Netherlands
The Journal of Medicine

REVIEW

Ischaemic preconditioning: from molecular characterisation to clinical application - part II

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This article is the second part of two papers on ischaemic preconditioning. The first part of this review was published in the November issue of this journal.[1]

ABSTRACT

Ischaemic preconditioning was originally described in animal hearts as histological infarct-size limitation by a previous brief episode of ischaemia. In humans, ischaemic preconditioning has been demonstrated in several *in vitro* and *in vivo* models, including coronary artery bypass grafting and percutaneous transluminal coronary angiograplasty, using surrogate markers of ischaemia and reperfusion injury. Increasing knowledge of the molecular signalling pathways mediating protection by ischaemic preconditioning has provided rational targets for pharmacological intervention. Several widely used drugs are able to mimic ischaemic preconditioning (e.g. adenosine, adenosine-uptake inhibitors, ACE inhibitors, angiotensin II antagonists, statins, opioids, volatile anaesthetics and ethanol), whereas others inhibit ischaemic preconditioning-induced protection (e.g. sulphonylureas and adenosine antagonists). The present review focuses on these different classes of drugs. Prudent use or avoidance of these drugs in patients who are at risk for myocardial infarction could theoretically limit ischaemia and reperfusion injury.

INTRODUCTION

In the first part of this review on ischaemic preconditioning, we described the infarct size limiting effects of the naturally occurring phenomenon of ischaemic preconditioning and the time windows in which this effect occurs.[1] Moreover, the interesting observation that a short period of ischaemia also renders distant organs resistant to a subsequent prolonged period of ischaemia was discussed. Finally, the most important triggers, mediators and end-effectors of ischaemic preconditioning that have been identified so far were summarised. However, most data described in this part were derived from animal experiments. Because these studies have convincingly shown that ischaemic preconditioning is the strongest form of *in vivo* protection against myocardial ischaemic injury other than early reperfusion, the possibility of using this phenomenon in clinical practice would be very desirable. Despite state-of-the-art reperfusion strategies, 30-day mortality of myocardial infarction is still around 7%.[2] In addition, the prevalence of cardiac failure is rapidly increasing and is often caused by (ischaemic) death of cardiomyocytes. Thus, there is a need for additional therapeutic strategies that increase tolerance to ischaemia and reperfusion. Exploitation of ischaemic preconditioning may offer such a strategy.

To adequately exploit this mechanism in the everyday clinical setting, three more issues need to be addressed. First, the evidence that preconditioning also occurs in the human heart needs to be discussed. Secondly, if indeed protection can be seen in humans, could it be exploited to develop therapeutic strategies to protect the human heart against ischaemic injury? In clinical practice, it is often not desirable or feasible to precondition myocardium with ischaemia. Fortunately, the accumulating knowledge about the molecular mechanisms mediating preconditioning has provided us with the possibility to modulate ischaemia and

** P. Smits was not involved in the handling and review process of this paper.

reperfusion injury pharmacologically, thus limiting infarct size in the same way as ischaemic preconditioning. Finally, it is essential to identify those patients who may benefit from preconditioning and the situations in which preconditioning could be applied. In the present part of this review, we will consecutively discuss these three issues. Considering pharmacological preconditioning, special emphasise will be put on drugs that are used frequently in internal medicine.

DOES PRECONDITIONING OCCUR IN THE HUMAN HEART?

Analogous to the previously discussed animal studies, the evidence that ischaemic preconditioning also occurs in humans has been derived from various experimental models, which are summarised in *table 1*. The most important difference between animal studies and human studies on this subject concerns the endpoint that is used to estimate ischaemic injury. Also, the design of the experimental protocol often differs substantially. In animal models, in contrast to human clinical practice, coronary occlusion is often induced and ended abruptly in otherwise healthy animals. Traditionally, in animal studies, the endpoint is histological infarct size. For obvious reasons, this endpoint cannot be used in human studies. Therefore, several models have been developed in which surrogate endpoints are used to evaluate ischaemic preconditioning in humans, recently reviewed by Tomai *et al.* and Kloner *et al.*[3,4]

In vitro, classical as well as delayed preconditioning has been shown in cultured cardiomyocytes, using tryptan blue exclusion as endpoint of simulated ischaemia and reperfusion injury.[5,6] The existence of ischaemic preconditioning has also been demonstrated in isolated human atrial trabeculae, obtained from patients undergoing open-heart surgery. In this model, using electrical field stimulation, recovery of contractile force after simulated ischaemia and reperfusion is used as endpoint.[7] Later it was found that preconditioning in this model is also critically dependent on protein kinase C (PKC) activation and adenosine-triphosphate sensitive potassium channel (K_{ATP} channel) opening and that adenosine A_1 and A_3 receptor stimulation can mimic preconditioning.[8,9]

These *in vitro* models are good candidates to screen drugs on their potential to mimic or modulate ischaemic preconditioning, but cannot directly be extrapolated to clinical practice. In addition to these *in vitro* models, there are several observations in daily clinical practice that might be explained by ischaemic preconditioning. The so-called warm-up phenomenon refers to the naturally occurring phenomenon, which is described in more than half of all patients with coronary artery disease, that performance is improved and ischaemia-induced symptoms are attenuated during a second period of exercise, when compared with the first exercise test. Ischaemic preconditioning has been suggested to be one of the possible causes of this phenomenon, particularly because the warm-up phenomenon lasts no longer than 90 minutes.[10] However, because adenosine receptor stimulation does not seem to

Table 1

Overview of the various in vitro and in vivo models of ischaemic preconditioning in humans with main endpoints and limitations

METHOD	MAJOR ENDPOINTS	PROBLEMS/LIMITATIONS
In vitro		
Cultured cardiomyocytes	Tryptan blue exclusion and lactate/ LDH release	Hypoxia instead of ischaemia Isolated cells, no infarct size
Isolated atrial trabeculae	Recovery of contractile function	Hypoxia instead of ischaemia No direct measurement of cellular death Endpoint determined by cell death and stunning
In vivo		
Warm-up phenomenon	Exercise tolerance	Role of ischaemic preconditioning as mediator controversial
Preinfarction angina	Clinical outcome after myocardial infarction	Confounded by more rapid thrombolysis
Repeated PTCA	ST-segment elevation, anginal pain, myocardial lactate extraction	ST-segment change determined by sarcK_{ATP} channels No direct measurement of cellular death Possible collateral recruitment
Aortic clamping before CABG	Postoperative troponin/CK-MB release, postoperative recovery	Confounded by perioperative drugs, which affect preconditioning No direct measure of cellular death
^{99m}Tc-Annexin A5 scintigraphy	Targeting of annexin A5	Skeletal muscle instead of myocardium

be involved in warm-up and because involvement of K_{ATP} channels is uncertain, a role for ischaemic preconditioning in warm-up remains controversial.[10,11] Another naturally occurring phenomenon that could be explained by ischaemic preconditioning is the possible infarct size sparing effect of preinfarction angina. Many patients with acute myocardial infarction have experienced angina in the hours or days preceding the infarction. Several studies have shown that indeed the presence of preinfarction angina, especially within 24 hours before infarction, is associated with improved clinical outcome after acute myocardial infarction, including death and the incidence of heart failure,[12,13] with reduced CK release[12,14] and with a smaller area of necrosis as assessed by nuclear imaging.[15] Also, Solomon *et al.* recently suggested that angina reported during the three months preceding myocardial infarction protects against left ventricular remodelling.[16] However, not all studies showed this association.[17] Moreover, Andreotti *et al.* showed that preinfarction angina is associated with a more rapid reperfusion of the infarct-related artery following thrombolysis, which is an attractive alternative explanation for the beneficial effect of angina.[18] This finding is in accordance with the observations that preinfarction angina only protects in patients treated with thrombolysis and not those treated with coronary angioplasty.[19] In conclusion, although there is strong evidence that preinfarction angina renders the myocardium more resistant to a subsequent myocardial infarction, the role of ischaemic preconditioning in this association remains controversial.

In addition to the above-mentioned naturally occurring forms of preconditioning, there are also two models in which active interventions are able to trigger preconditioning and which are therefore better suited to effectively study the modulation of this protection by external factors such as drugs. Firstly, in clinical practice, percutaneous transluminal coronary angioplasty (PTCA) offers the opportunity to electively and selectively apply ischaemia to a well-defined myocardial region. In theory, the first coronary occlusion in a series of occlusions could offer increased resistance to subsequent occlusions. Using this model, several studies showed that ST-segment shift on electrocardiography and subjective anginal pain are decreased during the second coronary occlusion, as well as wall motion abnormalities and lactate production,[20-22] although some studies showed no protection.[23,24] Subsequently, the finding that the nonselective adenosine receptor antagonist aminophylline could block this protection[25] and that intracoronary infusion of adenosine[26] as well as bradykinin[27] followed by a short period of wash-out before the first inflation could mimic preconditioning further strengthened the probability that indeed ischaemic preconditioning was responsible for the increased resistance to the second period of ischaemia. However, these results have to be interpreted with caution for two reasons. First, acute recruitment of collateral vessels is a major possible confounding factor.[28,29] Secondly, the most important surrogate endpoint used in this model of preconditioning is ST-segment elevation on electrocardiography. However, ST-segment elevation is determined by opening of sarcolemmal K_{ATP} channels,[30] which, as outlined in the first part of this review, are probably not necessary for ischaemic preconditioning to occur. Recently, it was clearly demonstrated that this parameter is not a good endpoint for preconditioning by showing a dissociation between this parameter and infarct size limitation.[31]

A second of the very few clinical scenarios in which cardiac ischaemia is planned is coronary artery bypass grafting (CABG). In this situation, ischaemic preconditioning can by studied while avoiding the possible confounding of recruitment of collateral vessels by applying global cardiac ischaemia instead of local ischaemia. The evidence that ischaemic preconditioning confers additional protection in CABG and the possible use of preconditioning in clinical practice has recently been comprehensively discussed.[32,33] Yellon's group were the first to show that pretreatment with two three-minute periods of cross-clamping and reperfusion before a ten-minute period of ischaemia and ventricular fibrillation induces better preservation of left ventricular ATP content and reduces postoperative troponin I release.[34-36] Whether ischaemic preconditioning is also able to confer additional protection to ischaemia when other techniques than intermittent cross-clamp fibrillation are used is more controversial. Illes *et al.* found improvement in postoperative cardiac index and reduced requirement for inotropics with one-minute aortic cross-clamping before cold blood cardioplegic arrest.[37] Moreover, Lu *et al.* found a reduction in postoperative CK-MB release and improved recovery of myocardial contractility in patients undergoing valve replacement with the use of cardioplegia.[38] However, other groups were not able to demonstrate beneficial effects of ischaemic preconditioning in the setting of cardioplegic arrest.[39,40] Considering pharmacological preconditioning, some studies have shown that pretreatment with adenosine instead of short periods of ischaemia and reperfusion before CABG is associated with better postoperative ventricular performance[41] and less CK-MB release,[42] whereas others did not show a benefit from pretreatment with a specific A1 receptor agonist[36] or adenosine.[43] The discordant results obtained with ischaemic and pharmacological preconditioning in the setting of open-heart surgery could well be caused by two important possible confounders. First, in this setting anaesthetics are always used concomitantly and, as discussed in a later section, it is known that most anaesthetics influence preconditioning in a positive or negative way. Moreover, there are indications that cardiopulmonary bypass itself is able to precondition the myocardium, leaving little room for additional protection.[44,45]

Although the beneficial effect of ischaemic preconditioning

on the incidence of ischaemia/reperfusion-induced arrhythmias remains controversial in animal models,[46,47] recent studies in man suggest clinical benefit. The incidence of ventricular tachyarrhythmias after declamping in CABG patients was shown to be significantly reduced by preconditioning with two two-minute periods of ischaemia and reperfusion.[48]

In conclusion, there is a wealth of evidence that ischaemic preconditioning also occurs in humans, but conclusive evidence and large-scale testing of the ability of drugs to mimic or inhibit preconditioning is still hampered by the lack of an optimal and easy-to-use human model. Ischaemic preconditioning is not confined to cardiac tissue, but has also been described for liver, brain and skeletal muscle.[49-51] Also, the mechanisms of ischaemic preconditioning in heart and skeletal muscle show many similarities.[52,53] Recently, our group developed and validated a new model of ischaemic preconditioning in forearm skeletal muscle. Fundamental to this model is that ischaemic exercise (isometric contraction of the finger flexors while the circulation is occluded with an upper-arm cuff) induces translocation of phosphatidylserines from the inside to the outside of cellular membranes of affected cells, which is considered an early marker of apoptosis. This process can be visualised by scintigraphic imaging of the arm and hand after injection of radiolabelled annexin A5, which selectively binds to these phosphatidylserine residues. With this model, we have shown that ten minutes of forearm ischaemia protects against increases in annexin A5 binding induced by a subsequent ten minutes of ischaemic exercise, that infusion of adenosine into the brachial artery of the experimental arm mimics protection (Rongen *et al.*, Circulation, in press), and that protection is inhibited by pretreatment with the adenosine receptor antagonist caffeine (Riksen, *et al.*, submitted). By infusing target drugs into the brachial artery, it is easy to test their influence on ischaemic preconditioning or ischaemia/reperfusion injury *per se*. Apart from a research tool, this model may eventually be used in a clinical setting to individualise pharmacological strategies that are aimed to improve tolerance against ischaemia and reperfusion.

PHARMACOLOGICAL PRECONDITIONING AND MODULATION OF ISCHAEMIC PRECONDITIONING

The elucidation, mostly from animal experiments, of great parts of the molecular machinery that is responsible for protection by ischaemic preconditioning, has provided us with several rational targets for pharmacological intervention. Various drugs have been shown to be able to mimic ischaemic preconditioning when applied instead of the preconditioning period of ischaemia. On the contrary, several drugs also interfere with ischaemic preconditioning and actually inhibit or reduce protection from ischaemic preconditioning. An overview of drug classes that are able to influence preconditioning is provided in *table 2*. In this section, we discuss human studies when possible. However, if these studies are unavailable, animal studies are used. It is important to realise that large interspecies differences exist with regard to preconditioning and the mechanism of preconditioning and, therefore, data derived from animal studies need to be interpreted with caution. In this section we will highlight several drugs that are already used in daily clinical practice and which have the potential of mimicking or modulating preconditioning. Consecutively, nucleoside uptake inhibitors, ACE inhibitors and AT1 receptor antagonists, HMG-CoA-reductase inhibitors, sulphonylureas, K_{ATP} channel openers, anaesthetics, and alcohol will be evaluated for their potential to modulate ischaemic preconditioning. Additionally, we will be discuss whether known positive or negative effects of these drugs on cardiovascular function or mortality could be explained by their preconditioning modulating effect.

Both animal and human studies have identified adenosine as one of the most important triggers of ischaemic preconditioning. However, because of its very short elimination time, adenosine itself is not suited for administration to serve this goal. Moreover, more stable specific adenosine receptor agonists are not yet available for human use in clinical practice. However, by inhibiting the cellular uptake of endogenous adenosine, dipyridamole is able to increase the extracellular concentration of endogenous adenosine. Indeed, intravenous pretreatment with dipyridamole significantly potentiated the infarct size limiting effect of ischaemic preconditioning in rabbit heart.[54] In humans, intracoronary administration of dipyridamole before balloon inflation during PTCA also reduced anginal pain and ST-segment shift[55] and prevented deterioration of ventricular function during balloon occlusion.[56] In clinical practice, efficacy of dipyridamole, given especially because of its presumed effect on platelet aggregation, has long been the subject of controversy. A recent meta-analysis concluded that in patients with vascular disease, there is no evidence that dipyridamole reduces the risk of vascular death, although in one study in patients after cerebral ischaemia, dipyridamole reduced the risk of further vascular events.[57] This lack of clinical benefit might be due to the fact that dipyridamole is not dosed high enough to adequately increase the endogenous adenosine concentration or because dipyridamole is often coadministered with acetylsalicylic acid, which might itself inhibit delayed ischaemic preconditioning,[58] offsetting the possible beneficial effects of dipyridamole.

Several studies have shown that bradykinin is also involved as a trigger in ischaemic preconditioning. In humans,

Table 2
Drugs with the ability to mimic or inhibit preconditioning

MIMICKING PRECONDITIONING	INHIBITION OF PRECONDITIONING
Adenosine receptor agonists Adenosine[9,26]	**Adenosine receptor antagonists** Theophylline, aminophylline, bamiphylline[7,25,157]
Nucleoside transport inhibitors *By increasing endogenous adenosine* Dipyridamole[56]	
K_{ATP} channel openers Nicorandil[158] Diazoxide[91]	**K_{ATP} channel blockers** Glibenclamide[79,91]
Opioid agonists[108] Morphine[107]	**Opioid receptor antagonists** Naloxone[109]
α_1-adrenergic receptor agonists Phenylephrine, norepinephrine[159,160]	**α_1-adrenergic receptor antagonists** Phentolamine[161]
β_1-adrenergic receptor agonists Isoproterenol[162]	**β_1-adrenergic receptor antagonists[163]**
B_2-bradykinin receptor agonists[27]	
ACE inhibitors *By increasing bradykinin concentration* Captopril, lisinopril[64]	
Angiotensin II receptor antagonists Losartan[66]	
Volatile anaesthetics Isoflurane, halothane, sevoflurane, enflurane, Desflurane[102]	**Intravenous anaesthetics** R-ketamine, thiopental and pentobarbital[105]
Nitric oxide donors Nitroglycerin[77]	
Statins Pravastatin[71]	
Ethanol[116]	
Corticosteroids[164]	**COX-2 inhibitors** *Inhibit only delayed preconditioning* High-dose ASA,[58] celecoxib[165]

References preferentially indicate human studies; if not available, animal studies are referred to.

bradykinin is able to mimic ischaemic preconditioning in the model of repeated PTCA.[27] Analogues to adenosine, direct bradykinin receptor agonists are not yet available for clinical human use. However, angiotensin-converting enzyme (ACE) inhibitors are known to inhibit the breakdown of bradykinin, thus increasing the concentration of endogenous bradykinin (*figure 1*).[59] Considering preconditioning of the myocardium, animal studies have demonstrated that pretreatment with ACE inhibitors reduces infarct size,[60] potentiates the acute[61] as well as delayed[62] infarct size limiting effect of subthreshhold ischaemic stimuli and attenuates myocardial stunning.[63] Moreover, selective bradykinin B2 receptor antagonists could inhibit these beneficial effects of ACE inhibitors.[61,63] Similar results were obtained in human atrial trabeculae, obtained during CABG, in which postischaemic recovery of contractile function was significantly increased by pretreatment with captopril and lisinopril in combination with a subthreshold ischaemic preconditioning stimulus. These beneficial effects were again completely prevented by a specific bradykinin B2 receptor antagonist.[64] These potentiating effects of ACE inhibitors on ischaemic preconditioning could be one of the mechanisms responsible for the favourable effects of these drugs on cardiovascular death and the incidence of heart failure in several clinical trials, such as the HOPE trial.[65] Surprisingly, AT1 receptor antagonists, initially presumed not to influence the kallikrein-kinin system, could also limit infarct size in rat[66] and pig[60] hearts and intriguingly, this effect could also be blocked by bradykinin antagonists.[66] This observation is in contradiction with earlier studies, showing inhibitory effects of AT1 receptor antagonism on the effect of ischaemic preconditioning.[67] One explanation for this beneficial effect of AT1 receptor antagonists could be that during blockade of the AT1 receptor, AT2 receptor stimulation by angiotensin II is enhanced (*figure 1*). AT2 receptor stimulation has recently been shown to activate the kallikrein-kinin system and thereby stimulate bradykinin release.[68] Indeed, it was subsequently shown that the

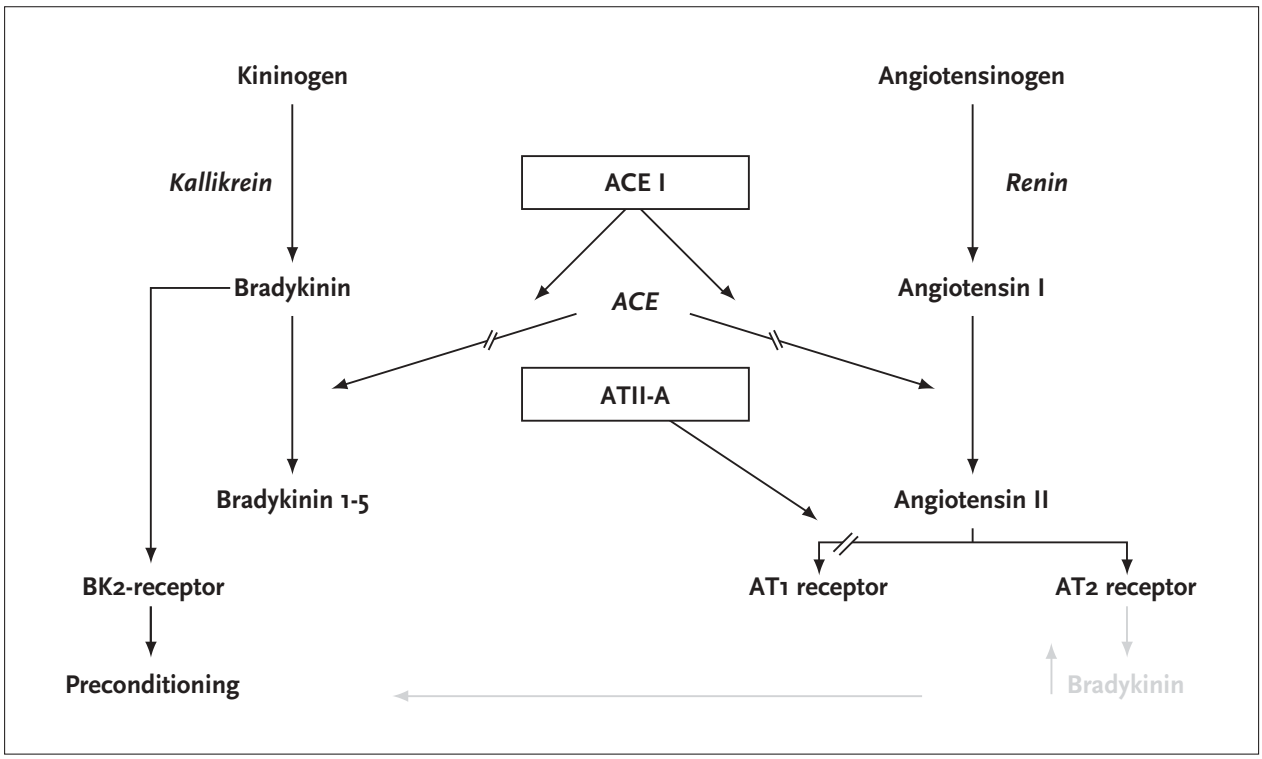

Figure 1

Schematic illustration of the interaction between the renin-angiotensin and the kallikrein-kinin system and the effects of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II antagonists (ATII-A)

This illustration shows how these drugs mimic ischaemic preconditioning. BK = bradykinin. ACE-I inhibit breakdown of bradykinin, which stimulates bradykinin BK2 receptors. ATII-A only block the AT1-subtype receptor. Subsequent increased stimulation of the AT2 receptor by endogenous angiotensin II could activate the kallikrein-kinin system, also leading to an increased release of bradykinin.

vascular effects of candesartan are blocked by bradykinin antagonism.[69] This effect might explain the similar effects on mortality of ACE inhibitors and ATII antagonists in patients who are at high risk for cardiovascular events after acute myocardial infarction.[70] It needs to be emphasised, though, that it is very difficult to investigate the preconditioning-mimicking effect of drugs in large clinical trials, because preconditioning would not influence the incidence of cardiovascular events, but rather the outcome, once an event has occurred.

3-Hydroxy-3-methylglutaryl (HMG)-Co-enzyme A (CoA) reductase inhibitors form another class of drugs, widely prescribed in cardiovascular compromised patients, that have been suggested to protect from ischaemia/reperfusion injury. The beneficial effects of HMG-CoA-reductase inhibitors on cardiovascular morbidity and mortality in patients at risk for cardiovascular disease are widely appreciated. Beyond their ability to halt the process of atherosclerosis, mimicking of ischaemic preconditioning has also been suggested to contribute to these major beneficial effects. Ueda *et al.* showed that the infarct size limiting effect of ischaemic preconditioning is blunted in hypercholesterolaemic rabbits and that pravastatin, added to their diet, completely restores this without affecting plasma total cholesterol, HDL and triglycerides.[71] This was explained by the finding that pravastatin also restores the activation of the enzyme ecto-5'-nucleotidase during the preconditioning ischaemia, which is attenuated in the untreated hypercholesterolaemic rabbits. As ecto-5'-nucleotidase converts adenosine monophosphate into adenosine, this could well contribute to the observed effect. Later it was shown that lovastatin and simvastatin also enhance ecto-5'-nucleotidase activity *in vitro*.[72] In the recent study by Lee *et al.*, hyperlipidaemic patients with coronary artery disease were randomised to pravastatin or placebo for three months before PTCA. Patients on pravastatin had less ST-segment shift, anginal pain and myocardial lactate production during the first balloon occlusion than the control group and this protection was abolished by pretreatment with the adenosine receptor antagonist aminophylline, suggesting that the cardioprotection offered by pravastatin was mediated by adenosine.[73] However, the treated patient group also had significantly lower plasma cholesterol levels. Because of these results, it is attractive to speculate that stimulation of ecto-5'-nucleotidase could be one of the

mechanisms that mediate the well-known protection of statins on the cardiovascular system. However, it needs to be stressed that other mechanisms of protection by this class of drugs might be present. Bell *et al.* recently showed very elegantly in mice hearts that administration of atorvastatin during reperfusion after a period of ischaemia significantly reduces infarct size independent of lipid lowering.[74] This protection was achieved by activation of a signalling cascade involving phosphatidylinositol 3-kinase (PI3K), the protein kinase Akt and eNOS. Alternative mechanisms of cardioprotection by statins include inhibition of neutrophil activation and preservation of NO-synthase activity after ischaemia and reperfusion,[75] which could result from inhibition of the mevalonate pathway and subsequent inhibition of the Rho/Rho kinase pathway.[76]

The last drug which has been shown to mimic preconditioning in humans *in vivo* and which acts on the level of the triggers of ischaemic preconditioning is the NO donor nitroglycerin. NO has been implicated especially in delayed preconditioning and this has been tested recently in the model of repeated PTCA.[77] Patients admitted for stable or unstable angina were randomised to receive a four-hour intravenous infusion of nitroglycerin or placebo 24 hours before PTCA. It appeared that nitroglycerin pretreatment, independent of collateral recruitment, rendered the heart resistant against ischaemia, as assessed by ST-segment shift, wall motion and subjective pain.

More distal to the trigger phase of ischaemic preconditioning, opening of mitochondrial K_{ATP} channels is essential for the occurrence of protection by ischaemic preconditioning. Drugs that interfere with K_{ATP} channel opening could therefore theoretically inhibit this protection. Indeed, using recovery of contractile function of human atrial trabeculae as endpoint of ischaemic injury, Cleveland *et al.* showed in an observational study that preconditioning is abolished in patients with type 2 diabetes using glibenclamide or glipizide compared with type 2 diabetics on insulin.[78] Moreover, it was shown that pretreatment with 10 mg of glibenclamide orally before PTCA abolishes ischaemic preconditioning as assessed by anginal pain and ST-segment shift in nondiabetics[79] and in the same model, ischaemic preconditioning was inhibited in type 2 diabetics who were chronically on glibenclamide.[80] Interestingly, in the same model, the newer sulphonylurea glimepiride did not abolish protection by ischaemic preconditioning,[80,81] possibly because it blocks extrapancreatic K_{ATP} channels to a lesser extent than glibenclamide. Finally, Scognamiglio *et al.* showed that type 2 diabetics randomised to the use of insulin have less myocardial dysfunction during dipyridamole stress echocardiography than patients on glibenclamide.[82] However, this model is not well suited for this purpose, because dipyridamole itself is able to provide cardioprotection, as mentioned earlier. Despite the limitations inherent to the human models used, glibenclamide does seem to inhibit ischaemic preconditioning. Does this mean that diabetics who take sulphonylureas are at increased risk for cardiovascular morbidity and mortality? This discussion was opened by the observation in the UGDP study that patients on tolbutamide have an increased cardiovascular mortality rate.[83] In the UKPDS, treatment with metformin decreased mortality, whereas treatment with glibenclamide did not reduce mortality.[84,85] Additionally, various smaller trials have provided conflicting results on the effect of sulphonylureas on cardiovascular morbidity and mortality.[86] In conclusion, there is no convincing evidence that use of sulphonylureas is associated with worse cardiovascular outcome in general clinical practice. Interestingly, in special situations of profound cardiac ischaemia, sulphonylureas may have detrimental effects: diabetics on sulphonylureas did have a higher in-hospital mortality after PTCA for acute myocardial infarction compared with diabetics not on sulphonylureas.[87] However, because most of these latter patients were on insulin, this could also point to a beneficial effect of insulin. At this point, it needs to be realised that insulin, independent of glucose lowering, can reduce myocardial infarction, when administrated early in reperfusion, acting via the Akt prosurvival pathway.[88,89]

In contrast to K_{ATP} channel blocking, pharmacological opening of these channels provides beneficial effects on myocardial ischaemia/reperfusion injury. Indeed, many animal studies have shown that pretreatment with the K_{ATP} channel opener diazoxide mimics the infarct size limiting effect of ischaemic preconditioning.[90] Similarly, ischaemic preconditioning mimicking effects of diazoxide have been shown in the human atrial trabeculae model.[91,92] Very recently, Wang *et al.* demonstrated that patients randomised to pretreatment with an intravenous bolus of diazoxide five minutes before commencing cardiopulmonary bypass for CABG have significantly better improvement of cardiac index after surgery.[93] More clinically oriented research has been done with nicorandil, a hybrid of a nitrate and a K_{ATP} channel opener, registered for use in patients with stable angina. This drug has been shown to reduce infarct size in several animal models via its opening of K_{ATP} channels, both acutely and after 24 hours.[94,95] In humans, pretreatment with an intravenous bolus of nicorandil before PTCA in patients with stable angina appeared to limit ST-segment shift independent of myocardial blood flow.[96,97] However, it needs to be emphasised that ST-segment shift is determined by sarcolemmal K_{ATP} channels, which are influenced by nicorandil but are probably less important in preconditioning, making this parameter highly unreliable for this goal. That these beneficial effects of nicorandil in the experimental setting could indeed also be applied to the clinical

setting is demonstrated by Patel *et al.*[98] They showed that patients with unstable angina who were randomised to nicorandil 20 mg orally twice daily added to an aggressive antianginal treatment with acetylsalicylic acid, β-blockers and diltiazem, suffer less myocardial ischaemia and ventricular arrhythmias in the first 48 hours after admission than the control group. The preconditioning mimicking effect of nicorandil could also have contributed to the results of the Impact Of Nicorandil in Angina (IONA) study, which showed a significant reduction in major coronary events in patients with stable angina and additional cardiovascular risk factors added to conventional antianginal therapy.[99] The role of preconditioning in this study, however, remains speculative.

Another class of drugs that are known for their potential to influence K_{ATP} channels are volatile anaesthetics. Because of the inherent timing before the start of operations and because of the relative ease of administration, this group of drugs would be especially suited to use for early cardioprotection. Indeed, in 1997 it was first described that isoflurane mimics the protective effect of ischaemic preconditioning in rabbits and dogs.[100,101] Today, it is known that most anaesthetics are able to mimic, enhance or inhibit ischaemic preconditioning, which was recently reviewed by Zaugg *et al.*[102,103] and Riess *et al.*[104] In animal studies, protective effects have been demonstrated for isoflurane, enflurane, halothane, sevoflurane and desflurane. Adenosine A1 receptor stimulation, PKC activation and opening of K_{ATP} channels have all been implicated in the mechanism of this protection.[103] On the contrary, various intravenous anaesthetics have been shown to inhibit opening of mitoK_{ATP} channels *in vitro* and it was demonstrated that R-ketamine, thiopental and pentobarbital inhibit diazoxide-induced protection in isolated rat ventricular myocytes.[102,105] Recently, a few small trials have investigated the effects of isoflurane, enflurane and sevoflurane preconditioning in patients undergoing CABG. These data provide evidence, although not always significant, that these anaesthetics are able to provide some protection as assessed by postoperative CK-MB and troponin I release and postoperative myocardial function.[102]

A recent randomised study even concluded that sevoflurane preconditioning in CABG patients preserves myocardial as well as renal function as assessed by postoperative plasma levels of N-terminal pro-brain natriuretic peptide and cystatin C, respectively. However, more traditional markers (CK-MB, troponin T and creatinine) were not improved by preconditioning.[106] Finally, considering anaesthesia, it has to be mentioned that opioid receptor agonists, which are frequently used in the perioperative timeframe, are also able to provide cardioprotection by preconditioning in animal models[107] and in isolated human atrial trabeculae.[108] Using the model of repeated PTCA in humans *in vivo*, Tomai *et al.* showed that pretreatment with the opioid-receptor antagonist naloxone completely blocks the protective effect of ischaemic preconditioning.[109] Interestingly, it was recently shown that volatile anaesthetics and opioids may work in conjunction to confer protection against myocardial infarction through potentiation of cardiac K_{ATP} channel opening.[110]

Besides pharmacological agents, compounds present in daily food and drink could also be able to provide protection against ischaemia/reperfusion injury. It is known that moderate alcohol consumption is associated with a decreased risk of cardiovascular disease.[111,112] Moreover, it was found that moderate drinking is associated with increased survival once acute myocardial infarction has occurred.[113] Besides beneficial alterations in lipid metabolism and platelet function, preconditioning of the myocardium by ethanol could contribute to this beneficial effect of alcohol consumption. Indeed, accumulating evidence from various animal models demonstrates that chronic as well as acute ethanol consumption reduces myocardial ischaemia/reperfusion damage by mimicking ischaemic preconditioning.[114-118] Hearts from guinea pigs drinking ethanol for 3 to 12 weeks showed improved functional recovery and reduced myocyte damage after ischaemia and reperfusion.[116] This preconditioning mimicking effect was completely abolished by adenosine A1 receptor blockade during the index ischaemia.[116] Indeed, it has already been shown that ethanol increases extracellular adenosine concentration by inhibiting cellular adenosine uptake,[119] and this mechanism could be involved in the previously described beneficial effect of ethanol. However, in rats, alcohol-induced cardioprotection was not blocked by adenosine receptor antagonists, whereas α-adrenergic antagonism did block this protection, suggesting species-specific signalling.[114] More recently, Miyamae *et al.* showed that chronic ethanol consumption induces a sustained translocation of PKC-ε from the cytosolic to the particulate fraction and that cardioprotection by ethanol is critically dependent on PKC activity during the index ischaemia.[115]

Acute ethanol ingestion shortly before the ischaemic insult, resulting in a concentration similar to that achieved after one to two alcoholic beverages, similarly provided protection by direct activation of PKC-ε.[118] Finally, the infarct size limiting effect of chronic ethanol ingestion in dogs was abolished by administration of glibenclamide during ischaemia, thus providing evidence that opening of K_{ATP} channels is crucial for this protection to occur.[117] In conclusion, chronic as well as acute consumption of alcohol provides protection against ischaemic injury in several animal species via adenosine and α-adrenergic receptor stimulation, PKC-ε translocation and opening of K_{ATP} channels.

THERAPEUTIC EXPLOITATION

From the evidence outlined in the present paper, it appears that also in the human myocardium, ischaemic preconditioning can significantly increase tolerance to ischaemia and reperfusion. However, in clinical practice, the application of short periods of ischaemia to induce preconditioning is in most circumstances not desirable or feasible. However, several classes of drugs have been described with the potential to enhance, mimic or inhibit ischaemic preconditioning. The prudent use, or avoidance, of these agents may be a more benign approach to elicit cardioprotection in clinical practice.

Because of the relatively tight time boundaries of protection by ischaemic and pharmacological preconditioning, it is essential to apply the pharmacological intervention shortly before the prolonged ischaemic period. However, myocardial ischaemia is seldom planned and accurately predicted. However, two situations in which temporary myocardial ischaemia can readily be predicted are PTCA and CABG. Although routine PTCA carries a small risk for complications, this risk is increased in a high-risk situation, such as unstable angina. Especially in these situations, pretreatment with preconditioning-mimicking drugs could be beneficial. Conversely, the temporary withdrawal of drugs which are known to interfere with preconditioning, such as K_{ATP} blockers or adenosine antagonists, could increase tolerance to ischaemia. Garratt *et al*. have shown in an observational study that diabetics taking sulphonylureas have increased in-hospital mortality after PTCA for acute myocardial infarction compared with diabetics who are not on sulphonylureas.[87] Again, it needs to be realised that this survival benefit could also be caused by the beneficial effect of insulin in the control group. Interestingly, in the setting of PTCA, preconditioning by repeated balloon inflations could also be used to stratify patients for their risk of adverse ischaemic events. Recently, Laskey *et al*. showed that 20% of patients undergoing PTCA fail to manifest ischaemic preconditioning, and that this is significantly associated with an increased risk of death or nonfatal myocardial infarction at one year of follow-up.[120]

A second situation in which cardiac ischaemia is planned and may consequently be preceded by a preconditioning stimulus is CABG, as described above. However, the protective effect of ischaemic or pharmacological preconditioning is still controversial, especially when other techniques than intermittent cross-clamp fibrillation are used.[32,33] It is argued that the protection afforded by cardioplegia and anaesthetics leaves little room for additional protection by preconditioning.[32,33] Moreover, it has been shown that cardiopulmonary bypass alone is able to provide cardioprotection comparable with classic ischaemic preconditioning in sheep hearts.[44] Similarly, in a recent study in humans, preconditioning with ischaemia only offered additional protection during CABG when no cardiopulmonary bypass was used.[45] Thus, preconditioning may only be indicated in settings in which conventional protection is anticipated against be suboptimal, for example in long duration or severe atherosclerosis.[33] Moreover, when considering protection against postoperative pump failure, it needs to be realised that stunning, more than discrete necrosis or apoptosis, might be responsible for this, and that early preconditioning probably does not protect against stunning. Perhaps a more successful, albeit less heroic, approach might be the elimination before surgery of factors with potential inhibiting effects on preconditioning, such as the use of sulphonylureas or caffeine.

Finally, considering anticipated periods of cardiac ischaemia, preliminary evidence exists that ischaemic preconditioning might be beneficial in transplantation. In sheep heart, recovery of systolic function was improved when a short period of ischaemia was applied before the explantation.[121]

Perhaps more benefit from pharmacological preconditioning could be expected when applied to patients at high risk for myocardial infarction, despite adequate conventional treatment. This would particularly concern patients with non-ST-segment elevation acute coronary syndromes, including unstable angina, who are at high risk of progression to complete coronary occlusion. More than 10% will die or suffer a myocardial infarction within six months, with half of these events occurring in the acute phase.[122] Pharmacological preconditioning during this phase could potentially reduce the amount of ischaemic damage. However, as the duration of protection afforded is limited, repeated dosing of the preconditioning drug is necessary to maintain the preconditioned state. Although a 72-hour continuous infusion of an adenosine A1 selective agonist in rabbits was not able to limit infarct size, suggesting receptor downregulation,[123] a more recent study in rabbits showed that repeated bolus injections of an adenosine A1 selective agonist at 48-hour intervals still provides strong limitation of infarct size at day 10.[124] Moreover, consumption of dipyridamole, added to the drinking water for two to six weeks, resulted in an attenuation of ischaemia/reperfusion injury in guinea pigs.[125]

Even more benefit from pharmacological modulation of preconditioning might be expected in large groups of patients with an increased baseline risk for cardiovascular disease, such as diabetics. Sulphonylureas are associated with an unexpected and unexplained small increase in cardiovascular mortality in several trials, as described previously. Reducing the use of sulphonylureas could potentially confer benefit to this patient group, with regards to cardiovascular morbidity and mortality.

Very recently, several studies have shown that pharmacological interventions during early reperfusion are also able

to limit infarct size.[126] This approach circumvents the problem that the ischaemic insult is mostly unpredictable, because these drugs can be given at reperfusion rather than before the event and are therefore more clinically applicable, but outside the scope of this review on preconditioning. Briefly, in the AMISTAD trial, it was shown that adenosine as an adjunct to thrombolysis results in a significant reduction of infarct size.[127] Also, infarct size limitation has been shown for insulin,[88,89] atorvastatin,[74] 5'-(N-ethylcarboxamido) adenosine and bradykinin,[128] all via activation of the PI3K/Akt pathway during reperfusion. Also, cyclosporine limits infarct size when administered during reperfusion by inhibiting opening of the mitochondrial permeability transition pore (MPTP).[129] Additional studies have to be performed to show whether this approach could offer clinical benefits.

A final word of caution regarding the potential therapeutic benefits of preconditioning concerns the reported effects of ageing and disease on ischaemic preconditioning.
In the literature, it is repeatedly mentioned that the protective effect of preconditioning may be lost in aged myocardium, in which cardioprotection is undoubtedly more relevant, although there is still no consensus on this subject. Studies on isolated hearts show that the effect of preconditioning is decreased in aged rats,[130-133] but not in aged rabbits[134,135] In humans, a similar controversy exists in the various models of ischaemic preconditioning.[136-139] Decrease in norepinephrine release during the preconditioning episode,[130] attenuated activation of K_{ATP} channels[137] and failure of adequate translocation of PKC isoforms[132] have all been implicated in this reduced protective effect of preconditioning in the aged heart.
A similar controversy exists as to whether protection by preconditioning is still present in the diseased heart, especially concerning diabetes and hypercholesterolaemia, the very conditions in which cardioprotection is particularly important. Although some studies indeed show protection by ischaemic preconditioning in diabetic rats,[140] most studies in rabbits and dogs demonstrated that diabetes abolishes protection by ischaemic preconditioning.[141-143] In dogs, it appeared that both streptozotocin-induced diabetes and hyperglycaemia by dextrose infusion inhibit the infarct-sparing effect of preconditioning, probably due to impaired activation of mitoK_{ATP} channels.[142,144,145] Similarly, in an observational study both preconditioning by ischaemia and by pretreatment with diazoxide was abolished in atrial tissue taken from patients with type 1 diabetes using insulin and from patients with type 2 diabetes on sulphonylureas but it was not abolished in patients with diet-controlled diabetes.[146] Finally, it is reported in the literature that the protective effect of preinfarction angina is diminished in patients with diabetes.[16,147] The lack of protection afforded by ischaemic preconditioning in patients with diabetes could well contribute to the consistently shown worse outcome after myocardial infarction in these patients compared with patients without diabetes.[148,149]
Considering hypercholesterolaemia, there is less evidence from the literature. There are studies that show preserved protective effects of ischaemic preconditioning[150,151] as well as studies that show reduced protection by ischaemic preconditioning in hypercholesterolaemic rabbits.[71]
Considering evidence in humans, it was recently shown that in patients with high plasma cholesterol, the preconditioning by repeated PTCA is reduced as compared with patients with normal cholesterol levels.[152] Considering other risk factors for atherosclerosis, little is known about the influence of smoking, hypertension and hyperhomocysteinaemia on the effect of ischaemic preconditioning. Regarding hypertension, it has been shown that protection is still present in spontaneously hypertensive rats and in hypertrophied myocardium from saline loaded rats.[153,154] When interpreting these data on aged and diseased hearts, one has to bear in mind that the exact signalling mechanism involved in preconditioning is dependent on the nature of the preconditioning stimulus.[155] Because the effects of ageing or disease might be limited to specific triggers, such as adenosine,[156] it is conceivable that failure to precondition these hearts is influenced by the choice of the preconditioning stimulus.

In summary, there is a wealth of both *in vitro* and *in vivo* evidence that ischaemic preconditioning also occurs in humans. Since the description of this phenomenon, several classes of drugs have been described which are able to mimic, enhance or inhibit ischaemic preconditioning. The use or avoidance of these drugs before procedures known to induce myocardial ischaemia or in patients at risk for myocardial infarction in general could theoretically reduce ischaemia and reperfusion injury and improve outcome. We recently developed a minimally invasive technique to monitor ischaemic tolerance in humans *in vivo*. Future clinical trials with this technique are needed to address the question whether this method can be used to individualise pharmacotherapy in order to optimise resistance to ischaemia-reperfusion and outcome in patients who are particularly vulnerable to ischaemic cell death: patients at risk for arterial thrombosis and patients with heart failure.

ACKNOWLEDGEMENTS

N.P. Riksen is a MD clinical research trainee financially supported by the Netherlands Organisation of Scientific Research (ZonMw). G.A. Rongen's contribution was made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences (KNAW).

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