Published in final edited form as:

Cardiovasc Drugs Ther. 2013 April ; 27(2): . doi:10.1007/s10557-012-6436-7.

Two Classes of Anti-Platelet Drugs Reduce Anatomical Infarct Size in Monkey Hearts

Xi-Ming Yang, MD, PhD¹, Yanping Liu, MD, PhD¹, Lin Cui, MD¹, Xiulan Yang, MD, PhD¹, Yongge Liu, PhD², Narendra Tandon, PhD², Junichi Kambayashi, MD, PhD², James M. Downey, PhD¹, and Michael V. Cohen, MD^{1,3}

¹Department of Physiology University of South Alabama College of Medicine, Mobile, AL

²Otsuka Maryland Medicinal Labs, Inc. Rockville, MD

³Department Medicine, University of South Alabama College of Medicine, Mobile, AL

Abstract

Recent studies in rabbits have demonstrated that platelet P2Y₁₂ receptor antagonists are cardioprotective, and that the mechanism is surprisingly not related to blockade of platelet aggregation but rather to triggering of the same signal transduction pathway seen in pre- and postconditioning. We wanted to determine whether this same cardioprotection could be documented in a primate model and whether the protection was limited to P2Y₁₂ receptor antagonists or was a class effect. Thirty-one macaque monkeys underwent 90-min LAD occlusion/4-h reperfusion. The platelet P2Y₁₂ receptor blocker cangrelor started just prior to reperfusion significantly decreased infarction by an amount equivalent to that seen with ischemic postconditioning (p<0.001). For any size of risk zone, infarct size in treated hearts was significantly smaller than that in control hearts. OM2, an investigational murine antibody against the primate collagen receptor glycoprotein (GP) VI, produced similar protection (p<0.01) suggesting a class effect. Both cangrelor and OM2 were quite effective at blocking platelet aggregation (94% and 97%, respectively). Thus in a primate model in which infarct size could be determined directly platelet anti-aggregatory agents are cardioprotective. The important implication of these investigations is that patients with acute myocardial infarction who are treated with platelet anti-aggregatory agents prior to revascularization may already be in a postconditioned state. This hypothesis may explain why in recent clinical trials postconditioningmimetic interventions which were so protective in animal models had at best only a modest effect.

Keywords

cangrelor; monkey; myocardial infarction; OM2; platelet; postconditioning

INTRODUCTION

Since the introduction of ischemic preconditioning as an intervention which could potentially minimize myocardial infarct size [1], many proposed interventions have been studied. Although a few interventions have shown promise in clinical studies, these have either involved small groups of subjects [2,3] or have shown only marginal benefit [4]. For

Correspondence: Michael V. Cohen, M.D. Department of Physiology MSB 3050 University of South Alabama College of Medicine Mobile, AL 36688 Tel 251-460-6812 mcohen@usouthal.edu.

DISCLOSURES Monkey studies were funded by a contract with Otsuka Maryland Medicinal Labs, Rockville, MD.

the most part, however, conditioning-mimetic interventions (interventions that should have invoked the protective signaling of pre- or postconditioning) have performed poorly in recent clinical trials despite being very protective in animal models [4,5]. One possible explanation is that one or more of the many drugs administered to patients with acute myocardial infarction about to undergo urgent coronary angioplasty might unknowingly have been a conditioning agent itself. If so, administration of the test agent would have yielded little additional effect, resulting in the mistaken conclusion that the agent was ineffective in humans. In the past decade there has been a dramatic increase in the use of antiplatelet agents in patients with acute coronary syndrome. Currently virtually all patients with myocardial infarction will receive some type of platelet anti-aggregatory agent before the revascularization procedure, most commonly one of the platelet P2Y₁₂ receptor blocking drugs: clopidogrel, prasugrel, or ticagrelor.

In a recent study in rabbits we noted an anti-infarct effect of clopidogrel and cangrelor (the intravenous analog of ticagrelor) that was similar in magnitude to that from conditioning [6]. What was surprising was that the protection did not appear to result from any effect on platelet aggregation, but rather from activation of the signal transduction pathway used by conditioning agents. We found that cardioprotection from P2Y₁₂ receptor blockers is dependent on the reperfusion injury salvage kinases (RISK) including Akt and ERK as well as adenosine A_{2B} receptors, mitochondrial K_{ATP} channels, and redox signaling, all of which are used by both pre- and postconditioning [7]. Importantly, none of the enzyme inhibitors or receptor or channel blockers of these signaling components which effectively aborted protection of the anti-platelet agents affected the ability of the latter to block platelet aggregation. Furthermore, when we combined ischemic postconditioning and cangrelor we could not achieve any additional reduction in infarct size [6]. We concluded that protective signaling rather than prevention of intravascular coagulation accounted for the protection.

Our initial report tested two P2Y₁₂ receptor antagonists in rabbits. We wondered if the effect could be seen in another species. Although platelet inhibitors have been reported to have an anti-infarct effect in patients [8,9], it is difficult to evaluate the magnitude of their tissue salvage since many of the factors that influence infarct size such as collateral flow, duration of ischemia, risk zone size, and co-morbidities could not be controlled. Also, infarct size was estimated only from cardiac enzyme release. Thus the first aim of this study was to test these drugs in a primate model in which anatomical infarct size could be directly measured and the magnitude of the cardioprotective effect of anti-platelet agents could be compared to that from postconditioning.

OM2, a murine antibody to human platelet glycoprotein (GP) VI receptors under development by Otsuka (Rockville, MD), is a potent blocker of platelet aggregation. In the initial steps of vascular injury the platelet becomes tethered to endothelial cells by binding to exposed collagen through GPVI and integrin $\alpha_2\beta_1$ receptors. This binding then triggers a complex signaling cascade leading to cross-linking of platelets and aggregation. OM2 binds tightly to human GPVI and inhibits ex vivo collagen-induced human platelet aggregation. It is important to note that this antibody did not bind to platelet collagen receptors of the usual experimental animal models, i.e., rodents, rabbits, pigs, and dogs. But it did bind to macaque receptors. We were, therefore, invited by Otsuka to investigate OM2 in monkeys. Not only did this invitation give us a rare opportunity to investigate whether P2Y₁₂ inhibitors provide cardioprotection in a primate species, but it also allowed us to pursue a second aim which was testing whether the protection seen with P2Y₁₂ receptor antagonists was unique to those agents, or whether it might be a class effect that included other platelet anti-aggregatory compounds. We, therefore, measured myocardial infarct size following a standardized ischemic insult in open-chest macaques treated with the platelet collagen receptor blocker,

OM2, and compared that protection to that seen following treatment with either the P2Y₁₂ inhibitor cangrelor or ischemic postconditioning or no treatment.

METHODS

All protocols were approved by the Institutional Animal Care and Use Committee of the University of South Alabama College of Medicine and conformed to published guidelines [10].

Surgical preparation

Cynomolgus (macaque) male monkeys from China were obtained through a licensed laboratory animal supplier, Covance in Denver, PA. They were warranted as pathogen free, weighed 2.0-4.0 kg and were approximately 2-3 years old. The monkeys were prepared as previously described [11]. Briefly, they were fasted overnight and then sedated with ketamine, 10 mg/kg IM. Additionally atropine, 0.05 mg/kg IM, was given. Anesthesia was achieved with sodium pentobarbital, 10-15 mg/kg IV, and additional doses were administered throughout the experiment. Through a cervical incision the trachea was intubated and the animal was ventilated with positive pressure and a gas mixture of 40% O₂/60% N₂. A carotid artery was cannulated for pressure measurement and blood sampling, and a jugular vein cannulated for pentobarbital and drug administration. A left thoracotomy was performed in the fourth intercostal space and heart exposed. A 2-0 suture on a needle was passed beneath the vascular bundle in the interventricular groove **close to the coronary artery's origin**, and the ends threaded through a length of polyethylene catheter to form a snare. In each heart we tested for cyanosis and cessation of contraction of the anterior wall of the heart when the snare was pulled for 10 s. ECG leads were attached. Rectal temperature was maintained at 38°C with the aid of a heating pad.

Coronary artery occlusion and monitoring

After equilibration for 20 min arterial blood was withdrawn for measurement of pO₂, PCO₂, and pH with a CG4+ cartridge and the I-STAT system (Haska Corp., Loveland, CO), platelet count (Automated Hematology Analyzer, ABX Micros 60-CT, ABX, Montpellier, France), and platelet aggregometry (see below). ECG and hemodynamics were continuously recorded. The coronary artery was occluded for 90 min. Immediately prior to reperfusion arterial blood was obtained for platelet count and aggregometry. If ventricular fibrillation occurred, electrical defibrillation was attempted without releasing the occlusion. After 2 h of reperfusion, arterial blood gases were measured with I-STAT. After the 4-h reperfusion period platelet count and aggregometry were remeasured.

Infarct area and risk zone

After heart extirpation risk zone and infarct size were measured with fluorescent microspheres and triphenyltetrazolium chloride (TTC), resp. Briefly, the heart was suspended on a Langendorff apparatus and the aorta retrogradely perfused with Krebs buffer. The snared coronary branch was reoccluded and 2-9 µm green fluorescent microspheres (Microgenics Corp., Freemont, CA) were added to the perfusate. The risk area was demarcated as myocardium without fluorescent microspheres. The heart was placed on dry-ice to freeze it and then cut into 2-3 mm slices perpendicular to its long axis. The slices were incubated for 8-10 min in 1% TTC (GFS Chemicals, Powell, OH) warmed to 37°C and then put into 10% formalin for tissue preservation and enhancement of contrast. TTC stains living tissue brick red, whereas infarcted tissue is either white or dark brown if intramyocardial hemorrhage has occurred. Risk zone regions identified under UV light and infarct regions identified under white light were traced on plastic overlays. Areas were

measured by planimetry and volumes calculated by multiplying areas by slice thickness. Infarct size is presented as a % of risk zone volume.

Platelet aggregometry

Aggregation was determined by measuring impedance with a whole blood aggregometer (Chrono-log Corp., Havertown, PA) interfaced with AGGRO/LINK (Chrono-log Corp.) and computer. One-half ml of saline and 0.5 ml of heparinized arterial blood were combined in a plastic cuvette and continuously stirred. For OM2, aggregation was initiated by adding 5 μ l of Chrono-par collagen (Chrono-log Corp.), 100 μ g/ml, diluted in SKF Horm Puffer Buffer (Nycomed, Melville, NY). If after several minutes no aggregation was detected, 2 μ l of a more concentrated collagen solution (1 mg/ml) were added. For cangrelor, aggregation was initiated with ADP, 1 mM. Either 2.5, 5, or 10 μ l were added to produce final concentrations of 2.5, 5, and 10 μ M.

Protocol

Control monkeys were subjected to only 90-min coronary artery occlusion/4-h reperfusion. OM2 was studied in 2 groups. In the first group 2 mg/kg was administered intravenously 2 min prior to the 90-min occlusion and again 2 min prior to reperfusion. A second OM2 group received a single 2 mg/kg bolus 10 min before reperfusion. A final group received 60 μ g/kg cangrelor intravenously 10 min before release of the coronary occlusion followed by continuous infusion of 6 μ g/kg/min until animal sacrifice.

Statistics

Baseline hemodynamics for all groups were compared by one-way ANOVA. Hemodynamic changes within any given group were evaluated by ANOVA for repeated measures. Infarct data were analyzed by one-way ANOVA. Because risk zone size is an independent determinant of the fraction of the risk zone that infarcts in monkey hearts [11] and because of widely differing risk zones in the monkeys, the significance of changes of infarct size with interventions was determined by plotting infarct size as a function of risk zone volume and then comparing regression lines for each intervention by ANCOVA. The platelet aggregation curves were analyzed by determining slopes and areas beneath the curves. Only the area data are presented. All curves were truncated at 5 min. Curves following administration of a drug were always compared to the baseline curve obtained before drug treatment, and paired t-test used to determine statistical significance. For all statistical analyses post hoc testing was done with Student-Newman-Keuls test. A p value of <0.05 was considered to be significant.

RESULTS

Experiments were completed in 14 control, 11 OM2, and 6 cangrelor animals. Although infarct data in 7 of these control hearts and in 5 ischemically postconditioned hearts have been previously reported [11], experiments in all control, postconditioned and drug-treated hearts were performed in the same period and conducted by the same investigator. Therefore, all control hearts are included in the analysis. The data in ischemically postconditioned hearts are presented only for comparison with data from the present interventional groups. Initially arterial pO₂ ranged between 130-170 mmHg, pCO₂ from 20-30 mmHg, and arterial pH from 7.45-7.65. Arterial pO₂ changed little by the second determination, but pCO₂ tended to be higher and pH lower.

Arrhythmias

All monkeys successfully completed the protocol and contributed data. Most monkeys had very infrequent premature ventricular contractions or brief runs of ventricular tachycardia which terminated spontaneously. Two monkeys, 1 treated with cangrelor, and 1 control animal developed ventricular fibrillation during coronary occlusion which was successfully converted to sinus rhythm by electrical defibrillation.

Hemodynamics

Baseline systolic blood pressures were generally above 100 mmHg and heart rates ranged from 150-200 bpm. There were no differences among groups (Table 1). During coronary occlusion blood pressure decreased, but recovered somewhat after snare release.

Platelets

Baseline platelet counts ranged from 180,000 to 400,000/ μ l and averaged 321,000/ μ l. There was little change during the protocol.

Adding 0.5 μ g collagen to 1 ml of suspended platelets collected at baseline in all animals caused vigorous platelet aggregation indicated by rapid rise in impedance (Fig. 1). At the end of 90-min coronary occlusion/4-h reperfusion aggregation in control hearts was equally rapid and vigorous (data not shown). OM2 attenuated aggregation, and at reperfusion 0.5 μ g collagen had no or negligible effect on aggregation (Fig. 1). As indicated in Table 2 OM2 decreased aggregation in response to 0.5 μ g collagen in all monkeys by more than 97%. An additional aliquot of 2 μ g collagen (arrow in Fig. 1) produced blunted aggregation. Results were similar after 4 h of reperfusion.

Figure 2 shows the effect of cangrelor on platelet aggregation. At baseline brisk aggregation occurred when 2.5 and 5 μ M ADP were added to blood. Ten μ M ADP had only a small additional effect. After cangrelor administration aggregation in response to either 5 or 10 μ M was completely blocked or barely evident. The average effect of 10 μ M ADP on aggregation was decreased by 94% after cangrelor treatment (Table 2). After 1 h of cangrelor infusion, no aggregation was apparent.

Infarct data—Table 3 and Fig. 3 present infarct data. Although risk zone volume varied widely from heart to heart there were no significant differences in average risk zone volumes among the groups. Previous work revealed that risk zone size is a strong independent determinant of extent of infarction in monkey hearts [11]. Therefore, testing for a shift in the plot of absolute infarct volume against risk zone volume is the most sensitive analysis in this model. The relationship between risk zone and infarct volume for control hearts is quite linear ($r = 0.97$). All points for either the OM2 pretreated hearts or those that received the drug just before reperfusion lie below the control regression line. Both OM2 regression lines ($r = 0.96$ for both) are significantly different from the control line ($p < 0.04$) indicating a cardioprotective effect. Likewise the regression for hearts treated with cangrelor ($r = 0.93$) is significantly different from the regression for control hearts ($p < 0.001$), but it's similar to those for OM2. Although reported earlier [11], the postconditioned (IPOC) hearts were part of the same study as the drug-treated hearts (we did not have permission from Otsuka to publish the OM2 data in that paper) and the data are presented in Fig. 3 again for comparison. If one considers a median risk zone size of 2.5 cm^3 , these treatments would produce a reduction of infarct size from 1.3 cm^3 in control to approximately 0.7-1 cm^3 in treated hearts for a salvage of nearly 25-50% of the ischemic tissue. The regression line for IPOC hearts is not different from those for OM2 and cangrelor.

DISCUSSION

As in rabbits [6], the P2Y₁₂ blocker cangrelor was a potent antagonist of platelet aggregation in monkeys and quite cardioprotective. Furthermore preventing aggregation by blockade of the GPVI receptor, totally different from the P2Y₁₂ receptor, was similarly protective suggesting that the protection is likely to be a class effect. Although OM2 and cangrelor produce similar cardioprotection, anti-GPVI antibodies reportedly have a smaller bleeding risk than other platelet antagonists which may make them a more desirable clinical choice [12,13]. Our observations that GPVI antibody and P2Y₁₂ antagonists decrease infarct size are compatible with studies in genetically engineered mice in which interference with platelet aggregation by removing either G_q [14], GPVI [15,16], or P-selectin [17] was accompanied by diminished infarction after ischemia/reperfusion.

Such protection would be expected if it resulted from prevention of intravascular obstruction and better reperfusion. However, our rabbit data suggested that the myocardial salvage from cangrelor resulted from protective signaling rather than any effect on aggregation and, therefore, blood flow [6]. Both cangrelor and OM2 protected hearts when administered just prior to reperfusion and pretreatment with OM2 offered no additional protection. This indicates that they prevented an injury occurring after reperfusion which is compatible with a conditioning type of protection.

The benefit from platelet inhibitors in primary angioplasty patients is well documented [18]. As a result they are now standard of care. What is equally important, however, is the mechanism of this benefit. The most promising cardioprotective interventions are those that invoke the protective signaling of postconditioning. But if the antiplatelet drugs already activate this signaling, then little would be gained by adding a postconditioning-mimetic in these patients. Of course, this study cannot prove that these agents were working through the same mechanism in the monkey heart, but it is consistent with the previous data in rabbits [6].

In rabbits there was no additional benefit from the combination of cangrelor and IPOC over that seen with each intervention separately [6], suggesting common mechanisms. It is interesting that Roubille et al. [9] did observe additional protection when IPOC was effected in patients already treated with a platelet anti-aggregatory agent, clopidogrel. We suggest that this magnified protection was observed because neither of the two interventions was optimized. Most of the patients (studied in 2004) received only a 300 mg loading dose of clopidogrel which is known to be suboptimal [8], and the optimal IPOC protocol for humans is unknown. It is interesting that in the study by Roubille et al. [9] neither clopidogrel nor IPOC alone caused significant salvage. This would explain the failure of Freixa et al. [19] and Tarantini et al. [20] to observe any effect of IPOC in their patients, all of whom had been treated with clopidogrel and a GPHb/IIIa blocker prior to recanalization.

There have been previous reports of a direct anti-infarct effect of platelet anti-aggregatory agents in animal models. Several reports in dogs have documented smaller infarcts after administration of GPIIb/IIIa agents [21,22,23], although in some of these investigations absence of the recommended stratification for collateral flow [24,25] diminishes the reliability of the conclusions. The response in collateral-poor pigs has not been consistent. Blockade of platelet adhesion with a selectin antagonist decreased infarct size after coronary injury and occlusion in pigs, but salvage of ischemic myocardium was probably related to attenuation of microvascular platelet deposition rather than signaling <<7229>>. Neither a GPIIb/IIIa antagonist [27] nor a regimen of clopidogrel and aspirin given to pigs [28] decreased infarct size. On the other hand, a study in isolated rat hearts showed that either cangrelor or abciximab could block the infarct magnifying effect of platelets from patients

with acute myocardial infarction [29]. All save one of these studies assumed that the observed protection was the result of blockade of platelet aggregation and postulated better reflow in the previously ischemic myocardium during reperfusion. Interestingly Kingma et al. [21] noted there was no effect of the GPIIb/IIIa agent on myocardial flow during ischemia or reperfusion, and, therefore, proposed a direct protective effect on the heart muscle by some unknown mechanism.

To our knowledge the present study is the first to demonstrate a direct anti-infarct effect of inhibitors of platelet aggregation in a primate model. These observations also suggest that the direct cardioprotective effect is not limited to just the P2Y₁₂ inhibitors.

Acknowledgments

This study was supported in part by grant HL-20648 from the Heart, Lung and Blood Institute of the National Institutes of Health and by funds supplied by Otsuka Maryland Medicinal Labs., Inc., Rockville, MD. Cangrelor was synthesized by a private firm.

REFERENCES

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74, 1124–1136.
2. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, et al. Postconditioning the human heart. *Circulation*. 2005; 112:2143–2148. [PubMed: 16186417]
3. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med*. 2008; 359:473–481. [PubMed: 18669426]
4. Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T, et al. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet*. 2007; 370:1483–93. [PubMed: 17964349]
5. Mochly-Rosen D, Grimes KV. Myocardial salvage in acute myocardial infarction-challenges in clinical translation. *J Mol Cell Cardiol*. 2011; 51:451–3. [PubMed: 21851825]
6. Yang X- M, Liu Y, Cui L, Yang X, Liu Y, Tandon N, et al. Platelet P2Y₁₂ blockers confer direct postconditioning-like protection in perfused rabbit hearts. *J Cardiovasc Pharmacol Ther*. 2013 (in press).
7. Tissier R, Cohen MV, Downey JM. Protecting the acutely ischemic myocardium beyond reperfusion therapies: are we any closer to realizing the dream of infarct size elimination? *Arch Mai Coeur Vaiss*. 2007; 100:794–802.
8. Patti G, Bárczi G, Orlic D, Mangiacapra F, Colonna G, Pasceri V, et al. Outcome comparison of 600- and 300-mg loading doses of clopidogrel in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: results from the ARMYDA-6 MI (Antiplatelet therapy for Reducton of MYocardial Damage during Angioplasty-Myocardial Infarction) randomized study. *I Am Coll Cardiol*. 2011; 58:1592–9.
9. Roubille F, Lairez O, Mewton N, Rioufol G, Ranc S, Sanchez I, et al. Cardioprotection by clopidogrel in acute ST-elevated myocardial infarction patients: a retrospective analysis. *Basic Res Cardiol*. 2012; 107:275. [PubMed: 22718009]
10. National Research Council. Guide for the Care and Use of Laboratory Animals. 7th. National Academy Press; Washington, D.C.: 1996.
11. Yang X- M, Liu Y, Liu Y, Tandon N, Kambayashi J, Downey JM, et al. Attenuation of infarction in cynomolgus monkeys: preconditioning and postconditioning. *Basic Res Cardiol*. 2010; 105:119–28. [PubMed: 19669077]
12. Kleinschnitz C, Pozgajova M, Pham M, Bendszus M, Nieswandt B, Stoll G. Targeting platelets in acute experimental stroke: impact of glycoprotein Ib, VI, and IIb/IIIa blockade on infarct size, functional outcome, and intracranial bleeding. *Circulation*. 2007; 115:2323–30. [PubMed: 17438148]

13. Li H, Lockyer S, Concepcion A, Gong X, Takizawa H, Guertin M, et al. The Fab fragment of a novel anti-GPVI monoclonal antibody, OM4, reduces in vivo thrombosis without bleeding risk in rats. *Arterioscler Thromb Vase Biol.* 2007; 27:1199–205.
14. Weig H- J, Bott-Flügel L, Städele C, Winter K, Schmidt R, Gawaz M, et al. Impaired platelet function reduces myocardial infarct size in G_q knock-out mice in vivo. *J Mol Cell Cardiol.* 2008; 44:143–50. [PubMed: 18021799]
15. Takaya N, Katoh Y, Iwabuchi K, Hayashi I, Konishi H, Itoh S, et al. Platelets activated by collagen through the immunoreceptor tyrosine-based activation motif in the Fc receptor γ -chain play a pivotal role in the development of myocardial ischemia-reperfusion injury. *J Mol Cell Cardiol.* 2005; 39:856–64. [PubMed: 16246361]
16. Li H, Takizawa H, Gong X, Concepcion A, Kambayashi J, Tandon N, et al. Platelet glycoprotein VI (GPVI) deletion reduces myocardial infarction in mice. *Circulation.* 2007; 116(Suppl II):II–200.
17. Xu Y, Huo Y, Toufektsian M- C, Ramos SI, Ma Y, Tejani AD, et al. Activated platelets contribute importantly to myocardial reperfusion injury. *Am J Physiol.* 2006; 290:H692–9.
18. Damman P, Woudstra P, Kuijt WJ, de Winter RJ, James SK. P2Y₁₂ platelet inhibition in clinical practice. *J Thromb Thrombolysis.* 2012; 33:143–53. [PubMed: 22183178]
19. Freixa X, Bellera N, Ortiz-Pérez JT, Jiménez M, Paré C, Bosch X, et al. Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. *Eur Heart J.* 2012; 33:103–12. [PubMed: 21846677]
20. Tarantini G, Favaretto E, Marra MP, Frigo AC, Napodano M, Cacciavillani L, et al. Postconditioning during coronary angioplasty in acute myocardial infarction: the POST-AMI trial. *Int J Cardiol.* 2012 (in press).
21. Kingma JG Jr, Plante S, Bogaty P. Platelet GPIIb/IIIa receptor blockade reduces infarct size in a canine model of ischemia-reperfusion. *J Am Coll Cardiol.* 2000; 36:2317–24. [PubMed: 11127479]
22. Kunichika H, Ben-Yehuda O, Lafitte S, Kunichika N, Peters B, DeMaria AN. Effects of glycoprotein IIb/IIIa inhibition on microvascular flow after coronary reperfusion. A quantitative myocardial contrast echocardiography study. *J Am Coll Cardiol.* 2004; 43:276–83. [PubMed: 14736449]
23. Sakuma T, Sari I, Goodman CN, Lindner JR, Klibanov AL, Kaul S. Simultaneous integrin $\alpha_v\beta_3$ and glycoprotein IIb/IIIa inhibition causes reduction in infarct size in a model of acute coronary thrombosis and primary angioplasty. *Cardiovasc Res.* 2005; 66:552–61. [PubMed: 15914120]
24. Reimer KA, Jennings RB. The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest.* 1979; 40:633–44. [PubMed: 449273]
25. Schwartz LM, Sebbag L, Jennings RB, Reimer KA. Duration and reinstatement of myocardial protection against infarction by ischemic preconditioning in open chest dogs. *J Mol Cell Cardiol.* 2001; 33:1561–70. [PubMed: 11549337]
26. Barrabés JA, Garcia-Dorado D, Mirabet M, Inserte J, Agulló L, Soriano B, et al. Antagonism of selectin function attenuates microvascular platelet deposition and platelet-mediated myocardial injury after transient ischemia. *J Am Coll Cardiol.* 2005; 45:293–299. [PubMed: 15653030]
27. Barrabés JA, Garcia-Dorado D, Mirabet M, Lidón R- M, Soriano B, Ruiz-Meana M, et al. Lack of effect of glycoprotein IIb/IIIa blockade on myocardial platelet or polymorphonuclear leukocyte accumulation and on infarct size after transient coronary occlusion in pigs. *J Am Coll Cardiol.* 2002; 39:157–65. [PubMed: 11755302]
28. Yang Y- J, Zhao J- L, You S- J, Wu Y- J, Jing Z- C, Yang W- X, et al. Different effects of tirofiban and aspirin plus clopidogrel on myocardial no-reflow in a mini-swine model of acute myocardial infarction and reperfusion. *Heart.* 2006; 92:1131–7. [PubMed: 16387825]
29. Barrabés JA, Inserte J, Mirabet M, Quiroga A, Hernando V, Figueras J, et al. Antagonism of P2Y₁₂ or GPIIb/IIIa receptors reduces platelet-mediated myocardial injury after ischaemia and reperfusion in isolated rat hearts. *Thromb Haemost.* 2010; 104:128–135. [PubMed: 20431845]

Figure 1. Platelet aggregation in monkeys before (A) and after (B) administration of OM2 antibody. Aggregation was initiated with collagen, 0.5 µg/ml. Whereas the control sample demonstrated robust aggregation, there was none in the post-antibody sample until an additional 2.0 µg/ml collagen (arrow) was added which led to markedly muted aggregation.

Figure 2. Platelet aggregation in monkeys before (A) and after (B) administration of cangrelor. Aggregation was initiated with ADP, 2.5-10 μM .

Figure 3.

Infarct vs risk zone volume plot for 5 monkey groups. Data for ischemic postconditioning (IPOC) was previously reported elsewhere [11] and is included for comparison. Regression lines for the two OM2 groups ($p < 0.01$) and cangrelor ($p < 0.001$) are significantly different from the control regression but not from that for IPOC.

Hemodynamics

Table 1

Groups	Baseline			90 min occlusion			30 min reperfusion			240 min reperfusion		
	HR (bpm)	sys BP (mmHg)	dias BP (mmHg)	HR	sys BP	dias BP	HR	sys BP	dias BP	HR	sys BP	dias BP
Control*	186±7	108±3	83±3	198±8	98±3 [‡]	72±3 [‡]	191±8	100±3 [‡]	76±2 [‡]	204±8	97±3 [‡]	75±3 [‡]
OM2-pretreatment	181±7	98±3	71±3	186±4	86±4 [‡]	62±3 [‡]	188±7	87±3 [‡]	60±3 [‡]	195±8	91±3 [§]	62±3 [§]
OM2-reperfusion	164±7	97±4	72±2	171±10	89±5	65±4	169±11	81±2 [‡]	60±3 [§]	179±10 [§]	83±2 [§]	61±3 [§]
Cangrelor	172±6	103±6	82±5	185±9	98±5	75±4	183±7	96±4	73±3	204±12	103±5	73±5

Abbreviations: BP = blood pressure dias = diastolic HR = heart rate sys = systolic. **Seven of the 14 hearts in this group were reported in a prior publication [11].** Statistical significance of difference between baseline and other time point in any given group:

[§] p<0.005

[‡] p<0.01

[‡] p<0.001

Table 2

Platelet aggregation

Aggregation curve area after 5 min, % of control		
	ADP, 10 μ M	Collagen, 0.5 μ g/ml
Control	100	100
OM2	N/A	2.6 $\pm$ 1.2 *
Cangrelor	6.0 $\pm$ 3.2 *	N/A

*
p<0.001 vs. Control

Table 3

Infarct data

Group	N	BW (kg)	HW (g)	Risk Zone (g)	Infarct Size (g)	IS/RZ (%)
Control *	14	2.8±0.2	14.0±0.8	2.54±0.29	1.37±0.23	49.3±3.9
OM2-pretreatment	6	2.4±0.1	13.3±0.8	2.20±0.34	0.85±0.25	33.4±7.3
OM2-reperfusion	5	2.2±0.1	11.1±0.7	1.83±0.14	0.55±0.13	28.8±5.2
Cangrelor	6	3.6±0.3 [†]	14.7±0.5	3.12±0.28	1.24±0.24	38.0±4.9

Abbreviations: BW = body weight HW = heart weight IS/RZ = ratio of infarct size to risk zone volume N = number of animals

Statistical significance of difference between experimental group and respective control:

* Seven of these animals were reported in a prior publication [11].

[†] p<0.01