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Pharmacokinetics of Aprotinin in Preoperative Cardiac Surgical Patients

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Background: **Aprotinin, a polypeptide protease inhibitor, reduces bleeding and transfusion requirements during cardiac surgery. To investigate aprotinin's pharmacokinetics, we administered therapeutic doses to patients scheduled to undergo cardiac surgery.**

Methods: **Preoperative doses of 500,000 and 1,000,000 kallikrein inhibitor units (KIU) were administered as an infusion over 30 min to 28 patients, and plasma concentrations were measured for 48 h. Aprotinin concentrations were determined using a sandwich–enzyme-linked immunosorbent assay. A three-compartment model was fit to the measured aprotinin concentrations using extended nonlinear least-squares regression.**

Results: **Plasma aprotinin concentrations at the end of the 30-min infusion were 147 ± 61 KIU/ml for the 1,000,000-KIU dose and 60 ± 19 KIU/ml for the 500,000-KIU dose. Elimination clearance was 35.5 ml/min, and volume of distribution at steady state was 26.5 l.**

Conclusions: **A 2,000,000-KIU dose is needed to produce the plasma concentration of 200 KIU/ml associated with kallikrein inhibition. Plasma concentrations in excess of 50 KIU/ml, the concentration required to inhibit plasmin, can be achieved by a significantly smaller dose. Based on the derived pharmacokinetic parameters, an infusion model was developed to appropriately administer and maintain effective plasma concentrations of aprotinin, depending on the plasma concentration desired and the target proteases to be inhibited. (Key words: Aprotinin. Pharmacokinetics; aprotinin: coagulation; fibrinolysis. Surgery: cardiac. Complications: bleeding.)**

APROTININ is a single-chain polypeptide isolated from cow lung. It has a molecular weight of 6,512 Daltons and consists of 58 amino acid residues, with three disulfide bridges. Therapeutic uses of aprotinin, including use in cases of acute pancreatitis, shock syndromes, and hyperfibrinolytic hemorrhage, are based on its ability to inhibit proteases such as trypsin, plasmin, and plasma and tissue kallikrein.[1] The activity of aprotinin is expressed in kallikrein inhibitor units (KIU), with 1 KIU defined as the amount of aprotinin that decreases the activity of 2 biologic kallikrein units by 50%.[1] One milligram of aprotinin is equivalent to 7,143 KIU.

Royston *et al.* first reported that administering aprotinin in large doses before initiation of surgery and cardiopulmonary bypass produced dramatic reductions in postoperative bleeding in patients undergoing repeat cardiac surgery.[1] The dose regimen currently used was devised on theoretical grounds by Fritz and Wunderer[2] and involves a loading dose followed by an infusion to obtain plasma aprotinin concentrations of 200 KIU/ml to inhibit plasma kallikrein.[1–3] Additional studies performed in Europe and the United States have confirmed the efficacy of aprotinin to reduce postoperative bleeding and transfusion requirements in patients during cardiac surgery and cardiopulmonary bypass.[3–7] Aprotinin's mechanism of action for reducing postoperative bleeding is incompletely understood, but it appears to reflect its ability to inhibit plasmin as well as kallikrein. The only published pharmacokinetic study evaluated aprotinin in healthy volunteers using iodine 125–labeled drug[8]; there are no data available describing the pharmacokinetics of aprotinin in cardiac surgical patients.

The purpose of the current study was to evaluate the preoperative pharmacokinetics of aprotinin in cardiac surgical patients. This information is necessary to refine dose schedules and may support reductions in aprotinin dose in this patient group, relative to the regimen described by Royston *et al.*[1] Because aprotinin can produce dose-related transient and reversible increases in serum creatinine, there may be beneficial effects of reducing the dose, especially in patients at risk for renal dysfunction. Therefore, the pharmacokinetics of apro-

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tinin were evaluated for 48 h preoperatively in patients who were to undergo cardiac surgery and receive it as a therapeutic dose.

Materials and Methods

After approval and informed consent had been obtained, patients scheduled for cardiac surgery were evaluated for 48 h preoperatively. Twenty-eight adult patients were randomized to receive either a 500,000- or 1,000,000-KIU dose of aprotinin administered *via* a peripheral intravenous catheter over a 30-min period. Blood samples were obtained from a peripheral vein in an opposite extremity before, 15 min during, and just before the end of infusion (30 min), and at the following time intervals after the infusion: 5, 15, 30, and 45 min and 1, 1.5, 2.25, 3, 4, 5, 10, 16, 24, 28, and 32 h. Blood samples were drawn into glass tubes containing sodium ethylenediamine tetraacetate, placed immediately on ice, and centrifuged. The plasma obtained was stored at −20°C until assayed.

Aprotinin concentrations were measured with a sandwich–enzyme-linked immunosorbent assay.[9] Microtiter plates were coated with 100 μl monoclonal antibodies and washed with a 0.05% Tween 80 in phosphate-buffered saline, and 200 μl 1% gelatin solution in buffer was left in the wells for 30 min. The plates were then washed with buffer, and each well was filled with 100 μl aprotinin at a standard concentration or a sample dilution in plasma. Diluting buffer was used as a negative control. The plates were covered, incubated at 37°C for 1.5 h, and washed with buffer, and each well was filled with 100 μl polyclonal rabbit anti-aprotinin immunoglobulin G (Bayer-Germany). The plates were covered, incubated at room temperature for 1.5 h, and washed. Anti-rabbit immunoglobulin G (100 μl) was placed in each well, and the plates were covered and incubated at room temperature for an additional 1.5 h. The plates then were washed, and 100 μl peroxidase substrate solution was placed in each well. After 10–20 min, when the negative controls were still colorless, the enzyme reaction was stopped by adding 100 μl 3 M sulfuric acid.

The sandwich–enzyme-linked immunosorbent assay was read at 450 nm against a reference of 570 nm and quantitatively evaluated. The intraassay coefficient of variation was 7.4% and the interassay coefficient of variation 15% for the plasma concentrations. Aprotinin can also be quantitated by enzyme-inhibition assays using trypsin, kallikrein, or related serine proteases. Comparison of the sandwich–enzyme-linked immunosorbent assay with the enzyme-inhibition assay for aprotinin reveals an adequate correlation for the two assay systems ($r = 0.779$; N = 178).

Extended nonlinear least-squares regression was used to fit a bi- or triexponential equation to the data obtained from each patient.[10] Calculations were implemented using the program MK-MODEL (available from Dr. Nicholas Holford, Department of Pharmacology and Clinical Pharmacology, University of Aukland, Aukland, New Zealand). The optimal model was evaluated using the criteria of Schwartz.[11] Calculated pharmacokinetic parameters for a three-compartment model included compartment volumes (V_1 [central compartment volume], V_2, and V_3); elimination clearance (C_1); distributional clearances from V_1 to V_2 and from V_1 to V_3 (Cl_2 and Cl_3, respectively); and the macro–rate constants and coefficients of the equations describing the concentration. Pharmacokinetic parameters for the two doses were compared among individual patients with a *t* test. Average pharmacokinetic parameters were derived by pooling data, with appropriate normalization for dose, as described by Dyck *et al.*[12]

Results

Demographics of the patients are listed in table 1.

The aprotinin concentration decreased rapidly after completion of the infusion, presumably because of distribution of the drug followed by a more prolonged elimination phase. Plasma concentrations at the end of the 30-min infusion were 147 ± 61 KIU/ml (mean ± standard deviation) for the 1,000,000-KIU dose and 60 ± 19 KIU/ml for the 500,000-KIU dose (figs. 1 and 2). A triexponential equation described the data better than did a biexponential equation for 21 of 28 patients (12 of 16 patients receiving 1,000,000 KIU and 9 of 12 patients receiving 500,000 KIU) and for pooled data for all patients. There was no difference in pharmacokinetic parameters for the two doses (for either the two- or three-compartment model) ($P \leq .05$). Av-

Table 1. Patient Demographics

Units Administered	Number	Men/ Women	Height (kg)	Age (yr)	Creatinine (mg/dl)
500,000	12	8/4	82 ± 18	63 ± 9	1.3 ± 0.5
1,000,000	16	12/4	75 ± 17	66 ± 12	1.2 ± 0.3

Values are mean ± SD.

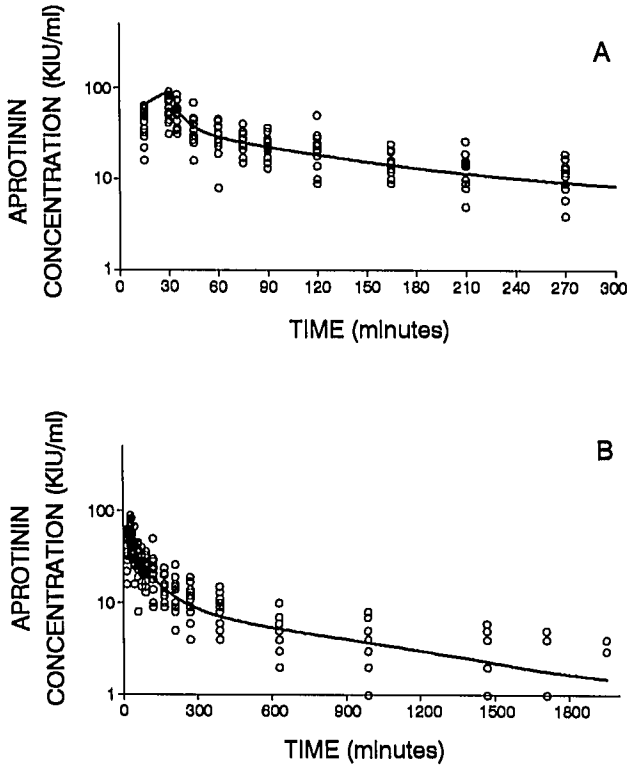

Fig. 1. Concentrations of aprotinin after a 500,000 KIU dose administered over 30 min. The solid line represents the concentrations predicted by the parameters presented in table 2. (*A*) Concentrations measured over 270 min after beginning the administration of aprotinin. (*B*) Concentrations measured over 32 h after the loading dose.

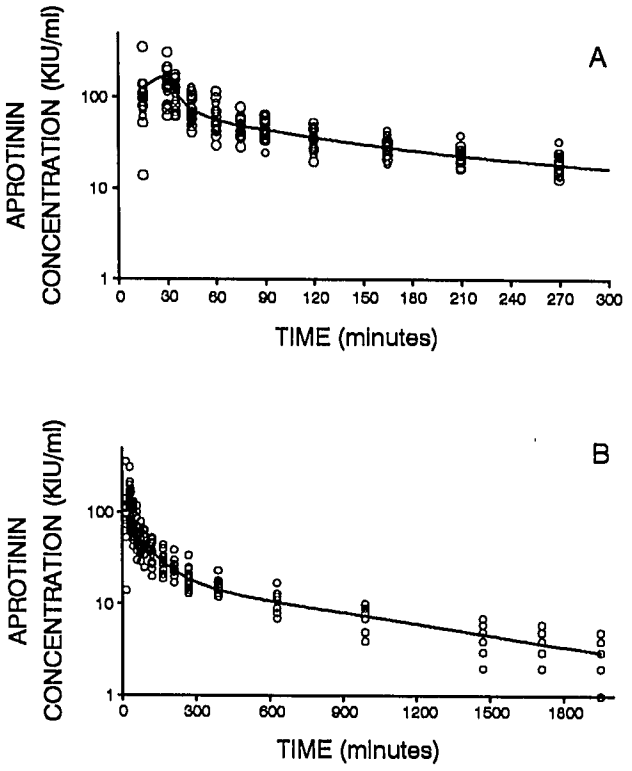

Fig. 2. Concentrations of aprotinin after a 1,000,000 KIU dose administered over 30 min. The solid line represents the concentrations predicted by the parameters presented in table 2. (*A*) Concentrations measured over 270 min after beginning the administration of aprotinin. (*B*) Concentrations measured over 32 h after the loading dose.

erage pharmacokinetic parameters derived from pooled data for a three-compartment model are presented in table 2. The concentrations predicted by these parameters are shown in figures 1 and 2. Values of the three-compartment regression parameters (V_1, V_2, V_3, Cl_1, Cl_2, and Cl_3) for individual patients are presented in table 3.

Discussion

Plasma aprotinin concentrations decline rapidly after intravenous injection because of redistribution to peripheral tissues. During the elimination phase, which is primarily renal, accumulation of aprotinin takes place within the proximal tubular epithelial cells of the kidney, where the drug may be gradually broken down over a several days.[9] Four hours after intravenous administration of iodine 125–labeled aprotinin, approximately 80% of an injected aprotinin dose is localized in the kidney.[9] In healthy volunteers receiving aprotinin as a single dose, the serum concentration (measured as total radioactivity) for 1 to 12 h after administration decreased in a biphasic pattern, with an initial half-life of 0.7 h and a terminal half-life of 7 h.

Table 2. Pharmacokinetic Parameters for Aprotinin

A	B	C	α	β	γ
438	61	19	0.14108	0.01071	0.00095
V_1	V_2	V_3	Cl_1	Cl_2	Cl_3
1.94	4.99	19.59	35.5	146.2	52.1

Parameters were calculated by pooling data from all patients. Parameters include the macro rate constants and coefficients of the equations describing the concentration (C) that would result from a bolus dose $C = A \exp(-\alpha \cdot t) + B \exp(-\beta \cdot t) + C \exp(\gamma \cdot t)$, compartment volumes ($V_1$, V_2, V_3), elimination, and intercompartment distribution clearances (Cl_1, Cl_2, Cl_3). Units are A, B, C = KIU/ml; α, β, γ = min^{-1}; V_1, V_2, V_3 = L; Cl_1, Cl_2, Cl_3 = ml/min. The macro rate constants were normalized to a dose of 1×10^6 kIU.

Table 3. Individual Pharmacokinetic Parameters

Patient No.	V_1	V_2	V_3	Cl_1	Cl_2	Cl_3
1	4.77	6.54	29.38	28.7	124.9	20.5
2	2.43	9.37	9.30	70.9	457.8	35.5
3	2.46	4.61	13.71	25.4	202.6	66.2
4	2.64	6.05	9.15	20.1	182.6	31.2
5	6.69	18.83	11.34	99.6	85.5	412.5
6	3.89	8.42	7.93	30.0	159.3	60.2
7	1.34	8.82	19.90	53.9	344.9	43.7
8	.65	10.00	11.65	39.9	939.5	100.8
9	2.93	6.33	12.37	39.6	242.4	31.2
10	1.86	8.36	17.38	31.5	733.6	24.0
11	2.20	11.64	16.02	51.2	469.8	28.0
12	3.74	6.52	6.79	27.1	92.2	17.5
13	3.10	8.79	29.42	26.3	131.4	34.6
14	2.18	6.69	15.75	35.5	158.3	53.8
15	6.04	6.84	23.46	49.1	130.6	58.7
16	.13	8.56	14.73	38.1	256.0	25.6
17	2.19	4.74	11.51	37.5	161.6	35.5
18	3.73	7.17	34.59	36.1	152.0	79.8
19	.18	2.47	26.66	33.5	205.2	139.5
20	.90	2.36	15.64	28.9	110.5	43.1
21	1.99	10.05	25.54	37.6	311.0	100.7
22	6.18	1.51	17.26	34.6	86.6	60.3
23	3.45	7.10	46.06	19.4	58.9	32.3
24	.32	1.55	19.38	30.8	54.9	45.3

Parameters are as described in table 2. Not included are results from four patients for whom convergence to positive parameter values could not be achieved.

It is difficult to compare fully our results with those of a study in healthy volunteers, because that study did not explicitly include a complete pharmacokinetic characterization of the results.[9] That study also did not consider a three-compartment model, which we found provided a better description of our data than did the two-compartment. The pharmacokinetic parameters that we derived are consistent with those expected for a hydrophilic polypeptide. Elimination clearance is near the expected glomerular filtration rate, and the distribution kinetics suggest diffusion-limited rather than flow-limited transfer to peripheral tissues.

We calculated "average" pharmacokinetic parameters by pooling data from all patients. The more conventional approach (two-stage) for calculating average pharmacokinetic parameters is to determine the mean of the individual parameters derived for each patient. When a two-stage analysis was applied to the individual regression parameters from our data, average compartment volumes of 2.4, 6.18, and 18.8 l (V_1, V_2, and V_3, respectively) and average clearances of 34.4, 213, and 45.8 ml/min (Cl_1, Cl_2, and Cl_3, respectively) were derived. The parameters derived by pooled data and two-stage analysis differ by as much as 30%. We have greater confidence in the robustness of the pooled data parameters.

Although the pooled data approach has the disadvantage of not distinguishing between interpatient and intrapatient variability, there are also disadvantages to the two-stage approach, discussed in detail by Dyck *et al.*[12] These include the assumption of a normal distribution of the parameters and a process of calculating the mean that does not account for variation in the uncertainty of individual parameter estimates. Furthermore, Shafer *et al.*,[13] Egan *et al.*,[14] and Dyck *et al.*[12] have shown that the pooled data technique can produce robust parameter estimates that are superior to two-stage estimates, when evaluated on the basis of how well the parameters can be used to dose the drug so that a desired target plasma concentration is achieved. In addition, in the current study, use of the two-stage approach was complicated because the same model (two *vs.* three compartments) was not optimal for all patients. For a two-stage calculation, we had either to report two sets of pharmacokinetic parameters (for both the two- and the three-compartment model) or to use the three-compartment model and either include parameters from patients for whom this model clearly did not fit the data optimally or simply disregard the data from these patients.

Given the documented utility of the pooled data technique, we selected it for primary analysis.[12-14] An alternative method for analyzing population pharmacokinetics, NONMEM, independently accounts for interpatient and intrapatient variability.[15] The "naive" pooled data approach used in this study does not characterize data variance as well as NONMEM, but structural model parameter estimates appear to be robust.[12-14]

A significant shortcoming of this study is that it was conducted before cardiac surgery and cardiopulmonary bypass. It is quite possible that the kinetics of this drug could be significantly altered by anesthesia, intraoperative hemodynamic changes, and the specific effects of cardiopulmonary bypass including hypothermia, hemodilution, nonpulsatile flow, and the exclusion of the heart and lungs from the circulation. Extrapolation of the results of this study to the calculation of aprotinin dosage for patients during cardiac surgery must be done cautiously.

Given this caveat, we can estimate the infusion rates needed to maintain plasma concentrations in excess of either approximately 200 or 50 KIU/ml using an al-

gorithm recently described by Bailey.[16] This technique produces the target plasma concentration at specific points in time using sequential infusions. Between these points the concentration is, in general, lower than the target. Because the plasma concentration needed to inhibit kallikrein is 200 KIU/ml or greater, we take 250 KIU/ml as a target and consider a triphasic infusion: 0–30 min (roughly corresponding to the time from induction to skin incision), 30–90 min (corresponding to the prebypass period), and a steady-state infusion for the duration of the procedure. The calculated infusion rates are 52,000 KIU/min for 30 min, 26,000 KIU/min for the next 60 min, and then a steady-state infusion at 10,400 KIU/min. In addition, 500,000 KIU should be added to the cardiopulmonary bypass circuit prime if we assume a priming volume of 2.5 l. The plasma concentrations predicted for this proposed regimen are shown in figure 3, demonstrating that plasma concentrations are predicted to range from 250–300 KIU/ml. To maintain plasma concentrations in excess of 50 KIU/ml (the value needed to inhibit plasmin), the infusion rates are adjusted by a factor of 5.

These calculations are, of course, only approximate guides to appropriate drug dosages, because the pharmacokinetic parameters were derived preoperatively and not during actual cardiac surgery with cardiopulmonary bypass. With the complexity of the interactions of aprotinin and the coagulation, fibrinolytic, and inflammatory cascades, the concentrations required for inhibiting kallikrein or plasminogen or plasmin may be different during extracorporeal circulation and in comparisons of *in vitro* and *in vivo* concentrations.

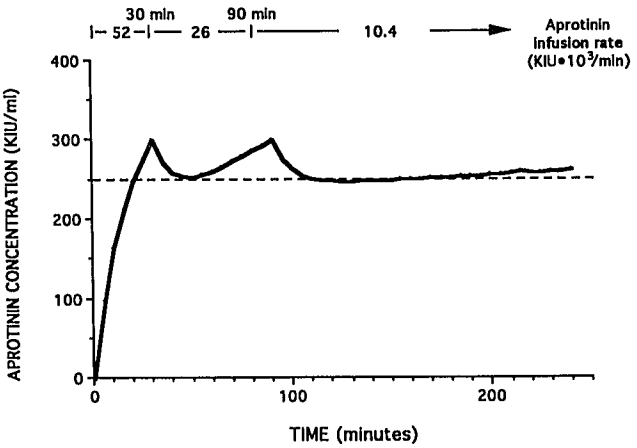

Fig. 3. Infusion model, based on derived pharmacokinetics, to obtain plasma aprotinin concentrations of 250 KIU/ml to inhibit kallikrein.

In summary, using a sandwich–enzyme-linked immunosorbent assay to assay aprotinin concentrations in plasma, we noted a shorter half-life for aprotinin in cardiac surgical patients than that reported in volunteers. A 2,000,000-KIU loading dose of aprotinin produces plasma concentrations effective to inhibit both kallikrein and plasmin. However, a 1,000,000-KIU loading dose produces plasma concentrations that are adequate to inhibit plasmin alone. This may explain the efficacy of smaller aprotinin doses.

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