Effects of long-term enalapril and losartan therapy of heart failure on cardiovascular aldosterone

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ABSTRACT. Plasma aldosterone escape is found during long-term ACE inhibitor therapy of chronic heart failure. Evidence for aldosterone production in cardiovascular tissues raised the question of whether aldosterone escape occurs or not in these tissues. Rats with infarction-induced chronic heart failure were treated with enalapril (20 mg/kg/d) and losartan (15 mg/kg/d) for 20 weeks. Untreated chronic heart failure and sham-operated rats were used as positive and normal controls, respectively. *Ex vivo* mesenteric artery and heart perfusion, high performance liquid chromatography, and RIA for aldosterone were performed. Chronic heart failure due to myocardial infarction was associated with tissue-specific activation of cardiovascular aldosterone synthesis. In the mesenteric artery, enalapril significantly inhibited aldosterone production compared to untreated, chronic heart failure rats, and losartan lowered aldosterone production to that of sham rats. In myocardium, enalapril failed to significantly inhibit aldosterone production, and losartan significantly inhibited aldosterone production compared to untreated, chronic heart failure rats. These results provide the first evidence that long-term ACE inhibition therapy induces aldosterone escape in myocardium but not in mesenteric artery of chronic heart failure. The angiotensin II subtype 1 receptor blocker losartan tranquilized aldosterone levels in the cardiovascular tissues of chronic heart failure rats.

(J. Endocrinol. Invest. *25:* 463-468, 2002)

INTRODUCTION

ACE inhibitors (ACEIs) are designed to block the renin-angiotensin system (RAS) and can represent an effective therapeutic approach to hypertension, congestive heart failure, and myocardial infarction (MI). In these cases, ACEIs produce an acute decrease in plasma aldosterone levels. However, long-term (>3 months) ACE inhibition is associated with so-called aldosterone escape (1-3). The Randomized Aldactone Evaluation Study (RALES) has shown that 25 mg of spironolactone added to ACE inhibition treatment is safe and reduces all-cause mortality by 30% in patients with severe heart failure due to systolic left ventricular dysfunction (4). Blockade of aldosterone in these patients may be necessary to overcome aldosterone escape during chronic ACE inhibition.

Angiotensin II (Ang II) receptor blockers were developed as agents that would better block the RAS and, thus, decrease the adverse effects seen with ACEIs. Several reports indicate that treatment with losartan, a kind of Ang II subtype 1 receptor (AT_1) blocker, lowered the level of plasma aldosterone, but the longest duration of observation has only been 3 months (5-7).

Until recently, it was assumed that aldosterone was derived solely from adrenal glands *via* the circulation; however, there is now convincing evidence, including our own, that cells of the heart and vasculature express CYP11B1 and CYP11B2 genes, which are responsible for 11β-hydroxylase and aldosterone synthase, respectively (8, 9). We undertook this study to clarify the hypothetical possibility of aldosterone escape in cardiovascular tissues during long-term ACE inhibition.

METHODS

Myocardial infarction model

Male Wistar rats (from the Chinese Academy of Medical Sciences Animal Center) were used in this study. Weighing 180-220 g at 12 weeks of age, they were kept in separate cages under standard conditions with respect to food, humidity and light peri-

Key-words: Aldosterone, myocardial infarction, chronic heart failure, ACEI, AT1 receptor blocker.

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Accepted January 31, 2002.

odicity. Left ventricular infarction was produced by ligation of the left anterior descending coronary artery as previously described (10). Sham-operated animals were treated similarly, except that the ligature around the coronary artery was not tied. Four weeks after the operation, an electrocardiogram was recorded under ether anesthesia in all surviving animals, and those (no.=15) with no obvious electrical signs of MI were discarded. The remaining rats with MI (no.=35) were randomly divided into 3 groups. Each group received one of the following treatments in their drinking water for 20 weeks: 1) enalapril (no.=10, 20 mg/kg/d, MSD, American), 2) losartan (no.=10, 15 mg/kg/d, MSD, American), 3) untreated MI (no.=15), and 4) sham-operated animals (no.=8) served as control groups.

Hemodynamic studies

After 20 weeks of treatment, the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg bw). Aortic and left ventricular pressures were recorded by inserting a catheter into the right carotid artery. The catheter was advanced into the aorta and then into the left ventricle. The fluid-filled catheter was connected to a pressure transducer (P23 ID, Gould Inc, U.S.A.). While the rat was breathing spontaneously, pressures were recorded on a physiological recorder (SJ-42, China). Carotid pressures were recorded as the mean values determined by electronic averaging, and left ventricular systolic pressure and left ventricular end-diastolic pressure (LVEDP) were obtained by averaging their respective values over 10 beats. Heart rate (HR) was determined from the tracing of artery pressure (11).

Ex vivo *mesenteric artery and heart perfusion*

After blood sampling, mesenteric arterial perfusion *ex vivo* was performed as we previously reported (12). Briefly, under pentobarbital anesthesia, the mesenteric arteries of the rats were excised using the Miyamori method (13). The isolated arteries were perfused with Krebs-Ringer solution (pH 7.4, at 37 C, and oxygenated with a 95% O_2 +5% CO_2 gas mixture) at a constant flow rate of 4 ml/min by an automated pump. Promptly, the hearts were isolated, cannulated from the aorta, and perfused with a modified Krebs-Henseleit solution using a Langendorff apparatus according to Curtis' method (14, 15). The perfusion pressure was constantly monitored by polygraph. Pre-perfusion (30 min) was performed for wash out, and then 240 ml of perfusate (60 min) was collected and extracted on a Sep-Pak C18 cartridge (Waters Associates, Milford, U.S.A.), which was pre-washed with 5 ml of methanol and 10 ml of distilled water.

High performance liquid chromatography for aldosterone

After washing the cartridge with 10 ml of distilled water, its contents were extracted with 5 ml of methanol and then freeze-dried. The dried eluate was dissolved in 30 µl of methanol, of which 10 µl was used for high performance liquid chromatography (HPLC), 10 µl for Ang II RIA.

The Sep-Pak C18 cartridge extract was chromatographed by reverse phase HPLC (Shimadzu, Japan) on an octadecyl silicea column with methanol (40%) and water (60%); the mobile phase had a flow rate of 1.5 ml/min for 60 min. The retention time of authentic aldosterone (Sigma) was confirmed to be 30 min at 280 mm on an SPD–10AV ultraviolet spectrophotometer. Each 1.5 ml fraction corresponding to authentic aldosterone was collected, freeze-dried, and assessed by RIA (kits from Bei Fang Company, Beijing) for aldosterone.

Determination of infarction size

Transverse myocardial sections (5 µm thick) were stained with Sirius red stain. Infarction size was determined by planimetric measurement with digital image analysis software (QU500, Germany), and the ratio of scare length to total length of each slice was measured and expressed as a percentage, as previously described (10).

Statistical analysis

Data are expressed as the mean±SE. One-way ANOVA was used to assess each parameter in the experimental groups. Student-Newman-Keuls comparisons of *post hoc* tests were then performed to identify which group differences accounted for the overall significant ANOVA. A value of $p<0.05$ was considered significant.

RESULTS

Infarction size, cardiac hypertrophy, and physiological data

Fifteen animals died during the experimental period. The numbers of remaining animals in each group were as follows: enalapril (no.=7), losartan (no.=6), untreated MI (no.=7), and sham-operated (no.=8). Mean infarction size and bw did not differ significantly between the experimental groups (Table 1). MI induced cardiac hypertrophy, as demonstrated by the increased ratio of heart weight to bw by 1.2

Table 1 - *Infarct size and anatomic and physiological parameters.*

	MI (%)	BW (g)	HW (mg)	HW/BW (mg/g)	MAP (mmHg)	LVSP (mmHg)	LVDEP (mmHg)	HR (bpm)
Sham (no.=8)	-	419±12	1029±27	2.47±0.06	121±3	134±4	3±1	374±10
MI (no.=7)	35±2	441±8	1311±34**	2.91±0.04**	111±4	127±5	30±2**	389±19
MI+Ena (no.=7)	36±2	414±12	1025±30	2.48±0.09°°	94±4**°°	108±3**°°	22±2**°°	408±4
MI+Los (no.=6)	38±4	406±15	1048±32	2.60±0.14°	97±3**°°	108±2**°°	23±1**°°	412±12

Ena: enalapril; HR: heart rate; HW: heart weight; Los: losartan; LVEDP: left ventricular end-diastolic pressure; LVSP: left ventricular systolic pressure; MAP: mean artery pressure; MI: myocardial infarction; Sham: sham–operated rat. *$p<0.05$ *vs* Sham-operated rat; **$p<0.01$ *vs* Sham-operated rat; °$p<0.05$ *vs* MI; °°$p<0.01$ *vs* MI; $^{+}p<0.05$ *vs* Ena; $^{++}p<0.01$ *vs* Ena.

fold. All treatments prevented such cardiac hypertrophy and decreased the mean arterial pressure. Heart failure was confirmed by the increase of LVEDP, which was decreased in both treatment groups compared with that of the untreated MI group, but higher than that of the sham group.

Levels of Ang II and aldosterone in plasma

Figure 1 shows that Ang II and aldosterone levels increased in the MI groups. Losartan significantly increased plasma Ang II compared to that of untreated MI rats.

Levels of Ang II and aldosterone in mesenteric artery perfusion

Figure 2 indicates that MI was associated with a 1.5-fold increase in Ang II content. Both treatments significantly inhibited the increase, and losartan lowered it to the sham-operated level. The level of aldosterone in the mesenteric artery of untreated MI rats was significantly higher than that of the other groups. Compared to sham rats, losartan lowered aldosterone to the sham level (p=0.135). The level of aldosterone in the mesenteric artery of enalapril is higher than that of sham-operated rats and losartan-treated rats.

Levels of Ang II and aldosterone in heart perfusion

Figure 3 illustrates that the level of Ang II in untreated MI rats was higher than that of sham-operated (p<0.01) and losartan-treated groups (p<0.05), but not significantly different from that of enalapril-treated. The level of aldosterone in the heart perfusion of untreated MI rats was higher than that of losartan-treated rats, but not significantly different from that of the enalapril-treated group (p=0.522). Compared to that of sham-operated rats, neither enalapril- nor losartan-treated groups lowered aldosterone to the sham-operated level (p<0.01). The level of aldosterone in the heart of enalapril-treated rats is higher than that of losartan-treated rats.

DISCUSSION

Aldosterone production was proved in vascular endothelial and smooth muscle cells, and in both homogenate and perfusate of isolated rat hearts (8, 9). Aldosterone receptors exist in cardiac myocytes, endocardial endothelial cells, cardiac fibroblasts, and vascular endothelial and smooth muscle cells. To rule out the possibility of release from the receptors, Takeda *et al.* (8, 15) carefully proved that it can reflect *de-novo* synthesis of aldosterone in cardiovascular

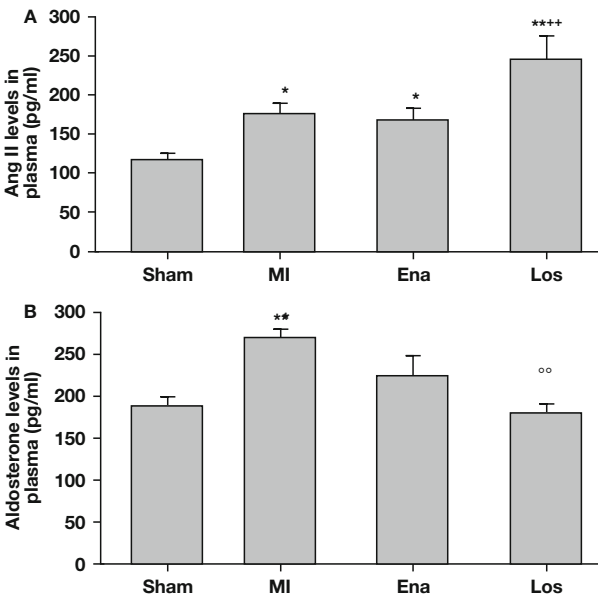

Fig. 1 - *Levels of angiotensin II (A) and aldosterone (B) in plasma. Values are the mean±SE (Sham no.=8; MI no.=7; MI+Ena no.=7; MI+Los no.=6; respectively).* *p<0.05 vs *sham-operated rat;* **p<0.01 vs *sham-operated rat;* °p<0.05 vs *MI;* °°p<0.01 vs *MI;* +p<0.05 vs *enalapril;* ++p<0.01 vs *enalapril. Ang II: angiotensin II; Ena: enalapril; Los: losartan; MI: myocardial infarction; Sham: sham–operated rat.*

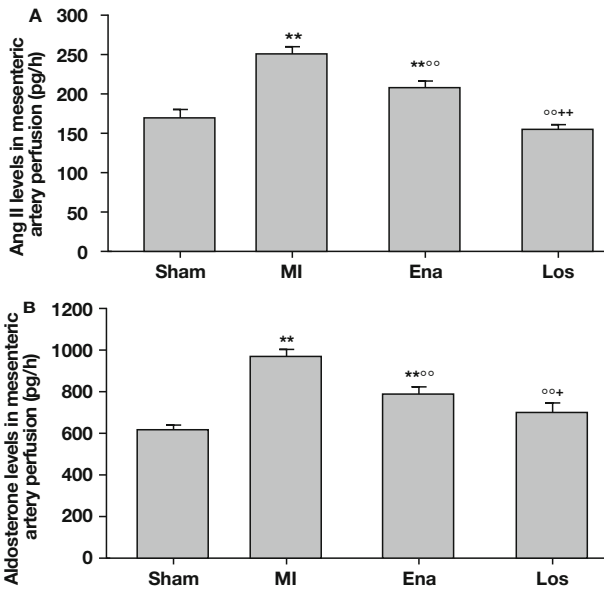

Fig. 2 - *Levels of angiotensin II (A) and aldosterone (B) in mesenteric artery perfusion. Values are the mean±SE (Sham no.=8; MI no.=7; MI+Ena no.=7; MI+Los no.=6; respectively).* *p<0.05 vs *sham-operated rat;* **p<0.01 vs *sham-operated rat;* °p<0.05 vs *MI;* °°p<0.01 vs *MI;* +p<0.05 vs *enalapril;* ++p<0.01 *vs enalapril. Ang II: angiotensin II; Ena: enalapril; Los: losartan; MI: myocardial infarction; Sham: sham–operated rat.*

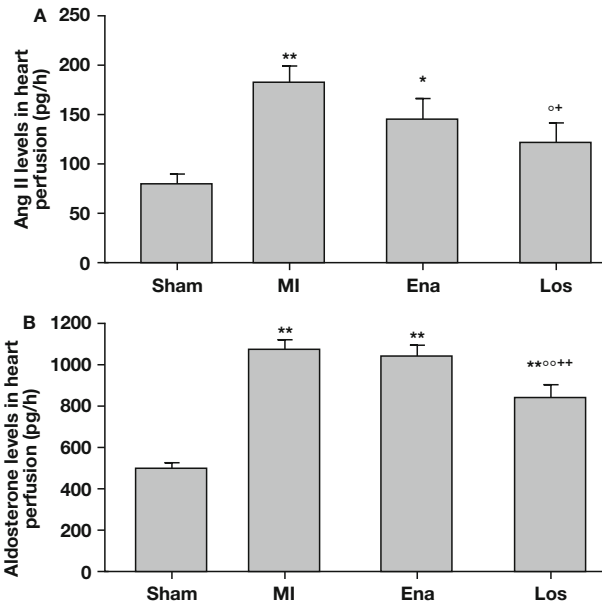

Fig. 3 - *Levels of angiotensin II (A) and aldosterone (B) in heart perfusion. Values are the mean±SE (Sham no.=8; MI no.=7; MI+Ena no.=7; MI+Los no.=6; respectively).* *p<*0.05* vs *sham-operated rat;* **p<*0.01* vs *sham-operated rat;* °p<*0.05* vs *MI;* °°p<*0.01* vs *MI;* +p<*0.05* vs *enalapril;* ++p<*0.01* vs *enalapril. Ang II: angiotensin II; Ena: enalapril; Los: losartan; MI: myocardial infarction; Sham: sham–operated rat.*

tissues by pre-wash out and adrenalectomy in their *ex vivo* cardiovascular perfusion experiments. Here, according to their methods, we proved that aldosterone in *ex vivo* cardiovascular perfusate was increased 24 weeks after MI induced heart failure, which was identified by the increase of LVEDP. Indeed, increased cardiac expression of angiotensinogen, ACE and AT_1 receptor proteins, ACE activity and Ang II content has been previously described in infarcted hearts (11, 16). The harmful effects of aldosterone in heart failure include magnesium loss, blockade of norepinephrine uptake by the myocardium, sympathetic activation, parasympathetic inhibition, myocardial ischemia, and myocardial fibrosis (4). Takeda *et al*. (17) observed that cerebral hemorrhage was significantly higher in the patients with aldosterone-producing adenoma when compared to the essential hypertension group. Pozzan *et al*. (18) believed that cardiomegaly was related to both hyperaldosteronism and hypertension.

Many clinicians have assumed that ACEIs would block both Ang II and aldosterone. However, there is data to suggest that plasma aldosterone may "escape" blockade and that Ang II plasma levels may rebound despite the use of an ACEI in patients with heart failure, hypertension, and acute MI (1-3, 19). One potential strategy to further improve morbidity and mortality in these patients may be blockade of aldosterone. It has been demonstrated that aldosterone can cause cardiac hypertrophy and fibrosis independent of the blood pressure (20). We do not know yet the effects of long-term ACEI and AT_1 antagonists on aldosterone produced in cardiovascular tissues. The present study indicated that long-term ACEI enalapril produced aldosterone escape in perfusate of myocardium but not in the mesenteric artery. As we know, several serine proteases, such as tonin and cathepsin G, have been shown to hydrolyze Ang II precursors. Recently, an aspartyl protease with cathepsin D–like properties was shown to convert angiotensinogen to Ang I in adult rat myofibroblasts isolated from ventricular scar tissue. The α-chymases include human, dog and rat chymase-3, which convert Ang I to Ang II by cleaving the Phe8-His9 bond in Ang I. The Ang II generated is not further degraded, because the Tyr4-Ile5 bond is resistant to cleavage by α-chymases (21). The aldosterone escape and Ang II rebound might be related to Ang II-forming pathways within tissues. Akasu *et al*. (22) examined tissue Ang II–forming activities and identified the responsible enzyme in several organs including heart and aorta in various species (human, hamster, rat, rabbit, dog, pig, and marmoset). In the heart, the highest total Ang II–forming activity was observed in humans, and a chymase-like enzyme was dominant in all of the species except rabbit and pig. Aorta exhibited a relatively high total Ang II–forming activity, with a predominance of chymase-like activity in all of the species except rabbit and pig, in which ACE was dominant. Their results indicate that there were remarkable differences in Ang II–forming pathways among the species and organs examined.

Activation on AT_1 leads to vasoconstriction and stimulation of the release of catecholamines, aldosterone, and antidiuretic hormone (23, 24). Here, we identified that an AT_1 blocker did decrease both plasma and cardiovascular tissue aldosterone levels in chronic heart failure during long-term therapy, although it increased the plasma Ang II levels. On the other hand, we are having difficulty interpreting the fact that Ang II levels were not increased in the mesenteric artery and cardiac effluent in the losartan group, which is in agreement with what Silvestre *et al*. (11) observed in their myocardial infarction rat study. Stimulation of the AT_2 receptor by means of Ang II is assumed to counteract vascular/myocardial remodeling and may possibly induce vasodilation (25). A combination of ACEI and AT_1 receptor antagonist may have an additive cardioprotective effect in heart failure, because these 2 agents could

have other mechanisms of action besides interrupting the RAS. For example, ACEIs prevent degradation of bradykinin and during treatment with AT_1 antagonists; increased angiotensin II could activate AT_2 receptors, with an antitrophic effect. The combination therapy with ACEI and AT_1 receptor antagonists in rats exerts more beneficial effects on cardiovascular diseases than monotherapy (26), but we did not find the report of combination therapy on cardiovascular aldosterone. The Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) is the first study to compare an AT_1 antagonist alone (candesartan) with the combination of an AT_1 antagonist plus an ACEI (enalapril) and an ACEI alone in chronic heart failure patients. The study found that candesartan had an effect similar to that of enalapril on a 6-minute walk distance, ventricular function, New York Heart Association functional class, and quality of life. Although there was an increase in Ang II with candesartan use, the impact on plasma aldosterone was similar to that of enalapril. Candesartan plus enalapril was most effective in prevention of left ventricular dilatation and suppression of plasma aldosterone, which decreased with combination therapy at 17 but not 43 weeks compared with candesartan or enalapril (27). It should be mentioned that the dosages of enalapril (20 mg/kg/day) (28) and losartan (15 mg/kg/day) (29) used in the experiment were much higher than those used in clinic.

In conclusion, we demonstrated that chronic heart failure due to MI was associated with tissue-specific activation of cardiovascular aldosterone synthesis. Long-term ACE inhibition induced aldosterone escape in myocardium but not in the mesenteric artery, while the AT_1 receptor blocker losartan did not induce escape in vessels and heart compared to those of sham rats, and untreated chronic heart failure rats, respectively.

ACKNOWLEDGEMENTS

This study was supported by the National Nature and Science Foundation of China (39870812 and 39870331) and the Nature and Science Foundation of Guangdong Province (980235). The Authors wish to thank Johan Baselius for checking the manuscript.

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