VEGF-mediated angiogenesis is impaired by angiotensin type 1 receptor blockade in cardiomyopathic hamster hearts

Toshihiro Shimizu, Hiroshi Okamoto*, Satoru Chiba, Yutaka Matsu, Takeshi Sugawara, Masatoshi Akino, Jia Nan, Hideki Kumamoto, Hisao Onozuka, Taisei Mikami, Akira Kitabatake

Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan

Received 5 August 2002; accepted 4 December 2002

Abstract

Objective: Coronary microcirculation plays an important role in the progression of cardiac remodeling. Among angiogenic factors, it has been reported that angiotensin II may contribute to neovascularization. However, it is unknown whether inhibition of the renin–angiotensin system suppresses angiogenesis, especially within the heart. Our aim was to evaluate the effects of the angiotensin-converting enzyme inhibitor enalapril and the angiotensin II receptor type I blocker valsartan on cardiac microvasculature, function, vascular endothelial growth factor (VEGF) expression, and survival in cardiomyopathic hamsters.

Methods: Male cardiomyopathic hamsters (BIO TO2) were administered either a placebo (group C), enalapril (30 mg/kg/day) (group E), or valsartan (40 mg/kg/day) (group V), starting at the age of 6 weeks. This continued until death. Hemodynamic study, histological analysis, and northern blot analysis were performed at 39 weeks.

Results: Group V showed significant increases in percent fibrosis, end diastolic pressure, and LV dP/dt min, and significant decreases in percent fractional shortening, LV dP/dt max, capillary density, and the level of mRNA expression of VEGF compared with group C. Group E showed significant increases in percent fractional shortening while the capillary density and level of mRNA expression of VEGF were unchanged. The 300-day survival rate was significantly lower in group V (25.0%) but higher in group E (100%) than that of group C (66.7%).

Conclusions: Therapy with valsartan may have adverse effects on survival rate concomitant with the progression of cardiac remodeling owing to impaired VEGF-mediated angiogenesis. Therapy with enalapril has a neutral effect on VEGF-mediated angiogenesis, leading to the suppression of cardiac remodeling and an increase in life expectancy.

Keywords: ACE inhibitors; Growth factors; Heart failure; Microcirculation; Renin angiotensin system

1. Introduction

Heart failure develops in a stepwise fashion owing to a progressive remodeling process. Remodeled myocardium is involved in cardiac hypertrophy, fibroblast proliferation, and extracellular matrix production, leading to the progress of heart failure. Moreover, interstitial fibrosis may increase the diffusion distances from capillaries to myocytes. The essential functions of the heart are performed at the level of the microvasculature, where oxygen, nutrients and hormones are delivered and catabolites are removed. Abnormalities of the microvasculature such as reductions...
in capillary density, thickening of the arteriole walls, and inadequate angiogenesis are involved in the pathogenesis of various forms of heart disease. Myocardial neovascularization is an important physiological process that frequently occurs in chronic myocardial ischemia. In the acute phase of ischemia, irreversible myocyte necrosis might cause failure of angiogenesis to compensate. In fact, significant collateralization has been demonstrated together with reduction in the size of the infarct after intracoronary administration of growth factors [1]. On the other hand, vascular endothelial growth factor (VEGF) knockout mice have impaired angiogenesis, leading to ischemic cardiomyopathy [2], suggesting that VEGF is a key molecule regulating the balance between cardiac oxygen consumption and vascular growth. The angiogenic cytokines may thus play a pivotal role in the progression of cardiac remodeling.

It is well known that cardiac remodeling is accompanied by local and systemic activation of the renin–angiotensin system (RAS). Among the neovascularization factors, angiotensin II (AII), the main effector peptide of the RAS may contribute to vessel growth regulation. The pro-angiogenic action of AII is mediated by the angiotensin type I receptor (AT1 receptor) through sustained activation of VEGF production within the ischemia [3]. The effects of angiotensin-converting enzyme inhibitor (ACEI) and AT1 receptor blockade (ARB) of failing myocardium on exclusively VEGF-mediated angiogenesis have not been elucidated. Because AII formation is suppressed by ACEI and blocked by ARB, it is likely that ACEI and ARB inhibit angiogenesis. However, there are several controversial reports regarding the effects of ACEI and ARB on angiogenesis. For example, an ACEI, perindopril, can improve impaired angiogenesis in the ischemic limbs of spontaneous hypertensive rats [4]. Likewise, an ARB, losartan, improves coronary angiogenesis in infarcted hearts [5]. In contrast, ramipril and losartan impair reparative angiogenesis in a murine model of limb ischemia [6]. Thus, little is known about whether RAS inhibition has a beneficial effect on coronary angiogenesis during the remodeling process.

In this study, we examined the effects of ACEI and ARB on VEGF-mediated angiogenesis in cardiomyopathic hamster hearts.

2. Methods

2.1. Experimental animals and protocol

At the age of 6 weeks, male BIO TO2 hamsters (Bio Breeders, Fitchburg, MA, USA) were randomly assigned to receive either enalapril (n=24, 30 mg/kg/day, p.o.), valsartan (n=24, 40 mg/kg/day, p.o.) or standard chow (n=24). All drugs were given to hamsters orally throughout their life. Of the 24 hamsters in each group, two animals were killed at 140 days of age for microvascular angiography, and 10 animals were sacrificed at 273 days of age for hemodynamic study, histological analysis, and Northern blot analysis. To examine the effects on survival, the remaining animals (12/group) were carefully monitored, and deaths were confirmed every day. The investigation conforms 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 1996). Because there were no significant differences in mRNA expression for VEGF or collagen I, capillary density, percent fibrosis, or cardiac function in F1B untreated control hamsters at the ages of 5, 13, and 20 weeks, and percent fractional shortening (%FS) and left ventricular end-diastolic dimension (LVDD) in F1B controls treated with ACEI or ARB were similar to those in untreated F1B controls in the previous study [7]. F1B control hamsters were administered neither enalapril nor valsartan in this study.

Enalapril was selected because of its general usage in heart failure. We had previously performed two experiments using cardiomyopathic hamsters, in which enalapril was used at two different doses, 20 and 25 mg/kg/day. Each experiment gave almost the same results macro- and microscopically. Fibrosis was suppressed and cardiac functions were preserved at the same levels. Serum concentrations of enalapril were measured in each group and found to be sufficient by comparing them with clinical data provided by Merck (Whitehouse Station, NJ, USA) (data not shown).

Valsartan was selected because of its higher selectivity for angiotensin type 1 receptors. We considered that valsartan had the possibility to be more effective for heart failure than other ARBs. It was very difficult to decide the dosages of valsartan suitable for this model. The bioavailability of valsartan to the cardiomyopathic hamster was not known. We used valsartan at 40 mg/kg/day. No adverse effect was observed, and the maximum hypotensive effects were exhibited at the same level with this dosage as in the enalapril group. We considered that this dose was adequate for cardiomyopathic hamsters.

To confirm that a sufficient dosage was actually received, serum AII levels were measured in the three groups.

2.2. Serum biochemical analyses

Serum asparaginate amino transferase (AST), creatinine, and AII levels were evaluated in 273-day-old hamsters (n=10/group). These laboratory tests were performed at Special Reference Laboratories (SRL, Sapporo, Japan). Serum AST levels were measured by the enzymatic method standardized by the Japan Society of Clinical Chemistry. The serum creatinine concentration was measured by enzymatic methods. The serum AII level was measured by radioimmunoassay methods. The serum concentration of enalapril was measured by radioimmunoassay
methods at Drug Monitoring Service/Medical Service, Banyu Pharmaceuticals (Tokyo, Japan).

2.3. Echocardiography

The method for echocardiography was described previously [8]. Briefly, at the age of 39 weeks, each hamster was anesthetized with intraperitoneal injection of urethane (50 mg/100 g of body weight) and α-chloralose (100 mg/100 g of body weight) and transthoracic echocardiograms (Hitachi EUB 8000) were obtained with a 13-MHz linear scanner. Urethane and α-chloralose do not have significantly depressive effects on cardiac function, as previously described [9]. 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.

2.4. Catheterization

The method for catheterization was described previously [10]. A 1.4-French microtip catheter manometer (SPR-677, Millar Instruments, 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 in left ventricular pressure (LV dP/dt max) and the maximum rate of fall in left ventricular pressure (LV dP/dt min) were derived from the left ventricular pressure by analysis with a computer system (MP-100WS, Biopac System, Santa Barbara, CA, USA) and the Acknowledged 2.0 program for the Macintosh (Biopac System).

2.5. Histological examination

Previous studies have shown that histochemical staining with the lectin *Griffonia simplicifolia* (GSA-B4) is a sensitive and reliable method to visualize the entire capillary vasculature within the skeletal and cardiac muscles of the hamster [11]. Therefore, left ventricular sections were stained with GSA-B4 (Sigma, St. Louis, MO, USA) to examine the structure of the capillary bed. Hearts were removed, dipped into OCT compound (Tissue-Tek™, Sakura Finetechicals, Tokyo, Japan), frozen in liquid nitrogen, and stored at −80°C until use. Sections 8 µm thick were obtained from cross-sections taken at the widest part of the left ventricle by means of a cryostat. They were fixed in acetone for 10 min and air-dried and placed in phosphate-buffered saline (PBS) for 15 min two times. The sections were treated with 3% hydrogen peroxide in methanol for 15 min at 4°C to inhibit intrinsic peroxidase activity, and washed two times for 5 min each in PBS. GSA-B4 was diluted 1:100 in PBS and incubated with the tissue sections overnight at 4°C. The slides were then reacted with streptavidin conjugated to peroxidase (Nich-irei, Tokyo, Japan) for 8 min and thoroughly rinsed in PBS for 5 min two times. Sites of bound lectin were visualized by incubation in a 3’,3’-diaminobenzidine (DAB)-hydrogen peroxide substrate medium (Nichirei) for 5 min, followed by two additional rinses. To enhance the DAB reaction, the sections were rinsed with 0.05 M sodium bicarbonate (pH 9.6) for 10 min and then incubated in DAB enhancing solution (Vector Laboratories, Burlingame, CA, USA) for 15 s. After counterstaining with hematoxylin, the tissue sections were dehydrated through a graded series of ethanol and xylene, placed on slides, and coverslipped. With the use of a light microscope (PM-10AK, Olympus, Japan) and a camera (C-35AD, Olympus), pictures of the sections were taken randomly in the subepicardial region, midventricular region, and subendocardial region. The numbers of coronary capillaries and cardiomyocytes were counted in a 11.25 mm² area per section.

2.6. Northern blot analysis

The cDNA probes used in the present study were cDNA for hamster VEGF, collagen I, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA for internal control. All cDNA probes were uniformly labeled with random primers using Klenow enzyme (Boehringer Mannheim) and [32P]dCTP (Life Science Products). Each preparation of total RNA was isolated from a left ventricular tissue sample using TRIzol reagent (Gibco BRL). Twenty micrograms of denatured RNA was size fractioned on 2% formaldehyde 1.2–1.5% agarose gels and then transferred to a nylon membrane (Hybond-N 1, Amersham Life Science). Northern blot analysis was carried out according to conventional methods. Each membrane was exposed at −80°C to X-ray films (X-OMAT, Eastman Kodak) with a single intensifying screen to increase exposure times and obtain signals in the linear range for densitometric analysis of each mRNA species. The GAPDH mRNA diffuse density score, used as an internal control, has been shown to be unchanged in the BIO TO2 heart. To evaluate mRNA levels, an optical scanner (GT-9500, Seiko, Tokyo, Japan) was utilized to digitize autoradiograms. The density of autoradiogram bands in the digitized image was measured with the NIH Image program.

2.7. Angiography

To visualize small intramyocardial coronary arteries, we performed angiography using synchrotron radiation (SR) at the Japan Synchrotron Radiation Research Institute (SRing-8, Hyogo, Japan). To prepare samples for visualization, we injected the contrast medium developed by Fujimoto et al. [12] into a Langendorff heart preparation of the BIO TO2 at physiological pressure. The heart was chilled rapidly to 0°C and was fixed in paraformaldehyde for 24 h at 4°C. Then, the heart was cut cylindrically to a
Table 1
Body weight, ventricular weight and biochemical data

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>LVW (mg)</th>
<th>LVW/BW (mg/g)</th>
<th>Cr (mg/dl)</th>
<th>AST (IU/l)</th>
<th>AII (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>110.6±4.993</td>
<td>236.3±24.49</td>
<td>2.136±0.191</td>
<td>0.180±0.042</td>
<td>179.7±58.90</td>
<td>3720±550.5</td>
</tr>
<tr>
<td>Enalapril (n=10)</td>
<td>107.0±11.50</td>
<td>202.3±23.15*</td>
<td>1.875±0.175*</td>
<td>0.180±0.063</td>
<td>161.1±47.81</td>
<td>608.3±249.4**</td>
</tr>
<tr>
<td>Valsartan (n=10)</td>
<td>110.6±4.992</td>
<td>269.1±8.281*</td>
<td>2.376±0.124*</td>
<td>0.200±0.089</td>
<td>173.2±78.81</td>
<td>6800±2049**</td>
</tr>
</tbody>
</table>

Values are means±S.E.; control=untreated BIO TO2; enalapril=BIO TO2 treated with enalapril; valsartan=BIO TO2 treated with valsartan; BW, body weight; LVW, left ventricular weight; Cr, creatinine; AST, asparaginate amino transferase; AII, angiotensin II

* P<0.01 vs. control; ** P<0.05 vs. control; † P<0.01 vs. enalapril.

Table 2
Echocardiographic parameters

<table>
<thead>
<tr>
<th></th>
<th>LVDd (mm)</th>
<th>%FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>73.6±6.46</td>
<td>11.6±3.18</td>
</tr>
<tr>
<td>Enalapril (n=10)</td>
<td>65.8±5.26</td>
<td>15.9±1.50*</td>
</tr>
<tr>
<td>Valsartan (n=10)</td>
<td>75.0±7.43*</td>
<td>6.88±0.70**</td>
</tr>
</tbody>
</table>

Values are means±S.E.; control=untreated BIO TO2; enalapril=BIO TO2 treated with enalapril; valsartan=BIO TO2 treated with valsartan; LVDd, left ventricular diastolic dimension; %FS, percent fractional shortening.

† P<0.01 vs. control; ‡ P<0.01 vs. enalapril; † P<0.05 vs. enalapril.

3. Results

3.1. Effect of long-term treatment with enalapril and valsartan

Survival curves for cardiomyopathic hamsters treated with enalapril and valsartan are shown in Fig. 1. The 300-day survival rate of BIO hamsters treated with enalapril (100%, 12 of 12 BIO hamsters survived) was significantly higher than that of BIO hamsters treated with the control placebo (66.7%, eight of 12 BIO hamsters survived). With valsartan, the rate (25%, three of 12 BIO hamsters survived) was significantly lower than that of BIO hamsters treated with the control placebo. All dead animals showed pleural effusion, a congested liver and significantly heavier lung weights (data not shown).

3.2. Body weight, left ventricular weight, serum creatinine and AST

As shown in Table 1, there was no significant difference in body weight (BW) among the three groups. However, left ventricular (LV) to body weight ratios were significantly decreased in the enalapril group and increased in the valsartan group compared to the control group. To examine the adverse effects of each treatment, serum creatinine levels and AST levels were examined and showed no significant difference among the three groups. The serum AII level was significantly decreased in the enalapril group and increased in the valsartan group compared to the control group.

3.3. LV function and hemodynamics

LV function and hemodynamics are summarized in Tables 2 and 3. No hamsters died during the final catheterization or echocardiographic study. Representative echocardiograms are shown in Fig. 2. LVDd were higher in the valsartan group than in the enalapril group. %FS decreased for valsartan compared with the control and enalapril.

Doppler-echocardiography revealed that the E wave was united with the A wave and one tall, sharp wave was observed in the control and valsartan groups; that is, these groups had diastolic dysfunction. The tall, sharp wave was divided into two waves which were the E wave and the A wave. The energy of SR left ventricular (L V) to body weight ratios were significantly decreased in the enalapril group and increased in the valsartan group compared to the control group.

As shown in Table 1, there was no significant difference in body weight (BW) among the three groups. However, left ventricular (LV) to body weight ratios were significantly decreased in the enalapril group and increased in the valsartan group compared to the control group. To examine the adverse effects of each treatment, serum creatinine levels and AST levels were examined and showed no significant difference among the three groups. The serum AII level was significantly decreased in the enalapril group and increased in the valsartan group compared to the control group.

3.3. LV function and hemodynamics

LV function and hemodynamics are summarized in Tables 2 and 3. No hamsters died during the final catheterization or echocardiographic study. Representative echocardiograms are shown in Fig. 2. LVDd were higher in the valsartan group than in the enalapril group. %FS decreased for the enalapril group compared with the control. %FS decreased for valsartan compared with the control and enalapril.

Doppler-echocardiography revealed that the E wave was united with the A wave and one tall, sharp wave was observed in the control and valsartan groups; that is, these groups had diastolic dysfunction. The tall, sharp wave was divided into two waves which were the E wave and the A wave. The energy of SR left ventricular (L V) to body weight ratios were significantly decreased in the enalapril group and increased in the valsartan group compared to the control group.

As shown in Table 1, there was no significant difference in body weight (BW) among the three groups. However, left ventricular (LV) to body weight ratios were significantly decreased in the enalapril group and increased in the valsartan group compared to the control group. To examine the adverse effects of each treatment, serum creatinine levels and AST levels were examined and showed no significant difference among the three groups. The serum AII level was significantly decreased in the enalapril group and increased in the valsartan group compared to the control group.

3.3. LV function and hemodynamics

LV function and hemodynamics are summarized in Tables 2 and 3. No hamsters died during the final catheterization or echocardiographic study. Representative echocardiograms are shown in Fig. 2. LVDd were higher in the valsartan group than in the enalapril group. %FS decreased for the enalapril group compared with the control. %FS decreased for valsartan compared with the control and enalapril.

Doppler-echocardiography revealed that the E wave was united with the A wave and one tall, sharp wave was observed in the control and valsartan groups; that is, these groups had diastolic dysfunction. The tall, sharp wave was divided into two waves which were the E wave and the A wave.
Table 3

Hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>HR (beats / min)</th>
<th>L VSP (mmHg)</th>
<th>L VEDP (mmHg)</th>
<th>LV $dP/dt$ max</th>
<th>LV $dP/dt$ min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>276.2±50.91</td>
<td>86.15±6.113</td>
<td>18.10±2.000</td>
<td>2548±315.5</td>
<td>−2007±220.8</td>
</tr>
<tr>
<td>Enalapril (n=10)</td>
<td>300.9±26.23</td>
<td>72.60±5.007*</td>
<td>17.30±1.904</td>
<td>2481±267.1</td>
<td>−1946±212.2</td>
</tr>
<tr>
<td>Valsartan (n=10)</td>
<td>291.7±24.02</td>
<td>74.17±4.842*</td>
<td>20.77±3.474**</td>
<td>2192±225.5*</td>
<td>−1704±194.3**</td>
</tr>
</tbody>
</table>

Values are means±S.E.; control=untreated BIO TO2; enalapril=BIO TO2 treated with enalapril; valsartan=LVSP; left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; HR, heart rate; LV $dP/dt$ max, maximum rate of rise of ventricular pressure; LV $dP/dt$ min, peak rate of fall of ventricular pressure.

* P<0.01 vs. control; ** P<0.05 vs. control; † P<0.01 vs. enalapril; †† P<0.05 vs. enalapril.

wave in the enalapril group; that is, diastolic function was more improved in the enalapril group than in the control and valsartan groups.

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 and in blood pressure between the treatment groups. LV systolic pressure (LVSP) decreased in the treatment groups compared with the control group.

There was no significant difference between LVSP in the enalapril group and the valsartan group. LV end-diastolic pressure (LVEDP) increased in the valsartan group compared with the control and enalapril groups. Although LVSP values were very low, control Flb as well as cardiomyopathic hamsters have low blood pressure by nature. LV $dP/dt$ max was decreased and LV $dP/dt$ min was increased in the valsartan group compared with the control and enalapril groups despite the reduction in LVSP.

3.4. Density of cardiomyocytes, capillary density, and percent fibrosis

Histological parameters are summarized in Table 4. The

Fig. 2. M mode and Doppler echocardiograms. The upper row shows M mode echocardiograms at the level of the chorda tendineae, and the lower row shows mitral valve inflow velocities. (C) Untreated BIO TO2 hamsters; (E) BIO TO2 hamsters treated with enalapril; (V) BIO TO2 hamsters treated with valsartan. IVST, interventricular septum; LVD, left ventricular dimension; LVPW, left ventricular posterobasal free wall.
numeric density of cardiomyocytes, which represents the viable myocytes per area, was more preserved from loss in the enalapril group than in the control group and was lower in the valsartan group than in the enalapril group. Capillary density was significantly lower in the valsartan group than in the control group and enalapril groups. There was no significant difference between the enalapril group and the control group. The percent of fibrosis was decreased in the enalapril-treated group and increased in the valsartan group more than in the control group (Fig. 3).

3.5. mRNA expression

Representative autoradiographs obtained by Northern blot analysis are shown in Fig. 4. The levels of mRNA expression of VEGF were significantly lower in the valsartan group than in the control group. There was no significant difference in the level of mRNA expression of VEGF between the enalapril group and the control (0.36±0.02 in the control group, 0.39±0.02 in the enalapril group, 0.28±0.02 in the valsartan group). The level of mRNA expression of collagen I was significantly higher in the valsartan group than in the control and enalapril groups. The level of mRNA expression of collagen I was significantly lower in the enalapril group than in the control group (0.60±0.04 in the control group, 0.46±0.04 in the enalapril group, 0.74±0.05 in the valsartan group).

3.6. Angiography

Representative angiograms are shown in Fig. 5. Microvasculature density was lower in the valsartan group than in the control group and the enalapril group.
Fig. 4. Northern blot analysis demonstrating expression of vascular endothelial growth factor (VEGF), collagen I, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in untreated BIO TO2 hamsters and those treated with enalapril and valsartan. The upper row shows representative Northern blot analysis of VEGF mRNA and a bar graph showing the abundance of VEGF mRNA relative to GAPDH mRNA. The lower row shows representative Northern blot analysis of collagen I mRNA and a bar graph showing the abundance of collagen I mRNA relative to GAPDH mRNA. (C) Untreated BIO TO2 hamsters; (E) BIO TO2 hamsters treated with enalapril (30 mg/kg/day); (V) BIO TO2 hamsters treated with valsartan (40 mg/kg/day). * P<0.05 vs. C; ** P<0.01 vs. C and E.

Fig. 5. Transmural coronary microangiography of untreated cardiomyopathic hamsters and cardiomyopathic hamsters treated with enalapril (30 mg/kg/day) and valsartan (40 mg/kg/day), done with the use of synchrotron radiation at SPring-8. (C) Untreated BIO TO2 hamsters; (E) BIO TO2 hamsters treated with enalapril (30 mg/kg/day); (V) BIO TO2 hamsters treated with valsartan (40 mg/kg/day).
4. Discussion

Our study demonstrated that ACEI had a neutral effect on VEGF-mediated angiogenesis and effectively inhibited the RAS, leading to inhibition of adverse cardiac remodeling. In contrast, ARB had an adverse effect on VEGF-mediated angiogenesis, leading to acceleration of cardiac remodeling in spite of the RAS inhibition.

Why did these changes occur? Bastien et al. [13] demonstrated that chronic blockade of AT1 receptors was associated with a significant reduction in the survival of cardiomyopathic hamsters. However, the mechanism of the deleterious effects of ARBs in the hamster model has remained unclear since their report. We confirmed results similar to theirs and revealed for the first time that the reason why ARB would be detrimental for the failing heart is impaired angiogenesis.

It is controversial whether RAS inhibition suppresses angiogenesis, especially within the heart. There is insufficient evidence to support a direct effect of the RAS on endogenous angiogenic cytokines. In fact, AT1 has previously been reported to induce a concentration- and time-dependent increase in VEGF expression by vascular smooth muscle cells as well as endothelial cells [14]. The differing consequences of RAS inhibition noted for enalapril versus valsartan in this study may be the result of following factors.

Heart failure and cardiac remodeling are characterized by activation of the RAS and other neurohumoral systems. ACEI not only inhibits the RAS but prevents breakdown of bradykinin (BK). BK enhances the angiogenic process through the activation of the B1 receptor, which is induced by tissue damage via activation of transcription factor nuclear factor kB and B2 receptor pathways, which are responsible for the majority of biological effects of BK [15,16]. Morbidelli et al. [17] reported that the BK-induced neovascularization was abolished by a B1 receptor antagonist. Furthermore, Emanueli et al. [18] found that B1 signaling is essential for developing new blood vessels, because the B1 knockout mouse exposed femoral artery showed a very high incidence of limb necrosis leading to auto-amputation compared with control mice, and local delivery of a B1 receptor agonist enhanced collateral vascular growth in ischemic limbs of control mice.

On the other hand, Silvestre et al. [19] showed that the proangiogenic effect of ACE inhibition mediated by the B2 receptor pathway, for neovascularization, vessel density and blood flow, was impaired in ischemic hind limbs of B2 knockout mice. Activation of B2 receptors causes vascular endothelial cells to release autacoids such as nitric oxide (NO) and prostaglandins (PG) [20].

Noiri et al. [21] demonstrated that endogenous NO production by endothelial cells is a prerequisite for the angiogenic effects. Leibovich et al. [22] have shown that production of angiogenic activity is dependent on L-arginine and NO synthase. In addition, PGE1 and PGI2 are known to stimulate angiogenesis in the cornea in rabbits and in the chorioallantoic membrane in chick embryos [23,24]. In clinical trials, PGE1 induces angiogenesis in the failing heart; that is, it causes CD34- and von Willebrand factor-positive cells to increase [25]. NO and PG could be potent inducers of angiogenesis. Thus, we speculated that enalapril had a neutral effect on angiogenesis in this experiment, because proangiogenic effects mediated by the activated B1 and B2 receptor pathways canceled out anti-angiogenic effects associated with RAS inhibition.

On the other hand, ARB selectively blocks the actions of AT1 mediated through AT1 receptors, yet potentially amplifies the effects mediated through AT2 receptors. ARB strongly induces angiogenesis in the microcirculation through the activation of AT1 receptor pathway, whereas, the stimulation of AT2 receptors mediates a growth-inhibitory response in the microvasculature in vivo and in vitro. LeNoble et al. [26] showed that CGP42112, acting as an AT2 agonist, blocked AII-induced angiogenesis in the developing chick chorioallantoic membrane. Munzenmaier et al. [27] showed that PD123319, an AT2 antagonist, cofused with AII increased vessel density compared with AII infusion alone. Thus, we considered that ARB had an adverse effect on angiogenesis, because the anti-angiogenic effect associated with RAS inhibition was enhanced by stimulation of the AT2 receptor pathway.

Many clinical trials have demonstrated that blockade of the RAS with ACEI modulates adverse left ventricular remodeling and prolongs survival. The ELITE II trial demonstrated that the effect of an ARB, losartan, on mortality was comparable to that of an ACEI, captopril [28]. However, there are several controversial reports regarding the effects of ARB on heart failure. Mortality was significantly increased in a subgroup receiving an ARB, ACEI, and a beta-blocker in the Val-Heft trial [29]. Blockade of the AT1 receptor did not improve LV remodeling and function in the early myocardial adaptive phase of mitral regurgitation [30]. The current study failed to demonstrate longer life expectancy caused by ARB and improvement of cardiac function because of impaired angiogenesis. Thus, treatment with ARBs might promote mortality and morbidity in a subset of heart failure.

BIO TO2 was an established model of progressive heart failure [31]. We used BIO TO2 hamsters to examine the effects of ACEI and ARB on VEGF-mediated angiogenesis. Because of the similarity of the chymase contributions to the diseased heart, it appeared likely that different class effects of ACEI and ARB could be compared and clarified [32].

Microvascular angiography was performed with synchrotron radiation at SPring-8, which is the largest and brightest third-generation facility in the world. Because the synchrotron radiation at SPring-8 is ultrabright and highly directional, we could visualize details of the microvasculature that are not discernible by conventional X-ray examination. The impairment caused by administering valsartan
and improvement induced by administering enalapril were confirmed angiographically as well as pathologically. It seems that the angiography detected more vessels in the enalapril group compared to the control. However, microvascular angiography is essentially a different method from histological evaluation. It is difficult to determine the quantity of the capillary density by microvascular angiography, for microvascular angiography using synchrotron radiation visualizes all vessels, including capillary vessels (the diameter is under 10 μm), pre-capillary vessels (10–100 μm), and small vessels (over 100 μm). On the other hand, the histological analysis evaluated only capillary vessels and revealed that there was no significant difference in the capillary density between the enalapril group and the control group. Thus, pre-capillary vessels and small vessels might have increased in the enalapril group. However, we could not assess the possibility in the manuscript because we could not distinguish pre-capillary and small vessels from capillary vessels.

In BIO TO2, myocyte loss progresses after birth and cardiac remodeling progresses after myocyte loss. TGF-β is a pivotal molecule in cardiac remodeling associated with cardiac fibrosis and hypertrophy. We reported that the expression of TGF-β was already higher in BIO TO2 than in control hamsters at 5 weeks [7]. RAS-inhibiting agents attenuate the expression of TGF-β and cardiac fibrosis. In our experiment, cardiac fibrosis was suppressed in the enalapril group. On the other hand, cardiac fibrosis developed in the valsartan group. Cardiac fibrosis might have been suppressed due to RAS inhibition in the valsartan group too, yet replacement fibrosis could strongly participate in cardiac remodeling after progressive myocyte loss owing to impaired angiogenesis.

In this experiment, we could not find favorable effects in the hemodynamic study in the enalapril group or evaluate the diastolic function using echocardiography in the valsartan group. We reported that %FS and dP/dt max were decreased in BIO TO2 compared with those in control hamsters, and pleural effusion or congestive liver was detected as a sign of heart failure in BIO TO2 at 20 weeks [7]. These analyses were performed at 39 weeks in order to evaluate the effect on survival. All BIO TO2 hamsters were in critical condition due to progressive heart failure. We speculate that the analysis occurred too late to detect the differences of hemodynamics. These differences may be detectable at a younger age, for example, at 20–25 weeks.

5. Conclusion

The present study showed that the therapy with enalapril had beneficial effects on survival rate concomitant with improvement of cardiac remodeling, especially a decrease in fibrosis and preservation of myocytes and microvascular-structure, which may lead to improvement in both systolic and diastolic functions. On the other hand, the therapy with valsartan had adverse effects on survival rate concomitant with worsening of cardiac remodeling, especially an increase in fibrosis and loss of myocytes owing to decreased VEGF-mediated angiogenesis, which may lead to deterioration in both systolic and diastolic functions in cardiomyopathic hamsters. It is suggested that we should pay careful attention when an ARB is administered to patients with heart failure.

Acknowledgements

We thank Professor F. Kajiya for his useful comments concerning angiography. This study was funded in part by Research Grants on Cardiomyopathy from the Ministry of Health and Welfare of Japan and Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, No. 10358020.

References


