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

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Summary

Aprotinin, a nonspecific serine protease inhibitor, has been primarily used as a haemostatic drug in cardiac surgery with cardio-pulmonary 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 2000000 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

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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

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(Franke *et al.* 2008, Maruna *et al.* 2008).

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 $mg \cdot kg^{-1}$ 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 $\mu g \cdot kg^{-1}$ + midazolam (Dormicum, Roche, CR) 3-5 mg + propofol (Diprivan, AstraZeneca, UK) 1 $mg \cdot kg^{-1}$ + rocuronium (Esmeron, Schering-Plough, France) 0.6 $mg \cdot kg^{-1}$ 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 TNFα, 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 $ng{\cdot}ml^{-1}$ (1.26-1.84) vs. 2.18 $ng{\cdot}ml^{-1}$ (1.90-2.62) in Group B (p=0.024).

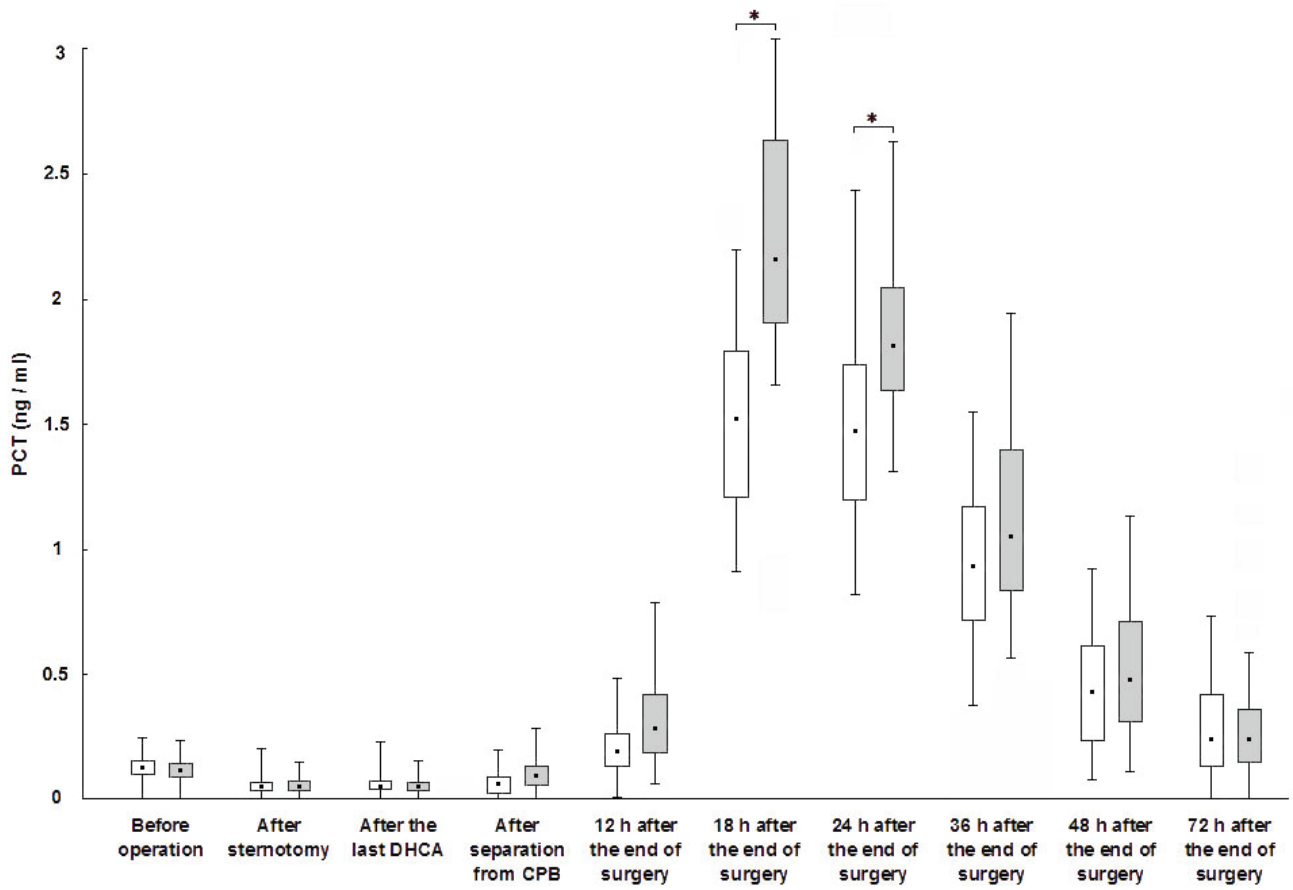

Fig. 1. The influence of Aprotinin on PCT level in perioperative period (medians and interquartile range). Box and whisker plot depicting the median, interquartile range and full range. Group A (Aprotinin) – white boxes, Group B (Tranexamic Acid) – grey boxes. * Statistically significant differences 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 (202.6-386.8) *	162.0 (92.4-209.2) *
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 post-surgical increase of PCT following major cardiac surgery with CPB and DHCA, in a similar manner to other pro-inflammatory 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

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Abbreviations

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

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