APROTININ IN FIBRIN TISSUE ADHESIVES INDUCES SPECIFIC ANTIBODY RESPONSE AND INCREASES ANTIBODY RESPONSE OF HIGH-DOSE INTRAVENOUS APPLICATION

Albertus M. Scheule, MD
Wolfram Beierlein, MD
Hans P. Wendel, PhD
Michael J. Jurmann, MD
Friedrich S. Eckstein, MD
Gerhard Ziemer, MD

Background: In cardiac operations, aprotinin therapy is used either locally as a component of commercially available fibrin tissue adhesives, intravenously, or combined. Our aim was to examine the formation of aprotinin-specific antibodies with regard to the application mode. **Methods:** Sera of 150 patients who had undergone cardiac operations and were receiving aprotinin therapy for the first time were sampled before the operation and at medians of 3.5 and 13.3 months after the operation. Aprotinin-specific IgG including all subgroups and aprotinin-specific IgE were analyzed. Aprotinin was given locally (as contained in fibrin sealant; $n = 45$; median dose, 6000 KIU), intravenously ($n = 46$; 2.000×10^6 KIU), and combined ($n = 59$; 2.012×10^6 KIU). **Results:** At 3.5 months, the prevalence of aprotinin-specific IgG antibodies was 33% (15/45 patients) after local, 28% (13/46 patients) after intravenous, and 69% (41/59 patients) after combined exposure ($P = .0001$). At 13.3 months, the prevalence of aprotinin-specific IgG antibodies was 10% (4/41 patients) after local, 31% (13/42 patients) after intravenous, and 49% (28/57 patients) after combined exposure. Total aprotinin dose was similar in patients who were antibody positive and negative. Before the operation, no aprotinin-specific antibodies were detected. Aprotinin-specific IgE were not found after the operation. **Conclusion:** Local aprotinin contact induces a specific immune response and reinforces that of intravenous exposure. The antibody spectrum is identical to the immune response induced by intravenous exposure. Any exposure should be documented. For use in cardiac operations as a hemostyptic, the necessity itself and alternatives for aprotinin as a stabilizing agent merit consideration. (J Thorac Cardiovasc Surg 1999;118:348-53)

Aproinin is a polyvalent proteinase inhibitor isolated from cattle lungs and has been in clinical use since the early 1960s. Its major indication was initially the treatment of acute pancreatitis. Today aprotinin has found its major application in cardiac operations for its well-documented beneficial effects on perioperative blood loss, transfusion requirements, and probably the inflammatory response to cardiopulmonary bypass.^{1,2}

As a foreign protein composed of 58 amino acid residues with a molecular weight of 6512 d, aprotinin is capable of inducing an immune response.³ The reactive-site region of the molecule represents the main immunogenic epitope.⁴ The frequency of severe allergic and pseudoallergic shock reactions after systemic re-exposure is estimated to be about 2% to 3%.⁵

Aprotinin is also contained in biologic tissue sealants, which have been commercially available in Europe since the late 1970s and in the United States since 1998. After the fibrinogen and thrombin components are mixed, a fibrin clot forms, the lysis of which is retarded by a small dose of aprotinin.⁶ Their efficacy as a hemostatic agent has been demonstrated in many surgical and nonsurgical disciplines.⁷⁻⁹ Reports of only a few cases of shock reactions induced by the aprotinin component have so far been published.¹⁰⁻¹²

Anaphylactic reactions are caused by mediator release from mast cells as the result of the cross-bind-

From the Department of Surgery, Division of Thoracic, Cardiac and Vascular Surgery, Tübingen University Hospital, Germany.

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Address for reprints: Gerhard Ziemer, MD, Department of Surgery, Division of Thoracic, Cardiac and Vascular Surgery, Tübingen University Hospital, Hoppe-Seyler Straße 3, D-72076 Tübingen, Germany.

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Table I. Operations and aprotinin dosages

Group	n	Operations: percent (total)						Median aprotinin dosage* (IQR)	
		CABG	IMA	CABG + IMA	Valve replacement	Combined [†]	Other [‡]	Trasylol, intravenous	Tissucol, local
Local	45	13.3 (6)	4.4 (2)	55.6 (25)	13.3 (6)	6.7 (3)	6.7 (3)	0	3,572 (3,168-5,680)
Intravenous	46	4.3 (2)	2.2 (1)	41.3 (19)	52.2 (24)	0	0	1.08×10 ⁶ (1.01-1.18×10 ⁶)	0
Combined	59	5.1 (3)	3.4 (2)	72.9 (43)	10.2 (6)	8.5 (5)	0	1.11×10 ⁶ (1.03-1.25×10 ⁶)	5,906 (3,318-9,088)

CABG, Coronary artery bypass grafting; IMA, internal mammary artery.

*KIU/m²BSA (Kallikrein inhibiting units per square meter of body surface area).

[†]CABG + valve.

[‡]Atrial septum defect, aneurysmectomy, aorta ascendens replacement.

ing of 2 surface-bound IgEs.¹³ IgGs can also trigger reactions clinically indistinguishable from anaphylaxis by involvement of the complement system.^{14,15}

The different antibody types are formed by plasma cells after stimulation of different populations of T-helper (T_H) lymphocytes by antigen-presenting cells: T_{H0}-lymphocytes induce a primary response with a broad spectrum of immunoglobulins. At repeated exposures, T_{H1}-lymphocytes may induce the formation of IgG₁ and IgG₃, whereas T_{H2}-lymphocytes may generate IgG₄ and IgE.^{16,17} Although the presence of specific IgE or IgG antibodies alone does not necessarily lead to hypersensitivity reactions,¹⁸ their measurement in the serum is an important tool in clinical practice. However, the serologic findings require clinical correlates such as characteristic symptoms or an evocative history to justify the diagnosis of sensitization to a specific allergen. Re-exposure tests such as skin tests are widely used for this purpose. For aprotinin, their reliability is questionable.⁵

Most shock incidents on repeated aprotinin application seem to be immune mediated, implying that preceding exposures must have induced aprotinin-specific antibodies and allergic sensitization. The literature contains many cases that occurred after only 1 previous contact with the drug.^{5,19,20} Former studies have revealed a prevalence of specific antibodies in about 50% of patients who had received 1 intravenous high dose of aprotinin.^{21,22}

In cardiac operations, aprotinin is used either locally as a component of commercially available fibrin tissue adhesives, intravenously, or in combination of both. Therefore it was our aim to examine whether the immunostimulatory potency of a first-time contact depends on the route of administration.

Patients, methods, and analyses

Patients. Patients undergoing cardiac operations and receiving first time aprotinin were eligible for the study

group. A complete history focusing on allergies was obtained from each patient. Informed consent was obtained from each patient; all patients were informed in writing about the results and implications.

Methods. Intraoperatively, patients received either a commercially available fibrin sealant (Tissucol Duo S; Immuno, Vienna, Austria), a saline aprotinin solution (Trasylol; Bayer, Leverkusen, Germany), or a combination of both (Table I).

Tissucol consists of 2 components: a plasma fraction (containing 70-110 mg fibrinogen, 2-9 mg plasma fibronectin, 10-50 units coagulation factor VIII, 0.02-0.08 mg plasminogen [all human], and 3000 KIU/mL bovine aprotinin) and a thrombin fraction (containing 500 U human thrombin and 5.88 mg/mL calcium chloride). Trasylol contains 10,000 KIU aprotinin per milliliter isotonic sodium chloride solution.

Neither high-dose intravenous aprotinin nor fibrin tissue adhesives are routinely used in our division. The decision to use 1 or both was only made intraoperatively because of surgical necessity. The aprotinin dosages in groups are presented in Table I.

Serum samples were obtained before the operation, at approximately 4 months (median, 3.5 months; interquartile range [IQR], 3.2-3.9 months) and 13 months (median, 13.3 months; IQR, 11.7-14.7 months) after the operation. The samples were centrifuged for 15 minutes at 10°C with 1200g and stored at -20°C until the in vitro anti-aprotinin-specific antibody screening tests were performed.

Antibody detection. Serologic analyses comprised an automatized fluorescence enzyme immunoassay (UniCAP System; Pharmacia & Upjohn, Uppsala, Sweden) for the detection of aprotinin-specific IgE and a standard enzyme-linked immunosorbent assay technique for quantitative detection of aprotinin-specific total IgG (tIgG) as previously described.²⁴ The same enzyme-linked immunosorbent assay technique was used for specification of IgG-subgroups 1 to 4 (mouse anti-human IgG_{1,2,3,4} monoclonal antibody; Pharmingen, San Diego, Calif; diluted 1:1000; serum dilution, 1:100) but, in a semiquantitative fashion, currently lacking established standards.

Statistics. Intergroup differences of tIgG prevalence and frequency of allergies were examined with the use of the χ^2 test. McNemar's test (2-tailed) was used to compare prevalence

Table II. Demographic data and allergic history

Group	n	Age (yr $\pm$ SD)	No. of male patients (%)	Allergic patients: percent (total)	No. of allergic diathesis* (%)				
					Food	Drugs	Inhalative	Contact	Insect venom
Local	45	60 $\pm$ 16	60.0 (27)	31.1 (14)	14.3 (2/14)	50.0 (7/14)	14.3 (2/14)	21.4 (3/14)	7.1 (1/14)
Intravenous	46	62 $\pm$ 8	67.4 (31)	34.8 (16)	6.3 (1/16)	50.0 (8/16)	12.5 (2/16)	37.5 (6/16)	0
Combined	59	60 $\pm$ 11	76.3 (45)	27.1 (16)	12.5 (2/16)	43.8 (7/16)	12.5 (2/16)	25.0 (4/16)	12.5 (2/16)
Total	150	60 $\pm$ 12	68.7 (103)	30.7 (46)	10.9 (5/46)	47.8 (22/46)	13.0 (6/46)	28.3 (13/46)	6.5 (3/46)

*Specific allergies listed multiple times.

Table III. Prevalence of aprotinin-specific IgG over time

Group	Patients positive* (%)		P value [†]
	3.5 Mo	13.3 Mo	
Local	33.3	9.8	.005
Intravenous	26.1	31.0	>.1
Combined	69.5 [‡]	49.1 [‡]	.03
Whole group	45.3	32.1	.002

*See Fig 1 for the number of patients.

[†]McNemar, 2-tailed.[‡]Combined versus local and intravenous: 3.5 months, $P = .0001$; 13.3 months, $P = .0004$ (χ^2 test).

within groups. Time-dependent drop in tIgG concentrations was examined with the nonparametric Wilcoxon matched pairs signed rank test. IgG values that were equal (or undetectable) at both examinations did not yield any relevant differences and were thus excluded from the test. The influence of aprotinin amount applied per square meter of body surface area on IgG formation as assessed by a positive screening test (IgG > 10 mg/L) was examined by logistic regression analysis with the statistical package JMP (SAS, Inc, Cary, NC). U-test was applied to examine differences of aprotinin-specific IgG levels in allergic and nonallergic patients. Not normally distributed values are presented as medians with interquartile ranges.²³

Results

One hundred fifty patients were selected consecutively from adult patients who had undergone operation electively for acquired heart disease in our division between August 1995 and July 1996. Twenty-two further patients were not eligible because aprotinin-specific serum IgG was present before the operation, and 11 additional patients were not eligible because of documented former aprotinin contact.

Seven patients with negative screening tests before the operation and who received no aprotinin therapy were reexamined after 3.8 months (IQR, 3.5-4.3 months). No aprotinin-specific antibodies were detected in this group.

Seven of the 150 patients underwent cardiac reoper-

ations without previous application of fibrin sealant or infusion of aprotinin. Table I details the operations and median aprotinin doses; Table II details the demographic data and allergic history.

The 150 preoperative sera were negative for all aprotinin-specific antibodies tested.

Each application mode of aprotinin was capable of inducing a specific antibody response after a first and unique application. Within the 3 groups, the aprotinin amounts that were standardized according to the body surface area did not differ significantly between patients who were deemed antibody positive and negative (local, $P = .36$; intravenous, $P = .52$; combined, $P = .60$; JMP).

The prevalence of detectable tIgG levels was highest in the group with combined exposure (approximately 70%) and significantly lower in the groups with local or intravenous contact (approximately 30% each) approximately 4 months after the operation ($P = .0001$; χ^2 test). After 13 months, the prevalence decreased significantly after local and combined contact but remained stable after intravenous contact (Table III; Fig 1). Most seroconverters in both directions were observed in the combined group (Table IV).

Except for 3 patients, all patients who had detectable levels of aprotinin-specific antibodies at 4 months had significantly lower levels after 1 year (Table IV). Patients with aprotinin-specific tIgG levels higher than the third quartile of positives had received aprotinin doses that did not differ significantly from those of patients with positive levels below ($P = .1$ at 3.5 months and $P = .24$ at 13.3 months; JMP).

The spectrum of aprotinin-specific IgG subgroups comprised mainly IgG₁ and IgG₄. In nearly all patients, IgG₁ contributed to tIgG. The contribution of IgG₄ to tIgG was lower, but its activity increased at approximately 13 months. Nearly all sera containing IgG₄ were also positive for IgG₁. IgG₂ and IgG₃, which played a minor role, were detectable at 4 months after combined application, but only in a few patients and with very weak activity. IgE was not found in any patient.

Fig 1. Prevalence of total aprotinin-specific IgG at 3.5 and 13.3 months, depending on application mode.

Table IV. Aprotinin-specific tIgG concentrations versus time after exposure

Group*	Median aprotinin-specific tIgG† (IQR)		Seroconverters‡ (n)		P value§
	3.5 Mo	13.3 Mo	Positive to negative	Negative to positive	
Local	34.1 (20.7-61.9)	45.0 (22.4-65.2)	9	0	.01
Intravenous	165.6 (96.5-321.6)	47.1 (30.6-70.9)	3	4	.01
Combined	78.4 (19.8-186.3)	25.2 (14.6-38.4)	14	3	<.0001
Whole group	77.5 (21.9-177.0)	26.3 (18.8-52.1)	26	7	<.0001

*See Fig 1 for number of patients.

†Measured as milligrams per liter in patients with detectable levels.

‡3.5 vs 13.3 months.

§Wilcoxon matched pairs signed rank test.

The incidence of aprotinin-specific antibodies in the 46 allergic patients was 43.5% (20/46 patients) within the observation period, which was nearly the same percentage as in nonallergic patients (46.2%; 48/104 patients). At both examinations aprotinin-specific tIgG levels did not differ significantly between allergic and nonallergic patients ($P > .2$; U-test). The median positive levels were 63 mg/L (IQR, 18-124 mg/L) in allergic patients and 78 mg/L (IQR, 29-186 mg/L) in nonallergic patients at 4 months and 36 mg/L (IQR, 19-58 mg/L) versus 26 mg/L (IQR, 19-50 mg/L) at 13 months. There were 42.9% of tIgG positive patients (6/14 patients) among the allergic patients who had received local aprotinin application, 31.3% tIgG positive patients (5/16 patients) among those with intravenous application, and 56.3% tIgG positive patients (9/16 patients) among those with combined application. Most tIgG-positive atopic patients were allergic to drugs (60.0%; 12/20 patients); but compared with the group of antibody-negative allergic patients

(38.5%; 10/26 patients), this preponderance of drug allergies was statistically not significant ($P = .15$; χ^2 test).

Comments

Study design. This prospective study examined the immune response induced by a single and first aprotinin exposure through fibrin tissue adhesive, infusion, or both. The design reflected the immune status that an aprotinin re-exposure would encounter after approximately 4 and 13 months. Because there is a certain risk of shock reactions on any re-exposure, even on skin tests, we had considerable ethical concerns about performing exposure tests without an imperative clinical need. Additionally, skin tests could have influenced the immune response in our patients. We therefore deliberately did not perform these tests.

We combined our data with the features of aprotinin-induced shock reactions known from literature to deduce recommendations for clinical practice.

Significance of antibodies. Immune-mediated shock reactions on re-exposure to proteins are most commonly induced by specific antibodies of the subtypes IgE and IgG. The pathophysiologic condition underlying true anaphylactic reactions is IgE triggered.¹³ The fact that we found IgE in none of the patients might reflect the rarity of such reactions on aprotinin re-exposure. However, we were surprised by this finding because in a former study we found a 14.3% incidence of IgE in children within 6 weeks after first-time exposure to fibrin sealant.²⁴ The absence of IgE in our adult patients may be that the 4-month interval between aprotinin exposure and blood sampling is too long to detect an IgE response. Immunoregulatory processes may be different in children and adults, because full immunocompetence matures within the first years of life.¹⁷

The role of antigen-specific IgG for anaphylactic and anaphylactoid reactions is still the subject of controversy. Its significance may depend on the serum level, the distribution of its subclasses, or the distribution of IgG and IgE.

According to Dietrich and colleagues,²⁵ a patient's reactivity might depend on a high level of aprotinin-specific IgG. The significant decrease of total IgG levels in our whole group over 1 year seems to be consistent with the decreasing risk of shock reactions.⁵ The absence of IgE in all patients at 4 months is also consistent with this conception. However, the high prevalence of aprotinin-specific IgG at both examinations is in contrast to the rareness of anaphylactic reactions on re-exposure. This finding renders an association of IgG antibody presence and shock reactions rather uncertain. Although we had patients with high anti-aprotinin IgG levels, our data do not have the potential to confirm or disprove the thesis of Dietrich and colleagues, because we did not re-expose our patients for the reasons mentioned earlier.

According to van der Zee and Aalberse,²⁶ the anaphylactic significance of IgG may depend on the "homocytotropic activity" of the IgG₄ subclass (ie, its capability of binding to mast cells and thereby acting as an anaphylactic antibody). Our results show an increase of aprotinin-specific IgG₄ over 1 year. This is contrary to the clinical observation of a risk decreasing with time. The phenomenon of rising IgG₄-levels is therefore possibly an in vitro artifact by secondary IgG₄ antibodies directed against aprotinin-specific IgG (anti-idiotypic antibodies).²⁷

In a case control study on patients with acute reactions to protamine, Weiss and colleagues²⁸ found an increased risk associated with the presence of both protamine-specific IgG and IgE. The relative risk associated with IgE was higher than that associated with IgG. Although protamine is a small molecular-weight for-

eign protein like aprotinin, these data must not be readily transferable to aprotinin.

Role of application mode. Any aprotinin exposure may induce a systemic immune response in adult patients. Local aprotinin additional to intravenous application has an over-additive effect on the incidence of aprotinin-specific IgG.

This phenomenon may be due to different pharmacokinetic properties of the application modes. After the systemic administration, the immune system is overwhelmed by high antigen doses for a short time (elimination half time, approximately 2 hours).³ In contrast, a fibrin sealant clot stabilized by aprotinin stays stable for up to 4 weeks⁶ and may thereby act as a temporary antigen-presenting deposit for the immunocompetent cells infiltrating the wound and the fibrin clot during the healing process.

Time course of immune response. The decrease in the combined group was nearly the same compared with that of the local group after 1 year. Considering the stability in the intravenous group, one may hypothesize that the decrease in the combined group is the effect of 2 superposed kinetics being each characteristic of a different immune compartment stimulated by the local and the intravenous application, respectively.

The decrease of aprotinin-specific tIgG reflects the absent reboosting effect in lack of further contacts with the immunogen. Although Table IV presents a higher median tIgG level after local exposure at 1 year, the individual levels decreased also in these 4 patients. Weipert and colleagues²² showed that aprotinin-specific antibodies may persist even up to 4 years after a single intravenous high dose during cardiac operations.

In a Medline search of literature from 1963 to 1998, we found 50 papers presenting 107 cases of shock reactions to aprotinin: 70 patients were pre-exposed (65.4%); 44 reports mentioned the re-exposure interval, which was less than 3 months in 30 cases (68.2%). Combined with our data, this underlines that immunoreactivity to aprotinin depends on time. Thus the re-exposure interval is an important determinant for the risk of shock reactions to aprotinin.

Allergic history. Our patients' histories of allergies did not predict the immune response to aprotinin. This can be attributed to the number of patients being too small for reliable epidemiologic calculations. Large clinical studies on anesthetic drugs revealed atopy and female gender as risk factors for IgE-mediated reactions.²⁹

Clinical impact and conclusion. This study demonstrates that a single local contact with aprotinin by commercially available fibrin tissue adhesives is capable to induce an immune response in adult cardio-surgical patients. Although the local dose represented only

permils of the intravenous dose, the antibody spectrum was identical with that induced by a single intravenous high-dose exposure.

The detectable antibody response to aprotinin weakens with time. This corresponds with the clinical observation that the incidence of shock reactions to aprotinin decreases after 6 months. As a measure of precaution, aprotinin administration in any form should be avoided within the first months after previous exposure. A patient's history of allergies is not indicative of the aprotinin-specific immune response.

In clinical practice, the use of commercially available fibrin tissue adhesives is commonly not documented. Before any aprotinin use, clinical evaluation should include the search for possible recent intravenous or local aprotinin exposures. To facilitate this search, we recommend a careful documentation of any aprotinin use.

Considering the potency to induce aprotinin-specific antibodies, it becomes questionable whether aprotinin should be added to fibrin tissue adhesives for all applications. The necessity itself and alternatives for aprotinin as a stabilizing agent merit detailed consideration.

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