

Effect of losartan and captopril on expression of cardiac angiotensin II AT₁ receptor mRNA in rats following myocardial infarction¹

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KEY WORDS myocardial infarction; gene expression; angiotensin-converting enzyme inhibitors; angiotensin receptors; messenger RNA; captopril; losartan

AIM: To study the effects of losartan (Los) and captopril (Cap) treatment on expression of cardiac angiotensin II (Ang) AT₁ receptor mRNA in rats following myocardial infarction (MI). **METHODS:** Twenty-four rats with MI after coronary ligation for 7 d were randomly divided into 4 groups: A) Cap in drinking water, *ad lib* (2 g·L⁻¹), B) ig Los 10 mg·kg⁻¹·d⁻¹, C) ig Los 30 mg·kg⁻¹·d⁻¹, and D) placebo for 6 wk. Sham-ligation rats (group E) served as controls. The levels of cardiac Ang AT₁ receptor mRNA expression in each group (*n* = 6) were examined by Dot blot using digoxigenin-labeled cDNA probes. **RESULTS:** Comparing with reflected peak areas of hybridization positive signals in group D (2640 ± 201 μm²), the expression of the cardiac Ang AT₁ receptor mRNA was much lower in the 3 treated groups (group A 1360 ± 134 μm², group B 1430 ± 244 μm², group C 1310 ± 95 μm²) (*P* < 0.01). But no difference was found between the 3 treated groups and sham-ligation group (1230 ± 233 μm²) (*P* > 0.05). **CONCLUSION:** Los and Cap attenuated the increase of cardiac Ang AT₁ receptor mRNA expression in rats following MI.

Ventricular remodeling following myocardial infarction (MI) contributes to the development of progressive clinical heart failure^[1]. The cardiac tissue type renin angiotensin system was activated after MI and heart failure^[2]. Ventricular remodeling could be attenuated by angiotensin converting enzyme inhibitors

(ACEI). ACEI and angiotensin II (Ang) receptor antagonist provided insights into the mechanism of ventricular remodeling after MI^[3]. At least 2 main Ang receptor subtypes, AT₁ and AT₂, were identified. The AT₁ receptor mediates virtually all of the effects of Ang in myocytes^[4]. Ang AT₁ receptor antagonist, TCV-116, could reduce the increase of Ang AT₁ receptor mRNA expression after MI in rats; however, in this study TCV-116 was maintained for only 7 d^[5]. There was no report on the effects of captopril (Cap), an ACEI, on Ang receptor regulation after MI in animal models and in humans^[6]. The present study was to assess the long term effects of Cap and Ang AT₁ receptor antagonist, losartan (Los), on expression of cardiac Ang AT₁ receptor mRNA after myocardial infarction in rats.

MATERIALS AND METHODS

Experimental preparation Sprague-Dawley ♂ rats, Class II, provided by Experimental Animal Center of Shanghai Medical University, weighing 240 ± s 10 g were anesthetized with ip pentobarbital 40 mg·kg⁻¹. Trachea was intubated and the rat was ventilated with a volume-cycled ventilator. After thoracotomy, the proximal left anterior descending coronary artery was ligated^[7]. All rats were screened. Large MI was conformed on d 7 after surgery by echocardiography and surface electrocardiogram recording^[8]. Sham-ligation rats served as controls. The rats were kept in temperature-controlled room (20 °C). Seven days after surgery, 24 rats with MI were randomly assigned to 4 groups and treated with Cap (2 g·L⁻¹ in drinking water, *ad lib*^[9,10], Sino-American Shanghai Squibb Pharmaceutical, Ltd, group A; *n* = 6), Los (10 mg·kg⁻¹·d⁻¹, dissolved in drinking water, ig, DuPont Merck Pharmaceutical Co, USA, group B; *n* = 6); Los (30 mg·kg⁻¹·d⁻¹, dissolved in drinking water, ig, group C, *n* = 6), and placebo (drinking water, group D; *n* = 6) for 6 wk. Sham-ligation rats were set as control group (group E, *n* = 6).

Determination of Ang in plasma Blood was collected in chilled polyethylene tubes at decapitation. The concentration of Ang was determined by radioimmunoassay using Ang radioimmunological kits (Northern Institute of Immunological

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Reagents, Beijing). Each plasma sample was measured thrice.

Analysis of cardiac Ang AT₁ receptor mRNA expression

Extraction of total RNA of myocardium Total RNA was extracted by the guanidinium thiocyanated/phenol chloroform^[11]. The RNA was determined by the absorbance at $\lambda_{260\text{ nm}}$ and $\lambda_{280\text{ nm}}$ (Uvikon 931).

Preparation of Ang AT₁ receptor-cDNA probe The primer was designed from the cDNA sequence of rat Ang AT₁ receptor^[12], and synthesized by Shanghai Institute of Cell Biology, Chinese Academy of Sciences: sense from the 5' noncoding region (5'-GAGTCCTGTTCCACCCGATCACCGA-TCAC-3') and antisense from the 3' noncoding region (5'-GGATGACGCCAGCTGAATCAGCACATCC-3'). The cDNA (1046 bp) was reversely transcribed with the template of total RNA, amplified by polymerase chain reaction (PCR) technique using the reverse transcript and PCR kit (Promega, USA). Denaturing, annealing, and extension were done 30 times at 93 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s. The purified cDNA was inserted into PGEM-T vector (3 kb), and the recombinated plasmid containing Ang AT₁ receptor gene was then transformed into JM109 cells according to the instruction of PGEM-T vector system (Promega, USA). After amplification, extraction and purification, digoxigenin-labeled cDNA probe by random primer method was obtained with digoxigenin DNA-labeling Kit bought from Boehringer Mannheim Company, Germany.

Dot blot hybridization and quantitative scanning of positive signals After denaturation, the RNA was spotted onto the nylon membrane. The nylon membrane was dried at 120 °C for 30 min. After prehybridized in hybridization solution (Boehringer Mannheim) without digoxigenin-labeled cDNA probe at 50 °C for at least 1 h, hybridization was performed at 50 °C for 16 h using the same hybridization solution containing digoxigenin-labeled cDNA probe. The strength of hybridization signals were quantitated by scanning densitometry (CS-910), and expressed as the ratio of the Ang AT₁ receptor mRNA over 20 μg total RNA. Reflected peak areas of dot blot hybridization positive signals corresponded to the amounts of the Ang AT₁ receptor mRNA. Each hybridization signal was quantitated thrice by scanning densitometry.

Statistic analysis Data were expressed as $\bar{x} \pm s$. Differences between groups were determined by ANOVA.

RESULTS

Effect of Los and Cap on the levels of cardiac Ang AT₁ receptor mRNA expression

The reflected peak areas per 20 μg of total RNA in MI group without any treatment ($2640 \pm 201 \mu\text{m}^2$) were significantly higher than those in sham-ligation group ($1230 \pm 233 \mu\text{m}^2$) ($P < 0.01$). The level of the

cardiac Ang AT₁ receptor mRNA in MI of placebo group was 2.2 times that in sham-ligation group.

Compared with placebo group, the expression of the cardiac Ang AT₁ receptor mRNA was lower in the 3 treated groups ($P < 0.01$). But no difference was found between the 3 treated groups and the sham-ligation group ($P > 0.05$) (Tab 1).

Tab 1. Cardiac Ang AT₁ receptor mRNA expression and concentrations of plasma Ang. $n = 6$ rats, $\bar{x} \pm s$. ^a $P > 0.05$, ^c $P < 0.01$ vs sham-ligation group; ^b $P > 0.05$, ^f $P < 0.01$ vs placebo group.

Group	Reflected peak areas of positive signals (μm^2)	Concentration of plasma Ang ($\mu\text{g} \cdot \text{L}^{-1}$)
-	2640 ± 201^c	0.28 ± 0.10^a
Cap	1360 ± 134^{bf}	0.22 ± 0.04^{ad}
Los 10 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	1430 ± 244^{bf}	1.22 ± 0.28^{cf}
Los 30 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	1310 ± 95^{bf}	1.45 ± 0.25^{cf}
Sham-ligation	1230 ± 233^f	0.20 ± 0.06

Effect of Los and Cap on plasma Ang The plasma Ang levels in MI placebo group ($0.28 \pm 0.10 \mu\text{g} \cdot \text{L}^{-1}$) showed no significant difference from those in Cap group ($0.22 \pm 0.04 \mu\text{g} \cdot \text{L}^{-1}$) and sham-ligation group ($0.20 \pm 0.06 \mu\text{g} \cdot \text{L}^{-1}$) ($P > 0.05$) 7 wk after MI. The plasma Ang levels in Los 10 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ group ($1.22 \pm 0.28 \mu\text{g} \cdot \text{L}^{-1}$) and Los 30 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ group ($1.45 \pm 0.25 \mu\text{g} \cdot \text{L}^{-1}$) were significantly higher than that in sham-ligation group ($P < 0.01$), while there was no significant difference between Los 10 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ group and Los 30 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ group ($P > 0.05$) at 7 wk after MI.

DISCUSSION

The major findings of our study were as follows: (a) the expression of the cardiac Ang AT₁ receptor mRNA increased significantly at 7 wk after MI in rats; (b) Cap and Los were equally effective in reducing the increased expression of Ang AT₁ receptor mRNA; and (c) the concentrations of plasma Ang did not significantly increase in the non-treated infarct group or decrease in Cap-treated group, but significantly increased in Los-treated group. These results indicated that a direct action of local cardiac Ang, mediated through its increased AT₁ receptor expression,

was involved in the remodeling process after MI.

Ang AT₁ receptor regulation by pharmacological interventions was complex. Previous studies reported that treatment with ACEI caused down-regulation of the AT₁ gene expression and Dup753, an AT₁ receptor antagonist, down-regulated the AT₁ receptor in the rat adrenal, but not in the kidney, nor in the aorta^[6]. Another AT₁ receptor antagonist, TCV-116, reduced the increase of the AT₁ gene in rats with MI^[5]. Our study demonstrated that the higher dose of losartan (30 mg·kg⁻¹·d⁻¹) did not produce additional effects of reducing the expression of Ang AT₁ receptor, but increased the concentration of plasma Ang further, though insignificant statistically. These results suggest that further circulatory neurohormonal activation might limit the efficacy of high dose (30 mg·kg⁻¹·d⁻¹) of losartan^[13]. In addition, the increase of the circulating Ang levels might activate the Ang AT₂ receptor, and thus produced the functional action of the latter. There is no way to assess the effects of this functional action of Ang AT₂ receptor because the role of Ang AT₂ receptor has not yet been clarified. Thus, in order to evaluate the effects of Ang AT₁ receptor completely, Ang AT₂ receptor antagonist must be used simultaneously.

The limitation of this study was that each rat might not take the same Cap dose in drinking water every day. Thus, this method of treatment (Cap in drinking water, *ad lib*) might produce bias of result to some extent.

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氯沙坦和卡托普利对大鼠梗死后心肌血管紧张素 II AT₁ 受体 mRNA 表达的影响

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关键词 心肌梗死; 基因表达; 血管紧张素转换酶抑制剂; 血管紧张素受体; 信使 RNA; 卡托普利; 氯沙坦

目的: 探讨氯沙坦(Los)和卡托普利(Cap)对大鼠心肌梗死后血管紧张素 II (Ang) AT₁ 受体 mRNA 表达的影响。 **方法:** 24 只梗死大鼠随机分为四组并分别用 Cap (2 g·L⁻¹ 加水中饮用)、Los (10 mg·kg⁻¹·d⁻¹ 和 30 mg·kg⁻¹·d⁻¹, 灌胃) 及安慰剂治疗 6 周, 假扎鼠作对照, 用地高辛标记的 cDNA 探针, 进行点杂交反应, 检测 Ang AT₁ 受体 mRNA 表达。 **结果:** 三个药物治疗组的 Ang AT₁ 受体

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mRNA 表达水平分别与安慰剂组比较, 均显著降低 ($P < 0.01$)。三个治疗组之间的 Ang AT₁ 受体 mRNA 表达水平及分别与假扎组比较, 均无显著

差异 ($P > 0.05$)。结论: Cap 和 Los 均可逆转大鼠心肌梗死后 Ang AT₁ 受体 mRNA 表达水平的增高。

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Effects of recombinant human endothelial-derived interleukin-8 on hemorrhagic shock in rats¹

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KEY WORDS interleukin-8; endothelins; epoprostenol; 6-ketoprostaglandin F_{1α}; hemorrhagic shock

AIM: To study the effects of recombinant human endothelial-derived interleukin-8 (IL-8) on hemorrhagic shock. **METHODS:** A profound hemorrhagic shock in rats was produced by exsanguination from femoral artery with mean arterial blood pressure (MABP) maintained at 5.32 kPa for 90 min. After transfusion, IL-8 250 μg · kg⁻¹ was iv injected. Plasma endothelin-1 (ET-1) and 6-ketoprostaglandin F_{1α} (6-KPGF_{1α}) contents were determined with radioimmunoassay. **RESULTS:** After iv IL-8, the MABP in IL-8 group was elevated obviously ($P < 0.01$), the rat survival 2 h after infusion was increased ($P < 0.05$). During profound shock the plasma ET-1 levels were higher (21 ± 4 vs 8.2 ± 1.8 ng · L⁻¹, $P < 0.01$) and the plasma 6-KPGF_{1α} contents lower than those in normal rats (107 ± 12 vs 157 ± 11 ng · L⁻¹, $P < 0.01$). IL-8 remarkably reduced the plasma ET-1 levels (10 ± 4 ng · L⁻¹, $P < 0.01$) and enhanced plasma 6-KPGF_{1α} contents (368 ± 16 ng · L⁻¹, $P < 0.01$). **CONCLUSION:** IL-8 has beneficial antishock effects.

Interleukin-8 (IL-8), a cytokine produced by endothelial cells and monocytes, plays an important

role in inflammatory response and immune regulation^[1,2]. IL-8 is a potent inhibitor of neutrophil adhesion to cytokine-activated endothelial monolayers and protects these monolayers from neutrophil-mediated damage^[3]. IL-8 leads to protective effects in myocardial ischemia and reperfusion^[4] and preserves vasorelaxant responses by promoting release of endothelium-derived relaxing factor^[5]. But the effect of IL-8 on hemorrhagic shock was not studied. The purpose of the present work was to study the effects of IL-8 on hemorrhagic shock.

MATERIALS AND METHODS

IL-8 (Department of Immunology, Beijing Medical University); murine endothelin-1 (ET-1) RIA kit (Peninsula Lab, USA); murine 6-ketoprostaglandin F_{1α} (6-KPGF_{1α}) RIA kit (Institute of Basic Medicine of PLA General Hospital, Beijing).

Profound hemorrhagic shock Adult Wistar rats (♂, $n = 32$, weighing 200 - 250 g) bred by the Animal Center of Beijing Medical University were anesthetized with ip urethane 1 g · kg⁻¹. A catheter filled with 5 % sodium citrate solution was inserted into the left carotid artery and connected to a pressure transducer and a polygraph to record the mean arterial blood pressure (MABP). The femoral artery was exsanguinated and the MABP was maintained at 5.32 kPa by further bleeding or autotransfusion for 90 min.

Experimental protocol Rats were randomly divided into 4 groups. 1) IL-8 group: After MABP being kept constant at 5.32 kPa for 90 min, all of the autologous blood was reinfused, and normal saline (15 mL · kg⁻¹) was infused into femoral vein, IL-8 250 μg · kg⁻¹ was added to normal saline. The changes of the MABP for 2 h and survival rate 2 h after transfusion were observed; 2) Control group: received the same amount of vehicle. 3) Sham operation group with IL-8: After

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