

## Original Article

# Expression of $\beta$ -tubulin in non-culprit arteries and effect of ramipril on lesion progression

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**Abstract:** Objective: The objective of this study was to determine the expression of  $\beta$ -tubulin in non-culprit arteries and investigate the effect of ramipril on progression of non-culprit lesions in rabbits. Methods: A myocardial ischemia-reperfusion model in the rabbit was prepared. Smooth muscle cells in non-culprit arteries were divided into 4 groups (10 samples in each group) at random: ① Hyperlipidemia control group, ② Myocardium ischemia group, ③ Myocardium ischemia reperfusion group, ④ Myocardium ischemia reperfusion and the ramipril intervention group. Immunohistochemistry was used to analyze for  $\beta$ -tubulin in non-culprit arteries, and expression of  $\beta$ -tubulin in each group was analyzed. Results: The thickness of atherosclerotic plaque in the myocardium ischemia reperfusion group was more than in then myocardium ischemia group ( $113.61 \pm 25.67 \mu\text{m}$  vs  $35.42 \pm 11.19 \mu\text{m}$ ,  $P < 0.0001$ ). The thickness of atherosclerotic plaque in the myocardium ischemia reperfusion and ramipril intervention groups was less than that in myocardium ischemia reperfusion group ( $82.79 \pm 17.24 \mu\text{m}$  vs  $113.61 \pm 25.67 \mu\text{m}$ ,  $P < 0.001$ ). Expression of  $\beta$ -tubulin in the myocardium ischemia reperfusion group was higher than that in the myocardium ischemia group, ( $413.61 \pm 50.46$  vs  $83.15 \pm 21.12$ ,  $P < 0.0001$ ). Expression of  $\beta$ -tubulin in myocardium ischemia reperfusion and ramipril intervention group was lower than that in the myocardium ischemia reperfusion group ( $312.79 \pm 37.33$  vs  $413.61 \pm 50.46$ ,  $P < 0.0001$ ). Conclusions: Non-culprit arteries may progress after primary PCI, which may be involved with  $\beta$ -tubulin, ramipril may inhibit the progression of non-culprit arteries by decreasing expression of  $\beta$ -tubulin.

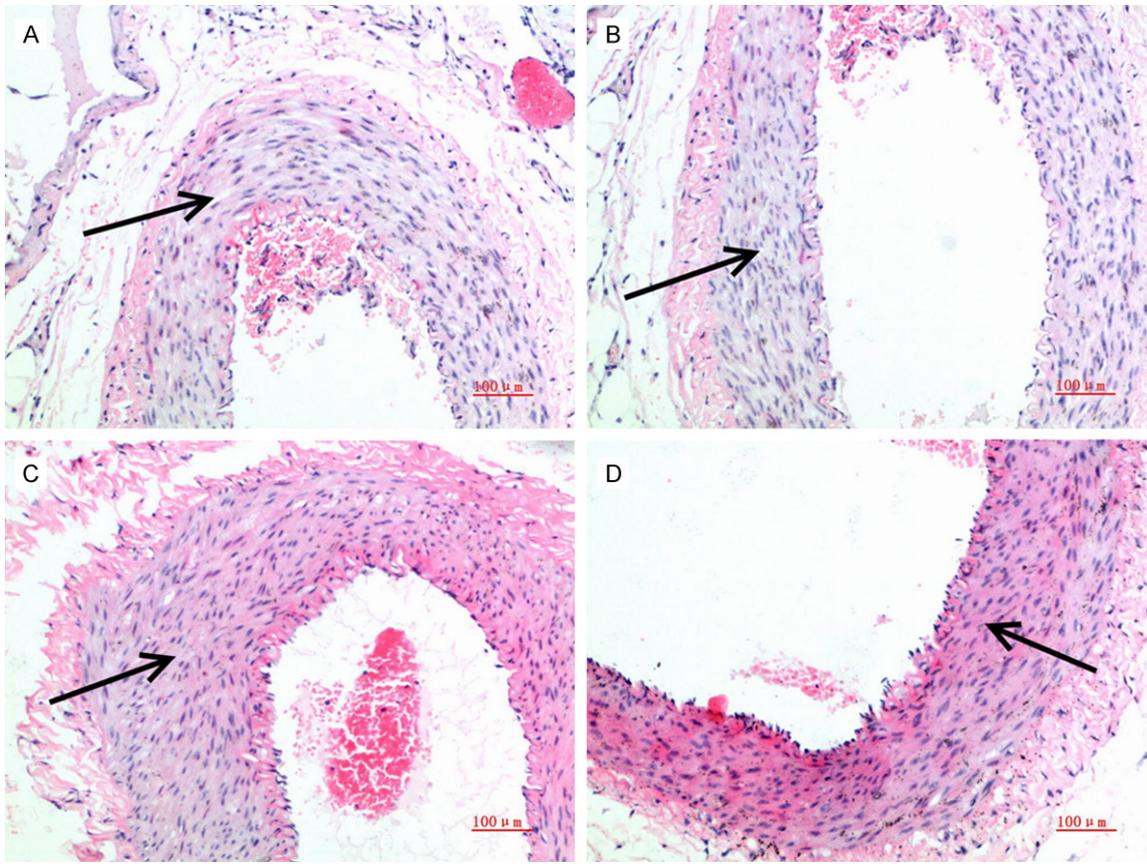
**Keywords:** Non-culprit arteries progression, ischemia reperfusion,  $\beta$ -tubulin, ramipril

## Introduction

Although primary percutaneous coronary intervention (PPCI) can rescue dying myocardium, reduce major adverse cardiovascular events, and improve prognosis of patients with ST elevation myocardial infarction (STEMI), unstable progression may occur in non-culprit arteries, which is a significant factor associated with prognosis [1-5]. However the mechanism of non-culprit arteries in progression has not been revealed yet. Our early stage research demonstrated that angiotensin II (AngII) was associated with the increasing expression of Cx43 in vascular smooth muscle cells by mitogen-activated protein kinase (MAPK) pathway [6]. This process increased the proliferation and migration of smooth muscle cells, and consequently accelerated the progression of non-culprit arteries. Therefore, progression of non-culprit arteries could result in increasing expression of

Cx43 [7-10]. However, the mechanism of Cx43 that accelerated the progression of non-culprit arteries remained unknown. Matsuuchi L et al. thought that Cx43 increased the proliferation effect by remodeling the cytoskeleton. Our early stage experiment showed that Cx43 protein level was positively related with  $\beta$ -tubulin level.  $\beta$ -tubulin may participate in the polarization, proliferation, and migration of smooth muscle cells as the main cytoskeleton protein. Therefore,  $\beta$ -tubulin protein remodeling mediated by Cx43 may participate in the progression of non-culprit arteries. In this study, we prepared a rabbit myocardium ischemia reperfusion model to observe expression of  $\beta$ -tubulin in non-culprit arteries, and to study whether angiotensin converting enzyme inhibitors (ACEI) (Ramipril) could inhibit expression of  $\beta$ -tubulin and inhibit progression of lesions in non-culprit arteries.

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**Figure 1.** Plaques in non-infarction related arteries after H&E staining. A: Hyperlipidemia group; B: Myocardium ischemia reperfusion group; C: Myocardium ischemia reperfusion group; D: Myocardium ischemia reperfusion and ACEI intervention group. Note: arrow indicates coronary atherosclerosis plaque.

### Materials and methods

#### *Hyperlipidemia animal model*

The animal experiments were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of Capital Medical University, and the study protocol was approved by the Institutional Ethics Committee of Capital Medical University. 50 healthy male rabbits were used in this experiment. All rabbits were adaptive fed for one week. Seven rabbits were randomly chosen into control group. Control group rabbits were fed with normal fodder for 80 days, while the other rabbits were fed with high-fat fodder. The vein blood sample was taken on the 81<sup>st</sup> day after fasting for 12 hours. The serum was separated after centrifugation to test the level of serum total cholesterol (TC). Rabbits whose Serum total cholesterol level was 3 times higher than the normal group (1-2 mmol/L) were selected into the hyperlipidemia group.

#### *Myocardium ischemia reperfusion animal model*

The acute myocardium ischemia animal models were subjected to the following operation. Due to the long and steady anesthesia maintenance, all the rabbits were given the intraperitoneal anesthesia of urethane sodium with the dosage of 1 g/kg. All rabbits were given lidocaine 1 mg/kg as prevention of ventricular fibrillation. Thoracotomy was performed on 8 hyperlipidemia rabbits, without coronary artery ligation as the control group. Another 8 hyperlipidemia rabbits were given a thoracotomy. After exposing the pericardium and the heart, the left anterior descending coronary artery (LAD) was clamped with 5/0 string and maintained for 30 minutes. ECG monitor was connected subcutaneously (25 mm/s, 10 mm/mv). The model was defined as successfully prepared under the following circumstances and maintained for 30 minutes. ① ST elevation or necrotic Q wave is observed in corresponding

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**Table 1.** Thickness of plaques in non-infarction related arteries

Group	n	Thickness of plaques ( $\mu\text{m}$ )
HL	10	40.13 $\pm$ 12.34
MI	10	35.42 $\pm$ 11.19*
MIR	10	113.61 $\pm$ 25.67**
MIR&ACEI	10	82.79 $\pm$ 17.24***

\* $P = 0.3831$ , compared with hyperlipidemia group; \*\* $P < 0.0001$ , compared with acute myocardial group; \*\*\* $P = 0.0055$ , compared with myocardial ischemia reperfusion group.

**Table 2.** Immunohistochemistry analysis of  $\beta$ -tubulin in non-infarction related arteries (mean integral optical density)

Group	n	Quantitative analysis of $\beta$ -tubulin
HL	10	89.67 $\pm$ 14.29
MI	10	83.15 $\pm$ 21.12*
MIR	10	413.61 $\pm$ 50.46**
MIR&ACEI	10	312.79 $\pm$ 37.33***

\* $P = 0.4293$ , compared with hyperlipidemia group; \*\* $P < 0.0001$ , compared with acute myocardial group; \*\*\* $P < 0.0001$ , compared with myocardial ischemia reperfusion group.

lead on ECG. ② The myocardium in the corresponding region turned into dark purple. Reperfusion was performed by releasing the clamping. The abdominal incisions were closed. All rabbits were fed a week after operation.

Animal groups: ① Hyperlipidemia group (HL) (n = 10, thoracotomy without coronary artery ligation). ② Myocardium ischemia group (MI) (n = 10, thoracotomy and LAD ligation for 30 minutes). ③ Myocardium ischemia reperfusion group (MIR) (n = 10, thoracotomy, LAD ligation for 30 minutes and reperfusion for 1 week). ④ Myocardium ischemia reperfusion and ACEI intervention group (MIR&ACEI) (n = 10, hyperlipidemia model with a dose of captopril, 2.5 mg/kg-d, thoracotomy, LAD ligation for 30 minutes and reperfusion for 1 week).

### H&E stain and observation

The coronary artery tissues were harvested for H&E stain and observation. The tissues were subsequently embedded in paraffin and discontinuously serial sectioned and stained with hematoxylin and eosin (H&E) and observed under light microscope. The thickness of each section was 4  $\mu\text{m}$ . All sections were analyzed by

Medical image analysis system (MIAS) to measure the thickness of plaques.

### Immunohistochemistry analysis

Immunohistochemistry analysis was used to evaluate the expression of  $\beta$ -tubulin protein. Some sections were collected for Immunohistochemistry analysis. Kit: First antibody: Anti-beta tubulin antibody, Sigma-Aldrich Corporation, USA. Second antibody: Gote Anti-Mouse IgG (H+L) Rhodamine (TRITC), Boster biological technology Corporation, China. High-definition medical imaging analysis system (HMIAS2000) was used for the quantitative assessment of the expression of  $\beta$ -tubulin protein.

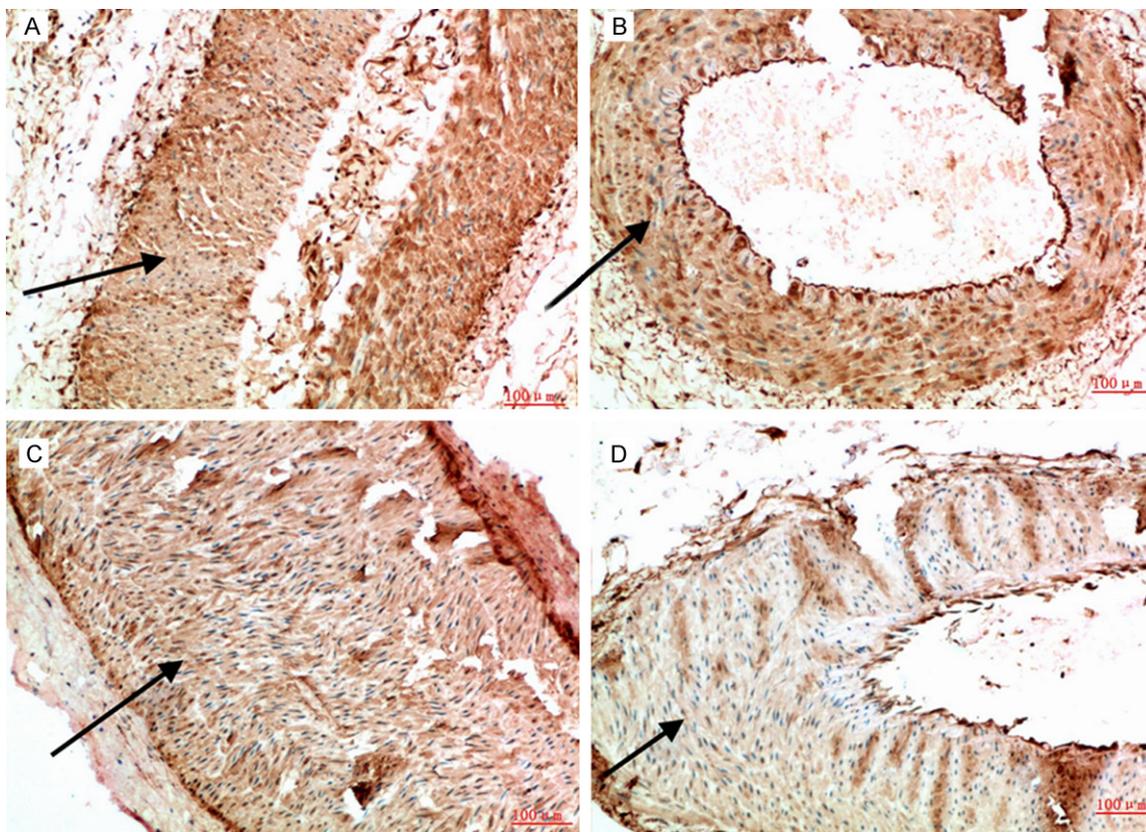
### Statistical analysis

Statistical analysis was performed using SPSS 10.0 software. The measurement data are presented as the mean  $\pm$  SD. Multiple group mean variance analysis was used to compare the differences in the mean among the groups. The  $q$  test was used to compare the differences in 2 mean between groups subsequently. The independent-samples  $t$  test was used to compare the differences in the mean between two groups.  $P < 0.05$  was considered to indicate a significant difference in all statistical tests.

## Results

The results demonstrate that atheromatous plaques could be found in non-infarction related arteries after ischemia reperfusion for 1 week (**Figure 1**). H&E staining quantitative analysis of non-infarction related arteries are presented in **Table 1**. The thickness of plaques in hyperlipidemia group is 40.13  $\pm$  12.34  $\mu\text{m}$ . The thickness of plaques in acute myocardium ischemia group is 35.42  $\pm$  11.19  $\mu\text{m}$  ( $P = 0.3831$ , compared with hyperlipidemia group). The thickness of plaques in the acute myocardium ischemia reperfusion group is 113.61  $\pm$  25.67  $\mu\text{m}$  ( $P < 0.0001$ , compared with the acute myocardium ischemia group). The thickness of plaques in myocardium ischemia reperfusion and ACEI intervention group is 82.79  $\pm$  17.24  $\mu\text{m}$  ( $P = 0.0055$ , compared with the acute myocardium ischemia reperfusion group) (**Table 1**).

Immunohistochemistry analysis of  $\beta$ -tubulin in non-infarction related arteries are presented in **Table 2** and **Figure 2**. The integral optical den-



**Figure 2.**  $\beta$ -tubulin in non-infarction related arteries after Immunohistochemistry staining. A: Hyperlipidemia group; B: Myocardium ischemia reperfusion group; C: Myocardium ischemia reperfusion group; D: Myocardium ischemia reperfusion and ACEI intervention group. Note: arrow indicates  $\beta$ -tubulin.

sity of plaques in hyperlipidemia group is  $89.67 \pm 14.29 \mu\text{m}$ . The thickness of plaques in the acute myocardium ischemia group is  $83.15 \pm 21.12 \mu\text{m}$  ( $P = 0.4293$ , compared with hyperlipidemia group). The thickness of plaques in the acute myocardium ischemia reperfusion group is  $413.61 \pm 50.46 \mu\text{m}$  ( $P < 0.0001$ , compared with the acute myocardium ischemia group). The thickness of plaques in the myocardium ischemia reperfusion and the ACEI intervention groups is  $312.79 \pm 37.33 \mu\text{m}$  ( $P < 0.001$ , compared with acute myocardium ischemia reperfusion group) (Table 2; Figure 2).

### Discussion

Previous research on non-infarction related arteries has been very limited. Our early research investigated 519 patients with STEMI undergoing direct PCI during a follow-up period of 6 months. The outcome has demonstrated that the main reason of postoperative remodeling after direct PCI in patients with STEMI is

non-infarction related arteries progression. Inflammatory and stress reactions may be involved in this procession. Elevation of catecholamine level plays an important role in the progression of non-infarction related arteries. However, the mechanism is yet to be fully studied.

The effect of connexins (Cxs) in the proliferation and migration of vascular smooth muscle cell in atherosclerosis has raised wide attention in the recent years. Connexins, or gap junction proteins, are structurally related transmembrane proteins, which assemble to form vertebrate gap junctions. It participates in the metabolic process and signaling by providing electric coupling and material exchange. Therefore, Cxs are of great significance in regulating some physiological processes, such as metabolism, stabilizing the internal environment, cellular proliferation, and cellular differentiation. The expression and distribution of Cxs how different changes during the proliferation and migra-

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tion of smooth muscle cell and plaque formation. The study which evaluates the coronary samples of patients undergoing heart transplant demonstrates that expression of Cx43 in the intimal thickening area is 10 times higher than the normal vessel in the early stage of atherosclerosis. As the disease progress, expression of Cx43 declines. In the end stage, expression of Cx43 is lower than the normal vessel. Inhibiting expression of Cx43 in vascular intima in the early stage can significantly inhibit the progression of this chronic inflammation. These findings suggest Cx43 may participate in progression of early stage atherosclerosis [6-8].

We tested expression of Cx43 in non-infarction related arteries in acute myocardium ischemia reperfusion rabbit model. The results demonstrate that expression of Cx43 in the non-infarction related arteries increase dramatically in the first week after reperfusion. We also found proliferation and migration of vascular smooth muscle cell in non-infarction related arteries. The outcomes also prove that angiotensin II-MARP signaling pathway and Cx43 participate in progression of non-infarction related arteries lesions [6]. While the mechanism that Cx43 resulting in proliferation and migration of VSMC remains unknown. Matsuuchi L et al. believe that the migration effect is caused by Cx43 through the cytoskeleton reconstruction. The main cytoskeleton includes F-actin,  $\alpha/\beta$  tubulin,  $\alpha$ -actinin, GTP enzyme activated protein with IQ motif, actin binding protein (ezrin protein, drebrin protein or cortactin protein). Our early research demonstrated the level of Cx43 is positively correlated with the level of  $\alpha/\beta$  tubulin. Tubulin, one of the main cytoskeleton proteins, is associated with the polarity, proliferation and migration of the cell. Therefore, tubulin reconstruction mediated by Cx43 may be associated with the progression of the non-infarction related arteries.

This study demonstrates that atherosclerosis plaques can be found in the non-infarction related arteries after ischemia reperfusion for 1 week (**Figure 1**).

H&E staining quantitative analysis of non-infarction related arteries show that the thickness of plaques in hyperlipidemia group was  $40.13 \pm 12.34 \mu\text{m}$ . The thickness of plaques in

acute myocardium ischemia group was  $35.42 \pm 11.19 \mu\text{m}$  ( $P = 0.3831$ , compared with hyperlipidemia group). The thickness of plaques in the acute myocardium ischemia reperfusion group is  $113.61 \pm 25.67 \mu\text{m}$  ( $P < 0.0001$ , compared with the acute myocardium ischemia group). The thickness of plaques in the myocardium ischemia reperfusion and ACEI intervention groups was  $82.79 \pm 17.24 \mu\text{m}$  ( $P = 0.0055$ , compared with the acute myocardium ischemia reperfusion group). Immunohistochemistry analysis of  $\beta$ -tubulin in non-infarction related arteries shows that the integral optical density of plaques in the hyperlipidemia group was  $89.67 \pm 14.29 \mu\text{m}$ . The thickness of plaques in the acute myocardium ischemia group was  $83.15 \pm 21.12 \mu\text{m}$  ( $P = 0.4293$ , compared with the hyperlipidemia group). The thickness of plaques in the acute myocardium ischemia reperfusion group is  $413.61 \pm 50.46 \mu\text{m}$  ( $P < 0.0001$ , compared with the acute myocardium ischemia group). The thickness of plaques in the myocardium ischemia reperfusion and ACEI intervention groups was  $312.79 \pm 37.33 \mu\text{m}$  ( $P < 0.001$ , compared with acute myocardium ischemia reperfusion group). The results suggest that expression of  $\beta$ -tubulin increases along with progression of non-infarction related arteries after AMI. Thus  $\beta$ -tubulin may play the key role in the progression of non-infarction related arteries. Expression of  $\beta$ -tubulin in the ACEI intervention group was significantly lower than that in the reperfusion group ( $312.79 \pm 37.33$  vs  $413.61 \pm 50.46$ ,  $P < 0.0001$ ). These results indicate that Ramipril can inhibit angiotensin II-MAPK signaling-Cx43- $\beta$ -tubulin pathway, and thus inhibit the progression of non-infarction related arteries.

The neuroendocrine activation caused by STEMI and cardiac hemodynamic changes often lead to the activation of renin-angiotensin system (RAS). Increased angiotensin II affect the signaling of MAPK pathway by autocrine, paracrine, emiocytosis (or by microcirculation between culprit and non-infarction arteries), and lead to the enhancement of the expression of Cx43. Increased Cx43 will lead to the proliferation and migration of vascular smooth muscle cell and progression of atherosclerosis by tubulin reconstruction [10, 11]. This study indicates that the sympathetic nervous system-catecholamine-angiotensin II-Cx43-tubulin pathway may participate in the progression of

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non-infarction related arteries. This finding may provide us with a novel target for therapeutic intervention in progression of non-infarction related arteries.

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### Disclosure of conflict of interest

None.

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