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Low dose carvedilol inhibits progression of heart failure in rats with dilated cardiomyopathy

*,[1]Kenichi Watanabe, [1]Yoshimi Ohta, [2]Mikio Nakazawa, [2]Hiroshi Higuchi, [3]Go Hasegawa, [3]Makoto Naito, [4]Koichi Fuse, [4]Masahiro Ito, [4]Satoru Hirono, [4]Naohito Tanabe, [4]Haruo Hanawa, [4]Kiminori Kato, [4]Makoto Kodama & [4]Yoshifusa Aizawa

[1]Department of Clinical Pharmacology, Niigata College of Pharmacy, Kamisin-ei-cho, Niigata 950-2081, Japan; [2]Department of Pharmacology, Niigata University School of Medicine, Asahimachi, Niigata 951-8510, Japan; [3]Second Department of Pathology, Niigata University School of Medicine, Asahimachi, Niigata 951-8510, Japan and [4]First Department of Medicine, Niigata University School of Medicine, Asahimachi, Niigata 951-8510, Japan

1 The cardioprotective properties of carvedilol (a vasodilating β-adrenoceptor blocking agent) were studied in a rat model of dilated cardiomyopathy induced by autoimmune myocarditis.

2 Twenty-eight days after immunization, surviving Lewis rats (32/43 = 74%) were divided into three groups to be given 2 mg kg^{-1} day^{-1} (Group-C2, $n=10$) or 20 mg kg^{-1} day^{-1} (Group-C20, $n=10$) of carvedilol, or vehicle (0.5% methylcellulose, Group-V, $n=12$).

3 After oral administration for 2 months, body weight, heart weight (HW), heart rate (HR), rat α-atrial natriuretic peptide (r-ANP) in blood, central venous pressure (CVP), mean blood pressure (mean BP), peak left ventricular pressure (LVP), left ventricular end-diastolic pressure (LVEDP), $\pm$dP dt^{-1} and area of myocardial fibrosis were measured. Values were compared with those for normal Lewis rats (Group-N, $n=10$).

4 Two out of 12 (17%) rats in Group-V died from day 28 to day 42 after immunization. No rat died in Groups-C2, -C20 and -N. Although the CVP, mean BP, LVP and $\pm$dP dt^{-1} did not differ among the three groups, the HW, HR and r-ANP in Group-C2 (1.14 ± 0.03, 339 ± 16 and 135 ± 31) and Group-C20 (1.23 ± 0.04, 305 ± 8 and 156 ± 24) were significantly lower than those in Group-V (1.36 ± 0.04 g, 389 ± 9 beats min^{-1} and 375 ± 31 pg ml^{-1}, respectively). The LVEDP in Group-C2 was significantly lower than that in Group-V (7.4 ± 1.4 and 12.2 ± 1.2 mmHg, respectively, $P<0.05$). The area of myocardial fibrosis in Group-C2 was smaller than that in Group-V (12 ± 1 and 31 ± 2%, $P<0.01$).

5 These results indicate that a low dose of carvedilol has beneficial effects on dilated cardiomyopathy.

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Keywords: Carvedilol; β-blocker; rat model; dilated cardiomyopathy; heart failure; myocarditis; atrial natriuretic peptide; myocardial fibrosis

Abbreviations: Mean BP, mean blood pressure; BW, body weight; CVP, central venous pressure; $\pm$dP dt^{-1}, rate of intraventricular pressure rise and decline; H B^{-1}, ratio of heart weight to body weight; HR, heart rate; HW, heart weight, LVEDP, left ventricular end-diastolic pressure; LVP, peak left ventricular pressure; r-ANP, rat α-atrial natriuretic peptide

Introduction

Dilated cardiomyopathy is a set of heterogeneous diseases of left ventricular dysfunction, of unknown aetiology. There are a variety of clinical courses and pathological findings (Taliercio *et al*., 1985; Roberts *et al*., 1987). Dilated cardiomyopathy is considered a sequel to myocarditis (Dec *et al*., 1985). Two mechanisms by which myocarditis develops into dilated cardiomyopathy have been proposed: one is a persistent viral infection, the other is a progressive autoimmune myocardial injury (Matsumori & Kawai, 1982; Klingel *et al*., 1992). Human myocarditis can be classified into lymphocytic myocarditis and giant cell myocarditis according to histopathologic findings. Giant cell myocarditis was believed to be a rare and fatal disease of unknown aetiology. However, a recent report indicates that it is more prevalent in human myocarditis than previously recognized (Davidoff *et al*., 1991). In that report, the left ventricular function of patients with lymphocytic myocarditis improved during long-term follow-up. On the other hand, a progressive decline in left ventricular systolic function was observed in patients with giant cell myocarditis. This observation implies that giant cell myocarditis is more likely than lymphocytic myocarditis to progress into dilated cardiomyopathy.

Carvedilol is a third-generation vasodilating β-blocker (Ruffolo *et al*., 1990; 1992; Nichols *et al*., 1991; Feuerstein & Ruffolo, 1995). Carvedilol has recently been shown to reduce morbidity and mortality in patients with congestive heart failure (Packer *et al*., 1996; Cohn *et al*., 1997). This reduction may occur in part *via* β- and α_1-adrenoceptor blockade, the latter resulting in vasodilation. More importantly, carvedilol and several of its metabolites are potent antioxidants (Yue *et al*., 1992; Bril *et al*., 1992).

In the present study, the effects of long-term treatment with carvedilol on the development of myocardial damage were examined in a rat model of dilated cardiomyopathy induced by autoimmune giant cell myocarditis recently developed by our laboratory (Kodama *et al*., 1991; 1994; Hanawa *et al*., 1992).

*Author for correspondence;
E-mail: watanabe@niigata-pharm.ac.jp

We found that a low dose of carvedilol had beneficial effects on the dilated cardiomyopathy after myocarditis.

Methods

Animals and medication

Nine-week-old male Lewis rats were obtained from Charles River Japan Inc. (Kanagawa, Japan). Cardiac myosin, which was prepared from the ventricular muscle of pig hearts according to a procedure described previously (Kodama *et al*., 1994), was dissolved in a solution of potassium chloride (0.3 mol l^{-1}) and phosphate buffered sodium chloride (0.2 mol l^{-1}) at a concentration of 10 mg ml^{-1}. The antigen solution was mixed with an equal volume of complete Freund's adjuvant supplemented with Mycobacterium tuberculosis H37Ra (Difco Lab, Detroit, MI, U.S.A.) at a concentration of 11 mg ml^{-1}. Forty-three rats were injected with the antigen-adjuvant emulsion (0.2 ml) in their footpads. The morbidity of experimental autoimmune myocarditis was 100% in the immunized rats when using this protocol (Kodama *et al*., 1994). Ten normal Lewis rats (21-week-old) comprized the age-matched normal control group (Group-N).

Rats of the myosin-immunized group became ill and immobile on day 14, but their activity gradually recovered beginning from the fourth week. Eleven (26%) of the 43 myosin-immunized rats died from day 19–28. All hearts from these rats showed extensive myocardial necrosis and massive pericardial effusion. Twenty-eight days after immunization, the 32 remaining rats were divided into three groups for the oral administration of either (1) carvedilol, 2 mg kg^{-1} per day (Group-C2, $n=10$); (2) carvedilol, 20 mg kg^{-1} per day (Group-C20, $n=10$); or (3) vehicle, (0.5% methylcellulose, Group-V, $n=12$) for 2 months.

Throughout the studies, all animals were treated humanely in accordance with the guidelines on animal experimentation of our institute.

Rat α-atrial natriuretic peptide (r-ANP)

The concentration of r-ANP in blood was measured using the rabbit anti-α-atrial natriuretic factor kit (Peninsula Lab. Inc., Belmont, CA, U.S.A.). Blood samples obtained from the vena cava inferior after the recording of the haemodynamic parameters were centrifuged ($700\times g$ for 20 min) and the supernatant was kept in a freezer (-80°C) until determination of r-ANP concentration.

Haemodynamic study

Rats were anaesthetized with 2% halothane in O_2 during surgical procedure to measure the haemodynamic parameters, and then this concentration was reduced to 0.5% to minimize haemodynamic effects. Mean blood pressure (mean BP) was recorded through a catheter introduced into the right femoral artery. Central venous pressure (CVP) was recorded through a catheter introduced into the confluence of the venae cava from the right jugular vein. These catheters (PE-50) were connected to Statham P50 pressure transducers (Gould Statham, Oxnard, CA, U.S.A.) and a carrier amplifier (Nihon-Kohden, Tokyo, Japan). A catheter-tip transducer (Millar SPR249; Millar Inst., Houston, TX, U.S.A.) was inserted into the left ventricle from the right carotid artery to measure peak left ventricular pressure (LVP) and left ventricular end-diastolic pressure (LVEDP). The rate of intraventricular pressure rise and decline ($\pm$dP dt^{-1}) was measured with a differential amplifier (NEC San-ei, Tokyo, Japan). Heart rate (HR) was calculated from electrocardiograms. All haemodynamic parameters were recorded on a thermostylus recorder (NEC San-ei, Tokyo, Japan), after a stabilizing period of 20 min.

Heart weight, heart size and histopathology

After measurement of the haemodynamic parameters, a blood sample was obtained from the vena cava inferior. The heart was removed and cleaned of the surrounding tissues. The heart weight (HW) was measured and the ratio of HW to body weight [H B^{-1} (g kg^{-1})] was calculated. Heart size for each rat was evaluated by two scales. The first was the length from the apex to the aortic root through the anterior intraventricular fissure (long-axis). The second was the external short-axis diameter of the left ventricle (short axis).

The removed hearts were cut into about 2 mm transverse slices and fixed in 10% formalin. After being embedded in paraffin, several transverse sections were cut from the mid-ventricle slice and stained with the hematoxylin-eosin and Azan-Mallory methods (Mallory, 1936). Using the specimens stained with Azan-Mallory at the middle level of the left ventricle, the area of myocardial fibrosis was quantified by a colour image analyser (CIA-102, Olympus, Tokyo, Japan), making use of the differences in colour (blue fibrotic area as opposed to red myocardium). The results were presented as the ratio of the fibrotic area to the area of myocardium (Kato *et al*., 1995).

Statistical analysis

Data were presented as the mean$\pm$s.e.mean. Statistical assessment of the groups was performed by the one-way ANOVA, followed by Tukey's method. The differences were considered significant at $P<0.05$.

Results

Clinical course

Two out of 12 (17%) rats in Group-V died from day 28–42. All hearts from these rats showed extensive myocardial fibrosis and massive pericardial effusion. No rat died in Group-C2, -C20 and -N. Although pericardial effusion was observed in most of the rats in Group-V, there was little effusion in Group-C2 and C20. Peritibial oedema was not observed in any group.

Body and heart weights, and heart size

The body weight, HW and H B^{-1} are shown in Figure 1. The body weight did not differ among the four groups. The HW and H B^{-1} were significantly larger in Group-V (1.36 ± 0.04 g and 3.35 ± 0.07 g kg^{-1}) than Group-N (1.02 ± 0.02 g and 2.45 ± 0.06 g kg^{-1}, both $P<0.01$). The HW and H B^{-1} were significantly decreased in Group-C2 (1.14 ± 0.03 g and 2.87 ± 0.05 g kg^{-1}, both $P<0.01$) and Group-C20 (1.23 ± 0.04 g and 3.03 ± 0.08 g kg^{-1}, $P<0.05$ and $P<0.01$) compared to Group-V.

The long-axis and short-axis external left ventricular diameter of Group-V (17.9 ± 0.4 and 13.7 ± 0.3 mm) were longer than those of Group-N (14.3 ± 0.3 and 11.3 ± 0.2 mm, both $P<0.01$). The long-axis and short-axis diameter of Group-C2 (16.6 ± 0.3 and 12.3 ± 0.3 mm, both $P<0.01$) and

-C20 (17.1±0.4 and 13.2±0.3 mm, $P<0.05$ and $P<0.01$) were shorter than those of Group-V.

Although most of these data differed between the three groups with heart failure and Group-N, HW and long-axis diameter in Group-C2 were decreased to near Group-N values.

Blood r-ANP concentration

The r-ANP concentration was significantly higher in Group-V (375±27 pg ml^{-1}) than Group-N (76±7 pg ml^{-1}, $P<0.01$, Figure 2). The r-ANP in Groups-C2 (135±25 pg ml^{-1}) and -C20 (156±26 pg ml^{-1}) were lower than that in Group-V.

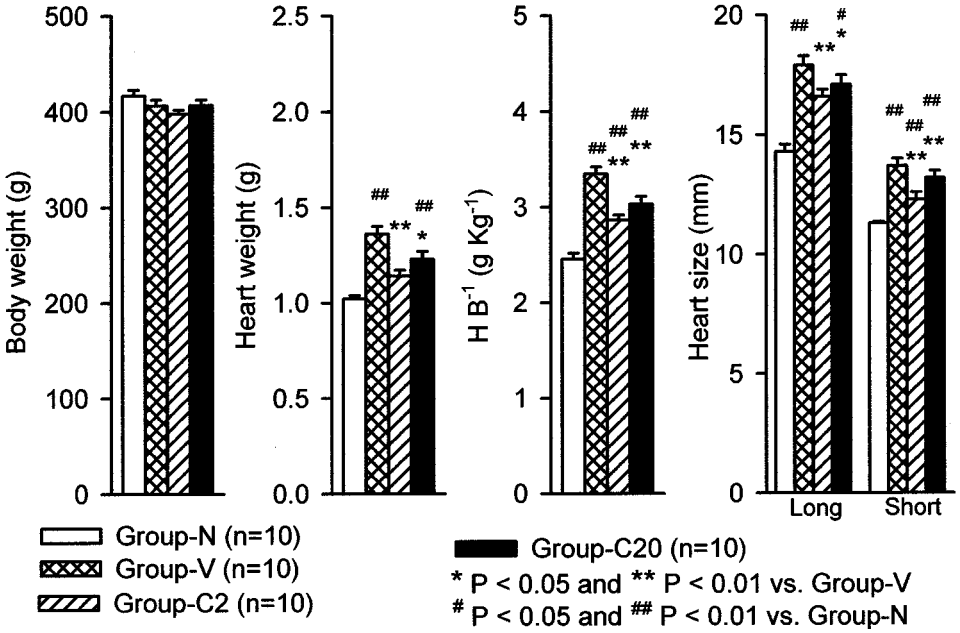

Figure 1 Effects of carvedilol on body weight, heart weight, ratio of heart weight to body weight (H B^{-1}) and heart size. Heart weight, H B^{-1} and heart size were significantly decreased in the carvedilol groups, though the effects were not dose-dependent. Although for the most part, these data differed between the three groups with heart failure and Group-N, HW and long-axis diameter in Group-C2 were nearly as low as that in Group-N.

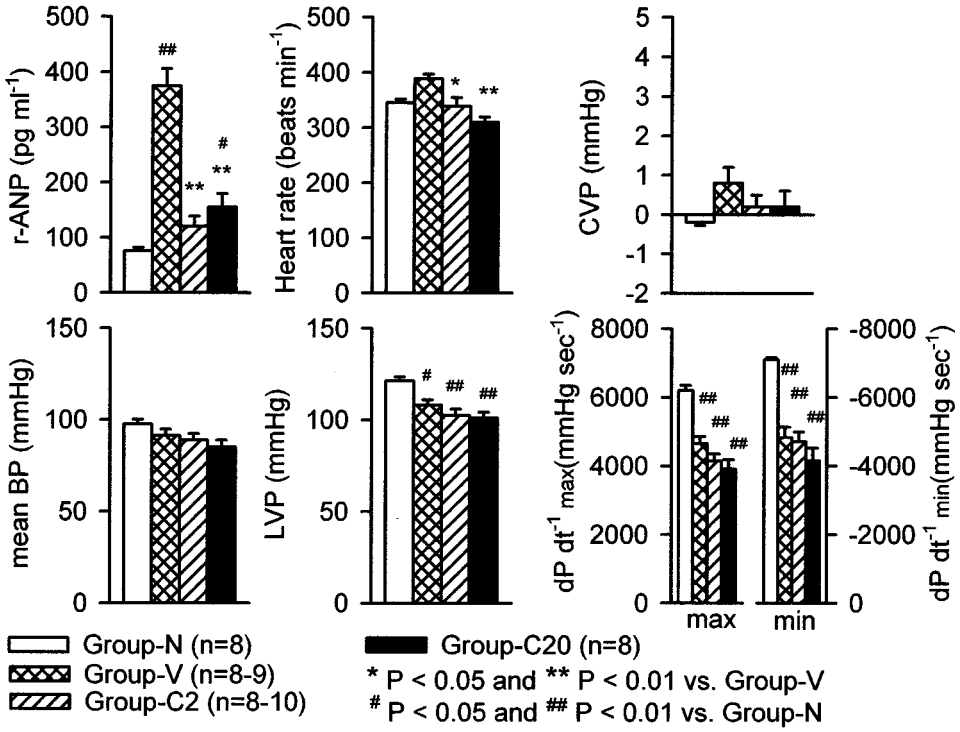

Figure 2 Effects of carvedilol on blood r-ANP concentration and haemodynamic parameters. The r-ANP concentration in carvedilol groups was significantly lower than that in Group-V. Central venous pressure (CVP), mean blood pressure (mean BP), peak left ventricular pressure (LVP) and ±dP dt^{-1} did not differ among the three groups with heart failure. Although most of these data differed between the three groups with heart failure and Group-N, r-ANP in Group-C2 was nearly as low as that in Group-N.

Haemodynamic parameters

Because of aortic insufficiency induced by inserting the catheter transducer in the left ventricle, one out of 10 rats in Group-V and two out of 10 rats in Groups-C20 and -N could not have their haemodynamic parameters measured.

The CVP and mean BP did not differ among the four groups. The LVP and maximum and minimum dP dt^{-1} were lower in Group-V (108 ± 3, 4649 ± 208 and 4827 ± 287) than Group-N (121 ± 2 mmHg, 6205 ± 143 mmHg s^{-1} and 7105 ± 61 mmHg sec^{-1}, Figure 2). The CVP, mean BP, LVP, and maximum and minimum dP dt^{-1} did not differ among Groups-V, -C2 and -C20. The HR was significantly lower in Groups-C2 and -C20 than Group-V (339 ± 16, 305 ± 8 and 389 ± 9 beats min^{-1}, respectively, Figure 2).

The LVEDP was higher in Group-V (12.2 ± 1.2 mmHg) than Group-N (4.6 ± 0.5 mmHg, $P < 0.01$, Figure 3). The LVEDP was lower in Groups-C2 (7.4 ± 1.4 mmHg, $P < 0.05$) and -C20 (9.6 ± 0.9 mmHg, NS) than Group-V. No significant difference in the LVEDP was observed between Group-C2 and Group-N.

Quantitative analysis of myocardial fibrosis

Figure 4 shows representative photographs of thin-sections stained with the haematoxylin-eosin and Azan-Mallory methods. Normal heart shows no fibrosis, but the heart from Group-V rats, were stained blue, which indicates fibrosis.

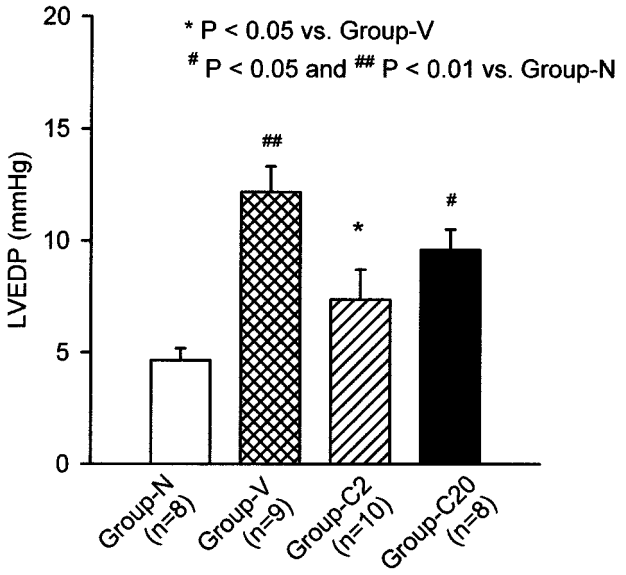

Figure 3 Effect of carvedilol on left ventricular end-diastolic pressure (LVEDP). LVEDP in Group-C2 was significantly lower than that in Group-V and nearly as low as that in Group-N.

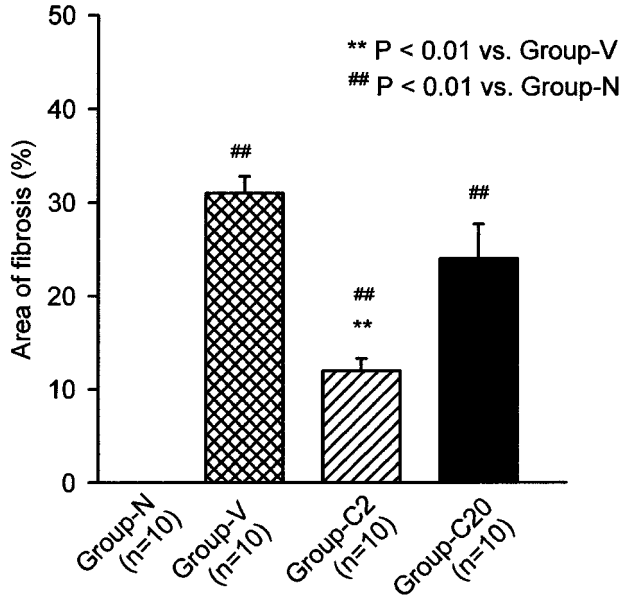

Figure 5 The effect of carvedilol on mycoardial fibrosis. Area of fibrosis was represented as per cent area of the ventricle. The area of fibrosis in Group-N was 0%. Among the three groups with heart failure, the area of fibrosis was the lowest in Group-C2.

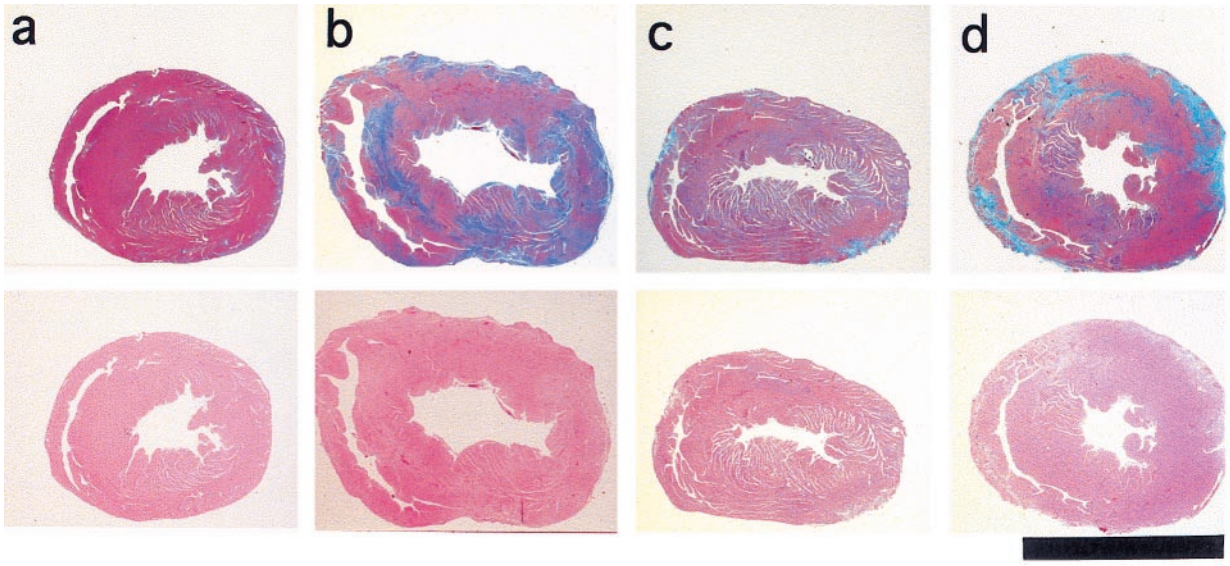

Figure 4 Effects of carvedilol on myocardial fibrosis and heart size. Figures show representative data for each group. Upper panel: Azan-Mallory stain. Lower panel: the haematoxylin-eosin stain. (a) Group-N; (b) Group-V; (c) Group-C2; (d) Group-C20. Scale bar is 1 cm.

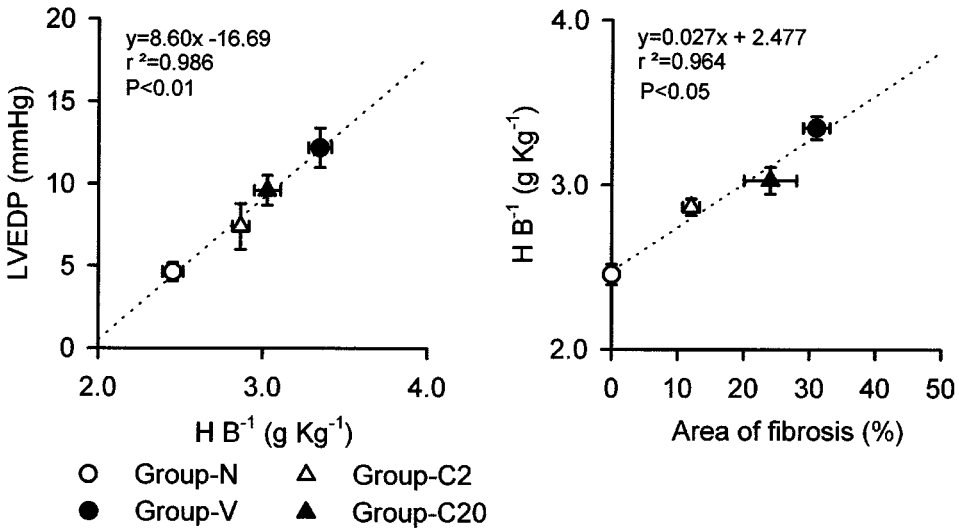

Figure 6 Correlations between ratio of the heart weight to body weight (H B^{-1}) and left ventricular end-diastolic pressure (LVEDP), and area of myocardial fibrosis and H B^{-1}. There were good positive correlations between H B^{-1} and LVEDP, and area of myocardial fibrosis and H B^{-1}.

The area of myocardial fibrosis was quantified by a colour image analyser. Among the three groups with heart failure, the incidence of fibrosis was the lowest in Group-C2 ($12 \pm 1\%$ in Group-C2, $24 \pm 4\%$ in Group-C20, and $31 \pm 2\%$ in Group V, Figures 4 and 5). The area of myocardial fibrosis in Group-C2 was smaller than that in Group-V ($P<0.01$). Although there was a tendency for the incidence of myocardial fibrosis to be lower in Group-C20, the difference between Group-V and Group-C20 was not significant.

The relations between H B^{-1} and LVEDP, and area of myocardial fibrosis and H B^{-1}

As shown in Figure 6, we plotted the relation between H B^{-1} and LVEDP, and area of myocardial fibrosis and H B^{-1} for the four groups. Positive correlations were found between H B^{-1} and LVEDP ($r^2=0.986$, $P<0.01$), and area of myocardial fibrosis and H B^{-1} ($r^2=0.964$, $P<0.05$).

Discussion

In the present study, using a rat model of dilated cardiomyopathy induced by autoimmune myocarditis developed by our laboratory, we examined the effects of carvedilol on progression of heart failure with dilated cardiomyopathy. We found that low-dose carvedilol (2 mg kg^{-1}) reduced HW, heart size and myocardial fibrosis, and lowered LVEDP more effectively than high-dose carvedilol (20 mg kg^{-1}).

Carvedilol is the only β-blocker that blocks β_1-, β_2-, and α_1-receptors without producing an intrinsic sympathomimetic effect, such as increasing cardiac noradrenaline, or causing an upregulation of β-receptors (Bril *et al*., 1992; Gilbert *et al*., 1993). Hence, the use of this drug is associated with a more complete antagonism of the sympathetic nervous system than is associated with other β-blockers. In humans, 25–50 mg of carvedilol is used to treat heart failure (Anderson *et al*., 1985; Bristow *et al*., 1996; Bohm *et al*., 1998). The reason why we used 2 mg kg^{-1} for one group was that this dose is equivalent to 25 mg in humans when one compares the dosage of carvedilol corrected by using body-surface area.

Interestingly, carvedilol improved the cardiac function and HW, in a dose-unrelated manner. The HW, LVEDP and area of myocardial fibrosis were significantly lower in Group-C2, but not in Group-C20, than in Group-V. The pA_2 values of the adrenoceptor blocking action was around 9 in the rat (Kawada *et al*., 1990). In addition to acting as an adrenoceptor blocker, carvedilol has antioxidant and antiproliferative effects (Yue *et al*., 1992; 1995; Ohlstein *et al*., 1993; Patel *et al*., 1995) the 50% inhibitory concentrations (IC_{50}) of which were about 1–3 μM. Moreover, some metabolites of carvedilol were also antioxidants whose IC_{50} was 10–100 times more potent than that of carvedilol (Feuerstein & Yue, 1994; Yue *et al*., 1994). Very recently, Cheng *et al*. (1999) reported that carvedilol blocks the rapidly activating component of the delayed rectifier K^+ current and L-type Ca^{2+} current with an IC_{50} of 0.35 and 3.59 μM, respectively. Thus, carvedilol has different pharmacological effects depending on its concentration.

Although the reasons for the dose-unrelated effect are not clear, it seems likely that a more complete adrenoceptor blocking effect and/or some additional pharmacological action(s), for example Ca^{2+} channel blocking action, which counteracted its beneficial effects on the heart failure, were induced at high dose. Indeed, a Ca^{2+} channel antagonist (Amlodipine) aggravated the heart failure in the present model (unpublished observation). Carvedilol displayed cardioprotective and renal protective actions even at the lower dose that had been demonstrated to be devoid of any significant effects on haemodynamics and the renin-angiotensin-aldosterone system, and the drug may have reversed or prevented the normal genetic predisposition to hypertrophy and remodeling in previous studies (Brooks *et al*., 1993; Barone *et al*., 1996; 1998).

Human dilated cardiomyopathy is thought to have a variety of causes. Therefore, the clinical courses and pathological findings in this cardiomyopathy are not uniform. Cardiac myosin-induced autoimmune myocarditis, which is not exclusively related to viral infection, develops clinicopathologically to resemble dilated cardiomyopathy in the chronic phase (Kodama *et al*., 1994; Hirono *et al*., 1997). Thus, the present results provide some insight in to the effectiveness of carvedilol treatment against dilated cardiomyopathy.

In the clinical stage, experimental observations suggest that patients with heart failure are under considerable oxidative stress, and that such stress may cause myocardial cells to undergo programmed cell death or apoptosis. The stress, furthermore, may start the process that has been implicated in the progression of heart failure (Belch *et al*., 1991; Sobotka *et al*., 1993). Carvedilol appears to be more effective than other β-blockers in attenuating the process of apoptosis. This action appears to be related directly to its antioxidant action, and not to its adrenergic-receptor-blocking effects. In *in vitro* experiments, carvedilol inhibits oxygen free radical-mediated lipid peroxidation more effectively than propranolol or labetalol. Moreover, carvedilol and its metabolites, but not propranolol, can scavenge oxygen free radicals (Yue *et al*., 1992; 1994; Feuerstein & Yue, 1994). These observations may explain why carvedilol reduces infarct size to a greater extent than propranolol in experimentally induced myocardial injury.

In addition to its antioxidant properties, carvedilol exerts antiproliferative effects that are also independent of its actions as a β-blocker. Carvedilol, but not celiprol, sotalol, or labetalol, inhibits the proliferation or rat aortic vascular smooth muscle cells under basal conditions and in response to endothelin-1 (Sung *et al*., 1993). Carvedilol suppresses the intimal proliferation that occurs in the rat carotid artery after balloon angioplasty and inhibits the proliferation of human vascular smooth muscle cells in vitro (Ohlstein *et al*., 1993; Patel *et al*., 1995). These antiproliferative effects may be important in preventing the progression of vascular remodeling which may contribute to the haemodynamic abnormalities seen in heart failure (Anderson *et al*., 1985; Bristow *et al*., 1996; Bohm *et al*., 1998). These actions could be important in preventing the progressive loss of myocardial cells that is characteristic of a failing heart. In this study, the area of myocardial fibrosis after administration of carvedilol was smaller than that in non-treated animals and carvedilol reduced HW and heart size. It is very likely that the beneficial effects of carvedilol were due to its antioxidant and antiproliferative activity.

ANP, which was first found in the atrium, is a potent vasodilator and diuretic agent (Burnett *et al*., 1986; Hirata *et al*., 1987). In patients with heart failure, the plasma level of ANP has been demonstrated to be elevated, the level being related to the severity of heart failure. Interestingly, in this study, carvedilol improved the cardiac function and the ANP levels were significantly lower in the carvedilol groups.

The present study indicates that carvedilol provides remarkable chronic cardioprotection, reducing or eliminating remodeling in a model of dilated cardiomyopathy and may help identify why this drug is efficacious at significantly improving outcome and survival when used for the treatment of heart failure in humans. In addition, a high dose treatment with carvedilol should be used with caution, as it may worsen heart failure.

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