

Role of endothelial apoptosis induced by LPS in myocardial no-reflow after ischemia and reperfusion

Yunwei Li¹, Yanyan Zhao¹, Guojie Yang^{1,*}, Qiutang Zeng², Jin Du¹

¹The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China; ²Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei 430022, China

Received January 12, 2008

Abstract

Objective. To evaluate the role of endothelial apoptosis in myocardial no-reflow during ischemia and reperfusion (I/R) injury. **Methods.** Coronary artery was occluded for 30 minutes followed by 2 hours reperfusion in group I/R. Rabbits were treated with lipopolysaccharide (LPS, 50 µg/kg, *i.v.*) for 5 hours before ischemia/reperfusion in group LPS. Size of no-reflow zone was determined by injecting thioflavin S immediately before the hearts were excised. Endothelial apoptosis were determined by *in situ* TDT-mediated dUTP nick end labeling. **Results.** LPS could induce coronary endothelial cell apoptosis. Compared with those in I/R group, both size of no-reflow zone and myocardial infarction zone in group LPS increased significantly. **Conclusions.** Apoptosis of endothelial cell of coronary artery induced by LPS could increase no-reflow in rabbit hearts. [Life Science Journal. 2008; 5(1): 35 – 37] (ISSN: 1097 – 8135).

Keywords: no-reflow; endothelial cell; apoptosis; myocardial infarction; LPS

1 Introduction

Coronary reperfusion is a main therapy for acute myocardial infarction, which saves myocardium, improves ventricular function and survival rate after infarction. But an acute reduction in coronary flow in the absence of epicardial vessel obstruction was frequently seen after ischemia and reperfusion. It was called as no-reflow phenomenon^[1]. No-reflow was seen in 0.6% to 3.1% of percutaneous coronary intervention (PCI) cases. As a result of no-reflow, patients were more likely to experience other cardiac events, including myocardial infarction (MI), left ventricular systolic dysfunction, ventricular arrhythmias and cardiac rupture^[2,3]. Although it is clear that abnormalities of the microvasculature cause the no-reflow, the exact mechanism is uncertain, and a variety of factors probably contribute to it.

Myocardial apoptosis is a genetically programmed form of cell death that primarily triggered during reperfusion through various mechanisms. Apoptosis has become increasingly recognized as one mechanism of cell death during ischemia/reperfusion (I/R) injury^[4]. However, the

contribution of myocardial apoptosis to myocardial no-reflow after reperfusion remains unknown. In this study, we determined the endothelial apoptosis after no-reflow induced by ischemia/reperfusion.

2 Materials and Methods

2.1 Animal preparation

Twenty-four New Zealand white rabbits, weighing 2.0 – 2.5 kg, provided by the Center of Experimental Animals of Henan.

Rabbits were randomly divided into 3 groups (8 rabbits of each group): control group (group C), group I/R and group LPS. Group C: rabbits were anesthetized for 120 minutes, and performed thoracotomy, without coronary occlusion. Group I/R: rabbits were subjected to left coronary artery occlusion (ischemia) for 30 minutes followed by restoring blood flow reperfusion for 120 minutes (reperfusion). Group LPS: rabbits were performed as group I/R, except transfusing intravenously lipopolysaccharide (LPS, 50 µg/kg) for 5 hours beforehand.

Rabbits were anesthetized with an intravenous injection of chloral hydrate (3 ml/kg). A left thoracotomy was performed in the fourth intercostal space, and the pericar-

*Corresponding author. Email: zhday@126.com

dium was opened. A silk thread was then passed around the left circumflex branch (LCX) of the coronary artery, with its ends being threaded through a small polyethylene tube. Electrocardiography was monitoring by bipolar limb leads. Coronary occlusion was by pulling the thread and clamping the artery with a mosquito hemostat. Reperfusion was by releasing the clamp. Myocardial ischemia was confirmed by ST-segment elevation of the ECG as well as observation of regional cyanosis over the myocardial surface. Reperfusion was confirmed by reactive hyperemia over the surface after the snare was released.

2.2 Measurement of the size of no-reflow zone

After reperfusion for 120 minutes, 4% thioflavin S (1 ml/kg) was injected into left ventricle (the zone of reperfusion was stained, while the zone of no-reflow could not be stained). Then occluded the LCX again and 10 ml of 1% Evan's blue was injected into left ventricle to stain the non-ischemic myocardium. The heart was quickly excised and placed on ice. After washing, the free wall apart from left ventricle were removed, and the left ventricle was then divided transmurally into ischemic and non-ischemic regions. Serial 5-mm sections were cut from the apex to bottom of the left ventricle, and slides were observed under the ultraviolet light. The zone of reperfusion appeared as fluorescence, but zone of ischemia was not stained.

2.3 Infarct size

Stained the ischemic myocardium with the 1% triphenyltetrazolium chloride (TTC, pH 7.4, 37 °C) for 15 minutes. Non-infarct myocardium was red, while infarct myocardium was pale. The infarct myocardium was separated from the non-infarct myocardium, and weighed them respectively.

2.4 Detection of apoptosis

Apoptotic myocardial nuclei were identified histologically using TUNEL. Immediately after the experiments, tissues which were not stained by thioflavin S from the hearts were treated with 4% formaldehyde for 24 hours, graded dehydrated (70% ethanol, 95% ethanol, 100% ethanol, and then xylene), and embedded in paraffin. Sections (5 μm) were prepared using the TUNEL kit (Zhongshan Jinqiao Co, Beijing). Slides were examined under an Olympus BX41 light microscope.

2.4 Statistical analysis

All data were presented as mean ± SD ($\bar{X} \pm SD$). Comparisons of two means were analyzed by Student's *t* test with groups. Analysis of variance was used for multiple comparisons among groups. Significance was evaluated

using SPSS10.0. A value of $P < 0.05$ was considered significant.

3 Results

3.1 Size of no-reflow zone

Myocardium in reperfusion zone appeared as fluorescence under the ultraviolet light, and no changes was observed in no-reflow zone. There was no significant difference between the size of ischemic myocardium in group I/R and group LPS, but the size of no-reflow zone in group LPS was significantly larger than that in group I/R (Table 1).

Table 1. Size of no-reflow and MI ($\bar{X} \pm s$)

Group	No-reflow	MI (mg)	MI (%)
I/R	21.24 ± 5.13	298.2 ± 18.2	29.7 ± 1.8
LPS	32.61 ± 5.36*	389.3 ± 15.7*	39.0 ± 1.5*

*: Compared with group I/R, $P < 0.05$.

3.2 Infarct size

The area was significantly bigger in group LPS than that in group I/R (Table 1).

3.3 Endothelial cell apoptosis

Apoptosis cell nuclei stained yellow and all normal nuclei were blue. No apoptosis was detected in control group; a few endothelial cell apoptosis appeared in group I/R, and significantly more endothelial cell apoptosis appeared in group LPS ($P < 0.05$)(Figure 1).

4 Discussion

No-reflow manifests as an acute reduction in coronary blood flow in the absence of epicardial vessel obstruction after ischemia followed by reperfusion. It was first described in humans almost 20 years ago. No-reflow was seen in 0.6% to 3.1% of PCI cases. Reperfusion therapy has become one of the pivotal goals in acute myocardial infarction, so the resulting no-reflow has obtained increasing attention in clinical practice. However, the exact cause of this phenomenon is unknown. Previous studies suggested that some factors, such as endothelial cell injury^[5], oxygen radical production, vascular spasm, platelet aggregation, activation of coagulation cascade^[6], and atherosclerotic plaque^[7], might be the causes. But several treatments to these factors, such as coronary vasodilators, thrombolysis, gave the limited therapeutic and preventa-

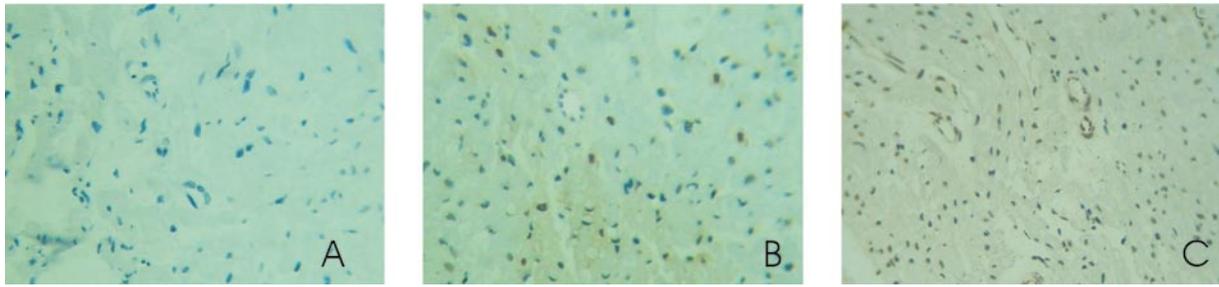


Figure 1. Effect of LPS on endothelial apoptosis ($\times 400$). A: No apoptosis cells in group C. B: Apoptosis cells in group I/R. C: Apoptosis cell in group LPS.

tive effect. It was reported^[8] that more endothelial cell apoptosis in coronary circulation with no-reflow than that with reperfusion, suggesting that endothelial apoptosis may play a role in the development of no-reflow.

Apoptosis in coronary endothelial cells of rabbits was induced by intravenous injection of LPS in this research. After 30 minutes of ischemia followed by 120 minutes of reperfusion, no-reflow appeared in all zone of ischemic myocardium. We also observed that the zone of no-reflow was smaller than that of myocardial infarction, and the zone of no-reflow was within MI region. All these suggested that the size of no-reflow was correlated with the size of MI. This study, using LPS inducing coronary endothelial apoptosis, elucidated the relationship between endothelial cell apoptosis and the development of no-reflow.

LPS induces endothelial apoptosis^[9], similar to the state after the rupture of atherosclerotic plaque. Our data showed that significantly more endothelial cell apoptosis appeared in group LPS including those in larger vessels than those in the group I/R, and the size of no-reflow zone in group LPS also exceeded the size in group I/R. It has been suggested that increased endothelial apoptosis led to atheroma denudation and subsequent coronary thrombosis^[10]. We speculate that no-reflow occur when the capacity of the regional coronary microvascular bed is exhausted. The size of infarcted myocardium in group LPS was more than that in group I/R, so the no-reflow might sharpen the myocardial necrosis, confirming the previous study^[2].

5 Conclusion

These data indicated that endothelial apoptosis induced by LPS played a role in the no-reflow. Strategies based on its inhibition may allow endothelial cell rescue and protect the myocardium. Further study is ongoing and the results from studies of inhibiting endothelial apoptosis are

eagerly awaited.

References

1. Schofer J, Montz R, Mathey DG. Scintigraphic evidence of the “no reflow” phenomenon in human beings after coronary thrombolysis. *J Am Coll Cardiol* 1985; 5: 593 – 8.
2. Kang S, Yang Y. Coronary microvascular reperfusion injury and no-reflow in acute myocardial infarction. *Clin Invest* 2007; 30(3): E133 – 45.
3. Heper G, Korkmaz ME, Kilic A. Reperfusion arrhythmias: Are they only a marker of epicardial reperfusion or continuing myocardial ischemia after acute myocardial infarction? *Angiology* 2007 – 2008; 58(6): 663 – 70.
4. Timurkaynak T, Arslan U, Balcioglu S, *et al.* Long term tirofiban infusion before percutaneous coronary intervention in patients with angiographically massive intracoronary thrombus. *Saudi Med J* 2008; 29(1): 42 – 7.
5. Brosh D, Assali AR, Maqer A, *et al.* Effect of no-reflow during primary percutaneous coronary intervention for acute myocardial infarction on six-month mortality. *Am J Cardiol* 2007; 99(4): 442 – 5.
6. Yaoita H, Ogawa K, Maehara K, *et al.* Apoptosis in relevant clinical situations: contribution of apoptosis in myocardial infarction. *Cardiovasc Res* 2000; 45: 630 – 41.
7. Black SC, Huang JQ, Rezaiefar P, *et al.* Co-localization of the cysteine protease caspase-3 with apoptotic myocytes after *in vivo* myocardial ischemia and reperfusion in the rat. *J Mol Cell Cardiol* 1998; 30: 733 – 42.
8. Bonderman D, Teml A, Jakowitsch J, *et al.* Coronary no-reflow is caused by shedding of active tissue factor from dissected atherosclerotic plaque. *Blood* 2002; 99(8): 2794 – 800.
9. Topol EJ, Yadav JS. Recognition of the importance of embolization in atherosclerotic vascular disease. *Circulation* 2000; 101: 570 – 80.
10. Wei YM, Hong M, Zeng QT, *et al.* Role of local coronary circulating endothelial cell apoptosis and prethrombosis in no-reflow phenomena in myocardial infarction patients under direct PTCA. *Journal of Clinical Cardiology* 2006; 22(5): 259 – 62.
11. Bombeli T, Schwartz BR, Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood* 1999; 93: 3831 – 8.