Journal of the American College of Cardiology Vol. 51, No. 14, 2008

Published by Elsevier Inc.
ISSN 0735-1097/08/$34.00
doi:10.1016/j.jacc.2007.11.070

Aging Mouse Hearts Are Refractory to Infarct Size Reduction With Post-Conditioning

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Objectives Our aim was to establish whether the efficacy of post-conditioning is maintained in aging hearts.

Background Post-conditioning, or relief of myocardial ischemia in a stuttered manner, has been shown to reduce infarct size, in part because of up-regulation of survival kinases (extracellular-signal regulated kinase [ERK] 1/2 or PI3-kinase/Akt) during the early min of reperfusion. All of these data have, however, been obtained in adult populations; the question of whether post-conditioning–induced cardioprotection is maintained in aging cohorts is unknown.

Methods Isolated buffer-perfused hearts were obtained from 3- to 4-month-old (adult) and 20- to 24-month-old C57BL/6J mice and subjected to 30 min of ischemia. For each cohort, hearts were randomized to receive standard, abrupt (control) reperfusion, or were post-conditioned with 3 or 6 10-s cycles of stuttered reflow. Primary end points were infarct size, cardiac expression of phospho-Akt, phospho-mitogen-activated protein kinase kinase 1/2 and phospho-ERK 1/2, and expression of mitogen-activated protein kinase-phosphatase-1 (MKP-1: phosphatase purported to play a primary role in ERK dephosphorylation).

Results In adult mouse hearts, post-conditioning significantly reduced infarct size via up-regulation of ERK (but not Akt) signaling. In contrast, in the 2-year-old cohort, post-conditioning failed to limit necrosis, possibly a consequence of the deficit in ERK phosphorylation and increased MKP-1 expression seen in old hearts. Indeed, infusion of sodium orthovanadate, a nonspecific MKP inhibitor, attenuated MKP-1 expression and restored the post-conditioned phenotype in old hearts.

Conclusions Old mouse hearts are refractory to infarct size reduction with post-conditioning, possibly because of an age-associated increase in MKP-1 and resultant deficit in ERK phosphorylation. (J Am Coll Cardiol 2008;51: 1393–8) © 2008 by the American College of Cardiology Foundation

Emerging evidence has revealed that post-conditioning, or relief of sustained myocardial ischemia in a stuttered manner, attenuates lethal myocardial ischemia-reperfusion injury and significantly reduces infarct size (1–5). Moreover, although the mechanisms contributing to post-conditioning–induced cardioprotection are as yet poorly understood, activation of extracellular-signal regulated kinase (ERK) 1/2 and/or phosphatidylinositol-3-kinase (PI3-kinase) during the early min after relief of ischemia has been implicated to play a pivotal role (2,6–8). Virtually all of these data have, however, been obtained in adult populations: with the exception of one recent report (9), there is currently no insight into whether the infarct-sparing effect of stuttered reflow is maintained in hearts from aging cohorts, a subset of particular relevance for the future clinical application of post-conditioning (10). To address this issue, we investigated the efficacy of infarct size reduction with post-conditioning in adult and 2-year-old mice.

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We report that in adult mouse hearts, post-conditioning evokes a robust reduction of infarct size that is mediated by up-regulation of ERK 1/2. In contrast, hearts from 2-year-old mice are refractory to post-conditioning–induced cardioprotection, possibly because of a deficit in ERK phosphorylation.

Methods

This study was approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School, and was performed in accordance with the Guide for the Care and Use of Laboratory Animals from the Institute of Laboratory Animals Resources (National Institutes of Health Publication Vol. 25 No. 28, revised 1996).

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Manuscript received September 4, 2007; revised manuscript received November 15, 2007, accepted November 19, 2007.

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April 8, 2008:1393–8

Abbreviations and Acronyms

ANOVA = analysis of variance

ERK = extracellular-signal regulated kinase

LV = left ventricle/ventricular

MEK = mitogen-activated protein kinase kinase

MKP = mitogen-activated protein kinase phosphatase

PI3 kinase = phosphatidylinositol-3-kinase

STAT3 = signal transducer and activator of transcription 3

Protocol 1: Post-Conditioning in Adult Mice

Infarct size. Infarct size was assessed in isolated buffer-perfused mouse hearts using standard methods described previously (11). In brief, adult (3- to 4-month-old) C57BL/6J mice (n = 44) were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), and the hearts were rapidly excised and mounted on an aortic cannula for retrograde perfusion (nonrecirculating) at a constant pressure of 55 mm Hg. The buffer was composed of (in mM): NaCl (118), KCl (4.7), $NaHCO_3$ (24), KH_2PO_4 (1.2), $MgSO_4$-$7H_2O$ (1.2), glucose (11), and $CaCl_2$ anhydrous (2.5) in distilled water at a pH of 7.4, and was continuously oxygenated with 95% O_2/5% CO_2. Care was taken to maintain both buffer temperature and heart temperature at 37°C. A balloon constructed of polyvinyl chloride plastic film was inserted into the left ventricle (LV), inflated to an end-diastolic pressure of 5 mm Hg, and used to monitor cardiodynamic function throughout the experiment.

After stabilization, all hearts underwent 30 min of sustained global ischemia. The first 34 hearts enrolled in the protocol were randomly assigned to undergo: 1) abrupt and complete reperfusion (control subjects); 2) post-conditioning with 3 cycles of [10 s reperfusion + 10 s reocclusion] followed by sustained restoration of flow; or 3) post-conditioning with 6 cycles of [10 s reflow + 10 s reocclusion] followed by sustained reperfusion. The remaining 10 hearts all received an infusion of PD98059 (5 μM final concentration), initiated at the time of reperfusion and administered via a side port immediately proximal to the heart. This dose of PD98059 was confirmed, in separate experiments in our laboratory, to inhibit up-regulation of mitogen-activated protein kinase kinase (MEK) and ERK (data not shown). The PD98059-treated hearts then underwent either abrupt (control) reperfusion or post-conditioning with 3 10-s cycles of stuttered reflow, with infusion of the inhibitor maintained for a total of 20 min.

At 2 h after the onset of reperfusion, each heart was cut into 4 to 6 transverse slices and the extent of necrosis was delineated by triphenyltetrazolium staining. All hearts were digitally photographed, and infarct size was quantified in a blinded manner (without knowledge of the treatment group) using image analysis software (11).

Immunoblotting. Buffer-perfused hearts were obtained from additional adult C57 mice (n = 12) as described for the infarct size protocol and assigned to undergo 30 min of global ischemia followed by abrupt, complete reperfusion (control subjects), 30 min of ischemia followed by 3 10-s cycles of stuttered reflow (post-conditioned), or time-matched uninterrupted perfusion (nonischemic shams). At 10 min after reflow, all hearts were frozen in liquid nitrogen and stored at −80°C until processed.

Hearts were prepared, and using methods described in detail previously (2,11), were probed by standard immunoblotting for the expression of phospho-Akt, MEK 1/2, and ERK 1/2, then stripped and re-probed for the expression of total Akt, MEK 1/2, and ERK 1/2 (all antibodies from Cell Signaling Technology, Danvers, Massachusetts). Bands of interest were quantified without knowledge of the group assignment using gel analysis software, and results for phospho-Akt, MEK, and ERK were normalized to the corresponding total kinase expression.

Protocol 2: Post-Conditioning in Old Mice

Infarct size. Hearts were isolated from 20- to 24-month-old C57BL/6J mice (n = 34) and buffer-perfused as detailed in Protocol 1. All hearts underwent 30 min of global ischemia and received either control (abrupt) reflow or post-conditioning with 3 or 6 10-s cycles of reperfusion-reocclusion. Infarct size was delineated and quantified as in Protocol 1.

Immunoblotting. Hearts from 20- to 24-month-old C57 mice (n = 12) were assigned to sham, control, and 3-cycle post-conditioning groups, and frozen at 10 min after relief of ischemia for immunoblot analysis of Akt, MEK, and ERK using the same methods described in Protocol 1.

Protocol 3: Post-Conditioning in Old Mice; Role of Mitogen-Activated Protein Kinase Phosphatases (MKPs)

Based on the outcomes of Protocols 1 and 2, supplemental experiments were conducted to investigate the concept of a possible causal relationship between failed up-regulation of ERK and loss of post-conditioning–induced cardioprotection in aging mouse hearts. First, because negative regulation of ERK is controlled primarily by members of the MKP family (12–15), tissue from adult and old hearts collected in Protocols 1 and 2 was probed by immunoblotting for the expression of MKP-1, MKP-3 (antibodies from Santa Cruz Biotechnology, Santa Cruz, California) and, for purposes of normalization, β-actin (Cell Signaling Technology). Second, additional hearts (n = 20) were harvested from 2-year-old mice and infused with sodium orthovanadate, a nonspecific inhibitor of MKPs (16,17) (final concentration in buffer, 2 μM). At 45 min after the start of treatment, hearts underwent 30 min of global ischemia followed by either abrupt reflow or 3 10-s cycles of post-conditioning, with infusion of vanadate maintained throughout the first 10 min of reperfusion. In 12 hearts, reperfusion was continued for 2 h and infarct size was delineated by triphenyltetrazolium staining. The remaining vanadate-treated hearts (4 control subjects, 4 post-conditioned) were frozen at 10 min post-reflow and processed for immunoblot analysis of MKPs, β-actin, phospho-ERK, and total ERK.

Statistics. Because Protocols 1, 2, and 3 were conducted consecutively rather than concurrently, separate statistical analyses were performed for each component of the study. For Protocols 1 and 2, infarct size (expressed as a percent of total LV weight) and kinase expression were compared among groups by analysis of variance (ANOVA) and, if significant F-values were obtained, post-hoc pairwise comparisons were made using the Newman-Keuls test. For Protocol 3, MKP expression (normalized to β-actin) was compared among adult hearts, old hearts, and old hearts infused with sodium vanadate by ANOVA followed by the Newman-Keuls test, whereas infarct size and ERK expression were compared between vanadate-treated control and post-conditioned groups by *t* test. All data are reported as mean ± SEM.

Results

Protocol 1

In adult hearts, infarct size averaged 53 ± 5%, 27 ± 3%*, and 29 ± 5%* of the total LV in hearts that received abrupt (control) reperfusion, post-conditioning with 3 10-s cycles of stuttered reflow and post-conditioning with 6 10-s cycles of interrupted reflow, respectively (Fig. 1A). (*$p < 0.05$ vs. control). That is, post-conditioning was cardioprotective, with no difference in benefit seen with 3 versus 6 episodes of reperfusion-reocclusion.

There were no differences in total Akt, MEK, or ERK among sham, control, and post-conditioned groups (data not shown). Hearts that received stuttered reflow showed an approximate 2-fold increase in expression of both phospho-MEK and phospho-ERK versus control subjects, whereas phospho-Akt was not up-regulated in response to post-conditioning (Fig. 1B). These data, implicating the involvement of MEK-ERK signaling in post-conditioning–induced protection, were corroborated in infarct size experiments showing that the infarct-sparing effect of post-conditioning was abrogated by co-administration of PD98059 (Fig. 1A).

Protocol 2

In contrast to results obtained in the adult cohort, post-conditioning failed to limit necrosis in hearts from 2-year-old mice: infarct sizes averaged 43 ± 3%, 40 ± 4%, and 41 ± 4% of the total LV in control, 3-cycle post-conditioning, and 6-cycle post-conditioning groups (Fig. 2A). Moreover, there was no evidence of an up-regulation of phospho-ERK (or phospho-Akt) in response to stuttered reflow; expression of phospho-ERK was attenuated, rather than augmented, in post-conditioned hearts versus both sham and control groups ($p = 0.065$ by ANOVA) (Fig. 2B). Paradoxically, this deficit in ERK phosphorylation was seen despite a persistent, post-conditioning–induced increase in expression of phospho-MEK in old mouse hearts (Fig. 2B).

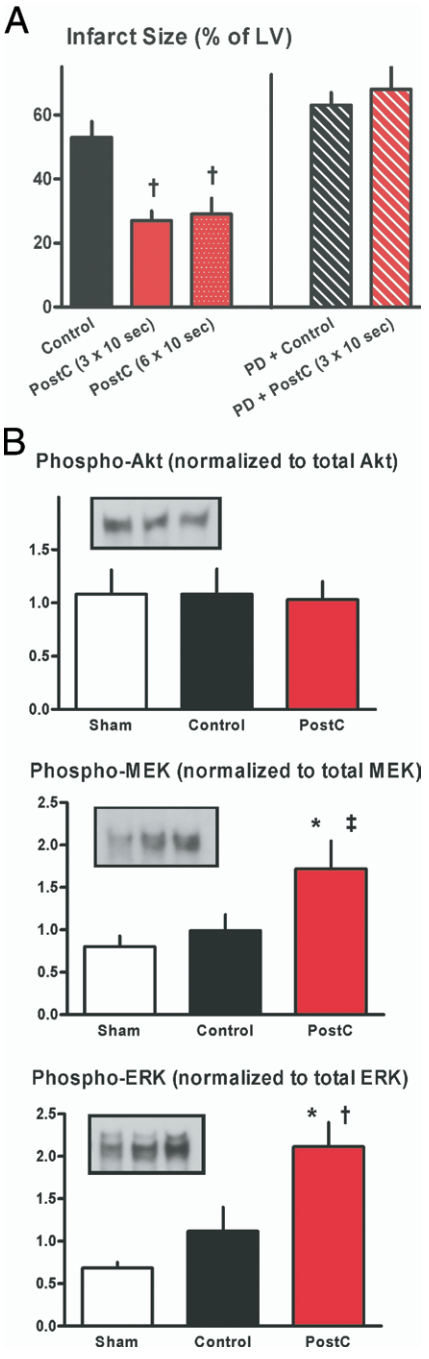

Figure 1 Protocol 1, Adult Mouse Hearts

(A) Infarct size (expressed as a percent of the total left ventricle [LV]). **(B)** Expression of phospho-Akt, phospho-MEK 1/2, and phospho-ERK 1/2. Data shown as mean ± SEM. ERK = extracellular-signal regulated kinase; MEK = mitogen-activated protein kinase kinase; PostC = post-conditioned; PD = PD98059. *$p < 0.05$ versus sham. †$p < 0.05$ versus control. ‡$p = 0.07$ versus control.

Protocol 3

Cardiac expression of MKP-1 was augmented in hearts from old mice versus adults (Fig. 3A), irrespective of the treatment

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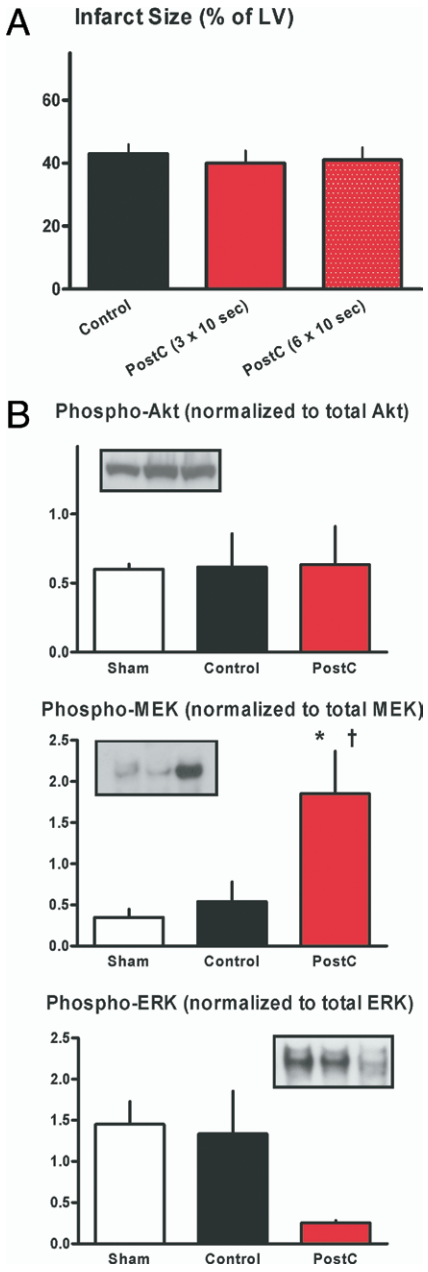

Figure 2 **Protocol 2, Old Mouse Hearts**

(A) Infarct size (expressed as a percent of the total left ventricle [LV]). **(B)** Expression of phospho-Akt, phospho-MEK 1/2, and phospho-ERK 1/2. Data shown as mean ± SEM. *p < 0.05 versus sham. †p < 0.05 versus control. Abbreviations as in Figure 1.

group (i.e., no differences among sham, control, and post-conditioned groups within each of the 2 cohorts). In contrast, expression of MKP-3 was comparable in hearts obtained from old and adult mice (data not shown). Infusion of sodium orthovanadate blunted the increase in MKP-1 expression seen in old hearts (Fig. 3A) and tended to re-establish the ability of stuttered reflow to evoke an increase in phospho-ERK (Fig. 3B). In addition, a modest but significant difference in infarct size was seen in orthovanadate-treated old post-conditioned hearts versus old control subjects (Fig. 3C).

Discussion

We report that post-conditioning reduces infarct size in adult mouse hearts, mediated in part via MEK-ERK signaling. Most notably, however, we provide evidence for a loss in efficacy of post-conditioning in hearts from 2-year-old mice.

Post-Conditioning in Adult Cohorts

Our observation of infarct size reduction with post-conditioning in hearts from adult C57BL/6J mice is consistent with the emerging paradigm of post-conditioning–induced cardioprotection described in multiple species including, most recently, a small number of studies conducted in mouse models

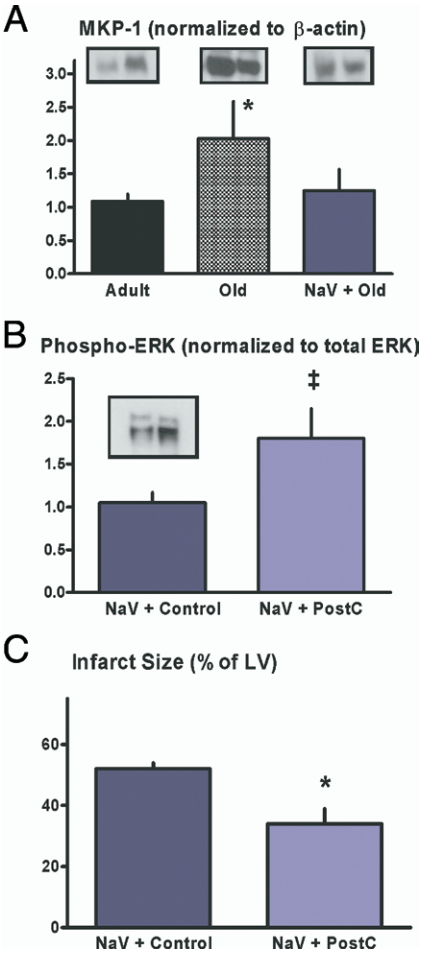

Figure 3 **Protocol 3, Role of MKPs**

(A) Expression of MKP-1 in adult mouse hearts, 2-year-old mouse hearts, and 2-year-old mouse hearts infused with sodium orthovanadate (NaV). *p < 0.05 versus adult. **(B)** Expression of phospho-ERK 1/2 in 2-year-old mouse hearts infused with NaV. ‡p = 0.08 versus NaV + control. **(C)** Infarct size (expressed as a percent of the total left ventricle [LV]) in 2-year-old mouse hearts infused with NaV. *p < 0.05 versus NaV + control. All data shown as mean ± SEM. Abbreviations as in Figure 1.

(4,5,9). There is growing evidence that the efficacy of the infarct-sparing effect of stuttered reflow is dependent on both number and duration of the constituent ischemia-reperfusion cycles, and that the optimal post-conditioning algorithm may vary among models and species (3,4,9,18,19). In this regard, our choice of 10-s cycles of stuttered reflow was based on pilot studies in which we found that repeated 30-s episodes of ischemia-reperfusion, shown to reduce infarct size in rabbit hearts (2), were ineffective in isolated mouse hearts. In terms of cellular mechanisms, there is general agreement that the infarct-sparing effect of post-conditioning is in part attributable to up-regulation of the classic survival kinases (ERK 1/2 and/or PI3 kinase/Akt) during the early minutes after stuttered reflow (2,6–8). We found no evidence of up-regulation of Akt in post-conditioned hearts versus control subjects, either at 10 min post-reflow (Fig. 1) or at earlier time points (data not shown), and in supplemental experiments, saw persistent infarct size reduction with post-conditioning in hearts infused with the PI3 kinase inhibitor LY294002 (mean infarct size of 32 ± 4% versus 51 ± 2% in inhibitor-treated control subjects; $p < 0.05$). In contrast, we did observe an increase in expression of phospho-MEK and phospho-ERK in response to stuttered reflow, and abrogation of post-conditioning–induced protection in hearts treated with PD98059. Thus, although the temporal profile of the up-regulation of MEK and ERK remains to be established, the current results support the involvement of MEK-ERK signaling in the infarct-sparing effect of post-conditioning in the mouse model.

We have focused on the assessment of infarct size rather than indices of LV function (i.e., acute recovery of LV developed pressure) as our primary end point of post-conditioning–induced cardioprotection. This is based on previous observations by our group and others that reduction of infarct size with post-conditioning is not accompanied by a significant improvement in LV cardiodynamics during the initial hours after relief of ischemia (2,4,7). The lack of improvement in function in post-conditioned hearts versus control subjects may reflect 2 related factors. First, global LV function measured in these models represents an integrated assessment of the contribution of the akinetic-dyskinetic necrotic region and the viable and salvaged but profoundly stunned (hypokinetic-akinetic-dyskinetic) peri-infarct tissue. Second, although stuttered reflow reduces infarct size, these initial studies suggest that post-conditioning does not have an independent, sustained beneficial effect on the acute recovery of function of post-ischemic stunned myocardium. Indeed, similar results were obtained in the current study: LV function recovered to 30% to 40% of baseline values during the reperfusion period, with no differences among control and post-conditioning groups.

Loss of Post-Conditioning–Induced Cardioprotection in Aging Mouse Hearts

Despite the consensus that relief of ischemia in a stuttered manner can limit infarct size in experimental models and recent, seminal evidence that post-conditioning may be of clinical benefit (20,21), the question of whether post-conditioning–induced cardioprotection is maintained in aging cohorts has to date received limited attention (9). This issue is crucial for future widespread therapeutic application of post-conditioning because the aging population is well recognized to be the specific subset in which acute myocardial infarction is most prevalent (10).

Accordingly, to assess the consequences of increased age on the efficacy of post-conditioning, we utilized 2-year-old mice, considered a standard model of aging (22–24) and confirmed in pilot experiments by our group to display classic morphologic and molecular features of cardiovascular aging and senescence (i.e., hypertrophy, increased fibrosis, and increased myocardial expression of $p16^{INK4a}$ (25,26) versus 3- to 4-month-old mice, data not shown). Comparison of Protocols 1 and 2 indicates that in control mouse hearts there was no age-associated exacerbation of infarct size. Rather, post-hoc analysis by 2-factor ANOVA (for age and treatment) indicates that infarct size was smaller in old-control versus adult-control cohorts ($p < 0.05$), in agreement with previous studies showing that the extent of necrosis was modestly reduced, or comparable, in aging cohorts versus adults (26–28). Most importantly, data obtained in Protocol 2 showed that in aging mouse hearts, post-conditioning failed to evoke an increase in expression of phospho-ERK and was ineffective in reducing infarct size. This concept of an age-associated loss in efficacy of infarct size reduction with post-conditioning was reinforced by the outcome of our post-hoc 2-factor ANOVA, i.e., $p = 0.022$ for the age × treatment interaction. Moreover, our findings made in hearts from 2-year-old mice showing definitive hallmarks of cardiovascular aging extend the recent observation of an attenuation (rather than complete loss) of the infarct-sparing effect of post-conditioning in mice of an intermediate, 13-month-old age (9).

Role of MKPs

If, as identified in Protocol 1, up-regulation of ERK plays a role in the infarct-sparing effect of post-conditioning, we reasoned that the loss-of-benefit of post-conditioning in old mouse hearts may in part be attributable to the observed deficit in ERK phosphorylation. Failed up-regulation of ERK in old post-conditioned hearts could be a consequence of a defect in upstream signaling; however, the persistent increase in phospho-MEK seen with stuttered reflow in the aging cohort argues against this concept. Accordingly, our goal in Protocol 3 was to investigate the logical, alternative possibility: that is, obtain preliminary insight into whether the loss of post-conditioning–induced protection is explained by an age-associated increase in ERK dephosphorylation.

Although negative regulation of ERK is a complex process that can be achieved by tyrosine-specific protein phosphatases, serine/threonine protein phosphatase, and/or dual-specificity phosphatases that dephosphorylate both tyrosine and threonine, members of the dual-specificity MKP family, including MKP-1 and -3, purportedly play a primary role in ERK dephosphorylation (12–15). Our experiments in Protocol 3 are among the few studies that

have assessed MKPs in the heart (13,29,30), and show a significant increase in cardiac expression of MKP-1 in 2-year-old mice versus adults. Moreover, we found that infusion of sodium orthovanadate both attenuated the increase in MKP-1 expression and restored the post-conditioned phenotype in the aging cohort. Given the nonspecific actions of orthovanadate (including inhibition of protein tyrosine phosphatases and adenosine triphosphatases [31–33]), these data do not constitute proof of a cause-and-effect relationship. Indeed, definitive resolution of this concept is hampered by the current lack of pharmacological and molecular agents capable of selectively targeting MKPs (34). In addition, our results do not exclude the possibility that age-related alterations in other signaling molecules (including, for example, attenuated expression of STAT 3 [9]) may contribute to the failure of stuttered reflow to limit infarct size in hearts from aging mice. Nonetheless, our results provide novel evidence that 2-year-old mouse hearts are refractory to infarct size reduction with post-conditioning, possibly because of a deficit in ERK phosphorylation caused in part by an age-associated increase in MKP-1.

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REFERENCES

1. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic post-conditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol 2003;285:H579–88.
2. Darling CE, Jiang R, Maynard M, Whittaker P, Vinten-Johansen J, Przyklenk K. Post-conditioning via stuttering reperfusion limits myocardial infarct size in rabbit hearts: role of ERK1/2. Am J Physiol 2005;289:H1618–26.
3. Vinten-Johansen J, Zhao ZQ, Zatta AJ, Kin H, Halkos ME, Kerendi F. Post-conditioning—a new link in nature's armor against myocardial ischemia-reperfusion injury. Basic Res Cardiol 2005;100:295–310.
4. Kin H, Zatta AJ, Lofye MT, et al. Post-conditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. Cardiovasc Res 2005;67:124–33.
5. Heusch G, Buchert A, Feldhaus S, Schulz R. No loss of cardioprotection by post-conditioning in connexin 43-deficient mice. Basic Res Cardiol 2006;101:354–6.
6. 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;44:1103–10.
7. Yang XM, Downey JM, Cohen MV. Post-conditioning's protection is not dependent on circulating blood factors or cells but requires PI3-kinase and guanylyl cyclase activation. Basic Res Cardiol 2005; 100:57–63.
8. Tsang A, Hausenloy DJ, Mocanu MM, Yellon DM. Post-conditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. Circ Res 2004;95:230–2.
9. Boengler K, Buechert A, Heinen Y, et al. Cardioprotection by ischemic post-conditioning is lost in aged and STAT3-deficient mice. Circ Res 2008;102:131–5.
10. Holmes DR Jr, White HD, Pieper KS, Ellis SG, Califf RM, Topol EJ. Effect of age on outcome with primary angioplasty versus thrombolysis. J Am Coll Cardiol 1999;33:412–9.
11. Przyklenk K, Maynard M, Whittaker P. First molecular evidence that inositol trisphosphate signaling contributes to infarct size reduction with preconditioning. Am J Physiol 2006;291:H2008–12.
12. Tamura S, Hanada M, Ohnishi M, Katsura K, Sasaki M, Kobayashi T. Regulation of stress-activated protein kinase signaling pathways by protein phosphatases. Eur J Biochem 2002;269:1060–6.
13. Olson AK, Protheroe KN, Segar JL, Scholz TD. Mitogen-activated protein kinase activation and regulation in the pressure-loaded fetal ovine heart. Am J Physiol 2006;290:H1587–95.
14. Dickinson RJ, Keyse SM. Diverse physiological functions for dual-specificity MAP kinase phosphatases. J Cell Sci 2006;119:4607–15.
15. Camps M, Nichols A, Arkinstall S. Dual specificity phosphatases: a gene family for control of MAP kinase function. FASEB J 2000;14:6–16.
16. Reddy ST, Nguyen JT, Grijalva V, et al. Potential role for mitogen-activated protein kinase phosphatase-1 in the development of atherosclerotic lesions in mouse models. Arterioscler Thromb Vasc Biol 2004;24:1676–81.
17. Gordon JA. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. Methods Enzymol 1991;201:477–82.
18. Tang XL, Sato H, Tiwari S, et al. Cardioprotection by post-conditioning in conscious rats is limited to coronary occlusions <45 min. Am J Physiol 2006;291:H2308–17.
19. Iliodromitis EK, Zoga A, Vrettou A, et al. The effectiveness of post-conditioning and preconditioning on infarct size in hypercholesterolemic and normal anesthetized rabbits. Atherosclerosis 2006;188:356–62.
20. Darling CE, Solari PB, Smith CS, Furman MI, Przyklenk K. 'Post-conditioning' the human heart: multiple balloon inflations during primary angioplasty may confer protection. Basic Res Cardiol 2007;102:274–8.
21. Staat P, Rioufol G, Piot C, et al. Post-conditioning the human heart. Circulation 2005;112:2143–8.
22. Headrick JP, Willems L, Ashton KJ, Holmgren K, Peart J, Matherne GP. Ischaemic tolerance in aged mouse myocardium: the role of adenosine and effects of A1 adenosine receptor overexpression. J Physiol 2003;549:823–33.
23. Peart JN, Gross GJ. Chronic exposure to morphine produces a marked cardioprotective phenotype in aged mouse hearts. Exp Gerontol 2004;39:1021–6.
24. Torella D, Rota M, Nurzynska D, et al. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. Circ Res 2004;94:514–24.
25. Anversa P, Rota M, Urbanek K, et al. Myocardial aging—a stem cell problem. Basic Res Cardiol 2005;100:482–93.
26. Przyklenk K, Li G, Whittaker P. No loss in the in vivo efficacy of ischemic preconditioning in middle-aged and old rabbits. J Am Coll Cardiol 2001;38:1741–7.
27. Schulman D, Latchman DS, Yellon DM. Effect of aging on the ability of preconditioning to protect rat hearts from ischemia-reperfusion injury. Am J Physiol 2001;281:H1630–6.
28. Fenton RA, Dickson EW, Meyer TE, Dobson JG Jr. Aging reduces the cardioprotective effect of ischemic preconditioning in the rat heart. J Mol Cell Cardiol 2000;32:1371–5.
29. Khan TA, Bianchi C, Ruel M, et al. Mitogen-activated protein kinase inhibition and cardioplegia-cardiopulmonary bypass reduce coronary myogenic tone. Circulation 2003;108 Suppl 1:II348–53.
30. Araujo EG, Bianchi C, Sato K, Faro R, Li XA, Sellke FW. Inactivation of the MEK/ERK pathway in the myocardium during cardiopulmonary bypass. J Thorac Cardiovasc Surg 2001;121:773–81.
31. Geraldes CF, Castro MM, Sherry AD, Ramasamy R. Influence of vanadate on glycolysis, intracellular sodium, and pH in perfused rat hearts. Mol Cell Biochem 1997;170:53–63.
32. Liem DA, Gho CC, Gho BC, et al. The tyrosine phosphatase inhibitor bis(maltolato)oxovanadium attenuates myocardial reperfusion injury by opening ATP-sensitive potassium channels. J Pharmacol Exp Ther 2004;309:1256–62.
33. Sakurai H, Tsuchiya K, Nukatsuka M, Sofue M, Kawada J. Insulin-like effect of vanadyl ion on streptozotocin-induced diabetic rats. J Endocrinol 1990;126:451–9.
34. Lazo JS, Skoko JJ, Werner S, et al. Structurally unique inhibitors of human mitogen-activated protein kinase phosphatase-1 identified in a pyrrole carboxamide library. J Pharmacol Exp Ther 2007;322:940–7.