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FORUM REVIEW ARTICLE

Postconditioning in Acute Myocardial Infarction Patients

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Abstract

Reperfusion therapy is the indispensable treatment of acute myocardial infarction (AMI) and must be applied as soon as possible to attenuate the ischemic insult. Evidence indicates that reperfusion is responsible for additional myocardial damage likely involving opening of the mitochondrial permeability transition pore. Ischemic postconditioning is a new way to dramatically reduce the lethal reperfusion injury. Several clinical studies using angioplasty postconditioning now support its protective effects in patients with an AMI. An interesting alternative is pharmacological postconditioning, which could be applied to a much larger number of patients. The mitochondrial permeability transition pore inhibitor cyclosporine A has been shown to generate a comparable protection in AMI patients. Future large-scale trials are needed to determine whether postconditioning may improve clinical outcome in ST-segment elevation MI patients. *Antioxid. Redox Signal.* 14, 811–820.

Introduction

Though incredible improvements in patients care with subsequent enhancement in prognosis, cardiovascular diseases remain the leading causes of death in the developed countries. Each year, acute myocardial infarction (AMI) is responsible for the death of millions of persons worldwide, with a mortality rate near 10%, and is the first cause of chronic heart failure (22). Several drugs and techniques demonstrated their ability to enhance the patient's survival in AMI, with reperfusion therapy as the first of all. Either by percutaneous coronary intervention (PCI) or the use of a fibrinolytic agent, successful reperfusion has been shown to dramatically reduce infarct size. The sooner it is performed, the larger the amount of salvaged myocardium. But even if it is fundamental to reperfuse the ischemic myocardium, there is an important price to pay: reperfusion *per se* is responsible for additional functional and structural myocardial damage named lethal reperfusion injury (Fig. 1) (48, 58). When reperfusion occurs, several metabolic and biochemical changes take place in the ischemic cardiomyocyte, including the generation of high quantities of reactive oxygen species, a fast restoration of normal pH, and a massive intracellular calcium overload, resulting in mitochondrial dysfunction and possibly cell death (21, 30, 48). Lethal reperfusion injury appears to represent from 20% to 70% of the total amount of irreversible myocardial damage according to the studied species and therefore constitutes a major therapeutic target (17, 43, 60).

The description of ischemic preconditioning (IPC) by Murry *et al.* in 1986 opened the way to a succession of numerous animal studies, which improved our understanding of the ischemia reperfusion phenomenon (38). This protection, triggered by applying a short sequence of ischemia reperfusion before an index prolonged ischemia, was shown to be greatly protective in terms of infarct size and functional recovery in the heart, at least partly by attenuating lethal reperfusion injury (Fig. 2) (41). Because of the fact that it is barely possible to predict episodes of prolonged ischemia, the applicability of IPC in the clinical setting is limited to some cases of surgical interventions (where IPC was shown to be efficient) (10).

Zhao *et al.* had the idea to apply the aforementioned "conditioning" regimen immediately after reperfusion following the prolonged ischemic insult (Fig. 2) (59). This technique, initially described in an open-chest dog model of myocardial ischemia reperfusion, proved its efficiency in most animal models of myocardial ischemia reperfusion in reducing reperfusion injury (3, 13, 25, 31). It appeared immediately more than ever interesting to the clinicians and raised great hope in further reducing the infarct size in patients and increasing their prognosis after an AMI. In this review, we will focus on the available published clinical data of postconditioning.

Postconditioning the Human Ischemic Myocardium

The first clinical studies assessing the feasibility, safety, and efficiency of ischemic postconditioning (IPost) in the setting of

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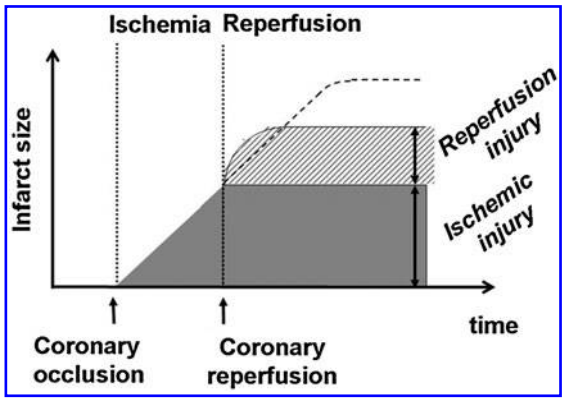

FIG. 1. Lethal reperfusion injury. Part of the cardiomyocytes constituting the final infarct size dies after the onset of reperfusion.

AMI were published in 2005, that is, just 2 years after the description of this concept by Zhao *et al.* They were small "proof-of-concept" studies. The IPost was performed during the time of primary PCI, just after the reopening of the culprit coronary artery by multiple balloon inflations and deflations. As there is no known optimal algorithm for IPost clearly defined in humans, the number and length of brief episodes of ischemia and reperfusion were chosen arbitrarily, yet considering, from animal studies, that the first brief ischemia should be applied within the first minute after reflow as the major part of the reperfusion-related cell death is irreversibly initiated during this time lapse (25, 47).

In the first clinical study on IPost, Staat *et al.* included patients with symptoms of ongoing ST-segment elevation MI (STEMI) eligible for primary PCI and no signs of spontaneous reperfusion, presenting <6 h after the onset of chest pain (51). Patients with complications such as cardiac arrest or cardiogenic shock and who had a previous history of AMI were not included, as were patients with evidence of coronary collaterals to the risk region at admission for coronary artery angiography. In this multicenter, open-label study, patients included were randomly assigned to either a control or the postconditioned group. The IPost protocol consisted of four cycles of 1-min inflation (at low pressure) of the angioplasty balloon; this protocol was applied within 1 min after reopening of the occluded culprit coronary artery using direct stenting. Meanwhile, patients in the control group underwent conventional PCI procedure with direct stenting, The primary endpoint was infarct size, assessed as the area under curve of total creatinine kinase (CK) release during the first 3 days following the reperfusion. The authors made a point of evaluating the size of the area at risk myocardium, as a major determinant of the infarct size, using left ventricular (LV) angiography and measuring the area of abnormal contraction (49). The duration of ischemia, another major determinant of infarct size, was estimated in all patients as the time elapsed between the onset of chest pain and direct stenting. The authors reported a significant 36% reduction in the area under curve of serum CK release (as well as the peak of CK release) in the postconditioned group compared with the control group (Fig. 3). The postconditioned patients exhibited a significantly higher blush grade after the procedure than the control patients, but there was no difference in terms of maximal ST-segment shift between the two groups at 48 h after PCI ($p = 0.09$). These data were consistent with an ability of the IPost to reduce the infarct size and enhance myocardial perfusion in the first minutes of reflow.

Later the same research group sought to determine whether this reduction in infarct size is persistent several months after the AMI and associated with an improved myocardial function (54). A new population of patients, admitted to the hospital for STEMI requiring PCI was included and assigned randomly to the postconditioned group or the control group. The procedure and IPost protocol were similar to the afore-

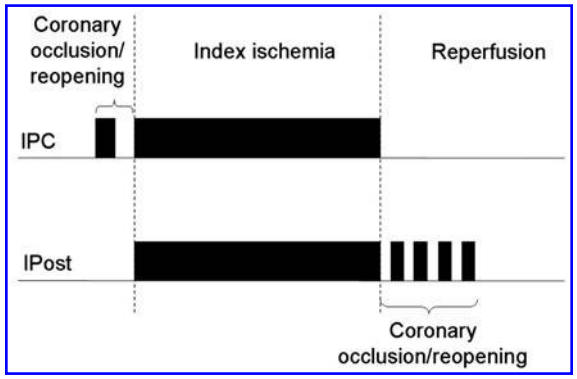

FIG. 2. Ischemic pre- and postconditioning.

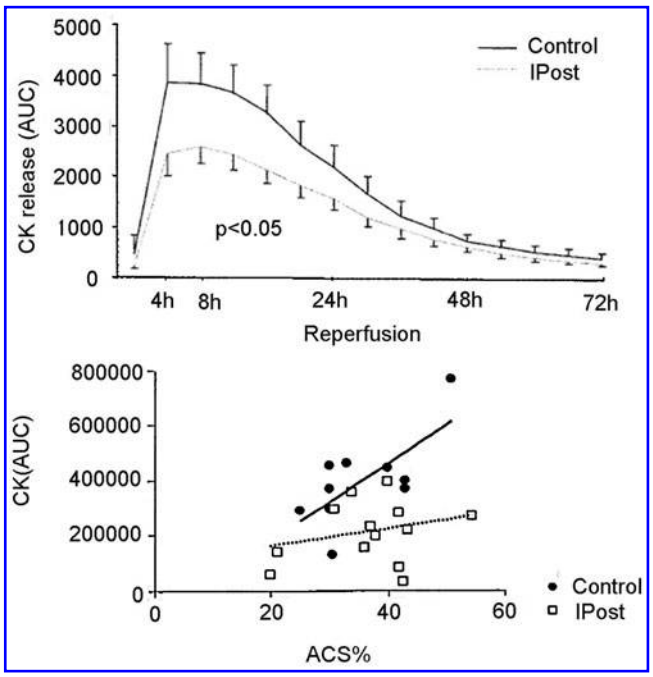

FIG. 3. Ischemic postconditioning does reduce the infarct size. The *upper figure* shows the creatinine kinase (CK) release (area under curve, AUC) in the postconditioned group (*solid line*) in comparison to the control group (*dotted line*) in favor of a 36% reduction in infarct size. The *lower figure* represents the CK release *versus* the area at risk (evaluated by the abnormally contractile segments [ACS] percentage) measured by left ventriculography in both control (●) and postconditioned (□) patients. The data points for the postconditioned group are below the control line.

mentioned Staat's study. In addition, ^{201}thallium-single-photon emission computed tomography (SPECT) and echocardiography were performed at 6 and 12 months, respectively, after PCI to determine whether infarct size reduction by IPost had persisted over time and to evaluate the effect of IPost on the recovery of myocardial contractile function at 1 year after infarction. Acute evaluation by cardiac enzymes release (both CK and troponin I) found a 40% reduction in infarct size, very similar to that reported in the initial study. A comparable effect was maintained at 6 months with SPECT imaging data showing a 39% reduction in infarct size. This effect was associated with a better recovery of myocardial contractile function at 1 year, as assessed by a greater LV ejection fraction (LVEF) and wall motion score index (WMSI). The authors concluded that the protective effects of IPost seem to be persistent and further resulted in a better recovery of contractile function.

Laskey performed a monocenter pilot study in which the IPost protocol consisted of a single 90-s inflation performed 3–5 min after reopening the culprit coronary artery by angioplasty (28). The endpoint was the ST-segment shift after the PCI and the coronary flow velocity reserve, as measured by a Doppler-tipped wire at the end of the PCI. The ST-segment decrease was significantly greater in the postconditioned group than in the control group, so were the distal coronary flow velocity parameters. The author concluded that IPost improved coronary reflow and vasodilator capacity of the distal coronary vascular bed. However, this vascular effect of IPost might have been severely underestimated in this study because of the fact that the IPost protocol was applied beyond the aforementioned first minute after reflow threshold. These data were confirmed in a second study by Laskey *et al.* including 24 patients and using the same IPost protocol (29).

In another study by Ma *et al.*, 94 patients with a first STEMI requiring primary PCI were randomized into either a control or a postconditioned group (34). IPost consisted of three cycles of 30-s re-inflation of the coronary balloon followed by 30-s reperfusion, started within the first minute of reflow. The authors measured the serum CK and CK–muscle and brain isoenzymes (MB) release during the first 3 days following the procedure and the results were consistent with those from Staat's study. The myocardial contractile function was determined at 8 weeks after the infarction, with the assessment of the WMSI by echocardiography. There was an improvement in the WMSI in both groups compared with baseline, but this increase was significantly greater in the postconditioned group than in the control group. The originality of this study consists in the fact that the investigators tried to evaluate the level of oxidative stress associated with reperfusion. Malhondialdehyde, a non-specific marker of lipid peroxidation (a consequence of the generation of reactive oxygen species at the time of reperfusion), was measured in blood several times during the first 7 days of reperfusion. At all time points, the level of malhondialdehyde was significantly lower in the postconditioned group than in the control group, suggesting that part of the beneficial effect of IPost could be explained by a reduction in oxidative stress as shown in some experimental studies (52, 59).

All these studies involving small numbers of patients contributed to confirm in the clinical settings of STEMI the cardioprotective effects of IPost reported in animal studies. PCI postconditioning is safe, easy-to-perform, and unexpensive and induces a persistent reduction in infarct size and improvement in myocardial contractile function. The question of whether it improves clinical outcomes has nevertheless not been assessed yet and would require a large-scale, multicenter, controlled, randomized study.

We must, however, keep in mind that many patients throughout the world who experience a STEMI do not have a fast access to a PCI-performing facility. For the patients in whom the STEMI is diagnosed at an early stage, a common reperfusion therapy is often thrombolysis. Whether IPost is efficient when reperfusion is obtained by fibrinolysis is still unknown. This mode of restoration of coronary flow may indeed modify the efficiency of postconditioning for at least two reasons. First, in animal preparations as well as during PCI, reflow is always complete and abrupt. Fibrinolysis may cause a slower reflow because of both a softer disaggregation of the thrombus and the persistence of a coronary artery stenosis, that is, a less abrupt reflow. Second, the timing of reflow by PCI is sharp, whereas the exact time of reopening of the coronary artery by thrombolysis may be difficult to determine accurately, only suggested by the reduction of chest pain and some signs on the electrocardiogram, such as ST-segment shift reduction or the occurrence of some types of arrhythmias. This is of course of major importance because experimental evidence indicates that any protective intervention must be performed before the first minute of reflow. This question is even more important when one considers that fibrinolysis may be started before hospital admission.

There is therefore an urgent need for the development of a drug that, given a few minutes before or at the time of reperfusion, would be able to mimic the effects of IPost and thus generate a pharmacological postconditioning (Fig. 4). Accumulating experimental data have allowed us to identify several potential molecular targets that may help design new treatments aimed at attenuating irreversible myocardial damage caused by reperfusion.

Pharmacological Postconditioning in AMI

Over the past 2 decades, several infarct size reduction studies have been performed, using various pharmacological agents, most of them being negative. We will focus here only on infarct size reduction studies in STEMI patients, which correspond to the concept of postconditioning, that is, with therapeutic interventions previously reported as postconditioning mimetic in animal models and aimed to

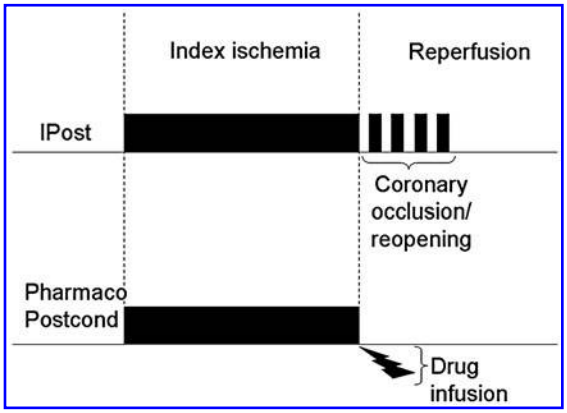

FIG. 4. Ischemic and pharmacological postconditioning.

reduce infarct size by specifically targeting lethal reperfusion injury.

Inhibition of mitochondrial permeability transition

Accumulating evidence suggests that the opening of the mitochondrial permeability transition pore (mPTP) plays a key role in lethal reperfusion injury. The opening of this nonspecific high-conductance channel localized in the inner mitochondrial membrane generates mitochondrial membrane depolarization, uncoupling of the respiratory chain, release of cytochrome *c* and other apoptotic factors in the cytoplasm, and adenosine triphosphate (ATP) depletion by hydrolysis (instead of synthesis) of ATP (20). Its structure is still not clearly defined but one of its key components is a protein found in the mitochondrial matrix called cyclophilin D. Animal studies showed that the inhibition or the depletion of cyclophilin D is associated with an increased resistance to cell death. Moreover, mice lacking cyclophilin D are protected against lethal reperfusion injury and develop significantly smaller MI compared with their wild-type controls (4, 5, 39). Cyclosporine A, which has potent immunosuppressor activity *via* its binding to the cytosolic cyclophilin A, also binds to the mitochondrial cyclophilin D and thereby inhibits mPTP opening. Cyclosporine A has been shown to reduce infarct size in various experimental conditions (2, 11, 19, 53, 56). Despite its described secondary effects on blood pressure and its immunosuppressive action, it then appeared as an interesting candidate for pharmacological postconditioning.

In a multicenter, single-blinded, controlled trial, Piot *et al.* sought to determine the safety and efficacy of the administration of cyclosporine A in patients referred for a STEMI (46). Fifty-eight patients presenting within 12 h after the onset of chest pain were randomized to receive either a single-dose intravenous injection of cyclosporine A (2.5 mg per kg of body weight) or an equivalent volume of saline solution. The dose of cyclosporine A was chosen considering the data available from animal studies showing its protective effects and was lower than the daily dose administered in cardiac transplant patients in prevention or treatment of acute graft rejection. The injection of cyclosporine A (or saline) was performed a few minutes before reflow to ensure that the drug reaches sufficient plasma concentration within the first minute of reperfusion. The primary endpoint was the size of the infarct as measured by cardiac enzymes release during the first 3 days following reperfusion. The secondary endpoint was also the infarct size as determined by cardiac magnetic resonance imaging (MRI) performed at 5 days after PCI in a subgroup of patients. The safety endpoints were the measurements of blood pressure, serum creatinine concentration during the first 3 days, hepatic enzymes, and white cell count at 1 day after the drug injection.

The infarct size as measured by the release of CK was significantly smaller in the cyclosporine group (Fig. 5). Cardiac MRI, performed in a subgroup of 27 patients, confirmed the reduction in infarct size shown by CK release (Fig. 6). The authors did not notice any adverse effect of cyclosporine A. It appeared then that the administration of cyclosporine A in the setting of AMI in conjunction with reperfusion therapy was safe and could significantly reduce the size of the infarction. In a follow-up study, Mewton *et al.* recently examined whether cyclosporine A might have

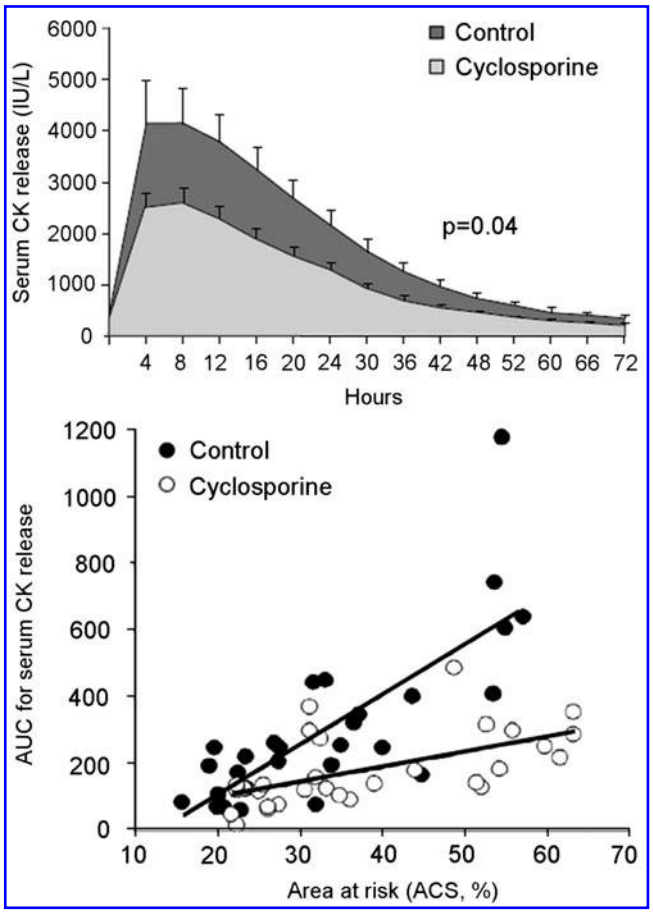

FIG. 5. Cyclosporine A significantly reduces the infarct size. The *upper figure* shows the CK release in the cyclosporine group (*light gray area*) in comparison to the control group (*dark + light gray area*) in favor of a 40% reduction in infarct size. The *lower figure* represents the CK release *versus* the area at risk in both control (●) and cyclosporine (○) groups. The data points for the cyclosporine group are below the control line.

modified LV remodeling in that subgroup of patients (37). Experimental reports in the rat model of acute MI had indeed suggested that administration of cyclosporine A after AMI may facilitate an adverse LV remodeling through the blockade of the calcineurin-nuclear factor of activated T-cell pathway involved in hypertrophy (16). Cardiac MRI was performed at 6 months and compared with that done at day 5. The authors reported first that infarct size reduction persisted at month 6 and that cyclosporine A did not exert any deleterious effect on LV remodeling. In addition, infarct size reduction by cyclosporine was associated with a lower LV dilatation at day 5, which was maintained at 6 months. Although these results are encouraging, a larger study is needed to determine whether inhibition of mPTP opening may improve clinical outcome in STEMI patients. However, even if the authors did not report any complication associated with the use of cyclosporine A, it remains questionable whether this drug might be one day commonly used as an adjunct to reperfusion therapy. More specific and non-immunosuppressive inhibitors of mPTP opening would certainly be a preferable option.

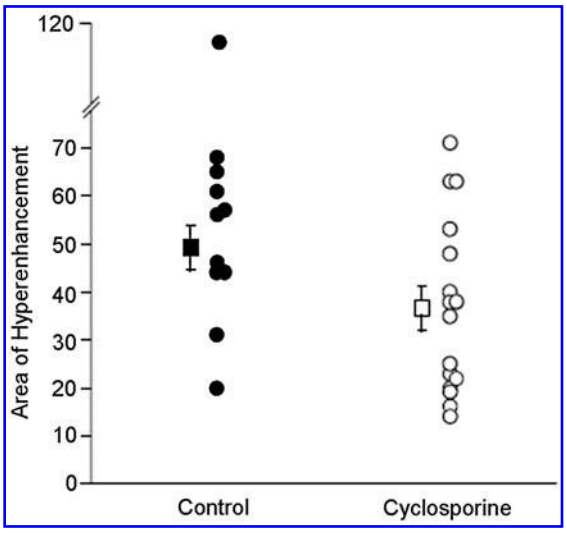

FIG. 6. The cardiac magnetic resonance imaging confirms the reduction in infarct size by cyclosporine A. The mean area of late hyperenhancement on magnetic resonance imaging representing the infarct size was significantly greater in the control group (○) than in the cyclosporine group (□). $p = 0.04$.

The J-WIND trial

In that study, the investigators addressed the protective effect of K_{ATP} channel openers on one hand and natriuretic peptides on the other hand. The protective role of K_{ATP} channel openers has been clearly demonstrated in experimental studies when used as preconditioning agents and also suggested when used as postconditioning agents (33, 44). The J-WIND study by Kitakaze *et al.* partly aimed to establish a cardioprotective role of nicorandil in 545 patients with an AMI (26). In this single-blinded, placebo-controlled, randomized trial, the patients received, before PCI, either a placebo or an intravenous infusion of nicorandil for 24 h. The primary endpoints were the infarct size determined by the CK and CK-MB release during the first 3 days, and the LVEF at 6–12 months after the infarction estimated by left ventriculography. Nicorandil failed to reduce the infarct size and did not improve LVEF, adding to the discrepancy of the literature about K_{ATP} channel openers as postconditioning agents.

The authors examined the protective role of the atrial natriuretic peptide (ANP) in a second arm of the study. Experimental evidence suggests that these peptides, in particular the ANP, may mimick IPost (6, 57). In the J-Wind study, a group of patients received a continuous intravenous infusion of ANP (0.025 mg/kg/min) during 3 days, generally started before reperfusion. The reperfusion strategy used was subjected to the practitioner's discretion. The infarct size was significantly smaller in the ANP group when the CK release was considered (−14.7%), but this difference did not reach significance when the troponin T levels were analyzed ($p = 0.084$). There was a trend toward a greater LVEF in the ANP *versus* the control group at 2–8 weeks after reperfusion and this difference became significant after 6–12 months (+5.1%). There was no difference between the two groups in terms of survival rate or cardiovascular events but the composite endpoint cardiac death and readmission for heart failure was significantly lower in the ANP group (hazard ratio 0.267), so was the occurrence of the authors' "reperfusion injuries" (corresponding to the occurrence of malignant arrhythmias, recurrence of ST-segment elevation, or worsening of chest pain before discharge of the patient) (hazard ratio 0.743). So it appears that the use of ANP as a reperfusion adjunct is associated with a small but significant reduction in infarct size and improvement in LVEF, corresponding to a better clinical outcome. However, it has to be recognized that the conditions of administration of both nicorandil and ANP, especially with respect to the timing of reperfusion, were not clear in that study.

Adenosine

Adenosine has long been considered as the ideal candidate for protection against ischemia and reperfusion damage, based on considerable experimental evidence of its protective effects in experimental preparations of preconditioning. The relationship between adenosine and IPost remains, however, controversial despite several well-conducted experimental studies, because of fickle effects of the drug or various adenosine receptor subtypes agonists in animal models of AMI (18, 40, 55). There are contradictory data concerning the adenosine receptors involved in the protection, with some research groups suggesting a critical role of the A1 receptors, the inhibition of which could abolish the protective effects of IPost, and some others reporting a protective role of the A2 or A3 receptors but not the A1 (12, 24, 45). Despite this contradictory literature and the fact that adenosine has unwanted hypotensive effects, it has been tried as an infarct size-reducing candidate in several clinical trials. In the AMI study of adenosine (AMISTAD)-I trial, a randomized, open-label study, Mahaffey *et al.* included 236 patients presenting within 6 h of the onset of chest pain and eligible for thrombolytic therapy (35). The patients were assigned to receive a peripheral intravenous infusion of adenosine (70 μg/kg/min for 3 h) or a placebo (saline solution for 3 h), which was started prior to the administration of the thrombolytic therapy. The primary endpoint was the infarct size, as assessed by SPECT imaging at 5–12 days after the infarction and the secondary endpoint was a composite clinical endpoint (major adverse cardiovascular events). According to the SPECT imaging, the infusion of adenosine resulted in a significant 33% reduction in infarct size. A subgroup analysis showed that only patients with an anterior wall infarction benefited from this therapy (67% reduction in infarct size, $n = 45$). Considering the secondary clinical endpoint, there was no difference between the two groups despite a trend toward a worse outcome in patients who received adenosine. So despite an apparent cardioprotective effect of adenosine as an adjunct of reperfusion therapy in the most severe patients (with anterior wall infarction), it is not clear at all whether this leads to a clinical benefit.

The safety of intracoronary administration of adenosine at the time of primary PCI was assessed by Marzilli *et al.* in a small randomized, double-blinded trial (36). Of the 54 patients included, 27 received a bolus of adenosine and the other 27 an equivalent quantity of saline solution during PCI. The primary endpoints were feasibility and safety of intracoronary injection of adenosine as well as its effects on coronary blood flow, that is, the occurrence of no-reflow. At the end of the procedure, the thrombolysis in MI flow grade was significantly higher in the adenosine group and significantly fewer patients experienced impaired microvascular reperfusion.

As secondary endpoints, the CK release was significantly lower in the adenosine group, and these patients developed less often Q-wave MI and had a significantly better LV function as assessed by echocardiography at 1 week after the infarction, compared with the control group. Moreover, significantly fewer adverse cardiac events (*i.e.*, cardiac death or cumulative clinical endpoints including cardiac death, recurrent ischemia, nonfatal MI, and heart failure) occurred in the adenosine group, suggesting a clinical benefit of this drug when administered at the appropriate time point.

In the ADMIRE (AMP579 delivery for MI reduction) trial, Kopecky *et al.* reported the effect of AMP579, a mixed adenosine agonist with A1 and A2 effects (27). This trial was designed as a double-blinded, multicenter, placebo-controlled study and included 311 patients undergoing primary PCI randomly assigned to four groups: a control group and three treatment groups receiving a peripheral intravenous infusion of AMP579 at different doses during 6 h, started before PCI. The authors chose the infarct size as the primary endpoint, measured by SPECT imaging at 5–9 days after the infarction. Unlike Mahaffey *et al.*, the authors did not report any difference between the different groups in terms of infarct size, even when considering only the patients with an anterior wall infarction. There was only a slight trend toward greater myocardial salvage in the patients with anterior wall infarction who received the higher dose of drug. It is, however, important to note that the serum levels of AMP579 approached the levels showing protection in the animal studies only in the higher dose infusion group, preventing from any further conclusion concerning the efficacy of this drug.

Last, Ross *et al.* sought to determine the clinical outcome associated with the infusion of adenosine in the large-scale, double-blinded, placebo-controlled randomized AMISTAD-II trial (50). Two thousand one hundred thirteen patients with ongoing acute anterior MI were included and assigned to receive either 70 μg/kg/min (as in AMISTAD-I) or 50 μg/kg/min of adenosine for 3 h or a placebo. The patients were reperfused either by fibrinolytic therapy or primary PCI and the treatment, administered intravenously, started within 15 min of the infusion of the thrombolytic agent or before PCI. The primary endpoint was the incidence of new congestive heart failure or rehospitalization for congestive heart failure or death during 6 months following the infarction. In a subgroup of 273 patients, the infarct size was also measured by SPECT imaging at 5–9 days after reperfusion. At the end of the follow-up, there was no difference between all groups with respect to the primary endpoint. Measurements of the infarct size revealed a trend toward smaller infarction in the adenosine groups *versus* placebo (17% *vs.* 27%, $p = 0.074$), with the higher dose of adenosine significantly more efficient in reducing infarct size *versus* placebo ($p = 0.0023$) than the lower dose ($p = 0.41$).

It is difficult to conclude as to a protective role of adenosine in STEMI patients. Additional studies are underway to address the role of adenosine in clinical conditions of a postconditioning intervention.

Erythropoietin and its analogs

Erythropoietin (EPO) has been shown to activate the reperfusion injury salvage kinase pathway in animal models of myocardial ischemia reperfusion injury (9). Lipsic *et al.*, in a small, monocenter, randomized study, included 20 patients who presented with symptoms of AMI. They were assigned to receive either a single intravenous bolus of darbopoietin alfa (300 μg) prior to primary PCI or no additional medication to standard therapy (control group) (32). This study was designed to assess the safety of this drug as an adjunct to reperfusion therapy and the investigators monitored the blood cell count until 30 days after the infarction. They also measured the CK and CK-MB release and the LVEF was determined after 4 months of follow-up by SPECT imaging. The authors did not report any significant change in the hematocrit level between the two groups. There was a trend toward a greater CK and CK-MB release in the darbopoietin group and the LVEF was comparable at 4 months.

Two other research groups investigated whether EPO could be an efficient adjunct to reperfusion therapy and contribute to reduce the infarct size. In a monocenter, randomized study, Binbrek *et al.* included 236 patients who were assigned to receive either a single intravenous dose of EPO (30,000 IU) or standard medical care (control group) just before the administration of a fibrinolytic agent (7). The investigators used the infarct size, measured by the CK release, as a primary endpoint. Hematocrit, myocardial contractile function before discharge assessed by echocardiography, and major adverse cardiovascular events (including death) were among the secondary endpoints described. The authors did not report any significant difference between the groups for the primary or the secondary endpoints detailed above, suggesting that, even if safe, the adjunction of EPO to reperfusion therapy did not have any beneficial effect.

In another monocenter, placebo-controlled, double-blinded study, Ferrario *et al.* enrolled 30 patients eligible to primary PCI who received either a single intravenous perfusion of EPO (33,000 IU) or a saline solution (control group) just before PCI (15). The investigators monitored every major adverse cardiocirculatory event during the first 12 months in addition to the CK release during the first 24 h and echocardiographic and cardiac MRI measurements at 6 months. No clinical events occurred during the follow-up period. The CK-MB release was significantly smaller in the EPO group. The MRI did not show any difference in the infarct size between the two groups but revealed a slight though significant decrease in LV end-systolic volume in the EPO group compared with controls, confirmed by echocardiography. Therefore, whether EPO, though safe, is efficient in reducing lethal reperfusion injury in man remains unclear.

The Remote IPost

Aside standard IPost and pharmacological postconditioning, it has been proven in animal studies that transient episodes of ischemia and reperfusion remote to the heart, either performed before the index ischemia (remote preconditioning) or at the onset of reperfusion (remote postconditioning), could reduce the lethal myocardial reperfusion injury (23). This amazing phenomenon, the mechanisms of which are not yet clearly understood, could be another outstanding alternative to IPost. Botker *et al.* reported recently the first clinical trial with the remote postconditioning performed on the upper limb using an arm cuff (8, 42). This monocenter randomized study included 251 patients who were assigned to a remote postconditioning group or a control group while on

their way to the hospital to receive primary PCI. The protocol consisted in four cycles of 5-min inflation followed by 5-min deflation of an upper-arm blood-pressure cuff. The primary endpoint was a myocardial salvage index estimated at 30 days after reperfusion by SPECT imaging. However, the calculation of this index required that a first SPECT imaging had been performed <24 h after admission, which was not possible in all patients. The analysis of the matched scan pairs revealed a significant increase in myocardial salvage by remote postconditioning, although the difference in infarct size did not reach significance between the two groups. These encouraging results tend to confirm the remote postconditioning as a serious candidate for the reduction of lethal reperfusion injury in the clinical setting, but we still need more data from other clinical trials.

Limitations

After 2 decades of disappointing negative infarct size reduction studies, some of these small-sized trials have renewed our interest in protective interventions for STEMI patients. Although there might be several causes for an infarct size reduction trial to be negative, a major one being the absence of efficacy of the tested pharmacological agent, some key point rules have to be followed to gain confidence into the final results (14).

It is essential to take into consideration several major confounding factors that have been shown to deeply influence the results of all infarct size reduction studies, namely the timing of the protective intervention with respect to reflow and the three major determinants of infarct size (duration of ischemia, size of the area at risk, collateral circulation) (49).

First, the time window for IPost is a critical factor (25). During Ovize's PCI postconditioning or for cyclosporine administration, the study treatment was always administered before or within the first minute after direct stenting of the culprit coronary artery. Second, only patients with a fully occluded culprit coronary artery (thrombolysis in MI flow grade at admission coronary angiography of 0 or 1) were included, thereby eliminating all patients who had undergone spontaneous reperfusion before PCI, that is, who had already been exposed to reperfusion injury before the protective intervention.

Third, the duration of ischemia is clearly a major determinant of infarct size. The symptom-to-balloon time must be measured and be comparable between the control and treated groups. Fourth, the assessment of area at risk size, the major determinant of infarct size in the mammalian heart, must be assessed, using either LV angiography or SPECT imaging with ^{99}Tc-sestamibi (1). Fifth, collateral flow is the third major determinant of infarct size. Patients with visible collateral circulation at admission coronary angiography are endogenously protected and develop small infarcts even in the absence of any protective intervention; these patients should be excluded from trials exploring the efficiency of infarct size reduction interventions. If not, they may severely decrease the power of future studies, preventing them to show any significant protective effect of the tested drugs.

Taking into account these major determinants of infarct size into the experimental design of future infarct size reduction trials will help us improve their accuracy and power to explore future treatments aimed to prevent lethal reperfusion injury.

Conclusions

The description of the lethal reperfusion injury phenomenon and the discovery of IPost revitalized cardiovascular research in this field and led to the development of very promising strategies that may dramatically enhance the prognosis of patients with an AMI. The IPost, although reserved for patients reperfused by primary PCI, is safe and provides outstanding results when the infarct size and the myocardial contractile function are considered, raising great hope for potential clinical benefits. Feasible in every patient, the pharmacological postconditioning would allow the expansion of IPost protection to almost all STEMI patients. Following cyclosporine A, several new drugs are being tested during the upcoming years, as postconditioning mimetics. We are now turned to the future to see whether pharmacological postconditioning will be able to significantly improve clinical outcome in large-scale trials that would lead to its addition to the standard medical therapy.

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Abbreviations Used

ACS = abnormally contractile segment
AMI = acute myocardial infarction
AMISTAD = acute myocardial infarction study of adenosine
ANP = atrial natriuretic peptide
ATP = adenosine triphosphate
AUC = area under curve
CK = creatinine kinase
CK-MB = creatinine kinase–muscle and brain isoenzymes
EPO = erythropoietin
IPC = ischemic preconditioning
IPost = ischemic postconditioning
LV = left ventricular
LVEF = left ventricular ejection fraction
MI = myocardial infarction
mPTP = mitochondrial permeability transition pore
MRI = magnetic resonance imaging
PCI = percutaneous coronary intervention
SPECT = single-photon emission computed tomography
STEMI = ST-segment elevation myocardial infarction
WMSI = wall motion score index

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