β-Blocker Treatment of Dilated Cardiomyopathy

Beneficial Effect of Carteolol in Mice

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Background. The effects of carteolol, a nonselective β-adrenergic receptor blocker with intrinsic sympathomimetic activity, were compared with those of metoprolol in a murine model of viral myocarditis and dilated cardiomyopathy caused by encephalomyocarditis virus.

Methods and Results. In the acute experiment, BALB/c and DBA/2 mice were inoculated with encephalomyocarditis virus. BALB/c mice were then given carteolol at 1 (n=10), 10 (n=10), 30 (n=11), or 100 mg/kg (n=9) daily, and DBA/2 mice were given carteolol at 1 (n=9) or 10 mg/kg (n=9) daily starting the day of inoculation. Controls were given distilled water (n=23 for BALB/c mice and n=8 for DBA/2 mice). BALB/c mice were killed on day 7, and DBA/2 mice were killed on day 14. In the subacute experiment, DBA/2 mice were inoculated with the virus and then given carteolol at 1 (n=12) or 10 mg/kg (n=16), or distilled water (n=27) daily, starting on day 14. Mice were killed on day 28. Virus replication, murine survival, heart weight to body weight ratio, and histopathological findings were similar in each group in the acute and subacute experiments. In the chronic experiment, DBA/2 mice were inoculated with the virus and were then given carteolol at 1 (n=13) or 10 mg/kg (n=9), metoprolol at 30 mg/kg (n=9), or distilled water (n=31) daily, starting on day 14. Mice were killed on day 104. Heart weight to body weight ratio and histopathological scores were significantly lower in mice given carteolol than in the infected control group. Furthermore, left ventricular cavity dimension, left ventricular wall thickness, and myocardial fiber diameter of the left ventricle were significantly reduced in mice given carteolol compared with the control group. Metoprolol did not cause any significant changes compared with the control group.

Conclusions. This study suggests that carteolol prevents the development of myocardial lesions similar to those in dilated cardiomyopathy after myocarditis in the chronic stage. (Circulation 1991;83:2021-2028)

Myocarditis is caused by various viruses. The therapeutic potential of several drugs has been investigated in experimental murine myocarditis.1–8 Traditionally, congestive heart failure in patients has been considered to be a contraindication for the use of β-adrenergic receptor blockers. However, recent studies have suggested that these agents can have beneficial effects9–15 and can improve survival16 in patients with dilated cardiomyopathy. Although myocarditis is thought to be one of the causes of dilated cardiomyopathy,17,18 few studies have investigated the role of β-adrenergic blockers in viral myocarditis.19 A few reports are available on the effects of β-adrenergic blockers with intrinsic sympathomimetic activity in dilated cardiomyopathy,20,21 and these reports do not indicate any beneficial effect of short-term regimens. However, long-term regimens with bucindolol have improved cardiac function.15 We have already reported an animal model of myocarditis induced by the encephalomyocarditis virus, which shows congestive heart failure in the acute stage22 and lesions similar to those caused by dilated cardiomyopathy in the chronic stage.23 In this study, we investigated the effects of carteolol on our animal model and compared them with the effects of metoprolol.

Methods

Experimental Infection and Treatment

Four-week-old inbred male BALB/c or DBA/2 mice were inoculated intraperitoneally with 0.1 ml of
the M variant of encephalomyocarditis virus diluted in Eagle's minimal essential medium to a concentration of 100 plaque-forming units (pfu)/ml. 

**Acute experiment.** To investigate the effect of carteolol on virus replication, BALB/c mice were given carteolol at a dose of 1 (n=10), 10 (n=10), 30 (n=11), or 100 mg/kg (n=9), or distilled water (n=23). Treatment was performed by daily subcutaneous injection starting on the day of inoculation. Mice were killed on day 7. The heart was used for plaque assay of the virus content. To investigate the effect of carteolol on survival and histopathological change, DBA/2 mice were given carteolol at a dose of 1 (n=9) or 10 mg/kg (n=9), or distilled water (n=8) orally daily for 14 days beginning on the day of inoculation and were then killed. After weighing the hearts, they were sectioned and histopathologically examined.

**Subacute experiment.** DBA/2 mice were inoculated with the virus and were given carteolol at a dose of 1 (n=12) or 10 mg/kg (n=16), or distilled water (n=27) subcutaneously daily starting on day 14 after inoculation. Mice were killed on day 28. After weighing the hearts, they were sectioned and histopathologically examined.

**Chronic experiment.** DBA/2 mice were inoculated and were given carteolol at a dose of 1 (n=13) or 10 mg/kg (n=9), metoprolol at 30 mg/kg (n=9), or distilled water (n=31) orally daily starting on day 14 after inoculation. Mice were killed on day 104. After weighing the hearts, they were sectioned transversely and histopathologically examined.

**Myocardial Virus Titers**

Myocardial virus titrations were performed with a plaque assay method as previously described. Myocardial virus titers were expressed as log_{10} pfu/mg heart.

**Histopathological Examination**

Hearts were fixed in 10% formalin and sectioned along the long axis through the atria and ventricles in the acute and subacute experiments. In the chronic experiment, hearts were sectioned transversely (perpendicular to the long axis) at the maximal circumference of the ventricle after fixation in 10% formalin. The heart tissues were embedded in paraffin and stained with hematoxylin–eosin. The wall thickness and the cavity dimension of the left ventricle were measured to the nearest 0.01 mm with an ocular micrometer. Myocardial fiber diameter of the left ventricle was determined by measuring the shortest diameter at the level of the nucleus of 40 myocardial fibers in the stained cross-sections with an ocular micrometer. Myocardial cell necrosis, fibrosis, cellular infiltration, and calcification were graded by two observers, who were not aware of treatment regimens, and were scored as follows: 0, no lesions; 1+, lesions involving less than 25% of the myocardium; 2+, lesions involving 25–50% of the myocardium; 3+, lesions involving 50–75% of the myocardium; and 4+, lesions involving 75–100% of the myocardium. The scores obtained from two observers were averaged.

**Statistical Analysis**

Survival was analyzed by the Kaplan–Meier method. Statistical analysis of the data for body weight, heart weight, heart weight to body weight ratio, myocardial virus titers, histological score, left ventricular wall thickness, left ventricular cavity dimension, and myocardial fiber diameter of the left ventricle was performed by one-way analysis of variance (ANOVA). Values are given as mean±SD.

**Results**

**Acute Experiment**

Myocardial virus titers on day 7 were similar among the groups: 2.1±0.9 log_{10} pfu/mg heart in the
infected control group, 1.9±0.9 log$_{10}$ pfu/mg heart in mice given carteolol at 1 mg/kg/day, 2.5±1.0 log$_{10}$ pfu/mg heart at 10 mg/kg/day, 2.4±1.1 log$_{10}$ pfu/mg heart at 30 mg/kg/day, and 2.1±0.8 log$_{10}$ pfu/mg heart at 100 mg/kg/day. Viral replication in the heart was not influenced by carteolol. There were no significant differences between groups in regard to survival, heart weight to body weight ratio, and histopathological findings on day 14 (Table).

**Subacute Experiment**

Before the assignment of treatment of mice on day 14, 45.9% (40 of 87) of the mice had died. Survival rates after starting carteolol treatment were not significantly different among these groups. Body weight, heart weight, and the heart weight to body weight ratio were similar in each group. The histopathological findings were not significantly different among the groups (Table 1).

**Chronic Experiment**

**Survival rate.** Before the assignment of treatment of mice on day 14, 58.9% (89 of 151) of the mice had died. The survival of the infected control group after day 14 was 64.5% (20 of 31). The survival was 76.9% (10 of 13) in mice given carteolol at 1 mg/kg/day, 66.7% (six of nine) at 10 mg/kg/day, and 77.8% (seven of nine) in mice given metoprolol. There were no significant differences between the groups.

**Body Weight, Heart Weight, and Heart Weight to Body Weight Ratio**

Although body weight was similar in each group, heart weight was significantly lower in mice given carteolol at 1 (124±19 mg, p<0.01) or 10 mg/kg/day (114±19 mg, p<0.01) than that in the infected control group (167±23 mg) (Figure 1). Therefore, the heart weight to body weight ratio in mice given carteolol at 1 (5.3±0.4 x 10$^{-5}$, p<0.01) and 10 mg/kg/day (5.1±0.7 x 10$^{-5}$, p<0.01) was significantly lower than that in the infected control group (7.1±1.0 x 10$^{-5}$). Metoprolol did not cause a significant change in heart weight or the heart weight to body weight ratio.

**Histopathological Examination**

In the chronic stage, cellular infiltration was not found, and the extent of fibrosis and calcification was increased. The histological scores of myocardial fibrosis were 2.1±0.9 in the infected control group, 1.4±0.5 in mice given carteolol at 1 mg/kg/day, 1.2±0.8 at 10 mg/kg/day, and 2.1±1.1 in mice given metoprolol. Scores of calcification showed the same pattern as the fibrosis scores. Histological scores for mice given carteolol at 10 mg/kg/day were significantly lower (p<0.05) than those for the infected control group and the metoprolol-treated group (Figure 2). Representative histological sections are shown in Figures 3A–3C. On day 104, hearts of mice without carteolol treatment showed dilatation and hypertrophy (Figure 3D). However, carteolol prevented those findings (Figure 3E). Metoprolol did not cause any change of those findings (Figure 3F).

**Wall Thickness and Cavity Dimension**

Left ventricular wall thickness in mice given carteolol at 1 (1.13±0.20 mm, p<0.05) or 10 mg/kg/day (1.11±0.20 mm, p<0.05) was significantly lower than that in the infected control group (1.28±0.16 mm) (Figure 4A). Left ventricular cavity dimension was significantly greater in the infected control group (1.77±0.36 mm) than that in mice given carteolol at 1 (1.31±0.24 mm, p<0.01) or 10 mg/kg/day (1.37±0.17 mm, p<0.05) (Figure 4B). Metoprolol did not affect wall thickness and cavity diameter.

**Myocardial Fiber Diameter**

Myocardial fiber diameter of the left ventricle in the infected control mice (18.4±1.2 μm, p<0.05) was
was significantly greater than that in mice given carteolol at 1 (16.9±1.1 μm) or 10 mg/kg/day (15.5±0.3 μm). However, the fiber diameter in mice given metoprolol was similar to that in the infected control group.

**Discussion**

Carteolol is a nonselective β-adrenergic blocker with moderate intrinsic sympathomimetic activity and little membrane stabilizing activity.²⁴⁻²⁷ It has about a 20–30-fold stronger β-adrenergic blocking effect per unit weight than propranolol. Although the elimination half-life of carteolol is relatively short at 3–7 hours, the pharmacological half-life is long (approximately 17 hours),²⁵,²⁶ so a sufficient β-adrenergic blocking effect can be obtained with a single daily dose.

To our knowledge, there has been only one published report¹⁰ on the effects of β-adrenergic blockers on myocarditis. In that study, the effect of metoprolol on acute coxsackievirus B3 in a murine model of myocarditis was examined. There was an increase in the mortality of the mice and in severe pathological changes of the hearts of mice given metoprolol at 32.5 mg/kg/day. The deleterious hemodynamic and immunologic effects of metoprolol were considered to cause these findings because the myocarditis virus titer was increased. On the other hand, in our experiment, moderate doses of carteolol had no deleterious effect on survival, heart weight to body weight ratio, histopathological findings, or myocardial virus titer in the acute and subacute stages. Thus, carteolol did not worsen viral myocarditis in these mice. The difference may be due to differences in the strain of mice, the virus, or the β-adrenergic blocker.

In 1975, Waagstein et al. introduced the concept that β-adrenergic blockers may be useful in some forms of heart failure due to dilated cardiomyopathy. Since then, several groups of investigators have studied the effects of β-adrenergic blockers on the hemodynamics, clinical course, and survival of patients with dilated cardiomyopathy.¹⁰⁻¹⁶,²⁰,²¹,²⁸⁻³⁰ Several studies have reported a beneficial effect of these drugs in patients with dilated cardiomyopathy. Recent reviews³¹,³² have provided evidence to support the efficacy of β-adrenergic blockers both from a pathophysiological point of view and from accumulated clinical experience, including randomized, controlled trials.¹⁴,¹⁵ However, their value has been questioned by other groups of investigators who were not able to reproduce the effectiveness in the short-term study.²⁰,²¹,²⁸⁻³⁰

A variety of mechanisms of the effect of β-adrenergic blockers have been suggested, including 1) decreased myocardial energy demand, 2) improved diastolic relaxation, filling, and compliance, 3) protection of myocytes against the direct toxic effects of catecholamines, 4) inhibition of sympathetically mediated vasoconstriction through the release of prostaglandins and renin, 5) upregulation of β-adrenergic receptors,³³,³⁴ and 6) anti-ischemic and antiarrhythmic effects. Another recently suggested mechanism is that the immune function is regulated in part by the sympathetic nervous system.³⁵,³⁶ In view of the evidence for impaired cellular and humoral immunity in dilated cardiomyopathy such as defective natural killer and suppressor cell activities³⁷,³⁸ and of the existence of autoantibodies against the cardiac β-adrenergic receptors,³⁹ it is certainly possible that inhibiting the effect of catecholamines on the immune system by β-adrenergic blockers may lead to reversal of some of these immune abnormalities. However, the detailed mechanism of action of β-adrenergic blockers is unknown, and several mechanisms are believed to interact cooperatively. One of the reasons why the mechanism of their action has been difficult to define is that few reliable animal models of dilated cardiomyopathy are available. Although some investigators believe that the cardiomyopathic Syrian hamster is a model of human dilated cardiomyopathy,⁴⁰⁻⁴² congestive heart failure generally does not occur until after 250 days, and the occurrence of the disease is not uniform. Therefore, to examine whether drug therapy can effectively prevent cardiomyopathy in these hamsters is difficult. There are a few reports that propranolol therapy is partially protective in this model.⁴³,⁴⁴ However, many other
investigators believe that the Syrian hamster is not a good model of dilated cardiomyopathy. Our murine model was induced by viral myocarditis, and the pathological and clinical findings in the chronic stage were quite similar to those of human dilated cardiomyopathy. In the present study we did not examine hemodynamic parameters because mice are too small to obtain reliable data, and we did not examine the number of $\beta$-adrenergic receptors. However, we did observe improvement of the histopathological changes of the myocardium and reduction of heart weight, heart weight to body weight ratio, left ven-
tricular cavity dimension, left ventricular wall thickness, and myocardial fiber diameter of the left ventricle. Thus, carteolol definitely improved myocardial damage, inhibited cardiac hypertrophy, and resulted in improvement of congestive heart failure. Our murine model appears to be a good experimental model for assessing the effects of $\beta$-adrenergic blockers on dilated cardiomyopathy.

If $\beta$-adrenergic blockers have a beneficial effect on dilated cardiomyopathy, then what kind of $\beta$-adrenergic blocker is the most effective? Most previous studies used a $\beta_1$-selective agent, metoprolol, and data about

**FIGURE 4.** Bar graphs of effects of carteolol and metoprolol on the left ventricular (LV) wall thickness (panel A) and cavity dimension (panel B) in the chronic experiment. LV wall thickness and LV cavity dimension were significantly reduced in mice given carteolol, whereas these parameters were not affected by metoprolol.

**FIGURE 5.** Bar graph of effects of carteolol and metoprolol on the myocardial fiber diameter of the left ventricle (LV) in the chronic experiment. Carteolol treatment significantly reduced the fiber diameter, whereas metoprolol had no such effect. Effect of carteolol was greater at a dose of 10 than at 1 mg/kg/day.
the effects of β-adrenergic blockers with intrinsic sympathomimetic activity have been limited.16,20,21 In this experiment, carteolol definitely had a beneficial effect, and metoprolol did not cause any significant improvement. Although metoprolol at 30 mg/kg/day is nearly equivalent to carteolol at 1–10 mg/kg/day, the difference in effects may have been due to the doses administered or to the characteristics of carteolol such as intrinsic sympathomimetic activity and β-nonselectivity. Carteolol has a vasodilating action that is mediated in part by β-adrenoceptor stimulation,27 and intrinsic sympathomimetic activity has been shown to favor the upregulation of β-adrenergic receptors in the failing heart.45 Furthermore, intrinsic sympathomimetic activity may protect against the potential adverse effects of β-adrenergic blockers, and these characteristics may be related to the fact that xamoterol, a β1-selective partial agonist, improved the symptoms and cardiac functions in patients with dilated cardiomyopathy.64,41 Furthermore, because immunologic mechanisms may participate in the control of β-adrenergic receptors and because the release of immunoregulatory cells from the spleen is thought to be controlled by a β2- and not a β1-receptor,48 β-nonselectivity may be favorable for the immunoregulation. Carteolol may be superior to metoprolol because it has intrinsic sympathomimetic activity and β-nonselectivity.

It is difficult to compare doses used in different animal species, but on the basis of body surface area, a given dose in mice is comparable to a dose that is 12 times lower in humans.49 Thus, the doses of 1 and 10 mg/kg in mice are equivalent to doses of 0.08 and 0.8 mg/kg in humans, which are within the range of clinical dosage. Because even 1 mg/kg/day carteolol was effective in mice, this drug may be used for the treatment of dilated cardiomyopathy in clinical settings.

In conclusion, moderate doses of carteolol had no deleterious effects on mice with viral myocarditis in the acute and subacute stages and had a beneficial effect in the chronic stage when the disease picture was similar to human dilated cardiomyopathy. These findings suggest that nonselective β-adrenergic blockers with intrinsic sympathomimetic activity are applicable in treating patients with dilated cardiomyopathy.

References


KEY WORDS • carteolol • beta-adrenergic receptor blocker • myocarditis • dilated cardiomyopathy • mice • metoprolol
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