

## Ischemic postconditioning: a clinical perspective

Primary percutaneous coronary intervention is the recommended therapy for patients with ST-elevation myocardial infarction. However, restoration of coronary blood flow may lead to reperfusion injury, which has been suggested to account for 50% of the final myocardial infarct size. As an adjuvant therapy to primary percutaneous coronary intervention, ischemic postconditioning (IPost) has been shown to be safe and to underlie cardioprotection in several clinical trials. However, there remain important issues to be settled before IPost can be used routinely in patients undergoing primary percutaneous coronary intervention: first, determining which IPost protocol is the most optimal in humans; second, determining the effect of IPost on clinical outcome; third, determining in which patients should IPost be applied; and fourth, determining the effect on left ventricular function. This article discusses these issues with a clinical perspective and looks into alternative pharmacological cardioprotection.

**KEYWORDS:** acute myocardial infarction ■ cardioprotection ■ ischemic postconditioning ■ ischemic postconditioning protocol ■ pharmacological postconditioning ■ primary percutaneous coronary intervention ■ reperfusion injury

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Acute myocardial infarction is a major cause of death and heart failure in western society [1]. Introduction of reperfusion therapy in terms of thrombolysis and primary percutaneous coronary intervention (PPCI) has led to a significant decrease in morbidity and mortality [2]. Today, PPCI is the recommended therapy for patients with ST-segment elevation myocardial infarction (STEMI). However, generation of reperfusion is a double-edged sword because it has the potential to cause additional myocardial damage not caused by the ischemic insult. This injury occurs in the first minutes of reperfusion and is termed reperfusion injury [3]. Ever since the discovery of reperfusion injury, this damage has been a target of intensive investigation. The pathogenesis is very complex and not fully understood. In a clinical perspective, the reperfusion injury appears to be important because it has been suggested to account for as much as 50% of the final myocardial infarct size [4]. Consequently, it is highly relevant to look for means to protect the heart during the reperfusion phase, and ischemic postconditioning (IPost), defined as repetitive interruptions of the coronary blood flow applied after a period of ischemia, is one of the most promising methods. This article focuses on IPost and aims to give an understanding of IPost and a discussion of potential clinical applications during PPCI. Finally, this article will give a brief look into alternative cardioprotective treatments and future perspectives.

### Cardiomyocyte death: a central element of reperfusion injury

It is beyond the scope of the present article to discuss in detail the pathogenesis of reperfusion injury, which has been reviewed comprehensively elsewhere [4–7]. Briefly, reperfusion injury is a syndrome comprising several distinct pathophysiological components: a (reversible) myocardial contractility impairment (stunning), arrhythmias, no-reflow and cardiomyocyte death (the lethal component) [4]. No-reflow may contribute to the lethal component (necrosis and apoptosis) of the reperfusion injury syndrome [8]. However, extensive experimental evidence implicates a number of factors as responsible for the cardiomyocyte death during reperfusion independently of no-reflow (see the following section on cardioprotective mechanisms).

### Ischemic postconditioning: background

The predecessor of IPost was ischemic preconditioning (IPC), which is defined as repetitive interruptions of the coronary blood flow applied before a period of ischemia. In a landmark study in 1986, Murry and colleagues first described the phenomenon of IPC [9]. In a dog model, they found that four occlusions of the infarct-related artery, each lasting 5 min followed by 5 min of perfusion applied prior to a 40-min total occlusion of a coronary artery, led to

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cardioprotection [9]. The animals treated with IPC developed significantly smaller infarct sizes compared with the animals treated only with reperfusion, a difference that corresponded to a 75% decrease in infarct size. To date, IPC is the most potent protection against reperfusion injury of all investigated methods, and it is considered the gold standard for protection against reperfusion injury during PPCI [10]. However, the clinical application in patients with acute myocardial infarction is limited owing to the fact that IPC has to be applied prior to the ischemic period.

The potential cardioprotective benefits of altering the myocardial reperfusion in acute myocardial infarction were first reported by Okamoto *et al.* in 1986 and later by Mrak *et al.* in 1990 and Sato *et al.* in 1997 [11–13], but it was not until 2003 that this concept gained real attention. Inspired by IPC, Vinten-Johansen's group introduced the phenomenon of IPost [14]. In a dog model, three repetitive interruptions of the coronary blood flow applied after a period of ischemia, each occlusion lasting 30 s followed by a 30-s reperfusion period, led to a 44% reduction in infarct size [14]. Furthermore, the reduction in infarct size caused by IPost was similar to the reduction in infarct size in the dogs treated with IPC and, importantly, there was no synergistic effect of IPC and IPost [14]. However, despite the close relationships between IPost and IPC, it is still controversial whether they share mechanisms and protect equally. Since the introduction of IPost, the cardioprotective effect has been intensively investigated in different species using a variety of IPost protocols and evidence that IPost has the potential to protect the heart during reperfusion in experimental animal studies is consistent [15].

### Cardioprotective mechanisms mediating the effect of ischemic postconditioning

While the immediate result of any IPost procedure is a mechanical modification of the flow pattern at reperfusion, it is clear today that the mechanisms activated and responsible for the IPost infarct-sparing effect involve a complex set of signaling cascades in cardiomyocytes. A number of excellent reviews on this subject are available [7,16–21] and only a very brief outline will be presented here. The alternating sequence of short reperfusion–ischemia episodes of IPost appears to stimulate a release of a number of endogenous mediators into the ischemic area. Examples of such mediators include adenosine,

bradykinin, opioids and cytokines, whose receptors are known to engage a number of specific signaling kinase cascades. At least three signaling pathways have been recognized: the reperfusion injury survival kinase pathway [22], NO–PKG–PKC pathway [23] and the most recently identified survivor-activating factor enhancement pathway [24]. While all these pathways appear to be important, a number of key aspects remain unresolved, including between-species differences, the crosstalk between (and the relative significance of) the individual pathways and – perhaps most fundamentally – the cellular pathway target(s) directly responsible for cardioprotection. The reader is referred to specialized reviews discussing the cardioprotection targets [25,26]. Some of the major targets include sarcoplasmic reticulum proteins controlling  $\text{Ca}^{2+}$  fluxes (sarco-/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase pump and its regulator, phospholamban), proteins determining  $\text{Ca}^{2+}$  sensitivity of the contractile apparatus (troponin I),  $\text{Ca}^{2+}$ -activated proteases (calpains) and mitochondria. The prominent attention devoted to the role of mitochondria in the context of ischemia–reperfusion injury has been caused by the phenomenon of the mitochondrial permeability transition pore (mPTP; reviewed in [27]). mPTP (of uncertain significance under physiological conditions) is a large-conductance channel in the inner mitochondrial membrane, induced at the onset of reperfusion by a concurrence of several factors: postischemic low ATP and high phosphate levels, high intracellular  $\text{Ca}^{2+}$  concentration, a burst of reactive oxygen species production and a normalization of intracellular acidosis. These changes in mitochondria may precipitate energy failure and  $\text{Ca}^{2+}$  deregulation, with the latter compounding the deregulation owing to the failure of the normal  $\text{Ca}^{2+}$  release–uptake cycle from the sarcoplasmic reticulum. Cell death is ultimately caused by hypercontraction, disruption of sarcolemmal integrity and ensuing necrosis. Alternatively, mitochondrial swelling owing to mPTP opening may release cytochrome c to the cytosol and trigger apoptosis in those cells with sufficient preserved ATP. While the status of mPTP as an important contributor to lethal cardiomyocyte injury upon reperfusion seems indisputable, the clear-cut, unequivocal evidence of mPTP comprising a direct cardioprotection target in settings such as IPost has proven difficult to obtain [25,28]. In addition to the activation of intracellular signaling, the IPost-induced cardioprotection may also be in part due to a change

in washout kinetics of metabolites accumulated in the ischemic area prior to a reinstatement of the coronary vessel patency, thus altering some key aspect of the myocyte biochemical milieu. A prominent example of such a mechanism is a delay of acidosis normalization by IPost, thought to contribute to cardioprotection through mPTP inhibition [29].

In summary, an IPost protocol translates a series of balloon deflations and inflations into an array of sophisticated, biochemical processes, ultimately affecting the balance of death and survival responses in the cardiomyocytes. The procedure has been shown to underlie cardioprotection in several species of experimental animals (e.g., rats, mice, rabbits, dogs, pigs and monkeys) [7,30,31] and appears to be effective in humans as well (see later) [32,33].

### Ischemic postconditioning during PPCI

In light of the consistent positive data from experimental studies, it seemed justified to perform randomized human studies in patients with acute myocardial infarction. The first to report such a study was Laskey, who found that two cycles of balloon inflation, each lasting 90 s separated by a 3–5 min reperfusion period, led to improved ST-segment resolution and coronary flow reserve in 17 STEMI patients undergoing PPCI [34]. Later in the same year, Staat *et al.* randomized 30 patients with STEMI to either conventional PPCI or PPCI with IPost consisting of four repetitive total occlusions of the infarct-related coronary artery, each lasting 1 min separated by 1 min reperfusion [33]. This additional treatment resulted in a 36% reduction in area under the curve of creatine kinase (CK)-MB measured during the first 72 h after the procedure as well as an improved blush grade. Since the initial reports by Laskey [34] and Staat *et al.* [33], several studies have confirmed the cardioprotective effect of IPost in humans using ST-segment resolution [34], release of biomarkers [35–38], SPECT [38,39], echocardiography [36,39] and MRI [32]. Notably, not all studies measured infarct size, since some only reported surrogate measurements of infarct size (CK release, ECG resolution, left ventricular (LV) function and perfusion defect index by SPECT). To date, a total of 399 patients have been enrolled in randomized studies and an additional 200 patients in retrospective studies with IPost, all of which are summarized in TABLE 1. In addition, a meta-analysis from 2009 using the data from Laskey [34], Staat *et al.* [33],

Ma *et al.* [36], Yang *et al.* [38], Thibault *et al.* [39] and Laskey *et al.* [40] concluded that IPost reduces peak CK [41]. The long-term benefits of IPost have been demonstrated in terms of reduction in infarct size by MRI after 3 months [32], by SPECT after 6 months [39] and by improvement of LV ejection fraction (LVEF) and wall motion index after 1 year [36,39]. Furthermore, Lønborg *et al.* and Staat *et al.* compared the size of infarction with the size of the myocardial area at risk, and both discovered that IPost reduces the absolute infarct size further in patients with larger risk zones than it does in patients with smaller risk zones (FIGURES 1 & 2) [32,33]. However, the methods used to quantitatively measure the myocardial area at risk are problematic. Staat *et al.* used the extent of abnormal contracting myocardial segments, a method that has not been compared with the more accurate techniques of SPECT and cardiac magnetic resonance [33], whereas, Lønborg *et al.* used the endocardial surface area method [32], which, although it correlates well with T2-weighted cardiac magnetic resonance measurement of the area at risk, it also seems to underestimate the size [42]. Taken together, in terms of infarct size and surrogate measures of infarct size, results have been consistent overall, with relative reductions in infarct size ranging from 18 to 39%. Importantly, IPost seems to be a safe procedure, since no studies have reported any complications or adverse effects.

It is important to stress that the studies performed to date are relatively small and patients within the existing studies represent a selected cohort. In addition, data from existing studies have been inconsistent with regard to improvement in LV function. Ma *et al.* [36], Yang *et al.* [38] and Thibault *et al.* [39] have reported improved LV function with IPost. On the other hand, Lønborg *et al.* [32] and Laskey *et al.* did not find any improvement in LV function. Accordingly, the aforementioned meta-analysis did not find any significant benefit of IPost with regard to this parameter [41]. Therefore, how IPost affects LV function still remains to be settled.

The translation of surrogate measures following IPost to clinical outcome is also unclear. Of the existing studies, only Lønborg *et al.* reported the effect of IPost on clinical outcome [32]. In 118 patients, Lønborg *et al.* found a 38% reduction in the number of patients with New York Heart Association (NYHA) class II–IV after 3 months and no significant differences in death, reinfarction, stroke and angina pectoris rates [32]. However, this result must be taken with precaution, since the study is relatively

Table 1. Summary of clinical studies with ischemic postconditioning during primary percutaneous coronary intervention.

Study (year)	Patients (n)	Protocol	End points	Results	Ref.
Laskey (2005)	17	2 × 3–5-min periods of deflation separated by 2 × 90 s periods of inflation	ST-segment resolution; coronary flow reserve	Improved ST-segment resolution; increased coronary flow reserve	[34]
Staat <i>et al.</i> (2005)	30	4 × 60-s periods of deflations or inflations	72-h AUC CK-MB; blush grade	36% reduction in infarct size measured by AUC CK-MB; improved blush grade	[33]
Ma <i>et al.</i> (2006)	94	3 × 30-s periods of deflations or inflations	Peak CK-MB; wall motion score index by echocardiography at 8 weeks	Reduction in peak CK-MB; improved wall motion index score	[36]
Darling <i>et al.</i> <sup>†</sup> (2007)	115	1–3 inflations during PCI versus >four inflations	Peak CK; length of hospital stay	Reduced peak CK; increased length of hospital stay	[35]
Yang <i>et al.</i> (2007)	41	3 × 30-s periods of deflations or inflations	CK during 72 h; infarct size by SPECT at 1 week; LVEF by echocardiography	Reduction in CK; 27% reduction in infarct size by SPECT; improved LVEF (no significant difference)	[38]
Thibault <i>et al.</i> (2008)	38	4 × 60-s periods of deflations or inflations	Perfusion defect index by SPECT at 6 months; LVEF by echocardiography at 1 year	39% reduction in perfusion defect index by SPECT at 6 months; improved LVEF by echocardiography at 1 year	[39]
Laskey <i>et al.</i> (2008)	24	2 × 3–5-min periods of deflation separated by 2 × 90 s periods of inflation	ST-segment resolution; coronary flow reserve; LVEF by echocardiography	Improved ST-segment resolution; increased coronary flow reserve; no improvement in LVEF	[40]
Wang <i>et al.</i> <sup>†</sup> (2009)	85	1–2 or 1–3 inflations during PCI versus >three or >four inflations	LVEF by echocardiography; peak CK; ST-segment resolution	Improved LVEF (1–2 vs >three inflations); reduction in CK (1–3 vs >four inflations); no improvement in ST-segment resolution	[37]
Lønborg <i>et al.</i> (2010)	86/118 <sup>‡</sup>	4 × 30-s periods of deflations or inflations	Infarct size by CMR; infarct size/AAR by CMR; LVEF by CMR; clinical outcome after 3 months	18% decrease in infarct size; 19% reduction in infarct size/AAR; no improvement in LVEF; 38% reduction in number of patients with NYHA class II–IV after 3 months	[32]

<sup>†</sup>Retrospective trial.  
<sup>‡</sup>A total of 43 and 59 patients were evaluated by CMR and clinical outcome, respectively.  
AAR: Area at risk; AUC: Area under the curve; CK: Creatine kinase; CMR: Cardiac magnetic resonance; LVEF: Left ventricle ejection fraction; NYHA: New York Heart Association; PCI: Percutaneous coronary intervention.

small, NYHA classification is an inaccurate and subjective measurement and the authors do not report any improvement in LV function. Therefore, larger randomized studies are warranted to determine the effect of IPost on clinical outcome.

It is important to emphasize that existing studies on IPost have been designed as 'proof-of-concept' to determine whether IPost is cardioprotective in humans undergoing PPCI. In this context, most of the studies have been designed to mimic the experimental design and to include patients with large infarcts, because it is assumed that any potential effect will be more pronounced in these patients. Therefore, the patients within the existing studies represent a selected cohort in terms of duration of symptoms, absence of collaterals, number of diseased vessels, thrombolysis in myocardial infarction grade (TIMI) flow grade and so on. The patients have been selected by different inclusion and exclusion criteria, as shown

in TABLE 2. Therefore, it is uncertain in which patients IPost is potentially effective and should be applied (see later).

Existing studies have used a variety of IPost protocols (TABLE 1). Therefore, it is unknown which protocol is the most efficient. This issue will also be addressed later on in this article.

### In which patients should ischemic postconditioning be applied?

In order to identify the patients who will gain from IPost, it is important to remember that the reperfusion injury occurs in the very first minutes of reperfusion and thus cardioprotection must be applied within this narrow time frame. Therefore, it is highly unlikely that patients with acute myocardial infarction and spontaneous reperfusion before the intervention will gain from IPost. From a clinical perspective, this means that presumably only the patients with acute myocardial infarction and TIMI flow of less than 2 before intervention should be treated with IPost.

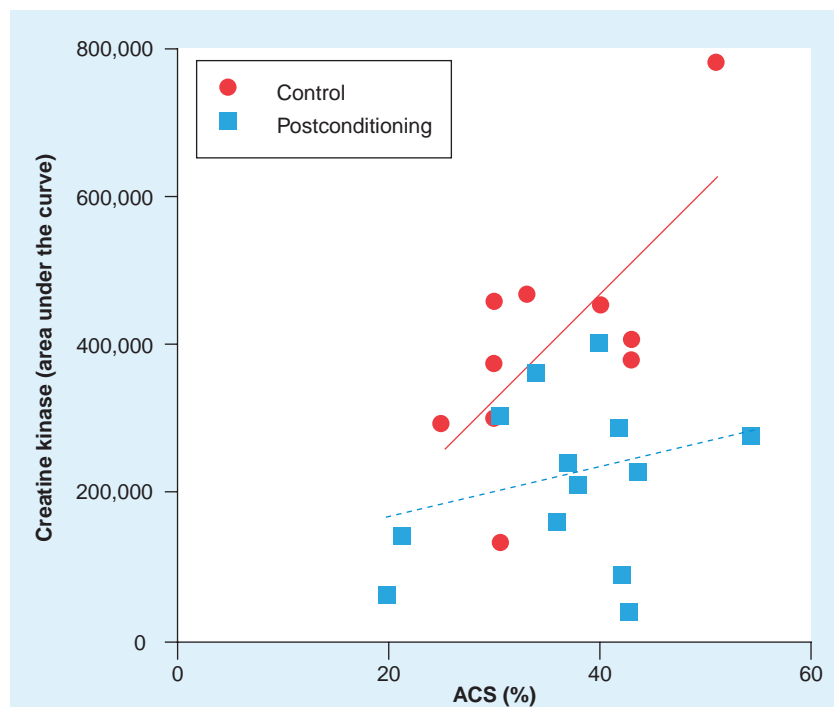
Data from experimental studies suggest that the cardioprotective effect of IPost is attenuated in the aged heart [43]. There are no existing data on this issue in humans. Future studies are warranted to investigate this. However, from a clinical perspective, it does not seem justified to use IPost according to the age of the patients, because in clinical settings it is impossible to identify the patients with an aged heart.

In a very elegant paper, Downey and Cohen concluded that only 25% of patients with acute myocardial infarction will have large enough infarction to benefit from any additional treatment to PPCI in terms of improved clinical outcome [44]. Therefore, even though IPost reduces absolute infarct sizes in patients with all risk areas [32], it is doubtful that patients with small areas at risk will benefit clinically from this treatment. The question remaining is how do we choose the 25% of patients who will benefit from the treatment? One approach could be to make a risk stratification based on the patient's medical history (duration of ischemia, previous myocardial infarction and preinfarct angina) and the angiographic result (collateral flow and location of lesion) in order to choose the patients with large-risk zones. Excluding patients with preinfarct angina seems to be justified, because these patients might have been inadvertently preconditioned and most animal studies have demonstrated no additional effect of IPC and IPost [14,45,46]. Furthermore, preinfarct angina has been shown to cause cardioprotection in humans [47], but may also represent micro-embolization, which possibly intermingles with the cardioprotective effects of IPost [48]. However, it is argued that patients who have symptoms for more than 6 h should be excluded, because the final infarct size seems to decrease only very little with PPCI between 6 and 12 h of ischemia and any additional treatment in this time window would have little potential benefit [49]. However, this approach is questionable for several reasons. With regard to collaterals, a recent study comparing patients with and without collaterals did not find any significant difference between the groups in terms of infarct size measured by SPECT 5–14 days after intervention [50]. Furthermore, based on the duration and character of the symptoms described by the patients, it can be very difficult to determine the precise duration of ischemia and to properly identify the patients with preinfarct angina, and it is almost impossible to know whether these patients have been adequately preconditioned. Therefore, this approach could be both inaccurate and

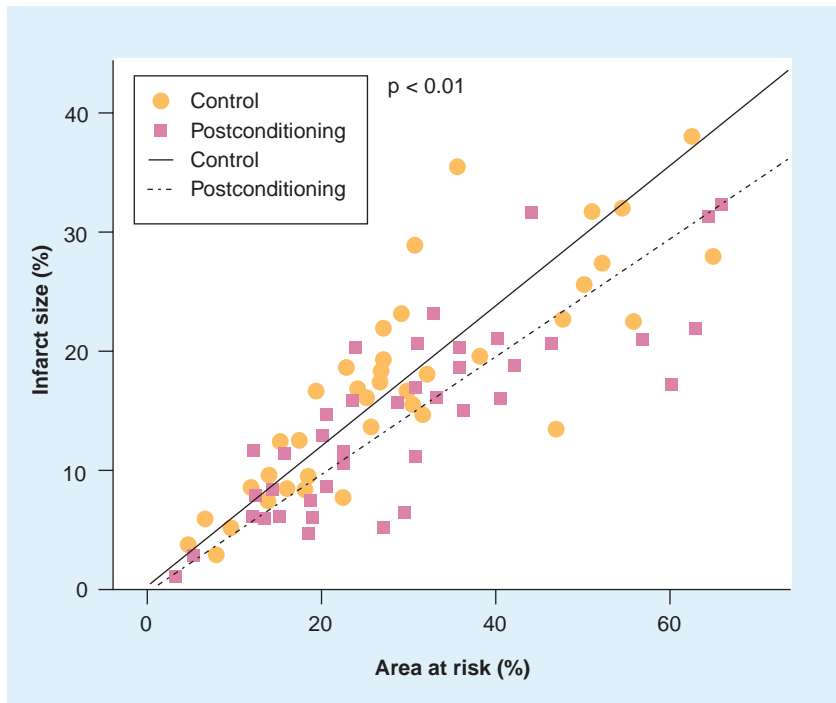
time-consuming. A better approach may be to apply IPost in all patients with STEMI admitted for PPCI and a TIMI flow of less than 2 in the infarct-related major coronary artery, and accept that only an estimated 25% of patients will benefit from the treatment. This approach seems to be reasonable because IPost is safe and inexpensive. In accordance, the recently published study by our group was designed to mimic the clinical 'everyday', using wider inclusion criteria than previous studies; for example, patients with collaterals and preinfarct angina were not excluded (FIGURE 2) [32].

### Which is the optimal ischemic postconditioning protocol?

An IPost protocol consists of a number of balloon deflations or inflations, which can be combined in numerous ways by varying the duration, number of repetitions and the total duration of the IPost procedure. In terms of creating the most effective cardioprotection IPost protocol, the interaction between these three variables is unknown and there is no consensus with regard to which IPost protocol is the most optimal in humans. This is clearly illustrated by the fact that in the seven randomized studies, a total of



**Figure 1. Linear regression of relationship between myocardial area at risk by ventriculography and infarct size by area under the curve of creatine kinase in patients treated with and without ischemic postconditioning in addition to primary percutaneous coronary intervention.** The difference between treatment groups is more obvious in the patients with a large area at risk. Reproduced with permission from [33].



**Figure 2. Linear regression of relationship between the myocardial area at risk and the infarct size measured by magnetic resonance in patients treated with and without ischemic postconditioning in addition to primary percutaneous coronary intervention.** The difference between treatment groups is more obvious in the patients with a large area at risk. Reproduced with permission from [32].

four different IPost protocols have been used. In addition, differences in inclusion and exclusion criteria and in means of evaluating the effect of IPost make comparisons between trials difficult. In order to design the optimal IPost protocol, it is tempting to look to animal studies. However, the effect of the IPost protocol seems to vary between species, illustrated by a protocol of three cycles of 30-s occlusions followed by 30-s reperfusion has been shown to be cardioprotective in dogs [14] and humans [32,33], but not in pigs [51,52]. Recently, Zhao and colleagues have randomized patients with STEMI to conventional PPCI or PPCI with either of two different IPost protocols consisting of three cycles of 30 balloon deflation or inflations and three cycles of 60 balloon deflations or inflations, respectively [53]. Measuring Fas and Fas-L (as surrogate markers for apoptosis), troponin I and LVEF, this study reported a marked reduction of apoptosis, a reduction in troponin I level after 7 days and an improvement in LVEF in the patients treated with the 60 protocol. The 30 protocol was beneficial compared with the conventional treatment in terms of troponin I level after 7 days and LVEF, but not in terms of apoptosis level. These results suggest that three repetitions of 60 deflations or inflations is superior to three repetitions of 30 deflations or

inflations. However, this study does not clarify whether the difference in effectiveness is due to the difference in duration of the deflations or inflations (60 vs 30) or is due to the difference in the total duration of the IPost procedure (6 vs 3 min). In an experimental study by Iliodromitis and colleagues, they found a further cardioprotection by increasing the total IPost period from 5 to 8 min, while the deflations or inflations remained unchanged at 30 [51]. If the findings in both of the aforementioned studies are true, then it seems that the key to cardioprotection by IPost might be the total duration of the procedure, whereas the exact numbers of repetitions and duration of deflations or inflations might be of less importance. However, it is important to emphasize that this has not been proven yet.

Theoretically, as previously discussed in this article, the cardioprotective effect of IPost depends on the inhibition of opening of the mPTP [54], which play an important role in mediation of the reperfusion injury [55]. The mechanism through which IPost inhibits mPTP seems to depend on a slow washout of  $H^+$  and subsequent slow normalization of the pH [29]. Furthermore, restoration of blood flow to the ischemic tissue leads to the generation of free radicals, which are important secondary messengers in cardioprotection in IPost [49]. Therefore, the key issues in designing the optimal protocol seem to be that the protocol should secure a slow normalization of the pH, but also allow enough time to generate sufficient free radicals. Accordingly, it has been reported that gradual, instead of abrupt, restoration of the coronary blood flow reduces the postinfarct myocardial injury [11–13,56]. This implies that the cardioprotective effects of IPost depend on the total duration of the procedure. Unfortunately, the mechanism of IPost seems to be much more complicated than that, because the cardioprotective effects also depend on other effects, such as an antioxidant effect [57,58] and activation of several intracellular molecular cascades [46,59,60]. Recently, it has been suggested that the cardioprotective effects of gradual restoration of the blood flow is independent of activation of the reperfusion injury signaling kinase pathway [56], which seems to be a mediator of the cardioprotective effects of IPost. However, it is still unclear whether gradual restoration of blood flow and IPost are equivalent procedures and share mechanistic characteristics. The relationship between the effects of slow restoration of blood flow and inflation or deflation duration and repetitions in IPost remains to be determined.

Taken together, in order to design the optimal IPost procedure, it is not possible to compare the existing studies, nor it is possible to directly transfer optimally designed protocols from animals. The cardioprotective effect of IPost seems to depend on the total duration of the IPost procedure, whereas the role of the duration of deflations or inflations and of the number of occlusions remains to be determined. In order to design the optimal protocol, future randomized studies are warranted and a better understanding of this mechanochemical coupling is needed.

### Pharmacological postconditioning & remote postconditioning

Even though IPost is a promising adjuvant therapy to PPCI, it has some limitations. First, it requires invasive treatment and is therefore only useful to those patients with acute myocardial infarction undergoing PPCI and not to those treated with thrombolysis. Second, spontaneous reperfusion or reperfusion mediated through prehospital anti-thrombotics before PPCI may induce reperfusion injury. Third, in some patients, IPost is not applicable for technical reasons. Pharmacological postconditioning or remote postconditioning might overcome these reservations, since they can be instituted immediately at first contact with the medical system and they are independent of technical limitations.

Accordingly, Bøtker *et al.* demonstrated that in 251 patients treated with PPCI, five cycles of ischemia of the arm, each period lasting 5 min, applied before arrival at the catheter laboratory (i.e., in the ambulance) led to better myocardial salvage compared with a standard reperfusion strategy [61]. The authors evaluated the postinfarct injury by measuring the salvage index

using SPECT, but did not find any effect on 30-day clinical outcomes. Results from animal work have suggested an additive effect of IPost and remote ischemic postconditioning, when compared with either of the procedures [62]. Combining the two treatments might have clinical implication in patients treated with PPCI, but this needs to be tested in randomized clinical settings.

During the last few decades, there has been intensive research for a drug that could be used to inhibit ischemia–reperfusion injury and protect the heart during reperfusion. Many drugs have been tried in clinical trials. Despite reported significant differences between the treatment groups in some of the performed trials, the results have been quite modest. An initial report on adenosine was promising, since pretreatment with adenosine leads to a significant decrease in infarct size [63]. However, later results were ambiguous since a larger study failed to demonstrate an effect on clinical outcome [64] and a synthetic adenosine agonist did not lead to cardioprotection [65]. Subsequently, the larger study has been reanalyzed and reports reduction in mortality in the patients with early reperfusion [66]. The most promising drug seems to be cyclosporine, which Piot *et al.* reported to reduce infarct size by 40% by means of area under the curve of CK and 20% by cardiac magnetic resonance (5 days and 6 months after initial treatment) [67,68]. Another promising drug is glucagon-like peptide-1 (GLP-1), which has been demonstrated to improve the LVEF by approximately 35% in patients with severely reduced LV function after acute myocardial infarction [69]. GLP-1 was administered continuously for 72 h and the

**Table 2. Summary of inclusion criteria in the clinical studies with ischemic postconditioning during primary percutaneous coronary intervention.**

Study (year)	Age (years)	Ischemia (h)	Preinfarct angina	Collaterals	Previous MI <sup>†</sup>	Vessel types	Comorbidity <sup>‡</sup>	Ref.
Laskey (2005)	58	<12	Yes	No	Yes	LAD, RCA and Cx	Yes	[34]
Staat <i>et al.</i> (2005)	56/58	<6	No	No	No	LAD and RCA	Yes	[33]
Ma <i>et al.</i> (2006)	64	<12	Yes	Yes	No	LAD, RCA or Cx	Yes	[36]
Darling <i>et al.</i> (2007) <sup>*</sup>	62/55	<12	No	Yes	No	LAD and RCA	Yes	[35]
Yang <i>et al.</i> (2007)	63/59	<12	Yes	No	No	LAD, RCA or Cx	Yes	[38]
Thibault <i>et al.</i> (2008)	56	<6	No	No	No	LAD and RCA	Yes	[39]
Laskey <i>et al.</i> (2008)	58/60	<6	Yes	No	No	LAD	Yes	[40]
Wang <i>et al.</i> (2009) <sup>§</sup>	59/57	<12	No	No	No	LAD, RCA and Cx	Yes	[37]
Lønborg <i>et al.</i> (2010)	62/61	<12	Yes	Yes	Yes	LAD, RCA and Cx	Yes	[32]

<sup>†</sup>Previous MI in the infarct related territory.

<sup>‡</sup>For example, diabetes and hypertension.

<sup>§</sup>Retrospective trial.

Cx: Circumflex artery; LAD: Left anterior descending artery; MI: Myocardial infarction; RCA: Right coronary artery.

treatment commenced 2–4 h after intervention. Interestingly, the GLP-1 analog, exenatide, has been demonstrated in experimental studies to reduce the infarct size when administered prior to and during the intervention [70,71]. Therefore, administration of GLP-1 and GLP-1 analogs in patients with acute myocardial infarction may have the potential to reduce the infarct size. However, the effects of cyclosporine and GLP-1 need to be investigated in larger clinical studies and the potential cardioprotective effect of GLP-1 needs to be demonstrated in humans. Furthermore, it is important to show that potential advantages of pharmacological postconditioning will translate into superior cardioprotection compared with IPost.

### Conclusion

At present, seven randomized trials with IPost have been performed in a total number of 399 patients undergoing PPCI. IPost appears to be a safe and effective adjuvant treatment to PPCI in terms of decreasing the infarct size. This treatment could potentially be applied in all patients with STEMI and TIMI flow less

than 2. However, before IPost is included in the treatment guidelines for patients with STEMI, important issues should be determined: first, the IPost protocol that is the most optimal in humans; second, the effect of IPost on clinical outcome; and last, the effect on LV function.

### Future perspective

Despite their relatively small sample sizes, current studies on IPost have shown this simple, inexpensive and safe procedure to be a potentially effective adjuvant therapy to PPCI. However, before IPost can be included in the future treatment guidelines for patients with STEMI, the effect on clinical outcome needs to be settled in larger randomized studies. Furthermore, future experimental research into the pathogenesis of reperfusion injury and the IPost mechanism is warranted to give us an improved knowledge of the cellular mechanism involved, which in turn will hopefully allow us to design the optimal IPost protocol, target new protective pathways and avoid an inadvertent pharmacological interference with some of the pathways, whose integrity might prove to be important.

#### Executive summary

##### **Cardiomyocyte death: a central element of reperfusion injury**

- Reperfusion injury comprises of a myocardial contractility impairment (stunning), arrhythmias, no-reflow and cardiomyocyte death.
- Opening of mitochondrial permeability transition pores is a pivotal event in reperfusion injury.

##### **Ischemic postconditioning: background**

- Ischemic preconditioning is the predecessor of ischemic postconditioning (IPost).
- IPost was introduced in 2003.

##### **Cardioprotective mechanisms mediating the effect of ischemic postconditioning**

- The effect of IPost seems to depend on prolongation of normalization of the pH value and activation of a number signal kinase cascades.
- The connections between manipulating reperfusion flow and activation of the survival signaling are not known.

##### **Ischemic postconditioning during primary percutaneous coronary intervention**

- IPost has been investigated in seven randomized trials and in a total of 399 patients.
- The effect has been evaluated by means of ST-segment resolution, release of biomarkers, SPECT, echocardiography and MRI.
- There are consistent results in terms of reduction of infarct size and surrogate measure of infarct size (creatinine kinase release, ECG resolution and left ventricular function), with relative reductions in infarct size ranging from 18 to 39%.
- There are issues that remain to be determined: first, the effect on clinical outcome; second, the effect on left ventricular function; third, the most optimal IPost protocol; and last, the patients in which IPost is effective.

##### **In which patients should ischemic postconditioning be applied?**

- It is assumed that IPost is only effective in patients with thrombolysis in myocardial infarction grade flow less than 2 before intervention.
- IPost is probably beneficial in 25% of the SPECT.
- IPost should be applied in all patients with SPECT and thrombolysis in myocardial infarction grade flow less than 2.

##### **Which is the optimal ischemic postconditioning protocol?**

- There is no consensus as to which IPost protocol is most effective.
- The effect of the IPost protocol depends on the total duration of the protocol.
- The role of the balloon inflation or deflation duration and repetition remains to be settled.
- The effect of an IPost protocol varies between species.

##### **Pharmacological postconditioning**

- In recent decades, there has been intensive research for a cardioprotective drug.
- Results from the clinical trials are quite modest.
- Glucagon-like peptide-1 and cyclosporine seem to be the most promising drugs.



An interesting perspective is to investigate the potentially additive cardioprotective effect of IPost and remote postconditioning. We hope and believe that future studies will identify cardioprotective pharmacological compounds. In this regard, it is important that future trials use adequate end points. It is not only important to evaluate the myocardial infarct size, but also to assess the myocardium area at risk. SPECT has for many years been considered the gold standard to measure the myocardium area at risk, but this method is limited because the tracer has to be injected before intervention. A promising method is T2-weighted MRI sequences, which are able to evaluate the myocardial area at risk retrospectively for up to 20 days after the initial treatment [42,72]. However, based on the limitations experienced by improper area-at-risk measurements and missing checks for collaterals in previous investigations, future trials should address these matters accordingly. In addition to evaluation of the infarct size and

area at risk, an interesting progressive method is molecular targeting using T2-weighted MRI sequences, which in animal studies have been demonstrated to be able to identify reperfusion injury, inflammation and apoptosis [73–75]. In the future, this technique will need to be developed in order to identify reperfusion injury in humans as well, which potentially allows a direct evaluation of an agent or mechanism for inhibiting reperfusion injury.

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