Olmesartan, a Novel Angiotensin II Receptor Type 1 Antagonist, Suppressed Cytotoxic Myocardial Injury in Autoimmune Heart Failure

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Running head : Olmesartan in autoimmune myocarditis

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Abstract

Some angiotensin II receptor type 1 antagonists are reported to inhibit proinflammatory cytokine production in vitro and in vivo. However, the effects of the drugs upon autoimmune diseases are unknown. We tested the hypothesis that olmesartan, a novel angiotensin II receptor type 1 antagonist, ameliorated experimental autoimmune myocarditis (EAM) in rats attributing to the suppression of inflammatory cytokines as well as to the immunomodulating action for the heart. We orally administered olmesartan 1mg/kg/day, 3mg/kg/day, and 10mg/kg/day to rats with EAM for 3 weeks. The results showed that olmesartan decreased blood pressure significantly compared with the untreated group, and markedly reduced the severity of myocarditis associated with the decrease of myocardial macrophage, CD4+, and CD8+ T cell expression by comparing the heart weight/body weight ratio, pericardial effusion scores, macroscopic and microscopic scores. Myocardial interleukin-1β (IL-1β)-positive staining cells by immunohistochemistry and IL-1β expression by western blotting were significantly lower in rats with EAM given olmesartan treatment compared with those of rats given vehicle. Cardiac myosin-specific delayed type hypersensitivity was significantly lower in olmesartan-treated rats than in control rats. The cytotoxic activities of lymphocytes in rats with EAM treated with olmesartan were reduced compared with untreated controls. In vitro study showed that both olmesartan and its active metabolite, RNH-6270, suppressed IL-1β production in U937 cells and cultured myocytes. Olmesartan ameliorates acute EAM in rats. The cardioprotection of olmesartan may be due to suppression of inflammatory cytokines as well as to suppressive effects for cytotoxic myocardial injury in addition to the hemodynamic modifications.

Keywords : Angiotensin II ; Myocardial inflammation ; Cardiomyopathy ; Heart failure
Introduction

In humans, acute myocarditis is a potentially lethal disease, and frequently precedes the development of dilated cardiomyopathy (DCM). Two mechanisms to explain how myocarditis develops into DCM have been proposed; one is a persistent viral infection, and the other is a progressive autoimmune myocardial injury \(^{8}\). The autoimmune giant cell myocarditis in rats mimics human fulminant myocarditis in the acute phase \(^{11}\).

The significance of renin-angiotensin system for the development of heart failure is now well-known \(^{2}\). Angiotensin II (Ang II) has deleterious effects on the heart and kidney. Heart failure patients with high plasma renin levels develop more severe myocardial dysfunction than those with low plasma renin levels. Recent reports indicated that some Ang II type1 receptor (AT\(_1\)) antagonists inhibited inflammatory reactions in macrophages \(^{2,16,24}\). AT\(_1\) antagonists have also been reported to inhibit interleukin (IL)-1 production \(^{25}\). These results imply that olmesartan, a novel AT\(_1\) antagonist, may be an effective agent in countering myocardial inflammation as a result of removal of overproduced cytokines \(^{2,6,12,16,24,25}\). However, the effects of olmesartan upon autoimmune heart failure are still unknown.

The purpose of the present study was to examine the effects of olmesartan \(^{15}\), a novel AT\(_1\) antagonist, on an experimental autoimmune myocarditis (EAM) model, focusing on both its inhibitory effects on the inflammatory cytokines and on the cytotoxic activities of lymphocytes against myocytes. We chose EAM as an animal model of heart failure in the present study.

Materials and Methods

Immunization

Acute EAM was induced by immunization with porcine cardiac myosin in 6-week-old Lewis rats as previously described \(^{19,20,27,28}\). Porcine cardiac myosin (Sigma) was injected subcutaneously in the foot pads with 0.1 ml of myosin (10 mg/ml) mixed with an equal volume of Freund's complete adjuvant (FCA) supplemented with Mycobacterium tuberculosis H37Ra (Difco) on days 1 and 8 \(^{19,20,27,28}\). Control rats
were immunized with FCA alone.

**Medication**

To analyze the hemodynamics of olmesartan on rats without myocarditis, control rats immunized with FCA alone were divided into four groups for the oral administration of either (1) vehicle (0.5% methylcellulose, Con/V, n=3), (2) olmesartan 1 mg/kg/day (Con/Olm-1, n=3), (3) olmesartan 3 mg/kg/day (Con/Olm-3, n=3), and (4) olmesartan 10 mg/kg/day (Con/Olm-10, n=3) for 3 weeks. The dosage of olmesartan was determined by the previous reports (9, 21, 26).

Rats immunized with cardiac myosin (myocarditis rats) were divided into other four groups and treated with either (5) vehicle (EAM/V, n=9), (6) olmesartan 1 mg/kg/day (EAM/Olm-1, n=8), (7) olmesartan 3 mg/kg/day (EAM/Olm-3, n=8), and (8) olmesartan 10mg/kg/day (EAM/Olm-10, n=8) for 3 weeks. These doses were chosen because of the previous studies (3). The blood pressure and heart rate (HR) were determined by the tail-cuff method using a photoelectric tail cuff detection system (Softron BP-98A, Tokyo, Japan) on days 1, 8, 15 and 22. All the rats were sacrificed on day 22 under ether anesthesia after hemodynamic measurements (28). The organs were weighed and the ratio of organ to body weight was calculated. Olmesartan and its active metabolite, RNH-6270 (15), were kindly provided by Sankyo Co. Ltd (Tokyo, Japan).

The protocol was approved by the Institutional Animal Research Committee of Kyoto University.

**Histopathology**

At sacrifice, macroscopic findings were graded on a scale of 0 to 4 and pericardial effusion was graded as 0 to 2, as previously described (19,20,27,28). Microscopic findings for myocardial damage and cellular infiltration were graded on a scale of 0 to 4, as previously described (19,20,27,28). After macroscopic examination, a part of the ventricles was embedded in OCT for immunohistochemistry; remnant tissues were kept at –80°C for Western blotting.

**Immunohistochemical assay**

We used an immunoperoxidase technique to perform immunohistochemistry for
anti-IL-1β and cell surface markers, as previously described (19,20,27,28). The following primary antibodies were used: anti-IL-1β (Serotec), ED1 (macrophages, Serotec), W3/25 (CD4, Serotec) and OX8 (CD8, Serotec). The positive-staining cells of heart tissue were counted blindly by two observers in six fields at × 400 magnification (within a 1-mm² grid), and the total positive-staining cells of the six fields were recorded as the number of infiltrating cells in the lesions.

**Western blotting**

The myocardial lysates were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and sequentially electrophoretically transferred to a membrane (Millipore). The membrane was incubated with an anti-IL-1β (Serotec) antibody, then with a peroxidase-linked secondary antibody (Amersham). Chemiluminescence was detected and semiquantitatively analyzed using the NIH Image system, as previously described (20,27,28).

**Delayed type hypersensitivity**

Myosin-specific delayed type hypersensitivity (DTH) was quantified on day 20 using a standard hindlimb’s foodpad swelling assay. Maximal swelling was found at 48 hours after injection.

**Cell culture**

To culture splenocytes and lymph node (LN) cells, spleens and LNs were harvested from Lewis rats at sacrifice, and single-cell suspensions were obtained by passing through a stainless steel mesh screen. Cells were suspended in RPMI-1640 supplemented with fetal calf serum (FCS), 1% sodium pyruvate, 1% nonessential amino acids, 5×10⁻⁵ M 2-mercaptoethanol and penicillin-streptomycin mixture.

To culture neonatal rat cardiomyocytes, cardiac ventricles from 1- to 4-day-old Lewis rats were minced, and dissociated with 0.125% trypsin. Cardiomyocytes (2×10⁴/well) were incubated in 96-well plates in 10% FCS-supplemented Dulbecco’s modified essential medium (DMEM) at 37 °C. Bromodeoxyuridine (100μmol/l) was added during the first 48 hours to prevent the proliferation of non-myocytes.
Cytotoxicity assays

Lewis rats were immunized with cardiac myosin, treated with various dosages of olmesartan or vehicle, and killed on day 22. LN cells from rats treated with olmesartan or vehicle were used as effector cells. F-2 cells (murine endothelial cells) and cardiomyocytes plated in 96-micro-well plates ($2 \times 10^4$ / well) were labeled with sodium chromate at $1\mu$Ci/well ($^{51}$Cr, Amersham International) for 1 hour. After labeled target cells had been washed with phosphate buffered saline (PBS) three times, LN cells were incubated at an effector-target ratio of 100 : 1 and 200 : 1 for 4 hours. The supernatant was collected and the radioactivity of $^{51}$Cr released into the supernatant was measured by a gamma counter. The percentage of cytotoxicity was calculated using the formula

$$\% \text{ cytotoxicity} = \frac{(E - S)}{(M - S)} \times 100$$

where $E$ is the counts per minute (cpm) released in the presence of effector cells, $S$ is the spontaneous cpm released from target cells incubated in the medium, and $M$ is the maximal cpm released from target cells incubated with 2 % Triton X-100.

Cytokine assays

U937 cells (human macrophages) and cultured rat myocytes were stimulated with 10µg/ml lipopolysaccharide (LPS). Olmesartan or its active metabolite, RNH-6270 (15), was added to the medium 30min before LPS stimulation. Forty-eight hours later, IL-1β in the medium was assayed by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (R&D System).

Statistics

All values were expressed as means±standard deviation (SD). One way analysis of variance (ANOVA), following by Fisher protected least significant difference test, was performed. A value of $p<0.05$ was considered statistically significant.

Results

Hemodynamics of olmesartan treatment in rats with and without EAM

In control rats, middle (3mg/kg/day) and high (10mg/kg/day) doses of olmesartan
decreased blood pressure significantly and tended to increase HR compared with the vehicle group (Table 1). But, there were no significant differences of blood pressure or HR in the low (1mg/kg/day) dose of olmesartan treated groups compared with the vehicle group.

In rats with EAM, none died during the course of the disease. The blood pressure was decreased in olmesartan treated groups (Table 1). There were no significant differences of HR among the four groups.

**Histopathology, heart weight/body weight (HW/BW) in rats with acute EAM**

On day 22 at sacrifice, the hearts showed severe and diffuse discolored myocarditis with massive pericardial effusion in rats immunized with cardiac myosin. Extensive injuries to myocytes with inflammatory changes and multinucleated giant cells (arrows, Figure 1B) were observed. Treatment with olmesartan 1mg/kg/day, 3mg/kg/day, and 10mg/kg/day reduced the severity of the disease, as assessed by measuring HW/BW, organ weight/BW, pericardial effusion, macroscopic and microscopic scores (Table 2 and Figure 1). The percentages of macrophages, CD4+, and CD8+ T cells recruited into the lesions were significantly reduced by the treatment (1mg/kg/day)(Table 2).

**Myocardial IL-1β expression**

Immunohistochemistry showed that IL-1β-positive cells were localized mainly in infiltrating inflammatory cells (Figure 2A, C, arrows). Olmesartan treatment markedly reduced the number of IL-1β-positive cells in the inflammatory lesions compared with rats with EAM treated with vehicle (Figure 2B, D). Western blotting showed that myocardial IL-1β was upregulated 2.4-fold in rats with EAM and treated with vehicle compared with the intact heart (Figure 2E, F). Olmesartan treatment decreased the upregulated IL-1β expression in EAM (Table 3). The findings of myocardial IL-1β expression in relation to the severity of lesions were constant.

**Myosin DTH**

We investigated whether the anti-inflammatory property of olmesartan suppressed the immune response by assaying DTH to cardiac myosin. As a result, myosin DTH was significantly lower in olmesartan (1mg/kg/day) treated rats with myocarditis than in
untreated rats with myocarditis (Table 3). Reduced DHT reaction was not seen in myocarditis rats treated with olmesartan 3mg/kg/day and 10mg/kg/day.

Cytotoxic activity of lymphocytes

The cytotoxic activities of lymphocytes against F-2 cells and cardiomyocytes were examined. At E/T ratios of 100:1 and 200:1, the cytotoxic activities of lymphocytes from rats immunized with cardiac myosin treated with olmesartan 1mg/kg/day, but not with olmesartan 3mg/kg/day and 10mg/kg/day, were significantly suppressed compared with those from rats immunized with cardiac myosin and treated with PBS.

Effects of the drugs on IL-1β production in vitro

IL-1β production was markedly increased by LPS stimulation (Table 4). Both olmesartan and RNH-6270 suppressed LPS-induced increased IL-1β production on U937 cells and rat myocytes in a dose-dependent manner (Table 4).

Discussion

The present findings clearly demonstrated that olmesartan, a novel AT₁ antagonist, reduced the severity of acute EAM in rats with EAM, and that the cardioprotection of olmesartan may be due to the suppression of both inflammatory cytokines and cytotoxic activity of lymphocytes against the heart in addition to the hemodynamic modifications.

Several clinical studies have described the participation of proinflammatory cytokines in the pathogenesis of cardiac diseases. The levels of circulating proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1 and IL-6 are elevated in patients with myocarditis. In a murine model of viral myocarditis, the intracardiac expression of TNF-α, IL-1β, interferon (INF)-γ, and IL-2 genes were increased. The degree of their expressions was correlated with the severity of the disease, suggesting that the overproduction of proinflammatory cytokines may aggravate the disease. This was supported in part by recent reports that the overexpression of TNF-α in the heart caused severe myocarditis and cardiomyopathy in transgenic mice (13), and that IL-1β as well as TNF-α promoted the aggravation of viral myocarditis in the virus resistant mice (10, 14). It was therefore demonstrated that the
suppression of inflammatory cytokines has beneficial effects in ameliorating acute myocarditis. Accordingly, we focused upon myocardial IL-1β expression as a significant marker to reflect the effects of olmesartan.

AT₁ antagonists such as olmesartan are reported to suppress the cytokine production and the transcription of cytokine genes in vitro and in vivo (2,6,12,16,24,25). In the present study, olmesartan treatment modified the increased IL-1β expression in acute EAM, and the immunohistochemical study showed that the numbers of macrophages, CD4⁺ and CD8⁺ T cells, and IL-1β-positive inflammatory cells was markedly reduced by olmesartan treatment. In addition, both olmesartan and its active metabolite, RNH-6270, suppressed IL-1β production in vitro. Accordingly, it may be that the beneficial effects of olmesartan in EAM may be partly due to the suppression of inflammatory events in the myocardium. The molecular mechanisms of olmesartan in the suppression of inflammatory cytokines are not fully understood. However, the inhibition of signal transduction pathways, such as nuclear factor kappa B (NF-κB), which is particularly important in the expression of cytokines, by AT₁ antagonists, has been postulated (2,6,12,16,24,25).

Another important role of olmesartan is immunomodulation. Potential immunoregulatory effects of Ang II, such as T-cell chemotaxis, have been reported (5,25). Accordingly, olmesartan, a novel AT₁ antagonist, may exhibit the immunosuppressive properties. Although whether olmesartan directly affects the host immune status or not is unknown, it was demonstrated in this study that DTH reaction of rats immunized with myosin treated with low dose of olmesartan (1mg/kg/day) was suppressed compared with those immunized with myosin and treated with PBS. It suggests that olmesartan treatment reduced angiten-specific delayed type hypersensitive activity in the host. Accordingly, the overall effects of olmesartan could induce the suppression of autoimmune myocarditis associated with the reduction of cytotoxic activity against myocytes; i.e., olmesartan suppressed the cytotoxic myocardial injury in autoimmune heart failure in rats. Indeed, the cytotoxic activities of lymphocytes against F-2 cells and cultured myocytes in rats with EAM treated with low dose of olmesartan (1mg/kg/day)
were reduced compared with untreated controls.

The reason why middle or high dose of olmesartan, but not low dose, was not so effective for the cardioprotection is unknown. However, as shown in Table 1, there was a relative hypotensive tendency in rats treated with middle and high doses, but not in low dose. That is, there might be a possibility that coronary perfusion pressure may fall below 90mmHg in mice-treated with middle or high dose, resulting in small improvement of cardiac pathology. Similar therapeutic dilemma of the drugs between hemodynamic and non-hemodynamic effects were also noted in benedipine, a calcium channel antagonist.\(^{(27)}\) Accordingly, primary factor for this drug might be at least in part related to the propensity of capacity of hemodynamic modifications. Although the actual effect might be different between drugs, dosages, and experimental models, lower or minimally hemodynamic doses appear warranted.

The rational for prescribing AT\(_1\) antagonists in patients with heart failure is based on the vasodilator action, anti-ischemic effects, anti-oxidant actions, and the capacity of these drugs to reduce left ventricular diastolic dysfunction, as well as to prevent progression of myocardial dysfunction.\(^{(2,4,18,22,23,28)}\) Despite initial studies reporting improvements in the hemodynamic profile, subsequent evaluation revealed controversial results, including deterioration in the hemodynamics, increased incidence of cardiac events, with progression of heart failure, and death.\(^{(1,17)}\) In our present study, olmesartan treatment for 3 weeks ameliorated myocarditis by comparing HW/BW ratio, pericardial effusion scores, macroscopic and microscopic scores by both anti-inflammatory and immunosuppressive actions. From our data, olmesartan treatment might be promising for new therapy for not only acute but also chronic myocarditis, particularly for ventricular remodeling where ongoing autoimmune process may play a role in the disease development. This is the first report that olmesartan, a novel AT\(_1\) antagonist, suppressed the cytotoxic myocardial damage in autoimmune heart failure.

Several studies have definitely established the key role of the renin-angiotensin system in the pathogenesis of heart failure by demonstrating that agents that inhibit the renin-angiotensin system confer cardiovascular benefit beyond the reduction of blood
pressure alone (2). It may be that Ang II promotes several critical processes in heart failure such as releases of cytokines, chemoattractant molecules, and growth factors, cell migration, and oxidative stress. Accordingly, AT1 antagonists are therapeutically effective for the treatment of patients with heart failure by reducing cytokines and oxidative stress, and by demonstrating antiinflammation (7, 9, 21, 26). At present time, the capacity of suppressing cytotoxic activities of lymphocytes might be unique for olmesartan.

In conclusion, olmesartan, a recently developed AT1 antagonist, ameliorates acute EAM in rats. The cardioprotection of olmesartan may be due to the suppression of inflammatory cytokines as well as immunosuppressive effects for cytotoxic myocardial injury in addition to the hemodynamic modifications.
Acknowledgments

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References


Figure legends

**Figure 1.** Histopathology and the effects of olmesartan treatment in rats with myocarditis

A and C: Histopathology in an intact heart (grade 0) immunized with FCA alone. B and D: Representative histopathology in a rat with myocarditis treated with vehicle. Diffuse myocardial necrosis and cellular infiltration with multinuclear giant cells (arrows) were shown in the inflammatory regions (grade 4). E: Representative histopathology in a rat with myocarditis treated with olmesartan 1 mg/kg/day (Olm-1) (grade 1). Treatment with olmesartan 1mg/kg/day reduced the severity of the disease. Hematoxylin and eosin, original magnification, ×80 (A and B inset, ×100), ×5 (C, D and E).

**Figure 2.** Immunohistochemical assay for IL-1β in the heart and myocardial IL-1β protein expression

Serial sections of heart tissue from rats with myocarditis were stained with hematoxylin and eosin (HE) (A, B) and antibodies to IL-1β (C, D). IL-1β-positive cells were localized mainly in infiltrating inflammatory cells (C, arrows). Olmesartan treatment reduced the number of IL-1β-positive cells in the inflammatory lesions (D). Original magnification ×80 (A, B, C and D).

E: Western blot analysis. Ten micrograms of each protein sample were loaded and electrophoresed on 15% SDS-PAGE. C, control rat; M, rat immunized with myosin and treated with vehicle; Olm, rat immunized with FCA and treated with olmesartan 1mg/kg/day; M+Olm, rat with myocarditis treated with olmesartan 1mg/kg/day. F: Densitometric analysis of relative protein levels. In rats with myocarditis, the IL-1β protein expression was markedly increased, and was decreased by the olmesartan 1mg/kg/day treatment. Values are derived from 4 animals and are presented as percentage of controls. **p<0.01 vs Myocarditis, †p<0.01 vs Control.
## Table 1. Hemodynamics of olmesartan on rats with/without EAM

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HR (beats/min)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>HW/BW (mg/g)</th>
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<tbody>
<tr>
<td>Normal rats</td>
<td></td>
<td></td>
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<tr>
<td>Vehicle</td>
<td>3</td>
<td>447 ± 55</td>
<td>127 ± 4</td>
<td>95 ± 4</td>
<td>3.25 ± 0.36</td>
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<tr>
<td>Olm-1</td>
<td>3</td>
<td>407 ± 39</td>
<td>110 ± 9</td>
<td>86 ± 7</td>
<td>3.18 ± 0.28</td>
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<tr>
<td>Olm-3</td>
<td>3</td>
<td>455 ± 18</td>
<td>108 ± 7**</td>
<td>78 ± 10*</td>
<td>3.32 ± 0.19</td>
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<tr>
<td>Olm-10</td>
<td>3</td>
<td>466 ± 26</td>
<td>104 ± 8**</td>
<td>77 ± 9*</td>
<td>3.30 ± 0.20</td>
</tr>
<tr>
<td>Rats with EAM</td>
<td></td>
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<tr>
<td>Myocarditis</td>
<td>9</td>
<td>443 ± 55</td>
<td>120 ± 10</td>
<td>89 ± 11</td>
<td>4.56 ± 0.21</td>
</tr>
<tr>
<td>Olm-1</td>
<td>8</td>
<td>446 ± 22</td>
<td>109 ± 13**</td>
<td>81 ± 8*</td>
<td>3.82 ± 0.31**</td>
</tr>
<tr>
<td>Olm-3</td>
<td>8</td>
<td>445 ± 30</td>
<td>94 ± 8**</td>
<td>67 ± 11**</td>
<td>4.02 ± 0.46*</td>
</tr>
<tr>
<td>Olm-10</td>
<td>8</td>
<td>468 ± 62</td>
<td>96 ± 8**</td>
<td>68 ± 15**</td>
<td>4.05 ± 0.48*</td>
</tr>
</tbody>
</table>

n indicates the number of rats in each group; Vehicle, rats treated with vehicle; Olm-1, rats treated with olmesartan 1mg/kg/day; Olm-3, rats treated with olmesartan 3 mg/kg/day; Olm-10, rats treated with olmesartan 10 mg/kg/day; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HW, heart weight; BW, body weight; Myocarditis, rats with myocarditis. *p<0.05, **p<0.01 vs Vehicle or Myocarditis.
### Table 2. Pathology of olmesartan on rats with EAM

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HW/BW (mg/g)</th>
<th>Lung/BW (mg/g)</th>
<th>Liver/BW (mg/g)</th>
<th>Kidney/BW (mg/g)</th>
<th>Pericardial Effusion Scores</th>
<th>Macroscopic Scores</th>
<th>Microscopic Scores</th>
<th>Myocardial Lymphocyte Subset (%)</th>
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</tr>
<tr>
<td>Myocarditis</td>
<td>9</td>
<td>4.56 ± 0.21</td>
<td>6.75 ± 0.96</td>
<td>48.2 ± 4.1</td>
<td>9.24 ± 0.39</td>
<td>1.5 ± 0.6</td>
<td>2.7 ± 0.9</td>
<td>2.8 ± 1.4</td>
<td>20.0 ± 1.9, 16.1 ± 1.9, 9.5 ± 2.3</td>
</tr>
<tr>
<td>Olm-1</td>
<td>8</td>
<td>3.82 ± 0.31**</td>
<td>5.55 ± 0.34**</td>
<td>39.4 ± 3.9**</td>
<td>9.39 ± 0.51</td>
<td>0.8 ± 0.3*</td>
<td>1.8 ± 1.0*</td>
<td>1.1 ± 0.4**</td>
<td>13.7 ± 1.9**, 11.1 ± 1.9**, 5.5 ± 1.6*</td>
</tr>
<tr>
<td>Olm-3</td>
<td>8</td>
<td>4.02 ± 0.46*</td>
<td>5.61 ± 0.57**</td>
<td>39.5 ± 3.1**</td>
<td>9.79 ± 0.95</td>
<td>1.2 ± 0.7</td>
<td>2.5 ± 0.9</td>
<td>1.8 ± 1.0*</td>
<td>16.8 ± 2.4, 14.3 ± 2.1, 7.6 ± 1.7</td>
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<tr>
<td>Olm-10</td>
<td>8</td>
<td>4.05 ± 0.48*</td>
<td>5.64 ± 0.59**</td>
<td>41.8 ± 6.4*</td>
<td>9.78 ± 0.70</td>
<td>1.4 ± 0.5</td>
<td>2.2 ± 0.9</td>
<td>2.0 ± 0.8*</td>
<td>17.8 ± 2.2, 14.4 ± 2.8, 7.1 ± 1.7</td>
</tr>
</tbody>
</table>

#The positive-staining cells of heart tissue were calculated.

*p<0.05, **p<0.01 vs Myocarditis.
Table 3. Myocardial IL-1β expression, myosin DTH and cytotoxic activities of olmesartan

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1β Expression#</th>
<th>Myosin DTH (Food pad response) mm × 10^2</th>
<th>Cytotoxic Activity (%) †</th>
<th>Against F-2 cells (E/T ratio)</th>
<th>Against myocytes 100:1</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100:1</td>
<td>200:1</td>
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<tr>
<td>Normal</td>
<td>1.0</td>
<td>15.9 ± 4.1 (n=5)</td>
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<tr>
<td>Myocarditis</td>
<td>3.1 ± 0.5</td>
<td>35.5 ± 4.6 (n=9)</td>
<td>16.0 ± 3.0</td>
<td>22.1 ± 4.1</td>
<td>17.2 ± 3.4</td>
</tr>
<tr>
<td>Olm-1</td>
<td>1.4 ± 0.7**</td>
<td>20.3 ± 4.1* (n=8)</td>
<td>11.3 ± 3.4*</td>
<td>14.4 ± 2.9**</td>
<td>8.6 ± 2.9**</td>
</tr>
<tr>
<td>Olm-3</td>
<td>1.7 ± 0.5*</td>
<td>30.5 ± 3.5 (n=8)</td>
<td>11.8 ± 3.4</td>
<td>17.0 ± 4.0</td>
<td>12.0 ± 4.0</td>
</tr>
<tr>
<td>Olm-10</td>
<td>1.8 ± 0.4*</td>
<td>31.1 ± 4.4 (n=8)</td>
<td>18.2 ± 3.1</td>
<td>24.0 ± 4.6</td>
<td>14.4 ± 4.1</td>
</tr>
</tbody>
</table>

(mean ± S.D.)

# Densitometric analysis of relative protein levels was shown. Values are derived from four animals and represented as percentage of controls.

†Values are derived from five animals.

*p<0.05,  **p<0.01 vs Myocarditis.  §p<0.01 vs Normal.
### Table 4. Effects of olmesartan and RNH-6270 upon IL-1ß production in vitro

<table>
<thead>
<tr>
<th>Cells</th>
<th>Conditions</th>
<th>Olmesartan (-)</th>
<th>Olmesartan 1pg</th>
<th>Olmesartan 10pg</th>
<th>Olmesartan 100pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>U937 cells</td>
<td>LPS (-)</td>
<td>1.56 ± 0.23</td>
<td>11.44 ± 0.26</td>
<td>4.04 ± 0.16**</td>
<td>3.10 ± 0.26**</td>
</tr>
<tr>
<td></td>
<td>LPS (+)</td>
<td>11.44 ± 0.26</td>
<td>4.04 ± 0.16**</td>
<td>3.10 ± 0.26**</td>
<td>2.69 ± 0.64**</td>
</tr>
<tr>
<td></td>
<td>RNH-6270 (-)</td>
<td>1.26 ± 0.14</td>
<td>10.76 ± 0.30</td>
<td>5.32 ± 0.40**</td>
<td>3.56 ± 0.50**</td>
</tr>
<tr>
<td></td>
<td>RNH-6270 (+)</td>
<td>10.76 ± 0.30</td>
<td>5.32 ± 0.40**</td>
<td>3.56 ± 0.50**</td>
<td>2.65 ± 0.43**</td>
</tr>
<tr>
<td>Myocytes</td>
<td>LPS (-)</td>
<td>1.58 ± 0.24</td>
<td>5.12 ± 0.42</td>
<td>3.24 ± 0.82*</td>
<td>3.11 ± 0.53**</td>
</tr>
<tr>
<td></td>
<td>LPS (+)</td>
<td>5.12 ± 0.42</td>
<td>3.24 ± 0.82*</td>
<td>3.11 ± 0.53**</td>
<td>2.24 ± 0.71**</td>
</tr>
<tr>
<td></td>
<td>RNH-6270(-)</td>
<td>2.01 ± 0.20</td>
<td>6.12 ± 0.40</td>
<td>4.42 ± 0.55*</td>
<td>3.86 ± 0.39**</td>
</tr>
<tr>
<td></td>
<td>RNH-6270 (+)</td>
<td>6.12 ± 0.40</td>
<td>4.42 ± 0.55*</td>
<td>3.86 ± 0.39**</td>
<td>3.36 ± 0.51**</td>
</tr>
</tbody>
</table>

U937 cells or cultured myocytes were stimulated with 10µg/ml LPS [LPS(+)]. Olmesartan or its active metabolite, RNH-6270, was added to the medium 30 min before LPS stimulation. 48 hours later, IL-1ß in the medium was assayed. Each value was derived from 4-6 trials. *P<0.05, **P<0.01 vs Olmesartan (-) or RNH-6270 (-).
Figure. 1

A: intact heart  

B: vehicle

C: intact heart  

D: Vehicle  

E: Olm-1
Figure 2.

A. HE

B. HE

C. IL-1β

D. IL-1β

E. Western blot for IL-1β

F. Bar graph showing:

- **p<0.01 vs Myocarditis
- †p<0.01 vs Control

Legend:
- C
- Olm
- M
- M+Olm