Management of myocardial dysfunction after a period of ischemia/reperfusion (I/R) is an essential part of cardiac surgery practice. Evidence indicates that reperfusion itself contributes to myocardial damage, as well as ischemia.1,2 The pathophysiology of I/R injury is complex and involves the production and releasing of inflammatory mediators and oxygen free radicals, activation of the coagulation cascade, kallikrein system, fibrinolytic system and the complement chain. The inflammatory component can trigger both necrosis and apoptosis. In order for a molecular drug to effectively attenuate this response, it would need to have a broad spectrum of activity to inhibit the multiple pathways and limit their cross-amplification.

Aprotinin (Trasylol®; Bayer Corp, Leverkusen, Germany), a nonspecific serine protease is an important attenuator of this response because it inhibits several important serine proteases such as kallikrein, plasmin, elastase and thrombin.

Cardioprotective effects of aprotinin in acute myocardial infarction without reperfusion or short-term reperfusion have been reported3,4. In myocardial tissue models of I/R, aprotinin has been shown to reduce uptake of tumor necrosis factor (TNF)-α, generation of nitric oxide and accumulation of leucocytes.5 It inhibits a variety of adhesion molecules, and therefore transendothelial migration of neutrophils.6

Clinically, aprotinin improves contractility, helps prevent ischemic contracture, and preserves the stores of adenine nucleotide.7 Further data indicate that the addition of aprotinin into the priming solutions during cardiopulmonary bypass (CPB) can reduce lung reperfusion injury.

In this study, we aimed to determine the effects of aprotinin on myocardial tissue damage that occurs during and/or after aortic cross-clamp application. We measured the levels of cardiac troponin-I (cTnI), creatine kinase (CK)-MB and lactate dehydrogenase (LDH) as markers of myocardial tissue damage occurring under the protection of cold-blood cardioplegia and systemic hypothermia in patients undergoing elective first-time coronary artery bypass grafting (CABG) procedure. Additionally we measured mixed venous oxygen saturation (SvO2) and cardiac index (CI) as hemodynamic outcomes.

Methods

After approval of Institutional Ethic Committee, 80 male patients with 3-vessel disease and good ventricular function undergoing elective first-time CABG surgery were...
Cardioprotective Effect of Aprotinin

Table 1 Patient Demographics

<table>
<thead>
<tr>
<th>Group</th>
<th>Aprotinin (n=40)</th>
<th>Control (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.5±7.5</td>
<td>60.8±6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.2±1.1</td>
<td>169.5±3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.9±12.6</td>
<td>79.8±4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>29 (73%)</td>
<td>31 (78%)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic hypertension (%)</td>
<td>19 (48%)</td>
<td>19 (48%)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>11 (28%)</td>
<td>14 (35%)</td>
<td>NS</td>
</tr>
<tr>
<td>β-blocker use (%)</td>
<td>26 (65%)</td>
<td>23 (58%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2 Perioperative Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Aprotinin (n=40)</th>
<th>Control (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-clamp time (min)</td>
<td>53.0±4.6</td>
<td>52.7±6.9</td>
<td>NS</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>72.2±13.8</td>
<td>75.0±7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Time to extubation (min)</td>
<td>285.0±17.4</td>
<td>285.5±27.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. of grafts</td>
<td>2.9±0.3</td>
<td>2.9±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Anesthesia time (min)</td>
<td>227.5±13.2</td>
<td>230.5±12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Postoperative bleeding (ml)</td>
<td>702.0±113.6</td>
<td>787.8±121.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Length of ICU stay (days)</td>
<td>1.1±0.4</td>
<td>1.3±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>5.4±0.9</td>
<td>5.5±0.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

CPB, cardiopulmonary bypass; ICU, intensive care unit.

Windows, version 10.0, Chicago, IL, USA) was used for all statistical analyses. Data are expressed as mean±standard deviation for continuous variables. Mann-Whitney U-test was used for the variables not distributed normally, and Student’s t-test was used for the variables distributed normally. Results are given with 95% confidence interval and a p-value of 0.05 or less was considered to indicate statistically significant differences.

Results

All patients had class III functional capacity with 3-vessel disease before elective surgery, and complete myocardial revascularization was achieved in all patients after CABG. The mean age of the patients was 59.5±7.5 years (range: 48–72 years) for group I and 60.8±6.6 years (range: 42–73 years) for group II. The patients’ demographic and perioperative data are summarized in Tables 1 and 2, respectively. No patient required inotropic or mechanical support during weaning from CPB or later in the ICU.

Myocardial protection during aortic cross-clamping was achieved in all patients by antegrade cold-blood cardioplegia and systemic hypothermia. There were no significant differences between the 2 groups in cross-clamping, CPB, anesthesia and mechanical ventilation times, length of ICU and hospital stays. The number of anastomosed grafts was also identical. Postoperative bleeding through the mediastinal and thoracic drains was lower in the aprotinin group (702.0±113.6 ml) than the in control group (787.8±121.3 ml) (p<0.05). Preoperative CK-MB levels were not different (20.4±5.5 U/L vs 20.3±4.3 U/L) in either group, but the level in the immediate postoperative period tended to be significantly lower in group I compared with group II (47.8±5.5 U/L vs 52.3±7.2 U/L; p<0.01). This difference between groups persisted for 6 h after operation (42.0±12.3 U/L in group I vs 46.9±8.6 U/L in group II; p<0.05) (Table 3). Postoperative CK-MB levels were significantly higher than preoperative levels in both groups (p<0.01), suggesting myocardial injury occurred during aortic cross-clamping.

cTnI levels measured before and immediately after surgery were similar in both groups. However, a significant difference was found between the study groups in the cTnI levels measured at the 6th (2.87±0.47 ng/ml in group I vs 3.11±0.46 ng/ml in group II; p<0.05), 12th (2.10±0.51 ng/ml in group I vs 2.39±0.53 ng/ml in group II; p<0.05) and 24th h after surgery (1.38±0.39 ng/ml in group I vs 1.60±0.36 ng/ml in group II; p<0.05) (Table 4). The release of cTnI was significantly lower in patients receiving aprotinin; however, this improvement in cTnI release disappeared by

Included in this double-blind, randomized, prospective study. The patients with ejection fraction <40% (according to echocardiography) were not included. Informed written and oral consent was given by all patients. Patients were randomized to receive aprotinin (Group I; n=40) or saline (Group II; n=40).

Anesthesia was standard for all patients: induction with fentanyl 15 μg/kg and propofol 2 mg/kg. Endotracheal intubation was facilitated by pancuronium 0.1 mg/kg. Anesthesia was maintained with sevoflurane 1–2% in 3 L/min of oxygen and fentanyl infusion. A pulmonary artery thermodilution catheter was placed in all patients. Hemodynamic measurements and mixed venous blood gas analysis were performed before surgery began, immediately after arrival in the intensive care unit (ICU) and at postoperative 6th, 12th and 24th h. Before cannulation, heparin (3.5 mg/kg) was given. After weaning from CPB, heparin was antagonized with protamine hydrochloride in the ratio of 1:1.3. All patients were given nitroglycerin (0.5 μg·kg⁻¹·min⁻¹) during the rewarming period. Surgical and bypass procedures were also standard.

Cardiac arrest was achieved using hyperkalemic cold (4°C) blood cardioplegia 10 ml/kg as the initial dose (1 L blood, 20 mmol/L K⁺, 16 mmol/L HCO⁻₃, 7.364 mg/L citrate, 16 mmol/L Mg²⁺ and 1 g/L glucose; pH = 7.54). Every 20 min during cross-clamping, the cardioplegic solution was repeated (5 ml/kg). Patients were cooled to a rectal temperature of 28°C. All patients underwent complete revascularization.

The full Hammersmith doses of aprotinin (2×10⁶ KIU pre-CPB, 2×10⁶ KIU at pump prime, 500,000 KIU/h during CPB) were given to the Group I patients during surgery. The patients in Group II received saline solution only at the same time points.

cTnI analyses were performed using an Access Immunoassay System Auto-analyzer (Beckman Coulter Corporation, Fullerton, CA, USA) and the cTnI levels were measured before surgery, immediately after surgery, at postoperative 6th, 12th and 24th h and on postoperative 5th day. cTnI levels were determined twice at each time point and the mean value was calculated and accepted as the cTnI value at that time. The CK fraction MB was measured by UV-spectrophotometric method (Sigma Chemical Corporation, St Louis, MO, USA) at the same time points except for postoperative 5th day. LDH measurements were performed before surgery, immediately after surgery and on the first postoperative day. Moreover, 12-lead ECG recordings were done before operation, and daily until hospital discharge.

SPSS (Statistical Package for Social Sciences for
the 5th postoperative day. The CI and S-O2 were higher in the aprotinin group at the 6th (2.73±0.28 vs 2.60±0.26 and 54.42±4.55 vs 51.75±4.75 (p<0.05)) and 12th h after operation (2.82±0.29 vs 2.69±0.25 (p<0.05) and 56.27±5.63 vs 52.65±6.09 (p<0.01)) (Table 5). There was no difference between the groups in the preoperative LDH levels; however immediately after operation and on the first postoperative day, LDH levels were lower in the aprotinin group (Table 5). Additionally, no ECG changes indicative of ischemia or development of pathologic Q waves suggesting newly developed ischemia or infarction were seen during the postoperative hospital stay. We did not observe any neurological or renal complications that would affect cTnI levels.

**Discussion**

In this study, the cardioprotective effect of aprotinin in myocardial I/R injury was tested in a controlled in vivo model. Primary outcomes were the release of cTnI and CK-MB, and secondary outcomes were LDH levels, CI and S-O2. Our results demonstrate that aprotinin does exert cardioprotective effects in patients undergoing first-time elective CABG.

Aprotinin has been in clinical use for over 30 years and generally regarded to be an effective hemostatic agent. In fact, it has been suggested that this agent is simultaneously hemostatic and antithrombotic. The activation of coagulation and inflammation is closely linked through a network of both humoral and cellular components. Aprotinin’s anti-inflammatory effects have been investigated by several in vitro and in vivo studies, recently anti-inflammatory effects on neutrophils, as well as on endothelial cells, were reported.

I/R injury is associated with an acute inflammatory response, which is mediated by cytokines, chemokines, and adhesion molecules that recruit neutrophils, monocytes, and other inflammatory cells and leads to damage of the ischemic myocardium. These are also the target areas of aprotinin for its anti-inflammatory effect.

In previous reports, aprotinin was found to improve myocardial ATP content and capacity for protein synthesis during cold storage with crystalloid cardioplegic solution. Bull et al placed adult rat hearts into 6 different solutions to determine whether or not the addition of aprotinin to a cardioplegic solution effects the ATP content and protein synthesis. They found that continuous exposure of the myocardium to aprotinin immediately after cardiectomy prevented rapid decline in ATP stores, which was seen both in the preparation and slicing in cardioplegic solution alone. Protein synthesis was also found to be higher in the slices stored in cardioplegic solution with aprotinin than in those stored in cardioplegic solution alone. This improvement in myocardial biochemical function correlated with suppression of the myocardial release, uptake, and activity of TNF-α when aprotinin was present. Locally produced TNF-α is known to contribute to postischemic myocardial dysfunction via depression of contractility and induction of myocyte necrosis, which is clinically expressed as an increase in intracellular markers. Thus, our findings of lower levels of myocardial-specific markers in patients receiving aprotinin are the clinical expression of what Bull et al found at the cellular level.

The generation of oxygen free radicals by inflammatory cells has been established as a fundamental process in the development of reperfusion injury in the myocardium and skeletal muscle. It was shown in a rabbit skeletal muscle model that carnitine and ascorbic acid improve reperfusion injury, and that aprotinin has a similar protective effect on skeletal muscle by a different mechanism. Formigili et al have measured the highest levels of TNF-α within the myocardium after reperfusion and demonstrated that the number of inflammatory cells within the myocardium increases 2.5-fold during ischemia, when compared with normal myocardium, and an additional 3-fold during reperfusion. These cells are the source of additional TNF-α and can
damage myocardium directly or through the induction of apoptosis.\textsuperscript{17} Aprotinin may play a role in myocardial protection by inhibiting this leucocyte-mediated apoptosis and preventing the capacity of leucocytes to transmigrate through the vascular endothelium.\textsuperscript{18–20} In a recent study at our center, it was shown that high doses of aprotinin can improve the oxidative burden after CPB by decreasing lipid hydroperoxides.\textsuperscript{21}

In rodent models of I/R, it has been shown that aprotinin, when administered 5 min before reperfusion (10,000–20,000 KIU/kg), reduces leucocyte infiltration into the myocardium and myocardial injury as measured by CK release.\textsuperscript{22} A similar study was done by Hoffmeister et al.\textsuperscript{23} in isolated Langendorff preparations in which they showed that aprotinin improves the recovery of myocardial contractility and decreases the release of troponin T (TnT), relative to saline.

Asimakopoulos et al.\textsuperscript{24} have shown that full-dose aprotinin (60,000 KIU/kg) decreases the expression of leucocyte integrin CD11b/CD18 in patients undergoing primary elective CABG surgery. They also reported that aprotinin inhibits in vitro leucocyte transmigration in response to interleukin-8 in a dose-dependent manner. However, it is unclear whether or not endothelial cell activation was related to myocardial I/R injury alone or related to the systemic inflammatory response secondary to CPB. It was also revealed that aprotinin inhibits intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression on TNF-\alpha activated endothelial cells, indicating that endothelial cells can be specifically targeted by aprotinin.\textsuperscript{14} In fact, it was shown in in-vitro models of ischemia that aprotinin preserves coronary endothelial adherens junctions and reduces myocardial tissue edema.\textsuperscript{15}

In one of the most promising reports, Wendel et al.\textsuperscript{4} reported that full-dose aprotinin caused a significant reduction in serum TnT and CK-MB levels at the 1\textsuperscript{st} and 3\textsuperscript{rd} post-operative days in patients undergoing CABG surgery. In that study, they used crystalloid cardioplegia and systemic hypothermia for myocardial protection. We used blood cardioplegia in both groups. It is known that blood cardioplegia has some advantageous effects on myocardial metabolism because of some of its components.\textsuperscript{26} If we consider that all patients were protected with blood cardioplegia, the lower leakage of cardiac enzymes in the aprotinin group might reveal an important message for us.

Supporting our findings of reduced release of myocardial specific markers, Sunamori et al.\textsuperscript{27} reported no elevation of CK-MB and aspartate aminotransferase levels following cardioplegia in patients receiving aprotinin. Aprotinin is also reported to be able to decrease the use of inotropes, vasopressors and antiarrhythmic agents after CABG.\textsuperscript{28}

The release of cTnI is aid to be monophasic, without an earlier peak. However, there is an early rise in cTnI, although not of the same magnitude as cardiac TnT (cTnT).\textsuperscript{29} The elevation of the duration is typically 5 days, but there might be some variation depending on infarct size. With the use of realistic cut-offs and improvement in assay sensitivity, it has been shown that the elevation of both cTnI and cTnL occurs in patients with end-stage renal disease.\textsuperscript{30} The proportion of patients with elevated cTnI is consistently greater (18–75\%) than the proportion of those with elevated cTnL (4–17\%) and the probability of elevation is greater in acute renal failure.\textsuperscript{31} Therefore, cTnL should be preferred in the determination of myocardial damage during CPB, considering the potential negative effects of aprotinin on renal system.

In our study, we observed less myocardial enzyme release and higher CI and S\textsubscript{O2} levels in the aprotinin group, although we did not see any major clinical reflection of these findings. We believe that this is because they all had fairly good ventricular function, as we intentionally excluded the patients with ejection fraction <40\%. Another factor may be the reasonable aortic cross-clamping and CPB times, so that the ischemic period was within acceptable limits. These 2 factors may have masked the clinical outcomes. The limited number of patients is also another factor. But we believe observing less enzyme leakage in even uncomplicated cases is an important message about the protective potential of aprotinin. The clinical benefits of aprotinin treatment may be achieved by choosing patients at greatest risk of exaggerated I/R injury, such as those undergoing combined procedures.

Our study design and data do not allow us to speculate if overall surgical costs and surgical admissions decrease when using a costly agent such as aprotinin for on-pump cases. However, we believe more benefit might be seen with more complicated cases and the cost/benefit ratio might be more reasonable with more risky cases.

\section*{Conclusions}

Our current findings indicate that aprotinin has some other protective effect on the myocardium than that achieved with baseline protection with blood cardioplegia and systemic hypothermia. As discussed, aprotinin interferes with more than one area of metabolism (improving oxidative burden, decreasing inflammatory reactions etc) to create its protective effects. Future studies will hopefully establish more rational and scientific approaches to the use of aprotinin in clinical practice.

\section*{References}

3. Lefer AM, Spath JA. Preservation of myocardial integrity by a protease inhibitor during acute myocardial ischemia. \textit{Arch Int Pharmacodyn Ther} 1974; 211: 225–236.


