Evidence for Preconditioning by Isoflurane in Coronary Artery Bypass Graft Surgery

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Background—Experimentally, isoflurane, a commonly used volatile anesthetic agent, mimics the cardioprotective effects of ischemic preconditioning via a mechanism that could involve the activation of protein kinase C. The present study was designed to assess the clinical relevance of this observation in patients undergoing elective CABG.

Methods and Results—Twenty patients were included in the study. In 10 of them, preconditioning was elicited after the onset of cardiopulmonary bypass via a 5-minute exposure to isoflurane (2.5 minimum alveolar concentration), followed by a 10-minute washout before aortic cross-clamping and cardioplegic arrest. Ten case-matched control patients underwent an equivalent period (15 minutes) of prearrest isoflurane-free bypass. Outcome measurements included troponin I and creatine kinase–MB isoenzyme (until the third postoperative day) levels and the activity of ecto-5′-nucleotidase, which contributes to adenosine production and is considered to be a reporter of protein kinase C activation, as assessed in right atrial biopsy samples taken before bypass and at the end of the preconditioning protocol (or after 15 minutes of bypass in control patients). Aortic cross-clamping times did not differ between the 2 groups: 52±14 and 48±14 minutes (mean±SD) in control and isoflurane-preconditioned patients, respectively. Likewise, prebypass values of ecto-5′-nucleotidase were similar in control (3.54±0.86 nmol · mg protein⁻¹ · min⁻¹) and isoflurane-treated (2.98±1.08 nmol · mg protein⁻¹ · min⁻¹) patients. The values subsequently remained unchanged in control patients (3.62±0.94 nmol · mg protein⁻¹ · min⁻¹), whereas they significantly increased after isoflurane preconditioning (4.74±0.50 nmol · mg protein⁻¹ · min⁻¹; P<0.002 versus baseline values, P<0.004 versus time-matched values in control patients). This was paralleled by a consistently smaller release of troponin I, which yielded an area under the curve and a peak value of 204±147 ng · mL⁻¹ · min⁻¹ and 3.98±2.83 ng/mL, respectively, versus 284±136 ng · mL⁻¹ · min⁻¹ and 5.88±3.64 ng/mL, respectively, in control patients. The release of creatine kinase–MB featured a similar pattern. There were no adverse effects related to isoflurane.

Conclusions—These data support a cardioprotective effect of isoflurane and, more generally, demonstrate the feasibility of pharmacologically preconditioning the human heart during cardiac surgery. (Circulation. 1999;100[suppl II]:II-340–II-344.)

Key Words: ischemia ■ bypass ■ anestheisa

Ischemic preconditioning is recognized as one of the most effective means of reducing cellular necrosis. As such, its implementation during cardiac surgery might be clinically relevant, particularly in high-risk patients in whom any additional myocardial ischemic injury induced with cardiopulmonary bypass and superimposed cardioplegic arrest can adversely affect postoperative outcome. Because in this setting it is particularly desirable to avoid an ischemic-type preconditioning stimulus,³⁴ extensive research is targeted toward identifying the pharmacological mediators of the preconditioning phenomenon in an attempt to exploit them therapeutically.

There is compelling evidence that the preconditioning signal activates various membrane receptors, which triggers a signaling pathway leading to the activation of several kinases,⁴ in particular, protein kinase C (PKC),⁵ and the subsequent opening of ATP-dependent potassium channels, possibly at the mitochondrial level.⁶⁷ How opening of these channels elicits cardioprotection is not yet established but might involve the limitation of calcium influx, better control of cellular volume, or both. This scheme provides a framework for rationalizing interventions targeted at mimicking preconditioning and, in this perspective, makes logical the use, among other drugs, of potassium channel openers.

Within this class of drugs, only nicorandil is currently available for human use. However, aside from the fact that nicorandil cannot be administered intravenously, this compound has marked nitrovasodilatory properties, which can be
of concern. Alternatively, isoflurane is a volatile anesthetic agent that has been reported in animal models of regional ischemia\(^8\)–\(^10\) to have infarct-limiting properties similar to those of ischemic preconditioning and presumably involving the activation of potassium channels.\(^8\) Although isoflurane is commonly used during cardiac surgery to relieve bursts of hypertension, the effects of its administration as a preconditioning agent have not yet been tested. The present clinical study was designed to address this issue.

**Methods**

**Protocol**

Twenty patients undergoing elective CABG were prospectively studied after approval by our institutional review committee. The conduct of anesthesia and surgery was similar in all patients. For anesthesia, fentanyl, furosemide, and pancuronium were used in a standard combination, but care was taken to avoid the administration of isoflurane until the onset of cardiopulmonary bypass. The heart was approached through a median sternotomy. After heparinization, cardiopulmonary bypass was established with a single 2-stage right atrial cannula and an ascending aortic cannula, and the left ventricle was vented through the right superior pulmonary vein. The extracorporeal circuit consisted of a nonpulsatile roller pump, a membrane oxygenator, and a 20-μm arterial line filter. Once bypass was run at full flow (2.2 L·min\(^{-1}\)·m\(^2\) body area\(^{-1}\)) with the heart totally decompressed, patients were randomly assigned to the control or preconditioning group. Preconditioning was achieved with a 5-minute exposure to isoflurane (2.5 minimum alveolar concentration), followed by 10 minutes of isoflurane-free bypass before aortic cross-clamping. Isoflurane was added to the gas mixture admitted in the oxygenator. Control patients underwent a time-matched (15-minute) period of isoflurane-free cardiopulmonary bypass. After aortic cross-clamping, myocardial protection was provided with minimally diluted blood cardioplegia delivered retrogradely through the coronary sinus in a continuous fashion, as previously described. The core temperature was allowed to drift spontaneously to 33°C to minimize diluted blood cardioplegia delivered retrogradely through the coronary sinus in a continuous fashion, as previously described. The core temperature was allowed to drift spontaneously to 33°C to 34°C, and blood cardioplegia was administered at this same tepid temperature.

**End Points**

The assessment of results was made on blood markers of myocardial necrosis and tissue markers of PKC activation. To detect perioperative myocardial necrosis, blood levels of creatine kinase–MB isoenzyme (CK-MB) and troponin I were serially measured with the mass technique and the Stratus II automated 2-site fluorometric enzyme immunoassay (Dade Diagnostika), respectively, after the induction of anesthesia, on arrival in the intensive care unit, and at 6 hours and 1, 2, and 3 days after surgery. To detect PKC activation, right atrial biopsy samples were taken before bypass and then either at the end of the 15-minute preconditioning cycle in isoflurane-treated patients or after an equivalent pump time in control patients. Tissue specimens were frozen and stored under liquid nitrogen, and the ectosolic and cytosolic 5′-nucleotidase activities were measured as previously described.\(^11\) In brief, the myocardium was separated into its membrane (ectosolic and cytosolic) fractions as follows. The tissue was homogenized for 5 minutes in 10 vol of ice-cold 10 mmol/L HEPES-KOH buffer (pH 7.4) containing 0.25 mol/L sucrose, 1 mmol/L MgCl\(_2\), and 1 mmol/L mercaptoethanol at 0°C. The crude homogenate was strained through a double-layer nylon sieve and homogenized again for 1 minute. To prepare a crude membrane fraction, part of the homogenate was centrifuged at 10000g for 10 minutes. The resulting pellet was washed 3 times and resuspended in the HEPES-KOH buffer. To prepare the cytosolic fraction, the remaining portion of the homogenate was first centrifuged at 3000g for 10 minutes, and the supernatant was centrifuged at 200000g for 1 hour. The membrane and cytosolic fractions were dialyzed at 4°C for 4 hours against 10 mmol/L HEPES-KOH (pH 7.4) containing 1 mmol/L MgCl\(_2\), 1 mmol/L mercaptoethanol, and 0.01% activated charcoal and were divided into aliquots that were frozen immediately and stored at −80°C. We have previously shown that with this procedure, the recovery of 5′-nucleotidase activity in the membrane fraction is 97%. 5′-Nucleotidase activity was assessed with the enzymatic assay technique and is reported in units of nanomoles per milligrams of protein per minute. Protein concentration was measured according to the method of Lowry et al\(^12\) with BSA used as standard.

**Statistical Analysis**

The release of CK-MB mass (ng·mL\(^{-1}\)·h\(^{-1}\)) and troponin I (ng·mL\(^{-1}\)·h\(^{-1}\)) over the first postoperative 72 hours was calculated as the area under the curve (AUC) by use of a curve-fitting application that generated a series of rectangles between consecutive points on the curve. The area of these rectangles was then summed. Postoperative enzymatic values were also compared with the use of a 2-factor ANOVA with repeated measures. Data on 5′-nucleotidase activity were compared with the use of paired and unpaired t tests. Significance was set at the 0.05 level. Results are reported as mean±SD values.

**Results**

The clinical profile and intraoperative data for the patients are summarized in the Table. The 2 cohorts were similar with respect to all parameters. As shown in Figure 1, prebypass values for ecto-5′-nucleotidase activity did not differ between the 2 groups. The values subsequently remained unchanged in control patients, whereas they significantly increased after isoflurane preconditioning (\(P<0.002\) versus baseline values, \(P<0.004\) versus time-matched values in control patients).

![Figure 1. Effects of isoflurane preconditioning on ectosolic activities of 5′-nucleotidase. For each patient (\(n=10\) per group), right atrial biopsy samples were taken before bypass (baseline) and either at the end of the preconditioning (PC) protocol or at a time-matched study point in control patients. Data are expressed as mean±SD values. CPB indicates cardiopulmonary bypass.](https://example.com/figure1.png)
Effects of isoflurane preconditioning on postoperative CK-MB release. Data are expressed as mean±SD.

Likewise, baseline activities for cyto-5'-nucleotidase were similar in control (9.1±0.9 nmol·mg protein⁻¹·min⁻¹) and isoflurane-preconditioned (9.3±2.3 nmol·mg protein⁻¹·min⁻¹) patients. However, in contrast to the ectosolic fraction, the cytosolic fraction did not change significantly thereafter and, after 15 minutes of bypass, averaged 8.1±2.5 and 9.2±1.7 nmol·mg protein⁻¹·min⁻¹ in control and isoflurane-preconditioned patients, respectively.

The postoperative release of CK-MB was consistently smaller in the isoflurane-preconditioned group than in the control group, with AUCs being 1043±338 and 1393±570 ng·mL⁻¹·h⁻¹, respectively (Figure 2). The release of troponin I displayed a similar pattern; it yielded an AUC of 204.5±146.7 and 284.5±136.4 ng·mL⁻¹·h⁻¹ in isoflurane-preconditioned and control patients, respectively (Figure 3). In keeping with these data, peak values of CK-MB (recorded on arrival in the intensive care unit) and troponin I (recorded on arrival in the intensive care unit) were no deaths, and no patient had a transmural myocardial infarction. Inotropic support was required in 1 group. The time to extubation was 106 hours in the control and isoflurane groups, respectively.

The major finding of this study is that ecto-5'-nucleotidase activity significantly increased in patients receiving isoflurane according to a preconditioning protocol. There is a large body of evidence implicating PKC as a critical mediator of the cardioprotective response to this preconditioning phenomenon. In the human heart, however, such an involvement has, until now, been exclusively based on in vitro studies that used cultured cardiomyocytes or right atrial trabeculae subjected to “simulated” ischemia/reperfusion (in fact, anoxia/reoxygenation) and showed that PKC agonists and antagonists trigger or blunt the preconditioning response, respectively. More direct evidence for a role of PKC has been the demonstration of the cytosol-to-membrane translocation of its α-isofrom induced through the exposure of human cardiomyocytes to adenosine. However, until the assay of the PKC isoforms most relevant to cardioprotection can be accurately performed in human biopsy samples taken during cardiac surgery, one has to rely on surrogate markers like ecto-5'-nucleotidase. This enzyme, which releases adenosine from 5'-cAMP, is 1 of the substrates that is phosphorylated by PKC, and elevation of its levels therefore stands as a reporter of PKC activation.

The more direct involvement of ecto-5'-nucleotidase in mediating the cardioprotective preconditioning response is a different and more controversial issue. The activity of ecto-5'-nucleotidase has been reported to increase after ischemic preconditioning, and this increase has been linked to an improvement in cardioprotection, because (1) the loss of the infarct-limiting effect of preconditioning correlates with the decay of activation of ecto-5'-nucleotidase and (2) this infarct-limiting effect is equally blunted after the administration of an inhibitor of ecto-5'-nucleotidase. Other studies, however, have failed to show that ecto-5'-nucleotidase is required for ischemic preconditioning to elicit an infarct-limiting effect. Several factors can account for these discrepant data, including differences in species, experimental protocols, and end points. This controversy also arises in large part from the fact that if ecto-5'-nucleotidase played a major role in mediating preconditioning, one would expect it to cause an elevation of adenosine levels in myocardial tissue, whereas an opposite pattern (ie, a decrease in tissue adenosine) has, until now, been reported during classic ischemic preconditioning. As well as after pharmacological preconditioning with isoflurane or potassium channel openers. In turn, one could argue that because of the predominantly endothelial location of ecto-5'-nucleotidase, endothelium-derived adenosine may be preferentially released into the vascular compartment and thus escape interstitial fluid sampling with microdialysis techniques (indeed, a reduction in ischemic changes occurring with repeated balloon occlusions during angioplasty procedures correlates with an increased release of adenosine in the coronary venous effluent). Together, these considerations suggest that it is important to make a clear distinction between ecto-5'-nucleotidase, taken as a surrogate marker of PKC activation, which sounds acceptable, and ecto-5'-nucleotidase, taken as an intrinsically cardioprotective compound mediating the preconditioning response, which remains more uncertain. The results of the present...
study cannot help clarify this issue, primarily because the small size of the patient groups made it predictable that statistical analysis would lack the power to demonstrate significant differences in postoperative enzymatic or clinical outcomes. One can only speculate that if elevated levels of ecto-5′-nucleotidase reflect PKC activation, then the intervention causing such an elevation should be cardioprotective because of the link between PKC activation and enhancement of cardioprotection.\textsuperscript{24,25} The mechanism of this relationship is thought to involve a modulatory activity of PKC on mitochondrial potassium channels, which are currently considered the likely cardioprotective end effectors of the preconditioning signal. The involvement of these channels in humans is strongly suggested by the finding that right atrial trabeculae harvested intraoperatively can be preconditioned by the potassium channel opener cromakalim,\textsuperscript{14} except for those retrieved from diabetic patients receiving preoperative sulfonlurea drugs known to block potassium channels.\textsuperscript{26}

Previous studies using rabbit\textsuperscript{9,10} and dog\textsuperscript{8} models of regional ischemia have shown that isoflurane could duplicate the infarct-limiting effects of ischemic preconditioning. The cardioprotection conferred by the anesthetic agent does not seem to be related to changes in myocardial oxygen consumption or coronary flow.\textsuperscript{9} A possible mechanism is that isoflurane causes an activation of potassium channels, as suggested by the abolishment of its infarct-limiting effects with potassium channel blockers.\textsuperscript{8} This hypothesis is more directly supported by the results of patch-clamp techniques showing that isoflurane increases the probability of potassium channel opening for any given concentration of ATP.\textsuperscript{27} This opening could then account for the increase in ecto-5′-ectosolic activity that has been reported after pharmacological activation of the potassium channels.\textsuperscript{28} Alternatively, the primary target of isoflurane could be PKC itself. The subsequent increase of the activity of 5′-nucleotidase would then cause a release of adenosine and, downstream, the adenosine-induced opening of potassium channels.\textsuperscript{28} Regardless of the precise sequence of events, the fact that simultaneous administration of ischemic preconditioning and isoflurane does not confer additional protection over that yet provided by each intervention alone\textsuperscript{3} suggests that the drug exerts its cardioprotective effects via the same pathway as classic ischemic preconditioning. Additional support for this hypothesis comes from the observation that in the present study, isoflurane increased ecto-5′-nucleotidase levels despite a 10-minute washout period, which suggests a “memory” phase consistent with a preconditioning pattern.

Because the primary effect of preconditioning is to reduce infarct size, outcome analysis focused on 2 sensitive markers of cellular necrosis: CK-MB and troponin I.\textsuperscript{30} Postoperative values of these 2 enzymes were consistently lower in preconditioned patients than in their control counterparts, which is consistent with the cardioprotective effects of isoflurane. That the between-group difference failed to achieve statistical significance can be explained by (1) the small sample size and (2) the already low levels of troponin I yielded by our control patients, which makes more difficult the demonstration of a further significant improvement. Thus, in this group, the 24-hour postoperative value averaged 4.4 μg/L, which compares favorably with the value of 5.2 μg/L reported at the same time point by Sadony and coworkers\textsuperscript{30} in patients classified as having minor myocardial damage. Likewise, the peak value observed (at 6 hours postoperatively) in our series (5.8 μg/L) is much lower than the cutoff value (13.4 μg/L) shown by Jacquet and associates\textsuperscript{31} to significantly separate those patients with an uneventful recovery from those with ischemia/infarction.

We acknowledge several limitations of this study. First, ecto-5′-nucleotidase activity was measured in right atrial biopsy samples, which does not necessarily reflect changes occurring in ventricular myocardium. Nevertheless, a study of the activity of 5′-nucleotidase in the human heart failed to detect differences between these 2 sites.\textsuperscript{32} Second, ecto-5′-nucleotidase activity was determined only at baseline and at the completion of the preconditioning protocol. A subsequent measurement during the period of aortic cross-clamping was not possible because of the limited amount of right atrial tissue available for sampling; consequently, the time course of ecto-5′-nucleotidase activity throughout the operation cannot be conclusively established. However, previous experimental studies\textsuperscript{37} have shown that the increase in ecto-5′-nucleotidase activity elicited by the preconditioning signal tended to be further enhanced during the subsequent period of prolonged ischemia, from which sustained protection can be expected. Third, because the primary objective of this study was to assess whether the use of isoflurane could elicit a biochemical event characteristic of preconditioning, no functional studies were performed. It is, however, noteworthy that experimentally, this anesthetic agent has demonstrated marked antistunning effects.\textsuperscript{33} This finding is consistent with the previously mentioned observation that interventions that cause PKC activation (as expected to have occurred in this study after isoflurane treatment in view of the elevated levels of ecto-5′-nucleotidase) improve the recovery of function after cardioplegic arrest.\textsuperscript{24,25} Despite these caveats, the present data demonstrate that even if cardiopulmonary bypass, by itself,\textsuperscript{34} and opioid-based anesthesia\textsuperscript{35} have a preconditioning effect, there is still some room left for additional compounds like isoflurane to act as preconditioning mimetics in the human heart. As such, these results support additional studies to determine how this drug or others can be optimally used to “turn on” the signaling pathway responsible for the cardioprotective effects of preconditioning.

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References


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