Effects of Imidapril and TA-606 on Rat Dilated Cardiomyopathy After Myocarditis

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SUMMARY

For the management of chronic heart failure, both angiotensin converting enzyme inhibitors (ACEI) and angiotensin II type 1 receptor blockers (ARB) are useful, however, the differences between the two groups of agents are unclear. We compared the effects of long-term treatment with an ACEI (imidapril) and an ARB (TA-606) in rats that had recovered from experimental autoimmune myocarditis (EAM). Forty-two Lewis rats were immunized with porcine cardiac myosin on day 0 and divided into 6 groups, group C (distilled water), group IL (imidapril 0.5 mg/kg/day), group IH (imidapril 2 mg/kg/day), group TL (TA-606 2 mg/kg/day), group TH (TA-606 6 mg/kg/day), and group IT (imidapril 0.5 mg/kg/day + TA-606 2 mg/kg/day). Drugs were administered from day 28. Hemodynamic parameters, heart weight/body weight ratio (HW/BW), and area of fibrosis were measured on days 70-74. Only the high dose of imidapril significantly decreased central venous pressure and significantly increased maximum dP/dt and the absolute value of minimum dP/dt. HW/BW was suppressed in groups IH, TH, and IT. Thus, in treatment of chronic heart failure in rats, a sufficient dose of ACEI was needed to improve hemodynamics and to prevent ventricular hypertrophy. The hemodynamic effects of ARB and combination therapy of both drugs at low doses were not significant. (Jpn Heart J 2003; 44: 735-744)

Key words: Angiotensin converting enzyme inhibitor, Angiotensin II type 1 receptor blocker, Dilated cardiomyopathy, Myocarditis, Heart failure, Hemodynamics, Ventricular remodeling

ANGIOTENSIN converting enzyme inhibitors (ACEI) are one of the most important classes of drugs in treating patients with chronic heart failure.1,2) Angio-
tensin II type1 receptor blockers (ARB) are now considered to be possible substitutes for ACEI. From a clinical point of view, we were interested in three issues, 1) determining which of the two was superior, 2) the optimal doses of each drug, and 3) the efficacy of their combination therapy. The Losartan Heart Failure Study (ELITE II), the Valsartan Heart Failure Study (Val-HeFT), and the Assessment of Treatment with Lisinopril and Survival Study (ATLAS) addressed these issues but only provided partial answers.

Myocarditis is a possible cause of dilated cardiomyopathy. We previously developed an experimental autoimmune myocarditis (EAM) model in rats which can be a model of human giant cell myocarditis. After the acute phase of myocarditis, it causes progressive dilatation of the left ventricle and deterioration of contractility, and ultimately leads to the dilated phase. Using this model, we compared the effects of long-term therapy with an ACEI (imidapril) and an ARB (TA-606) on hemodynamics and ventricular remodeling.

**METHODS**

**Experimental animals:** Forty-two male Lewis rats (10 weeks old) were purchased from Charles River, Japan (Yokohama, Kanagawa) and were maintained in our animal facilities.

**Active induction of EAM:** Purified cardiac myosin was prepared from the ventricular muscle of pig hearts according to a procedure previously described, and used as an antigen of autoimmune myocarditis. This model almost always develops myocarditis. Lewis rats were injected with 0.2 mL of antigen-adjuvant emulsion into their footpads on day 0.

**Drug therapy:** Imidapril and TA-606 were provided by Tanabe Seiyaku Co., Ltd. Imidapril was dissolved in distilled water and TA-606 in 0.25% carboxymethyl cellulose aqueous solution. The therapeutic doses were chosen according to the finding that 3 mg/kg/day of imidapril shows the same antihypertensive effect as 10 mg/kg/day of TA-606.

The rats were divided into 6 groups: group C (control, given only distilled water), group IL (imidapril low dose, 0.5 mg/kg/day), group IH (imidapril high dose, 2 mg/kg/day), group TL (TA-606 low dose, 2 mg/kg/day), group TH (TA-606 high dose, 6 mg/kg/day), and group IT (imidapril low dose, 0.5 mg/kg/day + TA-606 low dose, 2 mg/kg/day).

After the estimated fulminant phase, the drugs were administered orally once per day, 6 days per week, from day 28 until days 70-74 when hemodynamic measurements were performed (Figure 1).

**Hemodynamic measurements:** On days 70-74, the rats were anesthetized with 2% halothane in O₂ during the surgical procedure for catheterization, and then
anesthesia was maintained with 0.5% halothane for 20 minutes for equilibration. The heart rate, central venous pressure, mean arterial pressure, peak left ventricular pressure, left ventricular end-diastolic pressure, and both maximum and minimum dP/dt were recorded simultaneously.

**Sampling:** After hemodynamic measurements, blood samples were obtained from the inferior vena cava and centrifuged at 3,000 rpm for 10 minutes at 4°C. The serum fractions were stored at -80°C. The rats were then sacrificed and the hearts were removed and cleaned of atria and surrounding tissues. The ventricle was immediately weighed and cut transversely into three pieces. The apical one-third was freeze-clamped in liquid nitrogen and stored at -80°C to obtain mRNA. The middle one third was fixed in 10% formalin, and then embedded in paraffin for histopathological study.

**Serum ACE activity and aldosterone:** Serum ACE activity and aldosterone were measured by the method of Kasahara (Fujirebio Inc., ACE Color) and by RIA (Dainabot, aldosterone RIA kit), respectively.

**Histopathology:** The paraffin-embedded samples were stained by the Azan-Mallory method. The area of dark blue color, which indicates fibrotic tissue, was measured using computer software (Apple, MacSCOPE Ver2.5) and the ratio to the whole section area was calculated as the area of fibrosis.

**RT-real time PCR:** Total RNA was extracted from each apical third of the ventricles and reverse transcription was performed. Real time polymerase chain reaction (PCR) on a LightCycler (Roche Diagnostics) was performed using DNA Master SYBR Green. Primers for γ-actin (AGCCTTCCTTCTGCGGCTGAGT [sense] and TGGAGGGGCTGACCTAGCTACT [antisense]) (X52815), for atrial natriuretic peptide (ANP) (ATGGATTCAAGAACCCTGCTAGAC [sense] and GCTCCAACTCCTGCAATCTAC [antisense]) (E00698), for aldosterone synthase (AGAAACTCATGTTCTTTTCTGACC [sense] and ATATTTTTCCATTAAGGCAATCCCA [antisense]) (D14096, 14097), for angiotensin II type 2 receptor (AAAGTGATTCTTTTCTGACC [sense] and ATATTTTTCCATTAAGGCAATCCCA [antisense]) (D16840), for angiotensin converting enzyme (CAACTGGCATTAAAACCAACCAT [sense] and TTATATTTGGACATGCTC [antisense]) (U03708), for eNOS (TATTCTTTGAGGTAATCCC [sense] and TGTGGTGTAGGGTGAACA [antisense]) (AF085195), and for iNOS (TCACCTACTTCCTGGACATCACT [sense] and AGGGAGGAGCTGAGGATGAT [antisense]) (D44591) were used.

**Statistical analysis:** Values are presented as the mean ± SD. Statistical analysis was performed by the Tukey-Kramer method when the SDs of each group were considered equal by the Bartlett test. The Kruskal-Wallis test followed by the
Scheffé test were used with unequal SDs. A difference was considered statistically significant when the \( P \) value was less than 0.05.

**RESULTS**

**Course after immunization:** One rat died due to congestive heart failure during the fulminant phase, before treatment. All other rats survived until the hemodynamic measurements on days 70-74. Another rat, in which findings of myocarditis and fibrosis were not detected, was excluded from the following analysis because immunization in this rat was considered to have failed.

**Hemodynamic measurements:** Pulse rate, and systolic and diastolic blood pressure before treatment on day 0 and day 28 did not show significant differences among the groups (Table). On days 70-74, as shown in Figure 2, central venous pressure was significantly lower in group IH \((0.4 \pm 0.6 \text{ mmHg})\) than in group C \((5.5 \pm 3.9 \text{ mmHg})\). Maximum \(dP/dt\) and the absolute value of minimum \(dP/dt\) were significantly higher in group IH \((4,130 \pm 920 \text{ mmHg/sec}, \text{ minimum } dP/dt -3,614 \pm 984 \text{ mmHg/sec})\) than in group C \((2,348 \pm 856 \text{ mmHg/sec}, \text{ minimum } dP/dt -2,120 \pm 774 \text{ mmHg/sec})\) and group IL \((2,395 \pm 707 \text{ mmHg/sec}, \text{ minimum } dP/dt -2,209 \pm 624 \text{ mmHg/sec})\). Heart rate, mean arterial pressure, peak left ventricular pressure, and left ventricular end-diastolic pressure (LVEDP) did not show significant differences among the groups. However, LVEDP was highest in group C \((13.0 \pm 1.9 \text{ mmHg})\) and lowest in group IH \((8.8 \pm 2.7 \text{ mmHg})\).

**HW/BW and fibrosis:** Body weight on day 0 was not significantly different among the groups (Table). On days 70-74, the HW/BW ratio was significantly lower in groups IH, TH, and IT \((2.9 \pm 0.4, 3.3 \pm 0.3, \text{ and } 3.2 \pm 0.3 [\times 10^{-4}])\) than in group C \((4.1 \pm 0.5 [\times 10^{-4}])\). The differences were significant between the IH

| Table. Pulse Rate (PR, /minute), Systolic and Diastolic Blood Pressure (s-BP, d-BP, mmHg) of Tail Artery on Days 0 and 28, and Body Weight (BW, g) on Day 0. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Group C         | Group IL        | Group IH        | Group TL        | Group TH        | Group IT        |
| PR, day 0       | 355 ± 54        | 345 ± 28        | 340 ± 32        | 337 ± 16        | 347 ± 23        | 327 ± 14        |
| s-BP, day 0     | 118 ± 13        | 124 ± 9         | 117 ± 11        | 118 ± 7         | 126 ± 9         | 121 ± 10        |
| d-BP, day 0     | 92 ± 13         | 91 ± 9          | 87 ± 11         | 86 ± 7          | 94 ± 10         | 86 ± 6          |
| PR, day 28      | 374 ± 39        | 376 ± 27        | 350 ± 25        | 356 ± 41        | 409 ± 36        | 361 ± 25        |
| s-BP, day 28    | 96 ± 12         | 95 ± 10         | 106 ± 11        | 100 ± 9         | 99 ± 4          | 104 ± 8         |
| d-BP, day 28    | 73 ± 5          | 71 ± 11         | 81 ± 10         | 72 ± 6          | 77 ± 5          | 81 ± 4          |
| BW, day 0       | 326 ± 14        | 328 ± 12        | 327 ± 12        | 328 ± 9         | 327 ± 12        | 329 ± 9         |
and IT groups vs. the control group ($P < 0.01$, Figure 3). The area of fibrosis was highest in group C (12.4 ± 1.8%), and most rats showed a smaller area of fibrosis compared with the control (Figures 3 and 4).

**Serum ACE activity and aldosterone:** Serum ACE activity was decreased with imidapril dose-dependently, but not with TA-606 (Figure 5). Compared with group C, all other groups except the TH group showed a significant decrease in serum aldosterone (Figure 5).

**RT-real time PCR:** Sufficient cDNA was obtained from 36 samples. Four other samples were excluded because the calculated concentrations of cDNA of the housekeeping gene γ-actin were less than the lowest concentration of the standard template which was used to plot the concentration-PCR cycle number curve.
ANP mRNA/γ-actin mRNA did not show a significant difference among the groups. However, it was highest in group C (10.8 ± 9.2), and the values were equally low only in the IH group (4.0 ± 0.8) (Figure 6). mRNA of aldosterone synthase, angiotensin II type 2 receptor, eNOS, and iNOS were not detectable with even 40 cycles of PCR. ACE mRNA/γ-actin mRNA did not show significant differences among the groups.
DISCUSSION

For the management of patients with chronic heart failure, ACEIs and ARBs are used for improving mortality and morbidity rather than for reduction of blood pressure. In this study, we used equipotent doses of each drug to reduce blood pressure, although the optimal doses for organ protection are not known. Compared with the control group, imidapril and TA-606 showed no significant reduction in LV systolic pressure or mean arterial pressure, but almost equally reduced the serum level of aldosterone. In contrast, ACE activity was decreased in a dose-dependent manner with imidapril.

The beneficial effects on hemodynamics were achieved only at a high dose of imidapril: a fall in CVP and rises in maximum dP/dt and the absolute value of minimum dP/dt. With regard to the hemodynamic effects, imidapril was superior to TA-606 when the former was used in a sufficient dose. Combined administra-
tion of the two drugs at low doses was not as effective as a high dose of imidapril alone.

In groups IH and IT, the blood pressure on day 28 appeared to be higher than those in other groups, but there was no significant difference. The severity of myocarditis is not uniform among individual rats in this model. The blood pressure of age- and sex-matched normal Lewis rats is about 130 mmHg, while the maximum and minimum dP/dt are about 6,000 mmHg/s and -6,000 mmHg/s, respectively. Although there might be a slight, but not significant, difference in disease severity among our study groups, all groups had actually severe disease based on the hemodynamics and histology.

CVP, maximum dP/dt, and minimum dP/dt are indices of RV filling, LV contraction, and LV relaxation, respectively. They all are under the influence of ventricular stiffness or contractility and are altered by hypertrophy or fibrosis. In this study, HW/BW was suppressed by both imidapril and TA-606, dose-dependently, suggesting a preventive effect on remodeling. Combined administration of the two drugs was also effective in preventing cardiac hypertrophy.

On the other hand, the area of fibrosis was low in most treated rats although the difference was not significant: all of the areas of fibrosis in the control group were greater than 10%, and in 64% of the treated rats, the areas of fibrosis were less than 10%. The extent of fibrosis in the treated rats was very variable, and thus the differences were not significant.

The mechanisms of action are different between ACEI and ARB, but both drugs were suggested to have equal blocking effects on angiotensin II type 1 receptor in rats \(^\text{12}\) because chymase is not active in rats \(^\text{13,14}\). The reduction of serum aldosterone levels, which were determined through angiotensin II type 1 receptor, \(^\text{15}\) was not significant only in the TH group. We are not able to explain this result because we did not measure the concentrations of serum and tissue angiotensin II in this study. Increases in serum and tissue angiotensin II by the receptor blocker and an increase in the tissue distribution of TA-606 might be related to the phenomenon. Further studies are needed to determine a possible role for aldosterone escape with ARB.

In contrast, the role of angiotensin II type 2 receptors is unknown, and their roles in remodeling were investigated in previous studies \(^\text{16,17}\). Stimulation of angiotensin II type 2 receptors was suggested to promote cardiac hypertrophy \(^\text{17}\) and a significant reduction in HW/BW can be expected when we employ ACEI. However, the suppression of HW/BW with a high dose of TA-606 and combined administration can not be explained by angiotensin II type 2 receptors.

The heart has its own renin-angiotensin-aldosterone (RAA) system, namely the tissue-RAA system, which plays pathological roles in heart failure \(^\text{18}\). The bradykinin-NO system may also play a role in heart failure as an autocrine/para-
The renin-angiotensin-aldosterone system (RAA system) plays a crucial role in regulating blood pressure and cardiac function. In the fulminant phase of experimental autoimmune myocarditis (EAM), the deterioration of hemodynamics appeared to be associated with increased NOS activity. However, in chronic heart failure, the system may exert beneficial effects. The RAA system should be examined in cardiac tissue. RT-PCR was performed for this purpose. However, aldosterone synthase, ATII type 2 receptors, eNOS, and iNOS were undetectable, and ACE did not show a significant difference. On the other hand, every rat treated with the high dose of imidapril exhibited low expression of ANP mRNA, which appeared to reflect the hemodynamic benefits of the drug.

**CONCLUSIONS**

1) In rat dilated cardiomyopathy, imidapril had superior hemodynamic effects to TA-606 when used at a sufficient dose, 2) sufficient doses of each drug were necessary for inhibiting cardiac hypertrophy, and 3) combined administration of both drugs at low doses was not as effective as a high dose of imidapril alone.

**REFERENCES**


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