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Triple Therapy Greatly Increases Myocardial Salvage During Ischemia/Reperfusion in the *in situ* Rat Heart

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Abstract

Background: Cangrelor, a P2Y₁₂ receptor blocker, administered just prior to reperfusion reduced but did not eliminate myocardial infarction in rabbits. Combining cangrelor with ischemic postconditioning offered no additional protection suggesting they protected by a similar mechanism. To determine if cangrelor's protection might be additive to other cardioprotective interventions we tested cangrelor in combination with ischemic preconditioning, cariporide, a sodium-hydrogen exchange blocker, and mild hypothermia.

Methods: Open-chest rats underwent 30-min coronary occlusion/2-h reperfusion.

Results: Cangrelor, administered as a bolus (60µg/kg) 10 min before reperfusion and continued as an infusion (6µg/kg/min) for the duration of the experiment, decreased infarction from 45.3% of risk zone in control hearts to 25.0%. Combining cangrelor and ischemic preconditioning offered no additional protection. Mild hypothermia (32-33 °C) instituted by peritoneal lavage with cold saline just prior to coronary occlusion resulted in 25.2% infarction, and combining cangrelor and hypothermia nearly halved infarction to 14.1% of risk zone. Cariporide (0.5 mg/kg) just prior to occlusion resulted in 27.2% infarction and 15.8% when combined with cangrelor. Combining cangrelor, hypothermia and cariporide further halved infarction to only 6.3%. We also tested another P2Y₁₂ inhibitor ticagrelor which is chemically similar to cangrelor. Ticagrelor (20 mg/kg) fed 1 h prior to surgery reduced infarct size by an amount similar to that obtained with cangrelor (25.6% infarction), and this protective effect was abolished by chelerythrine and wortmannin, thus implicating participation of PKC and PI3-kinase, resp., in signaling.

Conclusions: Cardioprotection from a P2Y₁₂ receptor antagonist can be combined with at least 2 other strategies to magnify the protection. Combining multiple interventions that use different cardioprotective mechanisms could provide powerful protection against infarction in patients with acute coronary thrombosis.

Keywords

cangrelor; cardioprotection; cariporide; hypothermia; myocardial infarction; platelet aggregation; ticagrelor

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Conflict of Interest

None

Introduction

Ischemic postconditioning or pharmacological triggers of the conditioning mechanism applied just prior to reperfusion produce a potent anti-infarct effect in animal models. But when interventions based on the conditioning mechanism were subjected to large-scale clinical trials, results were disappointing [1-3]. Consequently none has been adopted as standard of care. There are multiple possible reasons for this failure [4], but one might be the possibility that one of the many drugs administered to patients with acute myocardial infarction prior to their revascularization had already protected the heart making additional intervention redundant and ineffective. Likely candidates for the interfering drugs are the antiplatelet agents since early administration of a P2Y₁₂ receptor antagonist significantly reduced infarct size in clinical trials [5,6]. We recently found that the P2Y₁₂ receptor blocker cangrelor given just prior to reperfusion was a potent anti-infarct agent in open-chest rabbits [7] and monkeys [8]. Surprisingly, when cangrelor administration was combined with ischemic postconditioning, there was no additional protection beyond that provided by either intervention alone [7]. We have presented evidence that cangrelor protection requires the same signaling pathways as conditioning [7]. However, we cannot totally exclude the possibility that cangrelor might inhibit the protection of conditioning while protecting by some other mechanism, e.g., prevention of coagulation or inflammation [7].

Because most patients with acute coronary syndrome now receive a P2Y₁₂ blocker prior to reperfusion, antiplatelet drugs could well be responsible for masking protection of other postconditioning interventions. P2Y₁₂ blockers, like ischemic pre- or postconditioning, reduce but, unfortunately, do not eliminate infarction, so muscle loss from infarction is still a clinical problem. However, increased salvage might be achieved if we could identify a non postconditioning-based anti-infarct intervention that could produce additive protection when combined with a P2Y₁₂ blocker. One candidate is the sodium-hydrogen exchange blocker cariporide. It has a strong anti-infarct effect if present during ischemia and has additive effects when combined with the conditioning agent AMP579 [9,10]. The same is true of mild hypothermia. Hypothermia exerts its most potent protection during the ischemic period and its protection can be added to that from ischemic preconditioning [11] which protects primarily during reperfusion [12]. In the present study we tested combinations of these two interventions with a P2Y₁₂ blocker to determine if any additive effects could be achieved.

We used the P2Y₁₂ blocker cangrelor (Fig. 1) to facilitate our animal protocols since it is given intravenously and thus has an immediate onset of action. Cangrelor, however, is currently not approved for clinical use. Ticagrelor (Fig. 1), a very similar oral form of the drug, does have clinical approval so we tested whether oral dosing of ticagrelor could offer similar protection in a rat model of ischemia/reperfusion.

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 [13].

Surgical preparation

Male Sprague-Dawley rats weighing 400-600 g were anesthetized with intraperitoneal pentobarbital (100 mg/kg). Subsequently anesthesia was supplemented with intravenous boluses of pentobarbital as needed to maintain the surgical plane. Through a midline cervical incision the trachea was incised and intubated. The tube was attached to a positive-pressure ventilator (MD Industries, Mobile, AL) and the lungs inflated with 100% oxygen. Respiratory rate and tidal volume were adjusted to keep arterial blood pH in the physiologic

range. A carotid artery was cannulated for measurement of pressure and withdrawal of blood. Cardiac temperature was monitored with an IT-18 thermocouple probe (Physiotemp, Clifton, NJ) that was advanced down the other carotid artery into the arch of the aorta. The pericardium was opened through a left thoracotomy in the fourth intercostal space to expose the heart. A 2-0 suture on a curved taper needle was passed beneath the left anterior descending artery near its origin, and the ends were pulled through a small vinyl tube to form a snare. The coronary artery was occluded by pulling the snare, which was then fixed by clamping the tube with a small hemostat. Arterial blood (0.6 ml) was withdrawn for measurement of platelet aggregometry in all animals after 19 min of coronary occlusion. In those treated with a blocker of platelet aggregation or signaling antagonist, blood for platelet aggregometry was also withdrawn 1 min prior to the end of the 30-min coronary occlusion.

Infarct area and risk zone

As previously described [14] the heart was removed at the end of the experiment and mounted on a Langendorff apparatus. The aortic root was perfused with saline at a pressure of 80 cm H₂O to wash out blood from the coronary arteries. The snared coronary artery was reoccluded, and 2-9 μ m fluorescent microspheres (Microgenics Corp., Fremont, CA) were injected into the aortic perfusate and retrogradely infused into the heart to demarcate risk zone as the tissue without fluorescence. The heart was weighed, frozen, and cut into ~2 mm-thick slices which were incubated in 1% triphenyltetrazolium chloride (GFS Chemicals, Powell, OH) in pH7.4 buffer at 37°C to differentiate living (stained) from infarcted (unstained) tissue. Risk zone and infarcted regions were traced on plastic overlays by a person blinded to the treatment, and areas measured by planimetry. Infarct and risk zone volumes were calculated by multiplying area by slice thickness and summing the products. Infarct size is expressed as a percentage of the risk zone.

Platelet aggregometry

Platelet aggregation was determined by measuring impedance with a whole blood aggregometer (Chrono-log Corp., Havertown, PA). One-half ml of saline and 0.5 ml of heparinized arterial blood were combined in a plastic cuvette and continuously stirred at 37°C. Aggregation was initiated by addition of 10 μ l of 1 mM ADP to produce a final concentration of 10 μ M. Nearly all aggregation curves showed early aggregation followed by slow disaggregation, a pattern normally seen in rat blood [15]. Area under each aggregation curve was measured and averaged for each group.

Protocols

The protocols are presented in Fig. 2. For all experiments the coronary artery was occluded for 30 min after which the snare was loosened, and reperfusion lasted for 2 h. The first control group received no treatment, no vehicle, and no introduction of intraperitoneal fluid. The many groups were studied serially since the results of one group often suggested an obvious follow-up experiment. To avoid including a redundant control group for each subsequent experimental group, control hearts were evaluated periodically throughout the study to ensure that all animals from our supplier had a similar baseline response to ischemia/reperfusion. A second control group labeled Triple Vehicle was subjected to the same ischemia/reperfusion protocol, but was also treated with a 0.5 ml bolus of intravenous saline 10 min before reperfusion followed by a continuous infusion of 1 ml/h saline until the end of reperfusion, a 30 μ l bolus intravenous injection of DMSO in 0.5 ml saline 5 min before reperfusion, and the intraperitoneal injection of 40 ml of saline warmed to 37°C 10 min before coronary occlusion and removed at the end of the occlusion. These maneuvers examined the effects of infusion of vehicle used in cangrelor and cariporide treatment groups and the intraperitoneal fluid injection protocol of hypothermia (see below).

To examine the possibility that interventions separately targeting the ischemic and reperfusion phases might yield additive protection, we first examined cangrelor which protects during early reperfusion [7]. Cangrelor, an intravenous preparation, affects platelet aggregation within 1-2 min and could thus be started shortly before reperfusion. Cangrelor was administered as an intravenous bolus, 60 µg/kg in saline, 10 min before reperfusion followed by an infusion of 6 µg/kg/min for the duration of the study. One group was treated with only cangrelor. In another group cooling was initiated by infusing 40 ml of ice-cold saline into the peritoneal cavity. This was done 10 min before occlusion and blood temperature had fallen to 32-33 °C by the time the coronary artery was occluded. The cold saline was removed at the end of the occlusion, and rewarming was effected with a heating pad. Core temperature measured by rectal probe returned to its control level after approximately 60 min. In the third group cariporide, 0.5 mg/kg dissolved in DMSO, a dose previously shown to produce cardioprotection [9], was injected intravenously 5 min before occlusion. In the next group cooling during ischemia was combined with cangrelor administration. In another group cangrelor and cariporide were combined. In the sixth group all 3 interventions, cariporide, cangrelor, and hypothermia, were combined. For comparison another group of rats was preconditioned with 3 cycles of 5-min coronary occlusion/5-min reperfusion before the 30-min occlusion. In the last group preconditioning was combined with cangrelor.

Ticagrelor is an oral P2Y₁₂ receptor antagonist. A 90 mg pill was crushed and suspended in water. Rats were treated with 20 mg/kg by gavage. One h later surgery for the ischemia/reperfusion protocol described above was started. Care was taken to cauterize all cut surfaces. Surgery was completed in 1 h. One group was treated with only ticagrelor. In two groups treated with ticagrelor either the PKC antagonist chelerythrine, 5 mg/kg, or the phosphatidylinositol 3-kinase inhibitor wortmannin, 60 µg/kg, was administered after 25 min of coronary occlusion. Two additional groups of rats were treated with only the two signaling inhibitors.

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 sizes were analyzed by one-way ANOVA. Post hoc testing was done with Student-Newman-Keuls test. The significance of changes of infarct size with interventions was also determined by plotting infarct size as a function of risk zone volume and then comparing regression lines for each intervention by ANCOVA. Platelet aggregation curves were analyzed by determining areas beneath the curves truncated at 5 min. Because we pretreated rats with oral ticagrelor 2 hours prior to coronary occlusion, it was not possible to obtain drug-free samples. The average of all aggregation curves in control animals was determined and this was used for comparison of samples from treated rats using an unpaired t-test. For rats treated with only chelerythrine or wortmannin, it was possible to compare aggregation in untreated and treated arterial samples with a paired t-test. A p value of <0.05 was considered to be significant.

Results

Experiments were completed in 95 rats in 15 experimental groups. Only 2 rats in the ticagrelor group were excluded from analysis because of very small risk zones of less than 0.3 cm³. In all normothermic groups blood temperature was closely monitored and maintained in a narrow range of 36.8-37.2°C for the duration of the experiment with the aid of a heating pad. In hypothermic groups there was a 3.5°C drop in blood temperature within 5 min of introduction of ice-cold saline into the peritoneal cavity (Fig. 3), and the target of 32-33°C was reached in less than 10 min. This low temperature was maintained for the

entire 30-min ischemic period. Removal of the cold saline and use of the heating pad at the onset of reperfusion produced rewarming and baseline blood temperature was reached after approximately 45 min. Blood pressure and heart rate data are presented in Table 1. There were no differences among groups at baseline. Neither chelerythrine nor wortmannin affected hemodynamics. Hypothermia significantly slowed heart rate without effect on blood pressure. Blood pressure decreased during coronary occlusion and tended to recover following reperfusion. Bleeding was more prevalent in rats treated with ticagrelor, and this often resulted in hypotension during later phases of reperfusion. The combination of ticagrelor and chelerythrine also produced hypotension. Average infarct sizes for the groups are summarized in Table 2. Risk zone volume did not differ among groups. The presented infarct sizes were normalized for risk zone. Importantly there was no difference between infarct size in Control and Triple Vehicle groups. As is our practice we also analyzed infarct size plotted against risk zone volume to test whether risk zone size might be an independent determinant of the infarct/risk zone ratio in any group. Risk zone size is a strong determinant of that ratio in rabbit [16] and humans [17], but less so in rat hearts [16].

Cangrelor plus ischemic preconditioning

In rabbits postconditioning has no additive effect when combined with cangrelor [7]. To see if conditioning and cangrelor might be additive in the rat, we combined ischemic preconditioning and cangrelor (Fig. 4A). Ischemic preconditioning is quite protective (19% infarction of risk zone compared to 45.3% in control hearts), but combining it with cangrelor does nothing additional (24% infarction). We, therefore, did not include a conditioning intervention with our multiple therapy analysis below. Ischemic preconditioning had no significant effect on platelet aggregation. Fig. 4B shows that risk zone size was comparable among the groups and did not contribute to the percent of the risk zone that infarcted. Additionally all points for the cangrelor, ischemic preconditioning and combination groups were significantly displaced downwards from the control points ($p < 0.001$) thus indicating a protected state.

Combination Therapy

Cangrelor blocks platelet aggregation in rats by ~ 90% and protects ischemic myocardium with only 25% of the risk zone infarcting (Figs. 4A and 5A). Fig. 5 shows the effect of combining cangrelor with other cardioprotective interventions. Whole-body cooling during myocardial ischemia has a similar effect on infarct size as cangrelor (25.2% infarction) (Fig. 5 A). When these two interventions are combined, protection is magnified and infarction nearly halved to 14.1% of myocardium at risk. Cariporide's protective effect is also comparable to that observed with cangrelor (27.2% infarction), and the combination of cangrelor and cariporide again halves infarcting tissue to 15.8% of risk zone. A combination of all 3 interventions reduces infarction to only 6.3% of risk zone. In some of these hearts infarction was confined to the contusion caused by the ligature around the coronary artery and thus infarction caused by myocardial ischemia was actually nil. To further illustrate the magnitude of the protection representative photographs of the stained heart slices from the cangrelor, cariporide, and combined therapy groups appear in Fig 6.

Oral Ticagrelor

Cangrelor is not approved for clinical use and currently only oral P2Y₁₂ blockers are available. We, therefore, tested the oral drug ticagrelor which is chemically similar to cangrelor as both are adenosine analogs (Fig. 1). Two and a half hours after gavage ticagrelor significantly blunted peak platelet aggregation by an average of 84%. Platelet aggregation curves in representative rats are shown in Fig. 7. Oral ticagrelor has an infarct-sparing effect identical to that of cangrelor. Whereas 45.3% of risk zone infarcted in control hearts, only 25.6% infarcted in animals treated with this P2Y₁₂ antagonist (Fig. 8A).

Ticagrelor's protection was eliminated by either chelerythrine or wortmannin, two signal transduction antagonists that abolish protection from pre- or postconditioning when administered shortly before reperfusion. Neither of these signaling blockers interfered with ticagrelor's inhibitory effect on platelet reactivity suggesting that protection from ticagrelor was not a direct result of blocked platelet aggregation. As depicted in Fig. 8B, the infarct-risk zone regression line for hearts treated with ticagrelor is significantly displaced downwards compared to all other groups ($p < 0.001$).

Discussion

Although a number of cardioprotective interventions have been subjected to large clinical trials, their results have been disappointing despite promising animal data. The only exception has been loading doses of P2Y₁₂ receptor inhibitors administered prior to reperfusion therapy. These agents have yielded significant reductions in infarct size [5] and fewer adverse major cardiovascular events in treated patients [5,18,19] and have become standard of care for patients with acute coronary syndrome. Yet infarction of myocardium has not been eliminated by P2Y₁₂ inhibition. We propose that no single known intervention is potent enough to adequately protect the heart against prolonged ischemic times that patients currently face prior to therapeutic reperfusion. Perhaps combining multiple cardioprotective interventions could impart more meaningful protection against infarction and achieve a greatly improved outcome in these patients.

We previously described the protective qualities of cangrelor [7,8], cariporide [9], cooling [11,20,21], and ischemic preconditioning [22]. We found that the protection of ischemic preconditioning could be added to that of cooling [11] and that the protective effect of pharmacological postconditioning with AMP579 could be added to that of cariporide [9]. We now show that combining cangrelor with either cooling or cariporide also results in additive effects. And the triple combination of cangrelor, cariporide, and cooling is even more protective with essentially no infarction since necrosis was mostly restricted to the tissue around the coronary ligature. Combining multiple therapies may be the key to greatly improving outcomes in patients.

The triple therapy examined here is technically feasible in patients. Platelet inhibitors are already in wide use although their current dosing may not be optimal. P2Y₁₂ inhibition must be active during the first minutes of reperfusion to protect against infarction [7]. Oral drugs are slow to be absorbed from the GI tract and platelet inhibition has been found to be suboptimal at time of ballooning in many treated patients [23,24]. A fast-acting intravenous preparation like cangrelor could remedy that situation.

We used cariporide as a sodium-hydrogen exchange inhibitor as it was readily available. Unfortunately, it increased the incidence of stroke when it was administered to surgical patients over a prolonged period [25] making its future clinical use questionable. However, other exchange blockers such as zoniporide or eniporide currently have no reported safety issues. We found that the exchange blocker was most protective when present during the ischemic period, while treatment starting at reperfusion was ineffective [9]. It would be impossible to administer an exchange blocker prior to coronary occlusion in a clinical setting. However, first responders could administer the drug which would then enter the ischemic tissue via collateral flow so that most of the critical ischemic period could be covered.

Mild hypothermia also seems to exert its protection during ischemia [11,20]. Hypothermia is more difficult to implement and significant cooling outside the hospital would be problematic. However, simply lavaging the peritoneal cavity with cold saline enabled us to

achieve the target temperature of 32-33 °C within 10 min. The temperature of blood returning to the heart from the lower half of the body readily equilibrates with that of the peritoneal fluid. Lavage could be begun soon after arrival at the hospital while the patient is being prepared for angioplasty. Since door-to-balloon times are typically 90 min, the heart could be exposed to mild hypothermia during much of the ischemic period as well. There is evidence that mild hypothermia actually protects via signaling pathways [21,26]. If so, it is likely that this form of protection could eventually be duplicated pharmacologically.

Cangrelor is most effective when administered just prior to reperfusion, and, therefore, likely targets reperfusion injury [7], while cariporide [9] and cooling [11,20] appear to target ischemic injury. These differing behaviors suggest that the protective mechanisms differ and we propose that this is the key to the additive protection of these interventions. Since cariporide and hypothermia protections are also additive, fundamental differences in mechanisms between these two are also likely. It is instructive to note that the mechanism and signaling of each of these three interventions have been extensively evaluated [7,21,26-29].

Mild hypothermia has been known to have a strong anti-infarct effect since the studies of Chien et al. [30]. Reducing the heart's temperature by just a few degrees during ischemia offers protection that is proportional to the degree of hypothermia [11] but does not impair its pump function. The assumed mechanism had been reduced utilization of energy stores from direct slowing of the heart's metabolic chemistry. However, recent evidence suggests that the protective mechanism is unrelated to reduced ATP utilization but rather involves signal transduction [21]. Cooling preserves ERK signaling during ischemia and an ERK blocker removes its protection but has no effect on ischemic injury in normothermic hearts [21]. Shao et al. [26] noted that Akt and nitric oxide signaling was also required for hypothermia's protection. These signals have also been shown to be required for protection from cangrelor [7] and conditioning [31], but in these cases the critical time for ERK signaling is during reperfusion rather than ischemia. Thus cooling prevents injury during ischemia while cangrelor prevents a reperfusion injury, most likely resulting from transition pore formation in the mitochondria.

Cariporide's mechanism of protection has been extensively studied in the past (for review see [32]), but its mechanism is not settled. One scenario proposes that at reperfusion Na^+ enters rapidly in exchange for the excess H^+ in the cell. The Na^+ then exchanges with Ca^{++} to produce Ca^{++} overload, and this would be attenuated by a Na^+-H^+ exchange blocker. Another proposed mechanism is a cariporide-induced delay in mitochondrial matrix acidification thus preserving ATP production during ischemia. The majority of investigators find cariporide administered at reperfusion to be ineffective, while virtually all find cariporide pretreatment to be very protective [33]. Whatever the actual mechanism, we found that protection from cariporide during ischemia is additive to that of cooling during ischemia suggesting their mechanisms differ.

P2Y_{12} receptor blockers have traditionally been thought to protect by preventing intravascular coagulation in the ischemic coronary vasculature [34]. It has also been proposed that activated platelets combine with activated neutrophils and directly attack ischemic myocardium [35,36]. It was concluded protection is directly related to inhibition of platelet aggregation. We previously found protection from cangrelor could not be added to that of postconditioning in rabbits [7]. Now we show protection from cangrelor could not be added to that from ischemic preconditioning in rats. To explain that lack of synergy we have proposed cangrelor protects by the same "conditioning" mechanism that is used by pre- and postconditioning. Although that hypothesis has yet to be proven, there is evidence to support that claim. Inhibiting signaling pathways required for conditioning's protection also blocks

protection from cangrelor as well as clopidogrel [7]. Yet none of these blockers interferes with the ability of P2Y₁₂ antagonists to inhibit platelet aggregation suggesting protection from these drugs is not simply the result of inhibiting aggregation. The present study extends that behavior to ticagrelor.

In a retrospective analysis of a clinical trial of ischemic postconditioning it was reported the combination of ischemic postconditioning and clopidogrel did have a synergistic effect [6]. But it's likely neither intervention was optimized to fully condition the hearts when applied individually. Patients for that study were recruited prior to 2005 when the beneficial effect of loading doses of P2Y₁₂ inhibitors was just beginning to be appreciated. Only one-half of the patients received a loading dose of clopidogrel, and most received only 300 mg which is clearly suboptimal [5]. The optimal postconditioning protocol for human hearts is unknown, and only one protocol was tested. Several subsequent clinical studies in which all patients received loading doses of a P2Y₁₂ inhibitor could find no additional protection from ischemic postconditioning [37,38].

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References

1. 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]
2. 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]
3. Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet*. 2013; 381:166–75. [PubMed: 23095318]
4. Cohen MV, Downey JM. Is it time to translate ischemic preconditioning's mechanism of cardioprotection into clinical practice? *J Cardiovasc Pharmacol Ther*. 2011; 16:273–80. [PubMed: 21821528]
5. 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. *J Am Coll Cardiol*. 2011; 58:1592–9. [PubMed: 21958886]
6. 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]
7. Yang X- M, Liu Y, Cui L, Yang X, Liu Y, Tandon N, et al. Platelet P2Y₁₂ blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J Cardiovasc Pharmacol Ther*. 2013; 18:251–62. [PubMed: 23233653]
8. Yang X- M, Liu Y, Cui L, Yang X, Liu Y, Tandon N, et al. Two classes of anti-platelet drugs reduce anatomical infarct size in monkey hearts. *Cardiovasc Drugs Ther*. 2013; 27:109–15. [PubMed: 23318690]
9. Xu Z, Jiao Z, Cohen MV, Downey JM. Protection from AMP 579 can be added to that from either cariporide or ischemic preconditioning in ischemic rabbit heart. *J Cardiovasc Pharmacol*. 2002; 40:510–8. [PubMed: 12352312]
10. Kristo G, Yoshimura Y, Ferraris SP, Jahania SA, Mentzer RM Jr, Lasley RD. The preischemic combination of the sodium-hydrogen exchanger inhibitor cariporide and the adenosine agonist

- AMP579 acts additively to reduce porcine myocardial infarct size. *J Am Coll Surg*. 2004; 199:586–94. [PubMed: 15454144]
11. Miki T, Liu GS, Cohen MV, Downey JM. Mild hypothermia reduces infarct size in the beating rabbit heart: a practical intervention for acute myocardial infarction? *Basic Res Cardiol*. 1998; 93:372–83. [PubMed: 9833149]
 12. Hausenloy DJ, Wynne AM, Yellon DM. Ischemic preconditioning targets the reperfusion phase. *Basic Res Cardiol*. 2007; 102:445–52. [PubMed: 17530316]
 13. National Research Council. Guide for the Care and Use of Laboratory Animals. 7th. National Academy Press; Washington, DC: 1996.
 14. 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]
 15. Sugidachi A, Asai F, Ogawa T, Inoue T, Koike H. The in vivo pharmacological profile of CS-747, a novel antiplatelet agent with platelet ADP receptor antagonist properties. *Br J Pharmacol*. 2000; 129:1439–46. [PubMed: 10742300]
 16. Ytrehus K, Liu Y, Tsuchida A, Miura T, Liu GS, Yang X- M, et al. Rat and rabbit heart infarction: effects of anesthesia, perfusate, risk zone, and method of infarct sizing. *Am J Physiol*. 1994; 267:H2383–90. [PubMed: 7528994]
 17. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, et al. Postconditioning the human heart. *Circulation*. 2005; 112:2143–8. [PubMed: 16186417]
 18. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, et al. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. *Lancet*. 2009; 373:723–31. [PubMed: 19249633]
 19. Kohli P, Wallentin L, Reyes E, et al. Reduction in first and recurrent cardiovascular events with ticagrelor compared with clopidogrel in the PLATO study. *Circulation*. 2013; 127:673–80. [PubMed: 23277305]
 20. Tissier R, Hamanaka K, Kuno A, Parker JC, Cohen MV, Downey JM. Total liquid ventilation provides ultra-fast cardioprotective cooling. *J Am Coll Cardiol*. 2007; 49:601–5. [PubMed: 17276185]
 21. Yang X, Liu Y, Yang X- M, Hu F, Cui L, Swingle MR, et al. Cardioprotection by mild hypothermia during ischemia involves preservation of ERK activity. *Basic Res Cardiol*. 2011; 106:421–30. [PubMed: 21399968]
 22. Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A₁ adenosine receptors in rabbit heart. *Circulation*. 1991; 84:350–6. [PubMed: 2060105]
 23. Sibbing D, Bernlochner I, Schulz S, Massberg S, Schomig A, Mchilli J, et al. Prognostic value of a high on-clopidogrel treatment platelet reactivity in bivalirudin versus abciximab treated non-ST-segment elevation myocardial infarction patients: ISAR-REACT-4 (Intracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment-4) platelet substudy. *J Am Coll Cardiol*. 2012; 60:369–77. [PubMed: 22682553]
 24. Parodi G, Valenti R, Bellandi B, Migliorini A, Marcucci R, Comito V, et al. Comparison of prasugrel and ticagrelor loading doses in ST-segment elevation myocardial infarction patients: RAPID (Rapid Activity of Platelet Inhibitor Drugs) primary PCI study. *J Am Coll Cardiol*. 2013; 61:1601–6. [PubMed: 23500251]
 25. Mentzer RM Jr, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B, et al. Sodium/hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg*. 2008; 85:1261–70. [PubMed: 18355507]
 26. Shao Z- H, Sharp WW, Wojcik KR, Li C- Q, Han M, Chang W- T, et al. Therapeutic hypothermia cardioprotection via Akt- and nitric oxide-mediated attenuation of mitochondrial oxidants. *Am J Physiol*. 2010; 298:H2164–73.

27. Miura T, Ogawa T, Suzuki K, Goto M, Shimamoto K. Infarct size limitation by a new Na⁺-H⁺ exchange inhibitor, Hoe 642: difference from preconditioning in the role of protein kinase C. *J Am Coll Cardiol.* 1997; 29:693–701. [PubMed: 9060913]
28. Miura T, Liu Y, Goto M, Tsuchida A, Miki T, Nakano A, et al. Mitochondrial ATP-sensitive K⁺ channels play a role in cardioprotection by Na⁺-H⁺ exchange inhibition against ischemia/reperfusion injury. *J Am Coll Cardiol.* 2001; 37:957–63. [PubMed: 11693777]
29. Toda T, Kadono T, Hoshiai M, Eguchi Y, Nakazawa S, Nakazawa H, et al. Na⁺/H⁺ exchanger inhibitor cariporide attenuates the mitochondrial Ca²⁺ overload and PTP opening. *Am J Physiol.* 2007; 293:H3517–23.
30. Chien GL, Wolff RA, Davis RF, VanWinkle DM. "Normothermic range" temperature affects myocardial infarct size. *Cardiovasc Res.* 1994; 28:1014–7. [PubMed: 7954586]
31. 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.
32. Karmazyn M. NHE-1: still a viable therapeutic target. *J Mol Cell Cardiol.* 2013 in press.
33. Avkiran M, Marber MS. Na⁺/H⁺ exchange inhibitors for cardioprotective therapy: progress, problems and prospects. *J Am Coll Cardiol.* 2002; 39:747–53. [PubMed: 11869836]
34. 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]
35. Lefer AM, Campbell B, Scalia R, Lefer DJ. Synergism between platelets and neutrophils in provoking cardiac dysfunction after ischemia and reperfusion: role of selectins. *Circulation.* 1998; 98:1322–8. [PubMed: 9751682]
36. Campbell B, Chuhuran CM, Lefer DJ, Lefer AM. in an isolated perfused rat heart model of ischemia and reperfusion. *Methods Find Exp Clin Pharmacol.* 1999; 21:529–34. [PubMed: 10599051]
37. Freixa X, Bellera N, Ortiz-Perez JT, Jimenez M, Pare 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]
38. 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; 162:33–8. [PubMed: 22494866]

Cangrelor

Ticagrelor

Figure 1.
Molecular structures of cangrelor and ticagrelor

Figure 2.
Protocols

Figure 3.

Changes in blood temperature in rats made hypothermic by introduction of ice-cold saline into the peritoneal cavity at time 0 (Cool). After 10 min the coronary artery was occluded (Cool + Ischemia). After 30 min of coronary occlusion, the myocardium was reperfused, the cold saline was removed from the peritoneal cavity, and the heating pad was turned on (Reperfusion + Rewarming).

Figure 4.

A. Infarct size as a percentage of risk zone in cangrelor-treated rats. Open symbols depict individual experiments while solid symbols depict group means $\pm$ SEM. Statistical significance of difference vs Control: * $p < 0.001$. Abbreviations: Cang = cangrelor PC = ischemic preconditioning. B. Infarct size plotted against risk zone size for all hearts studied in panel A. The lines are least squares fit through the origin.

Figure 5.

A. Infarct size as a percentage of risk zone in cangrelor-treated rats. Open symbols depict individual experiments while solid symbols depict group means $\pm$ SEM. Abbreviations: Cang = cangrelor, Carip = cariporide, Cool = mild hypothermia. The Triple Vehicle group combined exposure of hearts undergoing ischemia/reperfusion to the vehicles used in other groups: saline infusion in Cang, DMSO injection in Carip, and intraperitoneal injection of warm saline in Cool. Statistical significance of difference vs Control: * $p < 0.001$ Statistical significance of difference vs cangrelor or cooling or cariporide: † $p < 0.001$ Statistical significance of difference vs cooling + cangrelor or cangrelor + cariporide: ‡ $p < 0.001$ § $p < 0.001$ B. Infarct size plotted against risk zone size for all hearts studied in panel A. The lines are least squares fit through the origin.

Figure 6.

Representative hearts sliced from apex to base which had been exposed to either cangrelor or cariporide individually, their combination, or triple therapy of cangrelor + cariporide + cooling. The hearts are stained with triphenyltetrazolium and photographed under white light. The blue line shows the boundary between ischemic and normally-perfused tissue as determined from the distribution of fluorescent tracer microspheres when viewed under UV light (lower right panel). The latter heart photographed under UV light is not one of the hearts shown in the rest of the figure.

Figure 7. Platelet aggregation in an untreated rat (Control) and in one of the treated rats 2½h after oral ticagrelor. Platelet aggregation was initiated with 10 μ M ADP.

Figure 8.

A. Infarct size as a percentage of risk zone in ticagrelor-treated rats. Open symbols depict individual experiments while solid symbols depict group means $\pm$ SEM. Abbreviations: Chel= chelerythrine, Ticag = ticagrelor, Wort = wortmannin. Statistical significance of difference vs Control: * $p < 0.05$ ** $p < 0.001$ B. Infarct size plotted against risk zone size for all hearts studied in panel A. The lines are least squares fit through the origin.

Table 1**Hemodynamics**

Groups	Baseline		30 min occlusion		30 min reperfusion	
	HR (bpm)	mBP (mmHg)	HR	mBP	HR	mBP
Control	422±8	119±4	415±13	100±10 [*]	408±9	104±5
Triple Vehicle	424±17	112±13	420±12	111±3	409±14	111±4
Cangrelor	413±18	108±5	391±8	84±11 [‡]	401±14	92±10 [*]
Cooling	381±16	114±3	317±20 [§]	87±12 [*]	370±17	116±12
Cariporide	443±7	111±7	422±17	100±5	422±11	108±6
Cangrelor + Cooling	404±12	111±5	343±15 [*]	99±9	401±22	127±11
Cangrelor + cariporide	428±15	121±6	407±4	93±5 [*]	393±10 [*]	123±7
Cang + car + cool	418±7	118±9	368±11 [§]	104±9	431±13	138±10 [*]
PC	416±5	112±8	401±11	85±11 [‡]	400±12	93±9 [*]
PC + Cangrelor	389±11	106±9	393±15	100±7	376±15	92±3
Ticagrelor	423±15	116±8	406±11	92±9	402±11	95±7
Ticagrelor + chelerythrine	422±19	101±6	393±15	63±3 [*]	383±12	60±13 [*]
Chelerythrine	428±12	113±4	413±18	87±4	403±14	94±10
Ticagrelor + wortmanin	405±11	110±6	417±15	103±12	397±15	85±10
Wortmannin	430±18	109±7	417±19	93±14	425±18	94±14

Abbreviations: cang = cangrelor car = cariporide cool = cooling HR = heart rate mBP = mean blood pressure PC = ischemic preconditioning
mean±SEM

Statistical significance of difference between baseline and other time point in a given group:

^{*}
p<0.05

[§]
p<0.01

[‡]
p<0.005

Table 2

Infarct data

Group	N	BW (g)	HW (g)	Risk Zone (g)	Infarct Size (g)	IS/RZ (%)
Control	12	488±20	1.7±0.1	0.40±0.01	0.18±0.01	45.3±1.4
Cangrelor	7	455±5	1.6±0.0	0.44±0.02	0.11±0.01 *	25.0±1.8 *
Triple Vehicle	4	538±24	1.9±0.0	0.45±0.04	0.20±0.02	43.7±1.2
Cooling	6	524±13	1.7±0.0	0.49±0.01	0.12±0.01 †	25.2±0.9 *
Cariporide	6	519±8	1.7±0.0	0.50±0.02	0.14±0.01 §	27.2±2.8 *
Cangrelor + cooling	5	582±31 †	1.8±0.1	0.50±0.03	0.07±0.01 *	14.1±0.1 *
Cangrelor + cariporide	6	564±15 §	1.8±0.0	0.43±0.03	0.07±0.00 *	15.8±1.1 *
Cangrelor + cariporide + cooling	7	559±19 §	1.8±0.0	0.47±0.01	0.03±0.00 *	6.3±0.8 *
PC	6	549±13	1.9±0.1	0.48±0.02	0.09±0.02 *	19.4±3.5 *
PC + cangrelor	5	566±25 §	1.9±0.1	0.42±0.03	0.10±0.01 *	24.3±2.2 *
Ticagrelor	7	560±16 §	1.8±0.1	0.42±0.03	0.11±0.02 *	25.6±2.0 *
Ticagrelor + chelerythrine	6	541±13	1.9±0.0	0.48±0.03	0.21±0.02	43.9±1.6
Chelerythrine	6	566±10 †	1.9±0.0	0.46±0.03	0.20±0.02	42.9±1.9
Ticagrelor + wortmannin	6	530±9	1.9±0.1	0.49±0.03	0.26±0.02 *	52.2±3.7 §
Wortmannin	6	581±10 †	1.9±0.1	0.44±0.03	0.24±0.02 §	53.6±2.6 †

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

Statistical significance of difference between Control and other groups:

§ p<0.05

† p<0.01

* p<0.001