Therapy with the Nonpeptide Endothelin Receptor Antagonist 97-139 in a Murine Model of Congestive Heart Failure

Reduction of Cardiac Mass and Myofiber Hypertrophy

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SUMMARY

Endothelin-1 (ET-1) is a potent vasoconstrictor. This peptide exerts numerous effects on the heart, including regulation of cardiomyocyte growth during hypertrophy. The effects of the structurally novel, nonpeptide, ET-1-selective, competitive antagonist (ETA) 97-139 were investigated in mice with congestive heart failure (CHF) and myocardial hypertrophy. Morphological and microscopical analyses were conducted on day 56 after viral inoculation following 28 day treatment with 99-139. Eight week-old DBA2 mice were intraperitoneally inoculated with encephalomyocarditis virus at a dose of 500 pfu / mouse. The 30 mice were divided into two groups-an ETA treated group and an untreated group. Heart weight (HW) in the infected group was significantly ($p < 0.05$) increased compared to that in the uninfected group. HW and the HW / body weight (BW) ratio were significantly ($p < 0.05$) reduced in the ETA treated group compared with the untreated group (HW; 127.7 ± 6.2 mg vs 144.3 ± 4.2 mg, HW / BW; 4.9 ± 0.9×10^{-3} vs 5.4 ± 0.5 × 10^{-3}). Myofiber diameter in the ETA treated group was significantly reduced compared with the untreated group (12.1 ± 1.5μm vs 14.3 ± 1.9 μm). These results suggest the ET-1 receptor antagonist 97-139 has an effect on the reduction of cardiac mass and myofiber hypertrophy, and that 97-139 may be a useful agent for CHF due to viral myocarditis. (Jpn Heart J 2000; 41: 79-85)

Key words: Nonpeptide, Encephalomyocarditis, DBA2
ENDOTHELIN-1 (ET-1) is a potent vasoconstrictor. This peptide exerts numerous effects on the heart, including the regulation of cardiomyocyte growth during hypertrophy\(^1\). In isolated ventricular cardiomyocytes, the myocardial ET-1 system is upregulated in the failing myocardium.\(^2,3\) ET-1 induces myocardial cell hypertrophy and has positive inotropic and chronotropic effects \textit{in vivo}.\(^4,6\) In the treatment of congestive heart failure (CHF), ET-1 may play an inducible role in cardiac remodeling and may accelerate the severity of CHF. Therefore, this suggests that an ET receptor antagonist may reduce cardiac hypertrophy and congestive heart failure via decreased expression of ET-1.

Several reports have described the beneficial effects of ET-1 blockers in animal models of CHF. An ET-1 blocker improved hemodynamics in pacing-induced CHF\(^7\) and showed beneficial effects on survival and hemodynamics in models of cardiomyopathy\(^8\) and myocardial infarction.\(^2,9\) Viral myocarditis in a murine model causes CHF and myocardial hypertrophy.\(^10,11\) However, the beneficial effect of ET-1 blockade has not been reported in congestive heart failure due to viral myocarditis.

Recently, the structurally novel, ET-A receptor-selective, nonpeptide competitive antagonist (ETA) 97-139 was identified and found to have the same biological effect as same as that of BQ-123.\(^12\) ETA has not yet been used for the treatment of CHF induced by viral myocarditis.

We investigated ETA in mice with viral myocarditis, which induces severe CHF and myocardial hypertrophy after the onset of symptoms in the clinical setting. Morphological and microscopical analyses were investigated on day 56 after viral inoculation following 28 day treatment.

\textbf{METHODS}

\textbf{Animal:} Female DBA2 mice were obtained from Charles River Japan Co. Ltd, (Atsugi, Kanagawa, Japan).

\textbf{Virus:} A myocarditic variant of the EMC (encephalomyocarditis) virus was obtained from Dr. Yoshiko Seto of Keio University in Tokyo. Virus preparations were stored at 70°C as previously described.\(^11\) We injected each mouse intraperitoneally (i.p.) with an infective dose containing 500 plaque-forming units (pfu).

\textbf{Drug administration:} The 97-139 (Fujisawa Pharmacentical Co., Ltd., Tokyo) solution was prepared in phosphate solution. The doses were calculated according to the methods of Mihara, \textit{et al.}\(^12\)

\textbf{Treatment protocol:} Eight week-old DBA2 mice were inoculated i.p. with EMC virus (500 pfu / mouse). The 30 mice were divided into two groups, an ETA treated group and an untreated group. The drugs were administered with PBS by a
mini pump for 28 days starting 28 days after inoculation. Frequency of water consumption was determined twice a week. The mice were sacrificed at the end of the 28 day treatment period. They were administered ETA 1 mg / kg / day or PBS via an osmotic pump (Model 2ML2, CSI Japan, Co. Ltd, Tokyo) as mentioned previously. As uninfected control mice, 6 of each mice were treated with ETA at the same dose for 28 days and 6 were untreated.

Pathological examination: After sacrifice, the heart and other organs were weighed, fixed in 10% buffered formalin, and stained with hematoxylin-eosin. Myocardial sections were graded for severity of necrosis and inflammation, which were blindly scored by one pathologist from 1 to 4 as follows: grade 1, lesions involving less than 25% of the myocardium; grade 2, lesions involving 25 to 50%; grade 3, lesions involving 50 to 75%; and grade 4, lesions involving 75 to 100%. Myocardial fiber diameter in the lateral wall of the ventricle was determined by measuring the shortest diameter at the level of the nucleus of 50 myocardial fibers from each group using an ocular micrometer in the strained cross-sectional areas as mentioned previously.

Statistics: Statistical analysis was performed with StatView-J4.02 software for Macintosh. Data were examined using analysis of variance (ANOVA) for repeated measures and compared by the deviation method. A probability of 0.05 was chosen as the level of significance. All results are expressed as the mean ± standard deviation.

RESULTS

Organ Weight: Heart weight (HW) in the infected group was significantly \( (p < 0.05) \) increased compared to that in the uninfected group. HW and the HW / body weight (BW) ratio were significantly \( (p < 0.05) \) reduced in the ETA treated group compared with the untreated group (Figure 1). In the uninfected mice, ETA did not reduce the BW and HW as much as in the untreated uninfected control.

The lung, liver and kidney weights were not statistically significant between the ETA treated group and the untreated group (Table I).

Pathologic findings: In the 56 day post-virus inoculation, myocardial necrosis with calcification was evident in the untreated group. The inhibition of myocardial necrosis and calcification were not apparent in the ETA group (Table I, Figure 2).

Myocardial fiber diameter: The myocardial fiber diameter in the infected untreated mice with CHF was significantly \( (p < 0.05) \) higher than in uninfected controls. ETA treatment significantly reduced the myocyte diameter compared with the untreated infected group (Table II).
Table I. Body Weight (BW), Lung Weight (Lu W), Liver Weight (Li W) and Right Kidney Weight (K W) in Mice with Congestive Heart Failure due to Encephalomyocarditis Virus

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>Lu W (g)</th>
<th>Li W (g)</th>
<th>K W (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uninfected group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated control (n=6)</td>
<td>28.7 ± 0.7</td>
<td>133 ± 35</td>
<td>1233 ± 145</td>
<td>139 ± 10</td>
</tr>
<tr>
<td>ATA treated (n=6)</td>
<td>27.6 ± 0.6</td>
<td>130 ± 33</td>
<td>1245 ± 154</td>
<td>136 ± 13</td>
</tr>
<tr>
<td><strong>Infected group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated mice (n=13)</td>
<td>27.9 ± 0.8</td>
<td>153 ± 40</td>
<td>1226 ± 106</td>
<td>139 ± 18</td>
</tr>
<tr>
<td>ATA treated mice (n=10)</td>
<td>26.6 ± 0.6</td>
<td>156 ± 33</td>
<td>1333 ± 176</td>
<td>144 ± 11</td>
</tr>
</tbody>
</table>

*p<0.05 vs Untreated uninfected control.

Table II. Histopathologic Grade and Myocyte Diameter in Mice with Congestive Heart Failure

<table>
<thead>
<tr>
<th>Group</th>
<th>Necrosis</th>
<th>Inflammation</th>
<th>Myocyte diameter (µ m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uninfected group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated control (n=6)</td>
<td>ND</td>
<td>ND</td>
<td>11.8 ± 1.2</td>
</tr>
<tr>
<td>ATA treated Group (n=6)</td>
<td>ND</td>
<td>ND</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td><strong>Infected group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated mice (n=13)</td>
<td>2.2 ± 0.8</td>
<td>0.8 ± 0.5</td>
<td>14.3 ± 1.9 *</td>
</tr>
<tr>
<td>ATA treated mice (n=10)</td>
<td>2.1 ± 0.6</td>
<td>0.6 ± 0.4</td>
<td>12.1 ± 1.5 *</td>
</tr>
</tbody>
</table>

*p<0.05 vs Untreated uninfected control, *p<0.05 vs Untreated infected mice.
FIGURE 2. Morphological and microscopic findings in mice with congestive heart failure on day 56 after viral inoculation. The upper picture shows the reduction in cardiac mass treatment by with the endothelin receptor antagonist (ETA) 97-139. The lower picture shows the reduction in myofiber hypertrophy. Magnification: x800. Control; uninfected untreated control, Untreated; infected and untreated mice, ETA treated; infected and ETA treated mice.

DISCUSSION

Administration of the ETA 97-139 for 28 days reduced the heart mass and cardiac hypertrophy in CHF in a murine model of viral myocarditis. We believe that the ETA reduced cardiac hypertrophy and CHF.

The reductions in HW and the HW/BW ratio were prominent with ETA treatment. Myofiber diameter was also more decreased in ETA treatment than in the untreated infected mice.

The production of ET-1 is markedly increased in cardiac myocytes from the failing heart,14) and ET-1 is involved in stress-induced cardiac hypertrophy.15) ET-1 receptor antagonists significantly inhibit cardiac hypertrophy with congestive heart failure in animals2,7,8) and in humans.16) The reduction of cardiac ET-1 seems to be a key mechanism in the protection of cardiac remodeling. Our results showed that 28 day administration of ETA attenuated cardiac hypertrophy and CHF due to viral myocarditis. ETA may be useful for the treatment of viral myocarditis complicated by congestive heart failure and cardiomegaly in humans.

The beneficial effects of ETA have already been applied in patients with
heart failure and compared with captopril. An evaluation of bosentan in conventionally treated patients with symptomatic CHF over 2 weeks has revealed improved cardiac hemodynamics.17] Our results showed that cardiac mass was decreased to a greater extent in ETA-treated mice than in untreated mice. The reason for the lower mortality with ETA treatment may be due to the reduction in cardiac ET-1. The pathophysiological mechanism of ETA treatment in CHF requires further investigation.

The present study has demonstrated that the ETA 97-139 decreases relative heart weight and myocardial fiber diameter in a murine model of CHF due to EMC viral myocarditis. These findings suggest that the specific blockade of ET-1 plays an important role in the pathophysiology of cardiac hypertrophy and CHF induced by viral myocarditis.

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REFERENCES