Randomised placebo controlled double blind study of two low dose aprotinin regimens in cardiac surgery

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Abstract

Objective—To determine whether two low dose aprotinin regimens produce clinically significant reductions in postoperative blood loss compared with a control group.

Design—A randomised double blind placebo controlled study.

Setting—A regional cardiothoracic unit in London.

Patients—79 patients were consecutively allocated to one of three groups. All patients had primary elective surgery with standard anaesthetic and surgical techniques, and no patients were withdrawn from the study.

Interventions—Group K patients (n = 27) received aprotinin (10^6 kallikrein inactivator units (KIU)) into the pump prime whereas group L patients (n = 27) received an intravenous bolus of aprotinin (0.5×10^6 KIU) after induction of anaesthesia and 10^6 KIU was added to the pump prime. A third group (group J, n = 25) received 0.9% saline placebo.

Main outcome measures—After insertion of the chest drains at the end of cardiopulmonary bypass, blood losses were measured hourly until the drains were removed 18 to 24 h later. Total haemoglobin loss into the chest drains was calculated.

Results—Both aprotinin treated groups showed significantly less postoperative blood loss than controls (medians: group K, 400 ml; group L, 400 ml; v controls 780 ml; $p < 0.001$) and there was even less measured postoperative haemoglobin loss within the chest drains in both the aprotinin treated groups than in the controls (medians: group K, 16 g; group L, 19 g; v controls, 47 g; $p < 0.001$).

Conclusion—In primary cardiac surgery the dose of aprotinin may be reduced by about 80% from the recommended high dose schedule and still significantly reduce postoperative blood loss compared with placebo.

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Aprotinin (Trasylol, Bayer), a serine proteinase inhibitor, reduces postoperative blood loss after cardiac surgery with cardiopulmonary bypass.^{1,2} Most of the previous studies have involved the use of aprotinin in a high dose (about 6×10^6 kallikrein inactivator

units (KIU)), with the aim of reaching serum concentrations adequate for a full anti-kallikrein effect.

The aim of our study was to determine whether the dose of aprotinin could be greatly reduced and still produce clinically significant reductions in postoperative blood loss. We have not performed a dose-response study, but have simply assessed two low dose aprotinin regimens, and compared the effects with a control group. By the use of a much lower dose of aprotinin than that currently recommended we intended to reduce the cost per patient, maintain efficacy, and possibly extend the use of aprotinin to all patients needing cardiac surgery, not just high risk patients such as those needing repeat operations or complex procedures.

Patients and methods

Ethics committee approval and informed patient consent were obtained. A prospective, placebo controlled, double blind, randomised clinical trial was set up in patients scheduled to have elective cardiac surgery with cardiopulmonary bypass. Patients having repeat operations or with a known previous exposure to aprotinin were excluded from the trial.

DOSE OF APROTININ

Aprotinin is presented in clear vials, each containing 0.5×10^6 KIU in 50 ml 0.9% saline solution. One of the investigators (AKW) made up all the test solutions; 500 ml bags of sterile 0.9% saline solutions had a volume discarded and replaced with the same volume of test solution so that all bags contained equal volumes (500 ml). Two freshly drawn up bags were taped together, one labelled "patient bolus", and the other "pump prime" with either the letter J, K, or L. The other investigator (CRB) performed all the patients' measurements, unaware which groups the letters referred to.

With a computer generated random number table, 79 patients were consecutively allocated to one of the following three groups:

Group J: 0.9% saline placebo (n = 25). These patients received a 500 ml 0.9% bolus of saline solution at intravenous induction of anaesthesia, and a further 500 ml 0.9% saline solution was added to the pump prime.

Group K: 10^6 KIU aprotinin into the pump prime (n = 27). These patients received a bolus of 500 ml 0.9% saline solution at intravenous induction of anaesthesia, and 400 ml 0.9% saline solution plus 100 ml aprotinin

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(10^6 KIU) were added to the pump prime.

Group L: 0.5×10^6 KIU aprotinin intravenous bolus to the patient plus 10^6 KIU into the pump prime ($n = 27$). These patients received 450 ml 0.9% saline solution plus 50 ml aprotinin (0.5×10^6 KIU) as a bolus at intravenous induction of anaesthesia, and 400 ml 0.9% saline solution plus 100 ml aprotinin (10^6 KIU) were added to the pump prime.

All patients were premedicated with intramuscular papaveretum (10–20 mg), hyoscine (0.2–0.4 mg), and oral lorazepam (1–2 mg). After intravenous induction of anaesthesia with diazepam and etomidate, intermittent positive pressure ventilation was supplemented with phenoperidine (a narcotic analgesic), pancuronium bromide (a muscle relaxant), and isoflurane (an inhalational anaesthetic agent) as necessary. Porcine mucous heparin (300 IU/kg) was injected into a central vein before cannulation of the heart. Tests for activated clotting time were performed at regular intervals and further heparin given if times fell below 450 seconds.

A hollow fibre membrane oxygenator (Compactflo, Dideco) was primed³ with 500 ml Gelofusine, 500 ml 5% dextrose, 500 ml compound sodium lactate, 100 ml 50% dextrose, 100 ml 8.4% sodium bicarbonate, 60 ml 20% mannitol, 10 IU neutral insulin, 20 mmol KCl, 5000 IU mucous sodium heparin, and 500 ml trial drugs or placebo. Flows of 2.4 l/m²/min were obtained with a minimally occlusive roller pump and systemic hypothermia to 28–30°C was maintained while the aorta was occluded.

Myocardial preservation during aortic cross clamping was sustained with St Thomas' Hospital cardioplegic solution injected into the aortic root at 0°C. Mean arterial pressure during bypass was kept between 50 and 80 mm Hg with glyceryl trinitrate, supplemented with phentolamine or metaraminol as necessary. After rewarming to 37°C at the completion of the operation and discontinuation of bypass, the residual effects of heparin were neutralised with protamine sulphate.

After transfer to the intensive care unit, intermittent positive pressure ventilation was continued until the patient was warm periph-

erally, was not bleeding, and was otherwise stable. To maintain adequate filling pressures, blood was given if the measured packed cell volume was < 35% and Gelofusine if it was > 35%. Fresh frozen plasma was given if the measured clotting ratios were > 1.5 times the control value, platelet packs were infused if the platelet count was < $100 \times 10^9/l$, and 1 ml/kg/h crystalloid fluid was given.

MEASUREMENTS

Blood samples were taken before operation, after induction of anaesthesia, every 30 minutes during bypass, before and after being given protamine sulphate before transfer to the intensive care unit, and at regular intervals while in the unit, and at 24 hours and seven days after operation.

Haemoglobin concentration, platelet counts, and clotting studies were measured by routine laboratory methods. Blood loss during the operation into the swabs and suction apparatus was recorded both before and after bypass but shed mediastinal blood during bypass was reinfused together with all the remaining volume in the cardiopulmonary bypass reservoir at the end of bypass. After insertion of the chest drains, losses were measured hourly until their removal 18 to 24 hours later. Because individual drainage volumes can vary in haemoglobin content, total haemoglobin loss into the chest drains was calculated with a haemoximeter (OSM 2, Radiometer). No cell saver devices were used during the study.

STATISTICAL ASSESSMENT

Normally distributed data were analysed by analysis of variance. Intraoperative and postoperative losses were analysed by Mann-Whitney U tests, and nominal data by χ^2 tests or Fisher's exact tests. Significant differences were assumed at $p < 0.05$.

Results

Table 1 shows the demographic data of the patients studied. There were no significant differences between the groups for age, body surface area, preoperative haematological values, number of distal grafts, or percentage of patients receiving internal mammary artery grafts. Forty four per cent or more of the patients in each group had received regular aspirin within 10 days of their operation, usually up to two or three days before the operation date.

Operative times, intraoperative urine outputs, intraoperative blood loss, and protamine sulphate neutralisation dosages were similar in all groups (table 2).

Table 3 shows the data for postoperative drainage and replacement. There was a significant reduction in the median (interquartile range) of postoperative chest drainage in both the pre-treated groups compared with controls (group K, 400 (250–650) ml; group L, 400 (210–550) ml; v group J, 780 (575–1045) ml; $p < 0.001$), and there was an even greater reduction in measured haemoglobin loss

Table 1 Preoperative demographic data of study groups; mean (SD)

	Group J (n = 25)	Group K (n = 27)	Group L (n = 27)
Age (yr)	63(10)	62(12)	60(9)
Sex (M/F)	17/8	16/11	21/6
Body surface area (m ²)	1.78(0.20)	1.82(0.18)	1.87(0.18)
Haemoglobin (g/dl)	13.9(1.2)	13.4(1.5)	14.2(1.4)
Platelets ($\times 10^9/l$)	213(64)	243(85)	223(50)
APTT ratio;	1.01(0.11)	1.25(0.92)	1.06(0.19)
PT ratio;	1.02(0.07)	1.01(0.09)	1.00(0.10)
Patients receiving aspirin within 10 days of operation (n(%))	15(60)	12(44)	12(44)
No of days aspirin was stopped before operation (median (interquartile range))	3(2–4)	2(1–7)	3(1–7)
Patients receiving operation (graft/valve/combination, n)	18/3/4	19/8/0	22/4/1
Patients receiving internal mammary artery grafts (n (%))	14(56)	14(53)	20(74)
No of distal grafts	3.59(2.50)	3.26(1.41)	2.92 (1.12)

All differences were non-significant. APTT, activated partial thromboplastin time; PT, prothrombin time.

Table 2 Operative data

	Group J (n = 25)	Group K (n = 27)	Group L (n = 27)
Mean time from induction of anaesthesia to cardiopulmonary bypass (min)	83(24)	89(25)	78(23)
Mean cardiopulmonary bypass time (min)	72(21)	70(30)	77(40)
Mean ischaemia time (min)	38(14)	40(22)	39(21)
Mean time from end of bypass to transfer to ICU (min)	70(25)	68(14)	67(28)
Median intraoperative urine output (ml)*	830 (500–1150)	770 (500–1050)	820 (790–1080)
Median intraoperative blood loss (ml)*	950 (700–1280)	955 (660–1360)	900 (570–1095)
Mean protamine sulphate: heparin ratio (mg: 100 IU)	1.36(0.36)	1.43(0.33)	1.48(0.32)

All differences are non-significant. Values in parentheses are SD or interquartile range; ICU, Intensive care unit. Analysis of variance, except Mann-Whitney U test.*

Table 3 Postoperative data

	Group J (n = 25)	Group K (n = 27)	Group L (n = 27)	p Value
Median chest drainage (ml)	780 (575–1045)	400 (250–650)	400 (210–550)	< 0.001*
Median haemoglobin loss in drains (g)	47(25–99)	16(10–24)	19(6–28)	< 0.001*
Patients given blood replacement (n(%)):				< 0.002†
0 units	0(0)	0(0)	3(11)	
1 unit	0(0)	1(4)	1(4)	
2 units	5(20)	6(22)	9(33)	
3+units	20(80)	20(74)	14(54)	
Median No of blood units transfused/patient	3(3–5)	3(2–4)	3(2–4)	
Mean haemoglobin on day 7 PO (g/dl)	11.9(1.3)	11.6(1.5)	11.4(1.2)	NS
Mean platelet count on day 7 PO ($\times 10^9/l$)	280(87)	247(91)	262(72)	NS
Patients given fresh frozen plasma (n(%)):				< 0.01‡
0 units	10(40)	22(81)	21(78)	
2 units	13(52)	3(11)	6(22)	
4 units	1(4)	2(7)	0(0)	
6 units	1(4)	0(0)	0(0)	
Median No fresh frozen plasma units transfused/patient	2(2–2)	0(0–0)	0(0–0)	
Mean percentage increase in APTT ratio on day 1 PO	15(16)	8(13)	10(23)	NS
Mean percentage increase in PT ratio on day 1 PO	25(19)	12(18)	15(8)	< 0.02§

Analysis of variance. Values in parentheses are %, SD, or interquartile range; PO = postoperation. * Group J v groups K and L, Mann-Whitney U test; † group L v groups J and K, Fisher's exact test; ‡ group J v groups K and L, Fisher's exact test; § group J v groups K and L, analysis of variance.

within the chest drains in both the aprotinin treated groups (group K, 16(10–24) g; group L, 19(6–28)g, v group J, 47 (25–99) g; $p < 0.001$). Patients in group L received significantly less banked blood than both the control group and patients in group K. Percentage changes in haemoglobin concentrations and platelet counts on the first postoperative day and at discharge were similar in all three groups. Sixty per cent of patients in the control group received fresh frozen plasma compared with 19% in both the pretreated groups ($p < 0.01$), and there was a significantly increased mean prothrombin time ratio in control group patients on the first postoperative day (25% in group J v 12% in group K and 15% in group L, $p < 0.02$).

Discussion

There is now irrefutable evidence that aprotinin reduces blood loss after cardiac surgery.^{1,2} Cardiopulmonary bypass has extensive effects on the mechanism of coagulation that predispose patients to excessive bleeding after surgery, and although the physiochemical effects of aprotinin have been extensively investigated, its mode of action has not yet been fully elucidated. It may act in one or

more of the following ways. Firstly, preservation of platelet glycoprotein 1b (GP1b); von Willebrand Factor (vWF) combines with the GP1b receptor to promote platelet adhesiveness, and both plasmin and kallikrein are known to inhibit this reaction. Van Oeveren *et al* showed that GP1b concentrations are maintained in aprotinin treated patients,⁴ and so aprotinin may preserve platelet adhesiveness through its antiplasmin and antikallikrein effects. Secondly, attenuation of the intrinsic clotting pathway; kallikrein promotes clotting through the intrinsic pathway, and serum concentrations of aprotinin > 200 KIU/ml inhibit kallikrein and would thus be expected to reduce coagulation. Evidence for this is, however, conflicting. Dietrich *et al*,⁵ de Smet *et al*,⁶ and Lu *et al*⁷ all confirmed a reduction in coagulation by showing either preservation of antithrombin III concentrations or a reduction in thrombin-antithrombin III complexes when aprotinin was used. By contrast, Havel *et al* found no significant difference in plasma concentrations of thrombin-antithrombin III complexes between patients treated with aprotinin and controls.⁸ Thirdly, aprotinin may have an antifibrinolytic effect. By inhibiting plasmin-mediated digestion of fibrin, aprotinin reduces fibrinolysis as indicated by lower concentrations of fibrin degradation products and maintained α_2 antiplasmin activity. This has been confirmed by Havel *et al*⁸ and Blauhut *et al*.⁹

All these actions of aprotinin may combine to produce a more satisfactory coagulation profile in patients after bypass.

Most of the previous studies have involved the use of high dose aprotinin, the basis for this coming from studies performed by biochemists working in Germany; they aimed to reach a serum concentration of > 200 KIU/ml for full antikallikrein activity (antiplasmin activity is achieved at the lower concentration of 50 KIU/ml). Although aprotinin in high doses seems to be safe, Yared and Estafanos raised concerns over drug interaction precipitating renal failure,¹⁰ Bohrer *et al* reported thrombus formation associated with aprotinin¹¹ and Samama *et al* showed an increase in spontaneous thrombosis of the femoral artery in pigs treated with aprotinin compared with controls.¹²

In our cardiothoracic unit it is the surgeons' policy to continue to prescribe aspirin until admission to hospital, and consequently 50% of patients requiring primary cardiac surgery receive regular aspirin until two or three days before surgery. As Royston *et al* showed significant reductions in postoperative losses in patients pretreated with aspirin who received high dose aprotinin, we thought it was valid to study aprotinin in the population of patients we see who require primary cardiac surgery.¹³ In view of the concerns about high doses of aprotinin, which may be dose-dependent, we decided to investigate low dose aprotinin regimens.

Our results compare favourably with previously published studies of high dose aprotinin in primary cardiac surgery, with a

50% reduction in volumetric postoperative drainage and an even greater reduction in haemoglobin loss; for example, Fraedrich *et al* showed a reduction in mean postoperative blood loss from 984 ml (control group) to 488 ml (high dose aprotinin group).¹⁴ The results of our study were statistically indistinguishable for both treatment groups and indicate that the use of high dose aprotinin is unnecessary to reach significant reduction in postoperative blood loss after cardiopulmonary bypass in patients who need primary cardiac surgery.

With regard to the use of blood and blood products during the period of the study, it was the transfusion policy in our unit to give blood to achieve a packed cell volume of 35% irrespective of measured losses after operation. Consequently, all patients studied were overtransfused and we now believe, in collaboration with our haematology colleagues, that most patients given low dose aprotinin do not need autologous blood transfusions. Platelet packs, a more appropriate product to give to patients with non-surgical postoperative bleeding, were difficult to obtain in our unit and we acknowledge that some patients were probably given inappropriate fresh frozen plasma. The end point of our study was not to assess differences in the uses of blood and blood products between the groups, however, but simply to record and compare differences in postoperative losses.

From all the aprotinin studies undertaken so far we may speculate on certain features: (a) a patient bolus is required to reduce intraoperative losses, as, if the dose is left until the initiation of bypass, excessive bleeding may occur during dissection, especially in repeat operations. In our study, neither group of pretreated patients showed a reduction in intraoperative losses compared with the control patients, either because the dose was not given until the initiation of bypass (group K), or the bolus was inadequate (group L). Similarly, Benmosbah *et al* gave about 1.75×10^6 KIU at aortic cannulation and found no reduction in intraoperative losses, although postoperative losses were reduced compared with a control group.¹⁵ (b) A pump prime bolus seems to be needed to reduce activation of the clotting cascade through contact with the bypass circuit, and to maintain adequate serum concentrations by allowing for the dilutional effects of cardiopulmonary bypass. Royston showed that postoperative losses were related to serum concentrations at the end of bypass,¹⁶ and we postulate that the critical, as yet unknown, serum concentrations may be as low as 50 KIU/ml (the antiplasmin concentration).

Although serum concentrations of aprotinin were not measured, we speculate that if 10^6 KIU aprotinin is added to the pump prime, then assuming a circulating volume of 7 l on bypass, a bypass time of 75 minutes (the mean bypass time in this study was 73 minutes), aprotinin half life of 44 minutes, and zero order kinetics, then the serum concentration of aprotinin will decrease to about

50 KIU/ml by the end of bypass; a concentration sufficient for an antiplasmin, but not an antikallikrein, effect.

Carrel *et al* gave a patient bolus of 2×10^6 KIU at the start of the operation,¹⁷ whereas Locatelli *et al*¹⁸ gave an infusion of 0.5×10^6 KIU/hour with no bolus: both these groups showed no reduction in blood loss, possibly because serum concentrations of aprotinin were inadequate at the end of the bypass period. By contrast, when Carrel *et al* gave a single bolus of 2×10^6 KIU into the pump prime they found reductions in blood loss and transfusion requirements that were identical to reductions obtained with high dose aprotinin.¹⁷

Kallis *et al* have shown that aprotinin may be efficacious when given after operation to patients (not pretreated with aprotinin) who bleed excessively after surgery,¹⁹ and in the light of the gathered information, we think that a rational approach would be to give low dose aprotinin to all patients having primary cardiac surgery who have received regular aspirin within 10 days of their operation, and to reserve high dose aprotinin for high risk patients such as those needing repeat procedures. We believe that patients not taking aspirin and having primary myocardial revascularisation do not need intraoperative aprotinin. After operation, however, if significant non-operative bleeding occurs, then these patients may benefit from postoperative aprotinin.

In conclusion, this study shows that in primary cardiac surgery the dose of aprotinin may be reduced by about 80% from the recommended dose schedule and still significantly reduce postoperative blood loss.

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