

# Optimal Duration of Coronary Ligation and Reperfusion for Reperfusion Injury Study in a Rat Model

Shih-Tai Chang,<sup>1</sup> Chi-Ming Chu,<sup>2</sup> Teng-Yao Yang,<sup>1</sup> Li-Man Hung,<sup>3</sup> Kuo-Li Pan<sup>1</sup> and Wen-Jin Cherng<sup>4</sup>

**Background:** Reperfusion injury (RI) has an important impact on the clinical prognosis for patients with acute myocardial injury who had their coronary blood flow reestablished. However, no studies to date have investigated the timeframe of coronary occlusion and reperfusion effects on RI.

**Methods:** A total of 100 rats were divided into 4 groups based on the coronary ligation period: 30, 60, 120, and 180 min, and each group was further divided into 5 subgroups with different reperfusion periods: 0, 30, 60, 120, and 180 min. R0 was the baseline of each subgroup. All animals received the same protocols for designed ligation and reperfusion periods. Evans blue and 2,3,5-triphenyltetrazolium chloride were used to distinguish different myocardial injury areas: area at risk (AAR) and myocardial necrosis. The differences of the ratios of the necrotic area to AAR between each subgroup and baseline were further averaged to calculate an overall value of each heart.

**Results:** The relative RI percentages showed significant differences ( $0.8 \pm 2.3\%$ ,  $4.9 \pm 3.3\%$ ,  $10.8 \pm 3.1\%$ , and  $20.3 \pm 3.6\%$  respectively,  $p < 0.001$ ) at different time points of reperfusion but not at different time points of ligation ( $p = 0.593$ ). The effects of different time courses in RI showed that the L120R180 group ( $43.4 \pm 2.3\%$ ) had the highest RI difference with the baseline group.

**Conclusions:** Maximal RI occurred at the timeframe of L120R180 in our animal model. This result may be utilized to assess the substantial benefits of RI therapies in an experimental rat model setting.

**Key Words:** Acute myocardial infarction (AMI) • Coronary artery disease (CAD) • Primary coronary intervention • Reperfusion injury (RI)

## INTRODUCTION

Coronary artery disease (CAD) has been a global burden on healthcare resources and is the leading cause of morbidity and mortality worldwide.<sup>1</sup> Novel therapeutic strategies should take into consideration the consequences of CAD in order to reduce the global impact of this disease on society. Following an acute myocardial infarction, reestablishing coronary blood flow with a rapidly deployed reperfusion strategy, such as thrombolysis or primary coronary intervention, is essential to salvage viable myocardium. Prompt restoration of blood flow to the ischemic myocardium will limit the infarction size and reduce mortality. However, paradoxically, the

Received: April 28, 2015

Accepted: August 24, 2015

<sup>1</sup>Division of Cardiology, Chiayi Chang Gung Memorial Hospital; Chiayi School, Chang Gung Institute of Technology, Chaiyi Hsien; School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan; <sup>2</sup>Section of Health Informatics, Institute of Public Health, National Defense Medical Center and University, Taipei; <sup>3</sup>Department of Life Science, College of Medicine, Chang Gung University, Taoyuan; <sup>4</sup>Division of Cardiology, Chang Gung Memorial Hospital, Keelung, Taiwan.

Address correspondence and reprint requests to: Dr. Shih-Tai Chang, Division of Cardiology, Chang Gung Memorial Hospital, No. 6, Sec. West, Chai-Pu Road, Pu-Tz City, Chaiyi Hsien, Taiwan. Tel: 886-5-362-1000 ext. 2854; Fax: 886-5-362-3005; E-mail: cst1234567@yahoo.com.tw

return of coronary blood flow can also result in additional cardiac damage and complications, including life-threatening arrhythmia and myocardial necrosis, referred to as reperfusion injury (RI).<sup>2-7</sup>

Realizing the RI mechanism and reducing the RI extent are vital for coronary blood flow restoration. In the Deloche et al. study, the authors concluded that the size of the infarctions caused by temporary ischemia was found to be significantly smaller in 60% of the cases as compared to the infarctions caused by permanent ischemia.<sup>8</sup> Besides, Mulch et al. reported that deficient cardiac functional recovery was observed after ischemic periods extending beyond 30 minutes, in spite of reperfusion periods.<sup>9</sup> And Reimer et al.<sup>10</sup> provided evidence for the wavefront phenomenon of ischemic cardiac death in dogs. However, no studies illustrate the cardiac time sequence effect of coronary ligation and reperfusion. Furthermore, no study has explored the timeframes for coronary ligation and reperfusion that could result in the maximal RI. Moreover, whether experimental medication in this maximal RI period will interfere with the reperfusion therapy is not known. To our knowledge, no studies have investigated the relationship between RI extent and period of coronary ligation and reperfusion. If the animal ischemia/RI time-frame model can be achieved, the model may be utilized to assess the benefits of RI therapies in a clinical setting.

## MATERIALS AND METHODS

### Animal preparation

Normal 8-week-old male Sprague-Dawley rats (weight approximately 250-300 g) were fed a standard diet and acclimated to a quiet quarantine room for 7 to 10 days before the experiments were performed. All animals received human care, and the animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital. The animals were anesthetized with 2 ml/kg intraperitoneal injection of ketamine, atropine, and chloral hydrate (with a ratio of 1:3 each). If needed, a dose of 0.2 ml was repeated every 30 minutes. The rats were then placed in a supine position with paws taped to the operating table. Airway maintenance was conducted with an

endotracheal tube (PE-50), and the rats were ventilated at a rate of 66-120 beats/minute with a tidal volume of 0.6-1.25 ml/minute by a rodent ventilator (SAR-830/AP, CWE Inc., Ardmore, PA, USA). Oxygen saturations were maintained at greater than 95% during the experiment. The body temperature maintenance was monitored by a rectal probe connected to a digital thermometer to keep a constant core temperature of 37 °C.

### Induction of ischemia

A loading dose of 200 u/kg heparin was given to the rats before surgery, and a heparinized catheter was inserted into the right femoral artery for hemodynamic status monitoring. The heart was then exposed by way of a left vertical thoracotomy and pericardiectomy; the thoracotomy was conducted just to the left of the chest's midline. To reduce the mortality of rats during the experiment due to significant arrhythmia, the left anterior descending coronary artery (LAD) ligation should not be higher than the bottom of the left atrium. Regional ischemia was achieved by snaring the LAD with a 6-0 silk suture, and ischemia was confirmed by a visual assessment of cyanosis and dyskinesia of the myocardium supplied by the LAD. For the high prevalence of LAD reperfusion arrhythmia, the rats were given 100% oxygenation before and after the LAD ligation and reperfusion to reduce the number of lethal complications. Furthermore, the heart directly received a drop of 0.5% xylocaine just before ligation and after reperfusion. During the period of ischemia and reperfusion, the open wound was kept moist, and a parafilm covering was used to prevent dehydration. Additionally, 1 ml normal saline was administered through the right femoral artery catheter every 30 minutes. Because cardioversion for low blood pressure or arrhythmia during the experiment may affect the myocardial necrotic process, no cardioversion was performed during the study process, and the rats were removed from the study if the systolic blood pressure was lower than 50 mmHg.

### Study groups

The animals were divided into 4 groups: ligation 30 minutes (L30), 60 minutes (L60), 120 minutes (L120), and 180 minutes (L180). Each ligation group was further divided into 5 separate reperfusion sub-groups: reperfusion 0 minutes (R0, baseline group), reperfusion 30

minutes (R30), reperfusion 60 minutes (R60), reperfusion 120 minutes (R120), and reperfusion 180 minutes (R180). Therefore, this study protocol involved a total of 20 sub-groups, and every sub-group contained 5 rats.

### Assessment of infarction size and area at risk of the left ventricle

After the ischemia and reperfusion study protocol of each subgroup was conducted, the ligature around the LAD was retightened. Evans blue dye (1 ml of 2% solution) was injected into the left atrium to stain the area of the myocardium perfused by the patent coronary arteries for 5 minutes, and the unstained myocardium was defined as the area at risk (AAR). Then, the atria, right ventricle, and major blood vessels were subsequently removed from the heart. Phosphate buffer saline was injected into the isolated left ventricle for 5 minutes and the left ventricle was soon frozen at  $-80^{\circ}\text{C}$  until further utilized. The left ventricle was then sliced into 5 parallel sections (myocardial slice) of 1-mm thickness along the atrioventricular groove. The unstained portions of myocardium (the AAR) were separated from the stained portions of myocardium, and then the AAR portions were incubated in 1.0% 2,3,5-triphenyltetrazolium chloride (TTC) for 5 minutes at  $37^{\circ}\text{C}$  and fixed in 10% formalin. The area not stained with TTC dye was defined as the myocardial necrotic area. These serial slices were scanned using an Epson AL-CX11 flatbed scanner (Epson, Long Beach, CA, USA). The total left ventricular area, the necrotic area, and the AAR of the left ventricle (in arbitrary units) of each slice were measured using the NIH Image J software (computer-assisted planimetry with Image J-1.37 software). The percentages of the necrotic area/AAR were then averaged to calculate an overall value for each heart.<sup>5,6</sup> In each ligation group, the difference of necrotic area/AAR between each reperfusion sub-group and baseline (R0) was calculated as a relative RI difference with the baseline.

Myocardial apoptosis was qualified using a commercially available terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) kit. TUNEL staining was performed with fluorescein-dUTP for apoptotic cell nuclei, and 4',6-diamidino-2-phenylindole (DAPI) was used to stain all cell nuclei. Apoptotic index was measured by the number of TUNEL-positive myocytes/total number of myocytes stained by DAPI

from a total of 40 fields per heart in a blind manner by confocal microscopy at  $\times 400$  magnifications.

### Statistical analyses

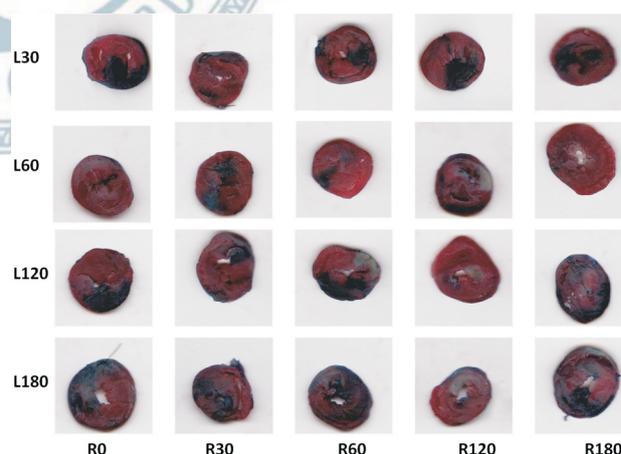
The Statistical Package for Social Sciences (SPSS) 14.0 for Windows was used to conduct the statistical analysis, and all the values were expressed in terms of mean  $\pm$  SE. Statistical analysis was performed using the generalized estimating equation (GEE) for groups of ligation and reperfusion. The 95% confidence intervals were used to identify which groups were significantly different in multiple comparisons. Time effects of RI changes in comparison with R0 for ligation and reperfusion were analyzed by using repeated measures ANOVA tests between subjects, and Bonferonni's post-hoc test was used to compare the effects of different timeframes in ligation or reperfusion. A p value  $< 0.05$  was considered significant.

## RESULTS

### Time effects of RI for ligation and reperfusion

Figure 1 and Table 1 show the effects of different ligation/reperfusion time courses on myocardial infarct size with respect to the left ventricle.

The reperfusion injuries vary in that they have different ligation and reperfusion periods. Figure 2A showed



**Figure 1.** Myocardial infarct size with the respect to left ventricle in different ligation/reperfusion time courses. Blue color represented as Evans blue stained myocardium (survival myocardium), and the other unstained myocardium was defined as the area at risk (AAR). White color represented as the area not stained with 2,3,5-triphenyltetrazolium chloride (TTC) dye and was defined as the myocardial necrotic area.

that the RI percentage depended on different time points of ligation. In the ligation periods of 30 minutes, 60 minutes, 120 minutes, and 180 minutes, the relative RI percentage showed no significant differences between groups ( $8.2 \pm 1.9\%$ ,  $7.3 \pm 2.7\%$ ,  $13.3 \pm 4.8\%$ , and  $8.0 \pm 3.8\%$ , respectively;  $p = 0.593$ ). However, in Figure 2B, the relative RI percentage that depended on different time points (in 30 minutes, 60 minutes, 120 minutes, and 180 minutes) of reperfusion showed significant differences ( $0.8 \pm 2.3\%$ ,  $4.9 \pm 3.3\%$ ,  $10.8 \pm 3.1\%$ ,

and  $20.3 \pm 3.6\%$ , respectively;  $p < 0.001$ ).

Table 2 shows the time effects of RI changes in comparison with R0 for ligation and reperfusion in multivariate ANOVA tests between-subject effects. In different ligation durations, the relative RI showed no differences between the groups. Conversely, the relative RI illustrated significant differences in groups of 180 versus 30 minutes ( $p < 0.001$ ) and 180 versus 60 minutes ( $p < 0.001$ ). The 180 versus 120 minutes group showed a trend of significance ( $p = 0.091$ ).

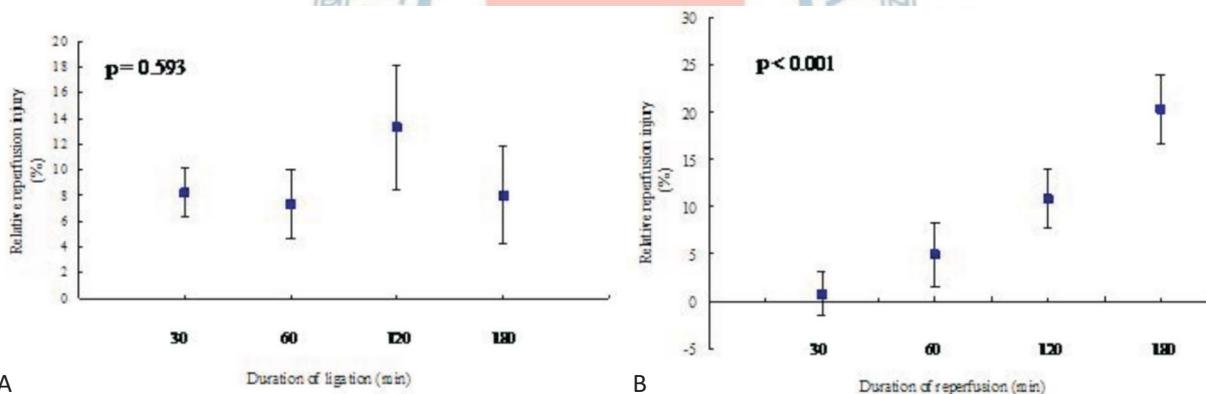
**Table 1.** Effects of different ligation/reperfusion time courses on myocardial infarct size with respect to the left ventricle

	R0	R30	R60	R120	R180
L30	$37.4 \pm 3.1$	$41.3 \pm 4.7$	$41.0 \pm 3.8$	$39.7 \pm 1.6$	$39.6 \pm 2.7$
L60	$41.2 \pm 3.1$	$40.9 \pm 3.1$	$41.7 \pm 2.9$	$47.8 \pm 6.5$	$46.3 \pm 4.2$
L120	$44.2 \pm 3.2$	$42.4 \pm 3.9$	$44.5 \pm 3.6$	$50.1 \pm 7.3$	$63.4 \pm 2.3$
L180	$51.4 \pm 8.7$	$50.0 \pm 2.4$	$55.5 \pm 14.1$	$55.3 \pm 8.6$	$61.3 \pm 2.7$

The data presented as mean  $\pm$  standard error of the ratio of myocardial infarct size to the left ventricle (%). N = 5 in each subgroup, total were 20 ( $4 \times 5$ ) subgroups.

**Effects of different time courses in RI**

Figure 3 shows the effects of different time courses in RI. The groups of L120R180 ( $43.4 \pm 2.3\%$ ), L180R180 ( $19.2 \pm 2.4\%$ ), L60R120 ( $16.1 \pm 7.1\%$ ), L120R120 ( $13.4 \pm 7.4\%$ ), and L60R180 ( $12.5 \pm 4.5\%$ ) had high mean RI differences with the baseline (R0). In comparison with the L120R180 group, the groups all showed significant RI differences. The apoptotic indexes are 6.76%, 5.81%, 5.49%, 5.58%, and 4.32%, respectively (Figure 4). According to the results, the L120R180 group had the highest RI difference with the other groups.

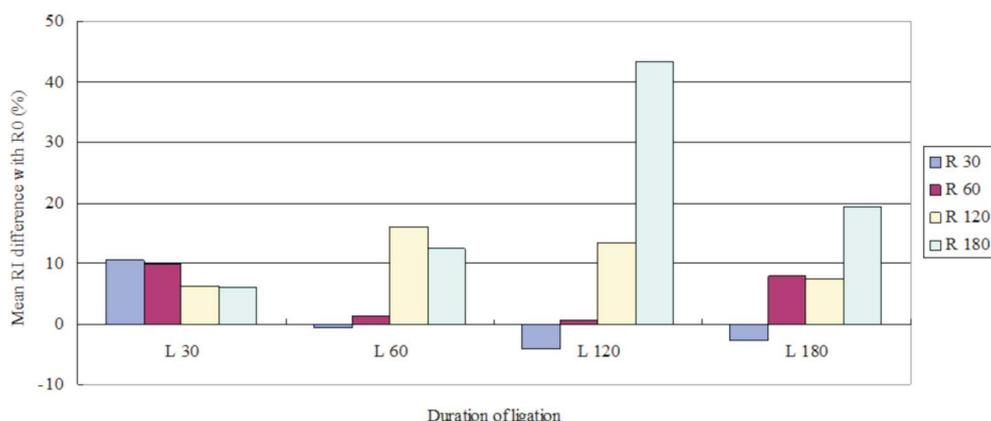


**Figure 2.** Time effects in ligation (A) and reperfusion (B) for reperfusion injury. It illustrated a linear relationship between RI extent and reperfusion duration.

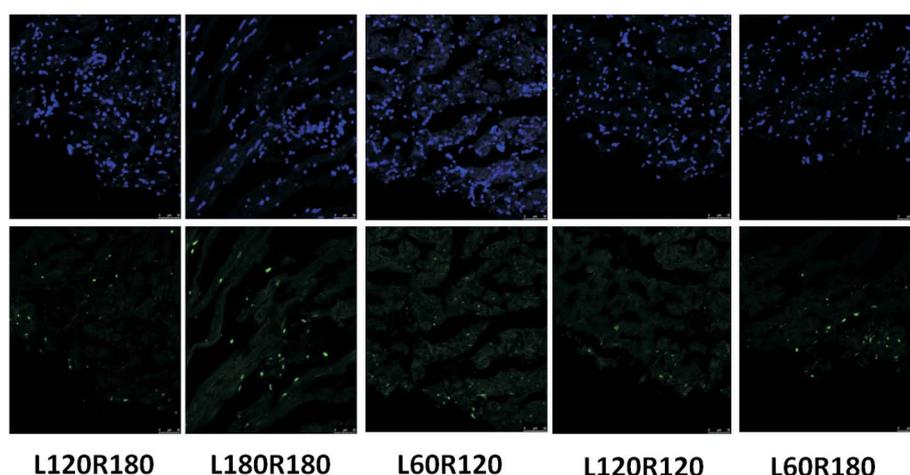
**Table 2.** Time effects of RI changes in comparison with R0 for ligation and reperfusion in multivariate ANOVA tests between subjects

Duration	30 min	60 min	120 min	180 min	p value			
					Overall	Post-HOC		
						180 vs. 30 min	180 vs. 60 min	180 vs. 120 min
Ligation	$8.2 \pm 1.9$	$7.3 \pm 2.7$	$13.3 \pm 4.8$	$8.0 \pm 3.8$	0.593	1.000	1.000	0.987
Reperfusion	$0.8 \pm 2.3$	$4.9 \pm 3.3$	$10.8 \pm 3.1$	$20.3 \pm 3.6$	< 0.001	< 0.001	0.001	0.091

The data presented as mean  $\pm$  standard error of the ratio of myocardial infarct size to the left ventricle (%).



**Figure 3.** Effects of different ligation/reperfusion time courses in reperfusion injury. The L120R180 group showed significant RI differences in comparison with all other groups.



**Figure 4.** The apoptotic indexes of different time courses in reperfusion injury. The L120R180 group had the highest RI difference with the other groups.

## DISCUSSION

In 1990, Tsao et al.<sup>5</sup> determined the time courses of RI in cats following occlusions of the LAD coronary artery for 90 minutes after reperfusion for times ranging from 0 to 270 minutes. Endothelial dysfunction, which presented as depressed acetylcholine response at the endothelium, occurred as quickly as 2.5 minutes after reperfusion. However, no significant myocardial necrosis occurred until 180 minutes of reperfusion, and  $10 \pm 3\%$  increased to  $28 \pm 3\%$  at reperfusion of 270 minutes. In 1991, Viehman et al. completed a similar trial in a cat model, except that no reperfusion was allowed.<sup>6</sup> They illustrated that a significant degree of myocardial necrosis was not observed until 360 minutes of permanent ischemia, and the degree of myo-

cardial necrosis was only  $18 \pm 4\%$  of the AAR. Nevertheless, other animal data suggested that up to 50% of infarct size may be attributable to RI.<sup>7,11</sup> Exploring the RI mechanism in order to reduce the RI extent is a matter of concern for acute myocardial injury coronary blood flow restoration therapy. However, different results occurred even in the same pharmacological trials found in previous studies.<sup>12-21</sup> These different studies developed various animal models. Therefore, these variations will affect the therapeutic results presentation and identification.

To our knowledge, no studies have investigated the relationship between RI extent and the period of coronary ligation and reperfusion. A number of studies have demonstrated the elusiveness of effective therapies for reducing RI. Nonetheless, most of the clinical trials to

prevent RI have been disappointing. Different experimental results obtained from the same pharmacological study were found frequently in previous reports.<sup>22-26</sup> Consequently, previous studies on RI therapy have some drawbacks. First, different studies developed different animal models. Second, the ligation and reperfusion duration of the coronary artery vary in different studies. Moreover, at present, no studies have confirmed the intervals of coronary ligation and reperfusion to obtain the maximal RI in an animal model. It is not known whether experimental medication in this maximal RI extent period will interfere with pharmacological effects in reperfusion therapy.

In our study, we tried to establish and confirm the intervals of coronary ligation and reperfusion to obtain the maximal RI in a rat model. We found that in different ligation durations, the relative RI showed no differences between groups. However, the relative RI percentages at different time points of reperfusion showed significant differences. This means that the reperfusion time interval has a more important role in RI than the ligation time interval does. Furthermore, after multivariate ANOVA tests between subjects on the time effects for ligation and reperfusion, the L120R180 group showed the highest RI difference in comparison with the L120R0 group. A comparison also showed significant RI differences between the L120R180 group and other groups. Although many other groups such as L180R180, L60R120, L120R120, and L60R180 showed a higher RI extent, the L120R180 group in the animal model seems to have a maximal RI extent.

Our study showed a linear relationship between RI extent and reperfusion duration. Therefore, during this study period, we conducted the animal model for reperfusion periods of more than 180 min, for example, 240 min, 300 min, or 360 min. However, even after electrical cardioversion, administration of xylocaine, and adequate hydration, high animal mortality rate due to arrhythmia, hypotension, or shock occurred during these long reperfusion periods. On the other hand, longer reperfusion periods also resulted in higher time costs. Hence, for having the highest relative RI extent ( $43.4 \pm 2.3\%$ ), higher animal survival rate during the experimental period, and optimal time cost, the L120R180 group may be the best choice for rat model.

## CONCLUSIONS

To our knowledge, no identical animal models have been developed in previous studies. Different studies developed various animal models. The variations will affect the results presentation and identification and produce a contrary conclusion. Besides, no study has explored the number of time intervals for coronary ligation and reperfusion to obtain maximal RI. For RI treatment, in our opinion, if we did not use experimental medications or interventional therapies at the largest RI extent time point, the experimental results would have vacillated and proven inconclusive. When medications or interventional therapies take the largest RI extent time point, the therapeutic effects will be well-illustrated.

Although the rat coronary anatomy is far from the human coronary anatomy, the rat model is relatively easier to manipulate and conduct experiments on than, for example, the pig model. According to our study, we established the optimal rat model for reaching the maximal RI, and in doing so, we obtained and confirmed more exact experimental outcomes for pharmacological or other therapeutic effects in RI reduction studies. This result may be utilized to assess the benefits of RI therapies in clinical settings.

## CONFLICTING INTERESTS

The authors declare that they have no conflicting interests.

## AUTHOR CONTRIBUTIONS

Conception: Shih-Tai Chang; Design and data collection: Teng-Yao Yang, Kuo-Li Pan; Revising the article for important intellectual content: Li-Man Hung; Data analysis and interpretation: Chi-Ming Chu; Final approval of the version to be published: Wen-Jin Cherng.

## SOURCES OF GRANTS

The authors would like to thank Chang Gung Memo-

rial Hospital for financially supporting this research under Contract No. CMRPG6A0241.

## REFERENCES

- Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997;349(9064):1498-504.
- Simoons ML, Serruys PW, van den Brand M, et al. Early thrombolysis in acute myocardial infarction: limitation of infarct size and improved survival. *J Am Coll Cardiol* 1986;7:717-28.
- White HD, Norris RM, Brown MA, et al. Effect of intravenous streptokinase on left ventricular function and early survival after acute myocardial infarction. *N Engl J Med* 1987;317:850-5.
- Sheehan FH, Doerr R, Schmidt WG, et al. Early recovery of left ventricular function after thrombolytic therapy for acute myocardial infarction: an important determinant of survival. *J Am Coll Cardiol* 1988;12:289-300.
- Tsao PS, Aoki N, Lefer DJ, et al. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. *Circulation* 1990;82(4):1402-12.
- Viehman GE, Ma XL, Lefer DJ, Lefer AM. Time course of endothelial dysfunction and myocardial injury during coronary arterial occlusion. *Am J Physiol* 1991;261(3 Pt 2):H874-81.
- Matsumura K, Jeremy RW, Schaper J, Becker LC. Progression of myocardial necrosis during reperfusion of ischemic myocardium. *Circulation* 1998;97:795-804.
- Deloche A, Fabiani JN, Camilleri JP, et al. The effect of coronary reperfusion on the extent of myocardial infarction. *Am Heart J* 1977;93(3):358-66.
- Mulch J, Schaper J, Gottwik M, Hehrlein FW. Recovery of the heart after normothermic ischemia. Part II: myocardial function during posts ischemic reperfusion. *Thorac Cardiovasc Surg* 1979;27(3):145-52.
- Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wave-front phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation* 1977;56:786-94.
- Arai M, Lefer DJ, So T, et al. An anti-CD18 antibody limits infarct size and preserves left ventricular function in dogs with ischemia and 48-hour reperfusion. *J Am Coll Cardiol* 1996;27:1278-85.
- Baran KW, Nguyen M, McKendall GR, et al. Double-blind, randomized trial of an anti-CD18 antibody in conjunction with recombinant tissue plasminogen activator for acute myocardial infarction: limitation of myocardial infarction following thrombolysis in acute myocardial infarction (LIMIT AMI) study. *Circulation* 2001;104:2778-83.
- Gonon AT, Gourine AV, Middelveld RJ, et al. Limitation of infarct size and attenuation of myeloperoxidase activity by an endothelin A receptor antagonist following ischaemia and reperfusion. *Basic Res Cardiol* 2001;96:454-62.
- Harlan JM, Winn RK. Leukocyte-endothelial interactions: clinical trials of anti-adhesion therapy. *Crit Care Med* 2002;30:S214-9.
- Faxon DP, Gibbons RJ, Chronos NA, et al., HALT-MI Investigators. The effect of blockade of the CD11/CD18 integrin receptor on infarct size in patients with acute myocardial infarction treated with direct angioplasty: the results of the HALT-MI study. *J Am Coll Cardiol* 2002;40:1199-204.
- Mahaffey KW, Granger CB, Nicolau JC, et al. Effect of pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to fibrinolysis in acute myocardial infarction: the COMPLEMENT inhibition in myocardial infarction treated with thrombolytics (COMPLY) trial. *Circulation* 2003;108:1176-83.
- Granger CB, Mahaffey KW, Weaver WD, et al. Pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to primary percutaneous coronary intervention in acute myocardial infarction: the COMPLEMENT inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial. *Circulation* 2003;108:1184-90.
- APEX AMI Investigators, Armstrong PW, Granger CB, et al. Pexelizumab for acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention: a randomized controlled trial. *JAMA* 2007;297:43-51.
- Simon DI, Xu H, Ortlepp S, et al. 7E3 monoclonal antibody directed against the platelet glycoprotein IIb/IIIa cross-reacts with the leukocyte integrin Mac-1 and blocks adhesion to fibrinogen and ICAM-1. *Arterioscler Thromb Vasc Biol* 1997;17:528-35.
- ISIS-4: a randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58,050 patients with suspected acute myocardial infarction. ISIS-4 (Fourth International Study of Infarct Survival) Collaborative Group. *Lancet* 1995;345:669-85.
- Magnesium in Coronaries (MAGIC) Trial Investigators. Early administration of intravenous magnesium to high-risk patients with acute myocardial infarction in the Magnesium in Coronaries (MAGIC) Trial: a randomised controlled trial. *Lancet* 2002;360:1189-96.
- Olafsson B, Forman MB, Puett DW, et al. Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and the no-reflow phenomenon. *Circulation* 1987;76:1135-45.
- Babbitt DG, Virmani R, Forman MB. Intracoronary adenosine administered after reperfusion limits vascular injury after prolonged ischemia in the canine model. *Circulation* 1989;80:1388-99.
- Babbitt DG, Virmani R, Vildibill HD, et al. Intracoronary adenosine administration during reperfusion following 3 hours of ischemia: effects on infarct size, ventricular function, and regional myocardial blood flow. *Am Heart J* 1990;120:808-18.
- Pitavys CJ, Virmani R, Vildibill HD, et al. Reduction of myocardial reperfusion injury by intravenous adenosine administered during the early reperfusion period. *Circulation* 1991;83:237-47.
- Homeister JW, Hoff PT, Fletcher DD, et al. Combined adenosine and lidocaine administration limits myocardial reperfusion injury. *Circulation* 1990;82:595-608.