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Original Research

Expression of cardiac angiotensin-converting enzyme after myocardial infarction

ZHU Yi-Chun[1], Mechthilt FALKENHAHN, Folker FRANKE[2], Rainer Maria BOHLE[2], Harald Martin STAUSS, Sergei DANILOV[3], Thomas UNGER
(*Department of Pharmacology, University of Kiel, Germany*; [2]*Department of Pathology, University of Giessen, Germany*; [3]*National Cardiology Research Center, Moscow, Russia*)

KEY WORDS peptidyl-dipeptidase A; myocardial infarction; immunohistochemistry; monoclonal antibodies; vascular endothelium; coronary vessels; macrophages

ABSTRACT

AIM: To localize cardiac angiotensin-converting enzyme (ACE) during left ventricular repair after myocardial infarction (MI). **METHODS**: Cardiac ACE was examined by immunohistochemical staining using monoclonal and polyclonal antibodies against ACE 24 h, 1 wk, 2 wk, 3 wk, and 6 wk after coronary artery ligation in rats. Immunofluorescent double staining technique was applied to distinguish the cells which express ACE. **RESULTS**: ACE staining was confined to the endothelial cells and distributed in normal cardiac tissue in a gradient pattern along the vascular tree: present around the whole circle of arterial endothelium, present in about 20 % of the capillaries, and absent in the veins. One week after MI, ACE expression was noted in the granulation tissue. Three weeks after MI, necrosis within the infarction was replaced by granulation tissue and fibrous tissue which showed strong over-expression of ACE. Six weeks after MI, the region with positive ACE staining regressed and the area with high collagen content on the endocardial side showed only weak ACE stain. Most of the ACE-positive cells in the ACE-over-expression-area were endothelial cells. A few macrophages seen in these regions were also ACE-positive. **CONCLUSION**: Cardiac ACE was over expressed during the process of tissue repair following MI, reaching a peak in 3 wk. Endothelial cells took the most part of ACE expression.

INTRODUCTION

Angiotensin Ⅱ (Ang Ⅱ) induces hyperplasia and/or hypertrophy on various cell lines in addition to its effects of vasoconstriction and sodium and water retention[1]. In certain cases, eg, during the progress of cardiac remodeling following an acute myocardial infarction (MI), cardiac angiotensin-converting enzyme (ACE) may play a role in the structural changes of the cardiac tissue.

On the other hand, numerous clinical and experimental studies have demonstrated the beneficial effects of ACE inhibitors on the failing heart following MI[1,2]. Evidence suggests that the cardioprotective action of ACE inhibitors may be partially mediated by the local action of the drug on the heart. Therefore, evidence for a local production of ACE in the heart may support this hypothesis. Previous efforts have been made to localize ACE in the heart using autoradio-

[1] Correspondence to Dr ZHU Yi-Chun, MD.
Now in *Department of Physiology, Shanghai Medical University, Shanghai 200032, China*.
Phn 86-21-6404-1900, ext 2410. Fax 86-21-6403-9987.
E-mail yczhu@shmu.edu.cn
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graphic methods[3]. Nevertheless, these methods fell short in specifying the intracellular localization of ACE.

The aim of the present study was to localize cellular distribution of ACE in both normal heart and heart with MI.

MATERIALS AND METHODS

Rat model of MI Male Wistar rats (Dr Karl Thomae GmbH, Biberach, Germany) weighing 250 – 300 g, were housed individually under controlled temperature, humidity, and light periods on a standard diet (0.6 % salt content) and drinking water. All experiments were performed in accordance with the National Animal Protection Law.

MI was induced by left anterior descending coronary artery (LAD) ligation[4]. In sham operated rats, the ligation was placed beside the coronary artery. Successful ligation of the coronary artery was verified by the occurrence of arrhythmia monitored by the ECG, and visually by the change of the color of the infarcted area. Acute mortality within 24 h was about 40 %.

Immunohistochemical study

1 Preparation of tissue samples Rats were killed 24 h, 1, 2, 3, and 6 wk after MI or sham operation (n = 2 – 3 at each time point). Hearts were stored in liquid nitrogen. Cryostat sections of fresh-frozen tissue were mounted on slides, air-dried, and stored at – 20 ℃.

2 Monoclonal antibodies/antisera Six monoclonal antibodies (MAb) (9B9, i2H5, 3G8, 5F1, 3A8, i/A8, all mouse IgG_1) against different epitopes of the N-domain of human ACE were generously supplied by Dr Serge DANILOV, Moscow, Russia, that had been tested for immunohistochemical staining of ACE in human and rat cardiac sections[5]. A polyclonal rabbit anti-mouse ACE antiserum (a gift from Dr Kenneth BERNSTEIN, Atlanta GA, USA) was used in double-staining procedures. ACE-positive cells were characterized by double-staining techniques using cell-type-specific markers: MRC OX-43 mouse IgG_1 anti-rat endothelial cell (Serotec, Wiesbaden, Germany) and KiM2R mouse IgG_1 anti-rat macrophage (Dianova, Hamburg, Germany).

3 Immunohistochemistry Immunoenzymatic detection was mainly performed by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique[6]: frozen tissue sections 5 μm were air-dried and fixed in acetone for 10 min. Sections were incubated with the primary MAb, then with rabbit anti-mouse immunoglobulins (Dako, Glostrup, Denmark) supplemented with either 12.5 % pooled human serum or 25 % pooled rat serum to inhibit cross-reactivity against human or rat immunoglobulin. This was followed by an incubation with the APAAP complex (Dako, Glostrup, Denmark). Each step took 30 min at RT. Samples were thoroughly washed in Tris-buffered saline (pH 7.6) between steps. The rabbit- "link" and the APAAP complex steps were then repeated for 10 min each. Alkaline phosphatase substrate reaction with new fuchsin and levamisole was performed for 20 min. Sections were counterstained with Mayer's acid hemalum and mounted in gelatin.

For double-staining immunofluorescent technique, sections were incubated with a mixture of monoclonal and polyclonal primary antibodies at 37 ℃ for 45 min, rinsed in PBS, and incubated with a mixture of fluorescein-conjugated goat anti-mouse immunoglobulins (Fitc, Cappel, USA) and rhodamine-coojugated pig anti-rabbit immunoglobulins (TRITC, Dako) at 37 ℃ for 30 min. Sections were rinsed in PBS and mounted in Mowiol.

RESULTS

The cellular distribution of ACE in rat cardiac tissue was localized by applying

immunohistochemical stain with the 9B9 MAb which was the only one of the MAb against human ACE reacting with rat ACE.

In healthy cardiac tissue of rat, ACE expression was confined to the endothelial cells of coronary vessels and capillaries (Fig 1A). Immunofluorescent double-staining for rat endothelial cells with MAb OX-43 (Fig 1B) and ACE with anti-ACE antiserum (Fig 1C) showed: 1) Most of the ACE-positive cells were endothelial cells; 2) Nearly all of the endothelial cells of the coronary vessels were ACE-positive while about 20 % of the capillary endothelial cells were ACE-positive; 3) Less than 5 % of ACE-positive cells were nonendothelial.

After coronary occlusion, a loss of 40 % – 50 % of left ventricular mass was observed. Sham operated hearts showed no sign of necrosis. One week after MI, ACE expression was found in granulation tissue. Signs of ACE over-expression in granulation tissue at the boarder of infarction were seen two weeks after MI (Fig 2A). Three weeks after MI, necrosis within the infarction was replaced by granulation tissue and fibrous tissue which showed strong over-expression of ACE (Fig 2B). Six weeks after MI, the region with positive ACE staining regressed and the area with high collagen content on the endocardial side showed only weak ACE staining (Fig 2C). However, the density of ACE expression in the ACE-positive region on the epicardial side six weeks after MI was still high and showed no significant difference compared with that 3 wk after MI (Fig 2B, C). In areas with over-expression of ACE, granulation and fibrous tissue showed a high density of ACE-positive capillaries.

Immunofluorescent double-staining using OX-43 MAb for endothelium and anti-ACE antiserum for ACE revealed that ACE expression was higher in endothelial cells of the capillaries within the area of ACE up-regulation (Fig 3A, B) compared with that in normal cardiac tissue in which about 20 % of capillaries endothelial cells expressed ACE (Fig 1B, C). Although most of the ACE-positive cells in the ACE-over-expression area were endothelial cells, some other cells were also ACE-positive in these regions. To further clarify the cell type of these ACE-positive cells, double-stain using KiM2R macrophage marker and anti-ACE antiserum in the ACE-positive granulation tissue 7 d after infarction demonstrated that some of the ACE-positive cells were macrophages (Fig 3C, D). Neither the cardiomyocytes of sham operated rats nor the cardiomyocytes of the infarcted rats showed any ACE expression. No significant change of ACE expression was observed within nonischemic myocardial tissue.

DISCUSSION

It has been shown that various stimuli, such as hormones and fluid shear stress, are able to modify the ACE expression in endothelial cells[7]. A more pronounced ACE expression in arterial endothelial cells than in venous vessels observed in our present study might be the result of the shear stress stimulation on endothelial cells. Thus, ACE expression in vascular endothelial cells might be mechanical load related. This, together with the fact that only part of the capillaries are perfused under physiological conditions[7] could also explain the heterogeneous pattern of ACE expression observed in myocardial capillaries.

Our findings also suggest that over-expression of ACE is involved in the process of cardiac scar repairing following MI. Increased ACE synthesis may lead to markedly enhanced Ang Ⅱ formation. Ang Ⅱ-stimulated growth of myocytes and fibroblasts was suggested as a basis for compensatory ventricular remodeling. In cardiac fibroblasts, elevated Ang Ⅱ levels have been shown to increase both, the mRNA expres-

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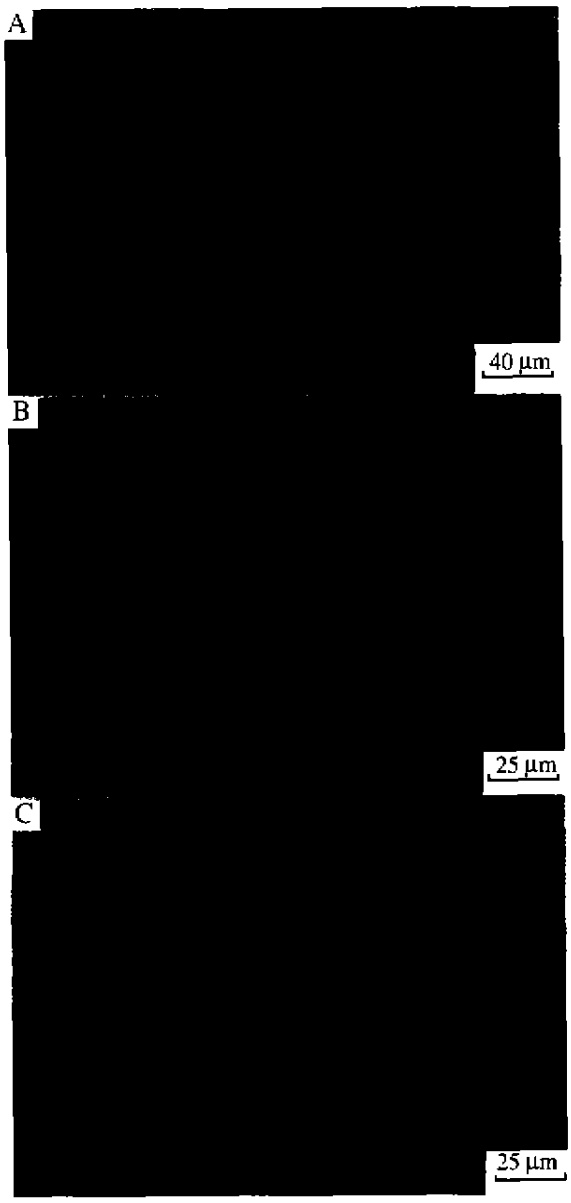

Fig 1. Immunohistochemical localization of ACE in rat heart using 9B9 monoclonal antibody against human ACE. A) Normal, showing ACE-positive coronary vessels and capillaries (APAAP, red). × 250. B and C) Immunofluorescent double-staining for rat cardiac endothelial cells (fluorescein labeled, green) ACE-positive cells (rhodamine labeled, red) in coronary vessel and capillaries. ×400.

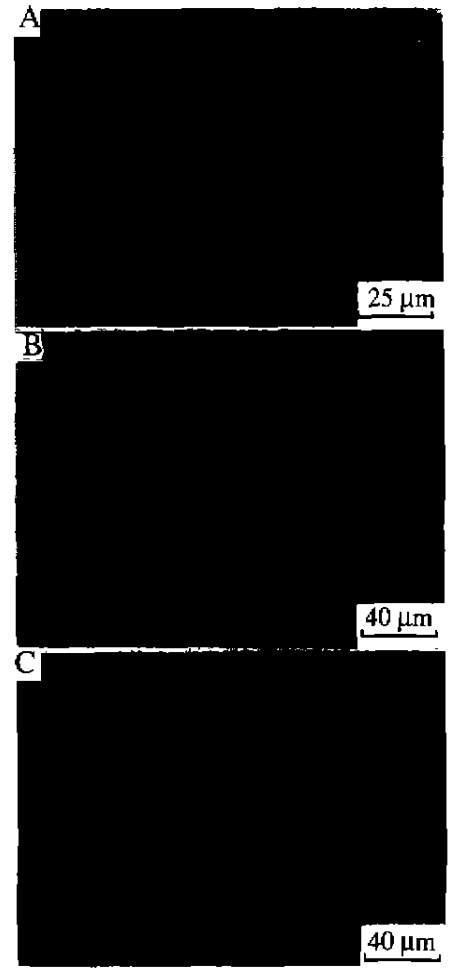

Fig 2. A) Two weeks after MI the granulation tissue showed signs of ACE over-expression (APAAP, red). ×400. B) ACE over-expression in repairing scar was visible 3 wk after MI (APAAP, red). ×250. C) The ACE-positive zone was restricted to the epicardium half of the infarction (on the right side, APAAP, red) and the scar with high collagen content showed only weak ACE expression 6 wk after MI. ×250.

sion and protein synthesis of type I collagen[8]. Therefore, high Ang II concentrations in the scar tissue of infarcted hearts might activate fibroblast growth and collagen synthesis. This is in line with the study in which treatment with losartan resulted in a reduction of cardiac mass and collagen content following MI[9].

Some investigators reported an ACE induction in the noninfarcted cardiac tissue following MI[10]. In the present study, ACE staining in the noninfarcted cardiac tissue was

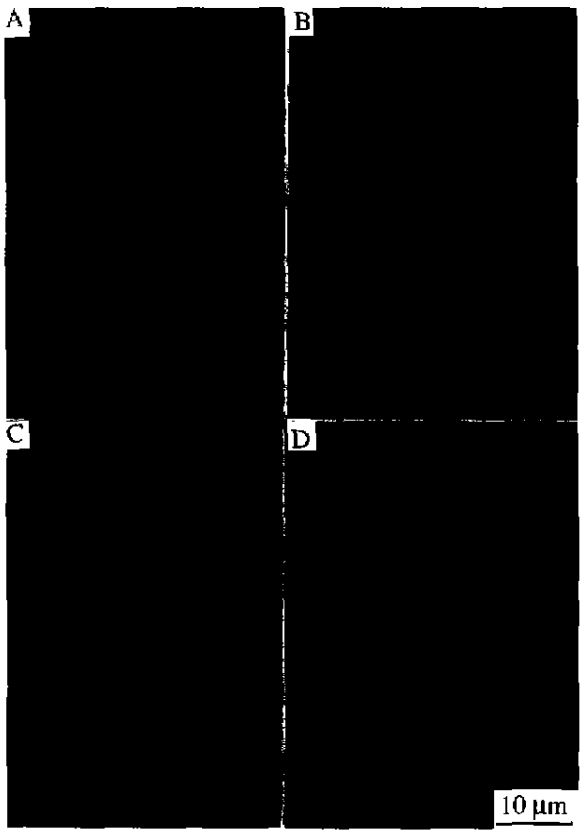

Fig 3. Immunofluorescent double-staining for rat cardiac tissue. ×1000. A, B) Endothelial cells (fluorescein labeled, green) and ACE-positive cells (rhodamine labeled, red) in the scar tissue 3 wk after MI. C, D) Macrophage (green) and ACE-positive cells (red) in granulation tissue 7 d after MI.

confined to the vascular endothelial cells and was certainly not present in cardiomyocytes. Therefore, ACE induction after MI was mainly located at the vascular endothelium of the heart.

In addition, some other cell types also revealed ACE expression. The initial increase of ACE expression in repairing scar tissue was associated with an influx of inflammatory cells. ACE-positive macrophages were detected in one-week old granulation tissue. *In vitro* studies have demonstrated that ACE synthesis is induced in macrophages when they were transformed from mononuclear cells[11]. It has been reported that ACE is involved in the presentation of antigens to MHC-class I-restricted T-lymphocytes[12]. This gave rise to the hypothesis that ACE may be involved in the process of activation and development of inflammatory cells. Since KiM2R only recognizes activated macrophages[13], it appears that the few macrophages, which were found to be ACE-positive, were at a certain stage of maturation in which they express ACE.

In conclusion, the level of endothelial ACE expression is associated with the anatomical location of the endothelial cells in normal cardiac tissue. Cardiac ACE was over expressed during the process of tissue repair following MI, reaching a peak in 3 wk. Endothelial cells took the most part of ACE expression.

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心肌梗死后心脏血管紧张素转换酶的表达

朱依纯[1], Mechthilt FALKENHAHN, Folker FRANKE, Rainer Maria BOHLE[2], Harald Martin STAUSS, Sergei DANILOV[3], Thomas UNGER
(*[1]Department of Pharmacology, University of Kiel, Germany; [2]Department of Pathology, University of Giessen, Germany; [3]National Cardiology Research Center, Moscow, Russia*)

关键词 肽基-二肽酶 A; 心肌梗死; 免疫组织化学; 单克隆抗体; 血管内皮; 冠状血管; 巨噬细胞

目的: 确定血管紧张素转换酶(ACE)在心肌梗死后左心室组织修复及重构过程中, 以及在假手术大鼠心肌中的分布. **方法**: 在冠状动脉结扎后 24 h, 1 wk, 2 wk, 3 wk, 6 wk 后用抗 ACE 单克隆及多克隆抗体通过免疫组化染色显示心脏组织 ACE 在上述时间点的表达. 通过免疫荧光双染色法进一步明确是哪一种细胞表达 ACE. **结果**: 在正常的心脏组织中 ACE 染色局限在内皮细胞上, 其密度随着血管树的分级呈阶梯状变化: 小动脉的全部血管内皮细胞都有 ACE 染色. 毛细血管内皮细胞仅有约 20 % 有 ACE 染色, 静脉内皮细胞则完全没有 ACE 染色. 心肌梗死 3 wk 后梗死区的坏死组织已被肉芽组织所取代并可观察到非常明显的 ACE 过度表达; 心肌梗死 6 wk 后 ACE 染色区缩小, 在有大量胶原沉积的区域仅可见微弱的染色. 绝大部分在 ACE 高表达区表达 ACE 的细胞是内皮细胞, 少量巨噬细胞上也有 ACE 染色. **结论**: 在正常心脏组织中, 内皮细胞表达 ACE 的程度与细胞所处的解剖位置有关. 心肌梗死后 ACE 在梗死区的组织修复过程中有过度表达. 其高峰在梗死后 3 wk, 过度表达的 ACE 主要来源于内皮细胞.

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