Long-Term Combined Therapy with an Angiotensin Type I Receptor Blocker and an Angiotensin Converting Enzyme Inhibitor Prolongs Survival in Dilated Cardiomyopathy

Toshihiro SHIMIZU,¹ MD, Hiroshi OKAMOTO,¹ MD, Satoru CHIBA,¹ MD, Yutaka MATSUI,¹ MD, Takeshi SUGAWARA,¹ MD, Hisao ONOZUKA,¹ MD, Taisei MIKAMI,¹ MD, Hideki KUMAMOTO,¹ MD, and Akira KITABATAKE,¹ MD

SUMMARY

The efficacy of ACE inhibitors (ACEIs) in the treatment of chronic heart failures is well documented. However, ACEIs may provide incomplete blockade of the renin-angiotensin system (RAS) because of the alternative pathways for angiotensin II (AII) production. We hypothesized that more complete blockade of RAS by adding an AT1 receptor blocker (ARB) may have greater potential to decrease mortality associated with heart failure and improve cardiac function than monotherapy with ACEIs.

The objective of this study was to evaluate the effect of combined therapy on cardiac functions and survival in cardiomyopathic hamsters.

Male cardiomyopathic hamsters (BIO TO2) were administered either placebo (group C), enalapril (30 mg/kg/day) (group E), or enalapril (30 mg/kg/day) + valsartan (500 mg/kg/day) (group EV), starting at the age of 6 weeks. Kaplan-Meier analysis was performed to assess the differences in survival. Cardiac functions were evaluated by echocardiogram and cardiac catheterization.

Group EV showed significant increases in fractional shortening, LV dP/dTmax, and deceleration time, and showed significant decreases in left ventricular diastolic dimension, LV dP/dTmin, and early diastolic mitral velocity/atrial systolic velocity. Treatment with enalapril resulted in longer survival compared with placebo. Moreover, life expectancy (median probability of survival: 433 days) increased significantly in group EV compared with group E ($P<0.05$) as well as group C ($P<0.001$).

It is concluded that combined therapy improved cardiac function and survival compared to placebo or enalapril monotherapy. (Jpn Heart J 2002; 43: 531-543)

Key words: Heart failure, Fibrosis, Myocytes, Renin angiotensin system, Ventricular function, Combined therapy

From ¹ the Department of Cardiovascular Medicine, Graduate School of Medicine, Hokkaido University, Japan.

Address for correspondence: Hiroshi Okamoto, MD, Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita 15-Nishi 7, Kita-ku, Sapporo 060-8638, Japan.

This work was supported in part by Research Grants on Cardiomyopathy from the Ministry of Health and Welfare of Japan and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, No. 10358020.

Received for publication February 21, 2002.

Revised and accepted April 25, 2002.

IN the clinical setting, many randomized controlled trials have revealed that ACEIs improve cardiac remodeling, leading to the prolongation of life expectancy.¹⁻⁴⁾ The ELITE II trial showed that AT1 receptor blockers (ARBs) have the same effects as ACEIs on heart failure.⁵⁾ Thus, combination therapy with ACEIs and ARBs offers more complete AII blockade of the RAS than can be obtained by ACEIs alone and preserves the benefits of bradykinin potentiation offered by ACEIs while providing the potential antitrophic effects of AT2 receptor stimulation. However, in the Val-HeFT trial, an ARB, valsartan, had a beneficial effect on mortality and morbidity as the primary endpoint compared with placebo treatment, although all causes of mortality could not be decreased by adding valsartan to conventional therapy that included ACEIs.⁶⁾ Some questions have arisen from this trial. First, the period of administration might have been too short, and second, the severity of heart failure too mild (62% of the patients enrolled were NYHA class II) to detect a mortality benefit. There are reports that combined therapy with ARBs and ACEIs improves exercise tolerance and diastolic dysfunction of the hypertrophied heart,^{7,8)} but it is not clear whether combined therapy results in improvements with regard to all causes of mortality. Thus, the synergistic benefits of long-term addition of different doses of valsartan to enalapril on mortality, cardiac function, and the cardiac remodeling process were examined using a dilated cardiomyopathic hamster model.

MATERIALS AND METHODS

Experimental protocol: This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). All experiments were carried out using male Bio TO2 (BIO) dilated cardiomyopathic hamsters (Bio Breeders Inc., Fitchburg, MA, USA).

Six week-old male BIO hamsters were randomly assigned to receive either enalapril (30 mg/kg/day, PO), valsartan (40, 100 and 500 mg/kg/day, PO), a combination of the two (enalapril; 30 mg/kg/day, valsartan; 500 mg/kg/day, PO), or standard chow. All drugs were given to hamsters orally throughout their life. To examine the effect on survival, animals were carefully monitored, and deaths were recorded every day. At the age of 39 weeks, some of the hamsters were sacrificed and necropsied.

Enalapril was selected because of its general usage in heart failure. We have previously performed two experiments using cardiomyopathic hamsters in which two different doses of enalapril, 20 mg/kg/day and 25 mg/kg/day, were administered. Each experiment yielded the same results. Fibrosis was suppressed and cardiac functions were preserved at the same levels. To confirm that a sufficient

dosage was actually received, plasma renin activities in the enalapril and control groups were measured by radioimmunoassay and found to be higher in the enalapril group than in the control group (12.0 ± 2.0 vs 2.38 ± 1.35 ng/mL/h; $P < 0.0001$). Serum concentrations of enalapril were measured in each group and found to be sufficient by comparing them with clinical data provided by Merck Research Laboratories, Lahway, NJ, USA (data not shown).

Valsartan was selected as it had the highest selectivity among ARBs. We hypothesized that valsartan may be more effective for heart failure than the other ARBs. It was very difficult to determine the dosages of enalapril and valsartan that are suitable for this model. The bioavailability of valsartan in the cardiomyopathic hamster is not known. We used valsartan at a dose of 500 mg/kg/day. No adverse effects were observed, and the maximum hypotensive effects were exhibited at the same level with this dosage as in the enalapril group. We concluded that this dose was not overly high and was effective in cardiomyopathic hamsters. First, we examined the mortality associated with the combination therapy with different doses of valsartan. Mortality was markedly improved by the combination of enalapril and a large dose of valsartan (Figure 1), so consecutive analyses were performed in the groups treated with placebo, enalapril, or a combination of enalapril and a high dose of valsartan.

Figure 1. Survival curves for cardiomyopathic hamsters treated with enalapril (30 mg/kg/day) and valsartan (40, 200, and 500 mg/kg/day). (EV40); BIO TO2 hamsters treated with enalapril (30 mg/kg/day) and valsartan (40 mg/kg/day), (EV200); BIO TO2 hamsters treated with enalapril (30 mg/kg/day) and valsartan (200 mg/kg/day), (EV500); BIO TO2 hamsters treated with enalapril (30 mg/kg/day) and valsartan (500 mg/kg/day).

Echocardiogram: The echocardiography method has been described in detail previously.⁹⁾ Briefly, at the age of 39 weeks, each hamster was anesthetized with an intraperitoneal injection of urethane (50 mg/100 g of body weight) and α -chloralose (100 mg/100 g of body weight) and then transthoracic echocardiograms (Hitachi EUB 565A, Tokyo) were obtained with a 13 MHz linear scanner. Urethane and α -chloralose have been found to not have significant depression effects on cardiac function.¹⁰⁾ M-mode echocardiograms were recorded and the left ventricular end-diastolic dimension (LVDd) and percent fractional shortening (%FS) were determined. Pulsed-wave Doppler echocardiograms of mitral flow velocity obtained with the transducer at the cardiac apex were recorded, and E/A and deceleration time (DT) were measured as indices of diastolic performance.

Catheterization: The catheterization method has been described previously.¹⁰⁾ A 1.4-French microtip catheter manometer (SPR-677, Millar Instruments, Inc., Houston, TX, USA) with a TC-510 control unit (Millar Instruments) was inserted into the left ventricle. As indices of hemodynamics, the maximum rate of rise of ventricular pressure (LV dP/dTmax) and the peak rate of the fall in ventricular pressure (LV dP/dTmin) were obtained from left ventricular pressure by analysis with a computer system (MP-100WS, BIOPAC System, Inc., Santa Barbara, CA, USA) and the AcqKnowledge 2.0 program for Macintosh (BIOPAC System).

Radioimmunoassay: Blood samples for the assay of creatinine and AST and AII were drawn via the left ventricular catheter at selected times immediately after completion of the hemodynamic recordings. The blood was drawn into tubes with EDTA on ice and then centrifuged at 4°C. The supernatant fluid was stored at -20°C until analyzed.

Histology: The left ventricle was fixed with 10% formaldehyde, embedded in paraffin after dehydration through a graded alcohol series, and then sectioned horizontally. The sections were stained with hematoxylin and eosin or azocarmine G.

Statistical analysis: Data are expressed as mean $\pm$ SEM. One-way analysis of variance (ANOVA) followed by a post hoc test was used for statistical comparisons among the various treatment groups. The survival data are presented as Kaplan-Meier curves and were compared by the log-rank test. Differences were considered significant at $P < 0.05$.

RESULTS

Effect of long-term treatment with enalapril and different doses of valsartan: First, we examined the effects of long-term treatment with enalapril and different doses of valsartan on mortality. The survival curves for cardiomyopathic hamsters treated with enalapril (30 mg/kg/day) and valsartan (40, 200, and 500 mg/kg/day)

are shown in Figure 1. The 50 percent survivals for the enalapril (30 mg/kg/day) and valsartan (40 mg/kg/day, 200 mg/kg/day, and 500 mg/kg/day) groups were 300.6 ± 26.0 , 375.6 ± 20.9 , and 439.2 ± 5.2 days, respectively ($P < 0.05$). Thus, the survival rate improved in a dose-dependent manner for valsartan in combination with enalapril (30 mg/kg/day), with 500 mg/kg/day of valsartan and enalapril exhibiting marked prolongation of survival, (Figure 1).

Effect of long-term treatment with enalapril and a high dose of valsartan: The 500-day survival rate for BIO hamsters treated with enalapril (16.7%, 2 of 12 BIO hamsters survived) was significantly higher than that of BIO hamsters treated with the control placebo (0%, 0 of 10 BIO hamsters survived). With enalapril and a high dose of valsartan, the rate (63.6%, 7 of 11 BIO hamsters survived) was significantly higher than that of BIO hamsters treated with enalapril alone, as shown in Figure 2. All dead animals had pleural effusion, congested liver, and significantly heavier lung weights (data not shown).

Body weight, left ventricular weight, serum creatinine and AST: As shown in Table I, there was no significant difference in body weight (BW) among the three groups. However, the left ventricular (LV) to body weight ratio was decreased in both treatment groups and showed no significant difference between enalapril and the combination group. Serum creatinine levels and AST levels were exam-

Figure 2. Survival curves for untreated cardiomyopathic hamsters, cardiomyopathic hamsters treated with enalapril (30 mg/kg/day), and cardiomyopathic hamsters treated with enalapril (30 mg/kg/day) and valsartan (500 mg/kg/day). (C); untreated BIO TO2 hamsters, (E); BIO TO2 hamsters treated with enalapril, (EV); BIO TO2 hamsters treated with enalapril and valsartan.

Table I. Body Weight, Ventricular Weight and Biochemical Data in Bio TO2 and F1b Hamsters

	BW (g)	LVW (mg)	LVW/BW (mg/g)	Cr (mg/dL)	AST (IU/L)	Ang II (μ g/mL)
Control (n=10)	110.6 $\pm$ 4.993	244.4 $\pm$ 25.373	2.209 $\pm$ 0.193	0.180 $\pm$ 0.042	179.7 $\pm$ 58.897	3720 $\pm$ 550.5
Enalapril (n=10)	107 $\pm$ 11.499	202.27 $\pm$ 7.321 (a)	1.875 $\pm$ 0.175 (a)	0.180 $\pm$ 0.068	161.1 $\pm$ 47.806	608.3 $\pm$ 249.4(a)
Combination (n=9)	104.111 $\pm$ 4.781	196.322 $\pm$ 4.131 (a)	1.886 $\pm$ 0.08 (a)	0.222 $\pm$ 0.088	202.44 $\pm$ 97.751	157.5 $\pm$ 20.54(a)

Values are means $\pm$ SD; control, untreated BIO TO2; enalapril, BIO TO2 treated with enalapril; combination, BIO TO2 treated with enalapril and valsartan; BW=body weight; LVW=left ventricular weight; Cr=creatinine; AST=aspartate aminotransferase; Ang II=angiotensin II. a) $P<0.01$ vs control, b) $P<0.05$ vs control.

Table II. Echocardiographic Parameters in Bio TO2 and F1b Hamsters

	LVDd (mm)	LAD (mm)	%FS (%)	E/A	DcT (msec)
Control (n=5)	74.6 $\pm$ 6.465	48.0 $\pm$ 4.472	10.618 $\pm$ 3.555	4.94 $\pm$ 1.047	45.95 $\pm$ 8.211
Enalapril (n=5)	65.8 $\pm$ 5.263(b)	41.8 $\pm$ 9.121	17.06 $\pm$ 2.124(b)	3.03 $\pm$ 0.839(b)	50.85 $\pm$ 8.248
Combination (n=4)	55.5 $\pm$ 4.796(a)(c)	29.25 $\pm$ 5.909(a)(c)	26.45 $\pm$ 7.911(a)(c)	1.738 $\pm$ 0.344(a)(c)	62.469 $\pm$ 5.386(b)(c)

Values are means $\pm$ SD; control, untreated BIO TO2; enalapril, BIO TO2 treated with enalapril; combination, BIO TO2 treated with enalapril and valsartan; LVDd= left ventricular diastolic dimension; %FS= percent fractional shortening; LAD= left atrial dimension; DcT= deceleration time; E/A= early filling velocity/atrial filling velocity. a) $P<0.01$ vs control, b) $P<0.05$ vs control, c) $P<0.05$ vs enalapril.

Table III. Hemodynamic Parameters in Bio TO2 and F1b Hamsters

	HR (/min)	LVSP (mmHg)	LVEDP (mmHg)	LV dP/dTmax	LV dP/dTmin
Control (n=5)	262.14 $\pm$ 64.477	87.3 $\pm$ 5.815	16.56 $\pm$ 6.685	2532.22 $\pm$ 421.926	-1924.22 $\pm$ 237.386
Enalapril (n=5)	301.320 $\pm$ 7.151	72.78 $\pm$ 5.817(a)	18.500 $\pm$ 2.74	2404.46 $\pm$ 391.764	-1748.42 $\pm$ 204.664
Combination (n=4)	302.1 $\pm$ 16.961	71.8 $\pm$ 4.116 (a)	12.46 $\pm$ 5.241 (a)	3064.860 $\pm$ 452.394 (b)	-2253.82 $\pm$ 276.076 (b)

Values are means $\pm$ SD; control, untreated BIO TO2; enalapril, BIO TO2 treated with enalapril; combination, BIO TO2 treated with enalapril and valsartan; LVSP=left ventricular systolic pressure; LVEDP=left ventricular end diastolic pressure; HR=heart rate; LV dP/dTmax, maximum rate of rise of ventricular pressure; LV dP/dTmin, peak rate of fall of ventricular pressure. a) $P<0.01$ vs control, b) $P<0.05$ vs enalapril

ined to determine the adverse effects of each treatment. No significant differences among the three groups were observed.

LV function and hemodynamics: LV function and hemodynamics are summarized in Tables II and Table III. No hamsters died during the terminal catheterization or echocardiographic study. Representative echocardiograms are shown in Figure 3. LV end-diastolic dimensions (LVDd) were lower in the combination group than in the enalapril and control groups. LVDd were lower in the enalapril group than in the control group. Percent fractional shortening (%FS) increased for both treatment with enalapril and the combination compared with the control. Doppler-echocardiography revealed the deceleration time (DT) increased and E/

Figure 3. M mode and Doppler echocardiograms. The top row shows M mode echocardiograms at the level of the chorda tendineae, the middle row shows M mode echocardiograms at the level of the aortic valve, and the lower row shows mitral valve inflow velocities. (C); untreated BIO TO2 hamster, (E); BIO TO2 hamster treated with enalapril, (EV); BIO TO2 hamster treated with enalapril and valsartan. IVST=interventricular septum; LVD=left ventricular dimension: LVPW=left ventricular posterobasal free wall; RV=right ventricle: AoD=aorta.

A decreased in the combination group compared with the enalapril group. The E wave was united with the A wave and one tall, sharp wave was observed in the control group. In other words, the control group had diastolic dysfunction. The tall, sharp wave was divided into two waves, an E wave and an A wave, in both the enalapril and combination groups. The E wave was taller and sharper in the enalapril group than in the combination group, indicating that diastolic function improved to a greater degree in the combination group than in the enalapril group. Left atrial dimensions (LAD) were smaller in the combination group than in the enalapril and control groups. LAD was slightly enlarged in diastolic dysfunction. The decrease in E/A, increase in DT, and enlargement of LAD indicated the improvement in diastolic function was a result of ARB and ACEI administration. The indices of diastolic function and LV dimensions were influenced by heart rate and blood pressure. However, these echocardiographic data were reliable because there were no differences in heart rate among the three groups or in blood pressure between the treatment groups. LV systolic pressure (LVSP) decreased in both the enalapril and combination groups and there was no significant difference in the decrease between the 2 groups, LV end-diastolic pressure (LVEDP) decreased in the combination group compared to the enalapril and control groups. Although the LVSP values were very low, control F1b as well as cardiomyopathic hamsters have low blood pressure by nature. Heart rate increased in the both the treatment group with enalapril and the combination group, although not significantly. LV dP/dTmax was increased and LV dP/dTmin was decreased in the combination group compared with the enalapril group. This improvement could not be explained based on differences in LVSP since there was no significant difference in LVSP between the enalapril and combination groups.

Nuclear density of cardiomyocytes, transverse diameter of cardiomyocytes, and percent fibrosis: The histological parameters are summarized in Table IV. The numeric nuclear density of cardiomyocytes, which represents the number of viable myocytes per area, was similar in the enalapril-treated and control groups, and was more preserved from loss in the combination group than in the control and

Table IV. Histologic Parameters in Bio TO2 and F1b Hamsters

	Nuclear density of cardiomyocytes (No./mm ²)	Transverse diameter of cardiomyocytes (μ m)	% fibrosis (%)
Control (n=5)	39.100 $\pm$ 7.015	21.02 $\pm$ 6.685	6.502 $\pm$ 1.065
Enalapril (n=5)	34.300 $\pm$ 3.144	22.6 $\pm$ 2.74	5.002 $\pm$ 0.727(b)
Combination (n=4)	54.5 $\pm$ 13.5(c)	21.18 $\pm$ 5.24	3.708 $\pm$ 0.812(a)(c)

Values are means $\pm$ SD; control, untreated BIO TO2; enalapril, BIO TO2 treated with enalapril; combination, BIO TO2 treated with enalapril and valsartan; a) $P < 0.01$ vs control, b) $P < 0.05$ vs control, c) $P < 0.05$ vs enalapril.

enalapril groups. However, there was no significant difference in the transverse diameter of cardiomyocytes among the three groups. The percent fibrosis was decreased in the enalapril-treated group and markedly decreased in the combination group compared to the control group (Figure 4). There were no apparent changes in cardiomyocyte size, and the suppression of fibrosis indicated a significant decrease in LVW. Significant correlations between LVdP/dTmax and nuclear myocyte density as well as between E/A and % fibrosis were found (Figures 5A and 5B).

Figure 4. Azan staining of sagittal sections of cardiomyopathic hamster hearts at the widest part of the left ventricle. * $P < 0.05$ vs control, ** $P < 0.05$ vs E, $P < 0.01$ vs C (C); untreated BIO TO2 hamster, (E); BIO TO2 hamster treated with enalapril, (EV); BIO TO2 hamster treated with enalapril and valsartan.

A **B**

Figure 5. **A:** Correlations between LV dP/dTmax and nuclear density of cardiomyocytes in BIO TO2 at 39 weeks. LV dP/dTmax, maximum rate of rise of ventricular pressure of left ventricle. **B:** Correlations between E/A and percent fibrosis in BIO TO2 at 39 weeks. E/A, early filling velocity/atrial filling velocity.

DISCUSSION

The major findings of this study are that combination therapy with ACEIs and ARBs improved systolic and diastolic function and survival due to the suppression of cardiac remodeling.

The BIO strain of cardiomyopathic hamsters was used as a model of heart failure. Because of the similarity of the chymase contributions to the diseased heart, it appeared likely that different class effects of ACEIs and ARBs could be compared and clarified.¹¹⁾ In this hamster model, it is well established that the disease process affects myocardial tissue in greatly inhomogeneous ways, as evidenced by focal cell loss, microvascular spasms,¹²⁾ inhomogeneous capillary flow¹³⁾ and resultant focal ischemic areas, marked heterogeneity in cellular calcium content,¹⁴⁾ and abnormalities of sympathetic nervous system activity.¹⁵⁾ In our previous experiments, it was confirmed that fibrillar collagen turnover is increased with abundant cardiac collagen steady-state mRNA both in early and late stages of cardiomyopathic hamsters. In these experiments, echocardiographic study revealed a typical heart failure state in this hamster model, for example, %FS was very low and the transmitral flow pattern exhibited a restrictive pattern.⁹⁾

In the current study, the serum AII concentration was higher in the control group and decreased in both treatment groups. AII is a critical mediator for myocyte hypertrophy and fibrosis, which contribute to cardiac remodeling. AII levels were lower in the combined group compared with the enalapril group ($P=0.08$), implying that a decrease in serum AII concentration resulted in improvements in cardiac remodeling and survival rate.

In the present study, we first confirmed a dose-effect for combination therapy on mortality. The higher the dose of valsartan, the more beneficial the effect on mortality. Combination therapy with enalapril and a high dose of valsartan was then compared with enalapril monotherapy. Although it is not known whether patients with heart failure can tolerate the high dose of valsartan used in the present study, the biochemical factors examined showed no abnormalities. Another question may arise from the present study. If the dose of enalapril is increased, can we determine the difference between the therapies? There have been some reports comparing the different effects of ACEIs and ARBs. Spinale, *et al* demonstrated that combined therapy with ACEIs and ARBs was more effective on LV systolic function of heart failure in pigs induced by rapid pacing than each drug therapy alone.¹⁶⁾ One factor contributing to the improved LV pump function with combined ACEIs and ARBs was a reduction in LV afterload. However, based on the vasodilator effect observed from LVSP, the dose-effect relationship was compatible between enalapril and combination therapy in our study.

The ATLAS trial showed no significant effect of another ACEI, lisinopril, on mortality when the dose was increased.¹⁷⁾ Thus, the longer life-span might be due to a synergistic class-effect based on the different actions of ACEIs and ARBs on the cardiac remodeling process.

Enalapril monotherapy significantly improved the percent fibrosis concomitantly with a decrease in E/A compared with the control group. However, their extents were limited compared with combination therapy. On the other hand, enalapril monotherapy had no effect on nuclear myocyte density or systolic function expressed by %FS and LV dP/dTmax. Combination therapy may preserve myocytes and suppress fibrosis, thus leading to improvements in both systolic and diastolic functions.

Conclusion: The present study has demonstrated that combination therapy with enalapril and a high dose of valsartan had beneficial effects on the survival rate concomitant with an improvement of cardiac remodeling, especially a decrease in fibrosis and preservation of myocytes, which may lead to improvements in both systolic and diastolic functions in cardiomyopathic hamsters. The results suggest that adding ARBs to ACEIs, which have been proven to prolong life in patients with heart failure, further improves the survival rate.

Study limitations: The present study has not clarified the molecular mechanisms responsible for the beneficial effects of the combination therapy. Previous studies have revealed that ACEIs suppress fibrosis through AII-mediated collagen synthesis, restore NO production via eNOS re-expression, and augment the effects of BK. Moreover, the benefits of ARBs with respect to cardiac remodeling and heart failure are not fully understood. Although mortality is the most important endpoint in evaluating new regimens, insistence on its use as the only endpoint in clinical trials necessitates that thousands of patients be enrolled. Accordingly, surrogate nonfatal endpoints might be clinically meaningful endpoints. However, in the case of animal models, a potential advantage for life expectancy is needed to evaluate new strategies. Many unknowns about combination therapy remain, and further investigations on the mechanisms of the tissue renin-angiotensin system and the relationships between other autocrine-paracrine systems are now underway.

ACKNOWLEDGMENT

The authors wish to thank Prof. K. Abe for his valuable comments concerning pathophysiology.

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