THE EFFECT OF $\alpha_1$-BLOCKER, BUNAZOSIN ON A MURINE MODEL OF CONGESTIVE HEART FAILURE INDUCED BY VIRAL MYOCARDITIS

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The purpose of this study was to investigate the therapeutic effect of an $\alpha_1$-blocker, bunazosin, using an experimental murine model of congestive heart failure induced by viral myocarditis. This model is characterized by a high incidence of severe myocarditis and subsequent congestive heart failure, and is suitable for the evaluation of the effect of drugs. To estimate myocardial damage objectively and quantitatively, we used antimony monoclonal antibody in addition to histopathological grading. Four-week-old BALB/c mice were inoculated with encephalomyocarditis virus. The mice were injected daily with bunazosin or saline as a placebo from the day of viral inoculation until day 7 (protocol-I) or day 14 (protocol-II), or from day 4 to day 14 (protocol-III). They were then injected with 1.5$\mu$Ci of indium-111 labeled antimony antibody and were killed 24 h later. The antimony cardiac uptake was counted and histopathological grading was performed. The heart-weight to body-weight ratio, left ventricular dimension, histopathological grades and antimony cardiac uptake were significantly lower in the bunazosin group than in the placebo group in protocol-II, but not in protocol-I or protocol-III. Bunazosin showed a protective effect against viral myocarditis only when it was started early after infection and continued until the stage of congestive heart failure.

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The usefulness of alpha-adrenergic blockers in treating patients with congestive heart failure is explained by its vasodilating effect. However, some investigators have reported that prazosin, the most well known $\alpha_1$-blocker, induced tolerance easily. In this study, we investigated the effect of bunazosin, a more selective $\alpha_1$-blocker than prazosin, in a murine model of viral myocarditis. This animal model is characterized by a high incidence of severe myocarditis and congestive heart failure, and is suitable for evaluating the therapeutic effects of drugs. Bunazosin is a balanced arterial and venous vasodilator, and the frequency of orthostatic hypotension during therapy using it is reported to be lower than that which occurs with prazosin therapy. Bunazosin has been reported to be useful in the treatment of congestive heart failure. It has also been reported that its vasodilating effect

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continued for a relatively long time\(^{13-14}\). In order to estimate myocardial damage objectively and quantitatively, we used radiolabeled antimyosin monoclonal antibody as a tracer of myocardial damage\(^{17-18}\) in addition to conventional histopathological grading.

**METHODS**

**A murine model of viral myocarditis**

The virus stock was prepared as described previously\(^7\) and had a titer of \(1 \times 10^7\) plaque-forming units (PFU)/ml determined in tissue cultures of FL (human amnion) cells. It was stored at \(-70\,^\circ\text{C}\) until use. Four-week-old male BALB/c mice were inoculated intraperitoneally with a 0.1 mlencephalomyocarditis virus suspension containing 100 PFU/ml. Uninfected age-matched BALB/c mice served as controls.

**Drug administration**

Bunazosin (Eizai Co., Ltd., Tokyo, Japan) solution was prepared by dissolving the drug powder in sterile saline. Mice in the bunazosin group were injected daily subcutaneously with 10 mg/kg body-weight of the drug in 0.1 ml saline. Mice in the placebo group and the uninfected control group were injected daily subcutaneously with 0.1 ml saline.

**Protocols**

A total of 97 inbred BALB/c mice were used in this study. In protocol-I, 27 mice were divided into 3 groups; an uninfected control group (\(n=5\)), and 2 groups infected with encephalomyocarditis virus. One group received bunazosin (\(n=12\)) and the other group received saline (\(n=10\)) daily from the day of virus inoculation (day 0) until day 7. In protocol-II, 34 mice were divided into 3 groups; uninfected control group (\(n=5\)) and 2 infected groups. The infected mice received bunazosin (\(n=15\)) or saline (\(n=14\)) daily from day 0 until day 14. In protocol-III, 36 mice were divided into 3 groups; uninfected control group (\(n=5\)), and 2 infected groups. The infected mice received bunazosin (\(n=16\))
or saline (n=15) daily from day 4 until day 14.

**Histopathological study**

After counting myocardial radioactivity in the antymyosin study (described later), the hearts were fixed in a 10% formalin solution, sectioned longitudinally at the midportion of the ventricle, embedded in paraffin, and stained with hematoxylin-eosin. The cavity dimensions of both ventricles were measured at the midportion of each ventricle to the nearest 0.01 mm with an ocular micrometer. The pathological grades of myocardial cell necrosis, cellular infiltration and calcification were assigned blindly by 2 observers and were scored as follows8: grade 0, no myocardial lesion; grade 1, lesions involving less than 25% of the myocardium; grade 2, lesions involving 25%—50% of the myocardium; grade 3, lesions involving 50%—75%; grade 4, lesions involving more than 75% of the myocardium (Fig. 1). The grades given by 2 observers were averaged.

**Antymyosin study**

Fab fragments of antymyosin murine monoclonal antibody labeled with indium-111 coupled with diethylene triamine pentaacetic acid18,19 were used (supplied by Daiichi Radioisotope Laboratories, Ltd., Japan). Mice were injected intravenously with 1.5 µCi of indium-111-antimyosin antibody through a tail vein on day 7 in protocol-I and on day 14 in protocol-II and protocol-III. Twenty-four hours later, mice were weighed and killed. The heart, liver and lungs were excised, weighed and the uptake of the tracer was determined with a well-type autogamma counter. The tracer uptake was expressed as a ratio of percent dose per gm of the organ to percent per milliliter of blood (organ to blood ratio). The weight of the mice was normalized to 20 g·mL⁻¹.

**Statistics**

Survival was analyzed by the Kaplan-Meier method. Statistical comparisons of the BW, HW, HW/BW ratio, pathological grades and antymyosin cardiac uptake data were performed by analysis of variance (ANOVA). Results are expressed as mean ± SD.

### TABLE 1. EFFECTS OF BUNAZOSIN IN MURINE MYOCARDITIS

<table>
<thead>
<tr>
<th>Protocol</th>
<th>BW (g·mL⁻¹)</th>
<th>HW (g·mL⁻¹)</th>
<th>HW/BW ratio</th>
<th>L/W ratio</th>
<th>LV dimension (mm)</th>
<th>RV dimension (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>17.8 ± 0.7</td>
<td>13.2 ± 1.0</td>
<td>0.76 ± 0.04</td>
<td>2.2 ± 0.1</td>
<td>4.2 ± 0.2</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>II</td>
<td>16.0 ± 1.4</td>
<td>12.0 ± 1.2</td>
<td>0.75 ± 0.03</td>
<td>2.1 ± 0.1</td>
<td>4.0 ± 0.2</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>III</td>
<td>14.4 ± 1.6</td>
<td>10.4 ± 1.1</td>
<td>0.73 ± 0.02</td>
<td>2.0 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>4.0 ± 0.3</td>
</tr>
</tbody>
</table>

*Re: period of the treatment: 0: n=5, 1: n=8, 2: n=7, 3: n=9, 4: n=5, 5: n=6, 6: n=8*
RESULTS

Survival rate
The survival rates at the end of each protocol were 58% vs 60% (bunazosin group vs placebo group) in protocol-I, 53% vs 50% in protocol-II, 50% vs 40% in protocol-III. There was no significant difference in the survival rate between the bunazosin group and the placebo group at any time during the study.

Body Weight (BW), Heart Weight (HW), HW/BW ratio, Lung Weight (LW), LW/BW ratio, and ventricular cavity dimensions
At the beginning of each study, there was no significant difference in BW between protocols (15.4±1.0 gm in protocol-I, 15.9±1.5 gm in protocol-II and 15.0±1.5 gm in protocol-III), or between groups in each protocol (uninfected group, infected bunazosin group and infected placebo group). Table I shows the BW, HW, HW/BW ratio, LW, LW/BW ratio and the cavity dimensions of right and left ventricles of all surviving mice.

The BW of the uninfected group was significantly higher than that of infected groups in all protocols except that of the bunazosin group in protocol-II. There was a significant difference in the BW between the bunazosin group and the placebo group in protocol-II (p<0.05), but not between the uninfected group and the bunazosin group. The HW in infected groups was significantly higher than that in the uninfected group in protocol-I (p<0.05), although there was no significant difference in protocol-II and protocol-III. The HW/BW ratio of infected groups was significantly higher than that of uninfected groups in all protocols except protocol-II. In protocol-II, the HW/BW ratio of the bunazosin group was significantly lower than that of the placebo group (p<0.05) and there was no difference compared with the uninfected group.

There was no significant difference in the LW between the uninfected group and the infected group in all protocols. However, the LW/BW ratio of infected groups was significantly higher than that of uninfected groups in all protocols except that of the bunazosin group in protocol-II. In protocol-II, the LW/BW ratio of the bunazosin group was significantly lower than that of the placebo group (p<0.05) and there was no difference compared with the uninfected group.

The left and right ventricular cavity dimensions of infected groups were significantly larger than those of uninfected groups in all protocols. In protocol-II, the left ventricular dimension of the bunazosin group was significantly smaller than that of the placebo group (p<0.05), but significantly
Fig. 3. Histopathologic grades of myocardial necrosis, cellular infiltration, and calcification (panel A), and antimony uptake (panel B) in protocol-II. Both pathologic grades and antimony uptake of the bunazosin group were significantly lower than those of the placebo group. There was no significant difference in antimony uptake between the bunazosin group and the uninfected control group.

Fig. 4. Histopathologic grades of myocardial necrosis, cellular infiltration, and calcification (panel A), and antimony uptake (panel B) in protocol-III. There was no significant difference in either pathological grades or antimony uptake between the bunazosin group and the placebo group.

larger than that of the uninfected group \((p<0.05)\). The right ventricular cavity dimension of the bunazosin group was also significantly smaller than that of the placebo group \((p<0.05)\), but significantly larger than that of the uninfected group \((p<0.05)\).

**Histopathologic grades and antimony cardiac uptake**

Fig. 2A, 3A and 4A show the pathologic grades and Fig. 2B, 3B and 4B show the antimony cardiac uptake in protocol-I, protocol-II and protocol-III, respectively. In protocol-I, there was no significant difference in the pathological grades of myocardial necrosis, cellular infiltration, and calcification between the bunazosin group and the
placebo group (Fig. 2A). The antmyosin cardiac uptake (heart-to-blood) radioactivity ratio was significantly higher in the hearts of infected groups compared with the uninfected group (p < 0.01), although there was no significant difference between the bunazosin group and the placebo group (Fig. 2B).

In protocol-II, there were significant differences between the bunazosin group and the placebo group in the pathological grades of myocardial necrosis (0.6 ± 0.6 vs 1.6 ± 0.7, p < 0.05), cellular infiltration (0.9 ± 0.9 vs 2.3 ± 0.9, p < 0.05) and calcification (0.4 ± 0.4 vs 1.6 ± 0.7, p < 0.01) (Fig. 3A). There was also a significant difference in antmyosin cardiac uptake between the bunazosin group (6.8 ± 3.4) and the placebo group (11.8 ± 4.2, p < 0.05) (Fig. 3B). There was no significant difference in the antmyosin uptake between the bunazosin group and the uninfected group (6.8 ± 3.4 vs 4.9 ± 1.5).

In protocol-III, there was no significant difference either in the pathological grades or in the antmyosin cardiac uptake between the bunazosin group and the placebo group (Fig. 4A and 4B). However, there was a significant difference in antmyosin cardiac uptake between the infected group and the uninfected group (Fig. 4B).

DISCUSSION

Animal model for congestive heart failure
There are few good animal models of congestive heart failure suitable for the evaluation of methods of therapeutic intervention. Matsumori et al developed a murine model of congestive heart failure induced by encephalomyocarditis virus myocarditis? In this model, mortality reaches its maximum 4 to 5 days after viral inoculation due to viremia, and then decreases gradually. The remaining mice then develop severe congestive heart failure, and the mortality increases again between day 11 and day 14. Left ventricular dilatation and wall thinning are pronounced on days 8 to 14 in comparison with conditions on days 5 to 7. The severe congestion in the lungs and liver is marked, pleural effusion and ascites are present. The development of congestive heart failure from acute myocarditis has been reported in clinical studies20–22 and myocarditis is considered to be a part of the etiology of dilated cardiomyopathy.23,24 This murine model is useful in the investigation of methods of preventing development of dilated cardiomyopathy following acute myocarditis.8–11

α1-blocker treatment in congestive heart failure

Alpha-blockers are clinically effective in patients with congestive heart failure!–3 They have some advantages which other vasodilators do not have, including beneficial effects on lipoprotein metabolism25 and on renal and cerebral blood flow.26–27 Bunazosin, an α1-blocker and balanced vasodilator, has been reported to be useful in the treatment of congestive heart failure.13–15 It has been reported to reduce mean aortic pressure, total vascular resistance index, left ventricular end-diastolic pressure and increased cardiac index in chronic congestive heart failure after experimental myocardial infarction13 and in patients with congestive heart failure.14–15 In this study, bunazosin did not reduce the survival rate of mice infected with the virus. However, bunazosin showed a protective effect in the subacute stage of viral myocarditis if injected from the day of viral inoculation to day 14 (protocol-II). With protocol-II, the parameters that indicate congestive heart failure (LW/BW ratio and right and left ventricular dimensions) were significantly improved and the histopathological lesions were significantly less in the bunazosin group as compared with those in the placebo group. Moreover, antmyosin cardiac uptake in the bunazosin group was significantly lower than that in the placebo group, indicating that bunazosin reduced myocardial damage. With protocol-I, bunazosin did not improve myocardial lesions at the stage of viremia, indicating that bunazosin itself may not have antiviral properties. The therapeutic effect of bunazosin seen with protocol-II may be due to its vasodilating effect and cardioprotective action.28 Bunazosin is reported to reduce histopathological myocardial damage in cardiomyopathic hamsters29 and spontaneously hypertensive rats (SHR)30. Tanaka et al reported that bunazosin treatment improved the restoration of adenosine triphosphate (ATP) and creatine phosphate in SHR after reperfusion following 30 min of ischemia.30
However, bunazosin had no significant effect if treatment was for shorter duration (protocol-I) or in the subacute stage, if injection was started from day 4 to day 14 (protocol-III). Therefore, the $\alpha_1$-blocker therapy in this murine model of congestive heart failure induced by viral myocarditis was considered to be of limited value.

**Antimyosin antibody cardiac uptake**

To date, the degree of myocardial lesions has been estimated mainly by semi-quantitative histopathological grading$^8$–$^{11}$ In this study, we used radiolabeled antimyosin antibody as a marker of myocardial damage$^{17}$–$^{18}$ in order to estimate myocardial damage objectively and quantitatively. This monoclonal antibody is clinically used to visualize and evaluate myocardial damage of myocardial infarction$^{31}$, myocarditis$^{33}$, cardiomyopathy$^{35}$, rejection after heart transplantation$^7$ etc. In our experimental studies using the murine model of viral myocarditis, we have demonstrated that antimyosin cardiac uptake correlates well with pathological grade of myocardial necrosis$^{17}$.$^{18}$ Antimyosin cardiac uptake of infected mice was greater than that in uninfected normal mice from day 5 to day 14 after viral inoculation, and was found to be related to myocardial necrosis in the indium-111 labeled antimyosin antibody study$^{18}$ Therefore, the therapeutic effects of drugs can be evaluated during this period of myocarditis.

In conclusion, the early and prolonged administration of bunazosin showed protective effect in murine congestive heart failure induced by viral myocarditis. The $\alpha_1$-blocker therapy in this animal model was considered to be of limited value.

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