The Coronary Circulation as a Target of Cardioprotection

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Abstract: The atherosclerotic coronary vasculature is not only the culprit but also a victim of myocardial ischemia/reperfusion injury. Manifestations of such injury are increased vascular permeability and edema, endothelial dysfunction and impaired vasomotion, microembolization of atherothrombotic debris, stasis with intravascular cell aggregates, and finally, in its most severe form, capillary destruction with hemorrhage. In animal experiments, local and remote ischemic pre- and postconditioning not only reduce infarct size but also these manifestations of coronary vascular injury, as do drugs which recruit signal transduction steps of conditioning. Clinically, no-reflow is frequently seen after interventional reperfusion, and it carries an adverse prognosis. The translation of cardioprotective interventions to clinical practice has been difficult to date. Only 4 drugs (brain natriuretic peptide, exenatide, metoprolol, and esmolol) stand unchallenged to date in reducing infarct size in patients with reperfused acute myocardial infarction; unfortunately, for these drugs, no information on their impact on the ischemic/reperfused coronary circulation is available. (Circ Res. 2016;118:1643-1658. DOI: 10.1161/CIRCRESAHA.116.308640.)

Key Words: coronary artery disease □ coronary occlusion □ hemorrhage □ myocardial infarction □ reperfusion injury

Myocardial ischemia/reperfusion injury affects not only the cardiomyocyte compartment but also all other cellular compartments, and the coronary circulation has a central role in it. Acute myocardial infarction most often arises from atherosclerotic plaque rupture/erosion with superimposed thrombosis (type 1 myocardial infarction). However, in the absence of coronary atherosclerosis, coronary vasospasm and endothelial dysfunction may also precipitate acute myocardial infarction (type 2). Reperfusion of the occluded coronary artery with restoration of coronary blood flow not only terminates myocardial ischemia but also inflicts additional injury, and interventional or surgical revascularization may actually induce periprocedural myocardial infarction (types 4 and 5 myocardial infarction). The spatial and temporal evolution of coronary occlusion and reperfusion determine not only the size of the affected myocardial region but also the nature of the outcome from myocardial ischemia/reperfusion, that is, reversible (stunning) or irreversible (infarction) injury and, vice versa, also protection from injury (hibernation and conditioning).

Cardioprotective interventions reduce myocardial ischemia/reperfusion injury, notably infarct size, but also arrhythmias, left ventricular dysfunction, and coronary vascular impairment. A complex signal transduction cascade underlies the cardioprotective effects of ischemic preconditioning, ischemic postconditioning, and remote ischemic conditioning. A variety of drugs that often recruit signaling steps of conditioning strategies have been used to achieve cardioprotection. The translation of cardioprotection from animal experiments to clinical practice has been difficult and largely disappointing to date, despite several positive proof-of-concept studies in humans. Neglect of the coronary circulation as a victim of myocardial ischemia/reperfusion injury and as a target for cardioprotection may have contributed to the lack of translation of cardioprotection to clinical practice.

A particular problem is acute myocardial infarction in women. On the one hand, the female heart is more resistant to myocardial ischemia/reperfusion than the male heart. On the other hand, women have nonobstructive coronary artery disease more often than men, and coronary vasomotion (coronary vasospasm, endothelial dysfunction, and microvascular dysfunction) may play a greater role in precipitating acute myocardial infarction in women.

Coronary Circulation as a Determinant of Myocardial Ischemic Injury

The perfusion territory of the coronary artery distal to the site of the occlusion is the area at risk of infarction because the coronary arteries are functional end arteries. Within a given area at risk, both the duration and the severity of coronary blood flow reduction determine the nature and amount of injury. A complete coronary occlusion of <20-minute duration results in reversible injury, that is, contractile dysfunction with a slow, but complete recovery during reperfusion, a phenomenon called myocardial stunning. The underlying mechanisms of the prolonged contractile dysfunction relate to the

Original received March 1, 2016; revision received March 17, 2016; accepted March 22, 2016.
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Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.116.308640
enhanced formation of reactive oxygen species during early reperfusion and impaired excitation–contraction coupling after oxidative modification of the sarcoplasmic reticulum and the contractile proteins. Repeated coronary occlusion of short duration or prolonged moderate reduction in coronary blood flow results in hibernating myocardium, a phenomenon of reduced contractile function with retained viability and thus eventual recovery after reperfusion. Hibernating myocardium displays signs of both injury (loss of contractile proteins, small doughnut-like mitochondria, and fibrosis) and adaptation (short-term energetic recovery, altered expression of mitochondrial proteins, and proteins related to cardioprotection). When the reduction in coronary blood flow is severe and lasts longer than 20 to 40 minutes, infarction in larger mammals develops first in the inner subendocardial layers of the core of the area at risk and then spreads in a wavefront to the outer subepicardial layers and the borders of the area at risk over time. The wavefront of infarct development reflects the lateral and transmural distribution of coronary blood flow, which is less in the inner than in the outer layers of the myocardium and less in the core than in the borders of the area at risk. The evolution of infarction varies with species and depends on the existence and extent of a collateral circulation. Rodents have a high heart rate and rapid development of infarction; only in guinea pigs there is such an extensive collateral circulation that no infarction develops for hours of coronary occlusion. Dogs have a well-developed native collateral circulation, and infarction starts after 40-minute coronary occlusion and spreads to affect 70% of the area at risk after 6 hours; in dogs, therefore, infarct size is best quantified as a fraction of the area at risk and normalized to the residual blood flow. Pigs have a negligible native collateral circulation, and infarction starts after 15 to 35-minute coronary occlusion and affects 80% of the area at risk after 60 to 180 minutes. Primates have few innate collaterals but are relatively resistant to myocardial ischemia; there is no infarction after 40- to 60-minute coronary occlusion, and even after 90-minute coronary occlusion, infarct size is smaller than that in pigs. Apart from such species differences in the native collateral circulation, coronary vasomotor mechanisms also differ between species. Pigs, in contrast to dogs, respond to acetylcholine with coronary vasoconstriction rather than vasodilation, and pigs have only negligible α-adrenergic coronary vasoconstriction. With respect to such coronary vasomotor mechanisms, humans are closer to dogs than to pigs; however, in the presence of coronary atherosclerosis in humans, the response to acetylcholine may also be reversed from vasodilation to vasoconstriction. Fortunately, infarct development in humans is slower than that in the above-mentioned large mammals. Even after 4 to 6 hours of coronary occlusion, 30% to 50% of the area at risk remain viable and thus salvageable, as one can estimate from magnetic resonance imaging (MRI) and from the amount of salvage by reperfusion. Salvageable myocardium remains even after 12 hours from symptom onset, and its salvage improves patients’ prognosis. It is unclear at present to what extent the resistance of the diseased human heart is attributable to a developed collateral circulation at the time of infarction, as in the native dog heart, or reflects an inherently greater resistance to ischemic injury, as in the primate heart, or reflects preceding episodes of myocardial ischemia/reperfusion with a preconditioning effect. Also, effective drug treatment (eg, by concomitant β-blockade, renin–angiotensin system inhibition, statins, or P2Y12 antagonists) may induce pre-existing cardioprotection and attenuate the consequences of acute myocardial ischemia/reperfusion. In contrast to previous notions, the hemodynamic situation has little impact on the development of myocardial infarction; only heart rate determines infarct progression to some extent. Variations in coronary blood flow not only determine the nature and extent of myocardial injury but paradoxically also protection from it. Repeated brief episodes of coronary occlusion preceding a prolonged coronary occlusion with reperfusion reduce infarct size, that is, there is ischemic preconditioning. Likewise, repeated brief coronary occlusion during early reperfusion reduces infarct size, that is, there is ischemic postconditioning.

### Coronary Circulation as a Determinant of Reperfusion and Reperfusion Injury

Although today it is unequivocally clear that timely reperfusion of an occluded coronary artery is the only way to rescue myocardium from impending infarction, this notion is a little more than 40 years old and goes back to the study by Ross and collaborators who first reported that reperfusion after 180-minute coronary occlusion reduced infarct size in dogs. These findings were quickly translated to humans with acute myocardial infarction who were reperfused by thrombolysis or percutaneous coronary interventions (PCIs). Even before the benefits of reperfusion were established, the dark side of coronary reperfusion became apparent, when Krug et al and then Kloner et al reported the coronary no-reflow phenomenon. And again only a few years later, Reimer et al and Reimer and Jennings reported in a series of detailed dog studies that signs of irreversible myocardial injury, such as rupture of the sarcolemma, become particularly manifest during reperfusion; at that time, it was not clear whether the irreversible injury was caused by reperfusion or only became better manifest during reperfusion. The long debate on the existence of lethal reperfusion injury was finally ended by the recognition of the ischemic postconditioning phenomenon when Vinten-Johansen and colleagues reported that repeated coronary reocclusion during early reperfusion reduced infarct size in dogs, and these findings were quickly confirmed in patients with reperfused acute myocardial infarction. The fact that a modified procedure of reperfusion could indeed attenuate irreversible injury once again revived earlier studies on gentle reperfusion, that is, the reduction of functional and morphological signs of injury by reperfusion at reduced perfusion pressure or reduced coronary blood flow.
the unequivocal notion that reperfusion is both mandatory for salvage from impending infarction and it causes irreversible injury per se, a complex picture arises in that both ischemia and reperfusion contribute to the ultimate injury, but their individual contribution to ultimate injury depends on the duration and severity of coronary blood flow reduction.\textsuperscript{52} Whereas ischemic injury increases with the severity and the duration of coronary blood flow reduction, there is a maximum of reperfusion injury at more moderate ischemic injury (Figure 1).

Technically, in the experiment, infarct size is best quantified by triphenyl tetrazolium chloride staining after sufficient reperfusion with washout of reductive equivalents.\textsuperscript{53,54} Infarct size is best normalized to the area at risk which is delineated by a water-soluble dye injected into the left atrium after reocclusion of the coronary artery at its culprit site (Figure 2). The area of no-reflow is delineated by injection of thioflavin S into the left atrium, which stains endothelial cells, such that lack of thioflavin fluorescence reflects no-reflow zones.\textsuperscript{55} In patients, infarct size can be estimated from cardiac enzyme release or imaging, notably late gadolinium enhancement in MRI. No-reflow is primarily an angiographic diagnosis immediately after reopening of the culprit coronary occlusion, and it is quantified by reduced thrombolysis in myocardial infarction (TIMI) flow grade, increased TIMI frame count, and reduced myocardial blush grade.\textsuperscript{56–59} More recently, microvascular obstruction is visualized by MRI as lack of contrast within gadolinium-enhanced areas. Area of risk can be best visualized by scintigraphy, and there are problems in estimating area at risk with \( T_2 \)-weighted edema imaging in MRI (see below).

Manifestations of Myocardial Ischemia/Reperfusion Injury in the Coronary Circulation

The manifestations of myocardial ischemia/reperfusion in the coronary circulation go from mild and reversible functional impairment to severe and irreversible destruction (Figure 3).

Vascular Permeability: Edema

Edema develops quickly within minutes of acute myocardial ischemia\textsuperscript{60–64} and is both intracellular and interstitial. Intracellular edema develops in cardiomyocytes and endothelial cells largely as a consequence of the rapidly developing energetic deficit and the reduced function of energy-dependent ion pumps.\textsuperscript{65} Interstitial edema develops as a consequence of increased interstitial osmolarity from increased ion and metabolite concentrations and a dysfunction of the endothelial barrier function during myocardial ischemia.\textsuperscript{66} The endothelial barrier function is made up by the glycocalyx,\textsuperscript{66} endothelial cells, and pericytes (particularly at the postcapillary venules\textsuperscript{67}). Endothelial cytoskeletal derangement and hypercontracture induce gap formation,\textsuperscript{68–71} which is enhanced by extracellular adenosine but attenuated by extracellular ATP.\textsuperscript{72} Degradation of the glycocalyx also contributes to reduced endothelial barrier function and edema formation\textsuperscript{66,73,74}; tumor necrosis factor \( \alpha \) is an important mediator of glycocalyx degradation,\textsuperscript{75} and glycocalyx degradation also promotes leukocyte\textsuperscript{76} and platelet adherence.\textsuperscript{77} Exogenous nitric oxide preserves vascular integrity and attenuates edema formation through protection of the glycocalyx.\textsuperscript{78} On reperfusion, interstitial edema is greatly enhanced by reactive hyperemia and the rapid washout of osmotically active molecules from the intravascular space. The cellular (as a
consequence of a reversed acidosis and intracellular sodium and calcium overload) and interstitial edema development during reperfusion follows a bimodal pattern where an initial maximum of water content after 120 minutes is associated with a beginning leukocyte infiltration, and a secondary peak after 7 days is associated with enhanced collagen deposition. Edema during reperfusion has been proposed to reflect the area at risk on MRI, but edema may artifactually increase the area at risk, and a bimodal pattern of edema further questions the use of T2-weighted edema measurement for area at risk delineation. Myocardial edema not only is a consequence of sustained myocardial ischemia/reperfusion but also contributes to the impairment of microvascular perfusion by extravascular compression.

Vasomotion
The coronary microcirculation distal to a severe coronary stenosis or coronary occlusion has traditionally been considered as maximally dilated after exhaustion of autoregulatory reserve. However, even during myocardial ischemia which limits regional contractile function, a pharmacologically recruitable vasodilator reserve persists. Reactive oxygen species contributes to the endothelial dysfunction and consequent impairment of coronary vasomotion. The impairment of endothelium-mediated vasodilation correlates to the severity of myocardial injury, that is, it is greater in infarcted than in reversibly injured myocardium. During myocardial ischemia/reperfusion, the coronary microcirculation remains responsive to vasoconstrictor mediators, notably α-adrenergic coronary vasoconstriction. Such α-adrenergic coronary constrictor impact is also seen in humans with chronic stable angina and during PCIs. The release of vasoconstrictor substances, such as thromboxane, serotonin, and endothelin from the rupturing culprit lesion into the microcirculation, in conjunction with the impairment of endothelial function by ischemia/reperfusion per se or by tumor necrosis factor α, can contribute to such enhanced vasoconstricor responsiveness during myocardial ischemia/reperfusion. With more prolonged ischemia in hibernating myocardium, there is structural remodeling of the microvasculature with hypertrophy of smaller and atrophy of larger vessels, reduced vascular distensibility, and increased vasoconstriction in response to endothelin.

Microembolization
Plaque fissure or rupture occur spontaneously and are induced traumatically/iatrogenically by PCIs. Atherosclerotic debris with superimposed thrombotic material is then dislodged and embolized into the coronary microcirculation where it induces patchy microinfarcts with an inflammatory reaction. Such microinfarcts add to the infarct size caused by sustained...
coronary occlusion with reperfusion\textsuperscript{101,103,104} and impair coronary dilator reserve.\textsuperscript{105,106} In patients, coronary microembolization is particularly seen with PCIs in saphenous vein bypass grafts, and it is here that protection devices are useful to prevent atherothrombotic debris from embolizing into the microcirculation.\textsuperscript{107,108}

Stasis and Intravascular Cellular Aggregates
Myocardial ischemia and reperfusion increase the expression of adhesion molecules, such as intercellular adhesion molecules, vascular cell adhesion molecules, and selectins, on endothelium and circulating cells and thereby promote the interaction of platelets, leukocytes, and endothelium and the adherence of platelet aggregates and platelet–leukocyte aggregates to the endothelium.\textsuperscript{109–115} Such aggregates are either released from the epicardial atherosclerotic culprit lesion and dislodged into the microcirculation or formed in the coronary microcirculation. These cellular aggregates contribute to the impairment of microvascular perfusion. In pronounced forms of no-reflow, also typical erythrocyte aggregates are found that block the capillaries.\textsuperscript{116}

Capillary Destruction: Hemorrhage
The most severe forms of coronary microvascular injury from myocardial ischemia/reperfusion, as already detailed in the original reports of coronary no-reflow by Krug et al\textsuperscript{144} and Kloner et al,\textsuperscript{45} are the massive swelling of capillary endothelial cells with consequent rupture of the vascular wall and leakage of circulating cells into the interstitium, that is, hemorrhage. Hemorrhage is associated with severe ischemia during coronary occlusion and with severe myocardial necrosis.\textsuperscript{117,118} Hemorrhage in reperfused myocardial infarction has received more attention by MRI because the hemoglobin metabolites are paramagnetic and attenuate the T\textsubscript{2}-weighted signal intensity within an otherwise high T\textsubscript{2}-weighted signal area (edema).\textsuperscript{119–122} No-reflow is seen in $\approx$35% of patients with optimal reperfusion therapy, and its incidence increases with the delay of reperfusion.\textsuperscript{123,124} No-reflow and hemorrhage carry an adverse prognosis for patients with reperfused myocardial infarction.\textsuperscript{124–126}

The above manifestations of coronary vascular injury by myocardial ischemia/reperfusion are attenuated by local ischemic pre- and postconditioning, as well as by remote ischemic conditioning and by various cardioprotective drugs and interventions. However, not for every manifestation of coronary vascular injury information is available for every form of cardioprotection. Often, only the resulting no-reflow or the area of no-reflow was assessed. In particular, data for patients with myocardial ischemia/reperfusion are not systematically available.

**Coronary Vascular Protection by Ischemic Preconditioning**
Ischemic preconditioning protects endothelial function and structure from myocardial ischemia/reperfusion injury.\textsuperscript{127} In Langendorff-perfused mouse hearts, ischemic preconditioning by 1 cycle of 2-minute global ischemia/5-minute reperfusion before 40-minute sustained global ischemia with reperfusion preserved the ultrastructure of endothelial tight junctions and attenuated edema.\textsuperscript{78} Similarly, ischemic preconditioning in rats attenuated the increase in microvascular permeability and edema development with sustained ischemia/reperfusion.\textsuperscript{128} Ischemic preconditioning with 5-minute coronary occlusion/10-minute reperfusion before 60-minute coronary occlusion and reperfusion in dogs reduced not only infarct size but also edema.\textsuperscript{38} An early study in anesthetized dogs found no protection by ischemic preconditioning on endothelium-dependent coronary vasodilation in response to acetylcholine and on low-reflow after 60-minute coronary occlusion with reperfusion.\textsuperscript{129} Another study in dogs with 60-minute coronary occlusion and reperfusion also reported no improvement in the coronary vasodilator response to acetylcholine but improved reflow with ischemic preconditioning by 2 cycles of 5-minute myocardial ischemia/5-minute reperfusion.\textsuperscript{130} However, the majority of subsequent studies reported protection by ischemic preconditioning on endothelial function, as assessed by endothelium-dependent coronary vasodilation in response to acetylcholine, serotonin, or ADP in rats,\textsuperscript{131–133} guinea pigs,\textsuperscript{134} dogs,\textsuperscript{135,136} pigs,\textsuperscript{137} and goats.\textsuperscript{138} Mechanistically, the preservation of endothelial function by ischemic preconditioning was related to adenosine,\textsuperscript{132,136} bradykinin,\textsuperscript{133} and nitric oxide.\textsuperscript{138,139} The preservation of endothelial function by ischemic preconditioning became apparent not only as improved endothelium-dependent coronary vasodilation but also as reduced leukocyte adherence.\textsuperscript{134,135,139} Coronary endothelial function recovers only slowly after myocardial ischemia/reperfusion and is still depressed after 1 month, but ischemic preconditioning also improves endothelium-dependent coronary vasodilation in response to acetylcholine and endothelial ultrastructure after 1 month.\textsuperscript{140} Vice versa, delayed ischemic preconditioning 24 hours before the sustained myocardial ischemia/reperfusion increases the activity of endothelial nitric oxide synthase, which mediates preservation of coronary vasodilator response to acetylcholine, carbachol, and bradykinin.\textsuperscript{141,142} Reactive oxygen species formation, although detrimental acutely for endothelium-dependent coronary vasodilation in response to acetylcholine, is mandatory for the delayed protection by ischemic preconditioning.\textsuperscript{143} Ischemic preconditioning not only preserves endothelium-dependent
coronary vasodilation but also attenuates the enhanced coronary vasoconstrictor tone after hyperkalemic cardioplegia through a ATP-dependent potassium channel–dependent mechanism in coronary vascular smooth muscle cells. Coronary microembolization with release of adenosine into the coronary vasculature does not induce acute preconditioning and reduce infarct size or, conversely, interfere with protection by ischemic preconditioning. Somewhat paradoxically, the increase in tumor necrosis factor α expression secondary to coronary microembolization can induce delayed protection and reduce infarct size from subsequent coronary occlusion/reperfusion, such that the actual impact of coronary microembolization on infarct size depends critically on timing and is difficult to predict. Preinfarction angina is considered a clinical correlate of ischemic preconditioning. Patients with preinfarction angina have reduced platelet reactivity, less monocyte–platelet aggregates, and better reflow and coronary flow reserve during reperfusion.

Coronary Vascular Protection by Ischemic Postconditioning

The classical study by Vinten-Johansen and coworkers in dogs undergoing an ischemic postconditioning protocol of 3 cycles of 30-second coronary reocclusion/30-second reperfusion at immediate reperfusion after 60-minute coronary occlusion reported not only reduced infarct size but also reduced edema; both infarct size and edema were reduced to the same extent as with ischemic preconditioning. Neither the reduction of infarct size nor the reduction of no-reflow with ischemic postconditioning was confirmed in a rabbit model. In a mini-pig model of 3 hours of coronary occlusion/reperfusion, ischemic postconditioning with 6 cycles of 10-second reocclusion/10-second reperfusion reduced infarct size and area of no-reflow, but hypercholesterolemia abrogated the protection by ischemic postconditioning. In patients with reperfused acute myocardial infarction, ischemic postconditioning protocols reduced infarct size, improved coronary blood flow (MRI, biomarker), and reduced edema and no-reflow. However, neither the reduction of infarct size nor a reduction of microvascular obstruction was confirmed in other trials. Reasons for such discrepancy are not really clear but may relate to use of direct stenting or lack of it and the increasing use of P2Y12 antagonists which induce cardioprotection per se such that the potential for further protection is diminished.

Coronary Vascular Protection by Remote Ischemic Conditioning

Remote ischemic conditioning was originally characterized as an interaction between two coronary vascular territories and has now been established as a powerful cardioprotective intervention which can be elicited from various vascular territories, including noninvasive occlusion/reperfusion of the limbs. Remote ischemic conditioning reduces infarct size when performed before (preconditioning), during (reconditioning), or after (postconditioning) sustained myocardial ischemia/reperfusion, and it has been confirmed in various species, including also proof-of-concept trials in humans undergoing elective interventional or surgical coronary revascularization or interventional/thrombolytic reperfusion of acute myocardial infarction. In pigs, remote ischemic preconditioning improved coronary blood flow through an ATP-dependent potassium channel–dependent mechanism. In healthy young volunteers, repeated remote ischemic conditioning twice a day for 1 week increased coronary flow reserve, and it did so also in patients with heart failure. In patients undergoing elective percutaneous coronary revascularization, remote ischemic preconditioning did not reduce coronary microvascular resistance in a nontarget vessel. In the Effect of Remote Ischemic Conditioning Before Hospital Admission (CONDI) trial, in patients undergoing primary PCI for acute myocardial infarction, remote ischemic perconditioning with 4 cycles of 5-minute arm ischemia/5-minute reperfusion during transport in the ambulance reduced infarct size but did not improve coronary blood flow. In patients with acute ST-segment elevation myocardial infarction undergoing PCI, remote ischemic perconditioning with 4 cycles of 5-minute arm ischemia/5-minute reperfusion at hospital admission reduced both infarct size and edema on MRI. Also in patients with acute myocardial infarction undergoing PCI, remote ischemic postconditioning by 3 cycles of lower limb ischemia/reperfusion reduced edema (MRI) and infarct size (MRI, biomarker), improved ST-segment resolution during reperfusion, but did not improve TIMI frame count or myocardial blush grading. In the recent LIPSIA (from Leipzig) conditioning trial in patients undergoing primary PCI for acute ST-segment elevation myocardial infarction, postconditioning alone with 4 cycles of 30-second reocclusion/reperfusion failed to improve myocardial salvage and microvascular obstruction by MRI, but combined postconditioning with remote ischemic perconditioning by 3 cycles of 5-minute upper arm ischemia/5-minute reperfusion improved myocardial salvage, albeit reduced microvascular obstruction only nonsignificantly.

Myocardial salvage by remote ischemic preconditioning, in turn, is greater when collateral blood flow is present, as evident from a retrospective analysis of the CONDI trial and supporting the notion of a humoral transfer of cardioprotective factors from the remote organ to the heart. Remote ischemic preconditioning reduces leukocyte adhesion and phagocytosis in healthy volunteers and it attenuates the increased platelet reactivity and formation of monocyte–platelet aggregates in patients undergoing ablation for atrial fibrillation and in patients undergoing coronary procedures. Remote ischemic preconditioning activates erythrocyte nitric oxide synthase and improves erythrocyte deformability, thus potentially antagonizing stasis.

Coronary Vascular Protection by Drugs and Cardioprotective Interventions

Experimental Studies

Most experimental studies on myocardial ischemia/reperfusion are performed in healthy and young animals which have a virgin coronary circulation without atherosclerosis, vascular remodeling, and endothelial dysfunction. Such studies accordingly cannot account for the presence of atherosclerosis with impaired endothelial function, exhaustion...
of autoregulatory mechanisms and coronary vascular re-modelling distal to coronary stenoses, the development of a significant collateral circulation, and also pre-existing myocardial disease (patchy microinfarcts and fibrosis) or adaptation (hibernation). These limitations contribute to the difficulties in translating data from animal studies to the patient with acute myocardial infarction undergoing reperfusion therapy, apart from and in addition to confounding comorbidities and comediations.

**Drugs**

As reviewed in detail elsewhere, many exogenous agents and drugs which often rely on the recruitment of signal transduction steps of conditioning maneuvers can reduce infarct size in various experimental models and in different species. In several such studies, the protection of the coronary circulation was also addressed. Adenosine, only when given at a high intracoronary dose for a prolonged period of time, reduced infarct size, no-reflow, and leukocyte infiltration in pigs. Also in pigs, cyclosporine A that inhibits opening of the mitochondrial permeability transition pore reduced not only infarct size when given just before reperfusion but also microvascular obstruction on MRI. Also, pretreatment with high-density lipoproteins from normocholesterolemic pigs which contain a load of sphingosine-1-phosphate reduced infarct size and the extent of no-reflow in pigs. Pretreatment with simvastatin reduced infarct size and the area of no-reflow in pigs, and such protection was related to activation of protein kinase A and of mitochondrial ATP-dependent potassium channels. Glucagon-like peptide 1 when given just before reperfusion in rats decreased infarct size and reduced the accumulation of leukocytes in the reperfused myocardium. Human recombinant angiopoietin-like peptide 4 reduced infarct size and area of no-reflow in mice and rabbits.

**Interventions**

Hypersmotic reperfusion with mannitol reduced edema and infarct size in pigs. Electric vagal nerve stimulation in pigs just before and into early reperfusion after 45-minute coronary occlusion reduced infarct size, area of no-reflow, and leukocyte accumulation, and the protective effects were related to nitric oxide synthase activity. Counterpulsation by an intra-aortic balloon pump during reperfusion after 60-minute coronary occlusion in pigs increased coronary blood flow and reduced infarct size and area of no-reflow. In contrast, partial clamping of the aorta to increase perfusion pressure augmented infarct size and no-reflow in pigs. Hypothermia (32°C) in rats and rabbits undergoing 30-minute coronary occlusion and reperfusion reduced infarct size and no-reflow area when initiated shortly after the onset of myocardial ischemia. Of note, whereas in rabbits undergoing 30-minute coronary occlusion with reperfusion, topical hypothermia (32°C) when initiated at 5 minutes before versus at 5 minutes after reperfusion tended to reduce infarct size only with hypothermia started before reperfusion, the area of no-reflow was reduced in both cases. When hypothermia was initiated no earlier than at 30-minute reperfusion, infarct size was not affected at all, but the area of no-reflow was still reduced. This particular study emphasizes the potential for a dissociation of effects on infarct size from those on area of no-reflow.

Finally, cellular postconditioning by intracoronary infusion of allogeneic cardiospheres derived in pigs at 30-minute reperfusion after 90-minute coronary occlusion reduced infarct size and area of microvascular obstruction 48 hours later.

**Clinical Studies**

As reviewed in detail elsewhere, many different interventions and drugs have been used, after successful preclinical studies, as adjunct therapy to reperfusion with the aim to reduce infarct size and possibly improve clinical outcome. Most of these trials have failed to provide convincing evidence for infarct size reduction. Hypothermia did not reduce infarct size (single photon emission computed tomography) or improve TIMI flow in patients with acute myocardial infarction undergoing primary PCI. Also, no reduction of microvascular obstruction and infarct size was found with MRI. Hypothermia, aspiration or mechanical thrombectomy, and intra-aortic balloon counterpulsation did not reduce infarct size or improve coronary blood flow. More recently, also the cardioprotection by cyclosporine A which had been shown in a small-scale proof-of-concept trial was not confirmed in another smaller study with prethrombolytic cyclosporine A and, importantly, not in 2 larger-scale phase III trials. Does Cyclosporine Improve Clinical Outcome in ST Elevation Myocardial Infarction Patients (CIRCUS) and CyclosporinE A in Reperfused Myocardial Infarct (CYCLE)? Many potential reasons for this discrepancy have been discussed, such as lack of direct stenting and greater use of P2Y12 antagonists, and also the use of a different vehicle in CIRCUS, but not in CYCLE.

In some of these studies, not only infarct size but also parameters reflecting coronary microvascular function were reported, and the effects of cardioprotective drugs on infarct size and coronary microvascular function were mostly concordant (Figures 5–8). Intracoronary abciximab as compared with intravenous abciximab reduced infarct size (creatine kinase and MRI) and microvascular obstruction (MRI). For intracoronary adenosine, concordantly reduced infarct size (creatine kinase and MRI) and less microvascular obstruction (TIMI flow on angiography) were reported in patients undergoing interventional reperfusion for acute myocardial infarction in but not in 3 other studies. Intravenous nitrite in patients with ST-segment elevation myocardial infarction and interventional reperfusion failed to reduce infarct size (bio-marker and MRI) or to affect TIMI flow (angiography). Intracoronary nitrite in patients with ST-segment elevation myocardial infarction and interventional reperfusion reduced infarct size (creatine kinase and MRI) only in a subgroup of patients with TIMI flow ≤1 at admission, and in this subgroup, intracoronary nitrite also reduced the area of microvascular obstruction (MRI). Erythropoietin in patients with ST-segment elevation myocardial infarction and interventional reperfusion neither reduced infarct size (creatine kinase and MRI) nor improved TIMI flow. Two different mitochondria-targeting drugs failed to reduce infarct size or to improve coronary microvascular function. Currently, only 4 drugs stand unchallenged to provide cardioprotection in term.
of infarct size reduction: atrial natriuretic peptide,\textsuperscript{218} metoprolol,\textsuperscript{12} esmolol,\textsuperscript{239} and exenatide,\textsuperscript{240–242} and no information on coronary microvascular function is available for these drugs.

**Coronary Microvascular Injury: Cause or Consequence of Myocardial Ischemia/Reperfusion**

The available studies indicate a close correlation between infarct size and that of no-reflow.\textsuperscript{243–245} Still, correlations cannot resolve questions of causality, and the lack of adequate techniques to make serial measurements of infarcted tissue and no-reflow with reasonable spatial resolution is largely responsible that causality between myocardial infarction and coronary microvascular no-reflow is not established.\textsuperscript{246} With microembolization of atherosclerotic debris, plugging of platelet/leukocyte aggregates and vasoconstriction in response to soluble mediators, the resulting coronary microvascular obstruction could be cause to myocardial infarction. With this rationale, thrombectomy, protection devices, and coronary vasodilators are used to reduce peri-interventional reperfusion injury.\textsuperscript{106} Also, recombinant angiopoietin-like peptide 4 has been demonstrated to stabilize the endothelial barrier and subsequently reduce infarct size and no-reflow.
no-reflow in mice. However, vice versa there may be primary damage to cardiomyocytes which only subsequently progresses to coronary microvascular damage, as seen in animal models with mechanical occlusion/reperfusion of virgin coronary arteries without a culprit lesion. Of particular interest to resolve a potential causality between infarct size and no-reflow are not the vast majority of studies where effects on both infarct size and no-reflow are concordant, but those few studies where they are dissociated. In pigs, edema at reperfusion after 48-minute coronary occlusion was reduced with both anoxic perfusion for 30-minute reperfusion following 30-minute coronary occlusion and ischemic preconditioning also reduced infarct size, but not reflow. Delayed hypothermia starting no earlier than after reperfusion, but only ischemic preconditioning by 2 cycles of 5-minute coronary occlusion/5-minute catabolite washout during coronary occlusion was reduced with both anoxic perfusion for 30-minute reperfusion following 30-minute coronary occlusion and ischemic preconditioning; SPECT, single photon emission computed tomography; and TnT, troponin T.

Figure 7. Forest plot of infarct size and corrected thrombolysis in myocardial infarction frame count (cTFC) in clinical trials reporting on both infarct size and microvascular dysfunction. The zero represents the mean value of the placebo group and gray bars the SEM of the placebo group. ■, mean values with significant difference from placebo; □, mean values without significant difference from placebo. Asp. Thromb. indicates aspiration thrombectomy; CK, creatine kinase; CK-MB, creatine kinase muscle brain; INT, intervention; Mech. Thromb., mechanical thrombectomy; MRI, magnetic resonance imaging; PLA, placebo; POCO, postconditioning; RIC, remote ischemic conditioning; SPECT, single photon emission computed tomography; and TnT, troponin T.

Figure 8. Forest plot of infarct size and edema and microvascular obstruction (MVO) on magnetic resonance imaging (MRI) in clinical trials reporting on both infarct size and microvascular dysfunction. The zero represents the mean value of the placebo group and gray bars the SEM of the placebo group. ■, mean values with significant difference from placebo; □, mean values without significant difference from placebo. Asp. Thromb. indicates aspiration thrombectomy; CK, creatine kinase; CK-MB, creatine kinase muscle brain; INT, intervention; PLA, placebo; POCO, postconditioning; RIC, remote ischemic conditioning; IABC, intra-aortic balloon counterpulsation; INT, intervention; PLA, placebo; POCO, postconditioning; RIC, remote ischemic conditioning; and TnT, troponin T.
ischemia/reperfusion injury but also to the impairment of metabolic coronary vasodilation, rendering it as a target not only to reduce infarct size but also improve coronary blood flow.20

Sources of Funding

G. Heusch was supported by the German Research Foundation (He 1320/18-3; SFB 1116/B8), the Hans and Gertie-Fischer Foundation, and the Heinz Horst-Deichmann Foundation.

Disclosures

None.

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Circ Res. 2016;118:1643-1658
doi: 10.1161/CIRCRESAHA.116.308640
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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