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Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery

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Abstract

***Objective*—To investigate whether ischaemic preconditioning could reduce myocardial injury, as manifest by troponin T release, in patients undergoing elective coronary artery bypass surgery.**

***Design*—Randomised controlled trial.**

***Setting*—Cardiothoracic unit of a tertiary care centre.**

***Patients*—Patients with three vessel coronary artery disease and stable angina admitted for first time elective coronary artery bypass surgery were invited to take part in the study; 33 patients were randomised into control or preconditioning groups.**

***Intervention*—Patients in the preconditioning group were exposed to two additional three minute periods of myocardial ischaemia at the beginning of the revascularisation operation, before the ischaemic period used for the first coronary artery bypass graft distal anastomosis.**

***Main outcome measure*—Serum troponin T concentration at 72 hours after cardiopulmonary bypass.**

***Results*—The troponin T assays were performed by blinded observers at a different hospital. All patients had undetectable serum troponin T (< 0·1 μg/l) before cardiopulmonary bypass, and troponin T was raised postoperatively in all patients. At 72 hours, serum troponin T was lower (P = 0·05) in the preconditioned group (median 0·3 μg/l) than in the control group (median 1·4 μg/l).**

***Conclusions*—The direct application of a preconditioning stimulus in clinical practice has been shown, for the first time, to protect patients against irreversible myocyte injury.**

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Keywords: myocardial ischaemia; coronary artery bypass grafts; ischaemic preconditioning; troponin T

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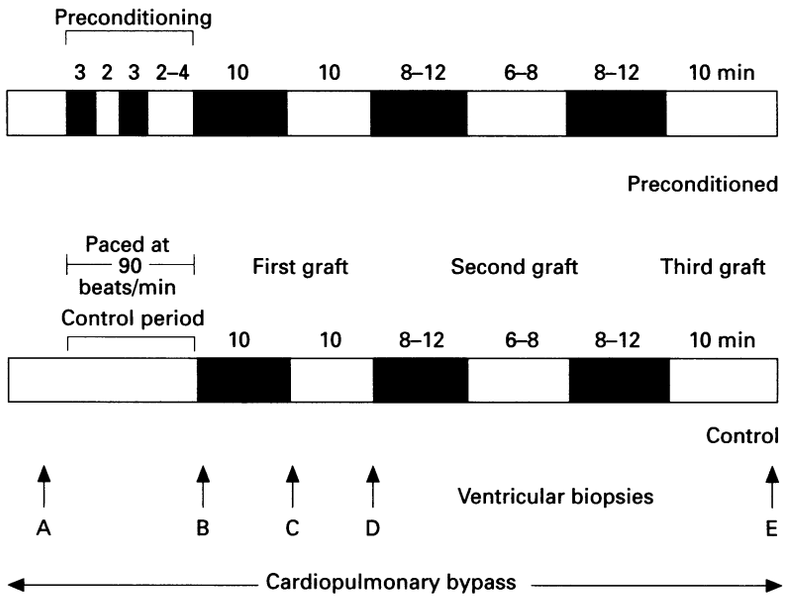
It is established that exposing the myocardium to brief periods of ischaemia and reperfusion induces greater tolerance to a subsequent more prolonged ischaemic insult. This endogenous adaptation to ischaemia, termed "ischaemic preconditioning", was initially shown to delay myocardial necrosis in an in vivo canine model of myocardial infarction.[1] Since then an extensive body of reports on preconditioning has accumulated, the phenomenon has been characterised, and its mechanisms explored, but despite increasing evidence for the existence of preconditioning in human myocardium,[2] few clinical studies have been performed.

It is our hypothesis that the protective effects of preconditioning can be induced in patients requiring cardiac surgery. The majority of cardiac surgical procedures involve the intentional interruption of coronary artery blood flow and myocardial ischaemia is therefore inevitable. Although techniques of myocardial protection during cardiac surgery have improved in the past 30 years, patients with more severe disease are now being offered surgery, and any additional treatment which attenuates myocardial injury should be investigated. It has been shown that ischaemic preconditioning may be induced in patients undergoing coronary artery bypass surgery.[3] In the latter study an ischaemic preconditioning protocol instituted before the longer ischaemic period (of 10 minutes) for fashioning the first saphenous vein to coronary artery anastomosis resulted in relative preservation of myocardial ATP levels at the end of the 10 minutes of ischaemia. The study was limited to the metabolic changes occurring at a single time point, but the similarity between the results in humans and the metabolic results from animal models[4] indicated for the first time that ischaemic preconditioning could be induced in human myocardium. However, the initial clinical study was not designed to evaluate myocardial protection throughout the whole course of the operation.

One of the difficulties in assessing interventions designed to protect the myocardium in patients undergoing cardiac surgery is the availability of end points with which to quantify ischaemic injury. Troponin T is a contractile apparatus regulatory protein that is only detectable in serum following severe ischaemic injury.[5] Measurement of cardiac troponin T can detect minor degrees of myocyte necrosis which had previously not been recognised using conventional cardiac enzymes and ECG tests.[6] Troponin T has been shown to increase significantly in all patients recovering normally after uncomplicated cardiac surgery,[7,8] and the amount of release correlates with the ischaemic time.[9,10] Therefore measurement of troponin T release into serum may be an appropriate marker of ischaemic injury following cardiac surgery and an effective means of assessing and comparing cardioprotective interventions.

In this study we investigated whether ischaemic preconditioning reduced myocardial



Protocol for operative management in control and preconditioned groups. Filled boxes indicate periods of ischaemia and empty boxes indicate periods of reperfusion. Temperature kept constant at 36°C during the preconditioning protocol and the first graft in both groups.

injury in patients undergoing routine coronary artery bypass surgery by measuring the release of troponin T into serum during the postoperative recovery. We also measured ATP levels in ventricular muscle samples taken on five occasions during both the ischaemia and reperfusion periods of the operation.

Methods

The investigation was approved by the local ethics committee and all patients entered into the study gave informed written consent. Patients with three vessel coronary artery disease and stable angina admitted for first time elective coronary artery bypass surgery were invited to take part in the study. Thirty three patients were randomised into control (n = 16) or preconditioning groups (n = 17) between March and October 1995. Patients with unstable angina, left ventricular aneurysm, or very poor left ventricular function (ejection fraction < 30%), valve disease, and those taking sulphonylurea antidiabetic drugs were not eligible for inclusion.

SURGICAL TECHNIQUE

All operations were performed by a single consultant surgeon (WBP) at the Middlesex Hospital and the anaesthetic and cardiopulmonary bypass techniques were standardised. The coronary artery bypass grafts were performed using the technique of intermittent ischaemic arrest with fibrillation for the distal vein to coronary artery anastomosis, and the heart reperfused and beating for the proximal vein to aorta anastomosis. Ventricular vents were not used. Whole body temperature was maintained at 36 ± 1°C for the period of the preconditioning protocol and first distal anastomosis in both groups; following this period all patients were cooled to 32°C.

Table 1 Patient demographics and operation data

	Control	*Ischaemic preconditioning*
Number of patients	16	17
Age, years	62 (2)	57 (2)
Sex	15 male: 1 female	15 male: 2 female
Previous MI	6	9
Number of grafts	3·2 (0·1)	3·1 (0·2)
LIMA usage	16	16
CPB time, min	96 (4)	96 (4)
Ischaemia time, min	34·1 (1·8)	33·3 (1·6)
Defibrillation energy, joules	58 (11)	58 (11)

Values are means (SEM), no significant differences between groups. CPB, cardiopulmonary bypass; LIMA, left internal mammary artery; MI, myocardial infarction.

PRECONDITIONING PROTOCOL

Patients randomised to ischaemic preconditioning were pretreated with the same preconditioning protocol as used in our previous study[3] (figure). After instituting cardiopulmonary bypass, two three-minute periods of ischaemia were applied by cross clamping the aorta, each separated by two minutes of reperfusion. During this 10 minute period, hearts were paced at 90 beats/min and myocardial and whole body temperature was maintained at 36 ± 1°C. Patients in the control group also received 10 minutes of normothermic cardiopulmonary bypass (without the preconditioning protocol) before the first anastomosis.

TROPONIN T ASSAY

Blood samples for troponin T assay were taken immediately before cardiopulmonary bypass, one hour after bypass, and at six, 24, and 72 hours. Blood was collected into plain tubes and the serum separated by centrifugation within one hour of collection. The serum was frozen and stored at –20°C until analysis.

Cardiac troponin T was measured in a blinded fashion using an enzyme linked immunosorbent assay (ELISA) at the Royal Brompton Hospital. A commercially available standard assay kit (ELISA troponin-T, Boehringer Mannheim) and batch ELISA analyser (Enzymun test system ES 300, Boehringer Mannheim) were used. The assay detected cardiac troponin T in the range 0·1–18 μg/l.

VENTRICULAR BIOPSIES AND ATP ASSAY

Samples of left ventricular muscle were obtained with a "Trucut" biopsy needle (Baxter) from the territory of the left anterior descending coronary artery. Biopsies were taken at the following time points: (A) baseline, before the 10 minute preconditioning protocol; (B) following the preconditioning protocol; (C) at 10 minutes of ischaemia, at the end of the first distal anastomosis; (D) after 10 minutes of reperfusion; (E) following 10 minutes of reperfusion after completion of the final anastomosis and before discontinuing cardiopulmonary bypass at the end of the operation (figure).

Samples were immediately frozen in liquid nitrogen and then freeze dried for at least 12 hours. Samples were assayed in a blinded fashion in random order. After accurately weighing each freeze dried sample, protein was extracted by homogenisation with 6% perchloric acid. ATP content was determined using

Table 2 Troponin T in serum

TnT μg/l	Pre CPB	1 h post CPB	6 h post CPB	24 h post CPB	72 h post CPB
Control	< 0·1	1·0 (0·4 to 1·5)	1·8 (0·8 to 3·5)	1·4 (0·5 to 2·2)	1·4 (0·7 to 3·0)
IPC	< 0·1	1·0 (0·5 to 1·4)	1·1 (0·5 to 3·3)	0·4 (0·3 to 1·7)	0·3 (0·2 to 2·0)*

Median and (interquartile range) of serum troponin T.
CPB, cardiopulmonary bypass; IPC, ischaemic preconditioning; TnT, troponin T.
*P = 0·05, Mann-Whitney U test.

an enzymatic assay and observing changes in optical density of the extracts with a spectrophotometer. ATP content of the biopsies was expressed in μmol/g dry weight.

ELECTROCARDIOGRAPHIC CHANGES

Twelve-lead electrocardiograms were recorded preoperatively, following surgery on return to the intensive care unit, and on the first and fourth postoperative days. Perioperative transmural infarction was defined on electrocardiographic criteria as the appearance of new persistent Q waves (one third QRS height and > 0·04 s duration). Other changes were noted (ST segment, T wave and reduction in R wave height of > 25%) if they persisted in two or more adjacent leads. Postoperative arrhythmias were also recorded.

STATISTICS

Demographic, operative, and ATP data are presented as mean (SEM). Differences within and between groups were analysed with a paired or unpaired *t* test as appropriate. The troponin T levels at 72 hours after bypass was preselected as the major end point. Troponin T results are presented as medians (with interquartile range) and between group differences were analysed by a non-parametric test (Mann-Whitney U) because of the non-Gaussian distribution of the data. Comparison of proportions was performed using the χ^2 with Yates correction for small sample size (χ^2_Y). Statistical significance was defined as a P value of 0·05.

Results

PATIENTS

Three patients who were eligible for inclusion in the study refused to consent. The demographic and operative data of the 33 patients completing the study are presented in table 1. There were no significant differences between the groups. No patients required inotropic or intra-aortic balloon support postoperatively. One patient (from the control group) developed a perforated peptic ulcer on the fifth postoperative day. This patient developed septicaemia and eventually died of multiple organ failure. There were no other major complications.

TROPONIN T

Troponin T concentrations were below the detectable range of the assay (< 0·1 μg/l) in all patients before cardiopulmonary bypass. There was a rise in serum troponin T in all patients postoperatively, indicating some myocyte injury during the operation (table 2). Peak troponin T release occurred at six hours after completion of cardiopulmonary bypass in both groups: preconditioned group, 1·1 μg/l; control group, 1·8 μg/l. At 72 hours troponin T was lower (P = 0·05) in preconditioned patients (0·3 μg/l) than in the control group (1·4 μg/l). Ten patients in the preconditioned group had troponin T values of < 0·5 μg/l at this time compared with only three patients in the control group (χ^2_Y = 5·54, P = 0·04). In the control group 10 patients had troponin T values of > 1·0 μg/l at 72 hours, but in the preconditioned group only five patients had levels above 1·0 μg/l (χ^2_Y = 3·64, P = 0·12).

ATP

ATP content in ventricular biopsies is shown in table 3. Baseline biopsies (A) were not significantly different between the groups. In the preconditioned group there was a decline (P = 0·016) in ATP after the preconditioning protocol (biopsy A to biopsy B) before the ischaemic period of the first anastomosis. The decline in ATP during the ischaemia of the first anastomosis (biopsy B to biopsy C) was 28% in control hearts and 18% in preconditioned hearts. However, there was no significant difference in ATP content between groups at any stage. The recovery in ATP on reperfusion following the first ischaemic period (biopsy D) was similar to that following the third ischaemic period (biopsy E) in both groups.

ELECTROCARDIOGRAPHY

No patient developed new Q waves on the ECG postoperatively. A reduction in R wave height occurred in five patients postoperatively (two from the control and three from the preconditioned group). ST segment changes were present in three patients postoperatively (one control and two preconditioned). Six patients developed atrial fibrillation during the first five postoperative days (four control and two preconditioned). These differences did not reach statistical significance.

Table 3 ATP content (μmol/g) of myocardial biopsies

	Biopsy A	Biopsy B	Biopsy C	Biopsy D	Biopsy E
Control	19 (1·2)	18 (1·0)	13 (1·0)	17 (1·0)	15 (1·1)
IPC	21 (1·0)	17 (1·4)*	14 (0·9)	17 (1·6)	17 (1·3)

Values are means (SEM).
IPC, ischaemic preconditioned. Biopsy A, baseline; B, after preconditioning protocol; C, at 10 minutes of ischaemia following first anastomosis; D, after 10 minutes of reperfusion; E, following 10 minutes of reperfusion after completion of final anastomosis.
*P = 0·016 compared with IPC biopsy A. No significant differences between groups.

Discussion

In patients with ischaemic heart disease undergoing myocardial revascularisation by coro-

nary artery bypass grafting, ischaemic preconditioning reduced perioperative myocardial necrosis, as manifest by cardiac troponin T release into serum. This is the first confirmation that ischaemic preconditioning may directly delay myocardial necrosis in humans.

In this study patients in the control and preconditioned groups had very similar operative ischaemia times (34 and 33 minutes, respectively) and troponin T concentration increased in all patients. However, 72 hours after the operation patients in the preconditioned group were releasing less cardiac troponin T into serum.

The subcellular compartmentation of troponin T is reflected by its release kinetics following ischaemia/reperfusion injury. A small unbound cytoplasmic pool accounts for the early peak in troponin T; the later sustained release of troponin T, over the 24 hours following ischaemia, reflects washout of structurally bound protein from continuing degradation of myofibrils in irreversibly injured cells.[11 12] The threshold for a positive troponin T release was set at 0·5 μg/l in a recent report investigating troponin T in brain dead organ donors[13] and all patients sustaining a Q wave or non-Q wave myocardial infarction had values > 1 μg/l in the original report of diagnostic efficiency.[13] In the present study no patients showed measurable troponin T in serum (< 0·1 μg/l, the detection limit of the assay) before cardiopulmonary bypass, and 72 hours postoperatively serum troponin T concentrations were < 0·5 μg/l in 59% of preconditioned patients but > 1 μg/l in 63% of the control group.

Troponin T is not present in the serum of normal individuals and unlike creatine kinase was not detectable in serum following orthopaedic and pulmonary surgery.[9] All studies measuring troponin T release after cardiac surgery, including those employing cardioplegia for myocardial protection, have reported significant increases, even in patients who have an apparently uncomplicated postoperative course.[7–10 14–16] Serum troponin T values at 24–72 hours after operation in the latter studies were similar to the results obtained in the control group reported here. Patients sustaining major perioperative myocardial infarction with development of new Q waves on the ECG form a clearly distinct group with very high troponin T levels and a worse prognosis.[7–9] The prognostic implications of moderate troponin T elevation are not known, but measurement of troponin T has shown that previously unrecognised myocardial damage occurs in all patients during cardiac surgery. This probably reflects diffuse tissue necrosis scattered throughout the myocardium, which would not be manifest as specific ECG changes. This concept is corroborated by the finding that troponin T is also detectable (median 0·5 μg/l) in a subgroup of patients with unstable angina—in whom it indicated a worse prognosis—and this was thought to be the result of localised myocyte necrosis caused by thrombotic microembolisation.[17] Taken together, these results suggest that the human myocardium is perhaps more vulnerable to irreversible ischaemic injury than previously assumed when less precise markers of myocardial injury were available.

The above findings emphasise the scope for improved myocardial protection during cardiac surgery. Although our study was performed in patients with stable angina and moderately good left ventricular function, we would expect that myocyte injury would be greater in patients with more unstable disease and longer ischaemic times. The present study was too small for morbidity or mortality to be used as end points and most patients had a completely uncomplicated postoperative course. Indeed, the clinical significance of the rise in troponin T after cardiac surgery is not yet understood, but since continuing release of troponin T at 72 hours indicates irreversible myocyte injury, and myocytes cannot be replaced, it must have biological significance.

Analysis of the ATP content in samples of ventricular muscle showed the expected depletion of ATP during the 10 minute ischaemic period of the first distal anastomosis (biopsy C) in both groups. There was equal recovery in ATP content following the 10 minute reperfusion for the proximal anastomosis (biopsy D). The ATP content following reperfusion at the end of the third period of ischaemia (biopsy E) was not different from that following reperfusion after the first ischaemic period (biopsy D). This indicates that there is no cumulative depletion in ATP with successive 10 minute ischaemic challenges in the human heart and corroborates the observations in the canine model.[18] Although there was a greater decline in ATP content during the ischaemic period of the first graft (biopsy B to biopsy C) in control hearts, there was no significant difference in ATP content between control and preconditioned groups at the end of this period (biopsy C). This finding is in contrast to our previous smaller study, which had shown a relative preservation in ATP in preconditioned hearts at the end of the first ischaemic period (biopsy C).[3] Indeed, in the absence of a measure of myocyte necrosis, the similarity between the ATP results in our previous study and those reported by Jennings in preconditioned dogs[4] had given us the confidence to believe that it was possible to precondition the human heart during cardiac surgery.

There are two potential reasons for the failure to observe differences in ATP content between control and preconditioned groups in the current study. (1) It is possible that preconditioning does not necessarily result in preservation of ATP, and that in this larger series of less selected patients no real difference in ATP content exists. Several recent experiments with isolated rat hearts, in which the time course of ATP changes has been followed in control and preconditioned hearts by nuclear magnetic resonance, have reported that protection from preconditioning occurs in the absence of significant ATP preservation.[19 20] (2) In spite of the latter findings there are many reports suggesting that preconditioning is at least associated with a relative preser-

vation of myocardial ATP content in larger animal models during the first 20 minutes of sustained ischaemia,[4 21 22] even if the ATP changes have not been proven to be the cause of the delay in infarction. The difference between these studies and ours in humans is that in the animal models it was possible to biopsy the myocardium for ATP analysis more often and plot the ATP changes in both groups over time. These curves do show a slower depletion in ATP during ischaemia in preconditioned hearts, so that the ATP content is transiently higher than in controls at about 10 minutes of ischaemia. However, with the limited number of samples possible in a human study, a single biopsy at 10 minutes of ischaemia (biopsy C) may have missed this transient difference. In this study it was not possible to measure other high energy phosphates and metabolites accurately or calculate the energetic charge on the small tissue samples available. It is unlikely that the availability of the latter data would alter our conclusions because in laboratory experiments with large animal models, the most notable difference between control and preconditioned groups is usually apparent in the ATP content data.

The fact that there was no difference in ATP content between the groups at the end of the operation does not contradict the troponin T results. Although ATP is the cellular energy source and it is rational to assume that more is better than less, it is not possible to correlate ATP or other metabolite content with cell viability, and the concept of a "critical" level of ATP below which cell death occurs is now known to be incorrect.[23]

In summary, we have shown that in patients treated by coronary artery bypass grafting, ischaemic preconditioning results in less perioperative myocardial necrosis as determined by serum concentrations of cardiac troponin T postoperatively. We believe this is the first time that preconditioning has been shown to offer patients some protection against irreversible myocyte injury associated with a therapeutic procedure in clinical practice. These results indicate that the direct application of a preconditioning stimulus at the beginning of an operation could result in better myocardial protection. It had been argued that the technique of intermittent ischaemic arrest in the performance of the bypass grafts or in the institution of cardiopulmonary bypass itself[24] may induce preconditioning; the improved myocardial protection in the preconditioned hearts compared with control hearts reported here suggests that these assumptions were incorrect. Although we are not recommending that patients should be subjected to additional ischaemia in the course of routine cardiac surgery, this observation highlights the potential of exploiting endogenous myocardial adaptation. If the adenosine A_1 receptor agonists and ATP dependent potassium channel openers, which initiate preconditioning in laboratory models,[25 26] are shown to be as effective in forthcoming clinical trials, then preconditioning might become a practical adjunct to myocardial protection during cardiac surgery.

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