Aprotinin combined with nitric oxide and prostaglandin E1 protects the canine kidney from cardiopulmonary bypass-induced injury

Lei Du, Jing Zhou, Jiali Tang, Qi An, Ke Lin, Xiaodong Wu, Kaiyu Tao, Jin Liu

Abstract

Objective: Aprotinin is frequently used to reduce blood loss during cardiac surgery; however, it also causes renal injury. Since aprotinin reduces nitric oxide (NO) and prostaglandin I$_2$ (PGI$_2$), and both cause vasodilation and inhibit activation of neutrophils and platelets, their reduction may be responsible for the injury. This study was to determine whether the combination of aprotinin with NO and prostaglandin E$_1$ (PGE$_1$, an analogue of PGI$_2$) can attenuate renal injury associated with aprotinin during cardiopulmonary bypass (CPB). Methods: Thirty mongrel dogs were equally divided into five groups, with each group receiving CPB and aprotinin, NO, PGE$_1$, a combination of the three or no treatment (control). Serum creatinine and creatinine clearance were determined. To elucidate the mechanism, neutrophil, platelet and thrombin activations were also assessed. Results: After CPB, serum creatinine increased and creatinine clearance decreased in all dogs. These changes were similar among the NO, PGE$_1$, aprotinin and control groups, but were significantly smaller in the combination group. Similarly, myeloperoxidase activities in tissues, assessed these substances, particularly NO and prostaglandin I$_2$, (PGI$_2$), cause vasodilatation and inhibit neutrophil and platelet activation by stimulating intracellular cyclic guanosine monophosphate and cyclic adenosine monophosphate. Unfortunately, at

1. Introduction

Postoperative haemorrhage is a major clinical challenge of cardiovascular surgery, and its complications include high consumption of blood products, prolonged intensive care hospitalisation and increased mortality and morbidity [1]. Pharmacologic intervention is, therefore, required to minimise blood loss during cardiac surgery. Aprotinin, a non-specific serine proteinase inhibitor derived from bovine lung, was approved by the Food and Drug Administration (FDA) in 1993. It reduces blood loss in a dose-dependent manner. Cochrane reviews [2] and meta-analysis studies [3] reported that high doses of aprotinin significantly reduce blood transfusion, blood drainage and re-operation due to bleeding when compared with other anti-fibrinolytic agents, such as tranexamic acid or aminocaproic acid. Unfortunately, at these doses, aprotinin also increases the risk of renal failure, myocardial infarction, stroke and death from cardiac causes [4,5], especially in high-risk cardiac surgery [6]. Furthermore, in a multicentre image study [7], the occlusion rate of saphenous grafts was higher with aprotinin therapy (15%) than in controls (11%). These data suggest that aprotinin may increase organ dysfunction and promote thrombus formation. Aprotinin not only inhibits fibrinolysis but also reduces PGI$_2$ [8], bradykinin [9] and endothelial nitric oxide (NO) [10]. These substances, particularly NO and prostaglandin I$_2$ (PGI$_2$), cause vasodilatation and inhibit neutrophil and platelet activation by stimulating intracellular cyclic guanosine monophosphate and cyclic adenosine monophosphate. During cardiopulmonary bypass (CPB), aprotinin-related decreases in NO and PGI$_2$ may aggravate inflammation via neutrophil and platelet coagulation. The interaction between neutrophils and platelets may amplify inflammation and coagulation, resulting in organ injury [11] and may explain why aprotinin is associated with a higher risk of microvascular and macrovascular thrombosis. Because of its high affinity for the kidney [12], aprotinin may preferentially cause renal dysfunction. In the present study, we hypothe-

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doi:10.1016/j.ejcts.2010.01.025
sised that aprotinin combined with NO inhalation and prostaglandin E₁ (PGE₁, an analogue of PGI₂) injection will attenuate renal injury associated with aprotinin during CPB.

2. Materials and methods

2.1. Surgical procedure

After approval by the local animal Ethics Committee, 30 adult mongrel male dogs, weighing 20–25 kg, were anaesthetised with sodium pentobarbital (25 mg kg⁻¹, intraperitoneal (i.p.)). After intubation with an Fr.7.5 endotracheal tube, the dogs were volume control ventilated (tidal volume 10 ml kg⁻¹) (Datex-Ohmeda Excel 210, Soma Technology, Cheshire, Connecticut, USA). Anaesthesia was maintained by inhalation of 0.5–1% isoflurane and intravenous (i.v.) midazolam (0.05 μg kg⁻¹ min⁻¹) and fentanyl citrate (0.02 μg kg⁻¹ min⁻¹). Muscle relaxation was achieved with i.v. pipercuronium bromide at 0.1 mg kg⁻¹ and maintained at 0.02–0.04 mg kg⁻¹ h⁻¹. A urinary catheter was inserted for collecting urine. All animals received humane care in compliance with the European Convention on Animal Care.

The heart was exposed through a mid-sternal incision. After heparinisation (3 mg kg⁻¹), the ascending aorta and the right atrial appendage were cannulated. The CPB circuit was composed of a rolling pump (Stöckert II, Munich, Germany), bubble oxygenator (95 type; Xi-Jing Medical Ltd., Xi’an, China) and an arterial filter. The CPB was primed with Ringer’s lactate solution (750 ml), 6% voluven (750 ml), heparin (20 mg) and 5% sodium bicarbonate (50 ml). The activated coagulation time was maintained for more than 480 s throughout the perfusion. A double-lumen cardioplegic catheter was inserted into the ascending aorta for delivery of hyperkalaemic blood 10 min after onset of CPB. The aortic cross-clamp was released after the heart was arrested for 60 min. The dogs were weaned from CPB after 30 min of reperfusion and observed for 6 h. After the experiments, the dogs were sacrificed with a bolus of i.v. sodium pentobarbital (120 mg kg⁻¹).

2.2. Experimental protocol

After CPB set-up, the dogs were randomly divided into five groups, with six dogs in each group. In the aprotinin group, each dog received full Hammersmith dose of 8000 KIU/kg (kallikrein inhibitor units) of aprotinin (Livzon Pharmaceutical Co., Nanyang, China) was given to each dog. In the PGE₁ group, 20 ng kg⁻¹ min⁻¹ of PGE₁ (Pukang Pharmaceutical Co., Nanyang, China) was given to each dog via the oxygenator during CPB. The control group received no treatment during CPB. The anaesthesiologists, surgeons and laboratory staff were blinded throughout the study.

2.3. Renal function

Renal function was assessed by serum creatinine levels and creatinine clearance (Ccr). Urine outputs were measured during and after CPB. Blood and urine samples were collected for the analysis of creatinine on an automated analyser at 0, 3 and 6 h after CPB. Ccr was calculated by using the following equation:

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Ccr = \frac{\text{urine creatinine} \times \text{urine volume}}{\text{plasma creatinine}}
\]

2.4. Blood cell counts and biochemical indices in plasma

Blood samples were drawn from the femoral venous line at 0, 3 and 6 h after CPB for blood cell analysis by a haematology automated analyser (Sysmex XE-2100, Sysmex Corporation, Nishi-ku, Japan). At the same time points, samples from the femoral vein were heparinised for analysing biochemical indices. They were centrifuged at 3000 rpm for 5 min at 4 °C. The plasma was transferred to another tube and immediately frozen and stored at −70 °C.

The plasma levels of elastase, platelet-activating factor (PAF) and prothrombin fragment 1 + 2 (PTF 1 + 2) were quantitatively determined by enzyme immunoassay developed by Bionewtrans Pharmaceutical Biotechnology Co., Ltd. (Franklin, MA, USA).

2.5. Flow cytometry analyses of CD11b

Leucocytes from femoral venous blood samples were diluted to 1 × 10⁶ cells ml⁻¹. Cells were incubated with purified monoclonal antibody CD11b mouse IgG1 (Serotec, MorphoSys UK Ltd., Oxford, UK) at 4 °C for 1 h. After washing three times with phosphate-buffered saline (PBS), the cells were incubated with rabbit anti-mouse secondary fluorescein isothiocyanate (FITC) conjugate (Serotec, MorphoSys UK Ltd.) for 30 min at 4 °C. Thereafter, the cells were washed twice and analysed using a flow cytometer (Esp Elite; Beckman Coulter, Chicago, IL, USA).

2.6. Neutrophil sequestration in tissues

Myeloperoxidase (MPO) in tissues was analysed for the determination of neutrophil accumulation. At the end of the experiments, tissue samples, weighing about 0.4 g each, were taken from the upper pole of the kidney, homogenised and dissolved in potassium phosphate. After centrifugation, supernatants were collected in phosphate buffer. The MPO levels were measured by enzyme immunoassay developed by Bionewtrans Pharmaceutical Biotechnology Co., Ltd.

2.7. Statistical analysis

All values are expressed as the mean ± standard deviation (SD). Statistical comparison was performed by one-way analysis of variance (ANOVA) using an SPSS software program (SPSS Inc, Chicago, IL, USA) with significance defined as \( P < 0.05 \). If significance was found, Tukey’s post hoc test was applied to locate the source of differences.
3. Results

3.1. Renal dysfunction was lessened by aprotinin combined with NO and PGE1

Renal dysfunction demonstrated by increased serum creatinine levels and decreased Ccr was observed after CPB in all groups (Fig. 1). Severity of renal dysfunction did not differ among the control and aprotinin groups. Aprotinin combined with NO and PGE1 (A + N + P group) significantly improved serum creatinine and Ccr in comparison to the other four treatments. In the aprotinin group, each dog received 8000 KIU/kg of aprotinin. Dogs in the NO group were given 40 ppm nitric oxide throughout the CPB. In the PGE1 group, 20 ng kg$^{-1}$ min$^{-1}$ of prostaglandin E1 was given throughout the CPB. Dogs in A + N + P group were given 40 ppm NO by inhalation, 20 ng kg$^{-1}$ min$^{-1}$ of PGE1 and 80,00 KIU/kg aprotinin by infusion. The control group did not receive any treatment. *, $P < 0.05$; **, $P < 0.01$ versus the other four groups; #, $P < 0.01$, and $\#$, $P < 0.05$ versus after induction.

3.2. Neutrophil activation was suppressed by the combination treatment

Systemic inflammation, especially neutrophil infiltration in renal tissues, causes renal injury during cardiac surgery. To investigate the role of inflammation, MPO as an index of neutrophil accumulation was measured. MPO activity was significantly lower in the combination group than in the other four groups ($P < 0.01$ vs the other four groups; Fig. 3). No difference was found in plasma elastase levels among the aprotinin, NO, PGE1 and control groups ($P > 0.05$). The inhibition of neutrophil activation by combination treatment was confirmed in the flow cytometry study. Mean fluorescent intensities of CD11b, an adhesion molecule expressed on activated leucocytes, were significantly lower in the combination group than in the other four groups ($P < 0.01$, Fig. 5). Again, there were no differences among the aprotinin, NO, PGE1 and control groups.

3.3. Inhibition of platelet activation by the combination treatment

Decreased platelet counts and increased plasma PAF levels were observed in all the groups after CPB ($P < 0.01$; Fig. 2). No difference was found in plasma elastase levels among the aprotinin, NO, PGE1 and control groups ($P > 0.05$).

The inhibition of neutrophil activation by combination treatment was confirmed in the flow cytometry study. Mean fluorescent intensities of CD11b, an adhesion molecule expressed on activated leucocytes, were significantly lower in the combination group than in the other four groups ($P < 0.01$, Fig. 5). Again, there were no differences among the aprotinin, NO, PGE1 and control groups.
Aprotinin slightly improved platelet counts 6 h after CPB, although it did not reach statistical significance compared with the control group ($P = 0.058$). The combination treatment of aprotinin with NO and PGE$_1$, however, improved platelet counts significantly ($P < 0.05$; Fig. 6(A)). PAF is released from activated platelets. In the present study, plasma PAF levels were lower in the aprotinin-treated group than in the control, NO and PGE$_1$ groups ($P < 0.05$), and further decreased in the combination treatment group ($P < 0.05$; Fig. 6(B)). These results suggest effective inhibition on platelet activation by aprotinin combined with NO and PGE$_1$. As thrombin is a potent platelet activator, PTF 1 + 2 level in the plasma was lower in the aprotinin group, and still lower in the combination group than in the control, NO and PGE$_1$ groups ($P < 0.05$; Fig. 7).

### 4. Discussion

In this CPB study, using serum creatinine and Ccr to assess renal damage, aprotinin did not show apparent renal impairment. On the contrary, when combined with NO and PGE$_1$, aprotinin prevented CPB-induced renal dysfunction and decreased CD11b expression, leucocyte sequestration in the kidney, plasma elastase, PAF and PTF 1 + 2. Aprotinin as the cause of renal toxicity has been questioned for decades [13]. Aprotinin has been reported to increase postoperative serum creatinine [2], the need for dialysis [4] and mortality in cardiac surgical patients [5,6]. However, other studies show that aprotinin does not increase the risk of renal injury [14—16]. These differences may result from variations in exclusion criteria, or in how renal function is defined or in patient selection. Aprotinin is used in patients with haemorrhagic tendency because of its ability to minimise bleeding [2,17], which may explain the higher morbidity in aprotinin users. Our current experiments were designed to eliminate variance, and the results showed no difference in renal function between the aprotinin and control groups (Fig. 1).

The cause of renal dysfunction following cardiac surgery is multifactorial, including decreased renal perfusion, lack of pulsatile perfusion during CPB, inflammatory response and microthrombus [18]. Aprotinin might aggravate renal injury by inhibiting kallikrein and endogenous vasodilators, such as PGI$_2$ [8] and NO [9,10], because both of them are important regulators of vascular tone and tissue perfusion. In this study, the severity of renal dysfunction was similar in aprotinin and control groups. The dysfunction was slightly alleviated by the combination of two vasodilators (NO and PGE$_1$), and was significantly alleviated by the addition of aprotinin to NO and PGE$_1$. The results suggest that reduced renal blood flow is not...
The main reason for CPB-induced renal injury and combination of the three agents may provide a synergistic, protective effect on the kidney. Our data do not show that aprotinin causes renal dysfunction. Although serum creatinine and Ccr are the most commonly used tests to assess renal function, they are insensitive indicators of tubular injury. Nevertheless, our data do demonstrate that aprotinin plays the most important role among the three agents tested in alleviating CPB-induced renal dysfunction.

Inflammatory response and coagulation cascade are initiated during CPB [18]. They are two intricate, cross-linked processes in defending injuries [11]. The interaction between inflammation and coagulation [19] facilitates organ injury during CPB. Neutrophil activation is one of the typical characters in CPB-induced systemic inflammatory response. The activated neutrophils adhere to the endothelium via interaction of CD11b/CD18 (Mac-1) and intercellular adhesion molecule-1 (ICAM-1), and then destroy tissues by releasing proteolytic enzymes [20]. Although NO, PGE 

- **References**


The investigation was carried out for 6 h after CPB. Thus, the data obtained can only be used to assess acute effects. However, aprotinin may accumulate within the cytoplasm, and increase mortality, even 5 years after its use [8]. Therefore, further studies are needed for the long-term assessment of this combination therapy.

In conclusion, our CPB studies demonstrate that aprotinin does not produce detrimental effects, but improves renal function when combined with NO and PGE 

The interaction among these agents may provide a synergistic protective effect on the kidney. Our data do not show that aprotinin causes renal dysfunction. Although serum creatinine and Ccr are the most commonly used tests to assess renal function, they are insensitive indicators of tubular injury. Nevertheless, our data do demonstrate that aprotinin plays the most important role among the three agents tested in alleviating CPB-induced renal dysfunction.


