

The Significance of Preformed Aprotinin-Specific Antibodies in Cardiosurgical Patients

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Acute hypersensitivity reactions are serious complications of reexposure to aprotinin. Previous contact via infusions or fibrin tissue adhesives can induce specific antibodies. In this study, we aimed to elucidate the preoperative prevalence of aprotinin-specific antibodies in patients scheduled for cardiac operations. Sera of 520 consecutive cardiosurgical patients were collected preoperatively and screened retrospectively for aprotinin-specific IgG using a standard enzyme-linked immunosorbent assay (ELISA). Positive sera were analyzed also for aprotinin-specific IgA (ELISA) and IgE (fluorescence enzyme immunoassay). The histories of all patients were reviewed with focus on aprotinin preexposure. Of 520 patients, 22 (4%) had specific IgG. Only three of these had a documented aprotinin preexposure. Of 448 patients exposed to aprotinin intraoperatively,

15 had preformed specific antibodies. The only patient presenting with severe anaphylaxis was positive for both IgG and IgE, and had a recent IV preexposure in cardiovascular surgery. The presence of aprotinin-specific IgG alone seems not to induce adverse reactions on exposure. Exposure history alone is not sensitive enough to identify patients with aprotinin-specific antibodies. **Implications:** Anaphylaxis on IV reexposure to aprotinin is a medical emergency. The clinical significance of preformed aprotinin-specific IgG remains questionable, whereas preformed IgE was present in the only patient who suffered from severe anaphylaxis on reexposure to aprotinin. Preformed antibodies are not reliably predicted by exposure history.

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Aprotinin, a polyvalent proteinase inhibitor isolated from bovine lungs, has been in clinical use since the early 1960s. Inhibiting fibrinolysis and preserving platelet function, it has found its major application in cardiac surgery for the beneficial effect on perioperative blood loss and transfusion requirements (1,2). Aprotinin may reduce the systemic inflammatory response after cardiopulmonary bypass (3) by antagonism to many of the enzymes involved in inflammatory processes. Aprotinin also has a stabilizing function in biological tissue sealants, commercially available in Europe since the early 1970s, and in the United States since 1998. Their efficiency for a great variety of applications has been demonstrated in many surgical and nonsurgical disciplines (4).

A foreign protein, composed of 58 amino acid residues with a molecular weight of 6,512 Da, aprotinin

can induce an immune response with formation of specific antibodies. The active region of the molecule is the main immunogenic epitope (5). Aprotinin-specific antibodies may precipitate allergic reactions on reexposure. Thus, former aprotinin exposure is considered to be a major risk factor for severe adverse reactions. The frequency of severe allergic and pseudoallergic reactions on IV reexposure is estimated to be about 2% (6). The incidence of adverse drug reactions to the aprotinin component of fibrin tissue adhesives is estimated to be about 0.5 per 100,000 applications (7). Most reactions with either form of reexposure occur within a few months after previous exposure.

This study was designed to assess the incidence of preformed aprotinin-specific antibodies in a cardiac surgical population and whether these antibodies have a clinical relevance regarding anaphylaxis. An additional goal was to determine whether exposure history was predictive of aprotinin-specific antibody status.

Methods

All adult patients scheduled consecutively for elective cardiac operations at our center between August 1995

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and November 1996 were eligible for the study. Informed consent for blood sampling was obtained from each patient.

Serological analyses consisted of a previously described standard enzyme-linked immunosorbent assay technique for detecting aprotinin-specific total IgG (8), which was slightly modified for the detection of aprotinin-specific IgA (peroxidase-conjugated affinity-pure goat anti-human serum IgA, α -chain-specific; Jackson ImmunoResearch Laboratories, West Grove, PA; diluted 1:3,000; serum dilution 1:500). The tests were performed in a semiquantitative fashion, because established standards are currently lacking. The cutoff was fixed at the mean $\pm$ 3 sds calculated from the optical densities of sera obtained from healthy volunteers without contact with aprotinin (optical densities: IgG 0.033, 20 volunteers; IgA 0.265, 10 volunteers). Serum levels of aprotinin-specific antibodies were classified as low when the optical density was below the threefold cutoff and as high when it was above. An automatized fluorescence enzyme immunoassay (UniCAP[®] System, Pharmacia & Upjohn, Uppsala, Sweden) was used to detect aprotinin-specific IgE (8). Intraoperatively, most patients received either a commercially available fibrin sealant (Tissucol[®] Duo S; Immuno, Vienna, Austria) or a saline aprotinin solution (Trasylo[®]; Bayer, Leverkusen, Germany), or both. Neither large-dose IV aprotinin nor fibrin tissue adhesive was used routinely. The indication to use one, both, or none was made intraoperatively based on surgical necessity.

The fibrin sealant consisted of two components: a plasma fraction (containing 70–110 mg of fibrinogen, 2–9 mg of plasma fibronectin, 10–50 units of coagulation factor VIII, 0.02–0.08 mg of plasminogen [all human], and 3,000 kallikrein inhibiting units [KIU] bovine aprotinin per mL) and a thrombin fraction (containing 500 units of human thrombin and 5.88 mg of calcium chloride per mL). The saline aprotinin solution contained 10,000 KIU aprotinin per mL of isotonic sodium chloride solution.

All patients were questioned for medical and surgical pretreatments with focus on former aprotinin exposures and the medical record reviewed. Additionally, a literature search was undertaken to check the pretreatments for a possible association with aprotinin contact in humans (1966–1997, free text mode):

#1 pretreatment (= term) and (aprotinin therapeutic use or aprotinin administration and dosage) in mesh

#2 pretreatment and (fibrin tissue adhesive in mesh) or fibrin sealant or fibrin glue or tissucol).

Any treatment earlier than 1959, the year in which aprotinin was introduced into clinical use (9), was *a priori* considered irrelevant. Conversely, any operation involving cardiopulmonary bypass during or after 1975 was considered relevant, because the earliest

aprotinin use in cardiac surgery was reported that year (10).

The interval between the first and the latest record was defined as relevant if the records allowed this classification. Each of our patients' pretreatments occurring within the intervals determined in this manner were considered as a pretreatment with possible aprotinin contact.

Values assumed binomially distributed are portrayed as means with standard deviation, whereas values nonnormally distributed are shown as medians with an interquartile range (IQR). Significances were calculated on the base of a χ^2 and a Wilcoxon's ranked sum test, respectively. The specific test is indicated where used. In both tests, significance was assumed for $P < 0.05$.

Results

Patients

The study group comprised 520 patients. The mean age was 63 ± 13 yr, with 375 male and 145 female patients scheduled to undergo the following operations: 341 coronary artery bypass graftings, 98 valve replacements or reconstructions, 9 replacements of the ascending or descending aorta, 39 combined procedures, 6 atrial septum defect closures, and 15 various procedures. Twelve patients primarily screened actually did not undergo surgery. The group included 44 patients who previously had undergone 50 cardiac operations (mean interval 134 ± 69 mo).

Aprotinin-Specific Antibodies

Screening for aprotinin-specific IgG antibodies identified 22 (4.2%) patients with preformed antibodies. According to the semiquantitative analysis, there were four sera with high, and 18 sera with low levels of aprotinin-specific IgG. Of the 22 patients positive for IgG, two were also positive for IgA (0.4%) and one for IgE (0.2%).

Aprotinin Exposure

Intraoperatively, 448 (86.2%) patients received aprotinin. Of these, 103 (19.8%) patients received a fibrin sealant containing aprotinin, 115 (22.1%) received IV large-dose aprotinin, 230 (44.2%) received a combination of both, and 72 (13.8%) received neither. The median local dose was 4,900 KIU/m² body surface area (IQR 3,226–7,059), and the median IV dose was 1,075,270 KIU/m² (IQR 1,014,200–1,162,790).

History-Taking and Former Aprotinin Contact

History-taking revealed 410 patients (78.8%) who had undergone a total of 952 surgical procedures, and five

Table 1. Preoperative Aprotinin-Specific Antibody Status and Former Aprotinin Contact

Aprotinin-specific antibodies	Aprotinin contact				
	Documented		Possible		None <i>n</i> (%)
	<i>n</i> (%)	Time lapse	<i>n</i> (%)	Time lapse ^a	
Present	3 (0.6)	3, 11, 81 ^b	9 (1.7)	51, 27-148	10 (1.9)
Absent	8 (1.5)	86, 65-108 ^a	215 (41.3)	87, 32-183	275 (52.9)

^a Months since last possible aprotinin contact, median with interquartile range.^b Months since last aprotinin contact, individual intervals.

patients had a history of acute pancreatitis. Eleven patients (2.1%) had a documented former IV aprotinin contact during cardiac operations (median dose 5×10^5 KIU, IQR $3.5-20 \times 10^5$ KIU). The median time lapse since the last documented aprotinin contact was 77 mo (IQR 23-98). According to the premises described in Methods, there were 224 patients (43.1%) who had undergone procedures with possible former aprotinin contact. The median time between these documented or possible aprotinin contacts was 85 mo (IQR 32-181).

Aprotinin Exposure in IgG-Positive Patients

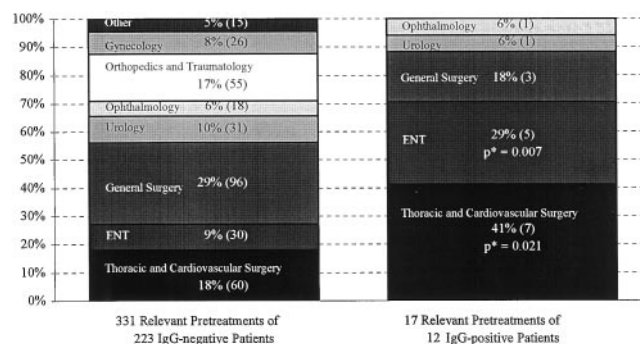
Fifteen of the 22 IgG-positive patients received aprotinin intraoperatively. Three of the IgG-positive patients received a fibrin sealant containing aprotinin, six received IV large-dose aprotinin, another six received a combination of both, and seven received neither. Of the four patients with high IgG levels, one received IV aprotinin, another received a combination of IV aprotinin and fibrin sealant, and two received no aprotinin. The results of the antibody screening tests were known only postoperatively.

Presence of Aprotinin-Specific Antibodies and History

Table 1 divides the 22 antibody-positive patients according to aprotinin exposure history. Patients with aprotinin-specific antibodies had significantly more frequent histories of thoracic, cardiovascular, and otorhinolaryngologic operations (Fig. 1). Compared with the operations in patients without aprotinin-specific antibodies, these thoracic and cardiovascular operations were significantly more recent. Operations in otorhinolaryngology and general surgery were also more recent in positive patients, but the difference was not significant (Table 2).

History and Aprotinin Exposure

Ten of the 11 patients with documented preexposure (90.1%) and 194 of the 224 patients with procedures with possible aprotinin exposure (86.6%) were intraoperatively exposed to aprotinin by infusion and/or

**Figure 1.** Relevant pretreatments in IgG-negative and IgG-positive patients. * χ^2 test. ENT = ear, nose, and throat.

fibrin sealant. The median time lapse since the documented or possible aprotinin contacts in this subgroup was 84 mo (IQR 32-181).

Anaphylaxis

One patient developed severe anaphylaxis on IV aprotinin exposure. The retrospective investigation revealed both preformed aprotinin-specific IgE antibodies and a high level of IgG. She had also been preexposed intravenously during a mitral valve reconstruction in a different hospital three months earlier (11).

Discussion

Anaphylactic reactions are caused by mediator release from mast cells due to cross-binding of two surface-bound IgEs (12). IgG can also trigger reactions clinically indistinguishable from anaphylaxis via involvement of the complement system (13). Dietrich et al. (14) demonstrated that high levels of aprotinin-specific IgG are associated with a higher risk of adverse reactions.

In our group, none of the patients with exclusively aprotinin-specific IgG exhibited allergic symptoms on exposure to aprotinin. The only patient having both aprotinin-specific IgG and IgE presented with severe anaphylaxis on IV aprotinin reexposure. This result suggests that the presence of aprotinin-specific IgG alone, even in high concentrations, has a questionable

Table 2. Time Lapses Since Pretreatments with Documented or Possible Aprotinin Contact According to Specialties

Specialty	IgG-positive patients				IgG-negative patients				<i>P</i> ^b
	<i>n</i> ^a	Pretreat- ments, <i>n</i>	Time lapse (months)		<i>n</i> ^a	Pretreat- ments, <i>n</i>	Time lapse (months)		
			Median	Interquartile range			Median	Interquartile range	
Thoracic and cardiovascular surgery	6	7	21	12–63	51	60	107	50–166	0.011
Otorhinolaryngology	5	5	156	125–245	30	30	189	48–286	>0.05
General surgery	3	3	54	51–142	79	96	94	31–195	>0.05
Other	2	2	32		117	140	72	30–145	>0.05

^a Total *n* smaller than sum of rows because of patients pretreated in several specialties.^b Wilcoxon's ranked sum test, one-tailed.

significance for the induction of adverse reactions. However, the subgroup of 15 patients with preformed aprotinin-specific antibodies and exposure to aprotinin is insufficient to derive statistically significant conclusions on the predictive value of antibody screening. Examining heparin, Kappers-Klunne et al. (15) found a high frequency of heparin-dependent IgG antibodies without concomitant occurrence of adverse effects, and concluded that they have a subordinate significance for inducing adverse reactions. The dominating role of allergen-specific IgE for allergic symptoms has been shown by Mark et al. (16) who found similar IgG levels in both cat-allergic and non-cat-allergic individuals, but IgE only in cat-allergic patients.

Former exposures to aprotinin represent an important risk factor for the occurrence of anaphylactic reactions (6). In the past 40 yr, aprotinin has been administered during procedures in a great variety of surgical disciplines and in acute pancreatitis (Table 2). Most exposures have not been documented and are therefore difficult to assess. We tried to resolve this problem by history-taking and by evaluating the data by the grid obtained by the Medline search. A weakness of this method is its focus on only possible and not on actual aprotinin exposures. A further shortcoming is the wide time intervals of the grid. For this reason, the number of possible aprotinin contacts has certainly been overestimated. Only the documentation of any aprotinin exposure allows precise identification of patients with former aprotinin contacts.

Patients with detectable IgG levels and a positive history have probably undergone an actual immune response. Most of them have been pretreated in cardiovascular surgery and otorhinolaryngology (Fig. 1). This becomes plausible when considering that, in these specialties, aprotinin is commonly used, in the former, mainly IV; in the latter, it is used primarily locally as a component of commercially available fibrin tissue adhesives.

The high percentage of antibody positives with a bland history (10 of 22 positive patients) indicates that it

is questionable to infer the presence of aprotinin-specific IgG from history. A lack of documentation and cross-sensitizations to allergenic epitopes from alimentary bovine proteins may account for these patients. Two of the IgG-positive patients also had IgA. Because they did not have a history of aprotinin contact, we cannot exclude cross-sensitizations.

The factor time plays an important role in the course of the aprotinin-induced immune response. In a former study, we demonstrated the time-dependent decrease of aprotinin-specific IgG prevalence: 45.3% and 32.1% of 150 patients were IgG-positive at median intervals of 3.7 and 13.3 months after previous local and/or IV aprotinin contact (17). In patients with a positive history, but absent antibodies, IgG may therefore have become undetectable over time. The median time intervals since the last possible aprotinin contacts is in favor of this interpretation (Table 1).

Reviewing published reports on hypersensitivity to aprotinin, we found that 30 of 44 reactions (68.2%) with a mentioned reexposure interval occurred within three months after previous contact.

We purposely avoided exposure tests and used antibody screening tests and clinical observation as several studies have shown that exposure tests (skin, eye, test dose) have a poor diagnostic value when performed with aprotinin, and bear even the risk of triggering acute reactions (6,18). In addition, we examined only IgG positive sera for IgA and IgE. This is first due to our experience that IgE was only present in IgG-positive sera (8) and second due to the high cost of the test. IgA was tested to verify the possibility of gastrointestinal cross-reactions.

We conclude that the presence of aprotinin-specific IgG alone seems not to induce adverse reactions on exposure. Whether the presence of aprotinin-specific IgE necessarily leads to anaphylaxis and the predictive value of screening tests for both aprotinin-specific IgG and IgE antibodies requires further investigation. Surgical history is not predictive of antibody status. Present data suggest that the presence of aprotinin-specific IgE

should be considered as a risk for anaphylaxis on reexposure. For us, this should be a contraindication to administer aprotinin.

References

1. Bidstrup BP, Royston D, Sapsford RN, Taylor KM. Reduction in blood loss and blood use after cardiopulmonary bypass with high dose aprotinin (Trasylol). *J Thorac Cardiovasc Surg* 1989; 97:364-72.
2. Miller BE, Tosone SR, Tam VKH, et al. Hematologic and economic impact of aprotinin in reoperative pediatric cardiac operations. *Ann Thorac Surg* 1998;66:535-40.
3. Royston D. Preventing the inflammatory response to open-heart surgery: the role of aprotinin and other protease inhibitors. *Int J Cardiol* 1996;53(Suppl):S11-37.
4. Schlag G, Redl H, Waclawiczek HW, et al. ed. Fibrin sealing in surgical and nonsurgical fields. Vol. 1: Wound healing (1994); Vol. 2: General and abdominal surgery, pediatric surgery (1994); Vol. 3: Plastic surgery, nerve repair, burns (1995); Vol. 4: Orthopedic surgery, maxillofacial surgery (1994); Vol. 5: Neurosurgery, ophthalmic surgery, ENT (1994); Vol. 6: Cardiovascular surgery, thoracic surgery (1995); Vol. 7: Gynecology and Obstetrics, Urology (1994); Vol. 8: Endoscopy (1995). New York: Springer-Verlag.
5. Siekmann J, Beckmann J, Mehlich A, et al. Immunological characterization of natural and semisynthetic aprotinin variants. *Biol Chem Hoppe-Seyler* 1989;370:677-81.
6. Dietrich W, Späth P, Ebell A, Richter JA. Prevalence of anaphylactic reactions to aprotinin: analysis of two hundred forty-eight reexposures to aprotinin in heart operations. *J Thorac Cardiovasc Surg* 1997;113:194-201.
7. Beierlein W, Scheule AM, Antoniadis G, et al. An immediate allergic skin reaction to aprotinin after reexposure to fibrin sealant. *Transfusion*. In press.
8. Scheule AM, Beierlein W, Wendel HP, et al. Fibrin sealant, aprotinin, and immune response in children undergoing operations for congenital heart disease. *J Thorac Cardiovasc Surg* 1998;115:883-9.
9. Beuchelt H. Trasylol, ein Proteinase-Inhibitor, in Experiment and klinischer Anwendung. *Med Chem* 1963;7:763-8.
10. Nagaoka H, Yamada T, Hatano R, et al. Clinical significance of bradykinin liberation during cardiopulmonary bypass and its prevention by a kallikrein inhibitor. *Jpn J Surg* 1975;5: 222-33.
11. Scheule AM, Jurmann MJ, Wendel HP, et al. Anaphylactic shock after aprotinin reexposure: time course of aprotinin-specific antibodies. *Ann Thorac Surg* 1997;63:242-4.
12. Ishizaka T, Ishizaka K. Activation of mast cells for mediator release through IgE receptors. In: Ishizaka K, ed. *Mast cell activation and mediator release*. Basel: Karger, 1984: 188-235.
13. Colten HR. Immunology. Drawing a double-edged sword. *Nature* 1994;371:474-5.
14. Dietrich W, Dalichau H, Kirchhoff PG, et al. The predictive value of preexisting specific antibodies to aprotinin for anaphylactic reactions—a multicenter study in 122 re-exposures to aprotinin [abstract]. *Anesth Analg* 1998;SCA29.
15. Kappers-Klunne MC, Boon DM, Hop WC. Heparin-induced thrombocytopenia and thrombosis: a prospective analysis of the incidence in patients with heart and cerebrovascular diseases. *Br J Haematol* 1997;96:442-6.
16. Mark PG, Segal DB, Dallaire ML, Garman RD. Human T and B cell immune responses to Fel d 1 in cat-allergic and non-cat-allergic subjects. *Clin Exp Allergy* 1996;26:1316-28.
17. Scheule AM, Beierlein W, Wendel HP, et al. Aprotinin in fibrin tissue adhesives induces specific antibody response and increases antibody response of high dose intravenous application. *J Thorac Cardiovasc Surg* 1999;118:348-53.
18. La Ferla GA, Murray WR. Anaphylactic reaction to aprotinin despite negative ocular sensitivity tests. *Br Med J* 1984;289: 1176-7.