Aprotinin does not impair renal haemodynamics and function after cardiac surgery†

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Patients undergoing cardiac surgery with moderate hypothermic cardiopulmonary bypass (CPB) were allocated randomly to receive either saline (control group, \( n=29 \)) or a high-dose regimen of aprotinin (aprotinin group, \( n=28 \)). In both groups, CPB was associated with similar and transient increases in effective renal plasma flow (+54% in controls and +48% in aprotinin-treated patients) and in fractional excretion of sodium and potassium, but glomerular filtration rate remained unchanged. Plasma and urinary ratios of 6-keto-PGF\(_{1\alpha}\) to thromboxane B\(_2\) (TxB\(_2\)) increased significantly, indicating systemic and renal release of vasodilatory prostaglandins. Osmolar clearance correlated with urinary excretion of cyclic GMP (\( r=0.79 \) and 0.86 in the control and aprotinin groups, respectively) and 6-keto-PGF\(_{1\alpha}\) (\( r=0.63 \) and 0.69 in the control and aprotinin groups, respectively). Compared with preoperative values, plasma atrial natriuretic peptide increased after weaning from CPB (+71% and +93% in the control and aprotinin groups, respectively). Aprotinin had no apparent adverse effect on renal function and it did not alter mechanisms involving prostanoids and atrial natriuretic peptide during cardiac surgery.

**Keywords**: polypeptides, aprotinin; surgery, cardiovascular; kidney, function; kidney, blood flow; hormones, prostaglandins; kidney, atrial natriuretic factor

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Aprotinin, a non-specific serine protease inhibitor extracted from bovine lung or porcine gut, can reduce bleeding and blood transfusion requirements in patients undergoing cardiac operations, liver transplantation and orthopaedic surgery.¹⁻³ The use of aprotinin has become widespread and is not limited to patients at increased risk of bleeding or those undergoing complex surgical procedures.⁴

However, concerns have been raised regarding its possible effects on renal function: in pigs, a large dose of aprotinin caused a reduction in renal blood flow during acute stress and a multicentre, randomized, controlled study⁵ reported more frequent increases in serum creatinine (>0.5 mg dl\(^{-1}\)) in patients given aprotinin compared with placebo (30% vs 8%, respectively). Aprotinin is a small molecule (6512 Da) and is freely filtered by the glomeruli and nearly completely absorbed. Some suggest that the breakdown capacities of the convoluted tubules could be overwhelmed, allowing nephrotoxic effects.⁷ In addition, aprotinin inhibits kallikrein, the key enzyme in the kinin-generating pathway, which triggers the release of potent vaso dilatory mediators, such as prostacyclin and nitric oxide, from endothelial and renal tubular cells.⁸⁻⁹ In contrast, aprotinin delays degradation of atrial natriuretic peptide (ANP),¹⁰ and greater plasma concentrations of ANP may counteract inhibition of the kallikrein–kinin system in the kidney and promote diuresis and renal vasodilatation.

We designed a randomized double-blind study to assess the effects of aprotinin on renal haemodynamics and function in patients undergoing cardiac surgery. In addition, we questioned if release of vasoconstrictor and vasodilatory prostanoids (thromboxane A\(_2\) (TxA\(_2\)) and prostacyclin) and plasma ANP and cyclic GMP (cGMP), its second messenger, were influenced by aprotinin pretreatment.

**Patients and methods**

After obtaining Institutional Ethics Committee approval and informed consent, we studied 60 patients undergoing elective coronary artery bypass graft surgery, aortic valve replacement and mitral valve replacement or repair. We excluded those with diabetes or creatinine clearance values less than 30 ml min\(^{-1}\). Patients in whom postoperative bleeding needed surgical haemostasis were also excluded.

The patient’s usual cardiac medications were administered up to the morning of surgery. Peripheral venous, radial arterial and central venous catheters were inserted for
fluid and drug administration in addition to haemodynamic measurements. Before operation, crystalloid 10 ml kg\(^{-1}\) was infused over 20 min. General anaesthesia was standardized using fentanyl 40–60 μg kg\(^{-1}\) and midazolam 0.03–0.06 mg kg\(^{-1}\) followed by pancuronium 0.10 mg kg\(^{-1}\) h\(^{-1}\) for neuromuscular block (0.15 mg kg\(^{-1}\) at induction and 0.05 mg kg\(^{-1}\) during bypass). Mechanical ventilation was adjusted to obtain normocapnia and normoxia.

Before cannulation of the aorta and right atrium, heparin sodium 300 μg kg\(^{-1}\) was administered; additional heparin was given if the kaolin-activated clotting time (Hemochrom 401, International Technidyne Corporation, Edison, NJ, USA) was less than 550 s. Non-pulsatile cardiopulmonary bypass (CPB) was started with a membrane oxygenator (Cobe Laboratories Inc., Lakewood, CO, USA) primed with crystalloid 2 litre; body temperature was decreased to achieve moderate hypothermia (26–30°C) with α-stat regulation for pH management. After aortic clamping, myocardial protection was provided with St Thomas’s solution 600–900 ml infused through the aortic root and repeated at 25–30-min intervals. During CPB, mean arterial pressure was maintained at 55–70 mm Hg using sodium nitroprusside, phenylephrine, or dopamine. After aortic clamping, myocardial protection was achieved using sodium 300 μg kg\(^{-1}\) dopamine were not given during the study. 

### Study procedure

Patients were allocated randomly to one of two treatment groups in a double-blind manner. Each patient received a similar volume of saline or aprotinin. In the aprotinin group, each patient received 2 million kallikrein inhibitory units (mkiu) over 30 min, 1 mkiu in the CPB priming volume and 0.5 mkiu h\(^{-1}\) i.v. from the start of surgery until skin closure. The mean dose was 4.1 mkiu/patient or approximately 50 000 kiu kg\(^{-1}\).

Before anaesthesia, priming doses of inulin 30 mg kg\(^{-1}\) (10% Inulin; Laeveson Gesellschaft mbH, Linz, Austria) and p-paraimmunohippurate 8 mg kg\(^{-1}\) (PAH, Merck, Sharp and Dohme, NJ, USA) were given i.v. followed by a continuous infusion of both drugs at 0.2 and 0.15 mg kg\(^{-1}\) min\(^{-1}\), respectively, up to the end of surgery. An equilibration period of 60 min was allowed before baseline measurements. Diuretics, mannitol or dopamine were not given during the study.

Kallikrein concentrations were measured in urinary samples before and after bypass using the method of Amundsen and colleagues. \(^{11}\) This assay measured urinary activity against the chromogenic substrate S-2266 (H-D-Val-Leu-Arg-pNA, AB Kabi Diagnostica, Stockholm, Sweden) for which urine kallikrein is highly specific. Results are expressed as nkat litre\(^{-1}\), where 1 nkt is the amount of glandular kallikrein which cleaves 0.05 mmol of substrate per minute. Intra- and inter-assay coefficients of variation were 4.2% and 7.1%, respectively.

### Measurements and calculations

Mean arterial pressure, heart rate and right atrial pressure were recorded. Glomerular filtration rate was assumed to equal creatinine or inulin clearances (C\(_{\text{IN}}\), C\(_{\text{creat}}\)) and effective renal plasma flow was estimated as PAH clearance (C\(_{\text{PAH}}\)). Other tests of renal function included osmolar clearance (C\(_{\text{Osm}}\)), water clearance (C\(_{\text{H2O}}\)) and fractional sodium and potassium excretion. Blood and urinary samples were obtained at the following times: (1) baseline measurement, 30 min before induction of anaesthesia (pre-op.), (2) before the start of bypass (pre-bypass), (3) before the second cardioplegia (bypass), (4) after administration of protamine (post-bypass) and (5) on day 5 after operation. During operation, urine was collected over 30 min and arterial blood was obtained in the middle of each sampling period. Before and after operation, urine was collected over 12 h and mean blood concentrations (for creatinine and electrolytes) at the beginning and end of the clearance period were calculated. Arterial blood was collected into cold tubes containing indomethacin and heparin (for prostaglandin assay) or dipotassium ethylenediamine-tetraacetic acid and aprotinin (for ANP and cGMP assay) and was centrifuged immediately at 4°C. C\(_{\text{IN}}\) and C\(_{\text{PAH}}\) were measured only during operation. Urinary and plasma inulin and PAH concentrations were assayed spectrophotometrically. Serum and urinary sodium and creatinine were measured with a standard flame emission photometer. All clearance values were corrected for a standard body surface area of 1.73 m\(^2\).

### Plasma concentrations of ANP and its second messenger cGMP

In addition to plasma and urinary concentrations of the stable metabolites of TxA\(_2\) (thromboxane B\(_2\) (TxB\(_2\))) and prostacyclin (6-keto-PGF\(_{1α}\)) were determined before anaesthesia and during operation (pre-op., pre-bypass, bypass and post-bypass). Plasma ANP was extracted from a 2-ml plasma aliquot on a C-18 octadecylsilane cartridge (Sep-Pak, Waters Associates, Milford, MA, USA) and measured by a specific radioimmunoassay; intra- and inter-assay variations were 3.9% and 13.7%, respectively. cGMP was measured after extraction by ethanol using a commercial kit (Amersham, UK); intra- and inter-assay variations were 4.5% and 10%, respectively.

TxB\(_2\) and 6-keto-PGF\(_{1α}\) were measured in 100-μl aliquots of plasma or urine by radioimmunoassay without extraction (Amersham, UK). The lower limit of sensitivity was 30 pg ml\(^{-1}\) for both measurements. Excretion of urinary prostanoïd metabolites was expressed as pg g creatinine\(^{-1}\).

Plasma concentrations of ANP, cGMP and prostanoïd were corrected for the haemodiluting effects of CPB according to the change in packed cell volume.

Intra- and postoperative i.v. fluid requirements (packed red blood cells, fresh frozen plasma, crystalloid and colloid...
solutions) were recorded for each patient. Postoperative blood loss was measured and recorded by observing the volume of drainage into volumetric chest tube drains until their removal (approximately 24–36 h).

**Statistical analysis**

Sample size calculation was based on estimates of urinary excretion of cGMP and prostanoids, determined in a preliminary experiment. The calculations indicated that 28 patients were needed in each group to show a difference of $\geq 1.2 \text{ SD}$ (power=90%, significance=5%).

All data are expressed as mean (SD or range); differences were considered significant if $P<0.05$. Two-way analysis of variance with Dunnett’s test was used for within-group comparisons with respect to baseline. Chi-square analysis with Yates’ correction was used to compare percentages of patients. Simple linear correlation (Pearson) or regression analysis was used to evaluate the relationship between variables. For ANP, cGMP and prostanoid analysis, data were log transformed and the concentration equal to the sensitivity of the assay was assigned the value of the statistical limit of significance.

**Results**

**Patient characteristics and outcome**

We studied 57 patients (29 in the control group and 28 in the aprotinin group); one patient in each group was subsequently excluded because of bleeding which required re-operation. Another patient in the aprotinin group had a massive myocardial infarct and died shortly after surgery.

Patient characteristics, preoperative treatment and surgical data did not differ between groups (Table 1). There were similar changes in mean arterial pressure, heart rate and right atrial pressure in the two groups (data not shown). Temporary inotropic and/or vasopressor support was needed for weaning from CPB in 14 patients in the saline group and in 15 in the aprotinin group; intra-aortic counterpulsation was required in one patient in each group. Postoperative myocardial infarct was diagnosed in two patients in the saline and in one patient in the aprotinin group. Duration of mechanical ventilation, stay in the ICU and time to discharge did not differ between groups.

In the aprotinin group, postoperative blood loss (775 (314) ml) and blood transfusion requirements (1.4 (0.7) u) were significantly less than in the control group (1185 (403) ml and 2.6 (1.4) u, respectively).

**Perioperative renal function**

In the control group, urinary kallikrein excretion did not change from before to after bypass (2.52 (1.94) and 3.14 (2.18) kat litre$^{-1}$, respectively) whereas aprotinin treatment was associated with significant lower urinary kallikrein excretion (0.24 (0.22) and 0.31 (0.30) kat litre$^{-1}$, respectively).

Renal function did not differ between groups at any time (Table 2). CPB was associated with significant increases in urinary flow, $C_{\text{Osm}}$ and fractional excretion of sodium and potassium; all of these variables were still significantly increased shortly after weaning from CPB and had recovered to preoperative values by day 5 after operation. During CPB, $C_{\text{PAH}}$ increased in the control and aprotinin groups (+54% and +48%, respectively). $C_{\text{creat}}$ and $C_{\text{IN}}$ did not change significantly throughout the study and were correlated with each other ($r=0.72$, $P<0.05$).

**Plasma ANP, plasma and urinary cGMP**

There were no differences between groups in plasma concentrations of ANP or plasma and urinary values of cGMP. In all
patients, plasma ANP decreased during bypass and increased after weaning from bypass (+71% in the control group and +93% in the aprotinin group), compared with preoperative baseline values (Fig. 1). There was a significant correlation between plasma ANP and plasma cGMP ($r = 0.52$ in controls and $r = 0.62$ in the aprotinin group) and between plasma ANP and right atrial pressure ($r = 0.41$ in controls and $r = 0.52$ in the aprotinin group).

Changes in plasma cGMP mimicked those of plasma ANP, although they did not reach statistical significance. Urinary excretion of cGMP increased significantly during and after bypass and was positively correlated with $C_{\text{Osm}}$ ($r = 0.79$ and 0.86 in the control and aprotinin groups, respectively).

### Plasma and urinary TxB2 and 6-keto-PGF1α

Patients in the aprotinin group did not differ from those in the control group in plasma concentrations and urinary excretion of 6-keto-PGF1α and TxB2 and their respective ratios. After the start of bypass, the ratio of plasma concentrations of 6-keto-PGF1α to TxB2 increased significantly because of an increase in 6-keto-PGF1α, while TxB2 remained unchanged (Fig. 2).

Urinary excretion of TxB2 and 6-keto-PGF1α increased significantly during and after CPB. The ratio of 6-keto-PGF1α to TxB2 was increased because of greater urinary excretion of 6-keto-PGF1α than TxB2 (Fig. 3). In the two groups, $C_{\text{Osm}}$ was directly related to urinary excretion of 6-keto-PGF1α ($r = 0.63$ and 0.69 in the control and aprotinin groups) and to the ratio of 6-keto-PGF1α to TxB2 ($r = 0.52$ and 0.54 in the control and aprotinin groups, respectively).

### Discussion

As acute renal failure is related to mortality in cardiac surgery, clinicians should be aware of the potential renal damage from drugs. In this regard, we found that in patients with good renal function, aprotinin treatment (with a mean dose of 4.1 mkiu) did not affect renal function during cardiac surgery with moderate hypothermic CPB. CPB impaired renal solute reabsorption, regardless of aprotinin pretreatment, and was associated with renal release of vasodilatory mediators (nitric oxide and prostacyclin), indicated by urinary excretion of their respective metabolites (cGMP and 6-keto-PGF1α).

### Aprotinin dose regimen

Different doses of aprotinin have different effects on coagulation, fibrinolysis and the inflammatory cascade. Compared with the standard high-dose aprotinin treatment (2 mkiu before bypass, 2 mkiu in the pump prime and an infusion of 0.5 mkiu h$^{-1}$), a smaller dose (using only 2 mkiu in the pump prime) can also reduce postoperative blood loss. At plasma concentrations greater than 50 kiu ml$^{-1}$, plasmin is inhibited, whereas 200 kiu ml$^{-1}$ is needed to suppress plasma kallikrein. According to a pharmacokinetic model proposed by Levy, Bailey and Salmenpera, target plasma concentrations of 200 kiu ml$^{-1}$ can be achieved with a bolus of aprotinin 2 mkiu in the pre-bypass period, 1 mkiu added to the CPB circuit and an infusion of 0.5 mkiu h$^{-1}$. We used a slightly higher dose regimen (1 mkiu instead of 0.5 mkiu in the pump prime) that gave a 34% reduction in postoperative blood loss and...
Fig 1 Changes in plasma atrial natriuretic peptide (ANP) and plasma and urinary cyclic guanidine monophosphate (cGMP) in the two groups of patients undergoing cardiac surgery. Data are mean (SD). *Significant difference from baseline (preoperative) (P<0.05). No differences between groups.

Fig 2 Changes in plasma thromboxane B₂ (TxB₂) and 6-keto-PGF₁α, and the ratio 6-keto-PGF₁α to TxB₂ in the two groups of patients undergoing cardiac surgery. Data are mean (SD). *Significant difference from baseline (preoperative) (P<0.05).

Reduced allogenic blood transfusion. As the affinity of aprotinin for renal tissue is greater than for plasma kallikrein, it was not surprising to observe 90% suppression of urinary excretion of kallikrein.

Aprotinin and ANP–cGMP and prostanoids pathways

Plasma concentrations of ANP and systemic and renal formation of TxA₂, prostacyclin and nitric oxide were similar in the two groups. The relationships we found between plasma concentrations of ANP, plasma cGMP and right atrial pressure confirmed that, even during operation, atrial release of ANP is triggered by changes in cardiac filling pressure¹⁶ ¹⁷ and that plasma ANP concentrations largely determine circulating concentrations of its second messenger cGMP.¹⁸ Although we observed no change in plasma concentrations of ANP after infusion of aprotinin, we cannot exclude the possibility that inhibition of ANP metabolism¹⁰ was compensated for by a reduction in ANP synthesis in atrial tissues.

Increased plasma and urinary ratios of 6-keto-PGF₁α to TxB₂ during CPB indicate that the balance of prostanoid synthesis was shifted towards prostacyclin-induced vasodilation which could prevent the vasoconstrictive and thrombogenic effect of TxA₂.

In clinically relevant concentrations (50–500 mkiu), aprotinin had no effect on endothelial and tubular release of prostacyclin¹⁹ and therefore it did not influence perioperative plasma and urinary 6-keto-PGF₁α concentrations.

In contrast with previous reports showing reduced platelet release of TxA₂ in aprotinin-treated patients, we found similar plasma TxB₂ concentrations in the two groups.²⁰ ²¹
Aprotinin and perioperative renal function

Characterized by impaired solute reabsorption, a brisk osmolar diuresis and increases in fractional excretion of sodium and potassium. Importantly, we observed a close relationship between osmolar diuresis and renal production of vasodilatory mediators during cardiac surgery. Urinary excretion of 6-keto-PGF₁α, TxB₂ and cGMP reflect renal synthesis of prostacyclin, TxA₂ and nitric oxide. During hypothermic CPB, enhanced salt and water excretion may result from several mechanisms: first, prostanoids antagonize the in vivo effect of antidiuretic hormone and inhibit Na⁺/Cl⁻ reabsorption in the proximal and distal nephron and in the loop of Henle; second, in the papilla, PGI₂- and NO-mediated medullary vasodilatation prevents the papillary Na⁺/Cl⁻ countercurrent exchange by disrupting the hyperosmolar gradient; third, tubular solute reabsorption is impaired by reduced activity of Na⁺/H⁺/Cl⁻ membrane pumps during hypothermia.

Aprotinin, cardiac surgery and renal dysfunction

The use of aprotinin in clinical practice has not been associated with an increased incidence of postoperative renal failure. In patients undergoing deep hypothermic arrest, the benefits and safety of aprotinin need further study. In a large multicentre study, the need for haemodialysis or filtration was strongly related to poor preoperative renal function, vascular disease and heart failure, type of surgery and use of intra-aortic balloon pump. Despite inhibition of the kallikrein–kinin pathways, aprotinin pretreatment did not influence prostaglandin synthesis, glomerular filtration, renal plasma flow or tubular transport mechanisms. This suggests that the kallikrein–kinin pathways play a minor role in renal homeostasis and that other factors (e.g. renin–angiotensin system) may stimulate the synthesis of prostanoids during cardiac surgery. Interestingly, in animal models of sepsis and in humans with cirrhosis, aprotinin can reverse systemic vasodilatory hypotension and improve renal function.

In contrast with our data and other studies, some investigators reported mild and transient postoperative increases in serum creatinine concentrations, COsm and TxB₂. However, these studies were not designed to assess renal outcome; changes in renal function were not great and no details were available on perioperative haemodynamic control and the use of fluids, diuretics and mannitol. For instance, in the study of Blauhut and colleagues, increased COsm was more likely related to significant higher arterial pressure values which resulted in pressure-induced natriuresis in aprotinin-treated patients.

In summary, we found no evidence that in patients undergoing cardiac surgery with hypothermic bypass, a high dose of aprotinin had an adverse effect on renal function or influenced its autoregulatory control mechanisms involving prostanoids and ANP. Nevertheless, until more data are available, a lower dose is still recommended in patients who have diabetes mellitus or renal insufficiency, and in those receiving chronic treatment with angiotensin-

In fact, maximal release of PGI₂ occurs shortly after the start of CPB whereas peak plasma TxB₂ concentrations occur during rewarming at the end of CPB. As we measured prostaglandins approximately 20 min after the start of CPB and before chest closure, we were unable to document the suppressive effect of aprotinin on plasma TxB₂.

Renal dysfunction and CPB

As reported elsewhere, we found no reduction in glomerular filtration rate and renal plasma flow during hypothermic extracorporeal bypass as long as mean arterial pressure and pump flow were maintained within the physiological range of autoregulation. In the two groups, increased CP₅ₐ was reduced. Transient tubular dysfunction was characterized by impaired solute reabsorption, a brisk osmolar diuresis and increases in fractional excretion of sodium and potassium. Importantly, we observed a close relationship between osmolar diuresis and renal production of vasodilatory mediators during cardiac surgery. Urinary excretion of 6-keto-PGF₁α, TxB₂ and cGMP reflect renal synthesis of prostacyclin, TxA₂ and nitric oxide. During hypothermic CPB, enhanced salt and water excretion may result from several mechanisms: first, prostanoids antagonize the in vivo effect of antidiuretic hormone and inhibit Na⁺/Cl⁻ reabsorption in the proximal and distal nephron and in the loop of Henle; second, in the papilla, PGI₂- and NO-mediated medullary vasodilatation prevents the papillary Na⁺/Cl⁻ countercurrent exchange by disrupting the hyperosmolar gradient; third, tubular solute reabsorption is impaired by reduced activity of Na⁺/H⁺/Cl⁻ membrane pumps during hypothermia.

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**Fig 3** Changes in urinary excretion of thromboxane B₂ (TxB₂) and 6-keto-PGF₁α, and the urinary ratio 6-keto-PGF₁α to TxB₂ in the two groups of patients undergoing cardiac surgery. Data are mean (SD). *Significant difference from baseline (preoperative) (P<0.05).
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