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Anaphylactic Reactions to Aprotinin Reexposure in Cardiac Surgery

Relation to Antiaprotinin Immunoglobulin G and E Antibodies

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Background: **Aprotinin, a serine proteinase inhibitor, reduces bleeding during cardiac surgery. As aprotinin is derived from bovine lung, it has antigenic properties. This investigation examined the incidence of anaphylactic reactions in patients reexposed to aprotinin and the relation to preformed antiaprotinin immunoglobulin (Ig)G and IgE antibodies.**

Methods: **This prospective observational study conducted at five centers in Germany evaluated patients undergoing repeat cardiac surgery reexposed to aprotinin between 1995 and 1996. Antiaprotinin IgG and IgE antibody measurements, using a noncommercial enzyme-linked immunosorbent assay and an immunofluorescence assay, respectively, were performed preoperatively and postoperatively. An anaphylactic reaction was defined as major changes from baseline within 10 min of aprotinin administration of systolic pressure 20% or greater, heart rate 20% or greater, inspiratory pressure greater than 5 cm H_2O, or a skin reaction.**

Results: **In 121 cases (71 adults, 46 children), a mean aprotinin reexposure interval of 1,654 days (range, 16–7,136 days) was observed. Preoperative antiaprotinin IgG (optical density ratio > 3) and IgE antibodies (radioallergosorbent test [RAST] score < 3) were detected in 18 and 9 patients, respectively. High concentrations of each (IgG, optical density ratio > 10; IgE, RAST score ≥ 3) were detected in five patients. Three patients (2.5%; 95% confidence interval, 0.51–7.1%) experienced an anaphylactic reaction after aprotinin exposure, followed by full recovery; these patients had reexposure intervals less than 6 months (22, 25, and 25 days) and the highest preoperative IgG concentrations of all patients ($P < 0.05$). Assay sensitivity was 100%, as no anaphylactic reactions occurred in IgG-negative patients (95% confidence interval, 0.0–3.1%); assay specificity was 98%. Preoperative IgE measurements were quantifiable in two of three reactive patients and in three nonreacting patients.**

Conclusions: **Quantitative detection of antiaprotinin IgE and IgG lacks specificity for predictive purposes; however, quantitation of antiaprotinin IgG may identify patients at risk for developing an anaphylactic reaction to aprotinin reexposure.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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APROTININ (Trasylol, Bayer Vital, Leverkusen, Germany) reduces perioperative bleeding during cardiac surgery.[1,2] A polypeptide (58 amino acids; 6.5 kd)[3] serine proteinase inhibitor, aprotinin is thought to reduce bleeding through inhibiting the contact phase activation of hemostasis, preventing fibrinolysis,[4] and reducing thrombin generation.[5]

As a protein derived from bovine lung, aprotinin possesses antigenic properties in humans.[6] After aprotinin administration, formation of antiaprotinin immunoglobulin (Ig)G and/or IgE antibodies has been demonstrated.[7–12] Anaphylactic and anaphylactoid reactions have been described for patients receiving the drug,[9,10,12–14] even after local application.[15] Incidence of these reactions after aprotinin reexposure is reported as 2.8%.[6] These observations suggest that patients who receive aprotinin treatment during an initial cardiac operation may develop antiaprotinin antibodies and thus be at risk for experiencing the antigen–antibody-dependent immune response termed *anaphylactic reaction* on reexposure to the drug.

Preoperative detection of antiaprotinin antibodies may help identify patients at risk for developing an anaphylactic reaction after aprotinin reexposure. The objective of the current study was to monitor antiaprotinin IgG and IgE antibodies before and after a second exposure to aprotinin during cardiac surgery and relate the presence of antibodies to anaphylactic reactions.

Methods

Study Design

This prospective and observational study was conducted at five sites within Germany. The study was subject to review and approval by local Ethics Committees (Ethikkommission der Bayerischen Landesärztekammer, Munich, Germany).

Patients

Patients undergoing reoperative open heart surgery, exposed previously to aprotinin and receiving aprotinin during the subsequent procedure, were eligible for enrollment. Those with coronary, valvular, or congenital heart disease were enrolled provided that they (or parents for pediatric patients) gave written, informed consent. Patients were excluded if they had a known hypersensitivity to aprotinin, received preoperative immunosuppressive therapy, or required emergency cardiac surgery. The population was divided into adults (n = 71) to receive unadjusted dosage regimens and pediatric patients (n = 46), arbitrarily defined as patients less than 12 years of age, to receive a weight-based dosage (see below).

Aprotinin Prick Test

Before induction of anaesthesia, administration of prophylactic antihistamines (see below), aprotinin treatment, and surgery, an aprotinin prick test was performed on the volar aspect of the forearm. Patient reaction to undiluted drug was compared with that to saline (negative control) and histamine (positive control). Test results were evaluated after 15 min. A positive reaction was defined as a local wheal 3 mm or greater in size and surrounded by erythema.[7] In cases of a weak reaction to histamine, an aprotinin-evoked wheal two thirds the size of the histamineevoked wheal was considered a positive reaction. The decision to use the prick test in pediatric patients was left to the investigator, as was the decision to include a patient with a positive prick test result.

Aprotinin Treatment

The study medication was supplied by the manufacturer (Bayer Vital). Each patient received a 1×10^4 kallikrein inactivator units (KIU) aprotinin test dose at least 10 min before administration of the first aprotinin bolus dose after skin incision. If no reaction occurred to the test dose, treatment of adult patients was initiated with a 2×10^6 KIU loading dose administered over 20 min, followed by continuous infusion of 5×10^5 KIU/h (50 ml/h). Additionally, the oxygenator was primed with a 2×10^6 KIU aprotinin bolus.

If no reaction occurred to the test dose, pediatric patients were treated using a weight-adjusted dosage—an aprotinin bolus dose of 3×10^4 KIU/kg of body weight over 15 min. The oxygenator was primed with the same dose. Aprotinin was not continuously infused in pediatric patients.

Prophylactic Treatment

Histamine H_1 and H_2 receptor antagonists were administered prophylactically at the induction of anesthesia to all patients. Adults received either 0.1 mg/kg dimetindine or 0.03 mg/kg clemastine together with 5.0 mg/kg cimetidine, 2.0 mg/kg ranitidine, or 20 mg famotidine. Additional corticosteroid treatment was administered at the discretion of the investigator.

Anesthesia and Cardiopulmonary Bypass

The protocol had no restrictions on the anesthetic and cardiopulmonary bypass (CPB) techniques used. Anesthesia was performed according to the local procedures of each study site.

Blood Sampling

Blood samples were obtained before induction of anesthesia and 5–7 days postoperatively. If an adverse reaction was suspected to have occurred, additional samples were collected on arrival of the patient in the intensive care unit and at 3 weeks and 3 months after surgery.

Antibody Measurements

Concentrations of antiaprotinin IgG antibodies were quantified using an enzyme-linked immunosorbent assay.[16] Quantification was performed by dividing the mean optical density (OD) value at each dilution of serum sample by the corresponding values for a control serum on the same 96-well micro–enzyme-linked immunosorbent assay plate coated with 200 KIU/ml (28.8 μg/ml) aprotinin. Free binding sites were blocked with 1% gelatin. After washing with phosphate-buffered saline–0.05% Tween 80, 100 μl/well diluted sera from patients and untreated healthy donors was added to the wells, sealed, and incubated at 37°C for 1.5 h. After washing, biotinylated affinity purified rabbit antihuman IgG (Jackson/Dianova, Hamburg, Germany; 1:80,000) was added and incubated for 1 h at room temperature. After washing, streptavidin-conjugated alkaline phosphatase (Jackson/Dianova; 1:2000) was added to the wells and incubated for 1 h at room temperature. Plates were developed by adding the substrate (p-nitrophenylphosphate, Sigma, Munich, Germany) in diethanolamine buffer. OD was measured at 405 nm after 30–60 min on a microreader (SLT 340, ATTC, Crailsheim, Germany). To exclude uncertain results that may be obtained by noise fluctuations at low OD ranges, an OD ratio greater than 3 at 1:800 and a consistent detectable pattern in all four sample dilutions was defined as a positive result. A serum sample with an OD ratio greater than 10 at a dilution of 1:800 was regarded as containing high concentrations of aprotinin-specific IgG antibodies. A fully automated analyzer, uniCAP, was used for semiquantitative determination of aprotinin-specific IgE antibodies with a commercial kit (Pharmacia, Feiburg, Germany).[15] IgE antibodies bound to aprotinin were automatically quantified by traditional radioallergosorbent test scores. A serum sample with a score of 3 or more was considered to have high concentrations of antiaprotinin specific IgE antibodies.

All antibody assay determinations were at least performed duplicate and deviated less than 10% between repetitive measurements. For every sample, at least four serial dilutions were tested. The assays were validated with respect to specificity (*i.e.*, competition binding experiments with antiaprotinin monoclonal antibodies and antiaprotinin–mutein monoclonal antibodies as well as Western blotting) and precision.

Outcome Measures

The primary outcome was the incidence of anaphylactic reactions in patients reexposed to aprotinin during cardiac surgery. Hemodynamic parameters (heart rate, systemic arterial pressure, pulmonary artery or pulmonary capillary wedge pressure, central venous pressure, ventilation pressure, fraction of inspired oxygen, arterial oxygen partial pressure, and arterial carbon dioxide partial pressure) were recorded during steady state after induction of anesthesia and at the end of the loading dose. For probable or suspected reactions, parameters were recorded every 15 min, after 1 h, every 30 min until recovery. All patients were carefully observed for skin reactions after aprotinin infusion. Secondary end points included measurements of antiaprotinin antibodies and their changes over time.

Adverse Reaction Definitions

An anaphylactic reaction was assumed when at least one of the following symptoms appeared within 10 min of aprotinin administration: (1) a decrease from baseline systolic pressure 20% or greater; (2) a change from baseline heart rate 20% or greater; (3) an increase from baseline inspiratory pressure greater than 5 cm H_2O; and (4) a skin reaction. Anaphylactic reactions were classified as mild (no intervention required), moderate (circulation restored within 15 min of reaction onset *via* use of vasopressors), or severe (longer-lasting circulatory depression and instability despite administration of vasopressors).

Statistical Analyses

Patients were categorized by an exposure–reexposure interval of less than or more than 6 months, as previous data indicate a time-dependent risk for developing a reaction approximating this interval.[6] Demographic data are presented as means with SDs. Differences in demographic characteristics were evaluated using the Student *t* test and chi-square or Fisher exact test for dichotomous variables, with $P < 0.05$ considered statistically significant. Categoric data are presented as frequencies.

The incidence of anaphylactic reactions was calculated as percent with 95% confidence intervals. Based on previous findings,[6] the incidence of anaphylactic reactions was estimated to be 2.8%. Thus, the 95% confidence interval of the occurrence of such an event was 0.3–10% as calculated for 120 reexposures.

The predictive value of the presence of quantifiable antiaprotinin IgG and IgE antibodies on occurrence of an anaphylactic reaction was determined. IgG or IgE test sensitivity was calculated by dividing the number of patients reacting adversely to aprotinin treatment and having high antibody results by the number of all patients experiencing an adverse reaction to aprotinin treatment. Test specificity was calculated by dividing the number of patients not reactive to aprotinin administration and having negative antibody results by the number of all patients reactive to aprotinin treatment but having negative antibody results. The predictive value for the occurrence of an anaphylactic event with a high antibody test result was calculated by dividing the number of patients experiencing an anaphylactic reaction to aprotinin and having high antibody test results by the total number of high antibody test results. Discriminatory power with respect to anaphylactic reactions for the assays, calculated as the proportion of predictive value for reaction to no reaction, were compared using the two-tailed Fisher exact test.

Results

Patients

Of the 118 patients enrolled, one did not receive aprotinin treatment because surgery was performed without extracorporeal circulation. Consequently, 117 patients (71 adult, 46 pediatric) were evaluated, for a total of 121 documented reexposures to aprotinin. Four patients each experienced two reexposures to aprotinin on study. Demographic data are summarized in table 1.

The preoperative aprotinin prick test, conducted in 70 adult and 14 pediatric patients, was not positive in response to undiluted aprotinin in any case. Because of rapid commencement of CPB, three patients did not receive an aprotinin test dose. The interval between aprotinin treatments was less than 6 months in 29 aprotinin reexposure cases. Prophylactic corticosteroid therapy was administered to 25% of adult and 83% of pediatric patients.

Measurement of Antiaprotinin Immunoglobulin G and E Antibodies

Preoperative concentrations of antiaprotinin IgG antibodies were high for 5 of 121 reexposures (4%), detectable for 13 of 121 reexposures (11%), and negative for 103 of 121 reexposures (85%). Of the five reexposures associated with high concentrations of IgG antibodies, three represented patients experiencing an anaphylactic reaction to aprotinin treatment. Notably, adverse reactions to aprotinin treatment were not observed in patients with no or only detectable concentrations of IgG antibodies. Using the high IgG antibody concentration, the diagnostic sensitivity of the assay was 100% (3/3).

Table 1. Patient Demographic and Surgical Characteristics*

Characteristic	Adults (≥ 12 yr)	Pediatrics (< 12 yr)
N (male:female)	71 (44:27)	46 (26:20)
Age [mean ± SD (range), yr]	58 ± 16 (12–79)	3.5 ± 3.1 (0.1–11)
Weight [mean ± SD (range), kg]	71 ± 13 (39–104)	14.7 ± 9.9 (3–46)
Presence of acquired allergies† [n (%)]		
Single	16 (23)	3 (6.5)
Multiple	6 (8)	0 (0)
Duration of CPB (mean ± SD, min)	107 ± 49	72 ± 36
Duration of surgery (mean ± SD, min)	288 ± 81	253 ± 71
Type of surgery		
Coronary artery bypass graft (CABG) (n)	26	0
Valve replacement (n)	33	0
Combined CABG–valve operation (n)	1	0
Congenital heart disease (n)	5	46
Other‡ (n)	6	0
Total aprotinin dose during current surgery [mean ± SD (range), KIU]	$3.9 \pm 1.2 \times 10^6$ ($0.1–8.5 \times 10^6$)	$0.11 \pm 0.4 \times 10^6$/kg ($0.037–0.22 \times 10^6$/kg)
Total aprotinin dose during preceding surgery [mean ± SD (range), KIU]	$3.7 \pm 1.2 \times 10^6$ ($0.1–8.0 \times 10^6$)	$0.074 \pm 0.052 \times 10^6$/kg ($0.004–0.20 \times 10^6$/kg)
Interval since last aprotinin exposure (mean ± SD, days)	1,654 ± 1,523	834 ± 857
Use of corticosteroids [n (%)]	18 (34)	38 (83)
Use of H_1 and H_2 receptor antagonists [n (%)]	70 (99)	44 (96)

* At surgery performed during the study. † Acquired allergies included allergies to penicillin and other antibiotics, contrast dye, bee or wasp poison, pollen, plaster, and the sun. ‡ Other procedures included heart transplantation, ascending aorta replacement, and mitral valvuloplasty.

CPB = cardiopulmonary bypass; KIU = kallikrein inactivator units.

Specificity was 98% (116/118). The predictive value of a high IgG assay result was 60% (3/5), whereas the predictive value of a negative result (116/116) was 100% (discriminatory power, $P < 0.05$).

Preoperative concentrations of antiaprotinin IgE antibodies were high in five patients. The diagnostic sensitivity of the assay was 67% (2/3) and the diagnostic specificity was 97% (111/114). The predictive value of a high positive IgE assay result was 40% and that of negative assay result was 99% (discriminatory power, $P < 0.05$). All high IgE results were associated with aprotinin exposure–reexposure intervals of less than 6 months.

The relation between antiaprotinin IgG antibody concentration and aprotinin exposure time interval is summarized in table 2. The three patients experiencing adverse reactions to aprotinin had the shortest treatment exposure–reexposure intervals and highest preoperative IgG concentrations. Preoperative and postoperative antiaprotinin IgG antibody concentrations for patients with detectable concentrations are shown in figure 1. Notably, patients 1 and 3 had the highest preoperative IgE concentrations, whereas patient 2 did not have detectable IgE concentrations before, or at any time during, the adverse reaction (data not shown).

In the postoperative period, concentrations of antiaprotinin IgG and IgE antibodies increased significantly relative to preoperative concentrations ($P < 0.05$). Five to seven days postoperatively, 13 patients (12%) had high IgG concentrations, and 12 patients (11%) had detectable IgG concentrations. Moreover, high IgE concentrations were found in eight patients (7%), and detectable IgE concentrations were observed in 10 patients (9%). Figure 2 depicts the course of IgG concentrations in the three patients experiencing an adverse reaction to aprotinin. Three weeks after the event, the IgG concentration was very high in patient 3, while after 3 months

Table 2. Relation between Antiaprotinin IgG-Antibody Concentration and Time Interval since Last Aprotinin Exposure

Exposure–Reexposure Interval (days)	Reexposures (n)	Negative IgG Concentrations [n (%)]	Detectable* IgG Concentrations [n (%)]	High IgG Concentrations [n (%)]	Adverse Reactions [n (%)]
≤ 90	17	10 (59)	3 (18)	4 (24)	3 (17.6)
91–180	12	7 (58)	5 (42)	0 (0)	0 (0)
181–270	6	3 (50)	2 (33)	1 (17)	0 (0)
271–365	6	4 (67)	2 (33)	0 (0)	0 (0)
> 365	80	79 (99)	1 (1)	0 (0)	0 (0)
Total	121	103 (85)	13 (11)	5 (4)	3 (2.5)

* Detectable but not high.

IgG = immunoglobulin G.

Table 3. Anaphylactic Reactions to Aprotinin: Descriptive Patient Data

Patient	IgE (RAST Score)		IgG (Ratio Mean OD Patient: Control Sera)*		Antihistamine Treatment before Test	Corticosteroid Treatment	Other Medications before Reaction	Aprotinin Prick Test	Aprotinin Test Dose	Time to Reaction	Other Known Allergies
	Before	After	Before	After							
1	2	2	16.8	8.5†	Clemastine–cimetidine	Remedial 250 mg pred	Cefuroxim, 935 mg, 45 min before reaction; pancuroniumbromid, flunitrazepam, fentanyl	Negative‡	5,000 KIU	Immediate with test dose	None
2	0	0	17.35	13.5§	Clemastine–cimetidine	Premedication 4 mg dex; intraoperative 250 mg pred	Cefuroxim, 1.0 g, 110 min before reaction; pancuroniumbromid, flunitrazepam, fentanyl halothane vol sub: dextran 40% 80 ml	Not tested	10,000 KIU well-tolerated	20 min after test; 5 min after load (630,000 KIU; plus 10^6 KIU pump prime)	Penicillin
3	2	2	22.95	23.2§	Clemastine–cimetidine	Remedial 80 mg dex	Vancomycin, 1.0 g/day, 60 min before reaction; refobacin 570 min before reaction; sufentanil, midazolam, pancuroniumbromid vol sub: HES 6% 500 ml	Negative‡	10,000 KIU well-tolerated	11 min after test; 1 min after load (100,000 KIU)	Penicillin

* Conducted at sample dilution of 1:800 which has a continuous ratio range of approximately 1–30. † Value 3 months postoperatively. ‡ High histamine-control-response observed. § Maximum value 3 weeks postoperatively.

IgE = immunoglobulin E; RAST = radioallergosorbent test; IgG = immunoglobulin G; OD = optical density; pred = prednisolone; dex = dexamethasone; vol sub = volume substitution; HES = hydroxyethylstarch.

it was still high in patient 2. The IgG concentration was detectable in patient 1 at 3 months.

Adverse Reactions

Of 121 aprotinin reexposures, three (2.5%) were associated with an immediate hypersensitivity reaction (table 2). Two patients had experienced a second reexposure to aprotinin within the study period. One patient reacted to the aprotinin test dose, whereas the other two reacted during the loading dose. All had cardiovascular collapse and demonstrated a skin flush. The time intervals since the last aprotinin exposure were 22, 25, and 25 days, respectively. The incidence of anaphylactic reactions occurring within an aprotinin exposure–reexposure interval of less than 6 months was 3 of 29 exposures (10.3%; 95% confidence interval, 2.2–27.4%); within an exposure–reexposure interval of 6 months or more, the rate was 0% (95% confidence interval, 0.0–3.9%).

Patient 1, a 7-yr-old boy diagnosed with pulmonary atresia, had two previous operations with aprotinin, the second of which was documented in the current study. A revision of the shunt was necessary 22 days later. Immediately after administration of the aprotinin test dose (1×10^4 KIU) after sternotomy, the patient developed an extended flush and hemodynamic instability (systolic blood pressure, 105–44 mmHg; heart rate, 105–185 beats/min). Concomitantly, severe bleeding from the venous cannulation site was evident, complicating the diagnosis. Perfusion pressure during CPB was remarkably low. Inotropic support with dopamine and norepinephrine was required after termination of CPB. The postoperative course was uneventful. The reaction was judged "severe," and relation to study drug assessed as "probable."

Patient 2, an 8-yr-old girl with an Ebstein anomaly, had undergone two previous valvuloplasties, receiving aprotinin during the first and second (1.7×10^6 KIU) procedure 2 yr later. A revision was necessary 25 days after the second operation. During the current operation, the patient received prophylactic dexamethasone treatment and did not respond adversely to the aprotinin test dose. After a 0.63×10^6 KIU aprotinin bolus dose, the patient manifested a facial flush, increased in inspiratory pressure (10–20 cm H_2O), and decreased systolic blood pressure (100–70 mmHg). At this time, the surgeon manipulated the heart. The oxygenator had been primed with aprotinin 1×10^6 KIU, which the patient received on initiation of CPB. Perfusion pressure during CPB was low despite vasopressor therapy. After termination of CPB, the patient recovered with inotropic support. The postoperative course was uneventful. The reaction was judged "moderate," and relation to study drug was assessed as "remote."

Patient 3 was a 55-yr-old woman who underwent a mitral valvuloplasty and received aprotinin 5.5×10^6 KIU. The patient required repeat operation 25 days later. The test dose was well tolerated. One minute after administration of 0.05×10^6 KIU of the aprotinin loading

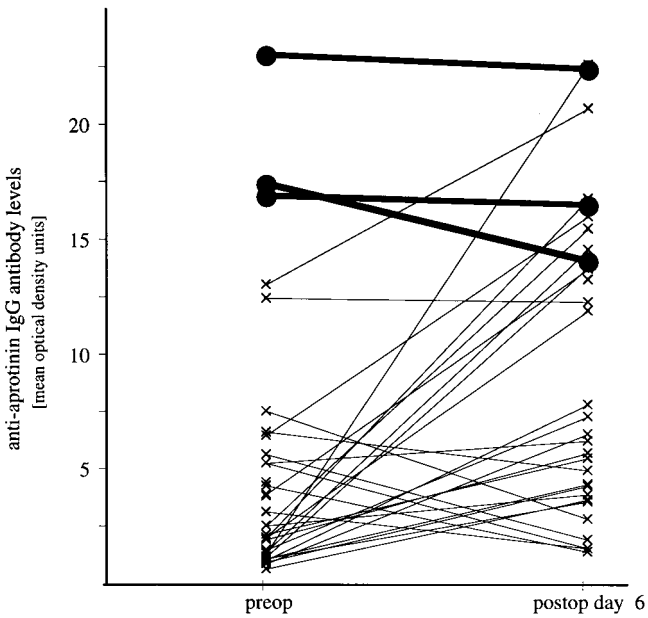

Fig. 1. Preoperative and postoperative antiaprotinin immunoglobulin (Ig)G antibody concentrations in all patients with measurable IgG concentrations. Although only patients with an optical density ratio greater than 3 were defined as positive for aprotinin-specific IgG antibodies, this figure includes all results with a measurable optical density. Patients experiencing an anaphylactic reaction to aprotinin reexposure are represented by the heavy lines and filled circles.

dose, systolic pressure decreased (110–25 mmHg), and a facial flush was observed. CPB was commenced within 5 min. Perfusion pressure remained remarkably low despite vasopressor therapy. Hemodynamic instability complicated termination of CPB. The clinical status improved slowly over the next 6 h. The reaction was judged "severe," and relation to study drug assessed as "probable."

Adverse events experienced by other patients included a case of renal insufficiency, which resolved, and a reaction to protamine. These events were not considered to be related to aprotinin treatment. Four patients died during their hospital stay. These deaths were considered unrelated to study drug. One patient developed an arrhythmia immediately after surgery and died with low cardiac output despite an emergency surgical reintervention. The other three patients died outside of the study phase, one at day 9 subsequent to a stroke, and the other two from multiple organ failure at days 29 and 49 postoperatively, respectively.

Discussion

Aprotinin, a protein derived from bovine lung, possesses antigenic properties. Immediate-type allergic reactions have been reported with the clinical use of this drug.[12,17,18] In addition, several case reports have described the presence of antiaprotinin IgG and/or IgE antibodies after exposure to aprotinin,[7–11] even in patients treated topically with fibrin glue containing aprotinin.[15] Weipert *et al.*[8] and Pfannschmidt *et al.*[7] reported detection of antiaprotinin IgG antibodies in approximately 50% of all patients and as long as 4 yr after exposure to aprotinin. Anaphylactic reactions after primary aprotinin exposure have been reported as a rare event.[19] Previously, the rate of anaphylactic reactions associated with aprotinin reexposure in heart surgeries was observed to be 2.8%.[6] Consistent with our previous report, in the current investigation the incidence of anaphylactic reactions associated with aprotinin reexposure is 2.5%.

The current results show that measurement of antiaprotinin IgG antibodies helps to identify patients at risk for developing an anaphylactic reaction on reexposure. During this study, the three patients with the highest preoperative IgG concentrations experienced an adverse reaction. Eleven percent of patients expressed detectable (but not high) preoperative IgG concentrations and did not react to aprotinin reexposure. Thus, the existence of antibodies *per se* is necessary but not sufficient for developing an anaphylactic reaction to aprotinin reexposure. The quantitative concentration of antiaprotinin IgG antibodies appears to dictate the emergence of this phenomenon.

Antiaprotinin IgG antibody concentrations were elevated at postoperative day 6 as compared with preoperative concentrations. Notably, two patients (patients 1 and 2) with a repeat reexposure to aprotinin and experiencing an adverse reaction showed even higher IgG concentrations 3 weeks after surgery as compared with those after the last operation. Thus, a booster effect may have caused the adverse reaction on the third exposure. Furthermore, our findings suggest that IgG antibody formation can take several days, which may explain the

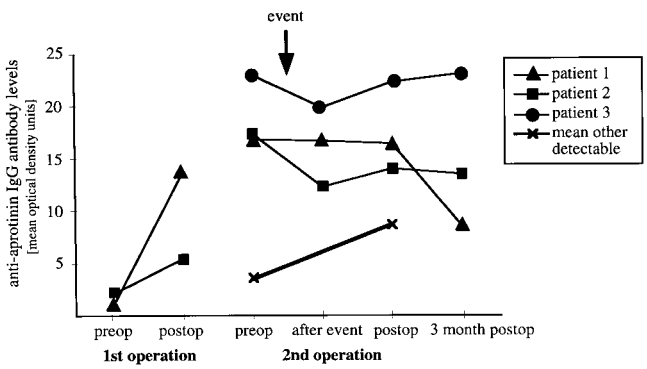

Fig. 2. Time course of antiaprotinin immunoglobulin (Ig)G antibody concentrations in three patients experiencing an anaphylactic reaction to aprotinin treatment. Notably, patients 1 and 2 had a repeat reexposure to aprotinin treatment during the study, and the data from the first (uneventful) treatment are included in the figure. For comparison, the mean IgG antibody concentrations in other patients with detectable antibody concentrations are also presented. IgG concentrations were measured using an enzyme-linked immunosorbent assay.

inability to detect IgG antibodies in some study patients postoperatively. This observation is consistent with results reported by Weipert *et al.*[8] showing that 33% of patients do not express antiaprotinin antibodies immediately after surgery. Furthermore, these data reinforce the authors' clinical observation that reexposure to aprotinin within 24–36 h of the initial exposure does not put the patient at risk for developing an adverse reaction to the drug.

As described previously,[6] the propensity to react adversely to aprotinin reexposure is dependent on the duration of the exposure–reexposure interval. In this study, all three reactions occurred within 4 weeks after the last exposure to aprotinin. Data from this study also indicate that the highest antiaprotinin IgG concentrations exist within 6 months after the last aprotinin exposure. Thus, an exposure–reexposure interval of less than 6 months appears to be critical for predisposing a patient to a potential adverse allergic reaction to aprotinin.

The three patients experiencing an adverse reaction to aprotinin reexposure underwent typical surgical procedures that include the risk of short-term repeat operation: failed valvuloplasties[9,15,20] and a shunt operation with inadequate postoperative oxygenation.[10] These types of procedures, as well as those using a left heart assist device followed by heart transplantation,[21,22] are typically associated with reports of adverse reactions to aprotinin treatment. As the incidence of adverse reactions with an aprotinin exposure–reexposure interval of greater than 6 months was observed to be 0%, determination of antiaprotinin IgG antibody concentrations is suggested for patients with an exposure–reexposure interval of less than 6 months and an expected clinical benefit from aprotinin use during surgery. Thus, within a reexposure interval of 6 months, aprotinin should be used only in exceptional cases, with definite caution, and then after testing for IgG antibodies, if possible. In these exceptional cases, recommended precautionary measures include having standard treatments for emergency hypersensitivity–anaphylactic reactions (*e.g.*, epinephrine, corticosteroids) readily available, administering test and loading doses when conditions for rapid aortic cannulation (if necessary) are present and delaying addition of aprotinin into the pump prime solution until after the loading dose has been safely administered.

The results of the preoperative aprotinin prick test were disappointing. Skin tests are the basic tool used to assess IgE antibody-mediated hypersensitivity.[23] No patient evaluated in this study showed positive preoperative aprotinin prick tests, a finding consistent with our previous observation.[6] As no difference was observed in sensitivity of intradermal testing *via* needle or prick test,[24] lack of positive results obtained with the prick test in this study most likely does not reflect a methodologic weakness. Only a few published reports of a positive aprotinin prick test result after an anaphylactic reaction to aprotinin treatment exist.[9,25] However, preformed IgE antibodies have been observed in patients presenting with severe anaphylaxis,[9,12,13] and the possibility exists that the patient showing no IgE antibodies produced a false-negative test result. Thus, aprotinin appears to be able to evoke both IgG- and IgE-mediated reactions.

Anaphylactic reactions are mediated by histamine[26] and other mediators, providing a rationale for the prophylactic use of H_1 and H_2 receptor antagonists with aprotinin treatment. Lorenz *et al.*[27] showed that prophylactic treatment with H_1 and H_2 receptor antagonists significantly reduced the incidence of anaphylactic reactions in response to gelatin infusion. Similarly, cutaneous responses to aprotinin may have been masked by administration of these drugs.[28] Whether prophylactic H_1 and H_2 receptor antagonism would similarly decrease the incidence or severity of anaphylactic reactions after aprotinin treatment cannot be inferred from current study results because all evaluated patients received this prophylactic therapy. Furthermore, 46% of patients received preoperative corticosteroid therapy. Whether this treatment attenuated or prevented the development of anaphylaxis also cannot be deduced from present results. However, note patient 2 received preoperative corticosteroid therapy and experienced the least severe reaction of all three reacting patients.

The data derived from this study have provided important information regarding the incidence of immediate-type anaphylactic reactions to aprotinin reexposure in cardiac surgery; however, this must be considered within the context of the study limitations. First, during the clinical situation of open heart surgery, unequivocal identification of an allergic reaction and its cause is no trivial matter. Many potentially antigenic drugs are administered, the hemodynamic situation at the time of aprotinin application is often unstable (as the surgeon may be manipulating the heart and the patient may be bleeding), and commencement of CPB completely alters patient hemodynamics. The case reports of the current study underscore this difficulty. However, even with these confounders, long-lasting low perfusion pressure during CPB despite vasopressor therapy seems to be indicative of an anaphylactic reaction. Second, the sample size of the study was too small to provide an exact number for the incidence of reactions in response to aprotinin reexposure. Third, the study was not designed to assess the effects of various prophylactic treatments on the development of adverse reactions to aprotinin therapy, as all patients were treated with H_1 and H_2 receptor antagonists. Future work could address this directly by monitoring for the response of IgE-sensitized cells with markers of mast cell degranulation such as mast cell tryptase.[29] Finally, a number of factors are thought to effect allergic responses, including age, gen-

der, genetics, previous drug reactions, concurrent illnesses, administration route and speed, and dose. Although this study documented many of these factors, the contribution of each is difficult to gauge in this small population.

In summary, this study found the incidence of anaphylactic reactions after aprotinin reexposure during cardiac surgery to be 2.5%. A preoperative aprotinin prick test was not predictive of response to aprotinin treatment. Detectable IgG or IgE antibodies to aprotinin were not always clinically relevant. All three patients experiencing an adverse reaction in response to aprotinin treatment had high concentrations of antiaprotinin IgG antibodies, whereas two of these three patients expressed high concentrations of IgE antibodies. The propensity of a patient to react adversely to aprotinin treatment was highly dependent on the length of the aprotinin exposure–reexposure interval. Within a reexposure interval of 6 months, aprotinin should be used with definite caution only in exceptional cases, such as in patients at high risk of bleeding expected to benefit clinically from aprotinin treatment during cardiac surgery. In these instances, quantitation of antiaprotinin antibody concentrations may identify those more likely to develop an anaphylactic reaction to this drug.

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