Serial Assessment of Myocardial Properties Using Cyclic Variation of Integrated Backscatter in an Adriamycin-Induced Cardiomyopathy Rat Model

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Although adriamycin (Doxorubicin) is one of the most effective and useful antineoplastic agents for the treatment of a variety of malignancies, its repeated administration can induce irreversible myocardial damage and resultant heart failure. Currently, no marker to detect early cardiac damage is available. The purpose of this study was to investigate whether an assessment of the acoustic properties of the myocardium could enable the earlier detection of myocardial damage after adriamycin chemotherapy. Forty Wistar rats were treated with adriamycin (2 mg/kg, i.v.) once a week for 2, 4, 6 or 8 weeks consecutively. Left ventricular ejection fraction (LVEF) was calculated using M-mode echocardiography data. The magnitude of cardiac cycle dependent variation of integrated backscatter (CVIB) of the myocardium was measured in the mid segment of the septum and in the posterior wall of the left ventricle, using a real time two dimensional integrated backscatter imaging system. LVEF was significantly lower in the adriamycin-treated 8-week group than in the controls (75 ± 9 vs $57 \pm 8\%$, $p < 0.05$). Myocyte damage was only seen in the 8-week adriamycin-treated group. However, no significant changes of CVIB were observed between baseline or during follow-up in the ADR or control group. In conclusion, serial assessment of the acoustic properties of the myocardium may not be an optimal tool for the early detection of myocardial damage after doxorubicin chemotherapy in a rat model.

Key Words: Anthracycline, cardiotoxicity, echocardiography

INTRODUCTION

Adriamycin, ADR (Doxorubicin HCl) is one of the most effective and useful antineoplastic agents for the treatment of a variety of malignancies. However, cardiotoxicity, which may ultimately lead to congestive heart failure, is a well known problem in the treatment of cancer and limits the therapeutic use of this group of effective antineoplastic agents 1. To optimize ADR treatment in patients and to prevent cardiac injury, the early detection of cardiotoxicity is mandatory. Although cardiotoxicity is frequently assessed by measuring left ventricular ejection fraction (EF), EF is known to be a rather insensitive measure of early cardiac damage.[1,2]

Ultrasonic tissue characterization by integrated backscatter offers a non-invasive means of determining the static and dynamic properties of the myocardium. Normal myocardium shows cardiac cycle dependent integrated backscatter variations (CVIB). Moreover, CVIB reflects regional and intramural contractile performance, and its magnitude decreases in both ischemic[3-8] and non-ischemic[9-13] myocardial diseases. We hypothesized that CVIB might be decreased earlier than EF in rats with ADR-induced cardiomyopathy. Therefore, we investigated whether the serial assessment of the acoustic properties of the myocardium enables the earlier detection of myocar-

dial damage after ADR chemotherapy.

MATERIALS AND METHODS

Experimental animals

ADR-cardiomyopathy was induced in 40 male Wistar rats weighing 230 ± 25g by the weekly administration of 2 mg/kg of ADR (supplied by Kyowa Hakko Kogyo Co. Ltd.) via a tail vein for 8 weeks. The control group was comprised 20 Wistar rats, which were treated with the same volume of normal saline. Batches of 3 rats were euthanized 24 hours after starting ADR or saline administration and also on the same day of weeks 2, 4, 6, and 8 (Week 0 was taken to be the time of the first administration of ADR). Echocardiographic examinations were performed after the induction of anesthesia with an intraperitoneal injection of ketamine (70 mg/kg). Left ventricular (LV) performance was examined by echocardiography at baseline and weeks 2, 4, 6, and 8 after the first injection of ADR or saline. LV dimensions (end-diastolic and end-systolic diameter) were measured by M-mode echocardiography at parasternal short axis image at the papillary muscle level. The EF was calculated by M-mode echocardiography, using a modification of the method described by Quinones et al.[14]

Integrated backscatter images

Integrated backscatter images were obtained using a real time two dimensional ultrasonic backscatter imaging device (Sonos 2500, Philips, Andover, Massachusetts) equipped with an acoustic densitometry measurement package for backscatter signal analysis. This system allows the operator to acquire and store a sequence of continuous two dimensional integrated backscatter images on an optical disk for subsequent analysis. Off-line analysis of the backscatter images was performed by retrieving a stored real time cine loop data from the system's built-in optical disc drive. Integrated backscatter was determined using the cine loop of the LV parasternal short axis view (Fig. 1). An elliptical region of interest of 31×31 pixels was placed in the mid anterior septum and at the posterior wall of the mid-LV. The largest region of interest was chosen to cover the myocardium whilst avoiding specular echoes from the endocardium or epicardium. All controls were held constant during the studies in each subject. The magnitude of CVIB was defined as the difference between minimum and maximum values in a cardiac cycle averaged over at least two consecutive beats. We used a specialized 5 - 12 MHz broadband transducer (S12 transducer, Philips, Andover, Massachusetts), which allows the high-resolution imaging of fine cardiac structures at shallow depths of 0 - 4 cm. Intraobserver
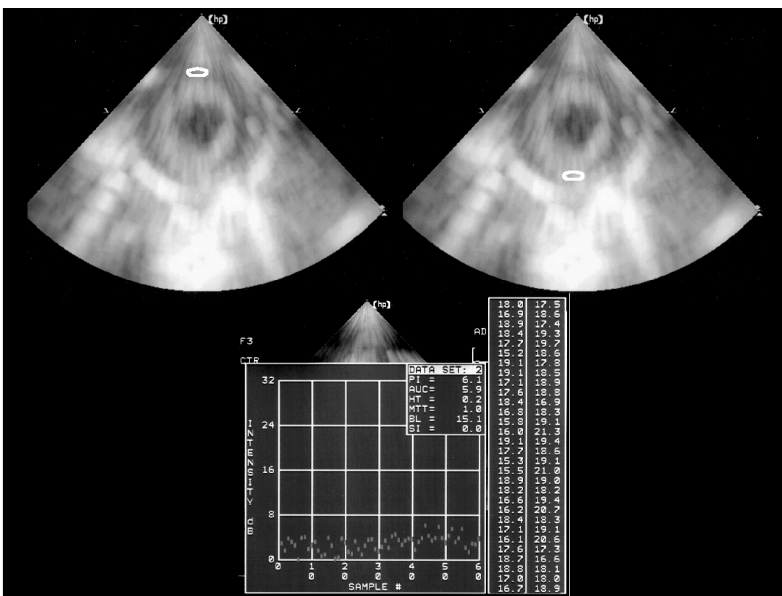
Fig. 1. Two-dimensional integrated backscatter image from parasternal short-axis view at the papillary muscle level and integrated backscatter measurements at the interventricular septum and posterior wall. An elliptical region of interest of 31×31 pixels was placed in the mid anterior septum (upper left) and the posterior wall (upper right) of the mid-LV. The largest region of interest was chosen to cover the myocardium whilst avoiding specular echoes from the endocardium or epicardium.

and interobserver variability of the magnitude of CVIB were assessed by having the study observer and two independent observers measure its magnitude in 10 randomly selected subjects. The mean absolute differences in the CVIB magnitudes were 0.3 ± 0.4 dB (intraobserver) and 0.4 ± 0.4 dB (interobserver).

Histological assessment of myocardial damage

After anesthesia with ether, chests were opened and hearts immediately isolated. Blood in hearts were removed and hearts weighed. Ventricles were cut horizontally and separated into 3 slices. Ventricle mid-slices were fixed in 4% paraformaldehyde at 4℃ for 8 hours, dehydrated in graded alcohol series, and then embedded in paraffin. The apical and basal portions of LVs were rapidly frozen in liquid nitrogen and preserved at -80℃ until use. A portion of each LV was also embedded in OCT compound and rapidly frozen. Paraffin sections (3 μm) were stained with hematoxylin and eosin and with Masson's trichrome stain.

Statistical analysis

Categorical data, expressed as percentages, were compared using the Chi Square test. Continuous variables, expressed as means $\pm$ SD, were compared by the 2-sample Wilcoxon rank sum test.

RESULTS

No significant difference in EF measured at baseline (80% vs 81%), 2 weeks (82% vs 81%), 4 weeks (80% vs 78%) or 6 weeks (82% vs 80%) was observed between the ADR group and the saline-treated controls. However, the LVEF of the ADR group measured at 8 weeks (57%), was significantly lower than that of the control group (75%) (Fig. 2). Similarly, no significant differences were observed between the two groups with respect to LV end-systole and diastolic dimensions, or in the wall thicknesses of the interventricular septum or posterior wall until 6 weeks after ADR. However, LV end-systolic and diastolic dimensions were significantly greater in

the ADR group subjects at 8 weeks. H&E staining revealed no significant abnormality in control group or in the ADR group until 6 weeks. Prominent pathologic abnormalities, such as focal fibrosis, were seen at 8 weeks only. CVIB of the septum and posterior wall did not change significantly with time (Fig. 3, 4) in either group. No significant differences in the CVIBs of septums or posterior wall were observed between the 2 groups at baseline or from 2 to 8 weeks after ADR or saline treatment.
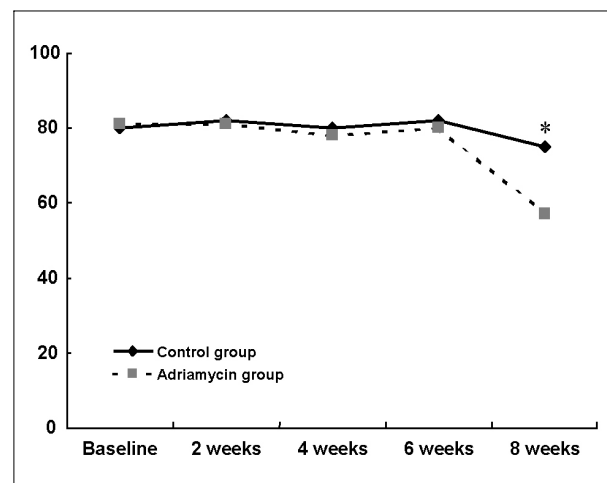

Fig. 2. Time-course left ventricular ejection fraction changes in the adriamycin and saline-treated control groups.
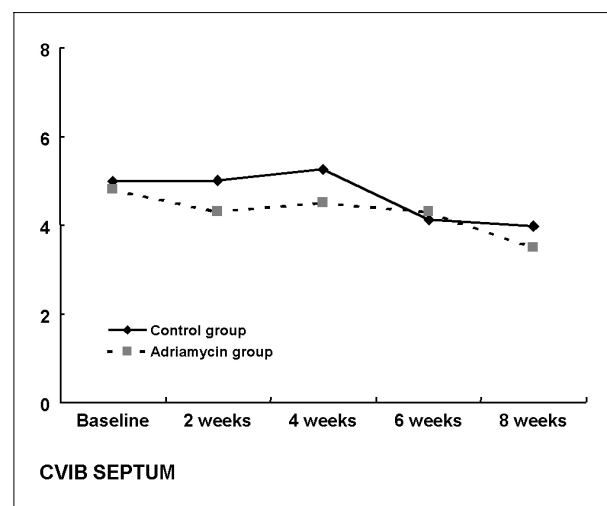

Fig. 3. Time-course of integrated backscatter cyclic variations of the integrated backscatter of interventricular septum in the adriamycin and the control groups.
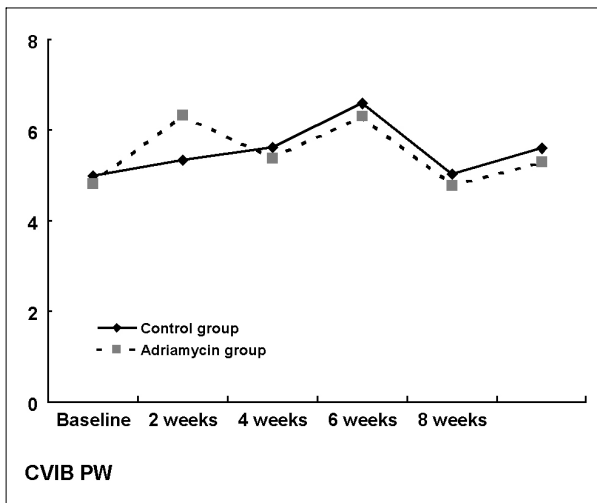
Fig. 4. Time-course of integrated backscatter cyclic variations of the posterior wall in the adriamycin and the control groups.

DISCUSSION

The principal finding of this study is that the serial assessment of the acoustic properties of the myocardium did not detect myocardial damage earlier after doxorubicin chemotherapy in a rat model. Thus, CVIB may not be an optimal or sensitive tool for the early detection of myocardial damage after doxorubicin chemotherapy. More sensitive techniques are thus needed to detect early ADR-induced myocardial damage.

The risk of inducing cardiac abnormalities including the development of heart failure even years after treatment as a result of doxorubicin therapy has been recognized for a long time 1. Suitable tests are needed to adapt chemotherapy schedules and to monitor supportive cardiac treatment. However, no highly sensitive screening method capable of predicting cardiac dysfunction exists. Currently, LVEF measured by radionuclide ventriculography or echocardiography is used to detect or exclude cardiac damage in cancer patients treated with doxorubicin. However, LVEF measurement is relatively insensitive for detecting doxorubicin induced cardiotoxicity at an early stage.[1,2] This is largely because no considerable change in systolic function occurs until a critical amount of morphological damage has taken place. Moreover, after this point deterioration proceeds rapidly and prognosis is poor.

Collagen is a primary determinant of both the scattering and the attenuation of myocardial tissue. A linear relationship was found between integrated backscatter and the hydroxyproline content in autopsied human heart, where fibrotic changes were associated with remote myocardial infarction. Echocardiographic tissue characterization can identify changes in myocardial acoustic properties related to myocardial histologic changes. CVIB is affected in ischemic heart disease,[3,5,6] cardiomyopathies,[9,10,15,16] cardiac allograft rejection,[11,17] and in the diabetic heart.[12,18] The relationship between myocardial wall thickening and CVIB magnitude is complex. CVIB magnitude is not linearly related to myocardial systolic wall thickening in dogs with coronary occlusion followed by reperfusion, 19 or in human hypertrophied hearts.[9] Thus, CVIB is dependent not only on wall thickening, but also on intrinsic myocardial properties, which cannot be visually assessed by conventional echocardiography. Our hypothesis was that histologic changes occur at lower doses of doxorubicin, and that these changes may alter myocardial acoustic properties but not LV global function. If true, myocardial tissue characterization would detect changes of myocardial dysfunction at an earlier stage. However, in our study, subjects treated with moderate doses of doxorubicin did not show a reduced CVIB despite a reduced systolic function. Recently, in contrast to our results, Nagai et al.[19] demonstrated, in patients who were receiving anthracycline for non-Hodgkin's lymphoma, that CV-IB was reduced in some patients treated with a moderate dose of doxorubicin, and thus they suggested that CV-IB analysis might be helpful for detecting early anthracycline cardiotoxicity. Further study is needed to confirm these findings.

In conclusion, the serial assessment of the acoustic properties of the myocardium failed to detect early myocardial damage after doxorubicin chemotherapy in a rat model. We believe that more sensitive techniques are needed for the early detection of ADR-induced myocardial damage.

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