Aprotinin Reduces the Procalcitonin Rise Associated With Complex Cardiac Surgery and Cardiopulmonary Bypass

P. MARUNA1, A. A. KLEIN2, J. KUNSTÝŘ3, K. M. PLOCOVÁ4, F. MLEJNSKÝ4, J. LINDNER4

1Institute of Pathological Physiology, First Faculty of Medicine, Charles University in Prague, 2Department of Anesthesia, Papworth Hospital, Cambridge, United Kingdom, 3Department of Anesthesiology and Intensive Care, General Teaching Hospital and the First Faculty of Medicine, Charles University in Prague, 4Second Department of Cardiovascular Surgery, General Teaching Hospital and the First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Received April 14, 2012
Accepted August 10, 2012
On-line November 22, 2012

Summary
Aprotinin, a nonspecific serine protease inhibitor, has been primarily used as a haemostatic drug in cardiac surgery with cardiopulmonary bypass (CPB). This study investigated the effect of Aprotinin on the post-operative levels of procalcitonin (PCT) and a set of cytokines in patients undergoing pulmonary artery endarterectomy (PEA). We analyzed 60 patients with chronic thromboembolic pulmonary hypertension undergoing PEA. 30 patients (Group A) were treated with Aprotinin (2000000 IU prior anesthesia, then 200000 IU in CPB prime and 50000 IU per hour continuously); a further 30 patients (Group B) received Tranexamic Acid (1 g before anesthesia, 1 g after full heparin dose and 2 g in CPB prime). PCT, TNFα, IL-1β, IL-6, and IL-8 arterial concentrations were measured from before until 72 hours after surgery. Aprotinin significantly affected early post-PEA plasma PCT. Patients treated with Aprotinin (Group A) had lower peak PCT levels compared to patients in Group B (1.52 ng/ml versus 2.18, p=0.024). Postoperative peak values of PCT and IL-6 correlated closely in both groups (r=0.78, r=0.83 respectively). Aprotinin attenuates the post-PEA increase of PCT in the same manner as other pro-inflammatory cytokines. Significant correlation between PCT and IL-6 post-surgery may be indicative of an indirect IL-6-mediated pathway of PCT alteration.

Key words
Aprotinin • Cardiopulmonary bypass • Procalcitonin • Pulmonary endarterectomy

Introduction
Aprotinin, a nonspecific serine protease inhibitor, has been used primarily as a haemostatic drug in cardiac surgery with cardiopulmonary bypass (CPB). In addition, Aprotinin may exert multiple biologically relevant effects attenuating the inflammatory response. Several studies have shown that high-dose Aprotinin can suppress the release of pro-inflammatory cytokines, particularly tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and IL-8 both in vitro and in vivo (Day et al. 2006, Türköz et al. 2001). Aprotinin acts on both pro-inflammatory and anti-inflammatory cytokines induced by ischemia/reperfusion injury, which is associated with CPB (Buerke et al. 2007), but the exact mechanisms of Aprotinin action require further clarification.

Procalcitonin (PCT) is a highly specific marker for the diagnosis of clinically relevant bacterial infection and sepsis, and is part of the pro-inflammatory network. Uncomplicated cardiac surgery induces a postoperative increase in serum PCT levels. The level of PCT seems to be dependent on the surgical procedure, with more invasive procedures associated with higher PCT levels.
This study was planned to investigate the effect of Aprotinin on post-operative PCT in patients undergoing major cardiac surgery, in the form of pulmonary artery endarterectomy (PEA), and compare this with the effect on other cytokines about which more is known.

Material and Methods

The clinical study was approved by the Research Ethics Committee of the First Faculty of Medicine, Charles University and General Teaching Hospital in Prague, in its session on April 27, 2006. Written informed consent was obtained from all subjects. Patients with chronic thromboembolic pulmonary hypertension scheduled for isolated pulmonary endarterectomy surgery (PEA) between January 2007 and June 2011 were eligible for inclusion into the study. Exclusion criteria were PEA combined with another procedure, severe postoperative bleeding requiring resternotomy, and local and systemic infection with SIRS score ≥2, defined according to Society of Critical Care Medicine Consensus Conference and guidelines of the Center for Disease Control and Prevention (Horan and Gaynes 2004).

Aprotinin was used routinely in all patients until January 2008. These patients were enrolled into the study as Group A. Dosage was 2000000 IU prior to induction of anesthesia, then 2000000 IU in CPB prime, and 50000 IU per hour continuously intravenously throughout the whole procedure. According to the recommendations of the European Medicines Agency published in November 2007 following a report by Mangano et al. (2007), Aprotinin use was discontinued and it was withdrawn from hospital formularies. From January 2008, patients (Group B) undergoing PEA surgery received Tranexamic Acid (TEA); 1 g before skin incision, 1 g after heparin dose and 2 g in CPB prime.

Surgical procedure

All patients underwent PEA via median sternotomy. CPB was established after cannulation of the ascending aorta and the inferior and superior vena cava. Deep hypothermic circulatory arrest (DHCA, 18-20 °C) was used (limited to 20 minute episodes), to ensure optimum operating conditions and facilitate accurate endarterectomy. Weaning from CPB was started with pressure control ventilation with positive end-expiratory pressure, atrio-ventricular epicardial stimulation, stepwise increased filling of the right heart and reduction of pump flow together with low doses of norepinephrine targeted to reach mean pulmonary artery pressure (MPAP) less than 20 mmHg and mean artery pressure over 70 mmHg. Dobutamine (Dobutrex, Lily, Germany) was administered only if inotropic support was needed during or after weaning of CPB.

Monitoring

Peripheral vein and radial artery cannulae were inserted before induction of general anesthesia. Femoral artery cannula, triple lumen central venous cannula, Swan-Ganz catheter, and single lumen jugular bulb catheter were also inserted for continuous monitoring of hemodynamic parameters and jugular bulb blood saturation. Left atrial catheter was surgically placed for both left atrium filling pressure measurement and norepinephrine administration.

Anesthetic management

All patients were premedicated with 0.1 mg·kg\(^{-1}\) of diazepam (Diazepam, Zentiva, SR) orally. After the transfer to the operating room the patients were given total intravenous anesthesia standard for this procedure in our institution. This consists of sufentanil (Sufenta, Janssen, Belgium) 0.5 µg·kg\(^{-1}\) + midazolam (Dormicum, Roche, CR) 3-5 mg + propofol (Diprivan, AstraZeneca, UK) 1 mg·kg\(^{-1}\) + rocuronium (Esmeron, Schering-Plough, France) 0.6 mg·kg\(^{-1}\) intravenously. Anesthesia was maintained with a continuous infusion of propofol and sufentanil with BIS targeted at 40 to 50. Further incremental doses of rocuronium 5-10 mg were administered if interference with the ventilator was noted.

PCT and cytokine analysis

Arterial blood samples were drawn from the femoral artery catheter before incision, after sternotomy, after DHCA, after separation from CPB, 12, 18, 24, 36, 48 and 72 hours following surgery. For all measurements, 5 ml of arterial blood was drawn into a vacutainer heparin tube and immediately centrifuged at 5000 rpm for 15 min. Plasma was stored at −80 °C until analysis. All plasma samples were analyzed retrospectively after the completion of the study in June 2011. Plasma levels of PCT were detected by Kryptor test (BRAHMS AG, Hennigsdorf, Germany) in duplicates. The sensitivity of the analytic method was 0.02 ng/ml. Plasma concentrations of TNFa, IL-1β, IL-6, IL-8 (ELISA, Immunotech, Paris, France) were measured in duplicates. The intra- and inter-assay
coefficients of variation were below 5%.

Statistical analysis

Statistical analysis was carried out using SPSS Statistics 18.0 for Windows (SPSS Inc., Chicago, USA). The power calculation was undertaken using Sample Power 2.0. The sample size calculation was based on a two-sided two-sample testing, a significance level of 0.05 and a power of 90%. The targeted sample size of 30 patients per group was specified. The normal distribution of all data was examined using the Kolmogorov-Smirnov normality test to determine subsequent use of tests for statistical comparison. As variables were not normally distributed, the data were reported as medians and interquartile range. Mann-Whitney test was applied for the data comparison between groups. Bonferroni correction was used to analyze simultaneous measurement at different time points.

Table 1. Pre-operative and intra-operative data (n=60).

| Parameter                           | Group A (n=30) | Group B (n=30) | p value |
|-------------------------------------|----------------|----------------|---------|
| Number of males (%)                 | 19 (63 %)      | 20 (67 %)      | 0.331   |
| Age (years)                         | 59.2 (13.6)    | 62.1 (7.0)     | 0.291   |
| NYHA classification                 | 3.13 (0.47)    | 3.09 (0.51)    | 0.457   |
| Pre-operative data                  |                |                |         |
| Mean pulmonary artery pressure (mm Hg) | 54.1 (9.6)    | 54.5 (9.9)     | 0.484   |
| Cardiac index (L.min⁻¹.m⁻²)         | 2.03 (0.33)    | 2.10 (0.39)    | 0.196   |
| Ejection fraction (%)               | 61.3 (9.2)     | 60.8 (6.9)     | 0.285   |
| Pulmonary vascular resistance (dynes.s.cm⁻⁵) | 878.3 (284.5) | 866.2 (292.8) | 0.217   |
| Intra-operative data                |                |                |         |
| DHCA time (min)                     | 36.8 (12.1)    | 35.5 (10.6)    | 0.284   |
| Cross clamp time (min)              | 121.8 (20.1)   | 116.9 (18.6)   | 0.171   |
| Minimum temperature (°C)            | 16.6 (0.7)     | 16.8 (0.6)     | 0.310   |

Variables are absolute number or mean (standard deviation). Abbreviations: DHCA – Deep hypothermic circulatory arrest; NYHA – New York Heart Association

Results

A total of 60 patients were enrolled. The patient demographic and surgical data are outlined in Table 1. All patients underwent satisfactory clearance of intra-arterial obstruction, and there were no intra-operative deaths. No patients required allogeneic blood transfusion. 30 patients (Group A) were treated with Aprotinin while 30 patients (Group B) received TEA in the perioperative period. In-hospital mortality was 1/30 in Group A (aspiration pneumonia, postoperative day 9) and 1/30 patient in Group B.

No significant differences between the groups were found for cross clamp time, DHCA time, catecholamine use perioperatively, and need for continuous renal replacement therapy post-surgery. There were no thromboembolic complications in either group. These were defined as a deep venous thrombosis diagnosed with venous Doppler or pulmonary embolism diagnosed either with computerized tomography (CT) or ventilation/perfusion scan. Tested groups did not differ in the baseline PCT, TNFα, IL-1β, IL-6 and IL-8. Mann-Whitney test was used to assess the impact of possible subsequent cytokine degradation in frozen samples. The test did not revealed significant relation between plasma levels of cytokines both preoperatively and postoperatively and the time between blood sample's collection and its analysis.

An initial decrease of TNFα, IL-1β, IL-6 and IL-8 was found in both groups in blood samples collected after the last DHCA. PCT increased postoperatively reaching a peak level 18 hours after the end of surgery in both groups (Fig. 1) with subsequent decline. Peak PCT was significantly lower in the Aprotinin group: 1.52 ng·ml⁻¹ (1.26-1.84) vs. 2.18 ng·ml⁻¹ (1.90-2.62) in Group B (p=0.024).
Fig. 1. The influence of Aprotinin on PCT level in perioperative period (medians and interquartile range). Box and whisker plot depicting the median, interquartile range and full range. Group A (Aprotinin) – white boxes, Group B (Tranexamic Acid) – grey boxes. * Statistically significant differences between groups, p<0.05

As expected, all inflammatory cytokines increased after surgery. Maximum TNFα, IL-1β, IL-6 and IL-8 levels were detected 12 h after the end of surgery in both groups. There were significantly lower peak concentrations of TNFα, IL-6, and IL-8 in Group A (Table 2).

Postoperative peak values of PCT and IL-6 correlated closely in both groups: r=0.78, p=0.008 for Group A, r=0.83, p=0.006 for Group B. Weaker correlation was found between TNFα and PCT peak values in both groups: r=0.64, p=0.036 for Group A; r=0.69, p=0.027 for Group B.

Discussion

We have found a significant alteration of plasma PCT levels in patients treated with Aprotinin undergoing major cardiac surgery with CPB compared to TEA. Uncomplicated surgical course was associated with increased PCT concentrations reaching a maximum 18 h post-surgery. PEA was chosen for this study as the surgical procedure is relatively invasive, requiring prolonged CPB and periods of DHCA, and induces systemic inflammatory response syndrome (SIRS) and hemodynamic instability (Thistlethwaite et al. 2010). 19 years after the discovery of PCT as an inflammatory marker, this is the first report of the influence of serine protease inhibitor on plasma PCT concentrations.

Multiple studies have demonstrated that cardiac surgery with CPB significantly increases proinflammatory and anti-inflammatory cytokines, and high-dose Aprotinin significantly reduces this increase. However, the actual effect of Aprotinin is still controversial. Greilich et al. (2001) reported lower levels of IL-10 associated with Aprotinin administration in patients undergoing cardiac surgery with CPB, other authors demonstrated that Aprotinin increased levels of IL-10 (Lei et al. 2003, Hill et al. 1998). Similarly, IL-1-receptor antagonist concentration was higher in patients using high-dose Aprotinin compared to patients without
Aprotinin (Tassani et al. 2000). Boeken et al. (1998) looked at the relationship between Aprotinin and PCT in patients undergoing cardiac surgery with CPB. However they did not find a significant elevation of PCT in patients after coronary artery bypass graft surgery, whether treated by Aprotinin or not. Their conclusion was that there was no influence on the levels of PCT in sepsis-free cardiosurgical patients was subsequently disputed by other studies (for review see Sponholz et al. 2006).

Table 2. Arterial blood concentrations of PCT and cytokines (medians and interquartile range).

| Parameter | Group | Before surgery | After separation from CPB |
|-----------|-------|----------------|---------------------------|
|           |       |                | 12 h | 18 h           |
| PCT (ng/ml) | A     | 0.18 (0.13-0.23) | 0.18 (0.11-0.24) | 1.52 (1.26-1.84) |
|           | B     | 0.16 (0.11-0.21) | 0.30 (0.19-0.44) | 2.18 (1.90-2.62) * |
| TNFα (ng/l) | A     | 16.4 (11.0-45.4) | 180.4 (147.2-241.4) | 114.7 (64.1-168.6) |
|           | B     | 18.6 (10.7-42.1) | 262.1 (217.2-342.1) | 162.0 (96.4-256.8) |
| IL-1β (ng/l) | A     | 224.4 (212.1-256.2) | 302.3 (249.4-348.8) | 266.9 (227.7-309.1) |
|           | B     | 236.2 (210.6-264.0) | 316.1 (258.9-366.2) | 272.4 (220.3-311.5) |
| IL-6 (ng/l) | A     | 22.6 (17.4-34.5) | 394.8 (311.4-487.2) | 374.1 (306.5-441.8) |
|           | B     | 24.8 (19.6-36.6) | 546.0 (476.3-652.6) * | 522.9 (442.0-613.7) * |
| IL-8 (ng/l) | A     | 82.8 (46.7-121.2) | 365.2 (324.6-426.8) | 334.3 (316.8-425.1) |
|           | B     | 79.0 (47.1-117.6) | 459.7 (395.0-562.4) * | 426.5 (367.2-537.4) * |

Abbreviations: CPB – Cardio-pulmonary bypass; IL – Interleukin; PCT – Procalcitonin; TNFα – Tumor necrosis factor-α. * Statistically significant differences between groups on p<0.05

The anti-inflammatory properties of Aprotinin have been recognized for more than 10 years, although its molecular mechanism is still unclear. Aprotinin is a broad-spectrum serine protease inhibitor. Serine proteases play a central role in the amplification of the inflammatory response through numerous pathways, including contact activation, coagulation, cytokine release, and complement cascades, all of which can be modulated by Aprotinin (Day et al. 2006, Tassani et al. 2000). Besides a direct effect on synthesis, Aprotinin may modulate PCT release indirectly via attenuation of the inflammatory cytokines TNFα and IL-6, which are known to mediate PCT release both in vitro and in vivo. Significant correlation between PCT and IL-6 concentrations post-surgery, demonstrated in our study, suggest this indirect pathway.

Recently, McEvoy et al. (2009) used murine myocardial ischemia-reperfusion model to demonstrate diverse effects of Aprotinin on various pro-inflammatory cytokines including IL-6. In the study, higher Aprotinin doses attenuated TNFα release, while IL-6 was unaffected. Authors suggest that Aprotinin may selectively affect cytokine release in the context of myocardial ischemia-reperfusion. To the contrary, our study showed significant influence of Aprotinin to IL-6 dynamics after cardiac surgery. McEvoy allow that the murine ischemia-reperfusion model does not necessarily recapitulate the situation occurring in the context of
cardiac surgery. Among other, the volume of distribution, pharmacokinetics and serine protease inhibitory profiles are likely to be different in the murine system than that of man.

In our study, the patients treated with TEA served as a control group to Aprotinin-treated patients. TEA is a synthetic derivative of the amino acid lysine with antifibrinolytic effects. It competitively inhibits the activation of plasminogen to plasmin by binding to specific sites of both compounds. Among other indications, TEA is used to prevent excessive blood loss in cardiac surgery. Recent studies comparing the effect TEA and Aprotinin in cardiosurgical patients revealed the different cytokine-proteolytic profile between both antifibrinolytics. It was shown that TEA has no significant effect of cytokine activation (Hsia et al. 2010) or cytokine-mediated inflammatory activities (Graham et al. 2012).

We conclude that Aprotinin attenuates the postsurgical increase of PCT following major cardiac surgery with CPB and DHCA, in a similar manner to other proinflammatory cytokines. These results also support previous findings that a rise of TNFα, IL-6 and IL-8 post-surgery is significantly altered by the use of Aprotinin.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Laboratory analysis was supported with a grant IGA MZ NT11210-4/2010 of the Ministry of Health, Czech Republic.

Abbreviations

| Acronym | Definition |
|---------|------------|
| CPB     | Cardio-pulmonary bypass |
| CTEPH   | Chronic thromboembolic pulmonary hypertension |
| DHCA    | Deep hypothermic circulatory arrest |
| ECC     | Extracorporeal circulation |
| IL      | Interleukin |
| MPAP    | Mean pulmonary artery pressure |
| PCT     | Procalcitonin |
| PEA     | Pulmonary endarterectomy |
| SIRS    | Systemic inflammatory response syndrome |
| TEA     | Tranexamic acid |
| TNFα    | Tumor necrosis factor-α |

References

BOEKEN U, FEINDT P, PETZOLD T, KLEIN M, MICEK M, SEYFERT UT, MOHAN E, SCHULTE HD, GAMS E: Diagnostic value of procalcitonin: the influence of cardiopulmonary bypass, Aprotinin, SIRS, and sepsis. *Thorac Cardiovasc Surg* **46**: 348-351, 1998.

BUERKE M, PRUEFER D, SANKAT D, CARTER JM, BUEKER U, RUSS M, SCHLITT A, FRIEDRICH I, BÜRGERMANN J, VAHL CF, WERDAN K: Effects of Aprotinin on gene expression and protein synthesis after ischemia and reperfusion in rats. *Circulation* **116**: I121-I126, 2007.

DAY JR, TAYLOR KM, LIDINGTON EA, MASON JC, HASKARD DO, RANDI AM, LANDIS RC: Aprotinin inhibits proinflammatory activation of endothelial cells by thrombin through the protease-activated receptor 1. *J Thorac Cardiovasc Surg* **131**: 21-27, 2006.

FRANKE A, LANTE W, KUPSER S, BECKER HP, WEINHOLD C, MARKEWITZ A: Procalcitonin levels after different types of conventional thoracic surgery. *Thorac Cardiovasc Surg* **56**: 46-50, 2008.

GRAHAM EM, ATZ AM, GILLIS J, DESANTIS SM, HANEY AL, DEARDORFF RL, UBER WE, REEVES ST, McGOWAN FX Jr, BRADLEY SM, SPINALE FG: Differential effects of aprotinin and tranexamic acid on outcomes and cytokine profiles in neonates undergoing cardiac surgery. *J Thorac Cardiovasc Surg* **143**: 1069-1076, 2012.

GREILICH PE, OKADA K, LATHAM P, KUMAR RR, JESSEN ME: Aprotinin but not epsilon-aminocaproic acid decreases interleukin-10 after cardiac surgery with extracorporeal circulation: randomized, double-blind, placebo-controlled study in patients receiving Aprotinin and epsilon-aminocaproic acid. *Circulation* **104**: 1265-1269, 2001.

HILL GE, DIEGO RP, STAMMERS AH, HUFFMAN SM, POHORECKI R: Aprotinin enhances the endogenous release of interleukin-10 after cardiac operations. *Ann Thorac Surg* **65**: 66-69, 1998.
HORAN TC, GAYNES RP: Surveillance of nosocomial infections. Appendix A. CDC definitions of nosocomial infections. In: Hospital Epidemiology and Infection Control. MAYAHALL CG (ed), Lippincott Williams and Wilkins, Philadelphia, 2004, pp 1659-1702.

HSIA TY, McQUINN TC, MUKHERJEE R, DEARDORFF RL, SQUIRES JE, STROUD RE, CRAWFORD FA, BRADLEY SM, REEVES ST, SPINALE FG: Effects of aprotinin or tranexamic acid on proteolytic/cytokine profiles in infants after cardiac surgery. Ann Thorac Surg 89: 1843-1852, 2010.

LEI Y, HAIDER HKH, CHUSNSHENG W, ZHIQIANG C, HAO C, KEJIAN H, QIANG Z: Dose-dependent effect of Aprotinin on aggravated pro-inflammatory cytokines in patients with pulmonary hypertension following cardiopulmonary bypass. Cardiovasc Drugs Ther 17: 343-348, 2003.

MANGANO DT, MIAO Y, VUYLSTEKE A, TUDOR IC, JUNEJA R, FILIPESCU D, HOEFT A, FONTES ML, HILLEL Z, OTT E, TITOV T, DIETZEL C, LEVIN J; INVESTIGATORS OF THE MULTICENTER STUDY OF PERIOPERATIVE ISCHEMIA RESEARCH GROUP; ISCHEMIA RESEARCH AND EDUCATION FOUNDATION: Mortality associated with Aprotinin during 5 years following coronary artery bypass graft surgery. JAMA 297: 471-479, 2007.

MARUNA P, LINDNER J, KUBZOVÁ K, KUNSTÝŘ J: Quantitative analysis of procalcitonin and cytokines after pulmonary endarterectomy. Prague Med Rep 109: 149-158, 2008.

McEVOY MD, SABBAGH MJ, TAYLOR AG, ZAVADZKAS JA, KOVAL CN, STROUD RE, FORD RL, McLEAN JE, REEVES ST, MUKHERJEE R, SPINALE FG: Aprotinin modifies left ventricular contractility and cytokine release after ischemia-reperfusion in a dose-dependent manner in a murine model. Anesth Analg 108: 399-406, 2009.

SPONHOLZ C, SAKR Y, REINHART K, BRUNKHORS F: Diagnostic value and prognostic implications of serum procalcitonin after cardiac surgery: a systematic review of literature. Critical Care 10: R145, 2006.

TASSANI P, AUGUSTIN N, BARANKAY A, BRAUN SL, ZACCARIA F, RICHTER JA: High-dose Aprotinin modulates the balance between proinflammatory and anti-inflammatory responses during coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth 14: 682-686, 2000.

THISTLETHWAITE PA, KANEKO K, MADANI MM, JAMIESON SW: Technique and outcomes of pulmonary endarterectomy surgery. Ann Thorac Cardiovasc Surg 14: 274-282, 2008.

TÜRKÖZ A, CİGLİ A, BUT K, SEZGİN N, TÜRKİÖZ R, GÜLCAN O, ERSOY MO: The effects of Aprotinin and steroids on generation of cytokines during coronary artery surgery. J Cardiothorac Vasc Anesth 15: 603-610, 2001.