Clinical cardioprotection and the value of conditioning responses

Jason N. Peart and John P. Headrick
Heart Foundation Research Centre, Griffith University, Queensland, Australia
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Peart JN, Headrick JP. Clinical cardioprotection and the value of conditioning responses. Am J Physiol Heart Circ Physiol 296: H1705–H1720, 2009. First published April 10, 2009; doi:10.1152/ajpheart.00162.2009.—Adjunctive cardioprotective strategies for ameliorating the reversible and irreversible injuries with ischemia-reperfusion (I/R) are highly desirable. However, after decades of research, the promise of clinical cardioprotection from I/R injury remains poorly realized. This may arise from the challenges of trialing and effectively translating experimental findings from laboratory models to patients. One can additionally consider whether features of the more heavily focused upon candidates could limit or preclude therapeutic utility and thus whether we might shift attention to alternate strategies. The phenomena of preconditioning and postconditioning have proven fertile in identification of experimental means of cardioprotection and are the most intensely interrogated responses in the field. However, there is evidence these processes, which share common molecular signaling elements and end effectors, may be poor choices for clinical exploitation. This includes evidence of age dependence, limiting efficacy in target aged or senescent hearts; refractoriness to conditioning stimuli in diseased myocardium; interference from a variety of relevant pharmaceuticals; inadvertent induction of these responses by prior ischemia or commonly used drugs, precluding further benefit; and sex dependence of protective signaling. This review focuses on these features, raising questions about current research strategies, and the suitability of these widely studied phenomena as rational candidates for clinical translation.

acute myocardial infarction; aging; cardiac ischemia; clinical translation; diabetes; obesity; preconditioning; postconditioning; reperfusion

THE EXTENT OF CELL DEATH during and after ischemia is the primary determinant of the outcome from acute myocardial infarction (AMI). Ameliorating this type of damage is therefore an important endeavor, with the potential to affect one of the world's leading causes of death and disability (the World Health Organization estimates ischemic heart disease will remain the leading cause of premature death globally over the next 2 decades). Although global prevalence is largely due to environmental/lifestyle factors and may thus be limited through their modification, it is essential we evolve effective means of reducing the effect of extant ischemic disease. The concept that myocardial cell death during ischemia-reperfusion (I/R) could be limited pharmacologically arose almost 40 years ago. In 1971, Maroko et al. (145) forwarded the novel idea that extent/severity of damage may not be predetermined but could be modified by therapeutic manipulations during I/R, prefacing widespread research efforts to identify and develop methods of “cardioprotection.” Nonetheless, early reperfusion remained the best strategy to limit infarction (52).

The cardioprotection field was invigorated by the discovery of the Precon phenomenon in 1986 (155), revealing an unsurpassed ability to experimentally improve postischemic myocardial outcome (and revealing that even O2− or I/R-sensitive species are able to mount effective adaptive responses). Since this discovery, of perhaps the most potent protective stimulus currently known, more than 3,000 published cardiovascular investigations have examined underlying mechanisms and assessed a means of harnessing Precon experimentally and clinically. Despite the elaboration of underlying signaling and development of a variety of triggers of this response in animal models, the therapeutic promise of Precon has not been realized. Mixed results have been acquired in clinical trials, and the extent of protection from cell death in cardiac patients is much less than in experimental models. More recently, powerful protection from Postcon was identified (244) and is also being actively studied (~250 papers since that study) in the hope that this response, effective after ischemia itself, may be a more relevant candidate from a temporal perspective. Clinical Precon may have relatively limited application (in cardiac surgery, for example) given the need to pretreat hearts before ischemia, whereas Postcon possesses the more desirable feature of conferring benefit when implemented at or after reperfusion (although the window of opportunity for effective postischemic protection may be narrow).

Accumulating evidence indicates that Precon and Postcon act via archetypal mediators, sometimes referred to as reperfusion injury salvage kinase (RISK) elements, including phosphatidylinositol 3-kinase (PI3K), Akt, and PKC (48, 77). These may regulate downstream elements such as GSK3β and modify mitochondrial targets, including ATP-sensitive K+ (KATP) channels and the mitochondrial permeability transition pore (MPTP) to provide protection or to generate further signaling...
intermediates such as free radicals that participate in the cascade (48, 75, 77). Despite poor translation and mixed outcomes from trials, these conditioning responses remain the most intensely investigated candidates for cardioprotection, the hope being that mimetics (pharmacological or surgical in nature) or modulators of essential signaling/effectors will limit ischemic damage in patients. Given the mixed success of this endeavor over the past two decades, one might consider whether the focus on Precon (and more recently Postcon) remains the most rational approach or whether drawbacks inherent to these responses render them rather poor candidates for clinical exploitation. A number of recent commentaries and reviews (17, 79, 115) have addressed poor translation of experimental cardioprotection, with most attention drawn to issues in clinical trial and experimental design and rather less to the features of these responses that are highly relevant to the target population (59). It is these latter properties we review here and that argue for a shift in research strategy to specifically target aged and diseased hearts of both sexes to identify responses that might indeed be exploitable for cardioprotection in the target patient population.

Manipulating Ischemic Tolerance via Conditioning Responses

The extent of injury and thus the ultimate outcome from I/R depends on the duration and severity of the insult and on the intrinsic resistance of the tissue to O2 deficit, energy/ionic/redox imbalances, and related features of I/R. The human myocardium is sensitive to ischemia, yet as in other mammals we possess adaptive processes that can transiently improve resistance to ischemic or hypoxic insult. Sublethal stressors, including cold, heat, exercise, calorie restriction, and transient periods of ischemia or hypoxia, can enhance cellular defense, limit age-related deterioration in cell function, and prolong lifespan. Precon is an example of such adaptation, documented in fish, birds, and mammals including humans (33, 64, 225).

Archetypal signaling in Precon/Postcon. The pathways triggering and mediating protection with Precon have been extensively studied, and a generalized signaling scheme has evolved, although features of these pathways remain controversial (48, 75, 77). Precon or Postcon stimuli (e.g., intermittent ischemia) enhance release of G-protein coupled receptor (GPCR) agonists such as opioids, adenosine, bradykinin, or catecholamines, which in turn activate prosurvival kinase or RISK pathways (e.g., opioids, adenosine, bradykinin, or catecholamines, although features of these pathways remain controversial (48, 75, 77). Despite poor translation and mixed outcomes from trials, these conditioning responses remain the most intensely investigated candidates for cardioprotection, the hope being that mimetics (pharmacological or surgical in nature) or modulators of essential signaling/effectors will limit ischemic damage in patients. Given the mixed success of this endeavor over the past two decades, one might consider whether the focus on Precon (and more recently Postcon) remains the most rational approach or whether drawbacks inherent to these responses render them rather poor candidates for clinical exploitation. A number of recent commentaries and reviews (17, 79, 115) have addressed poor translation of experimental cardioprotection, with most attention drawn to issues in clinical trial and experimental design and rather less to the features of these responses that are highly relevant to the target population (59). It is these latter properties we review here and that argue for a shift in research strategy to specifically target aged and diseased hearts of both sexes to identify responses that might indeed be exploitable for cardioprotection in the target patient population.

Clinical Application of Precon and Postcon

Although a number of small clinical trials have been performed on forms of Precon and Postcon, such interventions have not been adopted in mainstream clinical practice. This may stem from a number of factors, including fundamental differences between the realities and limitations of the clinical environment vs. the controlled experimental conditions of the laboratory. As we will outline in further detail, these candidate interventions have also been studied in young healthy animal models, a scenario quite distinct from patients in need of cardioprotection (older subjects whose hearts and/or vessels are diseased). The limited number of trials of Precon/Postcon-based interventions to date has generated mixed findings regarding efficacy in patients.

Yellon et al. (238) in the first small clinical trial of Precon tested effects of two episodes of intermittent reperfusion in patients undergoing coronary artery bypass graft (CABG) surgery. They detected a ~75% improvement in cardiac biopsy ATP content, supporting at least a metabolic protection. In a subsequent larger trial Illes and Swoyer (94) tested the effects of adjunctive ischemic Precon before cardioplegic arrest. Although no differences were evident in morbidity or mortality between groups, or in release of creatine kinase (CK), they detected a 32% improvement in functional outcome at 12 h postbypass. However, Jenkins et al. (100) did observe significant reductions in release of troponin T (by 80%) at 3 days post-CABG in an ischemic Precon cohort. These initial trials thus evidenced some benefit from forms of ischemic Precon. Nonetheless, there is also evidence Precon does not provide benefit over cardioplegia (37, 38) or as an adjunct to intermittent aortic cross-clamping during revascularization (167). Another study (170) acquired evidence of exaggeration of injury with ischemic Precon. Subsequent work by Teoh and colleagues (214, 215) indicates a superior protection with ischemic Precon vs. intermittent cross-clamping, cold cardioplegia, or pharmacological Precon, with 50–70% reductions in troponin T release compared with these interventions. Others present evidence that ischemic Precon is superior in limiting markers of cardiac cell damage vs. intermittent cross-clamp fibrillation (100, 207). Interestingly, trials of Precon stimuli in patients undergoing CABG with cold cardioplegia generally fail to alter enzyme markers of cardiac damage (37, 64, 233, 235, 236), although some detect reduced troponin I (101) or CK release (139).
Postcon has also been tested in humans in a small trial in patients reperfused within 6 h of myocardial infarction with four cycles of intermittent occlusion-reperfusion reduced CK release by 36% compared with the non-Postcon group (199). A retrospective analysis (41) of patients undergoing primary angioplasty also revealed a reduction in CK release in patients subjected to more than three balloon inflations vs. one to three inflations. In AMI, the application of Postcon stimuli 6–12 h after presenting with chest pain can reduce surrogate markers of infarction by 27% (237), 36% (143), or as much as 40–50% (216). Other small trials detect improvements in functional outcomes without alterations in markers of cell death (125).

Remote Precon, in which other tissues are subjected to intermittent I/R to indirectly promote recovery in the heart, has also been trialed in small studies. This stimulus has advantages over direct ischemic Precon, as it avoids added ischemic insult in
the diseased heart and major vessels. Günyaydin et al. (72) used a tourniquet around the right upper extremity of patients, which was inflated/dilated to affect 3-5 min periods of ischemia. They found no improvement in CK release but an elevation in lactate dehydrogenase levels and suggested an effect of remote Precon on anaerobic glycolysis. Subsequent trials support benefit from remote Precon in differing patient cohorts, including reductions in troponin T in pediatric patients (31) and 40–60% reductions in this marker at differing times in adults undergoing CABG (78), abdominal aortic aneurysm repair (7), and coronary stenting procedures (87).

Some of the drawbacks of ischemia as a Precon or Postcon stimulus may be limited by the use of pharmacological conditioning mimetics. Indeed, one of the aims of ongoing research into the molecular bases of these responses is to identify candidates for pharmacological manipulation. While Teoh et al. (214) acquired evidence of a superior effect of ischemic vs. pharmacological Precon, other support for benefit via conditioning pharmacomimetics has been acquired. Adenosine may play a key role in triggering cardioprotection with Precon and Postcon (77, 231), although it provides other beneficial effects that may be independent of these responses (166). Trials of adenosine as an adjunctive protective therapy have been successful in limiting postinfarct mortality (116), together with up to a 60% reduction in infarct vs. no treatment (182). Since adenosine was applied during ischemia and reperfusion, this protection may reflect Postcon rather than Precon effects. In the context of cardioplegic arrest, the adenosine intervention (adenosine + lidocaine) for nondepolarization preservation developed by Geoff Dobson (46) has proven very effective experimentally and in clinical trials. The IONA trial supports benefit from chronic K$_{ATP}$ opener treatment (nicorandil) in patients with known coronary artery disease (95). On the other hand, Kitakaze et al. (113) in the J-WIND trial observed infarct limitation with atrial natriuretic peptide but not nicorandil in infarct patients undergoing reperfusion therapy. Again, as these agents were not present before ischemia, this action can be deemed more akin to a Postcon stimulus. It is interesting in this respect that the K$_{ATP}$ opener failed to afford benefit, despite evidence for a role for this channel in both Precon and Postcon (48, 75, 77). On the other hand, other studies (96) do report some benefit with nicorandil under similar conditions.

Overall, clinical trials do offer evidence of some benefit from Precon- or Postcon-based therapies in certain clinical scenarios. However, evidence of infarct limitation is mixed, and such effects are generally much less than in experimental laboratory models. Reasons for these mixed responses are unclear, although as we discuss in more detail in subsequent sections, some of the intrinsic features of conditioning responses may be critical in limiting their efficacy and translation in the clinical environment. An added factor worth mentioning here is that the magnitude of benefit with cardioprotective stimuli may need to be very substantial before it is evident in reperfused myocardium. Early reperfusion alone (within 30–90 min) can limit infarction by 40–50% (152, 239). However, the outcomes with reperfusion vary widely, with salvage of >50% of at-risk myocardium in one out of two of AMI patients, while up to one out of four may still suffer >75% infarction despite effective reperfusion (152). The latter injury certainly argues for provision of adjunctive therapy, although only very potent responses would limit infarction to a goal of 20% of the effected ventricle (a threshold above which mortality and morbidity appear to rise; Ref. 152). Whether Precon or Postcon stimuli could independently affect this large reduction is not clear, and this may contribute to mixed efficacy in trials. Nonetheless, any graded reductions in infarction from 75 toward 20% should still improve outcome (while reductions below 20% may not further affect mortality). Moreover, early reperfusion remains unrealized in many cases: analysis of >27,000 US patients (27) and 3,000 hospital systems (232) documents means delays from symptom onset (or hospital arrival) to clinical reperfusion of 4–5 h, well outside the 30- to 90-min optimum for salvage and mortality reduction. Thus there remains a very good case for application of adjunctive protective therapy before or with reperfusion.

Age and Disease Dependence of Cardioprotection

It has been noted previously that protective strategies that fail in translation have historically been associated with inconsistent data regarding efficacy in experimental models (17). As we highlight below, there are key inconsistencies in observed efficacy of Precon- and Postcon-based interventions that should raise concern regarding their potential utility in patient populations. Unfortunately, there is a bias to publication of positive rather than negative findings in the scientific literature, which in turn may inadvertently bias views on the efficacy of a particular response. Our aim here is to play the role of “devils advocate” in promoting a critical appraisal of ongoing research strategies, presenting an overview of negative data relevant to the clinical applicability of Precon- and Postcon-based interventions. We suggest that poor clinical translation of conventional protective strategies may well reflect drawbacks inherent to Precon and Postcon, including age dependence and refractoriness in diseased patients (addressed here), interactions with common pharmaceuticals and environmental factors, and sex dependence (the latter addressed in subsequent sections).

Age dependence. A key problem with Precon responses, potentially mirrored in Postcon, is progressive loss of efficacy with age. Although this remains somewhat contentious, as some investigators do report preservation of Precon or related protection in aged hearts (23, 123, 138), the weight of evidence indicates that age reduces or abrogates Precon responses in humans (2, 12, 128, 136, 151, 159) and in animal models (1, 16, 50, 56, 85, 140, 186, 210, 211). Aging also inhibits protection via remote Precon (89), anesthetic Precon (197), in response to GPCR agonists such as adenosine (81, 173, 186, 231) or opioids (164, 165), and novel forms of Precon (82). Two recent studies (15, 174) also report that aging abrogates protection with Postcon. The negative influence of age on cytoprotection is not restricted to the cardiac system: aging impairs or eliminates Precon responses in brain (80, 184), liver (186, 190, 242), and platelets (173). Failure of these adaptive responses could be a general and integral component of the aging process, ultimately limiting the ability of tissues to adapt effectively to stress or withstand injurious insult (defining features of cellular senescence).

Despite a weight of evidence supporting age-dependent impairment of Precon, Postcon, and related stimuli, a number of investigations did detect preserved protective responses in aged myocardium. Loubani et al. (138) reported on effective Precon of aged human tissue, and Burns et al. (23) provided...
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support for preserved Precon in aged sheep. In addition, the antiarrhythmic effects of Precon may be effective in aging rats (91). Additionally, Shinmura et al. (193) presented evidence of a preserved delayed Precon response in aged rats. Other work supports conservation of the Postcon effect: Lauzier et al. (126) reported preserved Postcon in the Senescence Accelerated Mouse Prone 8 model, while Dow et al. (47) presented evidence that the antiarrhythmic effects of Postcon are conserved in aged rats. Other experimental interventions reported to proffer protection in aged hearts include Na+/H+ exchange inhibition (14, 158), prolonged opioid treatment (164), PKCε-activating peptide (119), PKCε inhibition (120), adenosine treatment (123), and CRYAB and HSPB2 deficiency (13).

The reasons for the preservation of protective responses in some studies but not others is not readily apparent, although some research suggests that perhaps the threshold for effecting cardioprotection with Precon or Postcon stimuli is elevated, necessitating a greater stimulus to overcome downstream defects (15, 128, 186). This would be consistent with graded responses to Precon stimuli (107, 187) and could explain variable data regarding age and Precon: studies of more profound Precon stimuli may detect efficacy in older hearts, while those using lower subthreshold stimuli may not. However, other investigations (86) identifying age-related abnormalities in cardioprotection have found that an enhanced stimulus is unable to overcome these changes. This issue requires further investigation, as it is critical to understand the basis of failed protection if these responses are to be harnessed for elderly patients.

An impaired sensitivity to initiating stimuli, noted above, is in part consistent with evidence that failed cardioprotection involves impaired activation of signaling, particularly at the level of p38-MAPK (165) (Table 1). Abnormal cell signaling is also in agreement with studies in diseased hearts (70, 76) and with observations that increasing the amplitude of the trigger stimulus may generate protection in older hearts, for example, with additional (186) or longer (15) conditioning cycles or with enhanced protective GPCR expression (81). These latter studies confirm that protective machinery is still present in older hearts but appears ineffectively activated and harnessed. Investigators have unmasked other relevant aspects of impaired signaling. Tani and colleagues (208, 212) provided evidence of failed activation and translocation of PKC in aged hearts. Przyklenk et al. (172) have shown that age selectively alters the nature of protective PKC signaling, with Precon responses PKCε-dependent in young but not aged myocardium. Hunter et al. (92) have acquired evidence of a role for altered kinase activation and GSK3β regulation in age-related ischemic intolerance. Other evidence of a signaling basis to failed protection includes shifts in phosphatase activity that may alter kinase phosphorylation (57) and impaired expression of connexin-43 (16), a gap-junction protein thought to be involved in protective signaling (188). There are parallels between the aging effects on signaling and the effects of disease (outlined in the following sections) (Table 1). For example, elevations in phosphatases may also be involved in impaired protection with obesity (18), while ineffective activation of kinases is also detected in diabetes (70, 219). It is important to continue these investigations into protective responses and signaling in aged myocardium, as this may not only reveal more effective protective strategies for the target patient population but may also provide insights into the molecular basis of age-related changes in both physiology and the pathogenesis of heart disease. Even at the level of randomized clinical trials in acute coronary syndromes, there is underrepresentation of elderly subjects and women (130).

Age dependence of Precon or Postcon responses is highly relevant to clinical utility, since the majority of patients to benefit from adjunctive cardioprotection are in the older age range: in the US, for example, coronary artery disease affects 50% of those >65, with up to 80% of deaths from the disease occurring in this age group. With continued aging of populations in coming decades, the proportion of people in this age range will grow considerably (to 1 in 4 in developed countries).

Influence of disease. A clinically useful cardioprotectant must be efficacious in diseased myocardium. Considerable evidence indicates that conventional responses derived from Precon and Postcon are negatively influenced by common conditions including diabetes, obesity, dyslipidemia, and possibly hypertrophy. Since diabetes and obesity are increasing in near epidemic proportions, are major risk factors for development of heart disease, and aggravate injury from ischemic insult, it is a decidedly negative feature for cardioprotective interventions to be sensitive to these conditions. The presence of such disease states, overlaid on an aged myocardial substrate, may render hearts insensitive to many conventional protective stimuli.

DIABETES. Ghosh et al. (65) demonstrated failed Precon in diabetic human myocardium and also in hearts with pathological reductions in the ejection fraction. Others demonstrated impairment of Precon-related responses in diabetic patients (127), consistent with experimental evidence of failed cardioprotection via Precon in diabetic animal models (42, 43, 110, 122, 141, 218). Responses to related or pharmacological stimuli are also impaired with diabetes, including anesthetic Precon (109, 209), opioid-mediated Precon (70, 175), and protection in response to heat stress (104), phosphodiesterase-5 inhibition (98), or via sphingolipid-dependent PKC modulation (73). Delayed or late Precon responses may also be eliminated (49).

The mechanistic basis of failed protection in diabetes has been investigated, with evidence that failure in human tissue arises from abnormalities in signaling, potentially upstream of PKC and p38-MAPK (76), consistent with impaired activation of p38-MAPK in aged mice (165). A signaling basis to failed protection in diabetic hearts is also consistent with decreased sensitivity of kinase signaling to Precon stimuli (219) and impaired activation of STAT3, Akt, and ERK1 with altered modulation of GSK3β (70). Additionally, there is evidence of KATP channel dysfunction (42, 43), mitochondrial dysfunction (76), excess accumulation of reactive oxygen species (109), impaired synthesis of stress-inducible heat shock protein (HSP) 70 (175), and impaired calcitonin gene-related peptide expression (140, 141). Overall, there is good evidence that some form of signaling dysfunction underlies poor protective efficacy in the diabetic myocardium. An obvious complicating factor is the inhibitory effect of hypoglycemic agents, particularly sulfonylureas that impair KATP channel activation (33, 90, 114, 189) and worsen cardiovascular outcomes (63). Curiously,
ischemic Precon may also impair the benefit with $K_{\text{ATP}}$ channel openers (28). Fortunately, some hypoglycemic drugs do not appear to exert these detrimental actions (114). Nonetheless, since hypoglycemic agents are not the primary cause of failed protection in diabetes, cardioprotection is unlikely to be restored solely through judicious choice of a hypoglycemic drug.

OBESITY. Obesity is increasing globally at alarming rates (doubling in both men and women in certain age groups in the last decade) and is one of the major risk factors for development of ischemic heart disease. Given common coexpression of obesity with other abnormalities, particularly other components of the so-called “metabolic syndrome” (glucose intolerance, hypertension, and dyslipidemia), it can be difficult to delineate the roles of individual disease constituents. Wagner et al. (224) have shown the loss of Precon in a rat model of established metabolic syndrome. In the $ob/ob$ mouse, a leptin-deficient obesity model, cardiac benefit from Postcon is impaired (18), while there is also evidence of failed Precon in obese insulin-resistant rats (106). Similarly, the protective effects of other stimuli are impaired in obese animals (73).

The molecular basis of the detrimental influence of obesity is unclear, although again dysregulation of kinase signaling may be involved. Bouhidel et al. (18) reported failed activation of Akt, ERK1/2, and p70S6K1 in $ob/ob$ mice, potentially involving enhanced expression of counteracting phosphatases. Similarly, Wagner et al. (224) acquired evidence of impaired ERK activation and failure to phosphorylate and inactivate GSK3β. Ineffective protection in obese insulin-resistant rats is associated with increased mitochondrial oxidant damage and impaired mitochondrial $K_{\text{ATP}}$ channel activation (106). As in aging and diabetes, there appear to be abnormalities in the activation of protective kinase cascades and mitochondrial $K_{\text{ATP}}$ channels in hearts from animals that are obese or suffer from the metabolic syndrome (Table 1). These similarities are suggestive of a common signaling dysfunction with age and disease, leading to poor tolerance and adaptation to injurious insult.

DYSLIPIDEMIA. The effect of atherosclerosis on dyslipidemia is equivocal, with opposing data acquired from different laboratories and in different models. Early evidence that hyperlipidemia might alter Precon responses arose from the study of Szilvássy et al. (206), where Precon was shown to be substantially inhibited in hypercholesterolemic rats. These investigators presented evidence that hyperlipidemia rather than resultant atherosclerosis is the key factor, since the effect was reversed by normalizing diet despite persistent atherosclerosis. Ferdinandy et al. (58) subsequently confirmed impaired Precon with hypercholesterolemia in the absence of atherosclerosis. The group also found that delayed protection with Precon was preserved despite elimination of the acute response, supporting selective inhibitory actions of hyperlipidemia. Ueda et al. (221) showed that hypercholesterolemia eliminated Precon in rabbits, an effect countered by treatment with a HMG-CoA reductase inhibitor (without altering plasma cholesterol substantially), while Kocić et al. (117) detected impaired functional protection with Precon in cardiac muscle from hyperlipidemic rats. Kyriakides et al. (124) present evidence of failed ischemic Precon in hyperlipidemic patients, a dysfunction supported by the more recent study of Ungi et al. (222), who found both worsened ischemic injury and inhibition of Precon in hypercholesterolemic patients.

Despite these latter findings in humans, recent studies in animal models have generated mixed results regarding dyslipidemias and cardioprotection. While Gircicz et al. (66) confirmed that hyperlipidemia eliminates protection with Precon in rats (an effect potentially related to dysregulation of matrix metalloproteinase-2), Jung et al. (105) and Kremastinos et al. (121) reported that hypercholesterolemia does not negate Precon in rabbits. Nonetheless, intrinsic tolerance to I/R was found to be reduced in the study of Jung et al. (105). Benefit from Precon is also evident in a genetic murine model (ApoE/LDLr knockouts on atherogenic diets) of atherosclerosis (132), and Iliodromitis et al. (93) detected preservation of Precon but failure of Postcon in hypercholesterolemic rabbits.

There is certainly a good theoretical basis for an influence of dyslipidemia on cardioprotection. Cell signaling is dependent on membrane makeup and function, which are sensitive to dietary lipids. Shifts in the makeup of lipid-rich caveolar domains may underlie age-dependent alterations in receptor signaling and cell function (163). Membrane makeup also dictates sensitivity to environmental factors and may govern gene expression for important regulatory elements (223). In this respect, hyperlipidemia has been shown to impair the expression of HSP70 (39). This protein may be important in tissue protection, and impaired expression is implicated in failed Precon in diabetes (175). Other factors potentially involved include reduced NO bioavailability (58, 88) and modification of caspase signaling and apoptosis (227). Overall, and despite ongoing controversy, the evidence does implicate a negative influence of hyperlipidemia on Precon and related protective responses.

HYPERTROPHY/HYPTERTENSION. Ebrahimi et al. (50) recently found that cardiac protection via Precon is impaired in aged hypertensive rats, involving the effects of both hypertension itself and of age. In other work, this group identified impaired cardioprotection with a GPCR stimulus (bradykinin) but preserved ischemic Precon in DOCA-salt hypertensive rat hearts.

Table 1. Observed alterations in phosphorylation/activation of key signaling components with age and disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>PI3K/Akt</th>
<th>ERK1/2</th>
<th>P70 S6K</th>
<th>PKC</th>
<th>p38-MAPK</th>
<th>GSK3β</th>
<th>Mito-K ATP</th>
<th>Phosphatases</th>
</tr>
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<tbody>
<tr>
<td>Aging</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>(92)</td>
<td>(165)</td>
<td>(57)</td>
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<tr>
<td>Diabetes</td>
<td>(70, 219)</td>
<td>(70)</td>
<td>(70)</td>
<td>-</td>
<td></td>
<td>(92)</td>
<td></td>
<td></td>
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<tr>
<td>Obesity/dyslipidemia</td>
<td>(18)</td>
<td>(18, 224)</td>
<td>(18)</td>
<td>(73)</td>
<td></td>
<td>(224)</td>
<td>(106)</td>
<td>(18)</td>
</tr>
</tbody>
</table>

Effects of different states are shown as − or + (for reduced or enhanced phosphorylation or activation, respectively). Data are taken from studies detecting altered responsiveness to protective stimuli and identifying differences in modulation of key elements of cardioprotective signaling. References are shown in parenthesis. PI3K, phosphatidylinositol 3-kinase. Note: impaired phosphorylation of GSK3β leads to activation of this detrimental signaling protein.
These findings are somewhat consistent with the earlier data of Reiss et al. (179), who showed that anesthetic Precon was also limited in larger or older hearts. Others (153) detected failed protection in hypertrophied myocardium. In contrast, there are also reports (24, 35, 157, 162, 198) of preserved protection in hypertrophy or hypertension, while others reported (178) enhanced protection in genetic hypertension. Related stimuli are reported to retain protective efficacy in hypertrophy (61, 84).

There is also mixed evidence regarding Precon in postinfarct remodeled myocardium. Miki et al. (150) found evidence of impaired Precon in remodeled hearts, although this was countered by more direct targeting of downstream mitochondrial K\textsubscript{ATP} channels (again supporting a limitation in signaling between triggering receptors and downstream effectors). Delayed or late Precon may also fail in remodeled hearts (55). However, Lucchini et al. (142) and Feng et al. (54) observed effective anesthetic Precon in remodeled myocardium. Thus selective aspects of protective signaling may be altered in the remodeled heart, which may be relevant to clinical utility in subjects who have experienced prior infarction.

Some of the inconsistency regarding the influences of hypertrophy and hypertension may arise from the fact that stretch itself can trigger cardioprotection (74, 157). Thus the ultimate outcome may reflect a mix of pathological induction of Precon overlaid on potentially detrimental effects of hypertrophy itself. Certainly, the protective response in hypertrophied hearts may differ from that in nonhypertrophied tissue. Butler et al. (25) found that hypertrophy shifts STAT signaling involved in Precon, with an important STAT-3 dependence in hypertrophied heart. Miki et al. (150) found evidence of impaired Precon in remodeled tissue, with an important STAT-3 dependence in hypertrophied heart. As with aging, it is possible that the molecular basis of these responses changes substantially with disease. This may be problematic in the development of clinical interventions, since molecular research in healthy tissue may not at all reflect the nature of these responses in diseased hearts (Table 1).

**Drug and Environmental Interactions**

**Pharmacological interactions.** A third important drawback to Precon or Postcon is that the implicated triggers and signaling paths are sensitive to common pharmaceuticals. These agents have the capacity to either interfere with essential signaling or inadvertently trigger protection (both actions precluding additional benefit from subsequent or adjunctive therapy targeting the same processes). Protective Precon/Postcon pathways may suffer interference from agents commonly used in coronary care, including β-blockers, nonsteroidal anti-inflammatory agents, Ca\textsuperscript{2+} channel blockers, and hypoglycemic sulfonylureas (as already discussed above).

**INHIBITORY INTERACTIONS.** The most obvious negative interaction is via drugs blocking the mediation or triggering of cardioprotection. Again, the results are mixed in terms of the effects of different agents on experimental cardioprotection. A number of studies raise concerns regarding the possible inhibitory effects of nonsteroidal anti-inflammatory agents such as aspirin. Gross et al. (69) reported blockade of opioid-dependent protection with aspirin but not ibuprofen (which actually augmented the benefit), while Jancso et al. (99) found that aspirin eliminates delayed Precon. Shinmura et al. (192) reported that such inhibition may be dose dependent, with no effect of low doses used for antithrombotic or analgesic purposes, whereas high levels used for antiinflammatory therapy did block delayed Precon. Thus although there is an inhibitory influence of aspirin, this might be reduced through choice of dose. Blockade of Ca\textsuperscript{2+} channels may also interfere with protection via Precon stimuli. Cain et al. (26) found that clinically used antagonists eliminate the Precon response in human myocardium, suggesting also that the detrimental effects of Ca\textsuperscript{2+} channel blockade on mortality (40) could stem from inhibition of intrinsic protective responses.

Both α- and β-adrenoceptor blockade can interfere with cardioprotection via Precon. β-Adrenoceptors are themselves implicated in protection, with studies demonstrating induction of a Precon-like state after β-adrenoceptor agonism (169). This is congruent with observations of impaired cardioprotection in animals treated with β-adrenoceptor blockers (203). Complicating matters, β-adrenoceptor blockade may also be protective (60, 97, 203). It is thus difficult to isolate the effects of the blocking agent on cardioprotection, independently from effects on intrinsic resistance. The α-adrenoceptor can also affect protection: Tomai et al. (217) acquired evidence of impaired Precon in patients treated with the α-selective blocker phenotamine, supporting a role for α-adrenoceptors in protecting human myocardium. It should be noted that issues of interference from different receptor blockers may be less of a concern than inhibitors of downstream signaling (as with aspirin, for example), since a clinically translated form of Precon or Postcon may well involve alternate or multiple GPCR stimuli or may target molecular events distal to receptor engagement.

**INTERFERENCE FROM CONDITIONING “MIMETICS.”** In addition to drugs inhibiting Precon or Postcon responses, patients are often exposed to agents that may actually trigger the same signaling pathways, including anesthetics (54, 241), opioid-based angesics (69, 164), and vasodilators (83, 103, 131). While such effects may provide acute benefit, they will simultaneously exclude further protection from therapy targeting Precon or Postcon mechanisms. Patients may additionally be exposed to prior sublethal ischemia before suffering AMI. Thus intrinsic Precon responses may already be endogenously triggered and/or have become refractory due to continued or frequent activation (both effects precluding added benefit from therapies targeting the same pathways). This possibility is supported by the work of Wu et al. (234), who found that coronary artery bypass patients who experienced angina ≤48 h before surgery were refractory to an ischemic Precon stimulus, whereas patients with angina ≥48 h before surgery responded to the same Precon stimulus.

The potential for this type of interaction is highlighted in Fig. 2, depicting postischemic functional recoveries of murine hearts that were untreated or received a mix of commonly applied agents (aspirin, morphine, and glyceryltrinitrate). The data show that these common drugs do trigger benefit, with ischemic Precon unable to provide added benefit in these hearts. Thus standard therapeutics already in place may induce the very protection one is seeking to trigger with conditioning-based intervention. From the perspective of clinical trials, which contrast effects of novel adjunctive therapy with placebo (i.e., best conventional treatment ± adjunctive cardioprotection), there may be no evidence of efficacy if ischemic patients have inadvertently been placed in a protected state via common pharmacotherapies or disease history. Best current practice may
already confer the benefit of conditioning responses, and extensive development of conditioning-based therapies may be pointless in the face of activation of the same paths by drugs almost invariably applied in ischemic patients (e.g., analgesics, anesthetics, nitrovasodilators).

Other interactions. Other types of interaction may limit the efficacy of conditioning-derived cardioprotection, including surgical procedures themselves and environmental factors. Ghosh and Galiñanes (64) found that Precon is ineffective in patients undergoing cardiopulmonary bypass and suggested that protection via bypass itself prevents additional benefit from Precon stimuli. This would significantly limit the value of strategies evolved from Precon or Postcon to only “off-pump” surgery. This also raises the possibility of inadvertent Precon via remote organ ischemia or hypoxia. In addition, it is not clear whether Precon-derived strategies are effective in the setting of cardioplegia: in a small clinical trial Perrault et al. (170) found that Precon did not enhance the protective effects of cardioplegia, which is in agreement with lack of effect of Precon on outcome from cardioplegic preservation in a rabbit model with limited infarction (53). The latter work additionally raises questions regarding the value of Precon-based interventions for reducing contractile dysfunction vs. infarction. Comparing cardioplegia and Precon, Kolocassides et al. (118) found that Precon did improve functional recovery yet actually accelerated contracture development and ATP depletion. Certainly most trials in CABG with cold cardioplegia report no protection from cell death/infarction by Precon stimuli (37, 64, 233, 234, 236).

Evidence has also been presented that environmental or lifestyle factors can negatively influence conventional protective responses. For example, inhaled microparticulates affect ischemic outcomes and cardioprotection (240), and alcohol has been reported to eliminate Precon in humans (160). Such observations not only reflect the potential for inhibition of conventional responses via environmental or modifiable lifestyle factors but also highlight the possible effect of such factors on pathogenesis of ischemic heart disease (e.g., 11).

Caffeine intake is another lifestyle factor that may influence I/R tolerance and cardioprotection. A recent study by Riksen et al. (180) found that the equivalent of two to four cups of coffee effectively eliminated the benefit with two differing Precon stimuli in human tissue (in vitro and in vivo). This likely occurs via blockade of adenosine receptors, as the extracellular levels achieved act primarily (if not solely) via adenosine receptor antagonism. Animal studies reveal caffeine consumption exaggerates injury with I/R, although it appears that acute caffeine ingestion is detrimental, whereas chronic caffeine intake is without effect (possibly due to development of tolerance; Ref. 181). Epidemiological evidence indicates that more than five cups of coffee per day increases the risk for cardiovascular disease (68, 108). Moreover, retarded caffeine metabolism exaggerates this association, with only two to three cups per day associated with an increased risk of infarction (34). These linkages may well reflect inhibition of intrinsic protective mechanisms revolving around adenosine receptor engagement, including Precon and Postcon (48, 77).

Sex Dependence of Conventional Protection

In the clinical setting, assumptions of general physiological parity between males and females have led to both being evaluated and treated similarly. However, experimental and clinical findings provide evidence of fundamental disparities between the sexes that in the cardiovascular system range from coronary vessel size through to intrinsic sensitivity to ischemia. Moreover, the etiology of myocardial ischemia may differ in females vs. males (147, 191). Thus sex-dependent approaches may be required for effective treatment of ischemic heart disease. Despite evidence of sex-dependent responses and the emerging notion of sex-dependent therapy, the vast majority of research into I/R and cardioprotection continues to be performed in males (typically young and healthy). Even randomized clinical trials in acute coronary syndromes still underrepresent females (130). Our knowledge of injury processes, intrinsic protective responses, and experimental cardioprotection in the female heart is thus limited. Available evidence regarding sex differences in ischemic tolerance and protection has been well reviewed recently (154).

Conflicting data continue to be acquired regarding the sex dependence of cardioprotective responses, whereas a less equivocal case has been presented for the sex dependence of intrinsic ischemic tolerance. A majority of evidence supports greater tolerance to ischemic insult in female animal models (10, 21, 62, 168, 220, 228–230) and in humans (191). None-
theless, sex dependence of ischemic tolerance is still debated, as a number of other studies (148, 149, 193) failed to detect differences in intrinsic sensitivity to I/R.

The mechanistic basis of sex-dependent responses to I/R and cardioprotection is under investigation, with several possible contributors. Estrogen itself is protective, contributing to ischemic tolerance in females. This involves a nuclear receptor-triggered genomic response, modulating the expression of proteins involved in protection and metabolic control (154). Nongenomic effects include activation of the protective PI3K pathway and modulation of MAPK signaling (112). Gabel et al. (62) and Wang et al. (229) provide evidence that the β-form of the estrogen receptor is specifically involved in ischemic tolerance in females vs. males. When contractility or Ca\(^{2+}\) is enhanced before ischemia, females also exhibit reduced injury vs. males, which may involve enhanced NO signaling and nitrosylation of Ca\(^{2+}\) channels to limit Ca\(^{2+}\) overload (205). Brown et al. (21) report that, in addition to greater intrinsic resistance to ischemia, females exhibit reduced sensitivity to exercise as a protective stimulus, both effects being linked to differences in sarcosomal K\(_{ATP}\) channel expression. In terms of the more recently uncovered Postcon phenomenon, Penna et al. (168) report that this response is sex dependent, being less efficacious in reducing infarction in females while less effectively limiting contractile dysfunction in males. They attribute these differential effects to differences in phosphorylation/activation of the RISK elements Akt and ERK.

Available evidence thus suggests that female hearts are in a partially protected state owing to the effects of estrogen, differences in protein expression, and activation of RISK elements. Although beneficial in itself, this tolerance may limit expression of additional protection via the same paths. As suggested in the findings of Wang et al. (228), enhanced nitric oxide synthase signaling may mediate greater intrinsic tolerance in female hearts, yet it is insensitive to further activation via Precon mimetics. This raises the question of whether these pathways and molecular targets, integral to the conventional protective responses of Precon and Postcon, are the most rational candidates for clinical cardioprotection in females. Certainly, evolution of a clinically effective protectant will require specific attention to sex differences in responses to both I/R and protective stimuli.

Restoration of Conventional Protection

Although considerable evidence supports age, disease, drug, and sex dependence of Precon-based interventions (and to a lesser extent more recently discovered Postcon), there is reason to believe that some of these drawbacks might be countered by “complementary” methods. Indeed, these complementary approaches may themselves confer more benefit than the cardioprotective stimulus. Specifically, exercise and caloric restriction offer the ability to restore failed protective responses in aged hearts (3, 5, 135), while simultaneously enhancing intrinsic ischemic tolerance (194, 201). It is estimated that ~80% of coronary artery disease is attributable to modifiable lifestyle/environmental factors (200), particularly activity level and calorie intake. Exercise and caloric restriction may thus not only augment efficacy of protective strategies but in themselves will substantially limit the pathogenesis and incidence of heart disease. In addition to these modifiable factors, other interventions have been linked experimentally to benefit. For example, the wine component n-tyrosol was recently reported to confer protection from ischemia by inducing survival and longevity proteins that may be considered “antiaging” for the heart (183). Similarly, manipulation of caveolins has the capacity to preserve protective signaling and prevent cellular senescence (163), while overexpression of protective GPCRs can restore protection in aged hearts (81). Thus molecular-based interventions may also have the capacity to restore protective signaling and efficacy in aged (potentially diseased) tissue.

Caloric restriction. A reduction in calorie intake, whether long (19, 196) or short term (194, 195), can significantly influence the ability of the heart to withstand injury during I/R and restore protective responses in aged tissue (4, 5, 135). The cardioprotective effects of caloric restriction may be additive with those of exercise (36). Research suggests a role for reduced oxidative damage (30), particularly to mitochondrial DNA (67, 137), in the beneficial effects of caloric restriction. In keeping with mitochondrial targeting, Broderick et al. (20) present evidence for an effect of caloric restriction on mitochondrial respiration, improving oxidative phosphorylation and energy production. Transcriptional reprogramming may contribute to these effects, with microarray interrogation revealing inhibition of age-related gene changes, together with shifts in expression of transcripts involved in protection of DNA, control of apoptosis, regulation of cytoskeletal structure and function, and fatty acid metabolism (129). Masternak et al. (146) suggested a role for modified expression of genes involved in insulin signaling, while repressed inflammation may also play a role (30). A more detailed understanding of the cellular effects of calorie restriction may pave the way to the development of effective cardioprotective strategies or of a means of enhancing conventional responses in relevant patients. Such research would also more rationally underpin dietary prescription in specific disease states or risk groups.

Exercise. The cardiovascular benefits of exercise are well established, although the underlying mechanisms remain to be elucidated. It is relevant to highlight that exercise capacity is a more powerful predictor of mortality than other established risk factors for cardiovascular disease (156), with cardiorespiratory fitness a critical determinant of mortality in older patients (204). Exercise training substantially limits cardiac injury with I/R (201), can induce Precon-like responses in human myocardium (136), and can reverse age-related abnormalities in cardioprotective responses (3, 5). Reduced activity levels may well underlie or contribute to the pathogenesis of major chronic diseases including cardiovascular diseases (29).

The molecular bases of the protective and restorative properties of exercise are not fully established, with most attention fixed on shifts in antioxidant status (201) and HSP expression (176). Several studies have identified improved expression of antioxidants in hearts from exercised animals, including elevations in glutathione (44, 133, 161), glutathione redox cycle enzyme activities (8), catalase (202), and Mn-SOD (41, 202). A number of studies (44, 134, 176) have also identified beneficial shifts in HSP expression, particularly HSP72. However, much of this work is associative in nature, with changes in antioxidant expression generally not established as causally linked to shifts in ischemic tolerance. Moreover, conflict exists in terms of the effect of exercise on antioxidant systems, with...
some investigators observing exercise-induced reductions in expression/activity of proteins, including glutathione S-transferase (111), glutathione, and glutathione reductase (9).

An alternate or additional mechanism involves modification of protective signaling, with some parallels with pathways involved in Precon and Postcon. Evidence has been presented that exercise-mediated protection involves PI3K and Akt (243), may require nitric oxide synthase (6), and involves GPCRs that also trigger Precon (45). The question arises then as to how and why exercise retains efficacy with age or disease whereas Precon responses involving common signal elements fail? However, exercise may not only enhance kinase signaling via conventional protective pathways but may also generate shifts in signaling (102). For example, Brown et al. (22) have shown that protection with exercise involves an increased expression and contribution from sarcolemmal $K_{ATP}$ channels, in contrast to mitochondrial $K_{ATP}$ involvement in conventional Precon. Other studies (213) support distinct signaling in exercise vs. Precon. Further interrogation of the exercise-dependent effects in cardiac cells may not only yield novel targets for cardioprotection and identify ways of augmenting conventional responses, but may ultimately limit the incidence and progression of heart disease and other chronic illnesses. A cogent case is presented by Chakravarthy and Booth (29) that increased caloric intake and reduced activity level in modernized societies leads to dysregulation of our Paleolithic transcriptome and a maladapted phenotype that underlies major chronic diseases.

Summary

In this brief review, we have highlighted some of the features of the most intensely studied protective phenomena (Precon and Postcon) that are highly relevant to translation and clinical utility. While research into conditioning responses continues to generate important knowledge regarding cellular injury and protection during I/R, they share features that may limit their utility and efficacy in the clinical setting. No pharmacotherapy is without drawbacks, interactions, or side effects. Nonetheless, in the search for effective cardioprotective strategies, a number of fundamental requirements should be met, most notably efficacy in the target population. In the context of ischemic heart disease, this population encompasses males and females, the majority $>50$ years of age, suffering comorbidities including diabetes/dyslipidemia/obesity, and often experiencing a progressive onset of disease. Each of these descriptors raises a concern regarding Precon/Postcon-based strategies: the efficacy of these responses appears to be sex dependent, the responses are negated or inhibited with aging, disease states such as diabetes additionally impair efficacy, and the underlying molecular signaling and effectors may already be activated by prior ischemia, surgical intervention, and drugs that cardiac patients might commonly be exposed to (anesthetics, anti-inflammatories, that cardiac patients might commonly be exposed to). Nonetheless, it is pertinent at this juncture to reexamine our rationale for studying the molecular mechanics of conditioning responses in a quest for clinical cardioprotection. Although some of the abovementioned drawbacks might be countered by complementary exercise or calorie restriction regimes, one is left considering whether these latter interventions should be a greater focus in primary prevention, rather than as post hoc manipulations to enhance the efficacy of secondary or tertiary pharmacotherapy. Either way, it is imperative that we modify our experimental approach, away from analysis of responses in young healthy males, to focus on the mechanisms and efficacy of cardioprotection in aged diseased hearts from both sexes.

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REFERENCES

PROTECTION VIA CONDITIONING STIMULI


114. Lee TM, Su SF, Chou TF, Lee YT, Tsai CH. Loss of preconditioning by attenuated activation of myocardial ATP-sensitive potassium channels

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221. Wang M, Crisostomo PR, Market T, Wang Y, Lillemoe KD, Mel-

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