 (n=4), and 0.298 (n=1) for pathology scores 1, 2, and 3, respectively. As the severity of myocardial damage increased, there was a corresponding rise in myocardial uptake of tracer in both ventricles of the doxorubicin-treated rats.

Morphological Changes Versus Hemodynamics
The left ventricular end-diastolic pressure in the doxorubicin-treated rats was significantly higher for histopathology score 3 (19±3 mm Hg, n=5) than for score 2 (15±2 mm Hg, n=6, p<0.05) or score 1 (13±2 mm Hg, n=3, p<0.05). The difference between scores 1

### Table 1. Summary of Baseline Hemodynamics in Experimental Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n=11)</th>
<th>DOX (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>450±30</td>
<td>230±20*</td>
</tr>
<tr>
<td>HW/BW (g/g)</td>
<td>3.7±0.1</td>
<td>4.7±0.4**</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>348±31</td>
<td>328±35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RV</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP (mm Hg)</td>
<td>29±2</td>
<td>31±5</td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td>1.5±0.6</td>
<td>5.5±1.6*</td>
</tr>
<tr>
<td></td>
<td>130±9</td>
<td>116±12**</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>4±2</td>
<td>16±4*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ao (mean) (mm Hg)</th>
<th>Control</th>
<th>DOX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>110±4</td>
<td>95±5**</td>
</tr>
</tbody>
</table>

BW, body weight; HW, heart weight; HR, heart rate; bpm, beats per minute; RV, right ventricle; SP, systolic pressure; EDP, end-diastolic pressure; LV, left ventricle; +dP/dt, positive dP/dt; Ao, aortic pressure; DOX, doxorubicin-treated rats.

* p<0.001; ** p<0.01.
and 2 was not statistically significant. Right ventricular end-diastolic pressures were 4±1 mm Hg (n=9), 6±2 mm Hg (n=4), and 7 mm Hg (n=1) in scores 1, 2, and 3, respectively, showing a significant difference between scores 1 and 2 (p<0.05). No correlation was observed, however, between the severity of myocardial damage and hemodynamic parameters of the dP/dt and systolic pressure of the left ventricle and the mean aortic pressure.

**Myocardial Uptake Versus Hemodynamics**

As shown in Figure 3, the myocardial uptake of $^{111}$In antimyosin was strongly correlated with the left ventricular end-diastolic pressure ($r=0.84$, $p<0.001$) and the right ventricular end-diastolic pressure ($r=0.83$, $p<0.001$) in the doxorubicin-treated rats. There was little correlation of uptake of tracer with the dP/dt and systolic pressure of the left ventricle and the mean aortic pressure.

**Table 2. Summary of Tissue Distribution of $^{111}$In Antimyosin Fab in Experimental Rats**

<table>
<thead>
<tr>
<th>Heart</th>
<th>Blood</th>
<th>RV</th>
<th>LV</th>
<th>Lung</th>
<th>Liver</th>
<th>Muscle</th>
<th>Sternum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=11)</td>
<td>0.023±0.003</td>
<td>0.076±0.006</td>
<td>0.067±0.008</td>
<td>0.067±0.008</td>
<td>0.734±0.105</td>
<td>0.034±0.003</td>
<td>0.093±0.018</td>
</tr>
<tr>
<td>DOX (n=14)</td>
<td>0.017±0.004*</td>
<td>0.161±0.051*</td>
<td>0.260±0.077*</td>
<td>0.081±0.031</td>
<td>0.742±0.111</td>
<td>0.009±0.002*</td>
<td>0.045±0.008*</td>
</tr>
</tbody>
</table>

RV, right ventricle; LV, left ventricle; DOX, doxorubicin-treated rats. Values are percent kilogram dose per gram. *p<0.001.

**Figure 1.** Photomicrographs showing morphological changes in the heart of a doxorubicin-treated rat. Panel A: Light microscopy of the myocardium showing myocytolysis, sarcoplasm with vacuole formation, and interstitial edema. Bar indicates 20 μm. Panel B: Electron microscopy showing myofibrillar loss, remnants of Z bands, and mitochondrial changes. Bar indicates 2 μm.
TABLE 3. Summary of Heart-to-Blood and Heart-to-Lung Ratios of $^{111}$In Antimyosin Fab in Experimental Rats

<table>
<thead>
<tr>
<th></th>
<th>RV/blood</th>
<th>LV/blood</th>
<th>RV/lung</th>
<th>LV/lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.3±0.5</td>
<td>2.9±0.4</td>
<td>1.1±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>DOX</td>
<td>9.5±2.9*</td>
<td>15.3±4.0*</td>
<td>2.1±0.4**</td>
<td>3.4±0.6**</td>
</tr>
</tbody>
</table>

RV, right ventricle; LV, left ventricle; DOX, doxorubicin-treated rats.
*p<0.001; **p<0.01.

Immunoaautoradiography of the Heart

Figure 4 shows representative autoradiograms for the control and doxorubicin-treated rats. An increased cardiac uptake of $^{111}$In antimyosin was clearly evident as the severity of myocardial damage increased. On microscopic autoradiography (Figure 5), there was radioimmunolocalization of the radiolabeled antibody in the doxorubicin-induced injured myocytes, whereas the absence of radioactivity was shown in the control myocytes.

Discussion

This study indicates that myocardial imaging with $^{111}$In antimyosin antibody Fab can evaluate the severity of myocardial damage and cardiac dysfunction to provide evidence of doxorubicin cardiotoxicity in experimental rats, as reported clinically by Estorch et al. In addition, the specificity of the antimyosin antibody to the injured myocardium has been shown in this particular rat model by the use of an irrelevant Fab antibody and the immunoaautoradiography.

The incidence of overt congestive heart failure increases progressively in up to 30% of patients who receive a cumulative dose of doxorubicin exceeding 550 mg/m$^2$. Therefore, methods of monitoring this drug's cardiotoxicity have been sought. Although a variety of noninvasive methods have been tested in cancer patients receiving doxorubicin, they have had only limited value in predicting cardiotoxicity and cardiac dysfunction. Only radionuclide ventriculography has been shown to be useful clinically. In regard to invasive methods, endomyocardial biopsy is considered the best way of detecting doxorubicin-related myocardial damage.

Using this technique in patients, Billingham et al reported that the presence and severity of the pathological changes in the heart were closely related to the cumulative dose of doxorubicin. Despite its ability to monitor cardiotoxicity, cardiac biopsy, which is invasive, is usually not clinically acceptable for performing serial examinations in a patient over the long term.

Radionuclide imaging is both a sensitive and noninvasive method for detecting myocardial damage or necrosis. $^{99m}$Tc pyrophosphate cardiac scintigraphy has been used to detect the cardiomyocyte injury accompanying acute myocardial infarction. Myocardial damage has been assessed by Chacko et al, who reported that the uptake of pyrophosphate was distributed diffusely throughout the myocardium of nine of 15 patients (60%) with various malignant neoplasms undergoing doxorubicin treatment and also, in some cases, mediastinal irradiation.

Recently, the immunoscintigraphic agent $^{111}$In monoclonal antimyosin antibody Fab has been developed for imaging the myocardial damage in vivo. This radiolabeled antimyosin has been shown to bind specifically to intracellular myosin when sarcolemmal disruption of cardiac myocytes occurs and the cell is damaged irreversibly. Suggestions for the underlying mechanism of doxorubicin cardiotoxicity include the oxidation of cell membrane by free radicals, inhibition of mitochondrial enzymes, reduced synthesis of DNA, formation of a toxic metabolite of doxorubicin, and selective disturbance of muscle gene expression in cardiomyocytes. These factors may be responsible for the mitochondrial degeneration, myofibrillar loss, distended sarcotubular systems, and vacuolation of the sarcoplasmic reticulum observed in our study.

We clearly demonstrated a specific myocardial localization of the antimyosin antibody in rats administered doxorubicin by immunoautoradiography of the heart, indicating that the monoclonal antibody specific for human myosin heavy chains (R11D10) has high affinity for rat ventricular myosin. A more intense myocardial

TABLE 4. Summary of Tissue Distribution of $^{125}$I Anti-IgG Fab in Experimental Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart</th>
<th>Blood</th>
<th>RV</th>
<th>LV</th>
<th>Lung</th>
<th>Liver</th>
<th>Muscle</th>
<th>Sternum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>0.027±0.004</td>
<td>0.008±0.001</td>
<td>0.007±0.001</td>
<td>0.015±0.003</td>
<td>0.011±0.002</td>
<td>0.005±0.001</td>
<td>0.006±0.001</td>
<td></td>
</tr>
<tr>
<td>DOX (n=10)</td>
<td>0.020±0.007</td>
<td>0.007±0.002</td>
<td>0.006±0.002</td>
<td>0.015±0.006</td>
<td>0.011±0.003</td>
<td>0.005±0.001</td>
<td>0.008±0.002</td>
<td></td>
</tr>
</tbody>
</table>

RV, right ventricle; LV, left ventricle; DOX, doxorubicin-treated rats. Values are percent kilogram dose per gram.
uptake is caused by the more severe damage to the myocytes of both ventricles. Also, the severity of the pathological changes and the antimyosin uptake of the myocardium were much higher in the left ventricle, indicating that the left side of the rat heart is more sensitive to doxorubicin, although we could not identify the mechanism from our study. Other reports have also shown that morphological damage is more pronounced in the left ventricle on either endomyocardial biopsy or autopsy study.32-34 Little uptake of the radiotracer was shown in skeletal muscle, however, confirming that skeletal muscle was less sensitive than cardiac muscle to doxorubicin.35 Ito et al31 also reported in an in vitro study that the doxorubicin effect on muscle gene expression was limited to cardiac muscle.

Estorch et al18 first reported the usefulness of $^{111} \text{In}$ antimyosin antibody Fab in evaluating doxorubicin-induced cardiotoxicity in cancer patients. Those investigators performed myocardial imaging in 20 women with advanced breast cancer after treatment with a cumulative dose of 500 mg/m$^2$ of doxorubicin and other chemotherapy. They observed a myocardial uptake of this radiotracer in 17 of the 20 cases (80%); eight of the 20 (40%) with impaired cardiac function presented with a more intense uptake of antimyosin. However, the relation between the extent of cardiac uptake of the tracer and the morphological changes in the heart was not examined. The present study in rats shows that as the severity of myocyte damage increases, there is a corresponding significant increase in antimyosin uptake. Also, there was a strong correlation between antimyosin uptake and both ventricular end-diastolic pressures in rats. An increased ventricular end-diastolic pressure reflects an alteration in the ventricular pressure–volume relation or a decrease in diastolic compliance of the ventricle.36 In this rat model, an increment in end-diastolic pressure may occur with a failure of the dilated ventricles caused by doxorubicin-induced myocardial damage, resulting in a good correlation with the antimyosin uptake. But Insler et al12 had found that seven (35%) of 20 patients with anthracycline cardiotoxicity showed no specific histological signs. Moreover, histopathological abnormalities had been found in 23 (52%) of patients without clinical toxicity, even at lower doses. In the present study, there was a more definite correlation between the two parameters. The possible reasons for the discrepancies may include that we evaluated morphological findings in the large section of the heart instead of focal myocardial biopsy by both light and electron microscopies and that the cumulative dose of doxorubicin was very large to produce injury of the myocytes followed by congestive heart failure in all rats. On the contrary, there was little correlation between the left ventricular dP/dt and the degree of histological damage or the $^{111} \text{In}$ antimyosin uptake in the left ventricle. The possible explanation for the result may be that the positive dP/dt as one of the isovolumetric phase indexes is usually of little value in assessing this property of cardiac function, as are the ejection phase indexes, and in classifying contractility among different patients.37

![Figure 3](http://circ.ahajournals.org/)

**FIGURE 3.** Scatterplot showing correlation between myocardial uptake and ventricular end-diastolic pressure. There is a strong linear correlation. ■, histopathological score 1; ○, score 2; and ●, score 3.

![Figure 4](http://circ.ahajournals.org/)

**FIGURE 4.** Color autoradiography of the excised heart in control (left) and in doxorubicin-treated rats (middle and right) after administration of $^{111} \text{In}$ antimyosin antibody, 3.7 MBq. A higher uptake of radioactivity in the heart is indicated by the red color and a lower dose by the blue color. The autoradiogram of the doxorubicin-treated heart in the middle picture shows worse myocardial damage than that in the right. DOX, doxorubicin-treated rat.
The limitations of this study are as follows. Our experiment reported preliminarily the usefulness of \( ^{111} \text{In} \) antimyosin antibody for detecting the myocardial damage induced by doxorubicin in comparison with its hemodynamic effects. The cumulative dose of doxorubicin used in this study (15 mg/kg) was enough to induce morphological changes in the rat heart similar to the results reported by Jensen et al.\(^{38} \) From the clinical point of view, it is important to develop a means for the early detection of cardiac toxicity. Therefore, the relation between antimyosin uptake and the dose-dependent damage to the myocytes remains to be completely defined.

In conclusion, \( ^{111} \text{In} \) antimyosin antibody appears to be a valuable radiopharmaceutical agent in detecting early cardiac damage and in evaluating the severity of the doxorubicin-induced cardiotoxicity. Furthermore, myocardial imaging using the radiotracer is noninvasive, and it can provide information to guide the course of doxorubicin treatment in patients without other evidence of myocardial damage.

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References


35. Stinson V-81-V-84
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M Hiroe, Y Ohta, N Fujita, M Nagata, T Toyozaki, K Kusakabe, M Sekiguchi and F Marumo

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