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Original Research

Effects of ramipril on cardiac gene transcription levels of angiotensin Ⅱ receptors after myocardial infarction

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KEY WORDS myocardial infarction; angiotensin Ⅱ; polymerase chain reaction; angiotensin-converting enzyme inhibitors; heart ventricle; heart septum; ramipril

ABSTRACT

AIM: To study the early changes of cardiac angiotensin (Ang) Ⅱ receptor gene transcription after myocardial infarction (MI) in rats chronically treated with the angiotensin-converting enzyme (ACE) inhibitor ramipril. **METHODS**: MI was induced by left anterior descending coronary artery ligation in rats and sham-operated rats were used as control. Rats were treated daily with ramipril (1 mg·kg^{-1}) or water, initiated 1 wk before surgery. Quantitative RT-PCR was applied to determine the Ang Ⅱ receptors AT1, AT2 receptor gene mRNA levels in the non-infarcted myocardium. **RESULTS**: AT1 and AT2 mRNA levels increased time point-dependently in the cardiac septum after MI reaching a peak on d 1. There was no significant difference of the myocardial AT1 and AT2 receptor mRNA levels between the ramipril-treated and water-treated rats after MI. **CONCLUSION**: The AT1 and AT2 receptor gene transcription in the non-infarcted myocardium was associated with the process of cardiac remodeling after MI but not affected by ACE inhibition.

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INTRODUCTION

Angiotensin (Ang) Ⅱ has been demonstrated to be one of the important mediators of the process of cardiac remodeling[1]. Angiotensin-converting enzyme inhibitors (ACEI) have been used to treat hypertension and more recently as therapeutics for congestive heart failure following myocardial infarction (MI). When ACEI were used later than 24 h following an acute MI, significant cardioprotective effects were observed[2]. However, when the treatment was initiated earlier, ie, within 24 h following MI, an ACEI led to a strong trend towards higher mortality in the ACEI treated group[3]. Therefore, a hypothesis may arise that the cardiac effects of the ACEI may be time point-dependent. Moreover, blockade of angiotensin Ⅱ synthesis and bradykinin potentiation have been demonstrated to be the mechanism of actions of the ACEI[2]. However, Ang Ⅱ may exerts beneficial effects on cardiac function in the early remodeling phase to compensate for the decrease of cardiac output by its positive inotropic and chronotropic effects. On the other hand, Ang Ⅱ may show long term effects of stimulating cell growth resulting in hypertrophy of the cardiac cells. Therefore, the cardiac actions of Ang Ⅱ may be time point-dependent and cardiac Ang receptors may be up- or down-regulated to serve as an enhancer or depressor of the Ang Ⅱ actions. In the present study, we investigated cardiac gene transcription levels at a serial of time points following MI. To further clarify the mechanisms of regulation of the Ang receptors, we investigated the effects of an ACEI ramipril on these parameters as well.

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MATERIALS AND METHODS

MI and treatment protocols Wistar rats (♂, Grade Ⅱ, № 02-22-12, weighing 250 g – 300 g) were housed under climate-controlled conditions with a 12-h light/dark cycle and were provided with standard rat chow and tap water. MI was induced by LAD ligation[4]. Briefly, under ether anesthesia, rats were incubated, artificially respirated, and connected to ECG recorder. The thorax was opened and the third and forth ribs were cut. The LAD was ligated with a sterile 6-0 atraumatic suture (Ethibond, Ethicon, Norderstedt, Germany) at 2 – 3 mm from its origin through a microscope. In sham-operated rat, the ligation was put beside the coronary artery. At 15 min, 30 min, 3 h, d 1 and d 3 after MI or sham operation the rats were decapitated, left heart ventricle including septum was immediately put in liquid nitrogen and stored at – 80 ℃. Half of the rats were pretreated with either ramipril (1 mg·kg^{-1}·d^{-1}, ig) or water. Treatments were initiated 7 d before MI or sham operation and continued until sacrifice.

RNA isolation and reverse transcription polymerase chain reaction (RT-PCR) The intraventricular septum was homogenized in a 10-fold volume (wt/vol) of ice-cold Trizol reagent with Polytron (Janke & Kunkel, Germany) and total RNA was extracted according to manufacturer's instructions (Gibco, BRL). Following centrifugation (11 430 × g, 4 ℃, 20 min) of Trizol containing tissue homogenate, the aqueous phase was transferred to a fresh tube. Total RNA was precipitated through two consecutive ethanol precipitations separated by an additional phenol/chloroform extraction step. Finally, total RNA yield was quantified by UV spectrophotometer at 260 nm. RNA was then stored in ethanol at – 80 ℃. Total RNA 5 μg from the intraventricular septum was reverse-transcribed into first-strand complementary DNA (fs cDNA) using oligo-dT primers (Gibco, BRL). The fs cDNA was amplified by polymerase chain reaction (PCR). The PCR was carried out in a total volume of 100 μL containing Tris-HCl 20 mmol, KCl 50 mmol, $MgCl_2$ 1.5 mmol, dNTP 0.2 mmol, 0.6 mmol of each primer, and 2.5 units of Taq DNA polymerase (Gibco, BRL). The expression of the house keeping gene, β-actin mRNA, was considered as an internal standard. The following primer sequences were used: sense 5′-CAAGACGCAGGCTTTTTGGCC-3′, antisense 5′-ATACCGCTATGGAGTACCGCTGGC-3′ (804 bp) for AT1 and sense 5′-TTGCTGCCACCAGCAGAAAC-3′, antisense 5′-GTGTGGGCCTCCAAACCATTGCTA-3′ (1179 bp) for AT2 receptor cDNA, and sense 5′-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3′, antisence 5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′ (870 bp) for β- actin. PCR was run 30 cycles for β-actin, AT1 and AT2 receptors cDNA in a Perkin Elmer 9600 thermocycler. Three-step PCR of denaturing, annealing, and extension reactions proceeded at 94 ℃ for 1 min, at 60 ℃ (β-actin) or 57 ℃ (AT1-) or 53 ℃ (AT2-) for 1 min, and at 72 ℃ for 1 min, respectively.

Quantification of PCR products PCR products 15 μL were diluted with Tris/edetic acid (TE) buffer (Tris 10 mmol·L^{-1}, edetic acid 1 mmol·L^{-1}, pH 7.40) 35 mL and submitted to ionic exchange chromatography on a MiniQ-column (Pharmacia) using the SMART microchromatography apparatus (Pharmacia). For separation of PCR products, a linear gradient (0 – 100 %; 10 min) of NaCl in TE-buffer was developed. PCR products of β-actin, AT1- and AT2-receptor were quantified by peak integration (OD 260 nm) using a concentration standard for calibration. β-actin mRNA was used as internal standard since mRNA levels of this housekeeping gene did not change more than 15 % in all preparations. Ratios of the corresponding peak areas (AT1/β-actin and AT2/β-actin) were calculated for each sample and used for quantitative calculations and comparisons[5].

Statistical analysis Data were represented as $\bar{x} \pm s$. Differences between individual groups were analyzed by Student's t-test.

RESULTS

The gene transcription pattern of cardiac AT1 and AT2 after MI The mRNA levels of AT1 receptor began to markedly rise at 30 min reaching its peak at 24 h after MI. Then, it went down on d 3 after MI, but was still higher than that of sham-operated rats (Fig 1A, Tab 1).

A weak AT2 receptor gene expression was detected in the cardiac septum of control rats. Interestingly, a transient enhancement of AT2 receptor gene

transcription after an acute MI in a similar pattern as that of AT1 receptor was observed; started to increase 30 min after MI and was still high 3 d after MI (Fig 1B, Tab 1).

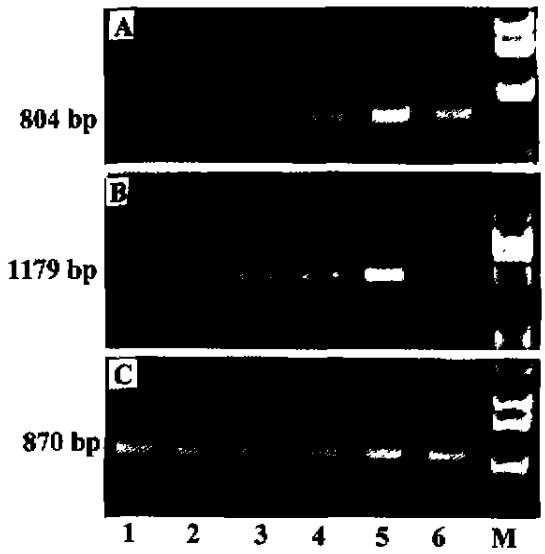

Fig 1. Gel electrophoresis in 1 % agarose gel of the RT-PCR fragments of cardiac AT1 receptor mRNA (A), AT2 receptor mRNA (B), and β-actin (C). M: molecular weight marker. Lane 1-6: before operation (0 min), 15 min, 30 min, 3 h, 1 d, and 3 d after operation, respectively.

Tab 1. Cardiac Ang receptor mRNA levels at a serial of time point after MI. $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* sham.

Time	AT1		AT2	
	Sham	MI	Sham	MI
0 min	1.00 ± 0.21 (n = 5)		1.00 ± 0.12 (n = 5)	
15 min	1.1 ± 0.3 (n = 6)	1.20 ± 0.16 (n = 6)	1.20 ± 0.23 (n = 6)	1.20 ± 0.67 (n = 6)
30 min	1.00 ± 0.26 (n = 6)	2.30 ± 0.19^c (n = 6)	1.40 ± 0.25 (n = 6)	5.6 ± 1.2^b (n = 6)
3 h	2.1 ± 0.5 (n = 6)	2.4 ± 0.6 (n = 7)	2.1 ± 0.5 (n = 6)	7.4 ± 1.8^b (n = 7)
1 d	1.5 ± 0.4 (n = 7)	12.6 ± 2.5^c (n = 7)	3.5 ± 0.7 (n = 7)	17.2 ± 3.8^c (n = 7)
3 d	2.1 ± 0.7 (n = 8)	5.0 ± 1.0^b (n = 7)	2.1 ± 0.5 (n = 8)	2.6 ± 0.6 (n = 7)

Effects of the ACE inhibitor ramipril on AT1 and AT2 receptor gene transcription In ramipril-treated rats, cardiac AT1 and AT2 receptor gene transcription showed a pattern similar to that of rats treated with water. In sham-operated rats, ramipril showed no significant effect on cardiac AT1 and AT2 receptor mRNA levels (Tab 2).

Tab 2. Cardiac Ang receptor mRNA levels at a serial of time point in ramipril-treated rats after MI. $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* sham.

Time	AT1		AT2	
	Sham	MI	Sham	MI
0 min	1.00 ± 0.15 (n = 5)		1.00 ± 0.22 (n = 5)	
15 min	0.80 ± 0.20 (n = 7)	1.20 ± 0.22 (n = 6)	1.3 ± 0.3 (n = 7)	1.3 ± 0.6 (n = 6)
30 min	1.0 ± 0.3 (n = 6)	1.4 ± 0.3 (n = 6)	1.40 ± 0.22 (n = 6)	4.8 ± 1.5^c (n = 6)
3 h	2.9 ± 0.5 (n = 6)	6.0 ± 1.3 (n = 7)	2.3 ± 0.4 (n = 6)	5.6 ± 1.6 (n = 7)
1 d	2.4 ± 0.7 (n = 8)	10.6 ± 1.9^c (n = 7)	3.8 ± 1.7 (n = 8)	15.3 ± 3.5^c (n = 7)
3 d	2.9 ± 0.8 (n = 7)	5.5 ± 1.3 (n = 7)	2.3 ± 0.6 (n = 7)	4.2 ± 0.6 (n = 7)

DISCUSSION

In the present study, we showed for the first time the complete gene transcription pattern of AT1 and AT2 receptors in ACEI-treated rats at a serial of points during the early phase of cardiac remodeling following an acute MI. The transient enhancement of AT1 and AT2 receptor gene transcription which we observed may correspond to the early phase of cardiac remodeling during which myocardial hypertrophy rapidly occurred in survived myocardium[6]. In other words, the gene transcription pattern of the AT1 and AT2 receptors seems to be in parallel with the extent of cardiac remodeling which is closely associated with the degree of the mechanical load imposed on viable myocardium. The current results agree with Reiss and coworkers who described an significant enhancement of Ang Ⅱ receptor mRNA levels in rat ventricle 2-3 d after LAD ligation[7]. Our findings strongly suggest that the time point-dependent increase of AT1 and AT2 receptor may serve as an time point-dependent enhancer of the actions

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of Ang Ⅱ on the viable myocardium and play a role during the early remodeling process following MI. Although we did not measure receptor density in the present study, elevation of AT1 and AT2 receptor mRNA levels has recently been demonstrated to be in parallel with the increase of AT1 and AT2 receptor density after MI in a rat model of coronary ligation[7,8].

In the current study, both the AT1 and AT2 receptor mRNA levels in the non-infarcted myocardium increased after MI in a similar pattern. The data may be interpreted as that the gene transcription level of both AT1 and AT2 receptor may be regulated by the same mechanism. Interestingly, chronic blockade of the Ang Ⅱ synthesis by ramipril had no influence either on the AT1 or the AT2 receptor gene transcription pattern in the viable myocardium in the MI as well as in sham-operated rats in the present study. These findings suggest that Ang Ⅱ reduction is not a regulator of the AT1 and AT2 receptor gene transcription under our current experimental conditions.

On the other hand, intracardiac pressure may be speculated to be another mediator for the AT1 and AT2 receptor gene expression. However, this hypothesis was not verified in our current observation. In our recent study using exactly the same experimental protocol as the present study, we observed that ramipril significantly changed the hemodynamic parameters such as mean blood pressure, left ventricular end-diastolic pressure and left ventricular dp/dt_{max}, but showed no influence on the up-regulated gene transcription pattern of the AT1 and AT2 receptors which was similar to that in the present study.

We assume that the changes of the gene transcription pattern of AT1 and AT2 receptors were associated with the early remodeling process of the survived myocardium after an acute MI. Ramipril-induced reduction of Ang Ⅱ and changes of myocardial workload may not be strong enough to block the association between the changes of the Ang receptor gene transcription pattern and the early remodeling process which was initiated by a big left ventricular infarction.

Our findings suggest that the time point-dependent increase of Ang receptor gene transcription may serve as an enhancer of Ang Ⅱ and play a role during the early remodeling phase of the survived myocardium following MI.

In conclusion, the time point-dependent pattern of up-regulation of cardiac AT1 and AT2 receptor gene transcription levels is associated with the early cardiac remodeling process following an acute MI and is independent of ACE inhibition.

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雷米普利对心肌梗死后心脏血管紧张素受体基因转录的影响

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关键词　心肌梗死；血管紧张素Ⅱ；多聚酶链反应；血管紧张素转换酶抑制剂类；心室；心间隔；雷米普利

目的：在慢性给予血管紧张素转换酶抑制剂雷米普利后的大鼠中观察心肌梗死(MI)后早期心肌组织中血管紧张素Ⅱ（Ang Ⅱ）受体基因转录的改变．**方法**：通过冠状动脉结扎建立大鼠 MI 模型，手术前 1 周即开始给予雷米普利或水，术后在存活大鼠中继续治疗至处死大鼠；用定量 RT－PCR 法检测大鼠心间隔组织的 Ang Ⅱ受体 mRNA 水平．**结果**：大鼠 MI 后心间隔组织中 AT1 和 AT2 受体 mRNA 水平升高，在 1 d 时达到高峰．给予雷米普利的大鼠 MI 后心间隔组织中 Ang Ⅱ受体 mRNA 水平与给予水的大鼠相比无显著差别．**结论**：非梗死区心肌组织中 Ang Ⅱ受体基因转录的特征性改变可能与 MI 后心肌重构过程有关而与 ACE 抑制无关．

（责任编辑　周向华）