LOWER CARDIAC TROPONIN T LEVELS IN PATIENTS UNDERGOING CARDIOPULMONARY BYPASS AND RECEIVING HIGH-DOSE APROTININ THERAPY INDICATE REDUCTION OF PERIOPERATIVE MYOCARDIAL DAMAGE

Nowadays in many European heart centers the activation of the fibrinolytic system, always occurring during cardiopulmonary bypass, is routinely reduced by high-dose application of the proteinase inhibitor aprotinin (total of >4 million KIU). In this study parameters of myocardial ischemic injury were investigated with the aim of identifying further benefits of aprotinin, particularly the protection of the myocardium during the ischemic period of aortic crossclamping. Forty patients with coronary artery disease who underwent aorta-coronary bypass grafting were randomly and in a double-blind fashion divided into two groups, one that received high-dose aprotinin therapy and one that received only saline solution. Markers such as troponin T, with high specificity for detection of myocardial ischemia and infarction, and markers with more general specificity such as creatine kinase, its isoenzyme, and lactate dehydrogenase showed significantly increased values after ischemia in both groups. In patients who received high-dose aprotinin therapy 3 days after cardiopulmonary bypass all parameters measured showed significantly lower levels compared with those in the control group. Therefore we can presume that the application of high-dose aprotinin provides myocardial protection from perioperative ischemic injury. (J THORAC CARDIOVASC SURG 1995;109:1164-72)

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Despite well-established procedures such as hypothermia and cardioplegia, perioperative myocardial infarction during cardiopulmonary bypass (CPB) still occurs more frequently than in 6% of the cases.¹⁻³ This incidence is still problematic and difficult to diagnose in less severe cases. It represents an unsolved phenomenon in clinical consideration of prognostic implications.¹ For diagnosis of perioperative myocardial infarction changes in plasma concentrations of creatine kinase (CK), its isoenzyme (CK-MB), and lactate dehydrogenase (LDH) are generally measured in connection with analysis of the electrocardiogram or myocardial scintigraphy.^{1,4} With the development of a new one-step enzyme immunoassay for cardiac troponin

T by Katus and co-workers⁵ a much more cardiac-specific and sensitive method for detection of perioperative myocardial ischemic injury is now available.⁶⁻⁸

Cardiac troponin T belongs to the tropomyosin-binding proteins of the troponin regulatory complex, which is located on the thin myofilaments (actin, tropomyosin, and troponin). It differs from skeletal troponin T by 6 to 11 amino acid residues^{9,10} and therefore if detected in serum is a highly specific marker for destruction of cardiac myocytes.

Postoperative bleeding is still a major complication in heart operations,^{11,12} the reasons for which are multifactorial: the contact of the blood with the artificial devices of the CPB circuit causes a drop in platelet count and a reduction in platelet functional activity.^{11,13-16} In addition, there is an activation of the contact pathway of intrinsic coagulation (that is, factor XII and the kallikrein-kinin systems),¹⁷⁻²² a reinforced activation of the fibrinolytic and complement systems,^{19,23,24} and degranulation of neutrophils.²⁵⁻²⁷

In recent years it has been demonstrated repeatedly that high-dose aprotinin therapy in heart operations significantly reduces postoperative blood loss by direct and indirect inhibition of fibrinolysis²⁸⁻³⁷

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along with a reduced, probably plasmin-mediated, degranulative effect on the adhesion glycoproteins (glycoprotein Ib) of the platelet membrane.³⁸⁻⁴⁵

In this randomized double-blind study findings in 40 patients undergoing CPB were investigated for further benefits of the serine proteinase inhibitor aprotinin with regard to myocardial protection from ischemic injury during the period of aortic cross-clamping.

Patients and methods

Forty patients with coronary artery disease who underwent elective aorta-coronary bypass grafting were entered in this study. Approval for this study was given by the local ethical committee and, after being fully informed verbally, all participating patients gave written consent.

Exclusion criteria were reoperations, preoperative low left ventricular function (ejection fraction $\leq 30\%$, left ventricular end-diastolic pressure ≥ 20 mm Hg), preoperative hemoglobin level 12 gm/dl or less, serum creatinine level 2 mg/dl or greater, acquired or hereditary thrombopathia, allergic diathesis, and previous application of aprotinin. Acetylsalicylic acid therapy was stopped at least 7 days before operation. Patients with preoperative autologous blood transfusion or intraoperative or postoperative infusion of fresh frozen plasma were not accepted.

Patients were randomly and in a double-blind fashion assigned to two groups: 20 patients received high-dose aprotinin (Trasylol) and 20 patients were administered physiologic saline solution (Table I). Aprotinin and the placebo were provided by the manufacturer (Bayer AG, Leverkusen, Germany) in identical bottles that differed only in the random numbers on their labels.

Anesthesia was standardized for all patients and involved induction by fentanyl (Fentanyl-Janssen), thiopental (Trapanal), midazolam (Dormicum), and vecuronium (Norcuron) and maintenance by fentanyl, midazolam, and vecuronium. A dose of 3.5 mg/kg body weight heparin (Liquemin) was given shortly before the start of CPB. Activated clotting time was measured with the use of Hemotec kaolin cartridges (Medtronic Cardiopulmonary, Anaheim, Calif.) to avoid artificially prolonged activated clotting time values in the presence of aprotinin.⁴⁶ If activated clotting time levels dropped below 480 seconds further heparin was administered. The heparin was antagonized with protamine hydrochloride in the ratio of 1:1 shortly after extracorporeal circulation (ECC) was completed.

Surgical and bypass procedures were standardized.

Oxygenation was done by a Cobe CML-Excel membrane oxygenator (Cobe Laboratories, Lakewood, Colo.) with a blood flow of 2.4 L/m² per minute in hypothermia (rectal temperature 28°C). Myocardial protection was achieved by 30 ml/kg body weight cold (4°C) cardioplegic solution (HTK Bretschneider, F. Köhler Chemie, Alsbach, Germany).

Postoperative blood loss was measured by thoracic drainage volumes in 2-hour periods until removal of chest tubes.

Serial preoperative and postoperative electrocardio-

Table I. Blood sampling time and aprotinin/heparin application schedule before, during, and after ECC

Sample No.	Sampling time	Aprotinin and heparin dosage
1	Preoperative	
	Skin cut, loading dose	30,000 KIU aprotinin/kg BW in 20 min
2	Thoracotomy, before heparin	7000 KIU/kg BW per hour permanent infusion
	Aortic crossclamping	3.5 mg heparin/kg BW
3	After 5 min ECC	30,000 KIU/kg BW in priming solution
4	After 15 min ECC	
5	After 30 min ECC	
6	After 45 min ECC	
	Release of aortic crossclamps	
7	Shortly after end of ECC	10 min after protamine
8	Shortly before end of operation	
9	1 day after operation	
10	3 days after operation	

BW, Body weight.

grams were registered on a 12-channel system and assessed for the occurrence of new Q waves or a greater than 50% R reduction in at least two chest leads.

Cardiac muscle troponin T levels were measured by a one-step enzyme immunoassay⁵ (Boehringer Mannheim, Mannheim, Germany) with use of two highly specific monoclonal antibodies directed against two different epitopes of cardiac troponin T molecule. Levels of enzymes CK, CK-MB, and LDH were determined with test kits from Boehringer Mannheim. Monitoring of aprotinin plasma levels was conducted according to the method of Mueller-Esterl and associates⁴⁷ with use of an enzyme-linked immunosorbent assay. Kallikrein inhibition capacity was measured by determining plasma inhibition of plasma kallikrein with the chromogenic peptide substrate S 2302 (Chromogenix, Mölndal, Sweden).

Statistical analysis was done by the SPSS statistical software package (SPSS Inc., Chicago, Ill.). Results are expressed as mean values plus or minus the standard deviation. Differences between mean values were assessed by univariate analysis of variance and Student's *t* test. *P* values of 0.05 or less were considered significant.

Results

Demography. The distribution of demographic data showed homogeneous collectives. The two patient groups did not differ significantly in age, weight, height, body surface area, red cell volume, crossclamping time, number of grafts, or duration of operation (Table II).

Infarction rate. In two patients of the entire population perioperative myocardial infarction

Table II. Demographic data and distribution of operative procedures

	<i>Aprotinin</i>	<i>Placebo</i>
Age (yr)	62.4 ± 7.4	60.6 ± 7.7
Weight (kg)	80.2 ± 9.4	76.8 ± 7.2
Height (cm)	173.3 ± 4.8	171.6 ± 3.8
Body surface (m ²)	1.9 ± 0.1	1.9 ± 0.1
Red cell volume (L)	2.2 ± 0.3	2.1 ± 0.2
Total operative period (min)	221.1 ± 56.6	218.7 ± 34.9
Crossclamping (min)	88.1 ± 23.3	93.6 ± 23.9
No. of venous grafts θ	2.95 ± 0.89	2.95 ± 0.76
1 Venous graft	1	0
2 Venous grafts	5	6
3 Venous grafts	8	9
4 Venous grafts	6	5
Arterial grafts	9	7

Values given as mean plus or minus the standard deviation.

could be diagnosed by new Q waves in electrocardiogram. Both patients came from the placebo group and were excluded from statistical analysis of all parameters reported. Both cases had the highest peak values of CK (628 and 1863 U/L), CK-MB (58 and 94 U/L), LDH (578 and 1788 U/L), and troponin T (18.2 and 12.1 ng/ml).

Aprotinin monitoring (Fig. 1). Serum aprotinin concentrations were determined 15 and 45 minutes after total ECC. At both sampling times the concentrations were much higher than the required 200 KIU/ml. Mean values of the samples were 341.8 ± 60.9 and 251.7 ± 53.5 KIU/ml, respectively. Therefore, analogous to these levels, very high levels of kallikrein inhibition capacity up to $387.8\% \pm 88.4\%$ (sampling time 3) were obtained in the aprotinin group. This demonstrates clearly that sufficient aprotinin for complete inhibition of plasma kallikrein with use of this regimen of application was obtained.

Blood loss and blood transfusion. Blood loss (Fig. 2) from the thoracic drainage volumes when the tubes were removed was significantly lower in the aprotinin group ($p = 0.021$). The total in the placebo group was 1353.6 ± 746.5 ml and in the group treated with aprotinin was only 878.9 ± 438.0 ml. By means of high-dose aprotinin therapy a reduction in blood loss of 35.1% could be achieved. In the aprotinin group 50% of the patients did not need any donor blood in comparison with only 18% in the control group. Therefore the aprotinin group received on average 1.25 units of blood and the patients in the placebo group 3.18 units.

Infarction markers

Troponin T (Fig. 3). Preoperative values of troponin T were below the detectable level. Myocardial perfusion after the total ECC period showed significantly elevated release of troponin T in both groups. In the aprotinin group only three patients had serum concentrations higher than 5 ng/ml, whereas seven patients in the control group (without the two excluded infarction cases) had concentrations higher than this level. In all postoperative determinations the placebo group without aprotinin showed significantly ($p \leq 0.01$) higher values on the first postoperative day.

Enzymes. Elevated CK levels were measured postoperatively in the placebo group (Fig. 4). Because of the high standard deviation and small group size no statistically significant differences could be detected, but there was a strong trend toward lower values in the aprotinin group. On the third postoperative day the control group had a mean level of 191.7 ± 446.4 U/L and the aprotinin group only 80.3 ± 62.6 U/L. Levels of both the isoform CK-MB (Fig. 5) and LDH (Fig. 6) showed a large increase after ischemia. Mean values on the third postoperative day were significantly lower ($p \leq 0.01$) in the aprotinin group.

Discussion

The results of reduced blood loss from thoracic drainages indicate the well-known beneficial effect of aprotinin in CPB. We therefore recommend the routine application of this proteinase inhibitor to minimize blood transfusion and the associated risk of infection.

In this study with high-dose aprotinin therapy we were able to demonstrate a suspected protective effect on the myocardium. Common enzymatic diagnostic indicators of perioperative myocardial infarction are not specific enough in cardiac operations to differentiate between destruction of skeletal and myocardial tissues.^{7, 48} With the use of electrocardiograms or myocardial scintigraphy it is also difficult to diagnose myocardial necrosis, especially in patients with previous myocardial infarction. We therefore only diagnose a perioperative myocardial infarction from the electrocardiogram in those patients in whom new significant signs of a transmural myocardial infarction were displayed after operation. Minor changes in the T wave that may be a result of multiple causes do not have a sufficient diagnostic accuracy. They were therefore not taken into consideration for the diagnosis of a periopera-

Fig. 1. Black columns (mean $\pm$ standard deviation) show significantly increased kallikrein inhibition (KKI, left ordinate) in aprotinin group in correlation with concentrations of aprotinin (line; KIU/ml, right ordinate).

Fig. 2. Cumulative blood loss (mean $\pm$ standard deviation). Patients treated with high-dose aprotinin had significant postoperative reduction in blood loss from thoracic drainage tubes from first 2 hours after arrival (A) in intensive care unit until drainage tube removal (DR).

tive transmural infarction. Thus a limitation of this study is the fact that the perioperative electrocardiogram does not provide a “gold standard” (which other studies confirm¹) with the result that minor nontransmural myocardial infarctions cannot be excluded in both groups.

With the introduction of a new one-step enzyme immunoassay for cardiac troponin T,⁵ an extremely sensitive method for detection of myocardial cell

necrosis is now available.⁶⁻⁸ With this specific marker we can detect the efficacy of different procedures of myocardial protection and surgical techniques. All the infarction parameters measured in this study showed corresponding results. Incidence of new Q waves correlated with markedly increased levels of enzymes and troponin T. In nearly all patients undergoing CPB ischemic tissue damage occurred, but in the group treated with high-dose

Fig. 3. Significantly increased troponin T values (mean $\pm$ standard deviation) in all patients after ECC in comparison with preoperative values. After aortic crossclamping period patients with high-dose aprotinin therapy showed markedly reduced serum concentrations of troponin T. Two patients with diagnosed myocardial infarction (*lines*) showed high elevated levels.

Fig. 4. After operation levels of CK (mean $\pm$ standard deviation) increased significantly in both groups; however, lower values were seen in patients receiving high-dose aprotinin therapy.

aprotinin a significantly smaller injury was detectable.

Fifteen years ago, Diaz and associates⁴⁹ conducted histologic investigations in dogs with regional ischemia and found both lower CK levels and less myocardial destruction in the aprotinin group. Up to now only a few publications have appeared that deal with this subject in human beings. Hjelm and colleagues⁵⁰ found no difference in treatment

with or without aprotinin in their investigations on human CPB. However, they only applied very small doses of 200,000 KIU initially and then 20,000 KIU/hour. On the basis of our experience and knowledge to date blood loss and presumably myocardial protection cannot be influenced with use of such a small dosage of aprotinin.²⁸⁻³⁶ However, corresponding to our results, Sunamori and associates⁵¹⁻⁵³ found lower enzyme levels in patients who

Fig. 5. After bypass, patients from control group showed higher levels of CK-MB (mean $\pm$ standard deviation) than those in aprotinin group. Difference was significant 3 days after CPB.

Fig. 6. LDH levels (mean $\pm$ standard deviation) in patients receiving high-dose aprotinin therapy were significantly lower than those in control group on third day after operation.

received aprotinin. With the administration of aprotinin by intravenous injections preceding aortic clamping, by intracardiac perfusion after aortic clamping, or with the use of both methods Gabri-lescu and colleagues⁵⁴ detected normalized histo-chemical reactions of proteases together with the ultrastructural organization of the myocardial fibers in experiments with monkeys, mice, and rats.

The mechanism of the protective effect on the myocardium cannot yet be explained. One possibil-

ity is that inhibition of local or circulating kinins, or both types of kinins, may be involved inasmuch as activation and aprotinin-dependent inhibition of kinin systems in CPB have been documented.³³ Our results show markedly and significantly enhanced kallikrein inhibition in the aprotinin group (Fig. 1). Higher levels of kallikrein would lead to increased release of bradykinin from high-molecular-weight kininogen. Elevated bradykinin levels are possibly responsible for increased myocardial capillary per-

meability and the occurrence of interstitial edema. Additionally, the inhibition of membrane serine proteinases by aprotinin may intercept membrane permeabilization, thus impairing the proteolytic cascades triggered by anoxia. Bayley and Fawzi⁵⁵ showed that the application of aprotinin protects the myocardial fibers from the lytic action of proteases on the sarcolemma by preventing the splitting of sarcolemmal fixing sites of Ca^{2+} and the extracellular Ca^{2+} penetration into the cell. In rabbits with experimental myocardial ischemia an aprotinin-dependent limitation of proteolysis in the cytoplasmatic and mitochondrial-lysosomal fractions was found⁵⁵ together with a membrane stabilizing effect.⁵⁶

Whatever the reason for its efficacy we suggest that high-dose aprotinin therapy in patients undergoing CPB is of therapeutic benefit not only in reducing blood loss, but also in protecting the myocardium against ischemic injury.

This paper is dedicated to Prof. Dr. W. Heller on his sixty-fifth birthday.

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