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Preconditioning with sevoflurane decreases PECAM-1 expression and improves one-year cardiovascular outcome in coronary artery bypass graft surgery

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Background. Cardiac preconditioning is thought to be involved in the observed decreased coronary artery reocclusion rate in patients with angina preceding myocardial infarction. We prospectively examined whether preconditioning by sevoflurane would decrease late cardiac events in patients undergoing coronary artery bypass graft (CABG) surgery.

Methods. Seventy-two patients scheduled for elective CABG surgery were randomized to preconditioning by sevoflurane (10 min at 4 vol%) or placebo. For all patients, follow-up of adverse cardiac events was obtained 6 and 12 months after surgery. Transcript levels for platelet–endothelial cell adhesion molecule-1 (PECAM-1/CD31), catalase and heat shock protein 70 (Hsp70) were determined in atrial biopsies after sevoflurane preconditioning.

Results. Pharmacological preconditioning by sevoflurane reduced the incidence of late cardiac events during the first year after CABG surgery (sevoflurane 3% *vs* 17% in the placebo group, log-rank test, P=0.038). One patient in the sevoflurane group and three patients in the placebo group experienced new episodes of congestive heart failure and three additional patients had coronary artery reocclusion. Perioperative peak concentrations for myocardial injury markers were higher in patients with subsequent late cardiac events [NTproBNP, 9031 (4125) *vs* 3049 (1906) ng litre^{-1}, P<0.001; cTnT, 1.31 (0.88) *vs* 0.46 (0.29) μg litre^{-1}, P<0.001]. Transcript levels were reduced for PECAM-1 and increased for catalase but unchanged for Hsp70 in atrial biopsies after sevoflurane preconditioning.

Conclusions. This prospective randomized clinical study provides evidence of a protective role for pharmacological preconditioning by sevoflurane in late cardiac events in CABG patients, which may be related to favourable transcriptional changes in pro- and antiprotective proteins.

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Preconditioning can provide protection against myocardial ischaemia and reperfusion damage and there is evidence to demonstrate its occurrence in humans.[1 2] Two phases of protection have been described. Early preconditioning occurs within minutes and is protective for 2–3 h, whereas delayed preconditioning becomes effective within 12–24 h and lasts for 3–4 days. Recently, nitroglycerin was demonstrated to effectively induce delayed protection in patients undergoing coronary angioplasty.[3] While rapid phosphorylation and translocation of cellular key proteins are required

in early preconditioning, transcriptional changes in pro- and antiprotective proteins predominantly form the basic mechanisms underlying the second window of protection.[4]

Previous experimental work and clinical observations support the concept that preconditioning may even exert longer-term cardiovascular effects over and above the classic early and delayed windows of protection. Brief ischaemic episodes improved endothelial function in dogs[5] and rats[6] for up to 1 month. In the clinical setting, the presence of preinfarction angina resulted in a lower in-hospital mortality rate in the TAMI[7] and TIMI-4[8] trials. However, to date, no clinical study has prospectively addressed the effect of pharmacological preconditioning on late cardiac events in high-risk patients. We have demonstrated previously that pharmacological preconditioning by sevoflurane increases postoperative myocardial function as assessed by the biochemical marker N-terminal pro-brain natriuretic peptide (NTproBNP) in patients undergoing on-pump coronary artery bypass graft (CABG) surgery.[9] In this study, sevoflurane, which has been shown previously to mimic preconditioning and to exert significant cardiac protection against ischaemia and reperfusion,[4] was used for preconditioning.

The principal aims of the present study were to prospectively evaluate the longer-term effects and potential molecular mechanisms of pharmacological preconditioning with sevoflurane on late cardiac events in a high-risk population. Specifically, we proposed that preconditioning by sevoflurane would decrease late cardiac events in patients undergoing CABG surgery.

Methods

Study population and preconditioning protocol

During 2001–02, 72 patients scheduled for elective CABG surgery were enrolled in this study at three Swiss hospital centres. The ethics committee at each centre approved the study protocol, and all patients gave written informed consent. We have previously reported on the immediate perioperative effects of preconditioning in these patients.[9] Patients aged 40–80 yr scheduled for elective CABG surgery on a cardiopulmonary bypass (CPB) circuit with cardiac arrest were eligible for this study. Exclusion criteria included concomitant aortic or valvular surgery, elevated cardiac enzymes <24 h before surgery, unstable angina, angina <24 h before surgery, haemodynamic instability requiring inotropic support and administration of diazoxide, nicorandil, sulfonylurea or theophylline.

Anaesthesia was induced in all patients with propofol or etomidate, opioids and muscle relaxants and maintained with a target-controlled infusion of propofol 2–5 $\mu g\ ml^{-1}$ to stabilize mean arterial pressure and heart rate within 20% of baseline values, with opioids, and muscle relaxants, as required. Patients were allocated randomly to preconditioning with placebo or sevoflurane, using a sealed envelope technique. Median sternotomy was performed to cannulate the right atrium and the ascending aorta. Sevoflurane at 4 vol% or placebo (oxygen in air) was administered during established CPB (2.4 litre min^{-1} m^{-2} body surface area) for 10 min. The surgeons, anaesthetists and perfusionists were blinded to the treatment by covering the anaesthetic liquid level of the vaporizer, which was either completely filled with sevoflurane or totally empty depending on the randomization. After preconditioning, the aorta was cross-clamped and the cold (4°C) cardioplegic solution was administered. No volatile anaesthetics were administered during the study except for the pre-ischaemic period in patients randomized to the sevoflurane group. Core temperature was actively lowered or was allowed to fall spontaneously. Atrial samples collected after preconditioning but before aortic cross-clamping were used for the determination of gene expression levels. The same postoperative care was given to patients from both treatment groups.

All study patients had prospectively scheduled medical consultations, independent of the patients' usual clinical care, and 12-lead electrocardiogram (ECG), at the hospital or with their general physicians 6 and 12 months after the operation. Sixty-three per cent of the patients (45/72) had their medical examinations in the hospital. For the remaining patients, the general physicians were asked to prospectively record information as detailed on the follow-up evaluation form, which was sent to them. Each patient's general physician was instructed on collection of the required data. Hospital charts, if applicable, were also reviewed. The study end-points were late adverse cardiac events including cardiac death, non-fatal myocardial infarction, unstable angina, intercurrent coronary angioplasty or CABG surgery, arrhythmias requiring rehospitalization, and new episodes of congestive heart failure occurring after the hospitalization for CABG surgery. Death was considered a result of cardiac cause if the patient died of myocardial infarction, arrhythmia or congestive heart failure. Myocardial infarction and unstable angina were defined as previously reported.[10] The following combination of criteria was required for the diagnosis of congestive heart failure: (i) symptoms and signs of pulmonary congestion; (ii) abnormal results on chest X-ray; (iii) a change in cardiovascular medication including at least the institution of treatment with a diuretic agent. The primary outcome variable combined all adverse cardiac events such as myocardial infarction, unstable angina, congestive heart failure, severe arrhythmias, coronary revascularization and cardiac death.

Blood samples were obtained before surgery, in the intensive care unit, and 24, 48 and 72 h after surgery for measurement of cardiac troponin T (cTnT) (normal range <0.01 μg $litre^{-1}$, sensitivity >0.01 μg $litre^{-1}$, intra- and interassay coefficient of variation <5%) and NTproBNP (normal range, males <227 ng $litre^{-1}$, females <334 ng $litre^{-1}$, sensitivity >5 ng $litre^{-1}$ intra- and interassay coefficient of variation <3%) (Roche Diagnostics, Mannheim, Germany).

Transcript levels of platelet–endothelial cell adhesion molecule-1 (PECAM-1), heat shock protein 70 (Hsp70) and catalase from atrial samples collected after preconditioning were determined after enrolment of all patients using the following primers: PECAM-1 (GenBank accession number NM000442), forward primer AGACGTGCAGTACACGGAAG, reverse primer GATGTCCTTCCAGGGATGTG; Hsp70 (GenBank accession number L12723), forward primer GGAGGTTCACAAGTCTGAGG, reverse primer CCTTGGATCCAGCTTGAGAG; catalase (GenBank accession number AY028632), forward primer CCACTGTTGCTGGAGAATCG, reverse primer CCGGATCCTTCAGATGTGTC. For each gene-specific amplification, 20 μl of cDNA was diluted in water before being used as a template for the QuantiTect SybrGreen RT-PCR kit (Qiagen, Hilden, Germany). RT-PCR quantification and determination of expression levels were performed on an ABI Prism 7700 Sequence Detector Real-Time PCR machine (Perkin-Elmer, Foster City, CA, USA). Amplification reactions were conducted with an initial step at 90°C for 3 min followed by 20–35 cycles. Intra- and interassay coefficients of variation, as measured for the samples, were <5%. All PCR reactions were performed in triplicate and α-tubulin was used as a reference control.

The method of Kaplan and Meier was applied to evaluate the occurrence of late cardiac events, and the Mantel–Cox test was used to determine statistical significance. Two-factor repeated-measures analysis of variance was used to evaluate differences over time between groups. All other data were analysed using unpaired *t*-tests or the Mann–Whitney test. Categorical data were analysed using the two-tailed Fisher exact test or the χ^2-test, as appropriate. If not otherwise indicated, values represent mean (SD). SPSS (SPSS, Chicago, IL USA) was used for statistical analyses.

Results

There were no major differences between the two groups with respect to clinical data, except for phenylephrine administration during the preconditioning process to maintain blood pressure above 50 mm Hg (Table 1). Also, as previously reported,[9] the use of opioids was similar between the groups, and there was no difference in the need for pharmacological inotropic support between groups at the time of admission to the intensive care unit. Only one placebo-treated patient required transient postoperative inotropic support with an intra-aortic balloon pump. There were no differences in cardiovascular medication between groups before surgery (Table 2) or during follow-up, with the exception of the use of diuretics, which was increased in the placebo group 12 months after surgery.

Table 1 Patient characteristics. Data are mean (SD) [minimum–maximum], number of patients or grafts, or median with percentiles (25th, 75th). **P*=0.0003 compared with placebo

	Placebo (*n*=35)	Sevoflurane (*n*=37)
Age (yr)	65 (10) [44–79]	62 (10) [44–80]
Sex (M/F)	28/7	31/6
Preoperative ejection fraction (%)	57 (11) [40–76]	54 (12) [30–78]
Cross-clamp time (min)	60 (24) [15–121]	66 (22) [37–131]
Number of grafts	3.4 (0.9) [2–5]	3.5 (1.1) [2–6]
Median phenylephrine use during preconditioning (μg): median (percentiles)	0 (0–100)	200 (50–525)*

Complete follow-up data was obtained in all patients. Late adverse cardiac events (cardiac death, non-fatal myocardial infarction, unstable angina, intercurrent coronary angioplasty, coronary artery bypass grafting, arrhythmias requiring hospitalization and new episodes of congestive heart failure) occurred in seven patients and consisted of three coronary artery reocclusions and three new episodes of congestive heart failure in the placebo group (6/35=17%), and one patient in the sevoflurane group (1/37=3%) experienced a new episode of congestive heart failure (log rank test, *P*=0.038) (Fig. 1). None of the patients died during the follow-up. One patient in the sevoflurane group and two patients in the placebo group had a perioperative non-transmural myocardial infarction.

Baseline values for NTproBNP and cTnT concentrations were similar in patients with and without late cardiac events (Fig. 2). However, peak perioperative serum concentrations for NTproBNP and cTnT were significantly higher (*P*<0.001) in patients with late cardiac events than in those without such events (Fig. 2). The data suggest that peak perioperative serum concentrations for NTproBNP and cTnT after preconditioning may predict late cardiac events.

Transcript levels for PECAM-1 were significantly (*P*=0.019) lower in the sevoflurane group (Fig. 3). In contrast, the expression level for catalase was higher in the sevoflurane group (*P*=0.001). Hsp70 expression was not different between the two groups (*P*=0.59).

Discussion

To our knowledge, this study is the first prospective randomized clinical trial evaluating the effect of cardiac preconditioning on late cardiac events. The salient findings of the study are as follows. First, pharmacological preconditioning by the commonly available volatile anaesthetic sevoflurane reduced late cardiac events in CABG patients and thereby improved 1-yr event-free survival. Secondly, pharmacological preconditioning activated a protective genetic programme in human myocardium, which may be linked to the observed late beneficial effects. Finally, perioperative peak concentrations for NTproBNP and cTnT were markedly increased in patients with subsequent late cardiac events.

Myocardial ischaemia and reperfusion induce a marked, sustained endothelial dysfunction in the coronary endothelium. In fact, long-term endothelial dysfunction was reported to occur for up to 12 weeks after 60 min of ischaemia in a

Table 2 Medical therapy at the start of the study and during 1 yr of follow-up. Data are number (%) of patients. The use of cardiovascular medication was similar in the two groups except for diuretics, which were used more often at 12 months in the placebo group (placebo *vs* sevoflurane: **P*=0.062; †*P*=0.035). Fisher's exact test and the χ^2-test, respectively, were used to compare groups. ACEI=angiotensin-converting enzyme inhibitors; ATA=angiotensin receptor antagonists; NSAIDs=non-steroidal anti-inflammatory drugs; COX-2-I=cyclooxygenase-2 inhibitors

	Placebo (*n*=35)				**Sevoflurane (*n*=37)**			
	Before hospital admission	**At hospital discharge**	**At 6 months**	**At 12 months**	**Before hospital admission**	**At hospital discharge**	**At 6 months**	**At 12 months**
β-Blocker	28 (80%)	30 (85%)	30 (85%)	30 (85%)	34 (92%)	35 (94%)	35 (94%)	35 (94%)
Ca^{2+} blocker	6 (17%)	6 (17%)	6 (17%)	6 (17%)	6 (16%)	7 (19%)	6 (16%)	6 (16%)
Nitrates	14 (40%)	8 (22%)	10 (28%)	10 (28%)	20 (54%)	15 (40%)	12 (32%)	12 (32%)
ACEI/ATA	10 (28%)	15 (42%)	17 (48%)	16 (45%)	19 (51%)	19 (51%)	19 (51%)	19 (51%)
Statins	28 (80%)	29 (82%)	27 (77%)	27 (77%)	31 (83%)	31 (83%)	33 (89%)	35 (94%)
Aspirin	35 (100%)	35 (100%)	35 (100%)	35 (100%)	37 (100%)	37 (100%)	37 (100%)	37 (100%)
Diuretics	9 (25%)	8 (23%)	13 (37%)*	14 (40%)†	11 (29%)	7 (19%)	6 (16%)	6 (16%)
NSAIDs	7 (20%)	20 (57%)	15 (43%)	14 (40%)	8 (22%)	16 (43%)	14 (38%)	14 (38%)
COX-2-I	0 (0%)	0 (0%)	4 (11%)	4 (11%)	0 (0%)	0 (0%)	6 (16%)	6 (16%)

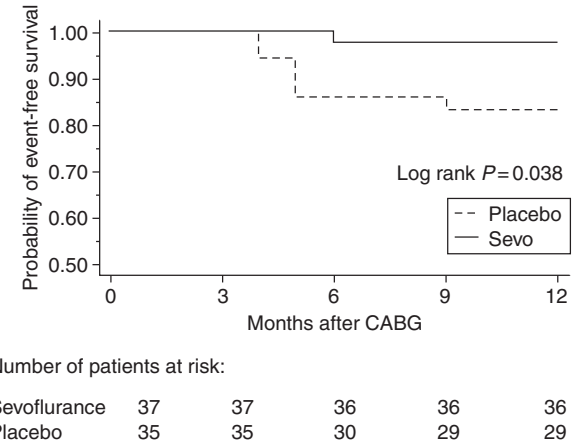

Fig 1 Kaplan–Meier curves for adverse cardiac events during 1 yr of follow-up after sevoflurane and placebo preconditioning in 72 patients undergoing CABG surgery. Each plot represents the cumulative percentage of patients remaining event-free. There is a significant difference in event-free survival in patients with sevoflurane preconditioning compared with patients with placebo (log rank test, *P*=0.038).

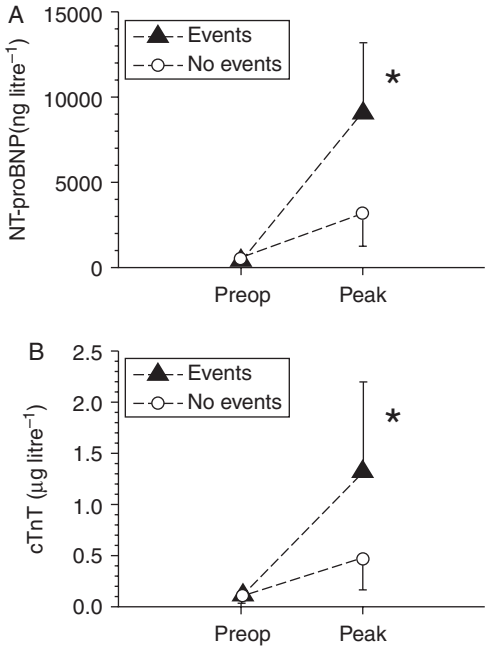

Fig 2 Biomarkers of myocardial injury in patients with and without late cardiac events. **P*<0.001 patients with or without late cardiac events. Of seven patients with late cardiac events, one was in the sevoflurane group and six were in the placebo group. 'Preop' denotes preoperative baseline concentrations and 'peak' denotes perioperative peak concentrations. Data are mean and SD.

dog model.[11] An increasing body of evidence supports the concept that preconditioning may have beneficial effects on coronary vasculature including the microcirculation[12] and, in particular, the endothelium.[13] Kaeffer and colleagues[6] showed in an *in vivo* rat model that preconditioning effectively prevents chronic reperfusion-induced coronary dysfunction. In their model, acetylcholine-dependent coronary dilation was preserved in preconditioned coronaries as opposed to non-preconditioned coronaries for up to 1 month after the ischaemic insult. More importantly, acute as well as chronic electron microscopic changes in the endothelium were absent after preconditioning. Collectively, these results suggest that unfavourable long-term ischaemia/reperfusion-induced functional and ultrastructural changes can be effectively prevented by preconditioning.

In accordance with these observations, clinical studies suggest that preinfarction angina, a clinical correlate of preconditioning, not only increases the chance of rapid reperfusion after thrombolytic therapy[14] and reduces the number of hypokinetic myocardial segments and infarct size,[15] but also affects the occurrence of delayed and late cardiac events.[7,8] Both the TAMI and TIMI-4 trials showed a lower in-hospital mortality rate in patients with preinfarction angina, which was not dependent on angiographically visible coronary collaterals. Moreover, increased 1-yr survival was reported in the TIMI-4 study for patients with preinfarction angina. Anzai and colleagues[16] demonstrated that among patients with first Q-wave anterior infarction, antecedent angina was associated with a lower incidence of cardiac ruptures and less need for readmission for

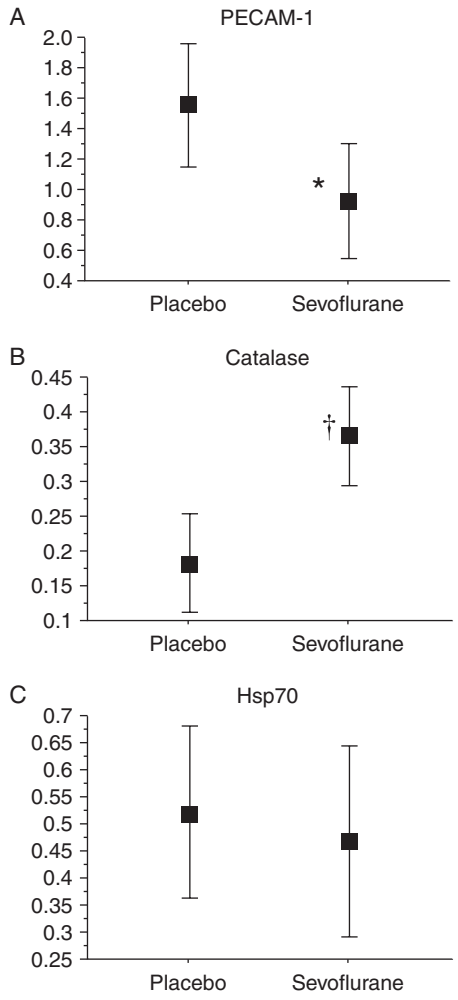

Fig 3 Transcript levels in atrial samples after preconditioning of (A) platelet–endothelial cell adhesion molecule-1 (PECAM-1), (B) catalase, and (C) heat shock protein 70 (Hsp70) expressed as fold changes with respect to α-tubulin. The transcript levels were determined for all patients (n=72). PECAM-1: placebo *vs* sevoflurane, *P=0.019. Catalase: placebo *vs* sevoflurane, †P=0.001. Hsp70, placebo *vs* sevoflurane, P=0.59. Data are mean and SD.

heart failure during follow-up. Similarly, Ishihara and colleagues[17] reported that prodromal angina, i.e. angina in the 24 h before infarction, but not previous angina, had a beneficial effect on the long-term prognosis after infarction and markedly improved 5-yr survival (83 *vs* 73%, P=0.009). These observations are in line with the results of a recent clinical study evaluating the biological significance of preconditioning in humans.[18] In this study, patients with coronary artery disease and an effective preconditioning mechanism, as measured by reduced ECG changes in response to the second balloon inflation during coronary angiography, showed a lower risk of death and non-fatal myocardial infarction during a 1-yr follow-up. The reported beneficial long-term effects may reflect a clinical counterpart of late beneficial effects by preconditioning.

The mechanism by which preconditioning may prevent late coronary events is not yet clear. However, it is tempting to speculate that preconditioning may directly modify the platelet–neutrophil–endothelial interaction by altering the synthesis of various protective and antiprotective proteins. Coronary endothelial dysfunction was previously reported to be linked to future cardiovascular events.[19 20] Using microarray technology, volatile anaesthetics were recently demonstrated to trigger a favourable genetic programme in the myocardium compatible with endothelial protection.[21] Decreased transcript levels for PECAM-1 and increased levels of catalase transcript were observed in response to anaesthetic preconditioning. PECAM-1 is an integral membrane protein on the surface of platelets and leukocytes and at the intercellular junctions of vascular endothelial cells controlling the rate and direction of leukocyte migration through the endothelium.[22] Indeed, a significant role of PECAM-1 in the transition of intracoronary atheromatous plaques from the stable to the unstable state has been suggested previously on the basis of the following observations. First, platelet-bound PECAM-1 is increased in patients with acute myocardial infarction,[23] and administration of anti-PECAM-1 antibodies reduced infarct size in animal models.[24] Secondly, increased platelet-bound PECAM-1 expression correlates with delayed and unsuccessful thrombolysis in patients with acute myocardial infarction.[25] Anti-inflammatory and anti-oxidant actions, including mitigation of leukocyte transmigration by preconditioning, may well prevent stable-to-vulnerable plaque transition and thereby delay long-term coronary reocclusion. Similar mechanisms have been suggested previously as part of the long-term beneficial effects of short-term administration of atenolol in cardiac risk patients undergoing surgery.[10] These effects may be particularly beneficial in the context of extracorporeal circulation with direct contact of circulating blood with the synthetic surfaces of the CPB, and inhibition of PECAM-1 has been proposed as an attractive target for reducing the inflammatory response to extracorporeal CPB.[26] Interestingly, in the current study catalase expression was significantly up-regulated after sevoflurane preconditioning. Scavenging of reactive oxygen species in ischaemia–reperfusion may significantly attenuate endothelial injury. Enhanced endothelial recovery after prolonged ischaemic cardioplegic arrest was demonstrated to depend on catalase activity.[27] Also, PECAM-1-directed delivery of catalase to the endothelium was shown to protect against vascular oxidative stress.[28] Hsp70, a chaperone which protects vital protein structure and function during ischaemia, was not differentially regulated. Collectively, these findings raise the interesting possibility that activation of a favourable genetic programme in the myocardium and particularly the endothelium may be mechanistically linked to the observed late beneficial effects.

There is an increasing number of studies,[29] including clinical ones, demonstrating a significant cardioprotective effect of volatile anaesthetics in patients undergoing CABG surgery.[2] Laboratory investigations also stress the concept that volatile anaesthetics may induce early and delayed

preconditioning in endothelial and smooth muscle cells,[30] implying that systemic administration of these agents may potentially protect a variety of other vital organs. Intriguingly, preconditioning by sevoflurane was reported to attenuate CPB-associated transient renal dysfunction in CABG patients.[9] Anti-inflammatory and anti-adhesive potentially plaque-stabilizing actions were previously demonstrated for sevoflurane and isoflurane in cardiac tissue,[31] and thus may delay the progression of coronary stenoses. The results of this study now confirm and extend these findings to reducing the incidence of late cardiac events in humans.

We have reported previously that sevoflurane preconditioning significantly decreased perioperative NTproBNP concentrations, but not cTnT concentrations.[9] We now show that late cardiac events were associated with significantly higher perioperative peak concentrations for NTproBNP and cTnT. Perioperative cTnT concentrations were previously reported to predict postoperative complications after cardiac surgical procedures.[32] Similarly, concentrations of brain natriuretic peptides, which directly reflect myocardial contractile state, predicted long-term cardiac function following CABG surgery.[33] In a recent study,[34] excess cardiovascular risk including cardiovascular events, heart failure, atrial fibrillation, and stroke was apparent at BNP concentrations well below the threshold indicating heart failure. The results of this study demonstrate an association of decreased perioperative peak concentrations for cTnT and NTproBNP in sevoflurane-preconditioned patients with a lower incidence of late adverse cardiac events, but these observations need further confirmation.

This study is the largest prospective randomized study on preconditioning evaluating late cardiac events. Previously reported 1-yr cumulative cardiac complication rates ranging from 1–20% after CABG surgery[35] give this study a power of 80% to detect a true difference of 20% between groups. Nonetheless, our observations are based on a small number of patients and thus require confirmation in future large-scale clinical trials. Furthermore, a small number of candidate genes was assessed after preconditioning. Future studies could use microarray technology at various intraoperative time points to confirm and extend our findings. Although no significant differences with respect to pre- and postoperative cardiovascular drug therapies were present between groups,[36] we cannot completely rule out a confounding effect of medication on cardiovascular outcome. Also, the anaesthetic regimen was not strictly standardized, with 15 patients in the placebo and 13 patients in the sevoflurane group receiving etomidate for the induction of anaesthesia. However, there is no evidence at present to suggest that the use of etomidate or propofol will affect the preconditioning mechanisms.[37] As more phenylephrine was given during the preconditioning process in the sevoflurane group to maintain arterial pressure, we cannot entirely exclude the possibility that the stimulation of α-adrenergic receptors contributed to the observed effects. However, in a separate multivariate analysis, the amount of phenylephrine administered did not prove to be a significant predictor of postoperative peak NTproBNP, making a significant contribution of phenylephrine to the observed protection unlikely.

Pharmacological preconditioning by sevoflurane improved 1-yr cardiac outcome in CABG patients. The present study suggests that myocardial preconditioning by sevoflurane may exert incremental long-term beneficial effects over and above the established early and delayed windows of protection, which may be linked to the activation of a favourable genetic programme in the heart.

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