Beneficial Effects of Angiotensin-Converting Enzyme Inhibition in Adriamycin-Induced Cardiomyopathy in Hamsters

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ABSTRACT—This study was performed to determine whether angiotensin (Ang) II-forming enzymes, angiotensin converting enzyme (ACE) and chymase might contribute to the development of adriamycin-induced cardiomyopathy in hamsters. Hamsters were administered adriamycin (2.0 mg/kg per day, i.p.) three times weekly for 2 weeks. In the ACE inhibitor-treated group, the hamsters received lisinopril (20 mg/kg per day, p.o.) for 2 weeks after the last injection of adriamycin. The 4-week mortality rates of the vehicle- and ACE inhibitor-treated hamsters were 44% and 12%, respectively. In comparison to the age-matched hamsters used as the control hamsters, a significant decrease in cardiac function and a significant increase in the ratio of the heart weight to the body weight were observed in the vehicle hamsters. Cardiac ACE activity, but not the chymase activity, in the vehicle hamsters was significantly increased in comparison to that in the control hamsters. In the ACE inhibitor-treated group, the increased ACE activity was reduced significantly, and the cardiac hypertrophy and dysfunction were improved significantly. In adriamycin-induced cardiomyopathic hamsters, cardiac ACE activity was increased and ACE inhibition significantly improved cardiac function and survival rate, indicating that cardiac ACE, but not the chymase, plays the pivotal role in the development of the adriamycin-induced cardiomyopathy.

Keywords: Adriamycin, Cardiomyopathy, ACE inhibitor, Chymase

Angiotensin (Ang) II is known to be generated from Ang I by angiotensin-converting enzyme (ACE) in the blood and in several tissues (1). However, an enzyme other than ACE was found in human cardiac tissues, and it was purified and identified as chymase (2). Chymase has been isolated and characterized in humans, hamsters and rats (2 – 4). However, the substrate specificities of these chymases are very different. Human and hamster chymases cleave the Phe^8-His^9 bond of Ang I to yield Ang II, while rat chymase hydrolyzes the Tyr^4-Ile^5 bond of Ang I to yield inactive fragments (5). Previously, we reported that cardiac chymase activities during a period of development of cardiac fibrosis in genetic cardiomyopathic hamsters and in chronic hypertensive hamsters were increased (6, 7). Recently, we also found that cardiac chymase was significantly increased after myocardial infarction in hamsters (8). These findings suggest that the activation of cardiac chymase may play an important role in the cardiac remodeling.

Adriamycin is one of the most effective anti-tumor antibiotics for the treatment of a variety of malignancies. However, its use is strictly limited by the acute or chronic onset of cardiotoxicities (9, 10). The chronic cardiotoxicity is dose-dependent, resulting in cardiomyopathy with fatal congestive heart failure. Although the participation of free radicals, the apoptosis of myocytes and the impairment of mitochondria in cardiomyopathy caused by adriamycin have been suggested, the detailed mechanisms were not fully understood (11 – 14). In the adriamycin-induced cardiomyopathy in the rat, ACE inhibitor treatment improved the cardiac dysfunction and the rate of mortality (15, 16). These reports suggest that Ang II formation by ACE may play an important role in the development of the cardiomyopathy. However, it has been unclear whether Ang II formed by cardiac chymase has a pathophysiological role in adriamycin-induced cardiomyopathy. In this study, we investigated the effects of an ACE inhibitor on adriamycin-induced cardiomyopathy in hamsters whose cardiac tissues contain chymase-dependent Ang II production and determined whether chymase in addition to ACE might contribute to the development of adriamycin-induced cardiomyopathy in hamsters.
MATERIALS AND METHODS

Animal model

Six-week-old male Syrian hamsters weighing 70–130 g were purchased from SLC Japan (Shizuoka). All hamsters were housed at room temperature (22°C) with a 12-h light-dark cycle and had free access to food and water.

On day 0, fifty animals were administered intraperitoneally 2.0 mg/kg of adriamycin (doxorubicin hydrochloride) as a single injection. They received the drug three times a week in six equal injections over a period of two weeks for a cumulative dose of 12 mg/kg body weight. Ten hamsters served as controls and received the same amount of saline intraperitoneally three times a week for 2 weeks. After the last injection of adriamycin (day 14), fifty adriamycin-treated hamsters were randomly divided into two groups: the vehicle group (n = 25) and the ACE inhibitor-treated group (n = 25). In the ACE inhibitor-treated group, hamsters were administered 20 mg/kg of lisinopril once a day by gastric gavage. The age-matched treated group, hamsters were administered 20 mg/kg of lisinopril once a day by gastric gavage. The age-matched treated group, hamsters were administered 20 mg/kg of lisinopril once a day by gastric gavage.

After measurement of hemodynamic parameters, the hearts were immediately removed and were immediately frozen in liquid nitrogen. Tissues were stored for measurement of ACE and chymase activities at −80°C until measurement.

Tissue preparation

Each heart was minced and homogenized in 10 vol (wt: vol) of 20 mM Na-phosphate buffer, at pH 7.4. The homogenate was centrifuged at 20,000 rpm for 30 min and the supernatant was discarded. The pellets were resuspended and homogenized in five vol (wt: vol) of 10 mM Na-phosphate buffer, at pH 7.4, containing 2 M KCl and 0.1% Nonidet P-40. The homogenate was stored overnight at 4°C and then centrifuged at 20,000 rpm for 30 min. The supernatant was used as the tissue extract for the measurement of ACE and chymase activities.

Measurement of ACE and chymase activities

ACE activity was measured using a synthetic substrate, hippuryl-His-Leu (HHL), specifically designed for ACE (Peptide Institute, Inc., Osaka) as previously described (17). Twenty-five microliters of tissue extract or plasma were incubated for 60 min at 37°C with 5 mM HHL in 100 µl of 10 mM phosphate buffer, pH 8.3, containing 0.6 M NaCl. The reaction was terminated by addition of 37.5 µl of 3% metaphosphoric acid, and the mixture was centrifuged at 18,000 rpm for 10 min at 4°C. After centrifugation of the mixture, we applied 50 µl of the supernatant to an octadecyl silica reversed-phase column (RP-18, 4 mm i.d. x 250 mm; IRICA, Kyoto), which had been equilibrated with 10 mM KH2PO4 and CH3OH (1:1, pH 3.0) beforehand and eluted it with the same solution at a rate of 0.5 ml/min. Hippuric acid was detected by ultraviolet absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 µmol hippuric acid/min.

Chymase activity was measured using the procedure as described previously (18). A 20-µl aliquot of tissue extract was incubated for 10 min at 37°C with 4 mM Ang I in 150 mM borax-borate buffer, pH 8.5 (final incubation volume of 100 µl) containing 5 mM ethylenediaminetetra-acetic acid, 8 mM dipiridyl and 0.77 mM diisopropyl phosphorofluoridate. Reactions were terminated by addition of 150 µl of 15% trichloroacetic acid, followed by centrifugation at 18,000 rpm for 10 min. For fluorometric quantification of His-Leu as an Ang I metabolite, 10% o-phthaldialdehyde was added to the supernatant under alkaline conditions, and then 6N HCl was added to stabilize the fluorescence for the measurement at 340-nm excitation and 455-nm emission. A blank was carried out with the addition of 0.5 mM chymostatin.

Hemodynamic studies

The hamsters were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the trachea was intubated. A polyethylene catheter that consisted of PE 10 tubing welded to PE 50 tubing (Clay Adams, Parsippany, NJ, USA) was introduced into the left carotid artery. Then the catheter was connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo) and the mean arterial blood pressure (MABP) was measured. After this procedure, the thorax was opened under positive-pressure respiration (NEMI Scientific, Inc., Medway, MA, USA) and a catheter was inserted into the left ventricular chamber via its apex, and the maximal positive and negative rates of pressure development (+dP/dt and −dP/dt) were measured.

Statistical analyses

Results are expressed as the mean ± standard error of the mean (S.E.M.). Survival rate was analyzed by the standard Kaplan-Meier analysis with a log-rank test. Significant differences among the mean values of multiple groups were evaluated by 1-way ANOVA followed by a post-hoc analysis (Fisher’s test). In all tests, values of P<0.05 were considered statistically significant.
RESULTS

Mortality

The survival rate during the 28-day observation period after the first injection of adriamycin was analyzed (Fig. 1). There were no deaths in the control group during the 28-day observation period. Nine hamsters in the vehicle group died during the fourth week (days 21 – 28). The total mortality rate of the vehicle group was 44%. On the other hand, three hamsters in the ACE inhibitor-treated group died during the third and fourth weeks. The ACE inhibitor-treated group exhibited a 28-day mortality rate of 12%. Kaplan-Meier survival analysis showed that treatment with the ACE inhibitor improved the mortality of adriamycin-induced cardiomyopathic hamsters in a statistically significantly manner.

Heart weight, body weight and ratio of heart weight to body weight

The heart weight in the control (n = 10), the vehicle (n = 14) and the ACE inhibitor-treated hamsters (n = 22) were 285 ± 9.1, 250.9 ± 7.3 and 203.5 ± 4.8 mg, respectively. The heart weight in the vehicle hamsters was significantly decreased compared with that in the control hamsters. Furthermore, the heart weight in the ACE inhibitor-treated hamsters was significantly decreased compared with that in the vehicle hamsters. On the other hand, the body weight in the control, the vehicle and the ACE inhibitor-treated hamsters were 124.2 ± 4.1, 85.4 ± 4.0 and 83.1 ± 2.3 g, respectively. The body weight in the control hamsters was significantly heavier than that of vehicle or ACE inhibitor-treated hamsters, while there is no significant difference between the body weight in vehicle or ACE inhibitor-treated hamsters. These findings were also observed in the rat adriamycin-induced cardiomyopathic model (19). The ratio of heart weight to body weight was significantly increased in the vehicle hamsters in comparison to the control hamsters, and the increase of the ratio of heart weight to body weight in the ACE inhibitor-treated hamsters was significantly smaller than that in the vehicle hamsters. There was no significant difference in the ratio of heart weight to body weight between the control and the ACE inhibitor-treated hamsters (Fig. 2).

Biochemical studies

As shown in Fig. 3, the cardiac ACE activity in the vehicle hamsters was significantly increased in comparison to that of the control hamsters (control hamsters, 56.29 ± 3.54 mU/g tissue; vehicle hamsters, 121.86 ± 23.62 mU/g tissue). However, the cardiac chymase activity of the vehicle hamsters did not significantly differ from that of the control hamsters (control hamsters, 2.59 ± 0.40 mU/g tissue; vehicle hamsters, 2.10 ± 0.27 mU/g tissue). Treat-
Hemodynamic studies

The MABP of the vehicle and the ACE inhibitor-treated hamsters were significantly lower than that of the control hamsters, while there was no significant difference between the vehicle and the ACE inhibitor-treated hamsters (control hamsters, 116 ± 8.1 mmHg; vehicle hamsters, 98 ± 5.9 mmHg; ACE inhibitor-treated hamsters, 98 ± 4.5 mmHg). As shown in Fig. 4, the positive dP/dt in the vehicle hamsters were significantly lower than the value in the control hamsters. The negative dP/dt in the vehicle hamsters tended to be lower than that in the control hamsters, although it did not reach statistical significance (P = 0.053). The ACE inhibitor treatment significantly improved the positive and negative dP/dt values.

DISCUSSION

In the present study, adriamycin treatment resulted in a significant deterioration of cardiac function as indicated by decreased positive and negative dP/dt values, and the total mortality rate of the adriamycin-treated hamsters during the 28-day observation period reached about 44%. Cardiac hypertrophy also appeared 28 days after the initiation of adriamycin treatment. In this model, the cardiac ACE activity was increased, and the ACE inhibitor treatment improved the mortality, cardiac hypertrophy and cardiac dysfunction in the developing period of adriamycin-induced cardiomyopathy in hamsters. Treatment of adriamycin increases the number of macrophages in cardiac tissues (20), and it is well known that macrophages express ACE (21). Therefore, the increased cardiac ACE activity in the adriamycin-induced hamsters may be dependent on the accumulation of ACE-expressing macrophages in the cardiac tissues. On the other hand, the cardiac chymase did not change by the treatment with adriamycin. These findings suggest that cardiac ACE rather than cardiac chymase may participate in the development of cardiomyopathy induced by adriamycin in hamsters.

In the present study, the cardiac ACE activity in the adriamycin-treated hamsters was increased about 2.3-fold compared with that in hamsters not treated with adriamycin. Previously, Venkatesan et al. (22) also found that cardiac ACE activity was gradually increased after it was reduced during the acute stage in rats. The present study showed that the ACE inhibitor treatment significantly inhibited cardiac ACE activity, normalized the ratio of heart weight to body weight and remarkably improved the cardiac dysfunction. These improvements are thought to be associated with the prolongation in survival. The appearance of these beneficial effects of the ACE inhibitor was independent of the reduction of systemic blood pressure because the MABP of the ACE inhibitor-treated hamsters did not significantly differ from that of the untreated vehicle hamsters. These findings suggested that beneficial effects of the ACE inhibitor in the adriamycin-treated hamsters were dependent on inhibition of cardiac ACE.

The local Ang II formation via activation of cardiac ACE might be involved in the autocrine or paracrine regulation of tissue pathological remodeling, because Ang II is known to promote cardiac hypertrophy, fibroblast proliferation and collagen synthesis (23–26). These adverse actions are considered to be the main reasons for the development of chronic heart failure following myocardial infarction or in the end stage of hypertension (27, 28). In contrast, the intervention of this Ang II action by treatments with ACE inhibitors or Ang II type 1 receptor antagonists improves cardiac function and this structural remodeling leads to a reduction of mortality rate in heart diseases (1). It was reported that the apoptosis of myocytes was obvious during the period of cardiomyopathy development following chronic treatment of adriamycin (5, 6). On the other hand, it was known that Ang II stimulation induces the apoptosis of ventricular myocytes (29). These reports suggest that Ang II may play a critical role in the development or progression of cardiomyopathy after adriamycin administration. In fact, recent clinical studies have demonstrated that ACE inhibitors, which were used to treat severe adriamycin-induced cardiomyopathy in addition to conventional heart failure treatment (diuretics and digoxin) (12, 13), improved the severity of symptoms, suggesting that the activation of ACE after adriamycin administration may be closely related to the progression of cardiomyopathy. However, ACE inhibitor also increases bradykinin levels.
via blockade of kininase II (ACE). Hartman (30) reported that the protective effect of ACE inhibitor after myocardial infarction may partially depend on the increased bradykinin-stimulated nitric oxide production in cardiac tissues. Therefore, in this study, the beneficial effect of ACE inhibitor may partially include increase of bradykinin levels in addition to suppression of the Ang II levels.

Recently, we reported that chymase played an important role in cardiac dysfunction after myocardial infarction in hamsters. However, in the present study, cardiac chymase did not contribute to the pathogenesis of cardiac dysfunction induced by adriamycin in hamsters. Chymase is stored in secretory granules in mast cells, and it has maximal activity immediately upon release into the extracellular matrix in cardiac tissues when mast cells are activated by a strong stimulus such as myocardial infarction (8). In the cardiomyopathic hamsters induced by adriamycin, we could not observe the activation of mast cells (data not shown). Therefore, this different mechanism may depend on whether or not mast cells in cardiac tissues are activated by a stimulus. On the other hand, we also reported that cardiac chymase activities in chronic stage of hypertensive hamsters were increased (7). In this study, we did not study the cardiac chymase activity in the chronic stage of adriamycin-induced cardiomyopathic hamsters. To clarify the involvement of chymase in the adriamycin-induced cardiomyopathic hamsters, further studies may be needed.

In conclusion, cardiac ACE activity, but not chymase activity, was increased after the adriamycin treatment and cardiac reactivity, was increased after the adriamycin treatment and following ventricular fibrosis during the chronic stage of hypertension. FEBS Lett 406, 301 – 304 (1997)

Role of ACE in Cardiomyopathy

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