Protection of the human heart with ischemic preconditioning during cardiac surgery: Role of cardiopulmonary bypass

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Objective: Studies on the effects of ischemic preconditioning in the human heart have yielded conflicting results and therefore remain controversial. This study investigated whether ischemic preconditioning was able to protect against myocardial tissue damage in patients undergoing coronary artery surgery with cardiopulmonary bypass and on the beating heart.

Methods: A total of 120 patients were studied and divided into 3 groups: group I: cardiopulmonary bypass with intermittent crossclamp fibrillation; group II: cardiopulmonary bypass with cardioplegic arrest using cold blood cardioplegia; group III: surgery on the beating heart. In each group ($n = 40$), patients were randomly subdivided ($n = 20$ /subgroup) into control and preconditioning groups (1 cycle of 5 minutes of ischemia/5 minutes reperfusion before intervention). Ischemic preconditioning was induced by clamping the ascending aorta in groups I and II or by clamping the coronary artery in group III. Serial venous blood levels of troponin T were analyzed before surgery and at 1, 4, 8, 24, and 48 hours after termination of ischemia. In addition, in vitro studies using right atrial specimens obtained before the institution of cardiopulmonary bypass, and then again 10 minutes after initiation of bypass, were performed. The specimens were equilibrated for 30 minutes before being allocated to 1 of the following 2 groups ($n = 6$ per group): (1) ischemia alone (90 minutes of ischemia followed by 120 minutes of reoxygenation) or (2) preconditioning with 5 minutes of ischemia and 5 minutes of reoxygenation before the long ischemic insult. Creatine kinase leakage (U/g wet weight) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction (mmol/l per gram wet weight), an index of cell viability, were assessed at the end of the experiment.

Results: There were no perioperative myocardial infarctions or deaths in any of the groups studied. The total release of troponin T was similar in groups I and II (patients undergoing surgery with cardiopulmonary bypass) and in the release profile; they were unaffected by ischemic preconditioning. In contrast, the total troponin T release for the first 48 hours was significantly reduced by ischemic preconditioning in group III (patients undergoing surgery without cardiopulmonary bypass) from 3.1 ± 0.1 to 2.1 ± 0.2 ng · h · mL. Furthermore, the release profile that peaked at 8 hours in the control group shifted to the left at 1 hour. In the in vitro studies, the atrial muscles obtained before cardiopulmonary bypass were protected by ischemic preconditioning (creatinine kinase = 2.6 ± 0.2 and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction = 152 ± 24 vs creatine kinase = 5.4 ± 0.6 and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction = 87 ± 16 in controls; $P < .05$); however, the muscles obtained 10 minutes after initiation of cardiopulmonary bypass were already protected (creatinine kinase = 0.8 ± 0.1 and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction = 316 ± 38), and ischemic preconditioning did not result in further improvements.

Conclusions: Ischemic preconditioning is protective in patients undergoing coronary artery surgery on the beating heart without the use of cardiopulmonary bypass, but it offers no additional benefit when associated with bypass regardless of the mode of cardioprotection used, because cardiopulmonary bypass per se induces preconditioning.

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Myocardial injury, manifested as transient cardiac contractile dysfunction ("stunning") and myocardial necrosis, is the most frequent complication during heart surgery.¹ Ischemic preconditioning (IP), a powerful form of endogenous protection against stunning and infarction, has been demonstrated in a variety of animal species²⁻⁴ and in vitro experiments involving isolated human cardiomyocytes⁵ and human atrial trabeculae.^{6,7} For logistic and ethical reasons, no clinical study can meet the strict conditions of experimental studies on preconditioning with infarct size as the end point. As a result, human in vivo studies have produced conflicting results, and the role of IP in humans remains controversial.

Yellon and coworkers⁸ were the first to report that IP protects the human heart in the setting of cardiac surgery by use of the conservation of myocardial adenosine triphosphate (ATP) content as the major end point. However, Perrault and colleagues⁹ reported that in the presence of cardioplegic arrest, there was no difference in the release of biochemical markers (CK-MB) between the preconditioned and control groups. The lack of additional protection conferred by IP was further confirmed by the absence of difference in the postarrest myocardial levels of ribonucleic messengers coding for cardioprotective heat shock proteins between the 2 groups. Similar negative results were reported by Kaukoranta,¹⁰ Di Salvo,¹¹ and their colleagues, who went on to report that in the presence of hypothermia, no beneficial effect of preconditioning was observed in similar patients undergoing surgery with intermittent fibrillation techniques.

The apparent discrepancy between results obtained with noncardioplegic and cardioplegic techniques could be reconciled if one takes into account a possible hypothesis: Preconditioning and its salutary effects are only observed in situations of unprotected ischemia. With this in mind, this study (1) investigates whether IP with 5 minutes of ischemia followed by 5 minutes of reperfusion is protective in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass (CPB) using cardioplegia and ventricular fibrillation techniques and in patients undergoing coronary artery bypass graft surgery on the beating heart without CPB and (2) elucidates the underlying cause of any protection.

Methods

In Vivo Studies

Patient selection and study groups. A total of 120 patients with stable angina who underwent operations performed by a single surgeon (M.G.) were entered in the study. They were divided into 2 groups: those undergoing surgery with CPB (ie, on-pump) and exhibiting 3-vessel coronary disease ($n = 80$) and those undergoing surgery on the beating heart without CPB (ie,

off-pump) and with single- or double-vessel coronary artery disease ($n = 40$). Patients in the CPB group were randomized by use of a computer-generated random table to undergo surgery (1) with CPB and intermittent crossclamp fibrillation $\pm$ IP ($n = 20$ in each subgroup) or (2) with cold blood cardioplegia $\pm$ IP ($n = 20$ in each subgroup). Similarly, patients undergoing surgery on the beating heart without CPB were randomized to receive or not receive IP ($n = 20$ in each subgroup). IP was induced by clamping the ascending aorta for 5 minutes at 37°C when CPB was used or by occluding the coronary artery to be grafted for the same period and then reperfusion for 5 minutes before the bypass graft was performed. We have demonstrated that this IP protocol is the most protective in the human myocardium.⁷ Patients with low ejection fractions ($<30\%$), unstable angina, recent myocardial infarctions (<1 month), additional cardiac diseases, severe noncardiac diseases, or diabetes, and who were receiving medication that included ATP-dependent potassium channel (K_{ATP}) openers were excluded. The study was approved by the local ethics committee, and all patients gave written informed consent to participate in the study. Preoperative characteristics of the patients are highlighted in Table 1.

Operative procedure. Anesthetic technique was standardized for all patients. Anesthesia was induced by fentanyl and maintained with enflurane in all patients. All operations were performed through a median sternotomy and with full heparinization (3 mg/kg intravenously). CPB was conducted with nonpulsatile perfusion flow ($2.2\text{--}2.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) with ascending aortic cannulation and 2-staged venous cannulation and moderate systemic hypothermia (32°C). When cardiac arrest was achieved with cold blood (6–10°C) cardioplegia, 1000 mL of the solution (composition in mmol/L: 16 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 2 CaCl_2 ; 20 KCl; 147 NaCl; 1.0 procaine HCl, pH 7.40) was mixed with blood from the pump in a ratio of 1:1 and injected into the aortic root immediately after aortic crossclamping to obtain a myocardial temperature of 12°C to 15°C. An additional dose of 500 mL of cardioplegic solution was injected after 30 minutes of ischemia. In the group in which intermittent aortic crossclamp fibrillation was used, reperfusion was performed for 3 minutes between each period. One single distal coronary artery anastomosis was performed during each ischemic period. Proximal anastomosis was completed on the beating heart with an aortic partial occlusion clamp in all groups.

In the off-pump group, coronary bypass grafting was performed on the beating heart using the Octopus myocardial stabilization device (Medtronic Inc., Minneapolis, Minn). The suction cups were placed on the epicardial surface on either side of the artery to be grafted, with a suction pressure of no more than 600 mm Hg. Small coronary clamps were applied to the proximal and distal sites of the anastomosis with just enough pressure to occlude coronary flow and therefore allow grafting in a bloodless field. The clamps were released when the anastomosis was complete. The proximal anastomosis was performed with a partial occlusion aortic clamp.

IP was applied before the first dose of cardioplegic solution or immediately before the aortic cross-application or the coronary artery occlusion. The use of a Khuri Tissue pH Analyzer (Vascular Tech, Boston, Mass) showed that the pH of the myocardium

TABLE 1. Patient characteristics and perioperative data

Variable	With CPB				Without CPB	
	Intermittent crossclamp		Cold blood cardioplegia		Control	PC
	Control	PC	Control	PC		
Number	20	20	20	20	20	20
Age (yr $\pm$ SD)	66.9 $\pm$ 5.4	63.7 $\pm$ 4.2	61.4 $\pm$ 9.2	63.8 $\pm$ 5.9	61.2 $\pm$ 2.7	59.2 $\pm$ 6.4
LVEF (% $\pm$ SD)	60.2 $\pm$ 5.5	61.8 $\pm$ 4.1	62.1 $\pm$ 5.7	63.7 $\pm$ 4.9	59.7 $\pm$ 9.2	65.1 $\pm$ 6.4
No. of grafts/patient	3.8 $\pm$ 1.5	3.6 $\pm$ 0.8	3.7 $\pm$ 1.2	4.1 $\pm$ 0.7	1.15 $\pm$ 0.6	1.2 $\pm$ 0.5
Ischemic time/graft (min $\pm$ SD)	16.1 $\pm$ 2.5	15.4 $\pm$ 3.1	17.2 $\pm$ 4.9	14.7 $\pm$ 5.7	17.8 $\pm$ 7.9	16.9 $\pm$ 4.7
CPB time (min $\pm$ SD)	66.4 $\pm$ 12.7	70.4 $\pm$ 11.4	71.4 $\pm$ 13.4	69.8 $\pm$ 6.7		

PC, Preconditioning; LVEF, left ventricular ejection fraction (expressed as a percentage $\pm$ standard deviation); CPB, cardiopulmonary bypass.

decreased from greater than 7.3 to less than 7.0 at the end of the 5-minute IP insult in all cases.

Assessment of myocardial injury. Serial venous blood samples were collected before induction of anesthesia and at 1, 4, 8, 24, and 48 hours after termination of ischemia in every patient for the assessment of troponin T (TnT). This was measured by use of the commercially available enzyme-linked immunoabsorbent assay kit TnT (Boehringer Mannheim, Mannheim, Germany). The lower detection limit of the assay was 0.05 ng/mL, and concentrations greater than the discriminator value of 0.1 ng/mL were considered elevated.

Hemodynamic measurements. Heart rate, mean pulmonary artery pressure, pulmonary capillary wedge pressure, and cardiac output were monitored with a pulmonary artery flotation catheter. Derived cardiovascular variables, including cardiac index and systemic and pulmonary vascular resistance, were calculated by standard formulas. Hemodynamic data were collected at 4 time points: baseline (just after induction of anesthesia) and 1, 3, and 24 hours after the termination of ischemia. Changes in derived variables were calculated and compared.

In Vitro Studies

Specimens of human right atrium appendage were obtained from patients undergoing elective coronary artery surgery with CPB. Samples were obtained before the institution of CPB and again 10 minutes after the initiation of CPB. Two 6-0 Prolene (Ethicon Inc, Somerville, NJ) encircling sutures were placed in the epicardial layer of the appendage once the pericardium was opened. During the entire period of harvesting, the atrium was exposed to the systemic circulation. The prebypass sample was then harvested, and a 2-stage venous cannula was inserted in the free wall of the right atrium 3 cm away from the appendage. CPB was then instituted, and after 10 minutes, a further sample of appendage was harvested. In each case, specimens were quickly immersed in cold (4°C) Krebs-Henseleit-HEPES medium, which comprised (in micromoles per liter) NaCl, 118; KCl, 4.8; NaHCO₃, 27.2; KH₂PO₄, 1; MgCl₂, 1.2; CaCl₂, 1.25; glucose, 10; and HEPES, 20. The medium was pre-bubbled with 95% O₂/5% CO₂ to attain a Po₂ of 25 to 30 kPa and pH 7.4. The atrial appendage was immediately sliced freehand with Swann-Morton skin graft blades (Swann-Morton Ltd, Sheffield, United Kingdom) to a thickness of 0.3 to 0.5 mm and a weight of 5 to

10 mg each, as previously described.¹² In brief, the tissue was placed with its epicardial surface face down on filter paper fixed to a rectangular glass base (5 $\times$ 25 cm). A ground glass slide (2.5 $\times$ 7.5 cm) was then pressed against the tissue, and the blade was drawn between the slide and tissue. The slicing apparatus and the tissue were kept wet at all times with medium that was stored on ice (4°C–10°C). The specimens were equilibrated for 30 minutes before being randomly allocated to 1 of the following 2 groups (n = 6 each from different patients/group): (1) ischemia alone (90 minutes of ischemia followed by 120 minutes of reperfusion) or (2) IP with 5 minutes of ischemia and 5 minutes of reperfusion before 90 minutes of ischemia followed by 120 minutes of reperfusion. For the induction of simulated ischemia, the medium was bubbled with 95% N₂/5%CO₂ (pH 6.80–7.00), and D-glucose was removed and substituted with D-2-deoxyglucose (10 mmol/L). The slices were maintained at 37°C during the entire ischemic period. Monitoring of Po₂ with an oxygen detector electrode (Oxylite; Oxford, United Kingdom) revealed that the Po₂ in the medium was 0 kPa. Tissue pH was not measured in the atrial sections. At the end of the ischemic period, the nonoxygenated medium was removed, and the slices were rinsed with oxygenated medium (O₂/CO₂, 19:1) and incubated in 5 mL of oxygenated medium containing 10 mmol/L glucose at 37°C for a further 120 minutes.

Tissue injury was determined by measuring the leakage of creatine kinase (CK) into the incubation medium during the 120-minute reperfusion period. This was assayed by a kinetic ultraviolet method based on the formation of nicotinamide adenine dinucleotide (Sigma Catalog No. 1340-K), and the results were expressed as U/g wet weight. Tissue viability was assessed by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) to blue formazan product at the end of the experimental time. In this assay, the yellow MTT is reduced to a blue formazan product by the mitochondria of viable tissue.

The tissue was loaded into a Falcon conical tube (15 mL) (Becton Dickinson Labware, Franklin Lakes, NJ) to which 2 mL of phosphate buffer solution (0.05 mol/L) containing MTT (1.25 mg/mL, 3 mmol/L at final concentration) was added and then incubated for 30 minutes at 37°C. After this, the tissue was homogenized in 2 mL of dimethyl sulfoxide (Homogenizer Ultra-Turrax T25, dispersing tool G8, IKA-Labortechnik, Staufen, Ger-

TABLE 2. Patients' hemodynamic data

Groups	Variable	HR	Mean MAP (mm Hg)	CVP (mm Hg)	Mean PAP (mm Hg)	PCWP (mm Hg)	CI (L · m ² · min ⁻¹)	PVRI (dyn · s · cm ⁻⁵ · m ⁻²)	SVRI (dyn · s · cm ⁻⁵ · m ⁻²)
Preoperative									
Crossclamp	-PC	68.4 ± 2.7	71.8 ± 5.7	10.3 ± 0.8	20.4 ± 3.1	5.8 ± 2.1	2.8 ± 0.2	195 ± 11	1568 ± 102
	+PC	75.8 ± 2.3	69.4 ± 3.5	9.8 ± 2.1	19.4 ± 5.2	6.4 ± 3.9	2.4 ± 0.4	182 ± 20	1602 ± 53
BCP	-PC	59.4 ± 9.4	65.7 ± 6.9	7.8 ± 5.7	22.6 ± 6.4	5.1 ± 2.6	2.9 ± 0.4	201 ± 13	1497 ± 33
	+PC	65.1 ± 6.8	70.2 ± 2.1	9.1 ± 2.4	25.1 ± 9.3	7.4 ± 1.3	3.05 ± 0.19	215 ± 25	1629 ± 98
Off-pump	-PC	52.4 ± 2.9	59.8 ± 8.7	5.6 ± 2.2	15.9 ± 11.4	9.3 ± 1.5	2.52 ± 0.54	156 ± 12	1307 ± 109
	+PC	49.5 ± 9.7	61.4 ± 11.2	6.7 ± 4.1	21.7 ± 4.8	5.7 ± 2.8	2.74 ± 0.29	168 ± 25	1567 ± 63
1 h after termination of ischemia									
X-clamp	-PC	101.3 ± 1.9	65.4 ± 4.7	9.4 ± 1.2	18.1 ± 2.7	10.4 ± 1.3	2.56 ± 0.31	217 ± 20	2001 ± 52
	+PC	95.6 ± 2.4	59.4 ± 7.2	11.2 ± 2.7	22.4 ± 5.1	8.5 ± 2.1	2.06 ± 0.28	271 ± 16	1959 ± 257
BCP	-PC	99.8 ± 10.4	71.2 ± 5.9	8.6 ± 3.5	16.4 ± 6.9	11.3 ± 1.4	2.76 ± 0.22	301 ± 27	2106 ± 554
	+PC	92.7 ± 4.9	64.8 ± 6.8	10.2 ± 2.9	21.9 ± 2.4	9.4 ± 3.8	2.98 ± 0.15	357 ± 12	1903 ± 261
Off-pump	-PC	105.5 ± 6.5	75.4 ± 8.7	11.6 ± 3.9	24.7 ± 9.4	12.1 ± 2.6	2.02 ± 0.26	298 ± 21	1506 ± 23
	+PC	112.2 ± 9.2	77.6 ± 6.2	13.5 ± 5.7	19.7 ± 3.1	10.5 ± 1.1	2.43 ± 0.18	312 ± 12	1709 ± 143
3 h after termination of ischemia									
Crossclamp	-PC	92.4 ± 11.5	67.5 ± 5.9	7.7 ± 3.2	20.9 ± 5.2	11.9 ± 6.4	2.52 ± 0.38	256 ± 17	1954 ± 106
	+PC	84.2 ± 12.7	71.8 ± 11.8	9.2 ± 2.7	19.8 ± 3.4	9.7 ± 2.9	2.21 ± 0.27	291 ± 67	2304 ± 328
BCP	-PC	95.3 ± 15.1	67.8 ± 11.9	9.8 ± 2.4	23.7 ± 6.8	10.2 ± 1.9	2.64 ± 0.12	333 ± 29	1796 ± 447
	+PC	105.3 ± 24.9	77.4 ± 4.9	10.7 ± 3.1	22.7 ± 3.6	11.6 ± 2.7	2.57 ± 0.25	401 ± 46	1667 ± 169
Off-pump	-PC	79.5 ± 12.9	71.5 ± 12.7	9.4 ± 2.5	21.9 ± 2.7	8.4 ± 2.5	2.11 ± 0.15	412 ± 29	1509 ± 225
	+PC	86.7 ± 14.7	75.1 ± 8.3	7.9 ± 4.2	26.4 ± 5.9	9.6 ± 1.6	2.92 ± 0.26	368 ± 36	1882 ± 203
24 h after termination of ischemia									
Crossclamp	-PC	61.5 ± 6.4	62.7 ± 2.9	6.4 ± 2.9	19.2 ± 2.7	5.8 ± 2.3	2.96 ± 0.26	208 ± 22	1804 ± 234
	+PC	72.9 ± 5.8	58.4 ± 9.1	7.6 ± 6.1	16.7 ± 4.9	10.4 ± 3.7	2.35 ± 0.36	214 ± 50	2004 ± 102
BCP	-PC	77.9 ± 9.6	61.9 ± 4.6	5.4 ± 3.8	21.6 ± 5.8	9.2 ± 1.5	2.55 ± 0.46	289 ± 16	1620 ± 98
	+PC	75.4 ± 11.3	71.3 ± 6.1	7.8 ± 1.6	24.9 ± 1.1	4.6 ± 2.8	3.19 ± 0.53	295 ± 26	1492 ± 207
Off-pump	-PC	91.6 ± 8.7	88.1 ± 5.3	4.4 ± 0.8	19.6 ± 4.6	7.8 ± 3.8	2.46 ± 0.34	312 ± 28	1623 ± 115
	+PC	84.3 ± 10.4	90.1 ± 2.8	6.8 ± 1.7	23.3 ± 3.3	10.2 ± 1.4	2.89 ± 0.41	256 ± 33	2006 ± 204

BCP, Blood cardioplegia; CI, cardiac index; CVP, central venous pressure; HR, heart rate; MAP, mean arterial pressure; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; SVRI, systemic vascular resistance index. Data are expressed as mean ± standard deviation.

many) at 9500 rpm for 1 minute. The homogenate was then centrifuged at 1000g for 10 minutes, and 0.2 mL of the supernatant was dispensed into a 98-well flat-bottom microtiter plate (Nunc Brand Products, Denmark). After this, the absorbance was measured on a plate reader (Benchmark; Bio-Rad Laboratories, Hercules, Calif) at 550 nm, and the results were expressed as micro-moles per liter per milligram wet weight.

Statistical Analysis

Statistical analyses were performed using the SPSS 9.0 statistical package program (SPSS Inc, Chicago, Ill) to investigate the efficacy of IP in the various groups of patients. Patients undergoing surgery without CPB demonstrated less severe coronary artery disease than those undergoing surgery with CPB (Table 2), and because of this, comparison between patients with and without CPB was not performed. A nonparametric test (Mann-Whitney *U*) was performed for non-Gaussian distribution of data. An unpaired Student *t* test was used for continuous data (2-tailed), and the χ^2 test for categorical data was used to compare variables between the groups. Repeated-measures analysis of variance was used to test the repeated observation variables postoperatively. The area under the curve was calculated by the method of Matthews and colleagues.¹³ Data were presented as mean ± SD.

Results

In Vivo Studies

There were no operative deaths (first 30 postoperative days) or perioperative myocardial infarctions in any of the study groups. Table 1 shows that the patient characteristics and perioperative data were similar within each study group, and that there was no difference between patients who were treated with or without IP.

Plasma Troponin T

Figure 1 (A and B) shows that the profile of TnT release in plasma was identical in patients undergoing coronary surgery with CPB whether they were protected with intermittent fibrillation or cold blood cardioplegia. Thus, there was a significant increase in plasma TnT by 1 hour after termination of ischemia that peaked at 4 hours, with mean values still remaining elevated at 48 hours. Notably, preconditioning did not alter this profile in both groups, indicating that preconditioning conveys no benefit to patients undergoing coronary surgery with CPB.

Figure 1A. Time course of release of plasma cardiac troponin T (TnT) concentrations in patients undergoing coronary artery bypass grafting with CPB and aortic crossclamping. In each group, patients were randomly subdivided into control and preconditioning groups ($n = 20/\text{group}$). Data are expressed as mean $\pm$ SD ($*P < .05$ vs control group).

In contrast, as shown in Figure 1C, the profiles of plasma TnT were different in patients with or without preconditioning and off-pump surgery. Peak release occurred by 8 hours after termination of ischemia in the control group. Peak release occurred by 1 hour in the preconditioned group with a sharp decrease during the next 3 hours and with significantly lower mean plasma TnT values than in the control group. The significance of the accelerated rate of enzyme leakage in the preconditioning group is not entirely clear, but it could be the result of changes within the biophysical properties induced by IP (thus allowing a greater transient leakage of intracellular enzymes) or of greater cumulative ischemic time in the preconditioned group compared with the control group. TnT levels were similar in both groups only by the end of 48 hours.

Figure 2 shows that the cumulative plasma release of TnT (ie, area under the curve) was similar in the patients undergoing surgery with CPB with no significant effect of preconditioning. It also shows that TnT release was lower in patients undergoing surgery without CPB, and that precon-

ditioning in this group significantly reduced the total TnT release by 33% when compared with the control group (2.1 ± 0.1 vs $3.1 \pm 2 \text{ ng} \cdot \text{h}^{-1} \cdot \text{mL}^{-1}$ $P < .05$).

Hemodynamic Data

Table 2 shows that the mean systemic arterial pressures, heart rate, mean pulmonary artery pressures, systemic and pulmonary vascular resistance, and cardiac indices fluctuated within normal ranges after the operation in both the control and preconditioned groups and in the groups with and without CPB.

In Vitro Studies

Figure 3 (A and B) shows the results of the CK leakage and MTT reduction of the atrial slices obtained before bypass and 10 minutes after initiation of CPB. The results demonstrate that the increase in CK leakage and the decrease in MTT reduction caused by ischemia and reoxygenation in the atrial muscles obtained before the institution of bypass were significantly improved in the slices obtained 10 min-

Figure 1. Cont'd. Time course of release of plasma cardiac TnT concentrations in patients undergoing coronary artery bypass grafting with CPB and cold blood cardioplegia (B) and on the beating heart without CPB (C). In each group, patients were randomly subdivided into control and preconditioning groups ($n = 20/\text{group}$). Data are expressed as mean $\pm$ SD (* $P < .05$ vs control group).

utes after the initiation of bypass, and that this level of protection was identical to that of preconditioning. Thus, muscles that were obtained 10 minutes after the initiation of bypass were already preconditioned, and the application of IP did not result in additional benefit compared with IP alone.

Discussion

The present study shows that the human heart is preconditioned by the institution of CPB, and that the use of IP in combination with other protective interventions such as cardioplegia does not result in additional protection. It also clearly demonstrates that the human heart can be protected

Figure 2. Area under the curve of the plasma release cardiac TnT in patients undergoing coronary artery bypass grafting with CPB and aortic crossclamping, CPB and cold blood cardioplegia, and on the beating heart without CPB (* $P < .05$ vs corresponding control group).

by IP when patients undergo surgery without the use of CPB. These findings have obvious important clinical implications, and they warrant further discussion.

Preconditioning of the Human Heart

Experimental findings on IP cannot be directly extrapolated to humans because the mechanisms may be different from other animal species. For both logistic and ethical reasons, no clinical study can meet the strict conditions of experimental studies on preconditioning in which infarct size is the primary end point; instead, surrogate end points have to be used. As a result, the demonstration of this phenomenon in the setting of cardiac surgery has been controversial. Yellon and colleagues⁸ were the first to examine the effect of two 3-minute ischemic episodes, in which each was followed by 2-minute reperfusion on myocardial high-energy phosphate content, in patients undergoing coronary artery bypass graft surgery with CPB. They claimed that the human myocardium showed the typical biochemical features of preconditioning observed by Murry and colleagues¹⁴ in their classic canine model of IP and thus could be preconditioned. There have been recent studies^{15,16} also highlighting the potential benefits of preconditioning in the cardiac surgery setting. However, Perrault and associates⁹ failed to show a beneficial effect of IP when this was induced with 3-minute aortic crossclamping followed by 2-minute reperfusion before the administration of warm blood cardioplegia. Similar findings have been reported by other investigators^{10,17} questioning the ability of preconditioning to protect the human heart.

The dispute on whether preconditioning confers cardioprotection during cardiac surgery is further fueled by a more recent study by Alkhalafi and colleagues.¹⁸ By use of a protocol identical to the one used in their first study,⁸ Alkhalafi and coworkers showed a reduction of TnT release at 72 hours in patients exposed to preconditioning, but not at 24 or 48 hours. Notably, the same authors reported an absence of protection in myocardial high-energy phosphates (in contrast with their first study). These opposing results are puzzling and contrast with the overwhelming evidence that preconditioning is cardioprotective during coronary angioplasty¹⁹ and in vitro experimental conditions using atrial trabeculae^{6,7} or isolated myocytes.²⁰ Our finding that CPB can act as a preconditioning stimulus in humans is supported by another study in sheep²¹ and sheds light on the previously mentioned controversy.

During cardiac surgery, there may be preoperative and intraoperative factors such as opioid agonists²² and anesthetic agents²³ that may mimic the protection of preconditioning. These include the use of opioid agonists, aprotinin, and, notably, CPB. It has been reported that inhalational anesthetics can induce cardioprotection, and that this effect differs with the agent used.^{24,25} In the present study, enflurane was used. Although enflurane is less effective than other inhalational anesthetics,²⁴ and IP was shown to be cardioprotective in the off-pump group, it may not be possible to completely rule out some protective effect caused

Figure 3. CK leakage (A) during the 120-minute reoxygenation period and MTT reduction (B) at the end of the reoxygenation period after 90 minutes of normothermic global ischemia with or without simulated IP. Data are expressed as mean $\pm$ SD of 6 experiments (* $P < .05$ vs corresponding group without preconditioning).

by this agent. Hypothermia is another cardioprotective factor²⁶ that may influence the cardioprotection of preconditioning. Recently, Takeshima and colleagues²⁷ also demonstrated that preconditioning was not protective with deep hypothermia. However, moderate hypothermia alone, as used in the present studies, does not inhibit the preconditioning response.

Mechanism of Preconditioning by Cardiopulmonary Bypass

Although the precise mechanism of IP still remains unclear, recent investigations have clearly identified a number of factors that are essential to achieve protection. CPB induces a systemic inflammatory reaction, and it is possible that some elements of this reaction may be responsible for the

observed protection. Yamashita and coworkers²⁸ recently reported that interleukin 1 and tumor necrosis factor α (the production of which is increased by CPB) cause an elevation in tissue manganese-superoxide dismutase, which was demonstrated when brief sublethal ischemia or anoxic insults were induced.²⁹ However, this thesis is unlikely, because the production of cytokines is a late event in response to CPB that requires more than 10 minutes.

Recently, our laboratory showed that the generation of free radical species occurs soon after the institution of CPB.³⁰ Therefore, it is possible to speculate that free radicals are the primary cause of cardioprotection by CPB. The relationship between free radicals and preconditioning was first suggested by Richard and colleagues,³¹ who showed that administration of oxygen free radical scavengers during

the first reperfusion period could block the beneficial effect of preconditioning on infarct size in dogs. They therefore proposed that the generation of low amounts of free radicals during the short ischemic episode is not sufficient to cause cell necrosis but enough to modify cellular activity and induce preconditioning. More recently, Pain and colleagues³² demonstrated that the opening of mitochondrial K_{ATP} channels triggers protection through the generation of free radicals that activate protein kinase C, an obligatory step in the signal transduction mechanism of IP. One of the potential limitations with our in vitro studies is the use of atrial myocardium as opposed to ventricular myocardium, and therefore any extrapolation must be conducted with caution; however, Speechly-Dick and coworkers³³ suggested that preconditioning exerts identical protection in both tissues. Undoubtedly, K_{ATP} channels are present in both atrium and ventricle,³⁴ although their density in both tissues is unknown.

The induction of CPB affects the body hemodynamics that may provoke a number of tissue responses. Thus, the loss of atrial and ventricular filling may stimulate a sympathetic-receptor-mediated release of local catecholamines, whereas the interruption of pulsatile systolic and diastolic blood flow to the adrenal glands may stimulate a systemic catecholamine release. Therefore, an altered adrenergic state may also be partially responsible for CPB-associated preconditioning in human myocardium. Several investigators,^{35,36} including the current authors,³⁷ have observed that norepinephrine or phenylephrine triggers preconditioning, and that this protection is prevented by adrenergic blockade. Similarly, Thornton and colleagues³⁸ demonstrated that tyramine, an agent that causes the release of endogenous catecholamines, reduced infarct size in rabbits when given before a sustained period of ischemia. Certainly, more studies are required to elucidate the mechanism of cardioprotection effected by CPB.

Clinical Implications

Cardiac surgical practice is rapidly evolving, and an increasing number of surgeons are adopting surgery on the beating heart, without the use of CPB, in their practice. Cardioplegic solutions cannot be used in this situation, and the demonstration that interventions such as IP are as effective can have important clinical implications. However, it should be recognized that the clinical application of IP may still be difficult and cumbersome, particularly if minimally invasive approaches are used. Because of this, the pharmacologic manipulation of the signal transduction cascade of preconditioning may seem to be a more appropriate alternative. In this regard, several investigators, including the current authors, are endeavoring to fully elucidate the mechanism of preconditioning in humans to make this intervention a clinical reality.

CPB is known to induce a systemic inflammatory reaction that is believed to be responsible for increased morbidity. The present studies demonstrate that CPB can also trigger preconditioning and be cardioprotective.

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