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. 

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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
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 is there 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, became 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.\(^\text{52}\) 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.\(^\text{53,54}\) 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.\(^\text{50}\) 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.\(^\text{56–59}\) 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\(^\text{60–64}\) 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.\(^\text{65}\) Interstitial edema develops as a consequence of increased interstitial osmolarity from increased ion and catabolite concentrations and a dysfunction of the endothelial barrier function during myocardial ischemia.\(^\text{66}\) The endothelial barrier function is made up by the glycocalyx,\(^\text{66}\) endothelial cells, and pericytes (particularly at the postcapillary venules\(^\text{67}\)). Endothelial cytoskeletal derangement and hypercontracture induce gap formation,\(^\text{68–71}\) which is enhanced by extracellular adenosine but attenuated by extracellular ATP.\(^\text{72}\) Degradation of the glycocalyx also contributes to reduced endothelial barrier function and edema formation\(^\text{66,73,74}\); tumor necrosis factor \(\alpha\) is an important mediator of glycocalyx degradation,\(^\text{75}\) and glycocalyx degradation also promotes leukocyte\(^\text{76}\) and platelet adherence.\(^\text{77}\) Exogenous nitric oxide preserves vascular integrity and attenuates edema formation through protection of the glycocalyx.\(^\text{78}\) On reperfusion, interstitial edema is greatly enhanced by reactive hyperemia and the rapid washout of osmotically active molecules from the intravascular space. The cellular

![Figure 1. Infarct size as a function of duration of ischemia and residual/collateral blood flow.](http://circres.ahajournals.org/content/early/2013/10/21/0000000-20134081.full)

With longer ischemia duration and less blood flow, ischemia-induced injury increases. The greater the ischemia-induced injury, the less myocardium is salvaged but also the less is damaged by reperfusion. Reprinted from Heusch,\(^\text{52}\) Copyright ©2013, American Physiological Society.
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 (Figure 4). 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 vasoconstrictor 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

Figure 2. Typical example for delineation of area at risk by Patent blue (left), of infarction by triphenyl tetrazolium chloride staining (middle), and of no-reflow by thioflavin S fluorescence (right). The no-reflow area was encircled by an incision.
that block the capillaries.116
of no-reflow, also typical erythrocyte aggregates are found impairment of microvascular perfusion. In pronounced forms microcirculation. These cellular aggregates contribute to the dislodged into the microcirculation or formed in the coronary
and Kloner et al, 45 are the massive swelling of capillary en-
metabolites are paramagnetic and attenuate the T2-weighted sig-
received more attention by MRI because the hemoglobin ca-
(depression, more in subendocardial than in subepicardial layers
coronary occlusion, the vasodilator response to acetylcholine is
coronary occlusion with reperfusion101,103,104 and impair cor-
ary dilator reserve.105,106 In patients, coronary microem-
bolization 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.107,108
Stasis and Intravascular Cellular Aggregates
Myocardial ischemia and reperfusion increase the expres-
sion 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 ag-
gregates to the endothelium.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.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 al44
and Kloner et al.45 are the massive swelling of capillary en-
dothelial 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 necro-
sis.117,118 Hemorrhage in reperfused myocardial infarction has
received more attention by MRI because the hemoglobin ca-
tabolites are paramagnetic and attenuate the T2-weighted sig-
nal intensity within an otherwise high T2-weighted signal area
(edema).119–122 No-reflow is seen in ≈35% of patients with op-
timal reperfusion therapy, and its incidence increases with the
delay of reperfusion.123,124 No-reflow and hemorrhage carry
an adverse prognosis for patients with reperfused myocardial
infarction.124–126
The above manifestations of coronary vascular injury by
myocardial ischemia/reperfusion are attenuated by local isch-
emic pre- and postconditioning, as well as by remote ischemic
conditioning and by various cardioprotective drugs and interven-
tions. However, not for every manifestation of coronary vascular
injury information is available for every form of cardioprotec-
tion. 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.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.78 Similarly, ischemic preconditioning in
rats attenuated the increase in microvascular permeability and
dema development with sustained ischemia/reperfusion.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
dema.38 An early study in anesthetized dogs found no protec-
tion by ischemic preconditioning on endothelium-dependent
coronary vasodilation in response to acetylcholine and on low-
reflow after 60-minute coronary occlusion with reperfusion.129
Another study in dogs with 60-minute coronary occlusion and
reperfusion also reported no improvement in the coronary va-
sodilator response to acetylcholine but improved reflow with
ischemic preconditioning by 2 cycles of 5-minute myocardial
ischemia/5-minute reperfusion.130 However, the majority of
subsequent studies reported protection by ischemic condi-
tioning on endothelial function, as assessed by endothelium-
dependent coronary vasodilation in response to acetylcholine,
serotonin, or ADP in rats,131–133 guinea pigs,134 dogs,135,136 pigs,137
and goats.138 Mechanistically, the preservation of endothelial
function by ischemic preconditioning was related to adenos-
ine,132,136 bradykinin,133 and nitric oxide.138,139 The preservation
of endothelial function by ischemic preconditioning became ap-
parent not only as improved endothelium-dependent coronary
vasodilation but also as reduced leukocyte adherence.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 endotheli-
um-dependent coronary vasodilation in response to acetylcho-
line and endothelial ultrastructure after 1 month.140 Vice versa,
delayed ischemic preconditioning 24 hours before the sustained
myocardial ischemia/reperfusion increases the activity of en-
dotheilum nitric oxide synthase, which mediates preservation
of coronary vasodilator response to acetylcholine, carbachol, and
bradykinin.141,142 Reactive oxygen species formation, although
detrimental acutely for endothelium-dependent coronary va-
sodilation in response to acetylcholine, is mandatory for the
delayed protection by ischemic preconditioning.143 Ischemic
preconditioning not only preserves endothelium-dependent

Figure 4. Increase in regional myocardial blood flow in response to intracoronary acetylcholine after 15- vs 60-minute coronary occlusion in dogs, separately for subendocardial, midmyocardial, and subepicardial layers. After 60-minute coronary occlusion, the vasodilator response to acetylcholine is depressed, more in subendocardial than in subepicardial layers and more in infarcted (triphenyl tetrazolium chloride [TTC] negative) than in viable (TTC positive) myocardium. Reprinted from Ehring et al.98 Copyright ©1995, the American Physiological Society.
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.144 Coronary microembolization with release of adenosine into the coronary vasculature does not induce acute preconditioning and reduce infract size or, conversely, interfere with protection by ischemic preconditioning.103,145 Somewhat paradoxically, the increase in tumor necrosis factor α expression secondary to coronary microembolization can induce delayed protection and reduce infract size from subsequent coronary occlusion/reperfusion,146 such that the actual impact of coronary microembolization on infract size depends critically on timing and is difficult to predict. Preinfarction angina is considered a clinical correlate of ischemic preconditioning.147,148 Patients with preinfarction angina have reduced platelet reactivity, less monocyte–platelet aggregates,149 and better reflow and coronary flow reserve during reperfusion.150,151

Coronary Vascular Protection by Ischemic Postconditioning

The classical study by Vinten-Johansen and coworkers38 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 infract size but also reduced edema; both infract size and edema were reduced to the same extent as with ischemic preconditioning. Neither the reduction of infract size nor the reduction of no-reflow with ischemic postconditioning was confirmed in a rabbit model,152 and no-reflow reduction by ischemic postconditioning was also not confirmed in pigs.153,154 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 infract size and area of no-reflow, but hypercholesterolemia abrogated the protection by ischemic postconditioning.155 In patients with reperfused acute myocardial infarction, ischemic postconditioning protocols reduced infract size.47,156–164 Improved coronary blood flow47,156,160,161,164 and coronary flow reserve,159 and reduced edema and no-reflow.163 However, neither the reduction of infract size nor a reduction of microvascular obstruction was confirmed in other trials.165–170 Reasons for such discrepancy are not really clear but may relate to use of direct stenting or lack of it48,171 and the increasing use of P2Y12 antagonists which induce cardioprotection per se such that the potential for further protection is diminished.172–174

Coronary Vascular Protection by Remote Ischemic Conditioning

Remote ischemic conditioning was originally characterized as an interaction between two coronary vascular territories175 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.176 Remote ischemic conditioning reduces infract size when performed before (preconditioning), during (perconditioning), 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.177 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.178 In patients undergoing elective percutaneous coronary revascularization, remote ischemic preconditioning did not reduce coronary microvascular resistance in a nontarget vessel.179 In the Effect of Remote Ischemic Conditioning Before Hospital Admission (CONDI) trial, in patients undergoing primary PCI for acute myocardial infarction, remote ischemic preconditioning with 4 cycles of 5-minute arm ischemia/5-minute reperfusion during transport in the ambulance reduced infract size but did not improve coronary blood flow.180 In patients with acute ST-segment elevation myocardial infarction undergoing PCI, remote ischemic preconditioning with 4 cycles of 5-minute arm ischemia/5-minute reperfusion at hospital admission reduced both infract size and edema on MRI.181 Also in patients with acute myocardial infarction undergoing PCI, remote ischemic postconditioning by 3 cycles of lower limb ischemia/reperfusion reduced edema (MRI) and infract size (MRI, biomarker), improved ST-segment resolution during reperfusion, but did not improve TIMI frame count or myocardial blush grading.182 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 preconditioning by 3 cycles of 5-minute upper arm ischemia/5-minute reperfusion improved myocardial salvage, albeit reduced microvascular obstruction only nonsignificantly.183 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.183 Remote ischemic preconditioning reduces leukocyte adhesion and phagocytosis in healthy volunteers,184 and it attenuates the increased platelet reactivity and formation of monocyte–platelet aggregates in patients undergoing ablation for atrial fibrillation185 and in patients undergoing coronary procedures.186 Remote ischemic preconditioning activates erythrocyte nitric oxide synthase187 and improves erythrocyte deformability,188 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 remodelling 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 comedications.

**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**

Hyposmotic 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 cardioprotection-derived cells 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. Hyperoxemia, 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 Repertused 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). 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{219} 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, thrombasmption, protection devices, and coronary vasodilators are used to reduce peri-interventional reperfusion injury.\textsuperscript{106} Also, recombinant angiopeitin-like peptide 4 has been demonstrated to stabilize the endothelial barrier and subsequently reduce infarct size and no-reflow.

### Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>PLA/INT</th>
<th>Infarct size</th>
<th>Intervention/Drug</th>
<th>Acronym</th>
<th>TIMI grade</th>
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Figure 5. Forest plot of infarct size and thrombolysis in myocardial infarction (TIMI) flow grade 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; hsTnT, high-sensitive troponin T; IABC, intra-aortic balloon counterpulsation; INT, intervention; MRI, magnetic resonance imaging; PLA, placebo; POCO, postconditioning; RIC, remote ischemic conditioning; SPECT, single photon emission computed tomography; and TnT, troponin T.

### Methods

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Figure 6. Forest plot of infarct size and myocardial blush grade (MBG) 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.
no-reflow in mice.²⁴² 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.²⁴³ 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 minutes reperfusion following 30-minute coronary occlusion that mitochondrial dysfunction is central not only to myocardial injury to the coronary circulation, and much research is needed here to help develop more effective and more specific therapeutic approaches to target coronary microvascular injury. It seems that mitochondrial dysfunction is central not only to myocardial

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-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-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 to not only reduce infarct size but also improve coronary blood flow.20

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Disclosures

None.

References

17. 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;40:633–644.


N. Intramyocardial haemorrhage after acute myocardial infarction.


ZQ. Reduction in myocardial infarct size by postconditioning in pa-


159. Laskey WK, Yoon S, Calzada N, Ricciardi MJ. Concordant improve-

ments in coronary flow reserve and ST-segment resolution during percu-

taneous coronary intervention for acute myocardial infarction: a benefit


10.1002/ccd.21583.


JR, Shroff GR, Moore L, Traverse JH. Long-term follow-up of pa-

tients undergoing postconditioning during ST-elevation myocardial


11225-010-9252-0.

161. Liu TK, Mishra AK, Ding FX. [Protective effect of ischemia postcon-

ditioning on reperfusion injury in patients with ST-segment elevation

acute myocardial infarction]. Zhonghua Xin Xue Guan Bing Za Zhi.


infarct size and edema in patients with ST-segment elevation myocar-


jacc.2012.03.026.


no-reflow in STEMI patients. Basic Res Cardiol. 2013;108:383. doi:


M, Grajek S. Postconditioning reduces enzymatic infarct size and

improves microvascular reperfusion in patients with ST-segment el-

evation myocardial infarction. Cardiology. 2014;129:250–257. doi:

10.1159/000367965.

165. Tarantini G, Favaretto E, Marra MP, Frigo AC, Napodano M, Cacciavillani


during coronary angioplasty in acute myocardial infarction: the LIPSIA


j.ijcard.2012.03.136.

166. Ugata Y, Nakamura T, Taniguchi Y, Ako J, Momomura S. Effect

of postconditioning in patients with ST-elevation acute myocardial


0112-0077-9.

167. Dwyer NB, Mikami Y, Hilland D, Aljizeeri A, Friedrich MG, Traboulssi

M, Anderson TJ. No cardioprotective benefit of ischemic postcondition-

ing in patients with ST-segment elevation myocardial infarction. J Interv


primary percutaneous coronary intervention: the effects of postcon-

ditioning on myocardial infarction in patients with ST-segment elevation


1896. doi: 10.1161/CIRCULATIONAHA.113.010690.


postconditioning and postconditioning in ST-elevation myocardial in-

farction: the randomized LIPSIA CONDITIONING trial. Eur Heart J.


YH, Gwon HC. Effect of ischemic postconditioning on myocardial sal-

vage in patients undergoing primary percutaneous coronary intervention

for ST-segment elevation myocardial infarction: cardiac magnetic reso-

nance substudy of the POST randomized trial. Int J Cardiovasc Imaging.


171. Loubeyre C, Morice MC, Lefèvre T, Piéchaud JF, Louvard Y, Dumas

White SK, Frohlich GM, Sado DM, Maestrini E, Downey JM. Status of P2Y12 treatment must be considered

of risk factors, comorbidities, and comedication with ischemia/reperfu-

sion injury and cardioprotection by preconditioning, postconditioning, and


10.1124/pr.113.008300.

172. Schwartz Longacre L, Kloner RA, Arai AE, et al; National Heart, Lung,

and Blood Institute, National Institutes of Health. New horizons in car-

dioprotection: recommendations from the 2010 National Heart, Lung, and


10.1161/CIRCULATIONAHA.111.032698.


The Coronary Circulation as a Target of Cardioprotection
Gerd Heusch

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