Aprotinin Reduces Cardiac Troponin I Release and Inhibits Apoptosis of Polymorphonuclear Cells During Off-Pump Coronary Artery Bypass Surgery

Christophe Bert, MD, PhD,* Frederik De Buck, MD,* Paul Sergeant, MD, PhD,† Jan Van Hemelrijck, MD, PhD,* Ahmad Kasran, MD, PhD,‡ Victor Van Duppen,§ Jan Ceuppens, MD, PhD,‡ Bart Meyns, MD, PhD,† Michel Delforge, MD, PhD,§ and Patrick Wouters, MD, PhD*

Objectives: **In addition to blood-sparing effects, aprotinin may have cardioprotective and anti-inflammatory effects during cardiopulmonary bypass–assisted cardiac surgery. In this study, the authors examined whether aprotinin had cardioprotective and/or anti-inflammatory effects in patients undergoing off-pump coronary artery bypass grafting.**

Design: **A prospective randomized clinical trial.**

Setting: **University hospital.**

Participants: **Fifty patients were randomized to control (n = 25) or aprotinin treatment (n = 25) groups.**

Interventions: **Aprotinin was given as a loading dose (2×10^6 KIU) followed by a continuous infusion at 5×10^5 KIU/h until skin closure.**

Measurements and Main Results: **Blood samples for cardiac troponin I; interleukin-6, interleukin-8, and interleukin-10; tumor necrosis factor α; and elastase were taken after anesthesia induction, completion of revascularization, and 6 hours, 12 hours, and 24 hours after revascularization. Blood samples were taken to assess for apoptosis in polymorphonuclear cells. Baseline plasma levels for cardiac troponin I did not differ between groups but were significantly lower in aprotinin-treated patients at the time of revascularization ($p = 0.03$) and 6 hours ($p = 0.004$) and 24 hours ($p = 0.03$) later. Aprotinin significantly reduced apoptosis in polymorphonuclear cells compared with control-treated patients ($p = 0.04$). There were no differences in plasma cytokine or elastase levels between groups.**

Conclusions: **The authors conclude that aprotinin reduces perioperative cardiac troponin I release and attenuates apoptosis in polymorphonuclear cells but has no significant effects on plasma cytokine levels in patients undergoing off-pump coronary artery bypass graft surgery.**

KEY WORDS: off-pump coronary artery bypass graft, aprotinin, inflammation, myocardial protection, neutrophils, apoptosis, blood loss

BOTH MECHANICAL INJURY during cardiac surgery and ischemia-reperfusion cause inflammation. In vitro experiments report anti-inflammatory effects of aprotinin by multiple mechanisms, including inhibiting nuclear factor kappa-B (NFκB) activation, protease activated receptor-1 (PAR-1) activation, phospholipase A2 activity, expression of L-selectin, intercellular adhesion molecule-1, and vascular cellular adhesion molecule.[1-6] Neutrophils mediate inflammation-associated tissue damage by binding to activated endothelial cells and migrating into the surrounding tissues where they release proteases and oxygen free radicals, leading to tissue damage. Inflammatory responses also induce apoptosis of tissue cells, potentially leading to altered organ function, and apoptosis of polymorphonuclear (PMN) cells is a key event in resolving acute inflammatory processes. Jimenez[7] reported that PMN cells become apoptosis resistant during systemic inflammatory responses prolonging the lifespan of these cells. This phenomenon is thought to contribute to the organ damage observed during systemic inflammatory response syndrome (SIRS) and related syndromes. Although cardiopulmonary bypass (CPB) is a major factor eliciting a generalized inflammatory reaction, inflammation also occurs in off-pump cardiac surgery, be it in a less pronounced way.[8-10] Aprotinin has been demonstrated to reduce inflammation in CPB-supported cardiac surgery.[11-19] Whether aprotinin can further reduce inflammation in off-pump coronary artery bypass graft (OPCAB) surgery has currently not been investigated. In addition, aprotinin has been reported to have cardioprotective properties during cardiac surgery with CPB by reducing ischemia-reperfusion injury, including reduced cardiac troponin I (cTnI) release in coronary artery bypass graft (CABG) patients.[20-24] Reducing inflammatory activation during surgical trauma and ischemia-reperfusion could lead to reduced myocardial tissue injury. If, in addition, activated PMN cells would become less resistant on apoptosis, their reduced lifespan could contribute to decreased tissue injury.

Little data are currently available on the potential cardioprotective and/or anti-inflammatory action of aprotinin in OPCAB surgery. The authors performed a prospective randomized clinical trial to evaluate aprotinin's effects in patients undergoing OPCAB surgery using cTnI as a marker of myocardial damage and interleukin (IL)-6, -8, -10, tumor necrosis factor α (TNF-α), PMN elastase, and PMN cell apoptosis as markers of inflammation.

METHODS

After approval by the institutional ethics committee, 50 patients undergoing elective OPCAB surgery were enrolled in the study after written informed consent was obtained. Fifty consecutive patients were assigned to the control group (n = 25) or to the aprotinin group (n = 25) by a computer-generated randomization list. Exclusion criteria were as follows: redo surgery, ejection fraction <30%, chronic immunosuppressive therapy (including corticosteroids), recent infection, unstable angina, use of coumarin derivatives and GPIIb-IIIa inhibitors, documented allergy, congenital coagulation disorders, and renal impairment (serum creatinine >1.5 mg/dL).

All patients received standard anesthetic care that included premedication with 0.05 mg/kg of lorazepam sublingually 1 hour before surgery. Anesthesia induction was with midazolam (0.03 mg/kg), sufentanil (1-1.5 μg/kg), propofol target-controlled infusion (starting

*From the Departments of *Anesthesiology, †Cardiac Surgery, and §Hematology, and ‡Laboratory of Immunology, University Hospital Gasthuisberg, Leuven, Belgium.*

Address reprint requests to Christophe Bert, MD, PhD, Dienst Anesthesie, OLV Ziekenhuis, Moorselbaan 164, B-9300 Aalst, Belgium. E-mail: christophe.bert@olvz-aalst.be

1053-0770/08/2201-0005$34.00/0

doi:10.1053/j.jvca.2007.01.018

target concentration of 0.5-1.0 μg/mL), and pancuronium (0.1 mg/kg). Anesthesia maintenance was with a continuous propofol infusion (up to target concentration of 2.5 μg/mL) and supplemental sufentanil (8-10 μg/kg) and pancuronium. Vaporizers for volatile anesthetics were removed from the ventilator. Patient monitoring included direct arterial pressure and pulmonary artery pressure monitoring. Patients allocated to the aprotinin group received a loading dose of 2×10^6 KIU before sternotomy, followed by a continuous infusion of 5×10^5 KIU/h until wound closure. Heparin was given as anticoagulation to maintain the activated coagulation times >400 seconds. All coronary bypass graft anastomoses were made with intracoronary shunts in place. The region to be operated on was immobilized by a regional vacuum stabilizer and, if needed, by an apical suction device. After revascularization was complete heparin was neutralized with protamine (1:1 ratio of active heparin) and final activated coagulation times were generally within 10% of baseline value. All patients underwent complete revascularization. Suctioned blood was washed (Autolog; Medtronic Inc, Minneapolis, MN) and returned to the patient if the collected volume exceeded 800 mL. All patients received routine postoperative care. Blood sugar concentrations were kept between 70 and 110 mg/dL.

Blood samples for determination of plasma levels of cTnI, IL-6, IL-8, IL-10, TNF-α, and elastase were taken after induction of anesthesia (T1), after revascularization but before protamine administration (T2), and 6 hours, 12 hours, and 24 hours after revascularization (T3, T4, and T5, respectively). The plasma fraction was isolated by centrifugation and stored at -20°C. Blood samples for determination of polymorphonuclear cell apoptosis (12 controls and 12 aprotinin-treated patients) were taken at the same time points except for T4 in EDTA-containing tubes and were immediately sent to the laboratory for analysis.

The inflammatory cytokines IL-6, IL-8, and IL-10 were measured by using cytometric bead array (CBA; BD Biosciences Europe, Erembodegem, Belgium) as described by the manufacturer. Acquisition of sample data was done by using a FACSort (BD Biosciences, San Jose, CA), and results were generated using the BD CBA Analysis Software. The lowest detection limit for all cytokines was 1.25 pg/mL.

Elastase was determined by sandwich enzyme-linked immunosorbent assay (Calbiochem; Merck Biosciences, Nottingham, UK). Samples were measured in duplicate. TNF-α was measured in an almost similar procedure to elastase by using the TNF-α EASIA kit purchased from Biosource Europe SA (Nivelles, Belgium). The lowest detection limits for elastase and TNF-α are 0.156 ng/mL and 14.7 pg/mL, respectively. cTnI plasma levels were determined by a 1-step sandwich enzyme-linked immunosorbent assay technique and analyzed on a Dimension clinical chemistry analyzer (Dade Behring Inc, Brussels, Belgium).

Phosphatidylserine translocation from the inner to the outer cellular membrane is one of the first phenomenon to occur once apoptosis is induced. Annexin V is a naturally occurring high-affinity ligand for PS. Because translocation also occurs during necrosis, annexin V is not an absolute marker for apoptosis. Therefore, it is often used in conjunction with vital dyes, such as propidiumiodide, which bind to nucleic acids only when it can penetrate cells through a damaged membrane, thus distinguishing necrotic from apoptotic cells.

Briefly, blood was collected in a 3-mL EDTA tube and polymorphonuclear cells separated from the whole blood by using density gradient centrifugation. Obtained PMNs were resuspended in cold Hepes buffer at a concentration of 1×10^6 PMNs per milliliter. Two hundred microliters of this cell suspension were used for analysis. Ten microliters of FITC-conjugated annexin V (stock: 20 μg/mL) and 20 μL propidiumiodide (PI) (stock: 50 μL/mL) (BD Biosciences Pharmingen, San Diego, CA) were added. After a gentle vortex, the PMNs were incubated for 15 minutes at 4°C in the dark before flow cytometric analysis.

Apoptosis and cell death were measured by using a FACScan flow cytometer (Becton Dickinson, Erembodegem, Belgium) equipped with a 488-nm argon-ion air-cooled laser (15 mW) and standard filter combination (FL1 = BP 530/30 nm, FL2 = BP 585/42 nm, and FL3 = LP 650 nm) provided by the manufacturer. A minimum of 20,000 events were collected for each sample. The PMN population was gated on a forward-scatter versus side-scatter dot plot, and quadrant statistics were used on an annexin V versus PI dot plot to express apoptotic (annexin V^{pos}/PI^{neg}) and dead (annexin V^{pos}/PI^{pos}) PMNs as fraction of the total number of PMNs. To calculate absolute numbers, these fractions were multiplied by the absolute PMN count obtained by a Micros 60 hematology analyzer (ABX Diagnostics, Deurne, Belgium). Persons involved in blood sample analysis (A.K., V.V.D., J.C., and M.D.) were not aware of the patient's allocation to either the control group or the aprotinin group.

Power analysis was performed on the primary outcome variable cTnI. A difference of 2 ng/mL was considered to be clinically important. For a power of 0.8 and $\alpha = 0.05$, a sample size of 10 patients for each treatment group seemed to be sufficient.

Demographic data and data on blood loss and transfusion were analyzed by Student t test. Because plasma levels of interleukins and elastase were not normally distributed, a Mann-Whitney U test was used to look for differences between both groups. Apoptosis results were also not normally distributed and treated likewise. cTnI values, however, did show a normal distribution and were analyzed by using a repeated-measures analysis of variance approach. Statistical analysis was performed with Statistica, release 6.1 (StatSoft Inc, Tulsa, OK).

RESULTS

Demographic data for patients from both study groups are listed in Table 1. Distribution of age, weight, height, sex, and preoperative medication were not different between groups. Also, operating time, number of distal anastomoses (venous and arterial), incidence of intramural dissections, and heparin and protamine doses were not statistically different (Table 2). The number of patients who needed pharmacologic support during the study period was not statistically different between groups (Table 2; 9/25 control v 7/25 aprotinin group, $p = 0.55$) nor was the total drug dose. Aprotinin administration did not

Table 1. Demographic and Preoperative Characteristics

	Control	Aprotinin
Age (y)	67.8 (±8.3)	65.7 (±10.2)
Weight (kg)	80.6 (±9.3)	76.8 (±13.9)
Height (cm)	171.5 (±9.4)	168.6 (±8.7)
Sex ratio (M/F)	20/5	20/5
β-adrenergic blockers	17/25	16/25
Calcium channel blockers	6/25	7/25
ACE inhibitors	8/25	5/25
AT II	3/25	2/25
Nitrates	16/25	15/25
Diuretics	3/25	3/25
Bronchodilating agents	0/25	1/25
Acetylsalicylic acid	19/25	18/25
LMWH	6/25	7/25
Statins	19/2	15/25

NOTE. Data are expressed as mean ± SD. Patient numbers are indicated for sex ratio and preoperative medication. No significant differences were found between groups.

Abbreviations: AT II, angiotensin II inhibitors; LMWH, low–molecular-weight heparins.