CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

ISCHEMIC PRECONDITIONING IN CARDIAC SURGERY: A WORD OF CAUTION

Louis P. Perrault, MD, FRCSC^a
Philippe Menasché, MD, PhD^a
Alain Bel, MD^a
Thierry de Chaumaray, MD^a
Jacqueline Peynet, MD^a
Adrian Mondry, MD^b
Patricia Olivero^b
Rodica Emanoil-Ravier, PhD^c
Jean-Marie Moalic, PhD^b

Objective: Ischemic preconditioning is now established as an effective means of reducing infarct size. However, it remains uncertain whether preconditioning can improve the myocardial protection afforded by cardioplegia. The present study was designed to address this issue. **Methods:** After the institution of cardiopulmonary bypass, 10 patients were preconditioned with 3 minutes of aortic crossclamping followed by 2 minutes of reperfusion before the onset of retrograde continuous warm cardioplegic arrest. Ten case-matched patients served as controls. Three blood samples were drawn simultaneously from the radial artery and the coronary sinus before bypass, at the end of the 5-minute preconditioning protocol or after 5 minutes of bypass in control patients, and at the end of cardioplegic arrest. These samples were assayed for creatine kinase MB isoenzyme and lactate. Right atrial biopsy specimens taken at the same time points were processed by Northern blotting for the expression of messenger ribonucleic acid of both *c-fos* and heat shock protein 70. **Results:** At the end of arrest, the release of creatine kinase MB from the myocardium was markedly greater in preconditioned patients than in the controls. The transmyocardial lactate gradient was shifted toward production in the preconditioned group ($+0.22 \pm 0.13$ mmol/L) and toward extraction in the control group (-0.06 ± 0.21 mmol/L). Molecular biology data did not suggest a protective effect of preconditioning. There were no clinical adverse events related to preconditioning. **Conclusions:** Preconditioning does not enhance cardioplegic protection and might even be deleterious. These results do not dismiss its use in cardiac operations. They rather emphasize the need for identifying pharmacologic mediators that could safely and effectively duplicate the cardioprotective effects of ischemic preconditioning. (J Thorac Cardiovasc Surg 1996;112:1378-86)

From the Department of Cardiovascular Surgery, Hôpital Lariboisière,^a INSERM U 127, Hôpital Lariboisière,^b and CNRS UPR A0043, Hôpital Saint-Louis,^c Paris, France.

This study was supported in part by a grant from the Biomed Concerted Action "The New Ischemic Syndromes" (Contract BMH4-CT950838 DG XII-SSMA) to Dr. Menasché.

Dr. Perrault is supported by the Clinician-Scientist program (phase I) of the Medical Research Council of Canada.

Read at the Seventy-sixth Annual Meeting of The American Association for Thoracic Surgery, San Diego, Calif., April 28–May 1, 1996.

Received for publication May 1, 1996; revisions requested May 13, 1996; revisions received June 17, 1996; accepted for publication June 17, 1996.

Address for reprints: Philippe Menasché, MD, PhD, Hôpital Lariboisière, 2 rue Ambroise Paré, Paris Cedex, France.

Copyright © 1996 by Mosby–Year Book, Inc.
0022-5223/96 \$5.00 + 0 12/6/75892

Ischemic preconditioning has been a powerful means of reducing infarct size in several animal models of myocardial regional ischemia.¹ In the setting of global ischemia, some studies have also reported that preconditioning improved functional recovery after hypothermic² or cardioplegic^{3,4} arrest. These observations have raised the possibility that preconditioning might enhance the efficacy of current methods of intraoperative myocardial protection. This prospect is appealing because cardiac surgery (along with percutaneous transluminal coronary angioplasty) offers the unique opportunity of planning the onset of aortic crossclamping and the attendant period of ischemic arrest. This allows timely implementation of the preconditioning stimulus and, therefore,

Fig. 1. Experimental protocol.

makes this form of protection particularly well suited for an intraoperative use.

At first glance, this hypothesis seems supported by the study of Alkhulaifi and coworkers.^{5,6} These authors subjected patients undergoing coronary artery bypass grafting to a preconditioning regimen consisting of two 3-minute cycles of aortic cross-clamping followed by 2 minutes of reperfusion before a 10-minute period of normothermic ventricular fibrillation. The major finding of this study was that at the end of the ischemic interval, myocardial tissue levels of adenosine triphosphate (ATP) were higher in preconditioned patients than in the control patients. However, whether this approach is relevant to cardioplegia-based preservation techniques remains unresolved. The present study was designed to address this issue. Along with standard biochemical and enzymatic indices, end points included measurements of myocardial levels of the protooncogene *c-fos* and of the heat shock protein (HSP) 70 messenger ribonucleic acids (mRNAs), because the expression of these two proteins is synergistically up-regulated by ischemia as part of the heart's genetic adaptive response to environmental stress.

Methods

Patients. Twenty patients undergoing isolated coronary artery bypass operations were prospectively entered into this study, which was approved by our institutional human experimentation committee. Anesthesia was uniform in all cases and consisted of a standardized combination of

fentanyl citrate, flunitrazepam, and pancuronium bromide. Cardiopulmonary bypass (CPB) was established with a crystalloid prime between the ascending aorta and the two venae cavae. CPB equipment consisted of a roller pump, a membrane oxygenator, and an in-line arterial filter. Left ventricular venting was accomplished in all patients through the right superior pulmonary vein. CPB was run at a flow rate of 2.2 L/min per square meter, and isoflurane (1%) or phenylephrine hydrochloride was used whenever required to maintain systemic pressures between 50 and 70 mm Hg. The core temperature, as assessed by a nasopharyngeal probe, was allowed to drift and fell to a nadir of 31° to 32° C. Myocardial protection was achieved with continuous retrograde blood cardioplegia maintained at the same temperature as the systemic perfusate and delivered according to our previously described "minicardioplegia" infusion technique.⁷ Administration of heparin before cannulation and its subsequent postbypass reversal by protamine sulfate were accomplished in a standard fashion.

Patients were randomized into one of two groups. After the onset of full-flow CPB with effective left ventricular decompression, patients assigned to the preconditioning protocol underwent a single 3-minute period of aortic crossclamping followed by 2 minutes of reperfusion before the onset of cardioplegic arrest. In control patients, a period of CPB of similar duration and under the same conditions of flow and left ventricular venting was instituted before the application of the aortic crossclamp (Fig. 1). Prearrest pacing was not used in either group.

End points. Blood samples were simultaneously drawn from the radial artery and the coronary sinus catheter before the institution of CPB, at the end of the 5-minute preconditioning sequence (transient aortic occlusion/reperfusion) or after an equivalent period of CPB in control patients, and at the end of cardioplegic arrest,

Fig. 2. CK-MB isoenzyme release during the course of the protocol in the preconditioning (PC) and control groups.

immediately before the removal of the aortic crossclamp. Samples were assayed for lactate by reflectance spectrophotometry (Eastman Kodak Company, Rochester, N.Y.) and for the MB isoenzyme of creatine kinase (CK-MB) by an immunometric technique (Ciba Corning, Medfield, Mass.).

Three sequential biopsies of the right atrial free wall close to the appendage were performed, at the same time intervals as the blood samples. The specimens were immediately frozen in liquid nitrogen and stored at -70°C . Total RNA was first extracted according to the technique described by Chomczynski and Sacchi.^{7a} Northern blot analysis was then performed using 25 to 50 μg total RNA per lane. Blots were successfully hybridized with probes specific for *c-fos*, HSP 70, and 18S rRNA. To this end, a 1.06 Kb *Pst* I fragment of a *v-fos* DNA cloned by Curran and associates^{7b} and an *EcoRI*-*Bam*HI fragment of genomic HSP 70 DNA cloned by Zakeri and colleagues^{7c} were labeled with (α - ^{32}P)deoxycytidine triphosphate (3000 Ci/mmol) using a random primer labeling reaction (Amersham Rediprime labeling system RPN 1633; Amersham Corp., Arlington Heights, Ill.). The 18S probe was a 24-mer oligonucleotide specific to the ribosomal 18S RNA. This probe was synthesized at the Institut Pasteur (Paris, France) and was 5'-end labeled using T4 polynucleotide kinase and (γ - ^{32}P)ATP (6000 Ci/mmol). Hybridization with labeled *fos* or HSP 70 DNA was performed for 12 to 16 hours at 42°C in 50% formamide, 5 $\times$ saline-sodium citrate buffer, 0.1% (weight/volume) sodium dodecylsulfate, 5 $\times$ Denhardt's solution, 250 gm/ml of sonicated herring sperm DNA, and 50 mmol/L NaH_2PO_4 at pH 6.5. A hybridization solution of the same composition, but without formamide, was used for the hybridization with the 18S rDNA probe. Membranes hybridized with the *fos* and HSP 70 probes were washed twice for 10 minutes in 2 $\times$ saline-sodium

Table I. Patient data

Variable	Control group (n = 10)	Preconditioned group (n = 10)
A (yr)	63 $\pm$ 4	68 $\pm$ 3
Sex ratio (M/F)	7/3	7/3
Unstable angina	6	5
LV ejection fraction (%)	55 $\pm$ 4	55 $\pm$ 3
CPB time (min)	107 $\pm$ 7	110 $\pm$ 8
CPB temperature ($^{\circ}\text{C}$)	32.1 $\pm$ 0.3	31.7 $\pm$ 0.4
Aortic crossclamping (min)	72 $\pm$ 6	64 $\pm$ 5
Cardioplegia off (min)	3 $\pm$ 1	4 $\pm$ 2
Spontaneous defibrillation	7	10

LV, Left ventricular; CPB, cardiopulmonary bypass. As applicable, data are shown as number of patients or mean $\pm$ the standard error of the mean.

citrate buffer, 0.1% (weight/volume) sodium dodecylsulfate at room temperature, and twice in 2 $\times$ saline-sodium citrate buffer, 0.1% (weight/volume) sodium dodecylsulfate at 50°C for 15 minutes. After hybridization with the 18S rDNA probe, membranes were washed three times at room temperature in 3 $\times$ saline-sodium citrate buffer for 10 minutes. To quantify hybridization signals, the blots were exposed (1 to 3 hours) to a BAS 1000-imaging plate (Bioimaging analyzer, Fujix, Tokyo, Japan) in an autoradiographic cassette. The images obtained after scanning were transformed to numerical data by means of the analysis function of MacBAS software (Fujix). The densitometric values corresponding to the *c-fos* or HSP 70 hybridization signals were normalized for the 18S rRNA signal, and results of *c-fos* and HSP 70 mRNA expression are presented as the percentage of the signal given by rat cardiac RNA sample extracted from a cycloheximide-treated animal⁸ used as an internal standard.

Statistical analysis. Comparison between the two groups was performed by two-way analysis of variance with repeated measures and unpaired two-tailed *t* tests. Paired two-tailed *t* tests were used for comparing data within each group. Statistical significance was set at the 0.05 level. Results are given as mean plus or minus the standard error of the mean.

Results

The major preoperative and intraoperative variables were similar in the two groups (Table I). Likewise, there was no significant difference between groups with regard to pre-CPB arterial and coronary sinus values for lactate and CK-MB. There was also no difference in *c-fos* and HSP 70 mRNA expression, assessed by the first pre-CPB biopsy, between preconditioned patients and those of the control group.

At the end of the crossclamp period, the release of CK-MB from the coronary sinus was greater in preconditioned patients than in control patients (Fig. 2). As assessed by repeated-measures analysis of variance, the difference between groups almost

Fig. 3. Lactate metabolism data during the course of the protocol in preconditioning (PC) and control groups. Each group consisted of 10 patients. Results are given as means $\pm$ standard error of the mean.

Table II. Quantitative expression of *c-fos* and HSP 70 mRNA in the preconditioned and control groups

Group	<i>c-fos</i> (% I.S.)			HSP 70 (% I.S.)		
Time points	Pre-CPB	End of PC/5 min of CPB	End arrest	Pre-CPB	End of PC/5 min of CPB	End arrest
Control (n = 7)	16 $\pm$ 6	66 $\pm$ 16*	93 $\pm$ 22*	118 $\pm$ 35	144 $\pm$ 46	148 $\pm$ 49
PC (n = 7)	26 $\pm$ 10	91 $\pm$ 22*	131 $\pm$ 24*	130 $\pm$ 32	148 $\pm$ 35	203 $\pm$ 47

PC, Preconditioning. Results are expressed as the percentage of the signal given by rat cardiac RNA sample extracted from a cycloheximide-treated animal used as an internal standard (I.S.).

* $p < 0.05$ versus Pre-CPB.

reached the level of statistical significance ($p = 0.05$). Whereas control patients extracted lactate at the end of the crossclamp period, consistent with the use of aerobic cardioplegia, lactate metabolism was shifted toward production in the preconditioned group (Fig. 3). This pattern was present at the end of the 2-minute period of intervening reperfusion that followed the 3-minute ischemic preconditioning challenge and remained unchanged thereafter.

Levels of *c-fos* significantly increased after the preconditioning period and the 5-minute CPB period (Table II), and this increase was similar in the two groups. The *c-fos* mRNA levels were significantly higher in atrial tissue excised immediately after preconditioning and at the end of cardioplegic arrest than in the pre-CPB atrial sample, but this increase was of similar magnitude in the two groups. In contrast to *c-fos*, HSP 70

mRNA levels measured at the end of the cross-clamp period were unchanged from baseline values in both groups (Table II). A representative Northern blot of *c-fos* and HSP 70 in control and preconditioned patients at the three time intervals is shown in Fig. 4.

Postoperative recoveries were uneventful in the two groups. There were no adverse clinical events related to preconditioning.

Discussion

Interpretation of results. The major finding of this study is that a single cycle of ischemic preconditioning does not improve the cardioprotective effects of continuous retrograde warm blood cardioplegia, as assessed by standard biochemical and enzymatic measurements. There is even some evidence that as short a period of aortic crossclamping

Fig. 4. Representative Northern blot of *c-fos* and HSP 70 mRNAs in one control and two preconditioned patients before CPB (1), after the preconditioning protocol or 5 minutes of CPB (2), and at the end of the cardioplegic arrest period (3). The *Ch* lane represents the signal given by a rat cardiac RNA sample extracted from a cycloheximide-treated animal used as an internal standard. The densitometric values corresponding to the *c-fos* or HSP 70 hybridization signals were normalized for the 18S rRNA signal.

as 3 minutes before the protracted period of cardioplegic arrest could have detrimental effects reflected by a greater release of CK-MB isoenzymes in the coronary sinus effluent and a shift of lactate metabolism toward production at the end of cardioplegic arrest. Lactate production persisted in the preconditioned group even though one would have anticipated the greatest changes in lactate extraction immediately after reperfusion after the brief preconditioning ischemic interval. We have no clear explanation for this observation. It is possible that the ischemic stress induced by preconditioning was of such magnitude that once cardioplegic arrest was superimposed after only 3 minutes of reperfusion, it could not be fully reverted despite continuous delivery of blood cardioplegic solution. The occurrence of such ischemic damage persisting throughout the period of arrest is indeed supported by the markedly greater CK-MB values found in the preconditioned group at the end of arrest. Alternatively, the wide scattering in the lactate data may have shifted the lactate gradient toward extraction in the control group and toward production in the preconditioned group, but the fact that the group

means were not significantly different ($p = 0.3$) should lead to caution in the interpretation of this finding. In addition, myocardial levels of the mRNA for the protooncogene *c-fos* significantly increased throughout the crossclamping period. This increase could be expected inasmuch as this protooncogene is readily expressed in response to stressful stimuli. However, the intraoperative rise in *c-fos* levels was of similar extent in the two groups, suggesting that the magnitude of stress induced by CPB and superimposed cardioplegic arrest had not been reduced by the prearrest preconditioning regimen. Furthermore, protooncogenes participate in the cellular response to stress by causing the transcriptional activation of genes that code for critical regulatory proteins. In this setting, *c-fos* is involved in the "switch on" of the HSP 70 gene. This protein is up-regulated in response to a variety of stressful stimuli including heat, pressure, and volume overload, myocardial stretch, hypoxia, and ischemia. The protective effects of HSPs are mainly attributed to their ability to prevent abnormal protein-protein interactions and might account for the correlation between increased expression of myocardial HSP 70

and accelerated functional recovery of stunned myocardium.⁹⁻¹¹ In the present study, the patterns of changes in HSP 70 mRNA differed from those of *c-fos* in that they did not increase significantly during the operation in either group, an observation consistent with that of McGrath and coworkers.¹²

At least four hypotheses can be raised to explain this finding. First, it is possible that the HSP content of diseased myocardium is already so elevated that it cannot be up-regulated further by a preconditioning stimulus. This assumption is primarily based on the fact that high levels of HSP 72 inhibit the transcription factor required for additional protein synthesis. Second, the last biopsy may have been performed too early to allow for the detection of a delayed increase in HSP 70 mRNA expression. However, this biopsy specimen was taken an average of 70 minutes after the preconditioning stimulus, which is within the time span previously reported for observing an increased expression of the mRNA coding for this protein.¹³ A third possibility could be that the adaptive capacity to stress decreases with advancing age.¹⁴ However, in the study of McGrath and associates,¹² the myocardial content of HSP 70 also failed to increase during the course of the operation in several young patients. Fourth, the duration of the preconditioning ischemic stimulus used in our study may have been too short to elicit an increase in HSP 70 mRNA, whereas the performance of the last biopsy before removal of the aortic crossclamp may have led to an underestimation of the accumulation of HSP 70 mRNA because the latter seems to involve a reperfusion-dependent component.¹⁵ However, although a delayed increase in myocardial levels of HSP 70 in the preconditioned group cannot be excluded, the analysis of patient outcomes indicates that, if it occurred, this increase did not translate into a clinically detectable improvement in postoperative recovery. On the other hand, the preconditioning ischemia may have been severe enough to cause cellular damage reflected by CK-MB release from the myocardium, which is known to down-regulate HSP 70 mRNA transcription. Consequently, our observations do not favor a role for the preconditioning protocol used in this study in eliciting the specific component of endogenous protection presumably mediated by stress-inducible proteins.

Preconditioning in the setting of cardiac surgery.

Actually, the lack of additional protection provided by preconditioning in the present study is consistent with several well-established features of this adap-

tive phenomenon. First, as previously stated, the major benefit of preconditioning is to reduce infarct size, and a large body of evidence confirms that the improvement in functional recovery seen in preconditioned hearts submitted to global ischemia is primarily due to a limitation of infarct size, not a reduction of stunning incurred by nonnecrotic myocardium.¹⁶ This was well demonstrated in the study of Illes and associates,³ in which the improvement in function found in preconditioned hearts after cardioplegic arrest correlated with a reduced leakage of CK. This finding may limit the relevance of preconditioning to cardiac operations, because early post-CPB pump failure is more commonly due to stunning than to overt infarction. Second, although the end-effectors of the intracellular signaling pathway elicited by the preconditioning stimulus are not yet fully characterized, it appears that a major mechanism whereby preconditioning exerts its cardioprotective effects is a slowing of the rate of ATP depletion during the protracted period of ischemia. This hypothesis has been challenged in experimental situations of total no-flow ischemia in which preconditioning was unexpectedly found to accelerate the fall of ATP.¹⁷ However, no-flow ischemia is not relevant to the clinical situation of aortic cross-clamping, because some residual myocardial flow originating from noncoronary collateral vessels persists and the heart is perfused continuously with a normothermic blood cardioplegic solution. Thus it is conceivable that preconditioning may afford some protection when used, as in Alkhulaifi's study,⁵ in conjunction with a technique such as normothermic ventricular fibrillation, which causes a drastic decline in myocardial levels of high-energy phosphates. Preconditioning could also contribute to successful results in techniques using intermittent aortic crossclamping¹⁸ because the first episode of warm ischemia could be equivalent to a preconditioning stimulus that limits cumulative deterioration of left ventricular function with subsequent ischemic intervals.¹⁹ Conversely, the ATP-sparing effect of preconditioning may become redundant with that of cardioplegia. This hypothesis is supported by the observation that, in a rat model of normothermic global ischemia of short duration (35 minutes), ischemic preconditioning and cardioplegia have been shown to be equally effective, but without additive effects.²⁰ Similarly, Steenbergen and coworkers²¹ have reported that preconditioning used in conjunction with magnesium arrest failed to improve protection beyond that provided by cardio-

plegia alone. It is noteworthy that magnesium is included in our cardioplegic formulation. The theory that any intervention that reduces myocardial oxygen consumption during the protracted period of ischemia may prevent the protective effects of preconditioning from occurring is further supported by the recent observation that preservation of ATP levels seen in patients kept normothermic during ventricular fibrillation⁵ is lost when patients preconditioned in a similar fashion are cooled to 32° C.²² Indeed, the two situations in which preconditioning has been shown to provide better protection than hypothermia or cardioplegia alone are long ischemic times² and maldistribution of cardioplegic solution because of proximal coronary artery occlusion.²³ We suspect that, in these two settings, suboptimal myocardial preservation may have caused some degree of myocardial necrosis, thereby accounting for the benefits of preconditioning through its infarct size-limiting effects. In the present study, aerobic perfusion was continuously maintained during cross-clamping periods of similar durations, while distribution of cardioplegic solution was optimized through the use of the retrograde route. These conditions may have left little room for preconditioning to "compensate" for a suboptimal myocardial preservation, hence its inability to yield additional cardioprotection. Our results lead us to be careful about speculating that intermittent warm blood cardioplegia could be beneficial, because periods of no flow could, presumably, precondition the heart. This hypothesis is intellectually attractive because it tends to justify the use of a technique more directed at improving the surgeon's comfort during the operation than at meeting the energy requirement of the myocardium. There is, however, no sound experimental or clinical information currently available to support this hypothesis.

A third confounding factor could be the ability of CPB to elicit, by itself, some form of preconditioning, perhaps through activation of adenosine and α -adrenergic receptors.²⁴ If such was the case, all our patients may have already been preconditioned by the brief CPB period preceding cardioplegic arrest, which could explain why a superimposed episode of ischemic preconditioning failed to provide additional protection.

Fourth, Bukhari and associates²⁵ have recently reported that aprotinin could negate the cardioprotective effects of preconditioning. This factor, however, is unlikely to have skewed our results, because aprotinin administration was evenly dis-

tributed in the two groups (three patients in each group).

A last intriguing observation is that not only did our preconditioned patients not show improved protection, but they even tended to have greater evidence of enzymatic and metabolic myocardial damage. We do not have a clear explanation for this finding. It is conceivable that the washout of adenosine, believed to be a key trigger of the intracellular signaling pathway that ultimately leads to preconditioning-related cardioprotection by continuous blood cardioplegic perfusion, may have prevented it from exerting its expected benefits and resulted in the damaging effects of a prearrest period of global ischemia. Additionally, the activation of ATP-dependent potassium channels, considered likely effectors of the signaling pathway elicited by preconditioning,²⁶ may have been attenuated by the hyperkalemic cardioplegic solution used, thus limiting their cardioprotective effects.¹ This scenario, however, is unlikely in this study because the serum potassium concentrations resulting from our cardioplegia delivery technique are by far too low (6 to 7 mEq/L) to abolish the transsarcolemmal potassium electrochemical gradient. Overall, the negative biochemical findings of this clinical study with ischemic preconditioning are consistent with the report by Bolling and coworkers,²⁷ which found an episode of no-flow ischemia before cardioplegic arrest to cause some adverse effects evidenced by an impairment of oxygen utilization efficiency and the absence of functional benefits during reperfusion.

Methodologic limitations. At least two limitations have to be addressed. First, the preconditioning protocol may have been inadequate. A 5-minute period (3 minutes of crossclamping followed by 2 minutes of reperfusion) was chosen to fit clinically acceptable time constraints. Experimentally, such a timing has been shown to elicit cardioprotection and, on the basis of our enzymatic and metabolic data, we would now consider a longer period of ischemic preconditioning before cardioplegic arrest ethically questionable. A second concern relates to the study of genetic markers. In addition to the previously discussed limitations inherent to the timing of the sampling protocol, it could be argued that assessment of HSP 70 mRNA levels is not as relevant as measurement of the protein itself. Nevertheless, a good temporal correlation has previously been reported between increases in levels of HSP 70 mRNA and those of the protein.²⁸ Alternatively, it is possible that right atrial tissue is not appropriate for detecting changes in stress proteins,

because these proteins, preferentially expressed in ischemic areas,¹⁰ may not be synthesized in great amounts in the right atrium, whose blood supply is not primarily dependent on coronary vessels and thus may not reflect adequately changes in ventricular stress proteins.

Conclusion

The present study leads us to advise caution about the clinical use of ischemic preconditioning before normothermic blood cardioplegic arrest. This conclusion does not preclude a possible use for some form of pharmacologic preconditioning which, based on current knowledge, could primarily rely on activation of adenosine receptors or opening of ATP-sensitive potassium channels.^{24, 29} Whether pharmacologic preconditioning may be beneficial in clinical practice still remains to be established; however, ischemic preconditioning by aortic crossclamping should be avoided because of its potential detrimental effects. Perhaps minimally invasive off-pump coronary artery bypass operations³⁰ and on-pump revascularization without aortic crossclamping will turn out to be appropriate elective indications for ischemic preconditioning. Indeed, the local isolation of the target blood vessels required to perform the anastomosis on the beating heart best reproduces animal models of regional ischemia that have yielded the most convincing evidence of the cardioprotective effects of preconditioning.

We acknowledge the help of Madame Roselyne Prioux in the preparation of the manuscript.

REFERENCES

- Parratt JR. Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation. *TIPS* 1994;15:19-25.
- Cave AC, Hearse DJ. Ischaemic preconditioning and contractile function: studies with normothermic and hypothermic global ischaemia. *J Mol Cell Cardiol* 1992;24:1113-23.
- Illes RW, Wright JK, Inners-McBride K, Yang CJ, Tristan A. Ischemic preconditioning improves preservation with crystalloid cardioplegia. *Ann Thorac Surg* 1994;58:1481-5.
- Engelman DT, Chen CZ, Watanabe M, Engelman R, Roussou JA, Flack JE, et al. Improved four and six hour myocardial preservation by hypoxic preconditioning. *Circulation* 1995;92(Suppl):II417-22.
- Alkhulaifi AM, Yellon DM, Pugsley WB. Preconditioning the human heart during aorto-coronary bypass surgery. *Eur J Cardiothorac Surg* 1994;8:270-6.
- Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. *Lancet* 1993;342:276-7.
- Menasché P, Touchot B, Pradier F, Bloch G, Piwnica A. Simplified method for delivering normothermic blood cardioplegia. *Ann Thorac Surg* 1993;55:177-8.
- Chomczynski P, Sacchi N. Single step method of RNA isolation by guanidium thiocyanate-phenol chloroform extraction. *Anal Biochem* 1987;162:156-9.
- Curran T, Peters G, Van Beveren C, Teich NM, Verma IM. FBJ murine osteosarcoma virus identification and molecular cloning of biologically active proviral DNA. *J Virol* 1982;44:674-82.
- Zakeri ZF, Wolgemuth DJ, Hunt CR. Identification and sequence of a new member of the mouse HSP70 gene family and characterization of its unique cellular and developmental pattern of expression in the male germ line. *Mol Cell Biol* 1988;8:2925-32.
- Bauters C, Moalic JM, Mouas C, Emanoil-Ravier R, Schiaffino S, Swynghedauw B. Coronary flow as a determinant of *c-myc* and *c-fos* proto-oncogene expression in an isolated adult rat heart. *J Mol Cell Cardiol* 1988;20:97-101.
- Black SC, Lucchesi BR. Heat shock proteins in the ischemic heart: an endogenous mechanism of protection. *Circulation* 1993;87:1048-51.
- Yellon DM, Latchman DS. Stress proteins and myocardial protection. *J Mol Cell Cardiol* 1992;24:113-24.
- Robinson BL, Morita T, Toft DO, Morris JJ. Accelerated recovery of postischemic stunned myocardium after induced expression of myocardial heat-shock protein (HSP70). *J Thorac Cardiovasc Surg* 1995;109:753-64.
- McGrath LB, Locke M, Cane M, Chen C, Ianuzzo CD. Heat shock protein (HSP 72) expression in patients undergoing cardiac operations. *J Thorac Cardiovasc Surg* 1995;109:370-6.
- Knowlton AA, Brecher P, Apstein CS, Ngoy S, Romo GM. Rapid expression of heat shock protein in the rabbit after brief cardiac ischemia. *J Clin Invest* 1991;87:139-47.
- Kregel KC, Moseley PL, Skidmore R, Gutierrez JA, Guerrero V Jr. HSP70 accumulation in tissues of heat stressed rats is blunted with advancing age. *J Appl Physiol* 1995;79:1673-8.
- Heads RJ, Latchman DS, Yellon DM. Differential stress protein mRNA expression during early ischaemic preconditioning in the rabbit heart and its relationship to adenosine receptor function. *J Mol Cell Cardiol* 1995;27:2133-48.
- Ovize M, Przyklenk K, Hale SL, Kloner RA. Preconditioning does not attenuate myocardial stunning. *Circulation* 1992;85:2247-54.
- Kolocassides KG, Galinanes M, Hearse DJ. Preconditioning accelerates contracture and ATP depletion in blood-perfused rat hearts. *Am J Physiol* 1995;269:H1415-20.
- Bonchek LI, Burligame MW, Vazales BE, Lundy EF, Gassman CJ. Applicability of noncardioplegic coronary bypass to high-risk patients. *J Thorac Cardiovasc Surg* 1992;103:230-7.
- Abd-Elfattah AS, Ding M, Wechsler AS. Intermittent aortic cross-clamping prevents cumulative adenosine triphosphate depletion, ventricular fibrillation, and dysfunction (stunning): Is it preconditioning? *J Thorac Cardiovasc Surg* 1995;110:328-9.
- Kolocassides KG, Galinanes M, Hearse DJ. Ischemic preconditioning, cardioplegia or both? *J Mol Cell Cardiol* 1994;26:1411-4.
- Steenbergen C, Perlman ME, London RE, Murphy E. Mechanism of preconditioning: ionic alterations. *Circ Res* 1993;72:112-25.
- Di Salvo C, Hemming A, Jenkins D, Yellon D, Oakley RE, Wright J, et al. Can the human myocardium be preconditioned?

- tioned with ischaemia under hypothermic conditions? Proceedings of the ninth annual meeting of the European Association for Cardiothoracic Surgery. Paris: September 25-7, 1995, page 324 [abstract].
23. Galinanes M, Argano V, Hearse DJ. Can ischemic preconditioning ensure optimal myocardial protection when delivery of cardioplegia is impaired? *Circulation* 1995;92(Suppl): II389-II94.
 24. Burns PG, Krukenkamp IB, Caldarone CA, Gaudette GR, Bukhari EA, Levitsky S. Does cardiopulmonary bypass alone elicit myoprotective preconditioning? *Circulation* 1995; 92(Suppl):II447-51.
 25. Bukhari EA, Krukenkamp IB, Burns PG, Gaudette GR, Schulman JJ, Al-Fagih MR, et al. Does aprotinin increase the myocardial damage in the setting of ischemia and preconditioning? *Ann Thorac Surg* 1995;60:307-10.
 26. Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K⁺ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model. *Circ Res* 1995;77:1030-5.
 27. Bolling SF, Olszanski DA, Childs KF, Gallagher KP, Ning XH. Stunning, preconditioning, and functional recovery after global myocardial ischemia. *Ann Thorac Surg* 1994;58:822-7.
 28. Currie RW, Tanguay RM. Analysis of RNA transcripts for catalase and SP71 in rat hearts after in vivo hyperthermia. *Biochem Cell Biol* 1991;69:375-82.
 29. Menasché P, Kevelaitis E, Mouas C, Grousset C, Piwnica A, Bloch G. Preconditioning with potassium channel openers: A new concept for enhancing cardioplegic protection? *J Thorac Cardiovasc Surg* 1995;110:1606-14.
 30. Subramanian VA, Sani G, Benetti FJ, Calafiore AM. Minimally invasive coronary bypass surgery: a multi-center report of preliminary clinical experience [abstract]. *Circulation* 1995;92(Suppl):I645.

Discussion

Dr. Steven F. Bolling (*Ann Arbor, Mich.*). I am also part of a group that studies preconditioning. Without a doubt, it is a very vexing area, as to its application to cardiac surgery.

I have a number of specific questions. Is your model actually a model of ischemic preconditioning? You used warm retrograde continuous cardioplegia and have published on this method, your miniblood cardioplegia, in an effort to reduce ischemia. Might this not be just a model of ischemia followed by no ischemia, which is not preconditioning? You do not have histopathologic data, not necessary inasmuch as this is a study in patients, but you do not show whether this is a model of myocyte necrosis or stunning.

Second, if this is preconditioning, did preconditioning occur? Perhaps there are other, better markers of preconditioning that could be followed as opposed to *c-fos* and HSP. Protein kinase C isoforms are perhaps better indicators of the occurrence of preconditioning, especially the delta and epsilon isoforms. Third, why were right atrial biopsy specimens used? The correlation between right

atrial myocyte changes and ventricular myocyte changes are not always the same.

Last, I think the title is appropriate: We must use a word of caution. In this kind of study a beta error of statistics can occur. If preconditioning is not shown in this particular model, it does not mean that preconditioning does not exist. This is a small number of patients. With no difference in clinical or biochemical outcomes, we cannot assume that preconditioning does not exist because we do not see it. I again agree with the authors that there are probably more effective pharmacologic interventions that would allow us to use preconditioning in cardiac surgery.

Dr. Perrault. First, we agree totally with your first comment. Initially we thought that perhaps we could improve the degree of ischemia or stunning that is sometimes seen even after warm normothermic continuous retrograde cardioplegia.

Looking at the lactate data on the control group, perhaps you are right that there seems to be very little ischemia occurring and then there is little room for preconditioning to improve the results. Obviously, because this study was performed in patients, histopathologic data were not easily available. We agree that we have not documented the presence of stunning after the procedure. The preconditioning stimulus did, however, cause acute electrocardiographic changes in a number of patients, but we did not study global or localized myocardial dysfunction. Also, although continuous perfusion of normothermic blood cardioplegic solution is associated in the majority of cases with good results, stunning and myocardial infarction leading to post-CPB pump failure still does occur at times. There may be subsets of high-risk patients perhaps with long crossclamp times or inadequate delivery of cardioplegia who might benefit from enhancement of cardioplegic protection. It is for this reason that we undertook this initial study in patients with normal ventricular function to see if any benefits could be detected after the preconditioning challenge. There is also no evidence that preconditioning has occurred, and we believe after performing this study that normothermic blood cardioplegia may not enable preconditioning to show any benefits.

As for our choice of the markers *c-fos* and HSP 70, obviously the study of the isoforms of protein kinase C would take our understanding of preconditioning in human beings one step further, but it also depends on the locally available resources for molecular biology markers. I am sure that other studies will be conducted with those markers and will show either evidence of preconditioning or perhaps more relevant information.

Right atrial biopsies were performed because the specimens were readily available near the cannulation site. We did not feel comfortable doing ventricular biopsies, and this was not acceptable to the ethical committee. In conclusion, we found that considering the good results obtained with normothermic cardioplegia, ischemic preconditioning, at least with this specific protocol, could not improve the protection in patients with normal ventricular function.