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The effect of β-blocker on hamster model BIO 53.58 with dilated cardiomyopathy determined using ^{123}I-MIBG myocardial scintigraphy

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Objective: ^{123}I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy is currently used to evaluate cardiac sympathetic nerve function, but MIBG also has the capacity to evaluate dilated cardiomyopathy (DCM) severity and therapeutic effectiveness. In this study, we administered β-blockers to a DCM hamster model and evaluated the effect of therapy using MIBG. We also pathologically compared the effects of myocardial fibrosis suppression. ***Methods:*** BIO 53.58 hamsters were divided into the following five groups based on β-blocker administration: vehicle (COT), 2 mg/kg/day carvedilol (CLT), 20 mg/kg/day (CHT) carvedilol, 4 mg/kg/day (MLT) metoprolol, 40 mg/kg/day (MHT) metoprolol. F1B hamsters were administered a vehicle (COF). Plasma catecholamine, noradrenaline (p-NADR), adrenaline (p-ADR), and dopamine (p-DOPA) were assayed, and MIBG was performed. The count ratio of the heart to the mediastinum (H/M) and left ventricle myocardial washout ratio (WR) were calculated. We then performed an autopsy and calculated the percent change in fibrotic area from myocardial sections. ***Results:*** H/M of the initial image in the COT group was significantly lower at 2.4 ± 0.2 than the 2.9 ± 0.7 in the COF group ($p < 0.05$). The CLT and CHT groups had higher H/M values compared to the COT group (3.1 ± 0.6, 3.0 ± 0.6 versus 2.4 ± 0.2: $p < 0.05$). Significant correlations were evident between the H/M of the delayed image and p-NADR and p-DOPA ($p < 0.05$, $p < 0.01$, respectively) as well as between WR and p-NADR and p-DOPA ($p < 0.05$). Percent change in fibrotic area was significantly lower in the β-blocker groups than in the COT group ($p < 0.05$). Significant negative correlations were seen between the H/M of the delayed image and the percent change in fibrosis area. ***Conclusions:*** The delayed image H/M and WR acutely reflected cardiac disorder and sympathetic nerve function disorder in BIO 53.58 hamsters. In the carvedilol-administered groups, there was improvement compared to the initial H/M image, indicating the efficacy of the β-blocker in DCM.

Key words: BIO 53.58 hamster, DCM, MIBG, β-blocker

INTRODUCTION

WAAGSTEIN et al. used β-blocker therapy to treat dilated cardiomyopathy (DCM), and reported an improvement in cardiac function as well as prognosis.[1–4] It has since been confirmed in several large-scale clinical studies that many different β-blockers are effective for cardiac failure. In patients with chronic cardiac failure, the norepinephrine (NE) level in the blood increases and cardiomyopathy or similar conditions result due to an accentuation of secretion or intake decrease of NE at the cardiac sympathetic nerve ending. β-blocker therapy is performed in order to normalize sympathetic nerve activity. However, ^{123}I-metaiodobenzylguanidine (MIBG) is taken up by the cardiac sympathetic nerve ending through a mechanism similar to NE and is washed out. The effect can thus be called a tray that reflects sympathetic nerve function.[5,6] Furthermore, reports indicate that the MIBG washout rate (WR) or myocardial uptake rate correlates with left ventricular function and that MIBG myocardial scintigraphy could be a useful modality for evaluating DCM severity

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Table 1 Hamster characteristics by group

Group	n	classification	β-blocker	dose
COF	5	F1B	(Vehicle)	–
COT	5	BIO 53.58	(Vehicle)	–
MLT	4	BIO 53.58	Metoprolol	4 mg/kg/day
MHT	4	BIO 53.58	Metoprolol	40 mg/kg/day
CLT	5	BIO 53.58	Carvedilol	2 mg/kg/day
CHT	5	BIO 53.58	Carvedilol	20 mg/kg/day

or determining therapeutic effectiveness.[7]

In this study, metoprolol and carvedilol were administrated to BIO 53.58 hamsters that functioned as an animal model of dilated cardiomyopathy. Neurohumoral factors such as plasma catecholamines and the myocardial fibrosis suppression were examined in the different groups and compared to the findings obtained from MIBG myocardial scintigraphy. We also examined the effect of β-blocker treatment.

MATERIALS AND METHODS

Experimental model

BIO 53.58 (Bio Breeders) hamsters were used as a dilated cardiomyopathy animal model. Both ventricles in these hamsters were supposed to dilate not though resulting in hypertrophy and subsequent cardiac failure.[8] F1B hamsters (F1B) of the same age were used as a normal control group.

Protocol

Five-week-old male BIO 53.58 hamsters (n = 23) and F1B (n = 5) hamsters were reared for two weeks and used in the experiments. Hamsters were assigned to one of six groups: BIO 53.58 hamsters administered a vehicle (COT group; n = 5), BIO 53.58 hamsters administered 2 mg/kg/day of carvedilol (CLT group; n = 5), BIO 53.58 hamsters administered 20 mg/kg/day of carvedilol (CHT group; n = 5), BIO 53.58 hamsters administered 4 mg/kg/day of metoprolol (MLT group; n = 4), BIO 53.58 hamsters administered 40 mg/kg/day of metoprolol (MHT group; n = 4), and F1B hamsters administered a vehicle (COF group; n = 5) (Table 1). Each drug was mixed with powdered feed for rats and continuously administered for fourteen weeks once the age of seven weeks was reached.

After the start of the experiment, body weight and caloric intake were measured regularly once a week. Body weight was measured with a top-loading electronic balance (BPS2100S; Sartorius AG, Germany) and recorded. Caloric intake was calculated from the food consumption that was measured twice a week for each cage. At the end of fourteen weeks of rearing, three fractions of plasma catecholamines were assayed, and MIBG myocardial scintigraphy was performed under anesthesia. At the same stage, limb-lead electrocardiography was carried out and heart rate (per minute) was measured. At autopsy, the myocardium was pathologically examined for fibrotic area.

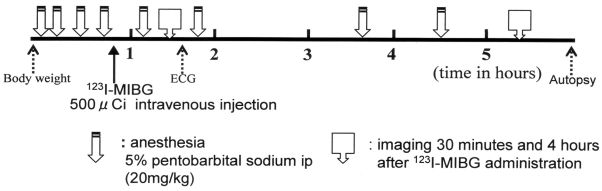

Fig. 1 ^{123}I-MIBG scintigraphy protocol. An initial image of the hamster back 30 minutes after ^{123}I-MIBG administrated as well as a delayed image was obtained 4 hours later. PRISM-2000 with an attached pinhole collimator was used to take the image.

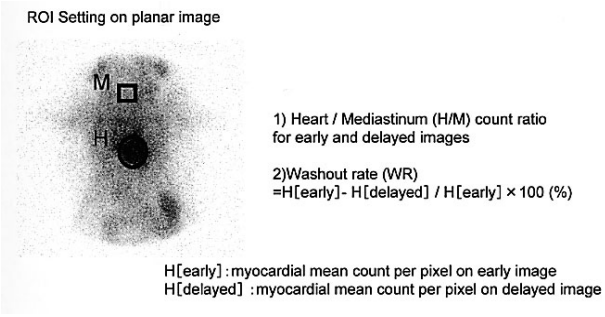

Fig. 2 MBG accumulation. From the planar image obtained, heart (H), mediastinum (M), and regions of interest (ROI) were set, and the mean count per pixel was calculated.

Neurohumoral analysis

At the end of rearing and after imaging with MIBG under adequate anesthesia, blood was sampled from the carotid artery or abdominal aorta and placed into EDTA-containing tubes. The samples were centrifuged (3,000 rpm) for ten minutes under refrigeration, frozen on dry ice, and the plasma catecholamines, namely noradrenaline (p-NADR), adrenaline (p-ADR) and dopamine (p-DOPA), were assayed.

Imaging approach

Hamsters were adequately anesthetized with a peritoneoclysis of 160 mg/kg 5% pentobarbital sodium. An incision was made in the femoral region, and 18.5 MBq (500 μCi) of ^{123}I-MIBG was administered into the femoral vein through this incision. The initial image was taken 30 minutes after injection and a delayed image after 4 hours had passed was taken of the backs of the hamsters (Fig. 1). A double-detector gamma camera PRISM-2000 (Shimadzu Corp., Kyoto) attached to a pinhole collimator (Picker Corp., Cleveland) was used to take the images. From the planar image obtained, regions of interest (ROI) were set at the heart (H) and mediastinum (M), and the mean count per pixel was measured to calculate the heart/mediastinum (H/M) ratio. The washout ratio (WR) for the entire myocardium of the left ventricle was also calculated from the initial and delayed image (Fig. 2).

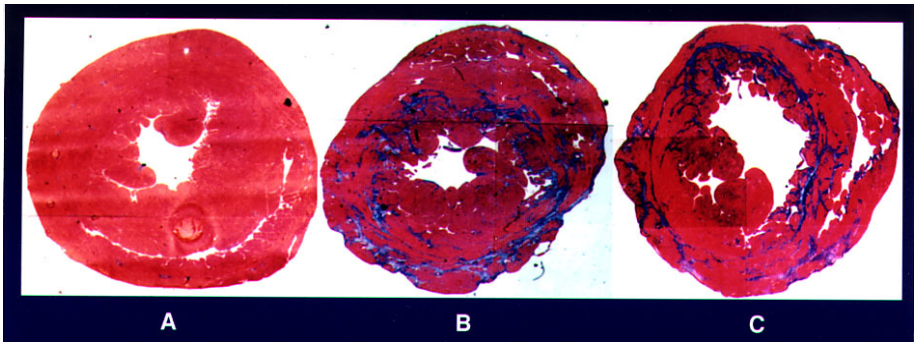

Fig. 3 Histology of the myocardium in each group. The myocardium was thinly sliced into 5 μm sections in the direction of the short axis using a cryostat. Azan-Mallory stained optical microscope images were photographed. (A: COF group, B: COT group, C: CHT group)

Table 2 Parameter changes in each group

Group	Body weight (g)		Heart rate/min
	Pre	Post	Post
COF	86.6 ± 10.5	169.5 ± 20.1	452.0 ± 59.0
COT	69.2 ± 5.7*	122.6 ± 12.8**	472.2 ± 17.9
MLT	71.8 ± 2.6*	127.4 ± 5.5*	434.8 ± 62.1
MHT	63.0 ± 18.0*	129.8 ± 9.3*	432.2 ± 52.5
CLT	69.1 ± 6.2*	133.4 ± 13.1**	428.2 ± 25.8$^{\#}$
CHT	69.8 ± 4.5*	131.9 ± 9.6**	415.6 ± 22.1$^{\#\#}$

Pre: pre β-blocker administration; Post: post β-blocker administration, *$p < 0.05$ versus COF group, **$p < 0.01$ versus COF group, $^{\#}p < 0.05$ versus COT group, $^{\#\#}p < 0.01$ versus COT group, mean ± SD

Table 3 Comparison of catecholamine content in each group

Group	p-NADR ($\times 10^3$ pg/m*l*)	p-ADR ($\times 10^3$ pg/m*l*)	p-DOPA ($\times 10^3$ pg/m*l*)
COF	5.2 ± 1.1	2.2 ± 0.7	0.19 ± 0.05
COT	22.8 ± 6.1*	9.3 ± 2.1*	0.37 ± 0.10
MLT	20.7 ± 5.3*	8.4 ± 2.2*	0.33 ± 0.06
MHT	15.3 ± 6.2	8.7 ± 3.3*	0.30 ± 0.12
CLT	14.4 ± 3.5	5.9 ± 1.2	0.36 ± 0.09
CHT	12.4 ± 4.6	4.9 ± 2.1	0.21 ± 0.04

p-NADR: noradrenaline; p-ADR: adrenaline; p-DOPA: dopamine, *$p < 0.05$ vs. COF, mean ± SE

Histological analyses

During autopsy the heart was extracted and the myocardium was thinly sliced into 5 μm sections in the direction of the short axis using a cryostat. The slices were immediately immersed in a 1 : 1 mixture of formalin and ethanol. After a ten-minute incubation period, the slices were washed with tap water and dried for fixation. Azan-Mallory stained optical microscope images were photographed (Fig. 3), and a negative of the prints was covered with a clear film to trace the region of myocardial fibrosis. Negatives were then scanned with the use of image processing software for quantitative analysis of the total cross sectional area of the myocardium and the area covered by fibrosis. These data were used to define "The percent change in fibrosis area."

Statistical analysis

The values in each group are expressed as means ± SD or means ± SE. Group comparisons were performed using the Student's t-test, and linear regression analysis was used for regression exponential testing. Differences were regarded as significant at $p < 0.05$.

RESULTS

The body weights of BIO 53.58 hamsters before and after administration of β-blockers were significantly less than the body weights of F1B hamsters ($p < 0.05$), but there were no significant differences among the BIO 53.58 hamster groups. Compared to the vehicle-administered group (COT), heart rate after β-blocker administration was significantly lower in the carvedilol-administered groups (CLT and CHT) ($p < 0.05$) (Table 2).

P-NADR was significantly higher in the COT and MLT groups than in the COF group ($p < 0.05$). P-ADR was significantly higher in the COT, MLT and MHT groups than in the COF group ($p < 0.05$). There were no significant differences noted between the groups in p-DOPA (Table 3).

We compared the MIBG myocardial scintigraphic findings with catecholamine levels and found a negative correlation between delayed image H/M and p-NADR, p-DOPA ($p < 0.05$, $p < 0.01$, respectively). The same significant positive correlations were seen between WR and p-NADR, p-DOPA ($p < 0.05$) (Fig. 4).

The H/M on the MIBG myocardial scintigraphy initial image was compared, and found to be significantly lower

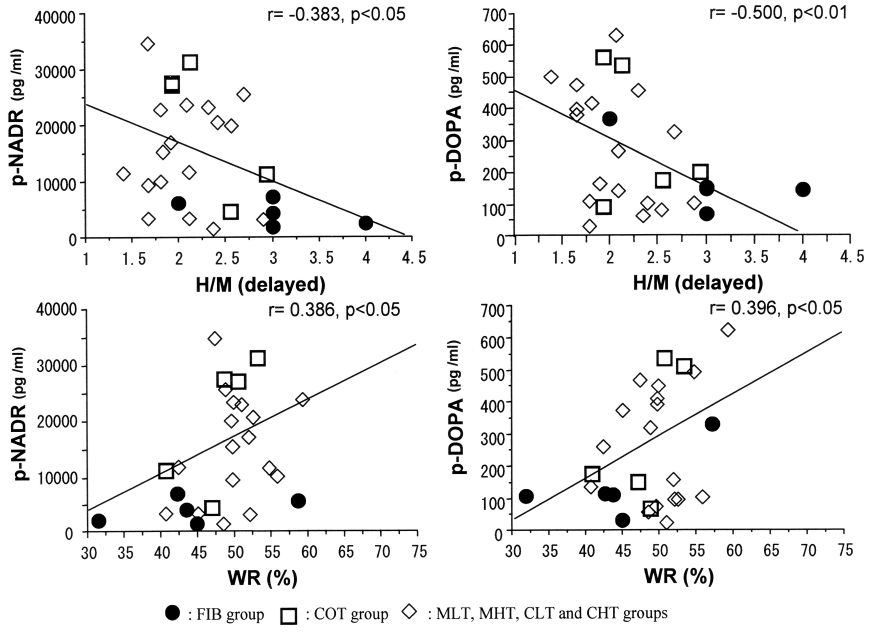

Fig. 4 Relationship between the delayed image H/M, washout rate and p-NADR, p-DOPA. Negative correlations were seen significantly between the delayed image H/M and p-NADR, p-DOPA ($p < 0.05$, $p < 0.01$, respectively). The same positive correlations were seen significantly between the delayed image H/M and p-NADR, p-DOPA ($p < 0.05$).

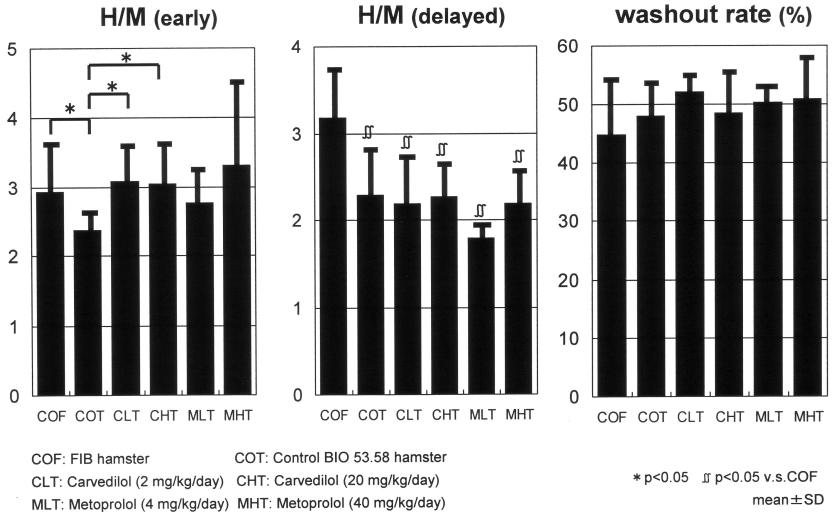

Fig. 5 Comparison of H/M ratio and washout rate of MIBG. The initial image H/M was found to be significantly lower in the COT group than in the COF group (2.4 ± 0.2 versus 2.9 ± 0.7: $p < 0.05$). In the carvedilol-administered CLT and CHT groups, H/M was significantly higher than in the COT group (3.1 ± 0.6, 3.0 ± 0.6 versus 2.4 ± 0.2: $p < 0.05$). In each of the BIO 53.58 hamster groups, we compared H/M on the delayed image and washout rate, but there were no notably significant differences.

in the COT group than in the COF group (2.4 ± 0.2 versus 2.9 ± 0.7: $p < 0.05$). In the carvedilol-administered CLT and CHT groups, H/M was significantly higher than in the COT group (3.1 ± 0.6, 3.0 ± 0.6 versus 2.4 ± 0.2: $p < 0.05$). In each of the BIO 53.58 hamster groups, we compared H/M on the delayed image and WR, but there were no notably significant differences (Fig. 5).

The percent change in fibrosis area was significantly higher in all BIO 53.58 hamster groups compared to the F1B group ($p < 0.01$), and significantly lower in the β-blocker-administered groups (CLT, CHT, MLT, and MHT groups) than in the vehicle-administered group (COT

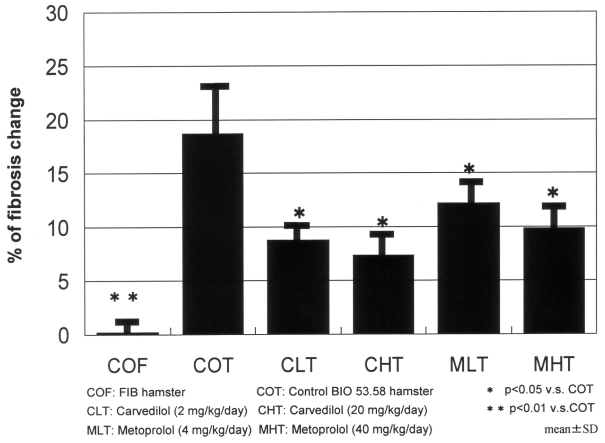

Fig. 6 Comparison of the percent change in fibrosis area in the myocardium. All BIO 53.58 hamster groups showed significantly higher values than the F1B group ($p < 0.01$), and significantly lower in the β-blocker-administered groups (CLT, CHT, MLT, and MHT groups) than in the vehicle-administered group (COT group) ($p < 0.05$).

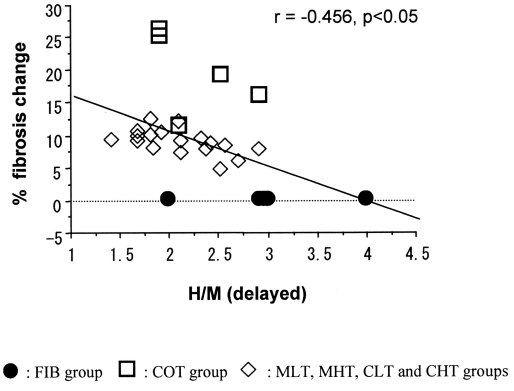

Fig. 7 Correlation of the percent change in fibrosis area and the delayed image H/M. Significantly negative correlation was seen between the percent change in fibrosis area and the delayed image H/M ($p < 0.05$).

group) ($p < 0.05$) (Fig. 6). A negative correlation was seen between H/M on the delayed image and the percent change in fibrosis area ($p < 0.05$) (Fig. 7).

DISCUSSION

DCM, a disease characterized by a failure of myocardial contraction and dilatation of the left ventricle, is an irreversible type of cardiomyopathy. Various etiologic factors such as genetic disposition, immunopathy, viral infection, and metabolic abnormality have been implicated.[8] Clinical symptoms such as dyspnea and shortness of breath occur due to low cardiac output, and pulmonary congestion occurs as a result of failed ventricular contraction. In terms of pathological condition, however, importance is placed on the involvement of neurohumoral factors. β-blocker therapy, which is conventionally contraindicated, has been used to treat cardiac failure cases and was reported as effective in improving cardiac function and prognosis.[1–4] Shortly thereafter metoprolol was reported to improve the left ventricular ejection fraction, pulmonary arterial wedge pressure, and exercise tolerance in patients with metoprolol dilated cardiomyopathy (MDC),[9] and bisoprolol significantly lowered overall mortality in CIBIS.[10] Furthermore, for severe cases of cardiac failure including ischemic heart disease, overall mortality significantly decreased with bisoprolol in CIBIS-II[11] and metoprolol in MERIT-HF.[12] In a US heart failure study using carvedilol, which has vasodilatation and antioxidation effects due to its α1 blocker mechanism, deaths from cardiac failure decreased, including sudden deaths from all cases of cardiac failure.[13]

In assessing the therapeutic efficacy of β-blockers for DCM, echo cardiography or cardiac pool scintigraphy is typically used to evaluate cardiac function along with clinical symptoms. However, the capability of MIBG myocardial scintigraphy to evaluate cardiac sympathetic nerve function may also make it a useful parameter for evaluating DCM severity or therapeutic effectiveness. In the present study, we administered the β-blockers metoprolol and carvedilol to DCM hamsters and monitored their therapeutic effectiveness using MIBG. The doses of metoprolol and carvedilol were determined from some other manuscripts and scientific meetings in which similar hamsters were used.[14] We also assayed neurohumoral factors such as plasma catecholamine content and pathologically compared their myocardial fibrosis suppression.

It is thought that hamsters with genetic cardiomyopathy—the DCM spontaneous onset model—contract the disease due to a genetic abnormality at δ-sarcoglycan in the myocardial cell system.[15] In BIO 14.6 hamsters demonstrating cardiac hypertrophy at about 20 to 30 weeks of age, and subsequent gradual ventricle enlargement and heart failure, the survival period was found to be about one year. In the present study we targeted BIO 53.58 hamsters, a mutated form of the BIO 14.6 hamster. Changes in the electrocardiogram should appear at approximately five weeks of age, and edema and breathing difficulty at about twenty weeks, but in these hamsters heart failure occurs along with the ventricular dilatation, myocardial degeneration, deficiency, and fibrosis soon after birth, and the mean survival period is about half a year.[15,16]

MIBG is taken in as an analog of norepinephrine (NE) by storage vesicles in the sympathetic nerve endings via uptake-1 and is released by exocytosis depending on the sympathetic nerve stimulus. Thus, MIBG is used as a tracer that reflects sympathetic nerve function. MIBG is also considered a good parameter for evaluating the prognosis of DCM,[17–19] and in particular, many reports have noted the usefulness of MIBG as a predictor of therapeutic effects. For example, delayed image H/M

predicts the improvement of left ventricular function, and the accentuation of WR reflects cardiac sympathetic nerve stress, which relates to left ventricular function.[20–22] Takatsu et al. reported that administration of an ACE inhibitor to BIO 14.6 hamsters improved MIBG accumulation.[23] Wakabayashi et al. administered cilazapril and verapamil to BIO 53.58 hamsters and found an improvement in myocardial MIBG accumulation.[24]

Previous reports indicate that factors involved in decreased MIBG accumulation in DCM included a functional decrease in uptake-1 due to ATP depletion, and a decrease and maintenance disorder in the number of storage vesicles or narrowing of the sympathetic nerve endings.[25–27] In the present study, the H/M value on the initial MIBG image in the BIO 53.58 control (COT) group was significantly lower than in the FIB (COF) group, and comparatively significantly higher in the carvedilol group than in the control group. Reports reflect that the effects of carvedilol include a suppressed decrease in the number of α1 and β receptors, a form of cardiac protection due to an antioxidant effect, and suppression of MIBG secretion from the storage vesicle.[20] There was also evidence that the anti-noradrenalin effect of carvedilol due to the β-receptor blockade is more potent than the effect of metoprolol.[28]

In the present study, the benefit of carvedilol use could be confirmed from the significantly reduced heart rate found in the carvedilol group compared to the control group. There was no decrease in plasma cathecholamine levels in the carvedilol group compared to the control group, suggesting that the H/M on the initial image was an important factor in evaluating β-blocker therapy.

There was no significant difference in WR between the control (COF) group and the other groups, possibly due to the effects of anesthesia. The WR influenced the H/M on the delayed image. Thus there was no significant difference in the H/M value on the delayed image. However, H/M on the delayed image and WR each significantly correlated with the plasma catecholamine levels, and these parameters also reflected the severity of the sympathetic nerve disorder and myocardial disorder. On the other hand, another report suggests that uptake-2, that is non-neuronal uptake, occupies a larger role in hamsters than in humans.[23] However, we found that uptake-2 had little effect from 30 minutes after MIBG administration. In the future, we would like to investigate the isolated effect of uptake-2.

Reportedly, when ACE inhibitor is administered to BIO 53.58 hamster, myocardial fibrosis is suppressed,[24] but in the present study myocardial fibrosis was significantly suppressed by even a small dose of β-blocker (Fig. 6). In terms of the underlying mechanism of this, it is thought that in addition to energy efficiency or ischemic improvement due to β-blocker bradycardiac effect, myocardium disorder, necrosis and fibrosis suppression due to direct catecholamine effect were intimately involved in the effectiveness. Also, the fact that H/M on the delayed image significantly correlated with the cardiac fibrosis rate suggests that the cardiac sympathetic nerve disorder was also involved in the onset of myocardial fibrosis.

In the present study we administered the β-blockers metoprolol and carvedilol to BIO 53.58 hamsters that functioned as a dilated cardiomyopathy animal model and evaluated the therapeutic effects. After β-blocker administration, we performed MIBG myocardial SPECT, and compared cardiac sympathetic nerve function with neurohumoral factors and myocardial fibrotic rates, and also evaluated the effects of β-blocker therapy. The H/M on the delayed image and WR calculated from MIBG myocardial scintigraphy correlated with plasma catecholamine levels, and H/M on the delayed image also correlated with myocardial fibrotic rate. Delayed image H/M and WR acutely reflected cardiac disorder and sympathetic nerve function disorder in BIO 53.58 hamsters. In the carvedilol-administered groups, initial image H/M was seen to improve, indicating the efficacy of the β-blocker in DCM.

APPENDIX

We used two kinds of drugs and there were two different dose groups for each drug, resulting in small sizes for the respective groups, and we calculated MIBG parameters while hamsters were under anesthesia. So we only could obtain comparatively rough correlations between MIBG parameters and catecholamines and fibrosis data. Also there were no differences seen in the therapeutic effects of β-blockers for delayed image H/M and WR.

MIBG image was taken only after administration of β-blockers and so we did not analyzed improvement induced by β-blocker therapy in the same hamsters.

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