Interference of propofol with signal transducer and activator of transcription 5 activation and cardioprotection by remote ischemic preconditioning during coronary artery bypass grafting

Eva Kottenberg, MD,[a] Judith Musiolik, PhD,[b] Matthias Thielmann, MD,[c] Heinz Jakob, MD,[c] Jürgen Peters, MD,[d] and Gerd Heusch, MD, PhD[e]

Objective: Remote ischemic preconditioning protects the myocardium from ischemia/reperfusion injury. We recently identified protection by remote ischemic preconditioning to be associated with the activation of signal transducer and activator of transcription 5 in left ventricular biopsy specimens of patients undergoing coronary artery bypass grafting during isoflurane anesthesia. Because remote ischemic preconditioning did not protect the heart during propofol anesthesia, we hypothesized that propofol anesthesia interferes with signal transducer and activator of transcription 5 activation.

Methods: In a randomized, single-blind, placebo-controlled, prospective study, we analyzed an array of established cardioprotective proteins during propofol anesthesia with or without remote ischemic preconditioning in 24 nondiabetic patients with 3-vessel coronary artery disease.

Results: Remote ischemic preconditioning (n = 12) compared with no remote ischemic preconditioning (n = 12) failed to decrease the area under the troponin I time curve (273 ± 184 ng/mL × 72 hours vs 365 ± 301 ng/mL × 72 hours; $P = .374$). Although phosphorylation of several protein kinases was increased from baseline to reperfusion, signal transducer and activator of transcription 5 phosphorylation was not increased and was not different between the remote ischemic preconditioning and no remote ischemic preconditioning groups.

Conclusions: Remote ischemic preconditioning during propofol anesthesia did not evoke either signal transducer and activator of transcription 5 activation or cardioprotection, implying interaction of propofol with cardioprotective signaling upstream of signal transducer and activator of transcription 5. (J Thorac Cardiovasc Surg 2014;147:376-82)

In classic ischemic preconditioning, defined by the protection mediated by brief episodes of ischemia/reperfusion, a whole array of trigger and mediator molecules are involved. For many of these molecules, pharmacologic recruitment has been attempted.[1] In addition to volatile anesthetics, opioids, the vasopressor phenylephrine, adenosine, and bradykinin have all been reported to evoke cardioprotection.[1] Remote ischemic preconditioning (RIPC),[2,3] an attractive alternate method to classic ischemic preconditioning for decreasing perioperative myocardial damage, can also protect the human myocardium from ischemia/reperfusion injury, as evidenced in patients undergoing elective coronary artery bypass grafting (CABG)[4-6] or percutaneous coronary interventions.[7,8] The lack of protection found in several recent studies[9-11] possibly relates to the anesthetic regimens used.[12] We recently reported decreased postoperative troponin I concentrations after RIPC only in patients undergoing CABG with isoflurane but not in those who received propofol anesthesia.[13]

The signal transduction of cardioprotection by RIPC, including the transmission of the protective signal from the distant organ and the intracellular pathways in cardiomyocytes, is still unclear. We recently observed cardioprotection by RIPC in patients undergoing CABG with previous RIPC under isoflurane anesthesia and identified an increase in tyrosine 694 phosphorylation of signal transducer and activator of transcription (STAT)5 in left ventricular (LV) biopsy specimens. STAT5 phosphorylation increased from baseline to early reperfusion only in the patients who had undergone RIPC but not in the control patients. However, a causal role for the observed STAT5 activation in cardioprotection remains to be established.[1,14]

Because we observed protection by RIPC during isoflurane, but not during propofol, anesthesia,[13] we hypothesized that propofol interferes with STAT5 activation, which was shown to be activated in our previous study of cardioprotection by RIPC with isoflurane anesthesia. Accordingly, we

From the Klinik für Anästhesiologie und Intensivmedizin,[a] Institut für Pathophysiologie,[b] Klinik für Thorax- und Kardiovaskuläre Chirurgie,[c] Klinik für Anästhesiologie und Intensivmedizin,[d] and Institut für Pathophysiologie,[e] Universität Duisburg-Essen, Universitätsklinikum Essen, Essen, Germany.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication Oct 14, 2012; revisions received Dec 9, 2012; accepted for publication Jan 11, 2013; available ahead of print March 4, 2013.

Address for reprints: Eva Kottenberg, DEAA, Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum Essen, Hufelandstr 55, Essen D-4511, Germany (E-mail: eva.kottenberg@uni-duisburg-essen.de).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2013.01.005

Abbreviations and Acronyms

CABG = coronary artery bypass grafting
cTnI = cardiac troponin I
cTnI AUC = area under the cTnI time curve
CPB = cardiopulmonary bypass
LV = left ventricular
RIPC = remote ischemic preconditioning
STAT = signal transducer and activator of transcription

assessed in a randomized, single-blind, placebo-controlled, prospective study, the effects of RIPC on STAT5 activation and the serum troponin I concentrations in nondiabetic patients undergoing CABG during propofol anesthesia.

METHODS

Patients

The present study is part of an ongoing multiprotocol study (ClinicalTrials.gov NCT01406678) addressing the effects and mechanisms of RIPC and anesthesia on the cardiac troponin concentrations in patients undergoing CABG. The institutional ethics committee approved the study, and all patients provided written informed consent. The present cohort of 24 patients with 3-vessel coronary artery disease scheduled for CABG (Table 1) was enrolled from June 2010 to December 2011. They were randomized on the day of surgery to receive, under propofol/sufentanil anesthesia, either a RIPC protocol, consisting of repetitive left upper arm ischemia (3 cycles of 5-minute cuff occlusion/5-minute reperfusion each), or no RIPC (cuff uninflated). None of the patients, data, or samples obtained in our previous studies of RIPC[6,13,14] were included in the present study.

All patients older than 18 years who were scheduled for elective, isolated, first-time CABG for 3-vessel coronary artery disease were eligible for the present study. The exclusion criteria were any type of diabetes mellitus (controlled by diet, oral drugs, or insulin), renal insufficiency (serum creatinine > 2 mg/dL), peripheral vascular disease affecting the upper limbs, acute coronary syndrome, acute or recent myocardial infarction, preoperative inotropic support before the induction of anesthesia, any type of mechanical assist device, any condition potentially increasing the preoperative cardiac troponin I (cTnI) level (eg, coronary interventions within the previous 6 weeks), or any type of emergency surgery, combined CABG/valve surgery, or any previous cardiac operations.

General Procedures

General anesthesia was induced by sufentanil (1 μg/kg, Sufenta; Janssen-Cilag, Neuss, Germany) and etomidate (0.3 mg/kg, Hypnomidat; Janssen-Cilag, Neuss, Germany), followed by rocuronium (0.6 mg/kg, Esmeron; Organon Teknika, Oberschleiβheim, Germany). Anesthesia was maintained by continuous propofol infusion (0.07-0.15 mg/kg/min), with additional sufentanil injected at the discretion of the responsible anesthesiologist, as required. During extracorporal circulation, the patients continued to receive a propofol infusion.

Surgical revascularization was performed in all patients after a median sternotomy. Hypothermic (30°C-33°C) cardiopulmonary bypass (CPB) was instituted through an ascending aortic cannula and a 2-stage right atrial cannula. After ascending aortic crossclamping, Bretschneider's cardioplegia was introduced. After completion of the distal CABG anastomoses, the aortic crossclamp was released, and cardiac reperfusion ensued. After rewarming the patient to 37°C and separation from CPB, reversal of heparin by protamine sulphate (3 mg/kg), and sternal closure, the patients were transferred to the intensive care unit.[13]

Troponin I

To assess myocardial injury, cTnI was measured (Immunoassay Dimension Flex, Dade Behring GmbH, Marburg, Germany) at specified times for 72 hours after CABG in an accredited laboratory. The serum cTnI levels were determined preoperatively and 1, 6, 12, 24, 48, and 72 hours postoperatively, and the area under the cTnI time curve (cTnI AUC) was determined.

Western Blot Analyses

In each patient, transmural myocardial biopsy specimens of 2 to 5 mg each were harvested using a Tru-Cut biopsy needle (Cardinal Health, Dublin, Ohio) from the LV perfusion territory undergoing revascularization, quickly frozen in liquid nitrogen, and stored at −80°C until analysis. The first biopsy specimen was taken at baseline before starting CPB, the second after 10 minutes of myocardial reperfusion after aortic declamping. Western blot analysis was performed with antibodies against the phosphorylated and total forms of several established signaling proteins, including protein kinase C, p38 mitogen-activated protein kinase, vasodilator-stimulated phosphoprotein as a substrate of protein kinase G, endothelial nitric oxide synthase, protein kinase B, extracellular-regulated kinase, p70 ribosomal S6 protein kinase, glycogen synthase kinase 3β, STAT3, and STAT5.[14] The immunoreactivities of the phosphorylated proteins were normalized to those of the respective total proteins.

Two aliquots of each sample were analyzed separately on 2 different gels; therefore, these data can differ. The baseline and reperfusion samples were compared within the no RIPC and RIPC group on 1 gel each, and the baseline and reperfusion samples were compared between the no RIPC and RIPC group on 1 gel each.

Study Protocol

After induction of anesthesia and endotracheal intubation, a standard blood pressure cuff was inflated to 200 mm Hg (ie, always in excess of the contralateral systolic radial artery pressure) for 5 minutes by a resident assigned to the case and not involved in either randomization or data assessment. As a control, a cuff was placed around the left upper arm but left uninflated. Three cycles of 5-minute left upper arm ischemia were separated by 5-minute intervals of reperfusion by deflating the cuff. The RIPC protocol was always completed before the skin incision.

The data from 24 patients fulfilling the enrollment criteria were analyzed in a single-blind, randomized protocol. Before the trial, computer-generated randomization schedules were generated and placed in sequentially numbered sealed envelopes. The laboratory personnel measuring the troponin concentrations, patients, surgeons, echocardiographers, and critical care teams were unaware of the treatments assigned for the study duration. The resident anesthetists, who applied the protocol by inflating or not inflating the cuff could not be blind to the group assignment but had no part in the data sampling or analysis.

Statistical Analysis

The patient characteristics and cTnI concentrations are expressed as numbers, frequencies, or mean ± standard deviation. The data were compared using a 2-way analysis of variance and Student's 2-sided *t* test for unpaired samples. A chi-square test was used to compare the categorical variables. The immunoreactivities on the same gels were compared by paired (within groups) or unpaired (between groups) *t* tests.

The following a priori null hypothesis was tested using the cTnI AUC as a primary and the STAT5 phosphorylation as a secondary criterion: (1) no difference exists in cTnI AUC with and without RIPC during propofol