Review

Ischemic preconditioning: a plea for rationally targeted clinical trials

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The concept of myocardial ischemic ‘preconditioning’ (PC) has been with us since 1986 [1]. A tremendous body of research over the last 15 years has documented this phenomenon in virtually every species tested, with both an early and late phase of PC-induced cardioprotection, manifest ~5–30 min and 12–24 h after the PC trigger, having been identified. A host of additional studies have yielded insight into the cellular mediators and complex second messenger pathways that purportedly contribute to the protection achieved with brief antecedent PC ischemia, with the currently favored paradigm involving initial stimulation of one or more G protein-coupled receptors; subsequent activation of multiple ‘in parallel’ or ‘in series’ kinases (i.e. including, among others, the \(\varepsilon\)-isoform of protein kinase C and one or more isoforms of p38 mitogen activated protein kinase); followed by phosphorylation and activation of a membrane-bound end-effector, possibly the sarcolemmal and/or mitochondrial \(K_{\text{ATP}}\) channel. These mechanistic studies have, as an ancillary benefit, provided a rational framework for the identification of pharmacologic agents that, when given in lieu of brief ischemia, may effectively mimic the benefits of conventional ischemic PC.

Perhaps most importantly, a substantial amount of evidence favors the concept that ischemic preconditioning (both early and delayed) can occur in human hearts. However, the translation of ischemic preconditioning from the experimental laboratory to clinical utility has been disappointing and slow. Our goals in this manuscript are to: (i) review the clinical models and scenarios in which conventional ischemic PC and/or pharmacologic PC-mimetic agents have shown promise in eliciting cardio-protection; (ii) summarize, for each of these multiple scenarios, the potential benefits that might be achieved in the implementation of PC as a therapeutic strategy; (iii) outline, for each of the relevant clinical settings, a proposed design for the multicenter trials that will be needed to prospectively test the clinical utility of PC-based interventions; and (iv) discuss the caveats and problems that may be encountered in the clinical application of ischemic preconditioning.

1. The ‘screening’ models

1.1. Isolated human myocardial samples

Among the numerous studies that suggest ischemic preconditioning can occur in the human heart, perhaps the most compelling and direct evidence has been obtained using isolated human atrial and ventricular tissue. For example, human cardiomyocytes exposed to a sustained episode of simulated ischemia showed enhanced survival when pretreated with an antecedent brief ischemic stimulus [2], isolated cardiac trabeculae exhibited enhanced recovery of function following reoxygenation when pretreated with brief bouts of pacing and hypoxia [3], and right atrial samples suffered less irreversible ischemia-induced injury (assessed by creatine kinase (CK) release and tetrazolium staining) when preconditioned with preceding bouts of brief ischemia [4].

Preconditioning of isolated human myocardial tissue clearly offers no direct benefit with regard to patient care. Nonetheless, this model has provided valuable insight into the contribution—and possible clinical relevance—of adenosine, protein kinase C (PKC), the ATP-sensitive

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potassium ($K_{ATP}$) channel and other purported mediators in the protection achieved with brief antecedent ischemia, as well as the potential efficacy of PC mimetic candidates [2,3,5,6]. Data obtained in isolated cardiomyocytes/cardiac tissue can thus be exploited to provide initial in vitro screening for agents earmarked for evaluation in clinical trials. However, as reduction of infarct size is the undisputed hallmark of PC-induced cardioprotection [7], it must be emphasized that the most meaningful application of this model in the screening of PC mimetics must include a direct or surrogate assessment of myocyte necrosis (Table 1).

1.2. Repeated balloon inflations during angioplasty

A second line of evidence that supports the concept that the human heart can be preconditioned comes from coronary angioplasty studies in which multiple, sequential balloon inflations (each occluding the coronary artery) are used to elicit repeated bouts of brief ischemia.

Specifically, most (but not all [8]) studies have reported an adaptation or increased tolerance to ischemia—i.e. significant attenuations in ST segment elevation, lactate production, regional wall motion abnormalities and/or ventricular ectopic activity—with sequential inflations [9–12]. Moreover, there is evidence that, in patients undergoing angioplasty, a single 90-s period of balloon occlusion prior to the angioplasty procedure preconditioned the heart and significantly reduced the likelihood of post-procedural CK release [13].

It must be acknowledged that the increased tolerance to ischemia seen with repeated balloon inflations may, in some patients, be due in part to increased collateral perfusion [14]. Efforts to control for this confounding variable do, however, reveal that collateral recruitment is not the sole explanation for the attenuated response to ischemia seen in this model [14,15]. Indeed, results obtained using agents that either stimulate or interfere with the adenosine–$K_{ATP}$ channel pathway support the concept that these salutary effects are, at least in part, a function of preconditioning. For example, some [16]—albeit not all [14]—studies have found that pretreatment with intracoronary adenosine reduced the magnitude of ST segment shifts during subsequent balloon inflations when...
compared with controls that received saline. Conversely, in patients given the adenosine receptor antagonists aminophylline [17] or bamiphylline [18] before angioplasty, there was no significant change in ST segment shift on consecutive balloon inflations. Other studies in patients undergoing coronary angioplasty have identified myocardial opioid and bradykinin receptors as functionally important in preconditioning, i.e. a placebo-controlled study of naloxone, an opioid receptor antagonist, given 15 min before angioplasty resulted in abolition of the preconditioning response as assessed by ST segment shift and chest pain severity [19]. In addition, involvement of the K<sub>ATP</sub> channel was shown in a report by Tomai et al. [20], where glibenclamide, a selective K<sub>ATP</sub> channel blocker administered prior to angioplasty abrogated the beneficial effect of sequential balloon inflations. Finally, Leesar and colleagues [21] recently demonstrated that the NO donor nitroglycerin could mimic the delayed preconditioning phenomenon in the setting of angioplasty. Specifically, patients receiving nitroglycerin showed less initial ST segment elevation and lack of a change in ST elevation on subsequent compared to a first balloon inflation, while no benefit was seen in placebo-treated controls. Taken together, these studies support the presence of both an early and late phase of PC- and PC mimetic-induced protection in patients undergoing angioplasty.

Would implementation of PC-based therapeutic strategies offer significant clinical benefit to the angioplasty patient? Reduction of chest pain, ventricular ectopic activity, etc. would clearly be of merit. However, from a practical standpoint, the current widespread use and documented efficacy of other protective modalities such as stents and glycoprotein IIb/IIIa inhibitors may, even in difficult angioplasty procedures, leave little scope for an additive improvement in clinical outcome with the adjuvant application of PC mimetics or conventional ischemic PC. Rather, as with the in vitro models, angioplasty is perhaps best suited to the initial screening of PC mimetic candidates aimed at either the early or delayed phase of cardioprotection, particularly if enzymatic indices of irreversible myocyte injury are incorporated into the study design (Table 1).

2. The ‘prophylaxis’ models

2.1. Repeated exercise and the ‘warm-up’ phenomenon

It is well-documented that patients who have two sequential and identical exercise tests with a ~10–30 min period of rest in between tend to perform better—i.e. have less chest pain, less ST segment elevation, and longer exercise duration—on the second test vs. the first [22–24]. Analogous findings have been observed in the catheterization laboratory when two episodes of pacing-induced ischemia were separated by a brief rest (no pacing) period: manifestations of ischemia were attenuated during the second phase of pacing compared to the first [25]. As with repeated balloon inflations during angioplasty, vasodilation and recruitment of collaterals may, in part, contribute to this enhanced tolerance to repeated demand ischemia. However, this observation, termed the ‘warm-up’ phenomenon, has been suggested to be a clinical analogue of ischemic preconditioning [23,24].

With regard to clinical utility, there is no doubt that a warm-up phase prior to heavy exertion is prudent in the coronary artery disease patient; this may precondition the heart and allow better exercise performance without ischemia. The scenario of exercise-induced demand ischemia may further represent a relatively simple and focused platform for clinical trials seeking to evaluate the efficacy of PC mimetic candidates (Table 1). While enthusiasm for this concept must be tempered by recent studies implicating possible mechanistic differences between conventional ischemic PC and the warm-up phenomenon—most notably with regard to adenosine and the K<sub>ATP</sub> channel [26–29]—continued investigation of adenosine receptor agonists, K<sub>ATP</sub> channel openers, opioids and other candidate mimetics in this model is nonetheless warranted. To address the early phase of protection, patients would be randomized to receive the test agent or placebo at 30 min prior to a standard exercise test. As in the conventional ‘warm-up’ protocols, typical endpoints would include: exercise duration; heart rate and blood pressure at peak exercise; the time required to achieve 1 mV of ST segment depression; and maximum ST segment depression. Although assessment of cardiac enzyme release (i.e. markers of necrosis) would probably not yield fruitful results in this model, a more comprehensive and invasive study design might further measure the development of thallium perfusion defects or regional wall motion abnormalities by echocardiography. A similar strategy could, in theory, be employed to evaluate the efficacy of agents targeted at evoking a delayed protective response by administering placebo or the PC mimetic drug 24 h before exercise. However, as the existence of a delayed exercise-induced ‘warm-up’ effect is in dispute [24], the relevance of this approach may be questionable.

2.2. Coronary artery bypass surgery

As PC-based strategies are, by definition, applied before an ischemic event, coronary artery bypass surgery is among the few clinical scenarios in which preischemic intervention is both logical and feasible (Table 1). However, studies to date with preconditioning in patients undergoing coronary bypass surgery have yielded apparently discordant results. Yellon et al. [30], using myocardial high energy phosphate content during sustained normothermic cross-clamp fibrillation as their primary endpoint, showed better preservation of ATP levels in patients preconditioned with two antecedent 3-min periods of aortic
cross clamping (the brief ischemic stimulus) vs. non-preconditioned controls. In a follow-up study by the same group [31], troponin release during cardiac surgery was reportedly attenuated with PC although, interestingly, no difference in ATP levels was seen. Beneficial effects of preconditioning have also been described using more conventional surgical methods and endpoints. For example, Illies and Swoyer [32] assigned patients to receive 1 min of aortic cross clamping during normothermic cardiopulmonary bypass (CPB) or a matched control period (CBP alone) prior to performing coronary grafts under cold cardioplegic arrest. While there were no differences in post-operative morbidity and mortality between groups, significant improvements in functional recovery (i.e. improved cardiac index; lower use of inotropic support) were seen in the preconditioned cohort.

In contrast to these favorable reports, others have found no benefit—and, indeed, worse outcomes—with PC. Specifically, Perrault and colleagues [33] observed that CK release was exacerbated and lactate production was enhanced in patients first preconditioned with 3 min of aortic cross-clamping before instituting warm retrograde cardioplegic arrest. Similarly, preconditioning with a 5 min period of global ischemia was associated with an increased depletion of ATP and a non-significant trend toward augmented CK and troponin release following the cross-clamp period, with no differences in hemodynamic recovery or post-operative complications between the control and PC groups [34].

Comparison of these studies and resolution of the seemingly disparate findings is confounded by differences in surgical technique (i.e. normothermic cross-clamp fibrillation, normothermic cardioplegic arrest, hypothermic cardioplegia) as well as the diversity of endpoints used (metabolic parameters, CK/troponin release, hemodynamic indices, need for inotropes, etc.). This latter issue may be especially important. While there is no question that preconditioning renders the heart resistant to infarction, it is equally well-recognized that preconditioning does not exert an independent beneficial effect on recovery of function of the viable but stunned myocardium [7]. As post-bypass pump dysfunction is primarily a consequence of stunning [35], studies relying exclusively on surrogate indices such as of functional recovery must be interpreted with caution.

Given these important caveats, what would constitute a rationally designed protocol to evaluate the efficacy of PC in patients undergoing coronary artery bypass surgery? This model is especially well-suited to the controlled administration of either brief ischemia per se (i.e. intermittent aortic cross-clamping) or a benign PC-mimetic agent prior to the onset of CPB. To test the early phase of protection, the PC stimulus would obviously be given immediately (~10–30 min) before the event. In this regard, recent studies have employed adenosine and niconardil (a K\textsubscript{ATP} channel opener) as the test agent [36–38]; however, adenosine agonists, delta opioids, bradykinin, and other compounds targeting the K\textsubscript{ATP} channel would also be logical choices. Similarly, to test the delayed phase of protection, a PC-mimetic candidate (such as, for example, nitroglycerin) could be administered 24 h prior to surgery. As discussed previously, definitive conclusions concerning the efficacy of these PC-based interventions will require an assessment of peri-operative Q wave myocardial infarction, as well as the post-operative release of CK-MB and troponin I. Ancillary clinical endpoints would include peri-operative mortality, the incidence of congestive heart failure, a detailed assessment of the requirement for post-operative inotropic support (duration, dose and total number of treatments), as well as a comprehensive analysis of post-operative arrhythmias (type and duration of arrhythmias and administration of antiarrhythmic drugs). Although assessment of functional recovery will not, in itself, yield conclusive results, a measure of post-operative function (i.e. ejection fraction from radionuclide ventriculograms or 2-D echocardiograms) should nonetheless be obtained.

Despite the relative ease with which the requisite pretreatment can be administered in patients undergoing bypass surgery, three fundamental issues will complicate the assessment of the efficacy of preconditioning in this model. First, the pre-operative administration of opioids may precondition the heart [39]. Similarly, insight from experimental animal models has revealed that anesthetic agents (i.e. halothane and others) and CPB per se are cardioprotective [40]. It is therefore possible that these two factors may attenuate or overwhelm any benefit of the test agent, particularly if all three stimuli elicit their protective effects via the same cellular pathways. Finally, modern surgical techniques—in particular, cardioplegia—are, in themselves, highly effective in limiting ischemic damage; as a result, with the exception of high risk coronary bypass surgery or instances in which the distribution of cardioplegia is heterogeneous, the incidence of peri-operative infarction is low [33,36]. Thus, it will be a challenge to achieve a statistically significant, additive benefit of an ancillary PC-based therapy in the setting of coronary artery bypass surgery.

2.3. Transplant

A third logical and feasible scenario in which PC-based interventions may provide benefit is the augmented preservation of hearts harvested for transplantation (Table 1). Although the efficacy of preconditioning in this setting is not well-explored, there is experimental evidence that PC and the use of PC-mimetics is beneficial. For example, Landymore et al. [41] recently showed that PC attenuated myocardial stunning and preserved high-energy phosphates after cardiac transplantation in sheep, while pharmacologic PC with phenylephrine improved functional recovery in transplanted rabbit hearts [42]. Favorable results have also
been reported in rat transplant models: ischemic PC was associated with improved functional recovery and reduced CK release, with further synergistic benefit achieved by combined administration of the Na\(^+\)--H\(^+\) exchange inhibitor cariporide together with brief antecedent ischemia [43]. Similarly, a brief pre-storage period of heat-enhanced ischemic PC translated into improved functional recovery in the transplanted rat heart [44].

Taken together these studies support a role for PC, or efforts to mimic its metabolic pathways, as a means of conferring clinical benefit. In view of the shortage of suitable donors relative to recipients there has been an interest to expand donor populations to include not only the brain-dead patient—in which, interestingly, antecedent PC ischemia may be rendered ineffective in eliciting protection [45]—but to those where cardiopulmonary ‘death’ has ensued (i.e. non-heart-beating donors). It is in this setting that PC-mimetics, as well as other modalities aimed at tissue preservation, may have their greatest impact. PC-mimetics would optimally be given to the heart-vital donor at ~30 min prior to harvesting the organ, or, alternatively, to the heart itself immediately after explantation. Meaningful clinical endpoints in the evaluation of PC in this setting would include transplant organ function (radionuclide ventriculogram, echocardiogram), surrogate enzymatic indices of myocyte necrosis, incidence of heart failure, patient survival, etc.

3. The ‘holy grail’: PC-based strategies for the treatment of acute myocardial infarction

If brief antecedent ischemia ‘preconditions’ the human heart, this benefit should, in concept, be manifest in the patient with angina prior to acute myocardial infarction (MI). In addition, the time course of this effect should reflect the established temporal characteristics of ischemic PC. Although disparate results have been obtained [46] considerable evidence suggests that preinfarct angina is, indeed, associated with smaller CK-determined infarct sizes [47–49], with protection only achieved in those patients experiencing angina within 24 h of the onset of infarction [50]. Reported ancillary benefits of preinfarct angina include significant reductions in mortality, attenuations in the incidence of heart failure, cardiogenic shock and serious tachyarrrhythmias, as well as improved recovery of LV contractile performance [47,49,51,52]. Interestingly, the favorable effects of preinfarct angina may extend beyond the cardiomyocyte, i.e. the smaller infarct sizes in the angina cohorts may also be due in part to an enhanced efficacy of thrombolysis, achieved via release of adenosine—a potent inhibitor of platelet aggregation—during the brief antecedent ischemic episodes [53,54]. However, as in instances of angioplasty and repeated exercise, it must be cautioned that the improved outcome described with preinfarct angina may, in some patients, be due in part to collateral recruitment.

Pharmacologic treatment of acute MI arguably represents the most attractive and lucrative conceptual target for PC-based therapies. Practical application of PC mimetics in this setting is, however, undermined by the fact that, in contrast to coronary artery bypass surgery and the other ‘prophylaxis’ models, acute MI is not a predictable or ‘planned’ ischemic event. As a result, the requisite preconditioning administration of the mimetic agent will be difficult to achieve (Table 1).

One compromise to this challenge might be to selectively focus on a clinical sub-population with a high probability of developing acute MI—i.e. patients with unstable angina—in an effort to determine whether treatment with PC-based therapies would avert infarction. At the onset of unstable angina, patients would be randomized to receive either placebo or the test agent. The primary endpoint would clearly be the development of Q wave or non-Q wave infarction, as determined by the measurement of CK and troponin I release, recognizing that these indices are relatively crude measures of infarct size. Surrogate indices of protection employed in previous angina-acute MI studies—i.e. incidence of congestive heart failure and shock, recovery of LV function, in-hospital and long-term mortality—would also be assessed. In addition, as some of the patients would undoubtedly go on to require angioplasty and/or stenting, coronary artery bypass grafting, etc. plans to prospectively compare the incidence and type of revascularization procedures in placebo versus PC mimetic-treated groups should also be incorporated into the study design.

It is important to recognize that, in addition to the challenges posed by the execution of such a study, interpretation of the results may also prove to be difficult. The assumption inherent in this approach is that the mimetic agent would be capable of augmenting any protective effect evoked by the angina per se, an issue which, as discussed previously, may be problematic if both protective stimuli share a common cellular mechanism. A second potentially confounding issue may be the anti-anginal drugs these patients would undoubtedly be receiving: it could be argued that pharmacologic resolution of the angina might abrogate its protective effect while, conversely, several anti-anginal drugs (i.e. nitroglycerin; nicorandil) are PC-mimetic candidates. Rational evaluation of PC mimetic strategies in the setting of acute MI may therefore still be a distant—albeit highly laudable—goal.

4. The underlying problem: preconditioning the aged and diseased heart

Among the host of experimental studies conducted to date on the topic of preconditioning, it is perhaps remarkable that the vast majority have employed healthy, juvenile
or adult animals. Only a handful of investigations have explored PC-induced cardioprotection in senesence or in the presence of underlying cardiovascular disease—the specific populations in which clinical application of PC would be most relevant.

Studies of PC in aged and diseased models have yielded discrepant results. There is evidence that, in the senescent rat heart, antecedent PC ischemia does not improve the recovery of post-ischemic ventricular function and, of greater concern, fails to elicit reductions in infarct size [55–58]. Of note, however, all of these previous studies were conducted in isolated buffer-perfused hearts. In marked contrast, in the in vivo rabbit model of acute MI, infarct size reduction with PC was manifest—with no loss in efficacy—in middle-aged and old cohorts exhibiting definitive hallmarks of cardiovascular aging [59]. A similar lack of consensus is seen in the clinical literature: of the four studies that have focused on preinfarct angina in elderly MI patients, two have concluded that antecedent ischemia continues to confer benefit (i.e. lower CK release; reduced incidence of death, heart failure or recurrent MI) [60,61], while the remaining two found no difference in outcome in the angina vs. no angina cohorts [62,63]. Of the limited number of experimental studies utilizing disease models, it is perhaps encouraging that reduction of infarct size with PC has been reported in the setting of hypertension-induced hypertrophy, atherosclerosis and streptozotocin-induced diabetes [64–66]. Disturbingly, however, recent data obtained from explanted right atrial appendages revealed that brief antecedent PC ischemia failed to limit irreversible injury (assessed by CK release) in diabetic and failing human myocardium [67], and prodromal angina failed to reduce CK-determined infarct size in patients with acute MI [68]. Future clinical application of PC-based therapeutic strategies clearly demands resolution of these discrepancies, with direct or surrogate assessment of infarct size being the endpoint of choice. In the interim, it may be prudent for any planned clinical trials incorporating large numbers of patients to consider including post-hoc subgroup analyses (i.e. age<vs.≥65 years; ejection fraction<vs.≥30%; presence vs. absence of diabetes, etc.) into the study protocol.

5. Summary

Ischemic preconditioning has emerged as among the most promising approaches to reduce ischemic cell death. While tremendous effort has gone into the determination of mechanisms of both the early and delayed phases of this phenomenon, progress toward the direct application of PC-based therapies in cardiovascular medicine has been lacking. Initial screening of potential PC-mimetic candidates is highly feasible in isolated myocyte models and in the setting of angioplasty, while prophylactic treatment with PC-based strategies could most readily be evaluated in clinical trials of heart transplant and coronary artery bypass surgery. In contrast, the requirement for pretreatment will make therapeutic application of PC-mimetics in the unpredictable setting of acute MI considerably more challenging. It is important that all clinical studies of PC and PC-based therapies endeavor to include, as a primary endpoint, a direct or surrogate assessment of myocyte necrosis, the undisputed hallmark of PC-induced cardioprotection. Moreover, any large-scale clinical application of PC-based strategies will clearly require definitive evidence for the continued efficacy of ischemic preconditioning in the aged and diseased heart.

References


