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Transient Limb Ischemia Induces Remote Preconditioning and Remote Postconditioning in Humans by a K_{ATP} Channel–Dependent Mechanism

Stavros P. Loukogeorgakis, MBBS, BSc, PhD; Rupert Williams, MBBS, BSc; Anna T. Panagiotidou, MBBS, BSc; Shyamsunder K. Kolvekar, MBBS, MS, MCh, FRCS, FRCSTh; Ann Donald, AVS; Tim J. Cole, BA, BPhil, MA, PhD, ScD; Derek M. Yellon, PhD, DSc, Hon FRCP; John E. Deanfield, BA, BChir, MB, FRCP; Raymond J. MacAllister, MA, MD, FRCP

Background—Transient limb ischemia administered before a prolonged ischemic insult has systemic protective effects against ischemia-reperfusion (IR) injury (remote ischemic preconditioning [RIPC]). It has been demonstrated that protection from IR can be achieved by brief periods of ischemia applied at a remote site during an injurious ischemic event (remote postconditioning [RPostC]). Using an in vivo model of endothelial IR injury, we sought to determine whether RPostC occurred in humans and whether it shared mechanistic similarities with RIPC.

Methods and Results—Endothelial function was assessed by flow-mediated dilation before and after IR (20 minutes of arm ischemia followed by reperfusion). RIPC was induced by conditioning cycles of 5 minutes of ischemia and reperfusion on the contralateral arm or leg before IR. For RPostC induction, conditioning cycles were administered during the ischemic phase of IR. Oral glibenclamide was used to determine the dependence of RIPC and RPostC on K_{ATP} channels. IR caused a significant reduction in flow-mediated dilation in healthy volunteers (baseline, 9.3±1.2% versus post-IR, 3.3±0.7%; $P<0.0001$) and patients with atherosclerosis (baseline, 5.5±0.6% versus post-IR, 2.3±0.5%; $P<0.01$). This reduction was prevented by RIPC (post-IR+RIPC: healthy volunteers, 7.2±0.5% [$P<0.0001$ versus post-IR]; patients, 4.5±0.3% [$P<0.01$ versus post-IR]) and RPostC (post-IR+RPostC: 8.0±0.5%; $P<0.0001$ versus post-IR). The protective effects of RIPC and RPostC were blocked by glibenclamide.

Conclusions—This study demonstrates for the first time in humans that RPostC can be induced by transient limb ischemia and is as effective as RIPC in preventing endothelial IR injury. RIPC and RPostC share mechanistic similarities, with protection being dependent on K_{ATP} channel activation. These results suggest that remote conditioning stimuli could be protective in patients with acute ischemia about to undergo therapeutic reperfusion. **(*Circulation*. 2007;116:1386-1395.)**

Key Words: ischemia ■ ischemic preconditioning ■ potassium channels ■ reperfusion injury

Ischemic preconditioning (IPC) and ischemic postconditioning (PostC) are mechanisms that protect tissues from injury during ischemia and subsequent reperfusion (ischemia-reperfusion [IR] injury).[1] IPC is initiated by short periods of noninjurious IR applied before a prolonged ischemic insult and reduces tissue damage caused by IR injury.[2] In contrast, PostC describes a modified schedule of reperfusion characterized by intermittent restoration of blood flow after a prolonged episode of ischemia.[3] When instituted after a potentially damaging ischemic insult, PostC causes a degree of tissue protection similar to IPC.[4,5] This has led to the concept that much of the reversible tissue damage that occurs in experimental IR injury is sustained during early reperfusion rather than during ischemia; PostC clearly causes tissue salvage by moderating damage during this phase, and it is possible that this also is true for IPC. Moreover, IPC and PostC share mechanistic similarities, with both interventions activating reperfusion injury survival kinases and mitochondrial K_{ATP} channels as part of the process whereby tissue protection is activated.[1,3]

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From the Vascular Physiology Unit, Institute of Child Health (S.P.L., R.W., A.T.P., A.D., J.E.D.), MRC Centre of Epidemiology for Child Health, Institute of Child Health (T.J.C.), Hatter Cardiovascular Institute and Centre for Cardiology (D.M.Y.), and Centre for Clinical Pharmacology and Therapeutics (S.P.L.), University College London; and Department of Cardiothoracic Surgery, UCL Hospitals NHS Foundation Trust (S.K.K.), London, United Kingdom.

Correspondence to Dr S.P. Loukogeorgakis, Vascular Physiology Unit, Institute of Child Health, University College London, 34 Great Ormond St, London, WC1N 3JE, UK. E-mail s.loukogeorgakis@ich.ucl.ac.uk

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The clinical applicability of IPC and PostC is limited by logistics; preconditioning requires advance warning of ischemic events, a circumstance that is unlikely to arise given their unpredictable nature in clinical practice. PostC can be performed after an ischemic event has occurred, but effecting intermittent reperfusion of vital organs requires mechanical intervention, which is possible in ST-elevation myocardial infarction, improbable in other acute coronary syndromes, and implausible in stroke.

However, systemic effects of ischemic conditioning stimuli exist that might be used to harness their protection in acute ischemic syndromes. Preconditioning the intestine,[6] kidney,[7] or limb[8] in advance of an ischemic insult protects the myocardium and other tissues from IR injury, a facet of IPC called remote IPC (RIPC) and extensively investigated in a range of animal models[9–11] and by our group in humans.[8,12] Remote PostC (RPostC) is a hybrid of RIPC and PostC in that the conditioning ischemic stimulus is applied at a remote site but contemporaneously with a prolonged, injurious ischemic insult.[13] The remote effects of an ischemic conditioning stimulus applied to an organ that is relatively resistant to ischemia (such as the limb) might protect tissues that are more sensitive to ischemia (such as the heart and brain).

We have used IR injury to the vascular endothelium as a model to characterize IPC and PostC in humans; in this model, IR injury causes transient endothelial dysfunction that is prevented by IPC[14] and PostC.[15] We also have used this model to demonstrate remote effects of IPC in humans.[12] The aims of the present study were to determine whether RPostC occurred in humans and, if so, whether RIPC and RPostC shared similar mechanisms.

Methods

Subjects

One hundred thirty-five studies were performed on 19 healthy volunteers (10 men, 9 women; mean±SD age, 24.9±5.4 years). Twelve studies were performed on 6 patients with atherosclerotic disease (4 men, 2 women; mean±SD age, 61.0±9.1 years). Patients were recruited from outpatient clinics at University College London Hospitals NHS Foundation Trust within 6 months after undergoing coronary artery bypass graft surgery. All volunteers gave informed consent. Studies were approved by the local research ethics committee and performed in a temperature-controlled laboratory (24°C to 26°C). All studies repeated in the same volunteers were at least 7 days apart and were performed in random sequence to avoid confounding learning effects.

Induction of IR

The nondominant arm was made ischemic by inflating a 9-cm-wide blood pressure cuff placed around the upper part of the arm to a pressure of 200 mm Hg for 20 minutes, as described previously.[12]

Induction of RIPC and RPostC

RIPC and RPostC were induced by inflating a 9-cm-wide blood pressure cuff placed around the upper part of the contralateral arm or the contralateral leg. The cuff was inflated to 200 mm Hg for 5 minutes (ischemia of the arm or leg, respectively), followed by a 5-minute deflation (reperfusion). This constituted a conditioning cycle, and 2 to 3 cycles were used in the protocols described below. The timing of the conditioning cycle with respect to the IR stimulus determined whether RIPC or RPostC was induced; for RIPC, the conditioning cycle was completed in advance of IR, whereas for RPostC, the conditioning cycle was induced during IR.

Assessment of Conduit Vessel Function

Conduit vessel endothelial function was assessed by measuring flow-mediated dilation (FMD) of the brachial artery in the nondominant arm, as described previously.[15,16] The dilator response of the brachial artery to glyceryl trinitrate (GTN; 25 μg sublingually) was used to assess endothelium-independent dilation.

Experimental Protocols

Effect of IR on Vascular Dilator Function

To determine the effect of IR on endothelial function, FMD was assessed before ischemia (baseline) and 20 minutes after reperfusion (n=11; Figure 1, protocol a). Similarly, the effect of IR on smooth muscle function was determined in separate studies by assessing the dilation of the brachial artery in response to sublingual GTN (25 μg) before and after IR (n=6), as described previously.[12]

Effect of RIPC on Endothelial IR Injury in Healthy Volunteers

FMD was assessed before and after IR but immediately preceded by RIPC. The following RIPC stimuli were tested for their potential to induce protection against IR-induced endothelial dysfunction: (1) 2 RIPC cycles applied on the arm (RIPCArm2C; n=9; Figure 1, protocol b), (2) 3 RIPC cycles applied on the arm (RIPCArm3C; n=9; Figure 1, protocol c), (3) 2 RIPC cycles applied on the leg (RIPCLeg2C; n=9; Figure 1, protocol b), and (4) 3 RIPC cycles applied on the leg (RIPCLeg3C; n=9; Figure 1, protocol c). In control studies, the dilation of the brachial artery in response to GTN 25 μg was measured before and after RIPC (3 cycles applied on the contralateral arm; RIPCArm3C) alone to determine whether RIPC had a direct effect on vascular smooth muscle function (n=6). We have demonstrated previously that such an RIPC protocol does not directly affect brachial artery endothelial function.[12]

Effect of RIPC on Endothelial IR Injury in Patients With Atherosclerosis

To determine whether the protective effects of RIPC against endothelial IR injury remain intact in patients with atherosclerosis, we recruited patients who had undergone coronary artery bypass graft surgery within the last 6 months with no evidence of active myocardial ischemia (angina) after surgery. Patients using nitrates, K_{ATP} channel openers (eg, nicorandil), or sulfonylureas (eg, glibenclamide) were excluded. FMD was measured at baseline and after IR alone (n=6; Figure 1, protocol a) or IR preceded by RIPC (RIPCArm3C; n=6; Figure 1, protocol c).

Mechanism of Protection by RIPC: Role of K_{ATP} channels

Ten healthy volunteers underwent repeat assessment of the effects of IR alone (Figure 1, protocol a) and IR immediately preceded by RIPC (RIPCArm3C; Figure 1, protocol c) on endothelial function. To assess the role of K_{ATP} channels, on a different study day, the same group of volunteers received 5 mg of the nonselective K_{ATP} channel blocker glibenclamide orally. To achieve peak blood concentration of the drug before the start of the study, glibenclamide was administered 45 minutes before baseline FMD assessment.[17] This was followed by the application of a 3-cycle RIPC stimulus on the contralateral arm, IR, and a final FMD assessment (Figure 2, protocol a). To exclude a direct effect of glibenclamide on endothelial IR injury, the protocol was repeated without RIPC (Figure 2, protocol b). In all studies, a high-carbohydrate meal (carbohydrates, 59 g; fat, 9 g; protein, 5 g; energy, 340 kcal) was given immediately and 3 hours (carbohydrates, 115 g; fat, 20 g; protein, 27 g; total energy, 750 kcal) after the administration of glibenclamide.[18] Blood glucose was monitored throughout the study.

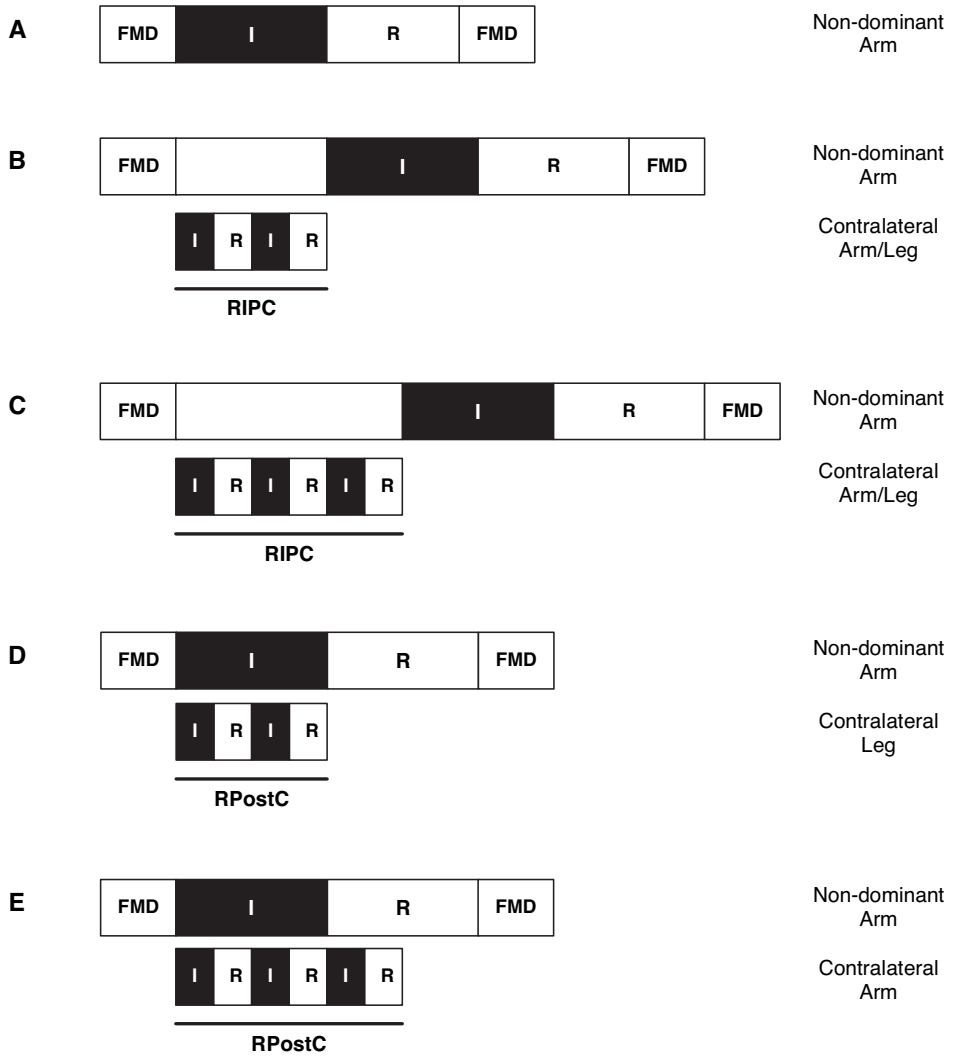

Figure 1. Protocol of studies to determine the effect of RIPC and RPostC on endothelial IR injury. FMD of the brachial artery was assessed before 20 minutes of arm ischemia (I) and at 20 minutes of reperfusion (R) (A). The effect of RIPC on endothelial IR injury was determined by applying 3 RIPC cycles (3 cycles of 5 minutes of I and 5 minutes of R) on the contralateral arm or leg (B) or 2 RIPC cycles (contralateral arm or leg) (C) immediately before IR. The effects of RPostC on endothelial IR injury were determined by applying 2 RPostC cycles (2 cycles of 5 minutes of I and 5 minutes of R) on the contralateral leg during index ischemia (D). To assess whether a sufficient RPostC stimulus needs to be applied in full during ischemia, 3 RPostC cycles were applied on the contralateral arm during ischemia and early reperfusion (E).

Effect of RPostC on Endothelial IR Injury

Nine healthy volunteers underwent assessment of the effects of IR on endothelial function (Figure 1, protocol a). To determine whether transient limb ischemia induces remote protection by modifying the reperfusion phase of endothelial IR injury (RPostC), 2 cycles of 5 minutes of ischemia and reperfusion were applied on the contralateral leg during the 20-minute ischemic insult (index ischemia) to the nondominant arm (RPostCLeg2C; n=9; Figure 1, protocol d). This stimulus was sufficient to prevent endothelial injury when applied in advance of IR (see the Effect of RIPC on Endothelial IR Injury in Healthy Volunteers section) and was chosen because it allowed the completion of the RPostC protocol during index ischemia. To establish whether it was necessary for the RPostC stimulus to be applied in full before the onset of reperfusion, a 3-cycle stimulus was administered on the contralateral arm during ischemia and early reperfusion (RPostCArm3C; n=9; Figure 1, protocol e). This protocol allowed the completion of 2 cycles of RPostC on the arm during index ischemia, but the final cycle was completed after reperfusion had begun.

Mechanism of Protection by RPostC: Role of K_{ATP} Channels

To establish whether protection by RPostC is dependent on K_{ATP} channel activation, the RPostCLeg2C protocol was repeated in the same group of healthy volunteers in the presence of systemic glibenclamide (n=7; Figure 2, protocol c). Glibenclamide (5 mg) was administered orally 45 minutes before baseline assessment of endothelial function, and high-carbohydrate meals were given to prevent hypoglycemia, as mentioned previously.

Calculations and Statistical Analysis

All data are expressed as mean±SEM unless otherwise stated. Brachial artery diameter was measured in millimeters, and dilation was expressed as percentage increase from baseline diameter. Data were compared by use of a paired Student *t* test or 1-way ANCOVA as appropriate. Comparisons by ANCOVA were performed between post-IR values adjusted for baseline FMD as the covariate. Adjustment for baseline FMD led to a nonsignificant between-subject term in the repeated-measures analysis. For multiple comparisons, prob-

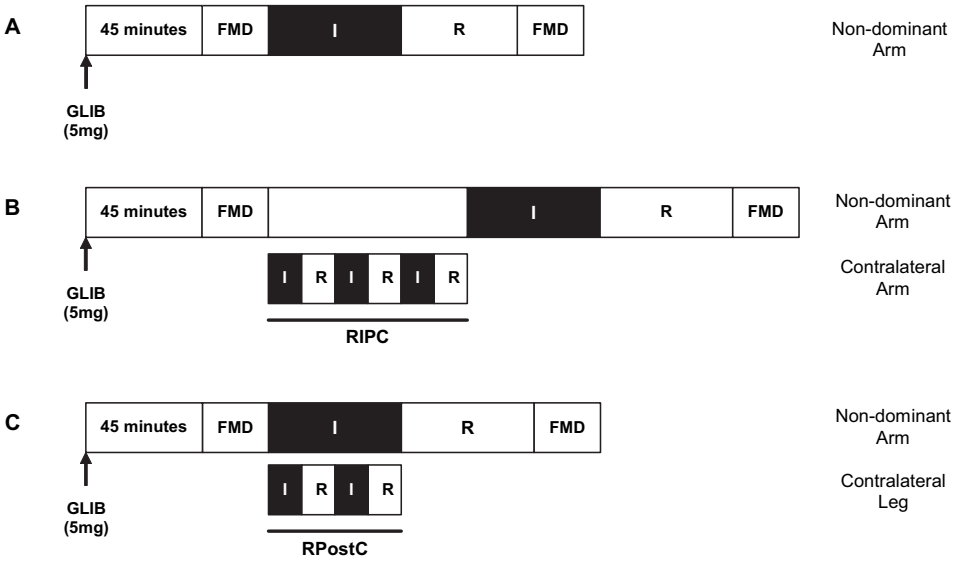

Figure 2. Protocol of studies to determine the role of K_{ATP} channels in the mechanism of protection by RIPC and RPostC. FMD of the brachial artery was assessed at baseline and after 20 minutes of arm ischemia (I) and 20 minutes of reperfusion (R). To determine the role of K_{ATP} channels in protection by RIPC, 3 RIPC cycles were applied on the contralateral arm immediately before IR in the presence of systemic glibenclamide (Glib; 5 mg) administered orally 45 minutes before baseline endothelial function assessment (B). Oral glibenclamide also was used to determine the role of K_{ATP} channels in protection by RPostC (2 RPostC cycles applied on the contralateral leg during index ischemia; C). Protocol A was designed to exclude any direct effects of glibenclamide on the endothelial response to IR.

ability values by ANCOVA were Scheffé adjusted. In all cases, values of $P<0.05$ using 2-tailed tests were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

All subjects tolerated the procedures without any complications. The procedures used to induce IR, RIPC, or RPostC had no effect on blood pressure, heart rate, brachial artery diameter, or FMD flow stimulus during reactive hyperemia (data not shown). Baseline blood glucose concentration was 5.3±0.1 mmol/L and fell slightly 2.5 to 3 hours after glibenclamide administration (4.2±0.1 mmol/L; $P<0.001$ versus baseline, paired *t* test). Blood glucose levels remained within normal limits in all studies, and no hypoglycemic events occurred. Glibenclamide had no effect on blood pressure, heart rate, brachial artery diameter, or FMD flow stimulus during reactive hyperemia (data not shown).

Effect of IR on Vascular Dilator Function

IR reduced brachial artery FMD (Figure 3a) but had no effect on arterial dilation in response to GTN (Figure 3b). A significant correlation existed between baseline and post-IR FMD (Pearson's $r=0.8$, $P<0.01$). This correlation justified using ANCOVA to adjust post-IR FMD values for baseline FMD in subsequent analyses.

Effect of RIPC on Endothelial IR Injury in Healthy Volunteers

RIPC (RIPCArm3C or RIPCLeg3C) immediately before IR preserved endothelial function (Table 1 and Figure 4). RIPC

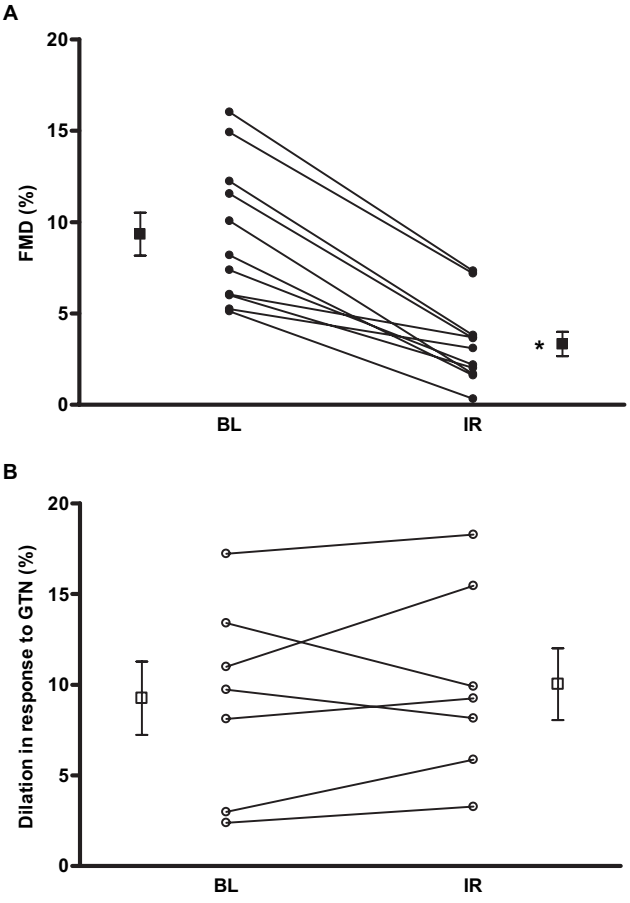

Figure 3. Effect of IR vascular dilator function. A, FMD was 9.3±1.2% at baseline (BL) and was reduced by IR (3.3±0.7%; *$P<0.0001$ vs BL, paired *t* test; n=11). B, GTN dilation was 12.6±1.7% at BL and was unaffected by IR (13.4±1.4%; $P=0.6$ vs BL, paired *t* test; n=6).

TABLE 1. Summary of Baseline and Post-IR FMD Data From Studies to Determine the Effect of RIPC on Endothelial IR Injury in Healthy Volunteers

Study	n	Baseline FMD	Post-IR FMD	Post-IR FMD (ANCOVA Adjusted)
IR	11	9.3±1.2	3.3±0.7	2.8±0.5*†
IR+RIPCArm2C	9	8.2±1.2	4.1±0.9	4.4±0.6‡§
IR+RIPCLeg2C	9	8.9±0.2	7.2±0.9	7.0±0.5
IR+RIPCArm3C	9	8.6±0.9	7.1±0.9	7.2±0.5
IR+RIPCLeg3C	9	8.0±1.2	7.6±1.3	8.0±0.7

Data are expressed as mean±SEM. No differences existed in baseline FMD. Post-IR FMD values were adjusted for baseline FMD by ANCOVA (regression coefficient, 0.70±0.08). For comparisons between the 5 groups, *P* values by ANCOVA were Scheffé adjusted.

*$P<0.0001$, IR vs IR+RIPCArm3C and IR+RIPCLeg3C; †$P<0.001$, IR vs IR+RIPCLeg2C; ‡$P<0.01$, IR+RIPCArm2C vs IR+RIPCLeg3C; §$P<0.05$, IR+RIPCArm2C vs IR+RIPCArm3C.

administered as 2 cycles was effective only when applied to the leg (Table 1 and Figure 4). RIPC had no direct effect on brachial artery smooth muscle function (dilation to GTN: baseline, 11.8±1.5% versus post-RIPC, 11.5±0.9%; $P=0.8$, paired *t* test; n=6).

Effect of RIPC on Endothelial IR Injury in Patients With Atherosclerotic Disease

IR caused a significant reduction in FMD in the patient group (baseline, 5.5±0.6% versus post-IR, 2.3±0.5%; $P<0.01$, paired *t* test; n=6). The IR-induced reduction in FMD was prevented by RIPC (RIPCArm3C) (Table 2).

Mechanism of Protection by RIPC: Role of K_{ATP} Channels

IR resulted in brachial artery endothelial dysfunction (Figure 5a). Glibenclamide did not affect the endothelial response to IR (Table 3 and Figure 5b). RIPC (RIPCArm3C) prevented endothelial dysfunction when applied immediately before IR but had no effect in the presence of systemic glibenclamide (Table 3 and Figure 5b).

Effect of RPostC on the Reperfusion Phase of Endothelial IR Injury

IR caused a significant reduction in brachial artery FMD (Figure 6a). RPostCLeg2C protected the endothelium of the brachial artery against IR injury (Table 4 and Figure 6b). Protection by RPostC required the completion of a sufficient stimulus during index ischemia (before the onset of reperfusion) because RPostCArm3C did not prevent an IR-induced reduction in FMD when applied during ischemia and early reperfusion (Table 4 and Figure 6b). Systemic glibenclamide blocked the protective effects of RPostC (RPostCLeg2C) against endothelial IR injury (Table 4 and Figure 6b).

Discussion

This study demonstrates for the first time in humans that transient limb ischemia is a remote conditioning stimulus that induces contemporaneous protection from the effects of injurious acute ischemia. The degree of protection by RPostC against endothelial IR injury in conduit vessels is similar to that achieved by RIPC, with evidence for a threshold remote conditioning stimulus to induce protection. Activation of K_{ATP} channels is critical for endothelial protection by RIPC and RPostC because the conditioning stimuli were ineffective when applied in the presence of the nonselective K_{ATP} channel blocker glibenclamide.

Vascular endothelial dysfunction is a possible pathogenic mechanism in organ dysfunction precipitated by IR injury, with potential to promote vasoconstriction and

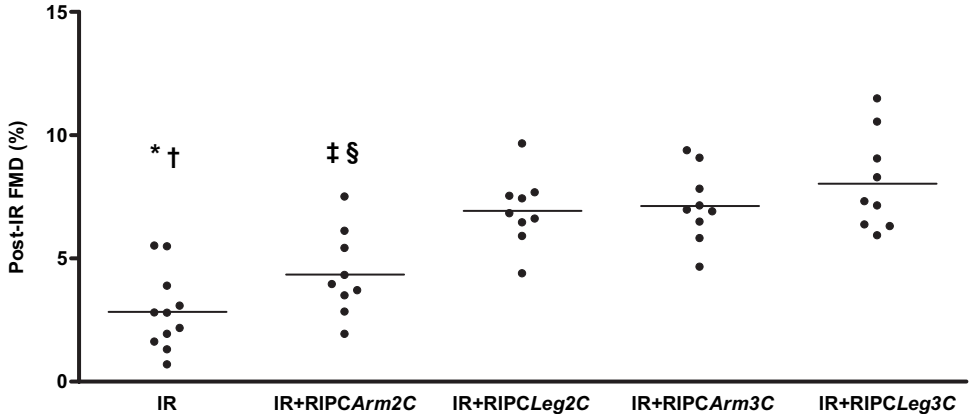

Figure 4. Effect of RIPC on endothelial IR injury in healthy volunteers. RIPCArm3C prevented IR-induced endothelial dysfunction (post-IR FMD, 7.2±0.5%; n=9), but no protection was observed when 2 RIPC cycles were applied on the arm (RIPCArm2C; post-IR FMD, 4.4±0.6%; n=9). RIPCLeg3C prevented the reduction in FMD caused by IR (post-IR FMD, 8.0±0.7%; n=9). RIPCLeg2C also protected from endothelial IR injury (post-IR FMD, 7.2±0.5%; n=9). Post-IR FMD values were adjusted for baseline FMD. *$P<0.0001$, IR vs IR+RIPCArm3C and IR+RIPCLeg3C; †$P<0.001$, IR vs IR+RIPCLeg2C; ‡$P<0.01$, IR+RIPCArm2C vs IR+RIPCLeg3C; §$P<0.05$, IR+RIPCArm2C vs IR+RIPCArm3C (ANCOVA).

TABLE 2. Summary of Baseline and Post-IR FMD Data From Studies to Determine the Effect of RIPC on Endothelial IR Injury in Patients With Atherosclerosis

Study	n	Baseline FMD	Post-IR FMD	Post-IR FMD (ANCOVA-adjusted)
IR	6	5.5±0.6	2.3±0.5	2.1±0.5*
IR+RIPCArm3C	6	4.5±0.6	4.3±0.5	4.5±0.3

Data are expressed as mean±SEM. No differences existed in baseline FMD. Post-IR FMD values were adjusted for baseline FMD by ANCOVA (regression coefficient, 0.54±0.23).

*$P<0.01$, IR vs IR+RIPCArm3C (ANCOVA).

thrombosis through the loss of endothelium-derived factors, including nitric oxide and prostacyclin.[19,20] In this study, the transient endothelial dysfunction of the brachial artery was used to model the protective effects of RIPC and RPostC. In agreement with previously published data from our group,[8,12] IR injury reduced FMD of the brachial artery by ≈50% and was largely prevented by RIPC. RIPCArm3C induced protection, but shortening the stimulus to 2 cycles was not protective. Tissue volume exposed to the preconditioning stimulus also determined the degree of protection because 2-cycle RIPC was effective against endothelial IR injury when applied to the leg. These data are consistent with the requirement for the preconditioning stimulus to cross a threshold[21–23] that may be determined by endogenous mediators, some of which (eg, catecholamines) are released during IR injury. Nonetheless, our small sample sizes cannot exclude a subtle beneficial effect of a subthreshold RIPC protocol on endothelial function after IR. Despite such uncertainties, 4-cycle RIPC of the arm has recently been shown to reduce myocardial injury caused by cardiac bypass surgery in children,[24] raising the possibility that when an ischemic event can be

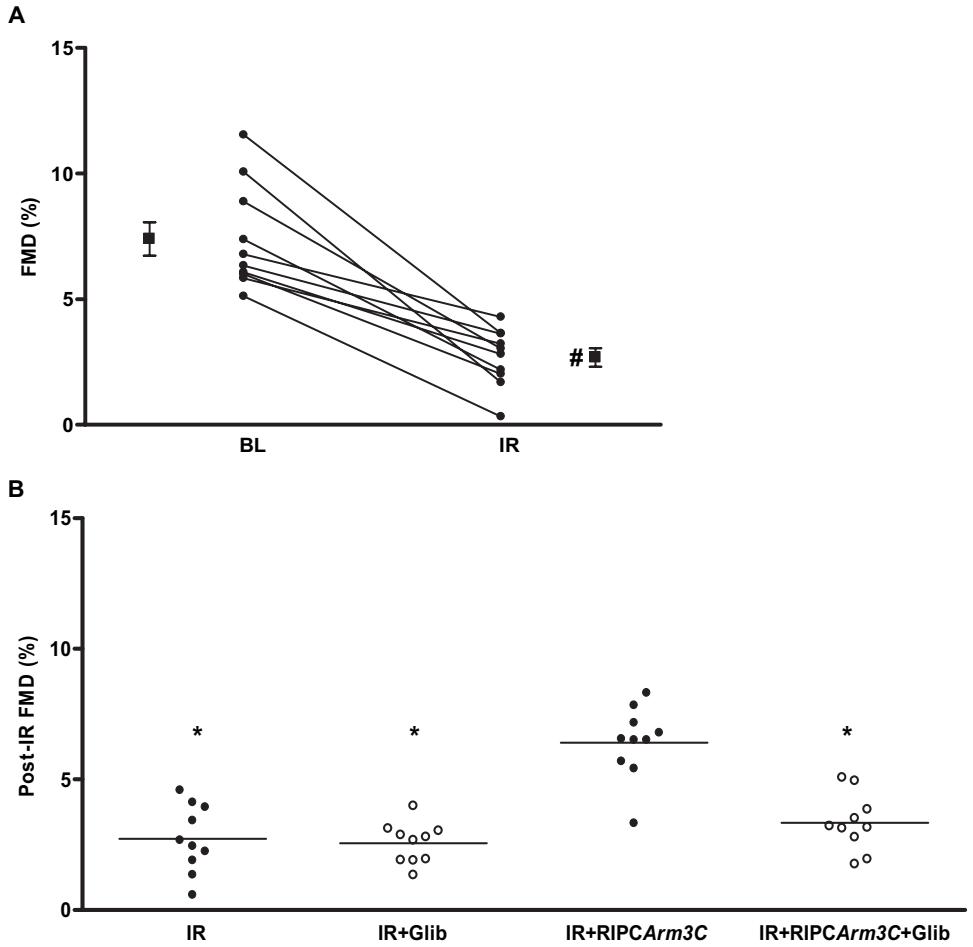

Figure 5. Role of K_{ATP} channels in the mechanism of protection by RIPC. A, FMD was 7.4±0.7% at baseline (BL) and was reduced by IR (2.7±0.4%; #$P<0.0001$ vs BL, paired *t* test; n=10). B, The IR-induced reduction in FMD was prevented by RIPCArm3C (post-IR FMD, 6.4±0.5%; n=10). The protective effects of RIPC were abrogated by systemic glibenclamide (Glib) (post-IR FMD, 3.3±0.3%; n=10). Glibenclamide did not have any direct effects on the endothelial response to IR (post-IR FMD, 2.6±0.2%; n=10). Post-IR FMD values were adjusted for baseline FMD. *$P<0.0001$ IR, IR+Glib, and IR+RIPCArm3C+Glib vs IR+RIPCArm3C (ANCOVA).

TABLE 3. Summary of Baseline and Post-IR Data From Studies to Determine the Role of K_{ATP} Channels in the Mechanism of Protection by RIPC

Study	n	Baseline FMD	Post-IR FMD	Post-IR FMD (ANCOVA Adjusted)
IR	10	7.4±0.7	2.7±0.4	2.7±0.4*
IR+Glib	10	7.6±1.2	2.6±0.4	2.6±0.2*
IR+RIPCArm3C	10	7.8±0.7	6.6±0.7	6.4±0.5
IR+RIPCArm3C+Glib	10	7.1±0.9	3.2±0.5	3.3±0.3*

Data are expressed as mean±SEM. Glib indicates glibenclamide. No differences existed in baseline FMD. Post-IR FMD values were adjusted for baseline FMD by ANCOVA (regression coefficient, 0.43±0.08). For comparisons between the 4 groups, *P* values by ANCOVA were Scheffé adjusted.
*$P<0.0001$, IR, IR+Glib, and IR+RIPCArm3C+Glib vs IR+RIPCArm3C.

predicted, a threshold remote conditioning stimulus might have a clinically demonstrable benefit.

Investigating the clinical utility of RIPC requires a demonstration that the phenomenon is intact in patients with atherosclerosis, who remain the principal group to benefit from tissue protection. To minimize the effect of drug therapy on the biology of preconditioning, we recruited patients who were asymptomatic of ischemic episodes after coronary artery bypass grafting. RIPC was as effective in this group as in healthy volunteers in preventing endothelial IR injury, demonstrating for the first time the preservation of RIPC in patients with established atherosclerosis.

However, most ischemic events are unpredictable, which limits the therapeutic potential of ischemic conditioning stimuli (remote or otherwise) that need to be applied in advance of such episodes.[25,26] This has stimulated interest in conditioning stimuli that are protective

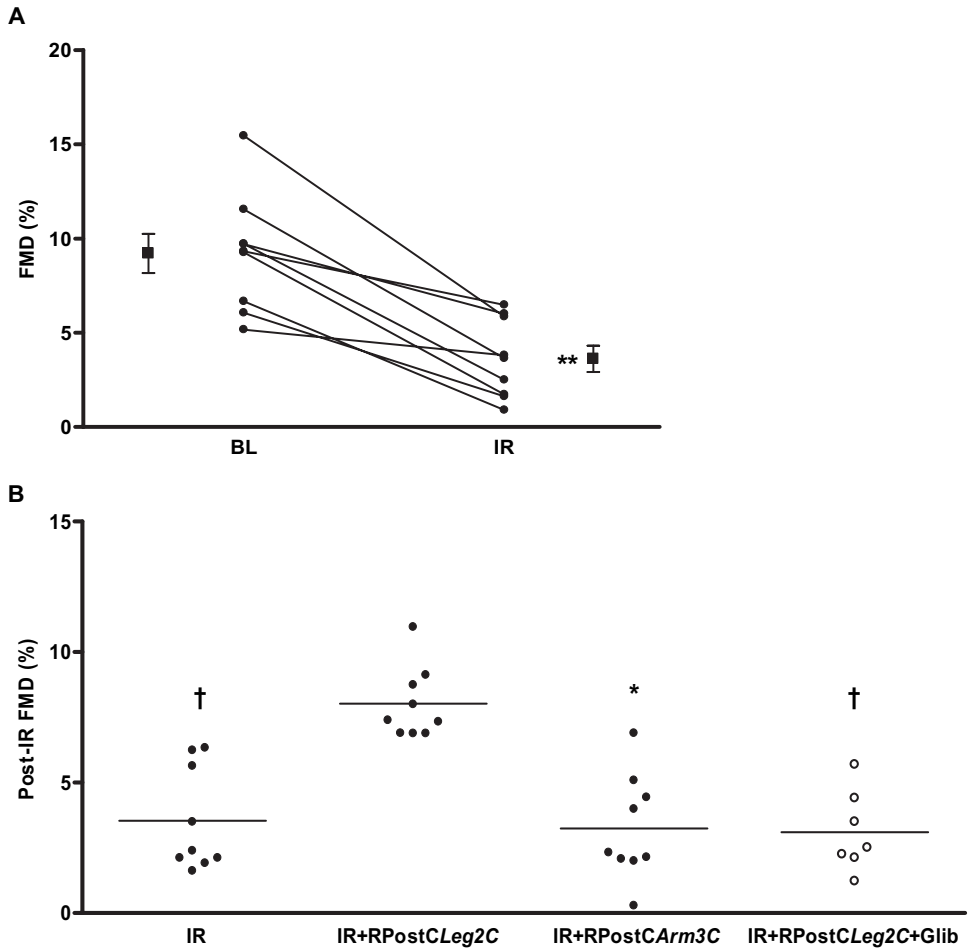

Figure 6. Effect of RPostC on endothelial IR injury and role of K_{ATP} channels in the mechanism of protection. A, FMD was 9.2±1.0% at baseline (BL) and was reduced by IR (3.6±0.7%; **$P<0.001$ vs BL, paired *t* test; n=9). B, IR-induced endothelial dysfunction was not observed with RPostCLeg2C (post-IR FMD, 8.0±0.5%; n=8). In contrast, RPostCArm3C did not prevent IR-induced endothelial dysfunction (post-IR FMD, 3.2±0.7%; n=9). Systemic glibenclamide (Glib; 5 mg) blocked the protective effects of RPostCLeg2C (post-IR FMD, 3.1±0.6%; n=7). Post-IR FMD values were adjusted for baseline FMD. †$P<0.001$, IR and IR+RPostCLeg2C+Glib vs IR+RPostCLeg2C; *$P<0.0001$, IR+RPostCArm3C vs IR+RPostCLeg2C.

TABLE 4. Summary of Baseline and Post-IR FMD Data From Studies to Determine the Effect of RPostC on Endothelial IR Injury and the Role of K_{ATP} Channels in the Mechanism of Protection

Study	n	Baseline FMD	Post-IR FMD	Post-IR FMD (ANCOVA Adjusted)
IR	9	9.2±1.3	3.6±0.7	3.5±0.7†
IR+RPostCLeg2C	9	9.2±1.1	8.1±1.0	8.0±0.5
IR+RPostArm3C	9	9.3±1.1	3.4±0.9	3.2±0.7*
IR+RPostCLeg2C+Glib	7	8.6±0.5	2.8±0.8	3.1±0.6†

Data are expressed as mean±SEM. Glib indicates glibenclamide. No differences existed in baseline FMD. Post-IR FMD values were adjusted for baseline FMD by ANCOVA (regression coefficient, 0.62±0.11). For comparisons between the 4 groups, *P* values by ANCOVA were Scheffé adjusted.

*$P<0.000,1$ IR+RPostCArm3C vs IR+RPostCLeg2C; †$P<0.001$, IR and IR+RPostCLeg2C+Glib vs IR+RPostCLeg2C.

during or after ischemia. Recent studies affirm that much of the reversible tissue damage occurring during IR injury is sustained during early reperfusion rather than during ischemia; modifying the conditions of reperfusion (gradual[27,28] or intermittent reperfusion [PostC][4,5]) reduces experimental IR injury to a degree similar to ischemic preconditioning stimuli. Beneficial effects of PostC in IR injury in humans have recently been demonstrated by our group in healthy volunteers[15] and by Staat et al[29] in patients undergoing coronary angioplasty who showed that 3 cycles of reinflation of the angioplasty balloon catheter in the first minute of reperfusion effected intermittent restoration of blood flow to the ischemic myocardium and reduced infarct size.

Nonetheless, mechanical intervention at the onset of reperfusion will be feasible in only a minority of ischemic events. An alternative is RPostC, defined as "an ischemic conditioning stimulus applied concurrently with an injurious ischemic episode but at a remote site,"[13] which combines facets of both RIPC and PostC. We observed that a conditioning stimulus consisting of 2 cycles of leg ischemia followed by reperfusion applied during index (20-minute) arm ischemia prevented brachial artery endothelial dysfunction caused by IR. These data demonstrate for the first time in humans that RPostC reduces experimental IR injury to the vascular endothelium. The degree of protection was similar to that seen with RIPC, provided that the ischemic conditioning stimulus crossed a threshold for protection before reperfusion occurred; a subthreshold conditioning stimulus (2 cycles on the contralateral arm during index ischemia) did not induce protection.

Emerging evidence from animal studies suggests that IPC/RIPC and PostC share common signaling pathways, including triggers (adenosine receptor stimulation[7,30]), mediators (protein kinase C activation[31,32]), and end effectors (opening of mitochondrial K_{ATP} channels,[7,33] activation of prosurvival kinases [PI3K-Akt, Erk1/2],[34,35] and inhibition of mitochondrial permeability transition pore opening[36,37]). In this study, we chose to investigate the role of K_{ATP} channels in the mechanisms of protection by RIPC and RPostC. Opening of K_{ATP} channels, particularly the mitochondrial subtype, has been shown to be a prerequisite for the induction of protection against IR injury by IPC,[38] RIPC,[7] PostC,[33] and RPostC.[39] Glibenclamide, a nonselective K_{ATP} channel blocker, abolished protection induced by RIPC and RPostC, the first such demonstration in humans of the involvement of K_{ATP} channels in these phenomena. These data do not identify the cellular location (sarcolemmal versus mitochondrial) or the site in the conditioning pathway of the channels involved in protection by RIPC or RPostC. K_{ATP} channel blockade did not affect baseline FMD and did not exacerbate IR injury. Thus, the effect of glibenclamide on RIPC and RPostC could not be attributed to direct actions of glibenclamide to alter baseline endothelial function or the endothelial response to IR. These findings using systemic glibenclamide are convergent with our previous results using local infusions of glibenclamide,[40] suggesting that minimal effects of systemic blockade of K_{ATP} channels on endothelial function are present in this model.

Uncertainties remain regarding the mechanism of transfer of protection from one limb to the other. If humoral factors are involved,[7,9,13,41,42] they need to be active during the critical early stages of reperfusion to induce protection.[13,43] An alternative, a neurogenic pathway, could be activated during conditioning ischemia and induce protection in tissues that are cut off from the circulation.[6,31,44,45] These are issues for further study. Regardless of these and other mechanistic uncertainties, this study demonstrates that ischemic protection can be induced in humans by the application of contemporaneous ischemic conditioning stimuli at remote sites. Such remote conditioning schedules could be used as adjuncts to current reperfusion strategies to protect from more substantial injury in patients with acute myocardial infarction or stroke.

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Disclosures

None.

References

1. Yellon DM, Hausenloy DJ. Realizing the clinical potential of ischemic preconditioning and postconditioning. *Nat Clin Pract Cardiovasc Med.* 2005;2:568–575.

2. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev*. 2003;83:1113–1151.
3. Vinten-Johansen J, Zhao ZQ, Zatta AJ, Kin H, Halkos ME, Kerendi F. Postconditioning: a new link in nature's armor against myocardial ischemia-reperfusion injury. *Basic Res Cardiol*. 2005;100:295–310.
4. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2003;285:H579–H588.
5. Halkos ME, Kerendi F, Corvera JS, Wang NP, Kin H, Payne CS, Sun HY, Guyton RA, Vinten-Johansen J, Zhao ZQ. Myocardial protection with postconditioning is not enhanced by ischemic preconditioning. *Ann Thorac Surg*. 2004;78:961–969.
6. Schoemaker RG, van Heijningen CL. Bradykinin mediates cardiac preconditioning at a distance. *Am J Physiol Heart Circ Physiol*. 2000;278:H1571–H1576.
7. Pell TJ, Baxter GF, Yellon DM, Drew GM. Renal ischemia preconditions myocardium: role of adenosine receptors and ATP-sensitive potassium channels. *Am J Physiol*. 1998;275:H1542–H1547.
8. Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R. Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation*. 2002;106:2881–2883.
9. Takaoka A, Nakae I, Mitsunami K, Yabe T, Morikawa S, Inubushi T, Kinoshita M. Renal ischemia/reperfusion remotely improves myocardial energy metabolism during myocardial ischemia via adenosine receptors in rabbits: effects of "remote preconditioning." *J Am Coll Cardiol*. 1999;33:556–564.
10. Patel HH, Moore J, Hsu AK, Gross GJ. Cardioprotection at a distance: mesenteric artery occlusion protects the myocardium via an opioid sensitive mechanism. *J Mol Cell Cardiol*. 2002;34:1317–1323.
11. Kharbanda RK, Li J, Konstantinov IE, Cheung MM, White PA, Frndova H, Stokoe J, Cox P, Vogel M, Van Arsdell G, MacAllister R, Redington AN. Remote ischemic preconditioning protects against cardiopulmonary bypass-induced tissue injury: a preclinical study. *Heart*. 2006;92:1506–1511.
12. Loukogeorgakis SP, Panagiotidou AT, Broadhead MW, Donald A, Deanfield JE, MacAllister RJ. Remote ischemic preconditioning provides early and late protection against endothelial ischemia reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol*. 2005;46:450–456.
13. Kerendi F, Kin H, Halkos ME, Jiang R, Zatta AJ, Zhao ZQ, Guyton RA, Vinten-Johansen J. Remote postconditioning: brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. *Basic Res Cardiol*. 2005;100:404–412.
14. Kharbanda RK, Peters M, Walton B, Kattenhorn M, Mullen M, Klein N, Vallance P, Deanfield J, MacAllister R. Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. *Circulation*. 2001;103:1624–1630.
15. Loukogeorgakis SP, Panagiotidou AT, Yellon DM, Deanfield JE, MacAllister RJ. Postconditioning protects against endothelial ischemia-reperfusion injury in the human forearm. *Circulation*. 2006;113:1015–1019.
16. Mullen MJ, Kharbanda RK, Cross J, Donald AE, Taylor M, Vallance P, Deanfield JE, MacAllister RJ. Heterogeneous nature of flow-mediated dilation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. *Circ Res*. 2001;88:145–151.
17. Niopas I, Daftsios AC. A validated high-performance liquid chromatographic method for the determination of glibenclamide in human plasma and its application to pharmacokinetic studies. *J Pharm Biomed Anal*. 2002;28:653–657.
18. Rydberg T, Jonsson A, Karlsson MO, Melander A. Concentration-effect relations of glibenclamide and its active metabolites in man: modelling of pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol*. 1997;43:373–381.
19. Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. *J Pathol*. 2000;190:255–266.
20. Laude K, Beauchamp P, Thuillez C, Richard V. Endothelial protective effects of preconditioning. *Cardiovasc Res*. 2002;55:466–473.
21. Li GC, Vasquez JA, Gallagher KP, Lucchesi BR. Myocardial protection with preconditioning. *Circulation*. 1990;82:609–619.
22. Schulz R, Post H, Vahlhaus C, Heusch G. Ischemic preconditioning in pigs: a graded phenomenon: its relation to adenosine and bradykinin. *Circulation*. 1998;98:1022–1029.
23. Jaberansari MT, Baxter GF, Muller CA, Latouf SE, Roth E, Opie LH, Yellon DM. Angiotensin-converting enzyme inhibition enhances a subthreshold stimulus to elicit delayed preconditioning in pig myocardium. *J Am Coll Cardiol*. 2001;37:1996–2001.
24. Cheung MM, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J, Holtby HM, Cox PN, Smallhorn JF, Van Arsdell GS, Redington AN. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol*. 2006;47:2277–2282.
25. Kloner RA, Speakman MT, Przyklenk K. Ischemic preconditioning: a plea for rationally targeted clinical trials. *Cardiovasc Res*. 2002;55:526–533.
26. Bolli R, Becker L, Gross G, Mentzer R Jr, Balshaw D, Lathrop DA. Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res*. 2004;95:125–134.
27. Okamoto F, Allen BS, Buckberg GD, Bugyi H, Leaf J. Reperfusion conditions: importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. *J Thorac Cardiovasc Surg*. 1986;92:613–620.
28. Sato H, Jordan JE, Zhao ZQ, Sarvotham SS, Vinten-Johansen J. Gradual reperfusion reduces infarct size and endothelial injury but augments neutrophil accumulation. *Ann Thorac Surg*. 1997;64:1099–1107.
29. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, Aupetit JF, Bonnefoy E, Finet G, Andre-Fouet X, Ovize M. Postconditioning the human heart. *Circulation*. 2005;112:2143–2148.
30. Yang XM, Philipp S, Downey JM, Cohen MV. Postconditioning's protection is not dependent on circulation blood factors or cells but involves adenosine receptors and requires PI3-kinase and guanylyl cyclase activation. *Basic Res Cardiol*. 2005;100:57–63.
31. Wolfrum S, Schneider K, Heidbreder M, Nienstedt J, Dominiak P, Dendorfer A. Remote preconditioning protects the heart by activating myocardial PKCepsilon-isoform. *Cardiovasc Res*. 2002;55:583–589.
32. Zatta AJ, Kin H, Lee G, Wang N, Jiang R, Lust R, Reeves JG, Mykytenko J, Guyton RA, Zhao ZQ, Vinten-Johansen J. Infarct-sparing effect of myocardial postconditioning is dependent on protein kinase C signalling. *Cardiovasc Res*. 2006;70:315–324.
33. Yang XM, Proctor JB, Cui L, Krieg T, Downey JM, Cohen MV. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J Am Coll Cardiol*. 2004;44:1103–1110.
34. Hausenloy DJ, Tsang A, Mocanu MM, Yellon DM. Ischemic preconditioning protects by activating prosurvival kinases at reperfusion. *Am J Physiol Heart Circ Physiol*. 2005;288:H971–H976.
35. Tsang A, Hausenloy DJ, Mocanu MM, Yellon DM. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. *Circ Res*. 2004;95:230–232.
36. Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res*. 2002;55:534–543.
37. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. *Circulation*. 2005;111:194–197.
38. Gross GJ, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res*. 1992;70:223–233.
39. Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, White PA, Kristiansen SB, Sorensen KE, Dzavik V, Redington AN, Kharbanda RK. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction: first demonstration of remote ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2007;292:H1883–H1890.
40. Broadhead MW, Kharbanda RK, Peters MJ, MacAllister RJ. KATP channel activation induces ischemic preconditioning of the endothelium in humans in vivo. *Circulation*. 2004;110:2077–2082.
41. Dickson EW, Lorbar M, Porcaro WA, Fenton RA, Reinhardt CP, Gysembergh A, Przyklenk K. Rabbit heart can be "preconditioned" via transfer of coronary effluent. *Am J Physiol*. 1999;277:H2451–H2457.
42. Weinbrenner C, Schulze F, Sarvary L, Strasser RH. Remote preconditioning by infrarenal aortic occlusion is operative via delta1-opioid

receptors and free radicals in vivo in the rat heart. *Cardiovasc Res*. 2004;61:591–599.
43. Kin H, Zhao ZQ, Sun HY, Wang NP, Corvera JS, Halkos ME, Kerendi F, Guyton RA, Vinten-Johansen J. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc Res*. 2004;62:74–85.
44. Gho BC, Schoemaker RG, van den Doel MA, Duncker DJ, Verdouw PD. Myocardial protection by brief ischemia in noncardiac tissue. *Circulation*. 1996;94:2193–2200.
45. Liem DA, Verdouw PD, Ploeg H, Kazim S, Duncker DJ. Sites of action of adenosine in interorgan preconditioning of the heart. *Am J Physiol Heart Circ Physiol*. 2002;283:H29–H37.

CLINICAL PERSPECTIVE

This study demonstrates for the first time in humans that transient limb ischemia administered during a prolonged ischemic insult reduces vascular ischemia-reperfusion injury (remote ischemic postconditioning). The degree of protection is similar to that achieved by a remote conditioning stimulus applied in advance of an injurious ischemic event (remote ischemic preconditioning), which is consistent with much of the reversible vascular injury occurring during reperfusion. Remote ischemic postconditioning and remote ischemic preconditioning share mechanistic similarities, with protection being dependent on activation of ATP-sensitive potassium channels. If the vasculoprotective effects of remote ischemic postconditioning can be translated to other organs, these results suggest that remote conditioning stimuli applied during infarction have potential for tissue salvage and might be useful as adjuncts to current reperfusion strategies. The clinical effect of such benign stimuli is readily testable in acute ischemic syndromes, including myocardial infarction and stroke.