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Effects of tirofiban on the reperfusion-related no-reflow in rats with acute myocardial infarction

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Abstract

Objective To investigate the effects of tirofiban on the no-reflow phenomenon of acute myocardial infarction (AMI) rats received reperfusion, as well as the underlying mechanisms. **Methods** Fifty-six male Sprague-Dawley rats were randomly divided into four groups: Sham operation group (Sham), AMI/reperfusion group (AMI/R), Tirofiban group (Tiro) and Tiro+N-nitro-L-arginine group (L-NNA; an endothelial nitric oxide synthase inhibitor). To generate the animal model mimicking the no-reflow phenomenon, the rats first received occlusion of the left anterior descending coronary artery for 60 min and then followed by reperfusion for 120 min. Area of no-reflow, area at risk and area of necrosis were measured by thioflavine S, Evans blue and triphenyl tetrazolium chloride staining, respectively. Haemodynamic function was measured at the end. In the meantime, nitric oxide synthase (NOS) activity was determined by a NOS assay kit. The expression of myocardial endothelial nitric oxide synthase (eNOS) was determined by an enzyme-linked immunosorbent assay (ELISA). The expression of phosphorylated eNOS at Ser^{1177} (p-eNOS Ser^{1177}) and vascular endothelial-cadherin (VE-cadherin) were determined by western blot. **Results** Compared with AMI/R group, tirofiban significantly reduced the no-reflow area and infarct size (all $P < 0.05$). Tirofiban elevated eNOS activity, lessen inducible nitric oxide synthase (iNOS) activity and increased the expression of Ser^{1177} phosphorylated eNOS and VE-cadherin in the ischemic myocardium (all $P < 0.05$). No statistical differences were found in the expression of eNOS among the four groups. Also, tirofiban improved cardiac function with significantly higher levels of left ventricular end systolic pressure, maximum change rate of left ventricular pressure rise and fall, heart rate, and lower level of left ventricular end diastolic pressure than those of the AMI/R group (all $P < 0.05$). Whereas, these effects of tirofiban were partially abolished by L-NNA. **Conclusions** Tirofiban could reduce the size of no-reflow and infarct. A possible mechanism underlying this effect is that tirofiban could protect the structural and functional integrity of microvascular endothelium which is partially regulated by eNOS activity.

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Keywords: Tirofian; Acute myocardial infarction; Nitric oxide synthase; Vascular endothelial-cadherin

1 Introduction

Coronary no-reflow phenomenon (NR) describes the situation when patients with acute myocardial infarction (AMI) are given reperfusion treatment, the occluded epicardial coronary artery undergoes surgical reconstruction, but the blood flow to some myocardial tissues may still be impeded.[1] As a complication symptom, NR increases the rates of in-hospital death and myocardial infarction by 5 to 10 folds. It is currently believed that the functional and structural damage to microvasculature is a key mechanism underlying NR.[2] Heitzer *et al.*[3] have found that inhibitor of platelet glycoprotein (GP) Ⅱb/Ⅲa receptor could protect endothelium, but it remains unknown whether its effects on coronary NR were due to its protection of the structure and function of micro-vascular endothelium. By establishing non-thrombus coronary NR and performing reperfusion in rats, our study aimed to investigate the effects of tirofiban, a GP Ⅱb/Ⅲa receptor inhibitor, on myocardial no-reflow and the structure and function of microvascular endothelium, as well as the underlying mechanisms.

2 Methods

2.1 Experimental animals and grouping

Fifty-six male Sprague-Dawley rats (form Experimental Animal Center of Liaoning Medical University), aged 10 to 15 weeks with weights ranging from 270 g to 330 g, were prepared. Rats were randomly divided to four groups: (1)

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rats underwent no ligation with only threading (Sham group, n = 8); (2) rats underwent 60 min of ischemia by ligation of the left anterior descending coronary artery and 120 min of reperfusion with no drug intervention (AMI/R group, n = 16); (3) 60 μg/kg tirofiban (Wuhan Yuanda Pharma Co, Wuhan, China) was given as a intravenous bolus at thirty minutes before reperfusion through tail veins (Tiro group, n = 16);[4] and (4) five minutes after tirofiban was administered, N-nitro-L-arginine (L-NNA), an endothelial nitric oxide synthase (eNOS) inhibitor, was given at a dose of 10 mg/kg to inhibit eNOS activity (Tiro+L-NNA group, n = 16). Same amount of saline was given to sham group and AMI/R group intravenously.

2.2 Reagents and instruments

Thioflavine S, Evans blue and triphenyl tetrazolium chloride (TTC) (Sigma-Aldrich, USA); rabbit anti-rat eNOS, p-eNOS ser^{1177} and β-actin polyclonal antibodies (Shenyang Beyotime Institute of Biotechnology); rabbit anti-rat VE-cadherin polyclonal antibody (Santa Cruz, USA); nitric oxide synthase kit (Nanjing Jiancheng Bioengineering Institute, China); rat eNOS ELISA kit (Rapidbio, America); DH-150 animal artificial respirator (Zhejiang University Medical Instrument Co. Ltd., China); balloons for vessel dilation (℄ 2.5–L14, Department of Intervention, the First Affiliated Hospital of Liaoning Medical University, China).

2.3 Establishing NR after giving reperfusion to AMI animals[5]

The rats were given celiac anesthesia with 20% urethane (5 mL/kg), and their median part of the neck was incised, the trachea intubated for mechanical ventilation (respiration rate: 60 breaths/min; tidal volume: 10–15 mL; respiratory ratio: 1: 3) and simultaneously linked to electrocardiographic (ECG) monitor and BL-420F biological function experiment system. The space between the second and third ribs along the left side of the sternum was incised vertically, the ribs were cut apart and the heart was exposed. The lower border of left atrial appendage and the root of the anterior descending branch of the left coronary artery within the conic part of the pulmonary artery were ligated with 6.0 Prolene thread. A water-filled (0.5 mL) balloon was placed between the blood vessels and the thread. A successful ligation will have these features: reduced movement of the front wall of the chest; the myocardium at the distal end of the thread turned purple; and the ST section on the electrocardiogram shows an arch-like elevation. Subsequently, the chest was closed for 60 mim and then the three-way stopcock was opened and the balloon was emptied with a needle. Then the rats were reperfused for 120 min before killed. For animals in the sham group, the thread was placed but without ligation.

2.4 Measuring the area of no-reflow (ANR), area at risk (ischemic, AAR) and area of necrosis (AN)[6]

At the end of reperfusion, the rats were given 6% thioflavine S (1 mL/kg) through tail veins. Area of reflow was marked by fluorescent lamp (wave length: 365 nm), while ANR was not. One minute later, the coronary artery was re-occluded by water-filled balloon and 2% Evans blue (1 mL/kg) was administrated through tail veins. AAR was defined as regions not stained with the blue dye while non-ischemic area was stained by Evans blue. After one minute, the heart was removed and washed with ice-cold physiological saline. Then, the atria and right ventricle were removed and the left ventricle was frozen for 10 min at -80℃. Next, the myocardium in parallel to atrioventricular groove was sliced into four to five transverse sections (1–2 mm). The slices were immersed in 1% TTC solution (pH 7.4) and put in the incubator for 30 min at 37℃. Non-AN appeared reddish-brown and AN pale. The stained slices were photographed. AAR (expressed as the percentage of the weight of the left ventricle), AN and ANR (expressed as the percentage of the weight of the AAR) were calculated with Image Pro. 6.0 software.[7]

2.5 Haemodynamic function

After 120 min of reperfusion, heart rate (HR), left ventricular end systolic pressure (LVESP), left ventricular end diastolic pressure (LVEDP), maximum change rate of left ventricular pressure rise and fall ($\pm dp/dt_{max}$) were measured.

2.6 Determining NOS activity in the ischemic myocardium

eNOS and inducible nitric oxide synthase (iNOS) activity were determined by a NOS assay kit. The NOS assay kit is to use the method of catalyzing L-arginine to measure NOS activity in myocardium. 100 mg myocardium sample from the anterior wall of the left ventricle were placed in a centrifuge tube, adding 10 volumes of ice-cold phosphate buffer saline (PBS, PH 7.4), and immediately homogenized for 1–2 min on ice, the homogenate was then centrifuged at 3000 r/min for 15 min at 4℃. The supernatant was collected and aliquoted. One of aliquots was used for detection and the rest were stored in −80℃ for future study. Then, the protein concentration was determined by BCA (bicinchonininc acid) Protein Assay Kit. The supernatant was incubated with 0.6 mL reaction buffer, when iNOS was determined, combined with the addition of inhibitor 1 mmol/L EGTA (iNOS Ca^{2+} independent, eNOS Ca^{2+} de-

pendent, EGTA can complex Ca^{2+} in samples). The reaction was terminated after 15 min at 37℃. The photometric measurement of the absorbance at 530 nm was applied to determine total NOS (tNOS) and iNOS activity. eNOS activity was calculated by subtracting iNOS from tNOS.

2.7 Detection of eNOS protein content in the ischemic myocardium by an enzyme-linked immunosorbent assay (ELISA)

ELISA kit was first equilibrated to room temperature (20–25℃), and then the sample supernatants collected in the above steps (section 2.6) were analyzed according to the instruction of manufacturer. Briefly, samples, standards and horseradish peroxidese (HRP) labeled detection antibody were sequentially added into the rat eNOS capture antibody pre-coated micropores; after incubation and thorough washing, TMB (3,3,5,5-Tetramethyl benzidine Solution liquid membrane substrate) was added for the color development which turns blue under TMB peroxidase and finally converses to yellow with acid treatment; The depth of color was positively correlated with eNOS contents. Optical density (OD) at 450 nm was measured using a microplate reader in less than 15 min after adding the stop solution. A standard linear regression curve was established using the concentration of the standard as abscissa and the corresponding OD value for the vertical axis, the concentration of each sample was calculated by the curve equation.

2.8 Calculating the contents of p-eNOS ser^{1177} and VE-cadherin in the ischemic myocardium by Western blot

One hundred milligram of myocardium from the anterior wall of the left ventricle was removed from the rats of each group. After pyrolysis by protein lysis buffer, the protein concentration was determined by BCA Protein Assay Kit. Equal amounts of protein were loaded per lane onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. After SDS-PAGE electrophoresis, the proteins were transferred to polyvinylidene fluoride (PVDF) membrane and blocked for one hour at room temperature. The membrane was then incubated in primary antibodies (1: 200 dilution for p-eNOS Ser^{1177} and VE-cadherin and 1: 400 dilution for β-actin) and hybridized overnight at 4℃. Next, the membrane was put in secondary antibody (1: 2000 dilution) and incubated for one hour at room temperature. After that, the membrane was developed using electo chemiluminescence (ECL) detection reagent. Using Image Pro. 6.0 Software, we determined the expression level of the target protein as the ratio of the gray values of target protein band to that of the internal control band.

2.9 Statistical analysis

All the data were analyzed by SPSS 17.0 software and were expressed as mean ± SE ($\bar{\chi} \pm s$). Comparisons between groups were tested by One-Way ANOVA analysis and least significance difference (LSD) test. A value of $P < 0.05$ (two sided) was considered significant.

3 Results

3.1 Effects of tirofiban on AAR, ANR and AN of the rats' myocardium

There was no significant difference of the AAR between different groups after 60 min ischemia and 120 min reperfusion ($P > 0.05$). Compared with AMI/R group, the ANR and NA were significantly reduced in both Tiro group and Tiro+L-NNA group ($P < 0.05$). In addition, Tiro+L-NNA group showed increased ANR and AN compared with Tiro group (all $P < 0.05$), suggesting that the effects of tirofiban were partially blocked by *L*-NNA. (Figure 1, Table 1).

3.2 Effects of tirofiban on hemodynamic in rats with ischemia/reperfusion

Compared with Sham group, AMI/R group, Tiro group and Tiro+L-NNA group showed decreased HR, LVESP, and $\pm dp/dt_{max}$, but increased LVEDP, all the differences were statistically significant ($P < 0.05$); compared with AMI/R group, HR, LVESP, $\pm dp/dt_{max}$ were higher in Tiro group and Tiro + L-NNA group, but LVEDP was lower, all the differences were statistically significant ($P < 0.05$); there was no significant difference of HR, LVESP, $\pm dp/dt_{max}$ or LVEDP between Tiro group and Tiro + L-NNA group ($P > 0.05$); (Table 2).

3.3 Effects of tirofiban on NOS activity of the rats' myocardial ischemic area

Compared with Sham group, eNOS activity in ischemic myocardium was decreased in AMI/R group, Tiro group and Tiro+L-NNA group, but iNOS activity was significantly increased ($P < 0.05$), the differences were statistically significant. Compared with AMI/R group, Tiro group showed significantly higher eNOS activity but lower iNOS activity ($P < 0.05$). Compared with Tiro group, eNOS activity was significantly lower in Tiro + L-NNA group ($P < 0.05$),

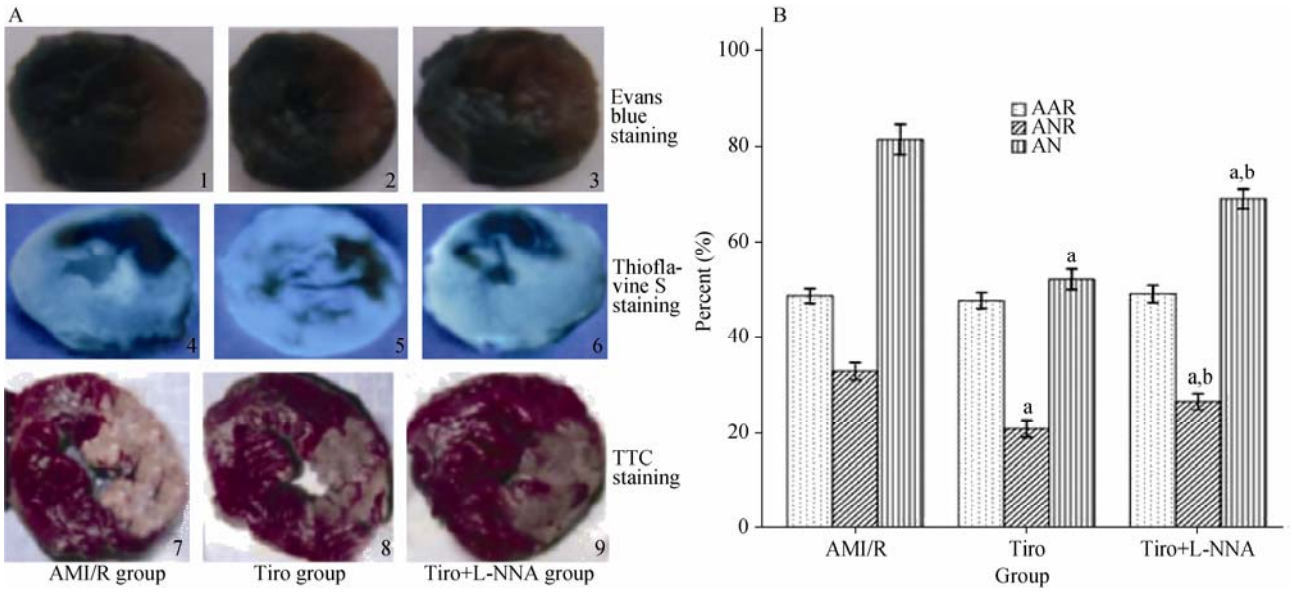

Figure 1. Sizes of the AAR, ANR, and AN. (A): Representative series of images of risk, no-reflow, and infarction at the left ventricular muscle. 1–3: the blue area was non-ischemic area after Evans blue staining; the other area is AAR. 4–6: the fluorescent area was reflow area after thioflavine S staining; the non-fluorescent area was ANR. 7–9: the pale area was AN after TTC staining; the reddish brown area was non-infarct size. Tirofiban (2, 5 and 8) decreased the ANR and AN compared with AMI/R group (1, 4 and 7) and Tiro+L-NNA group (3, 6 and 9). (B): AAR, ANR, and AN after 60 min of ischemia and 120 min of reperfusion. Tirofiban significantly reduced the ANR and AN compared with AMI/R group and Tiro+L-NNA group. [a]$P < 0.05$ *vs*. AMI/R group; [b]$P < 0.05$ *vs*. Tiro group. AAR: area at risk; AMI/R: acute myocardial infarction/reperfusion; AN: area of necrosis; ANR: area of no-reflow; L-NNA: N-nitro-L-arginine; Tiro: tirofiban; TTC: triphenyl tetrazolium chloride.

Table 1. The effects of tirofiban on AAR, ANR and AN (%, $\bar{\chi} \pm s$, $n = 8$).

Group	AAR	ANR	AN
AMI/R group	48.59 ± 1.78	32.81 ± 2.11	81.32 ± 3.68
Tiro group	47.68 ± 1.92	20.83 ± 2.13[a]	52.08 ± 2.62[a]
Tiro+L-NNA group	49.03 ± 2.17	26.56 ± 2.03[a,b]	68.87 ± 2.44[a,b]

[a]$P < 0.05$ *vs*. AMI/R group; [b]$P < 0.05$ *vs*. Tiro group. AAR: area at risk; AMI/R: acute myocardial infarction/reperfusion; AN: area of necrosis; ANR: area of no-reflow; L-NNA: N-nitro-L-arginine; Tiro: tirofiban.

Table 2. Effects of tirofiban on hemodynamic in rats with ischemia/reperfusion ($\bar{\chi} \pm s$, $n = 8$).

Group	HR (beats/min)	LVESP (mmHg)	LVEDP (mmHg)	+dp/dt$_{max}$ (mmHg/s)	-dp/dt$_{max}$ (mmHg/s)
Sham group	330.5 ± 3.7	106.9 ± 1.8	9.9 ± 1.2	4298 ± 208	3796 ± 178
AMI/R group	223.0 ± 5.6[a]	80.0 ± 2.4[a]	28.1 ± 2.5[a]	2784 ± 265[a]	2471 ± 202[a]
Tiro group	320.2 ± 5.3[a,b]	92.8 ± 2.2[a,b]	16.9 ± 2.2[a,b]	3476 ± 215[a,b]	3048 ± 135[a,b]
Tiro+L-NNA group	314.8 ± 4.5[a,b]	90.7 ± 2.1[a,b]	18.3 ± 2.1[a,b]	3298 ± 205[a,b]	2889 ± 215[a,b]

[a]$P < 0.05$ *vs*. Sham group; [b]$P < 0.05$ *vs*. AMI/R group. AMI/R: acute myocardial infarction/reperfusion; ±dp/dt$_{max}$: maximum change rate of left ventricular pressure rise and fall; HR: Heart rate; L-NNA: N-nitro-L-arginine; LVEDP: left ventricular end diastolic pressure; LVESP: left ventricular end systolic pressure; Tiro: tirofiban.

whereas there was no significant difference of iNOS activity between the two groups ($P > 0.05$). (Figure 2, Table 3).

3.4 Effects of tirofiban on eNOS, p-eNOS Ser^{1177} and VE-cadherin of the rats' myocardial ischemic area

ELISA results showed there was no significant difference of eNOS content in ischemic myocardium among four groups ($P > 0.05$). The western blot results suggested that, compared with Sham group, in ischemic region, expression of p-eNOS ser^{1177} was increased and expression of VE-cadherin was reduced in AMI/R group, Tiro group and Tiro + L-NNA group ($P < 0.05$). Compared with AMI/R group,

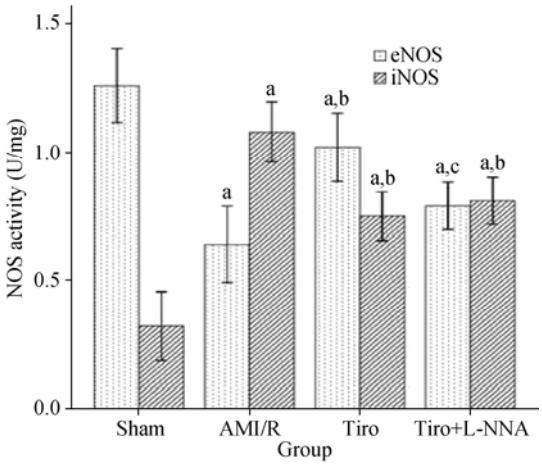

Figure 2. Comparison of NOS activity after 60 min of ischemia and 120 min of reperfusion in ischemic area of the myocardium. [a]$P < 0.05$ *vs*. Sham group; [b]$P < 0.05$ *vs*. AMI/R group; [c]$P < 0.05$ *vs*. Tiro group. AMI/R: acute myocardial infarction/reperfusion; eNOS: endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase; L-NNA: N-nitro-L-arginine; NOS: nitric oxide synthase; Tiro: tirofiban.

Tiro group had significantly higher level of p-eNOS ser[1177] and VE-cadherin (all $P < 0.05$). The expression of p-eNOS ser[1177] and VE-cadherin was then reduced in the Tiro+L-NNA group compared with Tiro group ($P < 0.05$), suggesting that L-NNA partially inhibited the effects of tirofiban (Figure 3, Table 4).

Table 3. Effects of tirofiban on NOS activity of the rats' myocardial ischemic area ($\bar{\chi} \pm s$, $n = 8$).

Group	eNOS (U/mg)	iNOS(U/mg)
Sham group	1.26 ± 0.17	0.32 ± 0.16
AMI/R group	0.64 ± 0.18[a]	1.08 ± 0.14[a]
Tiro group	1.02 ± 0.16[a,b]	0.75 ± 0.11[a,b]
Tiro+L-NNA group	0.79 ± 0.11[a,c]	0.81 ± 0.11[a,b]

[a]$P < 0.05$ *vs*. Sham group; [b]$P < 0.05$ *vs*. AMI/R group; [c]$P < 0.05$ *vs*. Tiro group. AMI/R: acute myocardial infarction/reperfusion; eNOS: endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase; L-NNA: N-nitro-L-arginine; NOS: nitric oxide synthase; Tiro: tirofiban.

4 Discussion

The present study showed that: (1) fluorescent dye deletion area can be observed during reperfusion in ischemic rats, suggesting there was no-reflow phenomenon in the rats underwent 60 min acute myocardial ischemia followed with

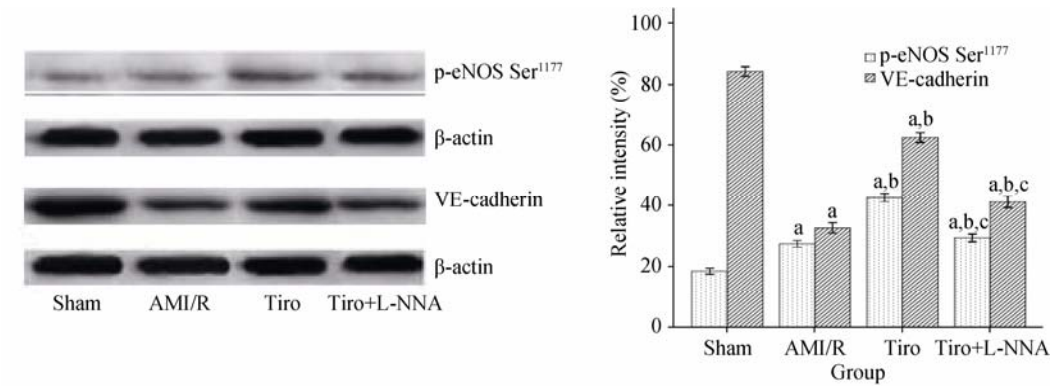

Figure 3. (A): Expression of p-eNOS Ser[1177], VE-cadherin and internal control β-actin of the rats' ischemic myocardium; (B) Expression of p-eNOS Ser[1177] and VE-cadherin was upregulated after 60 min of ischemia and 120min of reperfusion compared with AMI/R group and Tiro+L-NNA group. [a]$P < 0.05$ *vs*. Sham group; [b]$P < 0.05$ *vs*. AMI/R group; [c]$P < 0.05$ *vs*. Tiro group. AMI/R: acute myocardial infarction/reperfusion; eNOS: endothelial nitric oxide synthase; L-NNA: N-nitro-L-arginine; p-eNOS Ser1177: phosphorylated eNOS at ser[1177]; Tiro: tirofiban; VE: vascular endothelial.

Table 4. Effects of tirofiban on eNOS, p-eNOS Ser[1177] and vascular endothelial cadherin of the rats' myocardial ischemic area ($\bar{\chi} \pm s$, n=8).

Group	eNOS (ng/mg)	p-eNOS ser[1177](%)	Vascular endothelial cadherin (%)
Sham group	38.63 ± 2.14	18.45 ± 1.15	84.32 ± 1.84
AMI/R group	36.48 ± 2.07	27.36 ± 1.27[a]	32.54 ± 2.10[a]
Tiro group	38.20 ± 2.26	42.51 ± 1.63[a,b]	62.46 ± 1.92[a,b]
Tiro+L-NNA group	37.22 ± 2.62	29.30 ± 1.55[a,b,c]	41.08 ± 2.24[a,b,c]

[a]$P < 0.05$ *vs*. Sham group; [b]$P < 0.05$ *vs*. AMI/R group; [c]$P < 0.05$ *vs*. Tiro group. AMI/R: acute myocardial infarction/reperfusion; eNOS: endothelial nitric oxide synthase; L-NNA: N-nitro-L-arginine; p-eNOS Ser[1177]: phosphorylated eNOS at ser[1177]; Tiro: tirofiban.

120 min reperfusion, accompanied by cardiac microvascular endothelial structure and function destruction; (2) tirofiban treatment significantly reduced myocardial no-reflow and ischemia-reperfusion injury, improved heart function, by alleviating myocardial microvascular structural and endothelial dysfunction in the ischemic area; (3) NOS inhibitor L-NNA can partially eliminate the effects of tirofiban, suggesting the effects of tirofiban may related to the increased eNOS activity.

Although the main mechanism underlying the effects of tirofiban in reducing no-reflow were thought to be mediated by the inhibition of the formation of microthrombi,[8] there are other possible reasons behind triofiban's effects implicated by this and other studies. For example, Kunichika *et al.*[9] have found that tirofiban could remarkably reduce ANR and AN in dogs with non-thrombus coronary artery occlusion. Besides, Yang *et al.*[10] have noticed that tirofiban could reduce ANR and AN in pigs received reperfusion following AMI, while the combined use of clopidogrel and aspirin could not. The current study has proved that tirofiban could reduce ANR and AN of the rats' myocardium without coronary artery thrombi, indicating that inhibition of the formation of microthrombi may not be the only mechanism of GP Ⅱb/Ⅲa receptor blockade for reducing ANR.

Increasing amount of evidences had shown that the damage to the structure and function of microvascular endothelium had played a crucial role in no-reflow phenomenon. Endothelial dysfunction is characterized by the decreased production of endothelium-derived nitric oxide (NO),[11] which is produced from L-arginine by the NOS. NOS within myocardium has two major subtypes: eNOS and iNOS. eNOS is mainly found in vascular endothelial cells while iNOS is produced by inflammatory cells following stimulation. The NO derived from eNOS can protect the structure and function of vessels by regulating vascular tone, inhibiting the aggregation and adhesion of platelets and white cells, and decreasing oxidative stress; while the NO derived from iNOS can interact with oxygen, produce excessive peroxynitrite ($ONOO^-$), thus results in myocardial ischemia-reperfusion injury and endothelial dysfunction[12]. The current study has shown that in comparison to AMI/R group, tirofiban enhanced eNOS activity, decreased iNOS activity and reduced area of no-reflow after reperfusion following AMI, this is consistent with the findings of Liu *et al.*[4] This result suggests that tirofiban might improve vascular endothelial function and reduce NR by two means. On one hand, it could inhibit the activation of inflammatory cells during AMI/R, reduce iNOS activity and iNOS-derived NO and decrease endothelial damage. On the other hand, it could increase eNOS activity, increase the production of endothelium-derived NO and improve vascular endothelial function.

Our study has found that tirofiban increased the total activity of eNOS, but did not change its expression at protein level, indicating that tirofiban may affect the production of NO by enhancing the unit activity of eNOS. Studies have reported several factors that might affect eNOS activity, including the tyrosine phosphorylation of caveolin on endothelial cell membranes,[13] the increase of endothelial cell calcium concentration,[14] the phosphorylation of eNOS Ser^{1177},[15] and the increase of enzyme activity induced by tetrahydrobiopterin during ischemia-reperfusion.[16] Moreover, we have shown that tirofiban treatment can increase the expression of p-eNOS ser^{1177} in ischemic myocardium, indicating tirofiban may up-regulate the eNOS activity through the increase of phosphorylation of eNOS ser^{1177} during ischemia-reperfusion. We also found that the heart protective effects of tirofiban was related to the increased eNOS activity, as the protective effects of Tirofiban can be partially eliminated by eNOS inhibitor L-NNA. In current study, we further found that tirofiban up-regulate the eNOS activity by increasing the phosphorylation of eNOS ser^{1177}. eNOS, which plays an important role in ischemia-reperfusion injury, was mainly regulated by the PI3K/Akt pathway. However, it's still unclear that whether tirofiban- induced phosphorylation of eNOS ser^{1177} is related with the activation of the PI3K/Akt pathway.

As the most important adhesion protein of endothelial cells, VE-cadherin not only maintains the structural integrity of vascular endothelia, but also controls the signal transduction between cells.[17] The dysfunction of VE-cadherin is the most important cause of myocardial interstitial edema and inflammatory cellular exudation.[18]After myocardial ischemia-reperfusion, inflammatory cells aggregate locally and release proteinase, resulting in the decomposition of VE-cadherin, which is sensitive to proteinase, and the increase of vascular permeability.[19] We have found that VE-cadherin within ischemic myocardium decreased remarkably after ischemia-reperfusion, which is consistent with previous study.[20] Tirofiban administration during the Ischemia-reperfusion can inhibit the degradation of VE-cadherin and protect the structural integrity of endothelial cells, suggesting that the protective microvascular endothelial structure may be one of the mechanisms of tirofiban effects in reducing no-reflow. Combined use of L-NNA reduced the content of VE-cadherin, suggesting that VE-cadherin expression may be related to eNOS. The intracellular signaling pathway of VE-cadherin is not yet clear, many protein kinases and pathways are involved, including PKC, Ca^{2+}-PKG pathway, tyrosine kinase pathway, small G protein Rho signaling pathway and MAPK pathway.[21] We have found that tirofiban could decrease the activity of iNOS within ischemic myocardium in rats. Together, it could be

inferred that tirofiban increases the expression of VE-cadherin, protects the structural integrity of vascular endothelium and reduces inflammatory cellular exudation.

LVESP, LVEDP, dp/dt_{max} are commonly used indicators for the evaluation of myocardial function, decrease of LVESP and $+dp/dt_{max}$ reflect the decline in left ventricular systolic function, increase of LVEDP and reduction of $-dp/dt_{max}$ reflects the decline of left ventricular diastolic function. Hemodynamic parameters measured in this study showed that tirofiban increased HR, LVESP, dp/dt_{max}, and reduced LVEDP, suggesting tirofiban administration can increase contraction force, ventricular compliance, and improve heart function.

To summarize our current study, we found that tirofiban could induce the phosphorylation of eNOS within ischemic myocardium after AMI/R in rats, increase the activity of eNOS enzyme and enhances the production of endothelium-derived NO, leading to the protection of the microvascular endothelial function. Tirofiban could also increase the content of VE-cadherin, decrease inflammatory exudation and relieve the structural damage to vascular endothelium, thus reduce no-reflow, suggesting tirofiban-mediated reduction of myocardial no-reflow and ischemia-reperfusion injury might be related with the protection of microvascular endothelial structure and function, and the maintenance of structural and functional integrity of microvascular endothelial was partially regulated by eNOS activity. This study provides a new theoretical basis for the clinical application of tirofiban in prevention and treatment of NR. However, the mechanisms of no-reflow phenomenon are complicated. How tirofiban regulates NOS and VE-cadherin to protect the structure and function of microvascular endothelia still awaits further study to clarify.

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