Determination of myocardial ischemia and degree of reperfusion based on optical coherence tomography

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Received July 15, 2010; accepted September 10, 2010; posted online January 28, 2011

The feasibility of applying optical coherence tomography (OCT) in determining the degree of myocardial ischemia-reperfusion injury is assessed. The left anterior descending coronary artery of 90 Sprague-Dawley rats are ligated and reperfused at different times. The total attenuation coefficient obtained from the OCT images in the experimental group keeps increasing with reperfusion time and highly correlates with the histopathological characteristics ($P < 0.01$). We present evidence proving the feasibility of using OCT for evaluating myocardial ischemia reperfusion.

OCIS codes: 170.4580, 170.4500, 170.2655.
doi: 10.3788/COL201109.021701.

Cardiovascular disease is currently one of the most serious threats to human life. Myocardial ischemic disease is one of the most common forms of cardiovascular disease. Recently, a variety of new devices for minimally invasive diagnoses and treatment has been developed for myocardial ischemic disease, such as percutaneous transluminal coronary angioplasty (PTCA)[1], stent implantation, and transmyocardial laser revascularization (TMR)[2,3]. However, surgical revascularization after myocardial ischemia is still one of the most important treatments for myocardial ischemia disease. Therefore, determining the scope and degree of myocardial ischemic injury is important in the operation and to assess whether myocardial injury is aggravated after cardiac arrest and operation. These methods are of great importance for myocardial protection, surgery efficacy assessment, and postoperative care program development.

Several methods are currently used for detecting myocardial ischemia-reperfusion injury, such as positron emission tomography[4] and ^{201}Tl myocardial imaging[5]. However, these are expensive and require the use of hazardous radioactive substances, hence are not recommended for repeated use. A new imaging technology, optical coherence tomography (OCT), can capture real-time images of living organisms without the risk of radiation injury[6,7]. Considerable attention has been devoted to applying OCT coronary imaging. However, *in vivo* OCT applications relevant to myocardial ischemia and reperfusion are relatively few. We therefore aim to assess the feasibility of using OCT technology for determining the degree of myocardial ischemia reperfusion.

Considering the similarity of their myocardial ultrastructure to that of humans[8], rats are provide an ideal animal model for the study of human myocardial ischemic-reperfusion injury. The anterior descending arteries of rats were blocked and blood flow was reestablished in time to induce acute myocardial ischemia-reperfusion, simulating the treatment of myocardial ischemic disease. This mature experiment model is widely used in medical research. Healthy male Sprague–Dawley (SD) rats weighing 250−300 g were purchased from the National Rodent Laboratory Animal Resources, Shanghai Branch. All rats were randomly assigned into three groups: the short-term ischemia-reperfusion group (left anterior descending artery (LAD) ligation 15 min, S group) and the long-term ischemia-reperfusion group (LAD ligation 60 min, L group) as the experimental groups, and the control group (C group). Six rats were enrolled in each group at time points before and after ligation, and at different time points of reperfusion (5, 30, 60, 90, and 120 min). This study was performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85–23, revised 1996) and was approved by the Animal Care and Use Committee of the Fujian Medical University. Then, OCT images of the myocardial regions were obtained. The rat hearts were removed at the corresponding time point for each group and then fixed in 10% formalin. The general morphology of the sections was evaluated with hematoxylin-eosin (HE) staining and TdT-mediated dUTP nick end labeling (TUNEL) apoptosis detection (Roche, Germany). The semi-quantitative scoring system by Horwitz *et al.*[9] was used to evaluate cardiac pathology.

The OCT system used in this study is described in detail in our previous publication[10]. The OCT system is also briefly depicted in other studies[11−15]. The axial resolution of the OCT system in air is about 10 μm and the lateral resolution is roughly 20 μm. In a superficial layer of tissue, the OCT signal could be expressed as $I \propto \exp(-\mu_t z)$, $\mu_t = \mu_s + \mu_a$, where μ_t is the total attenuation coefficient; μ_s and μ_a are the scattering and absorption coefficients, respectively. The OCT signals are usually displayed logarithmically and are given by $s = x - \mu_t z + b$, where b is a constant. The total attenuation coefficient μ_t of tissue could be extracted using a linear

Table 1. μ_t on Each Set Time in Three Groups ($\overline{\chi}$±s)

Time	n	Group		
		S	L	C
Pro-Ligation	6	54.83±1.58	54.83±1.58	52.83±3.24
Post-Ligation	6	20.30±1.12	18.72±0.56	51.18±4.50
Reperfusion (5 min)	6	27.56±2.01*♠	20.56±0.23*♠	53.80±3.03△
Reperfusion (30 min)	6	34.15±1.17*♠	28.20±1.18*♠	52.51±2.10△
Reperfusion (60 min)	6	48.27±2.57*♠	40.47±1.50*♠	51.14±2.54△
Reperfusion (90 min)	6	51.17±1.35*♠	44.51±2.35*♠	52.16±1.94△
Reperfusion (120 min)	6	54.17±2.04♠	48.08±2.04*♠	51.14±1.04△

*Treatment effects: the μ_t values of the experimental group after reperfusion were significantly increased. Compared with the C group, the differences between the two groups were significant ($P<0.05$); ♠the difference of the experimental group at different time points after reperfusion was statistically significant (all $P<0.05$); △the difference of the C group at different time points after reperfusion was not statistically significant ($P>0.05$).

Table 2. Apoptotic Index on Each Set Time in the Three Groups ($\overline{\chi}$±s)

Time	n	Group		
		S	L	C
Pro-Ligation	6	0.10±0.02	0.15±0.02	0.13±0.04
Post-Ligation	6	8.99±0.49	13.98±0.19	0.18±0.07
Reperfusion (5 min)	6	7.42±0.27*♠	10.07±0.23*♠	0.17±0.03△
Reperfusion (30 min)	6	6.88±0.17*♠	8.98±0.18*♠	0.16±0.05△
Reperfusion (60 min)	6	3.27±0.17*♠	5.47±0.26*♠	0.18±0.06△
Reperfusion (90 min)	6	1.17±0.49*♠	4.56±0.35*♠	0.16±0.04△
Reperfusion (120 min)	6	0.17±0.04♠	2.48±0.04*♠	0.17±0.06△

*Treatment effects: the AI of the experimental group after reperfusion was significantly decreased. Compared with the control group, the differences between the two groups were significant ($P<0.05$); ♠the difference of the experimental group at different time points after reperfusion was statistically significant (all $P<0.05$); △the difference of the control group at different time points after reperfusion was not statistically significant ($P>0.05$).

Table 3. Hematoxylin-Eosin Injury Score on Each Set Time in the Three Groups ($\overline{\chi}$±s)

Time	n	Group		
		S	L	C
Pro-Ligation	6	0.03±0.01	0.02±0.01	0.03±0.02
Post-Ligation	6	4.60±0.32	6.16±0.29	0.03±0.01
Reperfusion (5 min)	6	4.42±0.37*♠	5.07±0.14*♠	0.02±0.01△
Reperfusion (30 min)	6	3.88±0.26*♠	4.35±0.23*♠	0.03±0.01△
Reperfusion (60 min)	6	1.52±0.17*♠	2.71±0.16*♠	0.03±0.02△
Reperfusion (90 min)	6	0.67±0.09*♠	1.46±0.36*♠	0.02±0.01△
Reperfusion (120 min)	6	0.07±0.06♠	0.81±0.04*♠	0.03±0.01△

*Treatment effects: the HE injury score of the experimental group after reperfusion was significantly decreased. Compared with the control group, the differences between the two groups were significant ($P<0.05$); ♠the difference of the experimental group at different time points after reperfusion was statistically significant (all $P<0.05$); △the difference of the control group at different time points after reperfusion was not statistically significant ($P>0.05$).

fitting procedure.

As shown in Table 1, with the anterior descending artery opening, μ_t is gradually increasing in both the S and L groups. This is because an increase in blood flow, and blood has a strong scattering and absorption characteristic at near-infrared light[16]. The C group exhibits no significant change at different time points after the treatment. Table 1 also shows that through a long period of reperfusion, μ_t is restored to normal in the S group, whereas it is not restored to normal in the L group.

Histopathology is the gold standard for determining the extent of myocardial injury. To understand the aforementioned results, HE and TUNEL staining were conducted to determine the myocardial injury score and the apoptosis index (AI), respectively. Tables 2 and 3 show that as treatment continues, the HE injury score and the AI gradually decrease in both the S and L groups, which shows that with the reopening of the anterior descending artery, the extent of myocardial injury is gradually

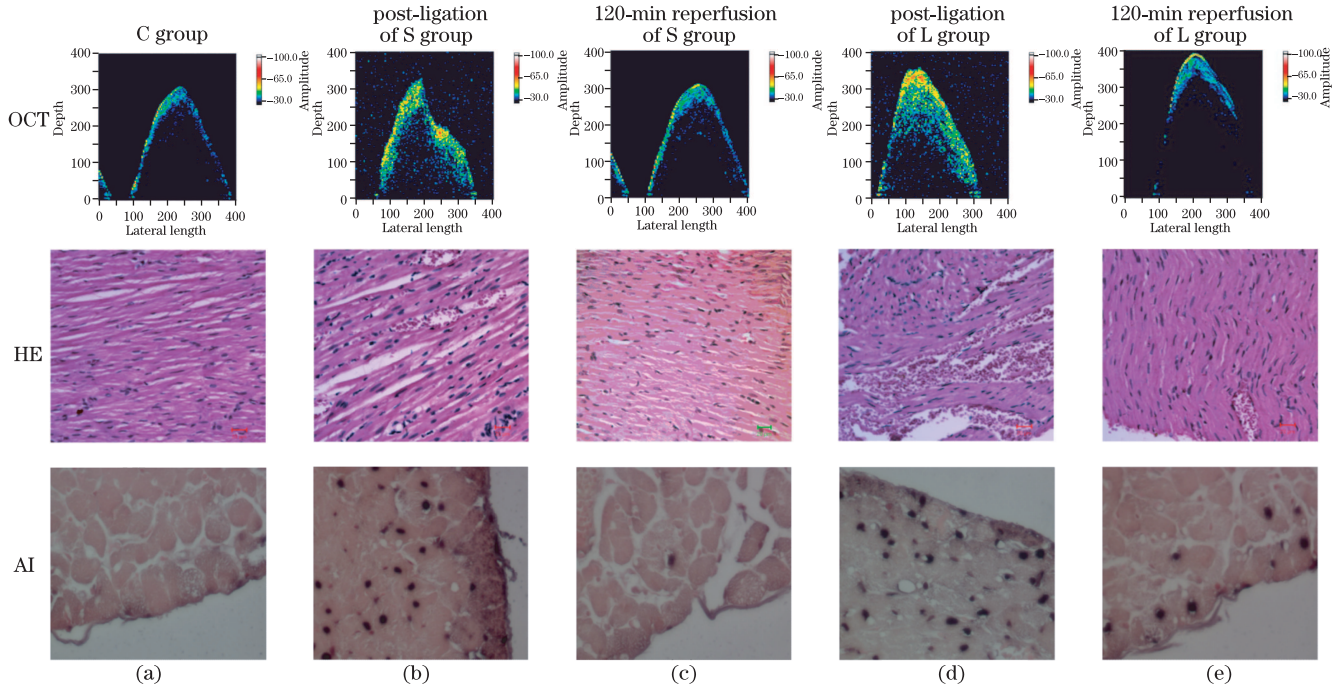

Fig. 1. OCT images and the corresponding HE and AI images of the three groups and the reperfusion group. (a) C group; (b) post-ligation of S group; (c) 120-min reperfusion of S group; (d) post-ligation of L group; (e) 120-min reperfusion of L group.

reduced. However, the L group at 120 min reperfusion had not yet recovered to the level before ligation. The C group showes no significant change at different times after the treatment.

Correlation analysis shows that μ_t is negatively correlated with the histopathological indices ($P < 0.01$). Figure 1 shows that the imaging depth of the OCT images decreases after reperfusion in the S and L groups, whereas the corresponding histopathology indices (HE and AI) are ameliorated. The OCT images in Fig. 1 show that after reperfusion, the S group is restored to its original state, whereas the L group could not be restored to its original level, which correlates with the HE and AI images. The results demonstrate that OCT imaging highly correlates with the histopathological indices during acute myocardial ischemia-reperfusion injury. Thus, OCT can be used to determine the extent of acute myocardial ischemia-reperfusion injury.

After reperfusion at different time points and with treatment time, the HE injury score and AI of the experimental groups were gradually reduced. However, the L group (120 min reperfusion) had not yet recovered to the level before ligation. The C group at different time points after the treatment showed no significant change. Compared with the C group, the differences between the experimental and L groups were significant ($P < 0.05$).

In the S group, the histopathological indices returned to normal after 120 min of reperfusion. However, the L group did not recover to the normal level. This suggests that early intervention is necessary to restore myocardial blood flow after myocardial ischemia. However, the histopathological indices do not reflect the extent of acute myocardial ischemia in real time. This can be by determining the attenuation coefficient from an OCT image, which favors myocardial protection.

In conclusion, OCT imaging highly correlates with the histopathological indices during acute myocardial ischemia-reperfusion injury. This suggests that OCT can be used to determine the extent of acute myocardial ischemia-reperfusion injury.

This work was supported by the Medical Innovation Topics of Health Department of Fujian Province under Grant No. 2009-CX-1.

References

1. K. Rasmussen, M. Maeng, A. Kaltoft, P. Thayssen, H. Kelbaek, H. H. Tilsted, U. Abildgaard, E. H. Christiansen, T. Engstrøm, L. R. Krusell, J. Ravkilde, P. R. Hansen, K. N. Hansen, S. Z. Abildstrøm, J. Aarøe, J. S. Jensen, S. D. Kristensen, H. E. Bøtker, M. Madsen, S. P. Johnsen, L. O. Jensen, H. T. Sørensen, L. Thuesen, and J. F. Lassen, Lancet **375,** 1090 (2010).
2. M. C. Heidt, D. Sedding, S. K. H. Stracke, T. Stadlbauer, A. Boening, P. R. Vogt, and M. Schönburg, Thorac. Cardiovasc. Surg. **57,** 79 (2009).
3. K. B. Allen, J. Kelly, A. M. Borkon, R. S. Stuart, E. Daon, A. F. Pak, G. L. Zorn, and M. Haines, Anesthesiol. Clin. **26,** 501 (2008).
4. M. Wiemer, J. P. Wielepp, O. Lindner, W. Burchert, C. Langer, D. Horstkotte, and T. Butz, Chin. Med. Journal **122,** 2807 (2009).
5. H. Iwado, Y. Iwado, K. Ohmori, K. Mizushige, K. Yukiiri, Y. Takagi, Y. Nishiyama, N. Tamaki, S. Senda, and M. Kohno, Am. J. Cardiol. **93,** 685 (2004).
6. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. G. Fujimoto, Science **254,** 1178 (1991).
7. J.-H. Han, X. Liu, J. U. Kang, and C. G. Song, Chin. Opt. Lett. **8,** 167 (2010).
8. J. Z. Yogaratnam, G. Laden, L. Guvendik, M. Cowen, A. Cale, and S. Griffin, J. Surg. Res. **149,** 155 (2008).
9. L. D. Horwitz, P. V. Fennessey, R. H. Shikes, and Y. Kong, Circulation **89,** 1792 (1994).
10. X. Cai, H. Li, H. Chen, B. Yu, and Z. Li, Acta Photon. Sin. (in Chinese) **37,** (S2) 180 (2008).
11. Z. Li, H. Li, Y. He, S. Cai, and S. Xie, Phys. Med. Boil. **53,** 5859 (2008).
12. A. M. Rollins and J. A. Izatt, Opt. Lett. **24,** 1484 (1999).
13. K. Wang, Z. Ding, T. Wu, and Z. He, Acta Opt. Sin. (in Chinese) **29,** (S) 32 (2009).
14. G. He, C.Liao, H. Jiang, X. Long, and L. Song, Acta, Opt. Sin. (in Chinese) **29,** (S1) 6 (2009).
15. T. Wu and Z. Ding, Chinese J. Lasers (in Chinese) **36,** 503 (2009).
16. Y. Wang, G. Han, Y. Liu, X. Cheng, and W. Frank, Chinese J. Lasers (in Chinese) **36,** 1595 (2009).