

IMMUNOLOGICAL STUDIES ON PATIENTS WHO RECEIVED APROTININ THERAPY

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INTRODUCTION

Aprotinin, a proteinase inhibitor isolated from the bovine lung, is a basic polypeptide with a molecular weight of 6,513 which strongly inhibits various serine proteinase such as kallikrein, trypsin, chymotrypsin and plasmin¹⁾⁻⁴⁾. This agent is clinically useful for the treatment of acute pancreatitis, pancreatic necrosis and shock⁵⁾⁻⁹⁾. Extensive clinical and toxicological studies have demonstrated that aprotinin is a very safe inhibitor of proteinase. Aprotinin, however, has been shown occasionally to cause anaphylactic or anaphylactoid reactions in humans¹⁰⁾⁻¹³⁾. The latter observations suggest that aprotinin may induce hypersensitivity reactions mediated by specific antibody. In fact, aprotinin has been found to provoke systemic shock possibly based upon immunological mechanisms¹⁰⁾⁻¹³⁾, but the immunoglobulin classes responsible for aprotinin-induced hypersensitivity reactions have not been reported on, so far as is known at present.

The present experiments were undertaken to test the specific IgE and IgG antibodies in sera obtained from patients who had received aprotinin therapy. The results obtained indicated that anti-aprotinin IgE antibody was detected in some sera of patients receiving aprotinin therapy.

MATERIALS AND METHODS

Serum donors

All patients who received aprotinin therapy had pancreatitis. Sera were obtained from 18 patients with hypersensitivity reactions to aprotinin and from 44 patients with no hypersensitivity reactions. The group showing hypersensitivity to aprotinin (group A) consisted of 8 male and 10 female patients; 8 out of the 18 patients were new to aprotinin therapy, 7 received a repeat administration of aprotinin and 2 were unknown. By contrast, the group showing no hypersensitivity reactions to aprotinin (group B) consisted of 29 male and 15 female patients; 42 out of the 44 patients were new to aprotinin therapy and 2 were unknown. As a rule, serum samples were collected three times in group A: within 1 week, 2-4 weeks and 8 weeks after hypersensitivity reactions. Serum samples were taken four times in group B: before aprotinin administration, immediately after the end of aprotinin therapy and 2 and 8 weeks after the end of aprotinin therapy. Control sera were obtained from 9 healthy adult volunteers as well as from 8 atopic patients with bronchial asthma who had positive skin tests to common air-borne allergens such as house dust mite or grass pollen.

Measurement of specific IgE antibody

Anti-aprotinin IgE antibody in serum was measured by radioallergosorbent test (RAST)¹⁴⁾. Briefly, CNBr-activated paper discs were coupled with aprotinin at a concentration of 1 mg/ml in 0.1M NaHCO₃ solution (100 discs/10 mg/10 ml) and were washed three times with 0.1M NaHCO₃ solution. Unbound sites in the discs were then blocked by 1M β -ethanolamine. Paper discs coupled with human serum albumin (HSA; Miles Laboratories) were used to determine non-specific binding. The discs were incubated at room temperature with 100 μ l of test serum. After overnight incubation, the discs were washed three times, incubated overnight with 100 μ l of ¹²⁵I-labelled rabbit anti-human IgE (Pharmacia) and washed extensively. The radioactivity bound to the paper discs was determined by gamma counter (Aloka JDC-772). The results were expressed as the ratio of the counts in the disc coupled with aprotinin to those in the disc coupled with HSA (RAST index). A RAST index of more than 2.0 was considered to be positive.

Measurement of serum IgE

Serum IgE levels were measured by paper radioimmunosorbent test (PRIST)¹⁵⁾ using a commercially available Phadebas IgE PRIST kit (Pharmacia). Paper discs coupled with anti-human IgE were incubated with 100 μ l of test serum

Abbreviations: PCA passive cutaneous anaphylaxis; PRIST paper radioimmunosorbent test; RAST radioallergosorbent test

diluted at a rate of 1: 10 for 3 hr at room temperature. After washing, the discs were incubated with ^{125}I -labelled anti-human IgE for 12 hr. The radioactivity bound to the washed discs was determined by gamma counter. The results were expressed as international units per milliliter (IU/ml).

Measurement of specific IgG antibody

Specific IgG antibody in serum was detected by agar-gel diffusion and/or passive cutaneous anaphylaxis (PCA) in guinea pigs. Agar-gel diffusion was carried out according to the method of Ouchterlony¹⁶⁾. We examined whether precipitin lines were formed between aprotinin solution (5 mg/ml) and test serum. Heterologous PCA was performed as described by Ovary¹⁷⁾. Briefly, the test serum was injected intradermally at a rate of 0.1 ml/site into shaved backs of male guinea pigs of Hartley strain. The guinea pigs weighed about 300 g. PCA was elicited by the intravenous injection of 1 ml of physiological saline containing 5 mg of aprotinin and 5 mg of Evans blue after the sensitization period of 3 hr. The blueing spot was examined at 30 min after antigen challenge.

RESULTS AND DISCUSSION

The first experiments were carried out to study whether aprotinin was capable of inducing non-specific skin reactions in humans and of having cross-reactivity with common air-borne allergens. As shown in Tables 1 and 2, both skin test to aprotinin at a concentration of 10^{-4} g/ml (7.143 KIU/ml) and RAST for detecting anti-aprotinin IgE antibody were negative in all 9 of the healthy volunteers who had no history of allergic disease and in all 8 of the atopic patients with bronchial asthma who had positive skin tests to common air-borne allergens. Note that the atopic patients had significantly higher

Table 1 Immunological findings of healthy adult volunteers

No. of volunteers	Sex	Skin test	Serum IgE (IU/ml)	Specific IgE antibody (RAST index)
1	F	—	53	0.9
2	M	—	231	1.0
3	M	—	31	1.1
4	M	—	28	1.0
5	M	—	22	1.2
6	M	—	13	1.2
7	M	—	59	1.3
8	M	—	110	0.9
9	M	—	92	1.0
Mean $\pm$ SE			71 $\pm$ 23	1.1 $\pm$ 0.05

Skin test was performed by intradermal injection with aprotinin at a concentration of 10^{-4} g/ml (7.143 KIU/ml). — : Negative.

Table 2 Immunological findings of atopic patients with bronchial asthma

No. of volunteers	Sex	Skin test	Serum IgE (IU/ml)	Specific IgE antibody (RAST index)
1	F	—	330	0.8
2	M	—	528	1.0
3	M	—	418	1.3
4	M	—	858	1.0
5	M	—	506	1.0
6	F	—	286	1.1
7	F	—	165	1.1
8	F	—	770	1.1
Mean $\pm$ SE			483 $\pm$ 84	1.1 $\pm$ 0.05

Skin test was performed by intradermal injection with aprotinin at a concentration of 10^{-4} g/ml (7.143 KIU/ml). — : Negative.

serum IgE levels than the healthy volunteers. None had a past history of aprotinin therapy. These findings indicated that aprotinin had no ability to induce histamine release from human skin mast cells or to share the common antigenic determinant with atopic allergens responsible for the production of specific IgE antibodies.

Subsequently, the sera obtained from the 18 patients sensitive to aprotinin were investigated, although the dosages of aprotinin were unknown. The clinical symptoms induced by aprotinin administration were as follows; shock, urticaria, erythema, fever, vomiting and so on. As indicated in Table 3, positive RAST was found in 6 out of the 11 patients (54.5%) immediately after hypersensitivity, in 8 out of the 17 patients (47.1%) at 2-4 weeks after hypersensitivity and in 1 out of the 4 patients (25.0%) at 8 weeks after hypersensitivity. Totally, 10 out of the 18 patients (55.6%) were positive to RAST. Note that the RAST index attained its maximum (40.3) in one patient (No. 6) in group A. In addition, 3 out of the 8 male patients (37.5%) and 7 out of the 10 female patients (70.0%) showed positive RAST. Our findings were in agreement with the survey of adverse reactions to aprotinin in which the frequency was shown to be approximately two times higher in female (1.92%) than in male individuals (0.88%)¹⁸⁾. The reasons, however, are still unknown. Whereas positive RAST was observed in 5 out of the 7 patients with a past history of aprotinin therapy (71.4%), 3 out of the 8 patients with no history (37.5%) and 2 out of the 3 patients with unknown history (66.7%) were positive to RAST. These results showed consistently that whereas the frequency of adverse reactions to aprotinin was 0.72% in 972 patients receiving their new administration, it increased to 9.09% in patients receiving a repeat administration (personal communication from Bayer Pharmaceutical Co., Osaka). In 2 patients with a past history of drug allergy (pyrazolone derivatives-induced hypersensitivity reactions), one showed positive RAST and the other negative RAST. More interestingly, a gradual decrease in anti-aprotinin IgE levels was observed in 5 out of the 6 patients showing positive RAST by 2-4 weeks after the provocation of hypersensitivity, suggesting that the specific IgE antibody might be transiently neutralized by specific antigen. Indeed, a similar phenomenon was often found in patients allergic to penicillin¹⁹⁾. By contrast, it is not clear whether aprotinin could induce the secondary IgE antibody

Table 3 Immunological findings of patients with hypersensitivity reactions to aprotinin

No. of patients	Age	Sex	Previous history of aprotinin therapy	History of drug allergy	Serum IgE ^{a)} (IU/ml)	Specific IgE (RAST index)			Specific IgG ^{b)}		Clinical symptoms of hypersensitivity
						A	B	C	Agar gel	PCA	
1	31	F	No	No	209	NT	13.1	NT	—	—	Urticaria, nausea, vomiting, fever
2	55	M	No	No	165	1.7	1.4	NT	—	—	Facial flush, fever, erythema
3	40	F	Yes	No	242	13.9	4.4	NT	—	—	Nausea, urticaria, chill
4	55	F	No	No	165	1.7	1.2	NT	—	—	Shock
5	45	M	No	Yes (pyrazolone)	121	NT	4.1	NT	—	—	Shock
6	63	F	Unknown	No	22	40.3	14.2	NT	—	—	Shock
7	27	F	No	No	110	1.5	3.8	3.5	—	—	Shock, coma
8	61	F	Yes	No	5	2.5	1.7	1.6	—	—	Shock
9	74	M	Yes	Unknown	143	3.0	NT	NT	—	—	Shock
10	69	M	Yes	No	156	NT	2.4	NT	—	—	Shock, rash
11	54	M	No	No	37	NT	1.6	NT	—	—	Vomiting, palpitation
12	53	F	Unknown	No	22	3.0	2.2	NT	—	—	Chill, fever, rash
13	56	F	No	No	30	NT	1.1	1.3	—	—	Shock
14	43	M	Yes	Unknown	121	NT	1.4	1.5	NT	NT	Anxiety of breast, erythema, convulsion
15	53	M	Yes	Yes (pyrazolone)	308	1.6	1.4	NT	NT	NT	Itching, papula, facial flush
16	52	M	No	No	13	NT	1.3	NT	NT	NT	Rash
17	44	M	Unknown	No	83	1.3	1.7	NT	NT	NT	Shock
18	43	F	Yes	No	814	7.8	2.0	NT	NT	NT	Shock

Time of sampling: A; Immediately after hypersensitivity, B; 2-4 weeks after hypersensitivity, C; 8 weeks after hypersensitivity. —: Negative. NT: Not tested.

^{a)}: Serum IgE measured within 2-4 weeks after the provocation of hypersensitivity.

^{b)}: Specific IgG antibody tested within 8 weeks after the provocation of hypersensitivity.

response in patients with a past history of aprotinin therapy, since RAST was not determined in them before aprotinin administration. On the other hand, the mean serum IgE levels of the 18 patients sensitive to aprotinin showed 154 ± 44 IU/ml within 2-4 weeks after the provocation of hypersensitivity, which were significantly lower than those of the atopic patients with bronchial asthma (488 ± 84 IU/ml). No significant correlation was observed between the serum IgE level and the RAST index in these patients. In addition, hypersensitivity reactions were clearly induced in some patients with low and negative levels of anti-aprotinin IgE antibody. In fact, no correlation has been known to exist between the serum level of specific IgE antibody and the severity of anaphylactic symptoms, especially in drug allergy¹⁹⁾²⁰⁾. It should be noted that 8 out of the 18 patients sensitive to aprotinin were new to aprotinin therapy and showed hypersensitivity reactions induced by a single administration of aprotinin. In contrast to above findings, 7 of them had a past history of aprotinin therapy. These results strongly suggest the possibility that aprotinin might be exerting its effect on some hypersensitivity reactions in the 8 patients receiving a single administration as a pseudoallergen and on those in the 7 patients with a past history of its therapy as a specific antigen. With respect to the specific IgG antibody, it was not detected in the sera obtained from 13 out of the 18 patients sensitive to aprotinin by agar-gel diffusion and heterologous PCA in guinea pigs within 8 weeks after the provocation of hypersensitivity (Table 3).

Finally, the sera obtained from the 44 patients insensitive to aprotinin were tested for specific antibodies before and up to 8 weeks after the end of aprotinin therapy. Tables 4 and 5 show the results obtained from the 44 patients with positive or negative RAST. 42 out of the 44 patients had no past history of aprotinin therapy and 2 were unknown; 14 of them (31.8%) were positive to RAST after aprotinin therapy and the remainder (62.8%) were negative. No significant difference was observed in serum IgE levels between patients showing positive and negative RAST, suggesting that the production of anti-aprotinin IgE antibody in patients was unconnected with their predisposition to atopy. The mean total dosages of aprotinin were $(336.6 \pm 76.6) \times 10^4$ KIU in the 14 patients showing positive RAST and $(64.5 \pm 18.5) \times 10^4$ KIU in the 30 patients showing negative RAST. They were significantly higher in patients with positive RAST than in those with negative RAST. Most patients with negative RAST who were considered to be unsensitized received a single administration of aprotinin, whereas most patients with positive RAST who were considered to be sensitized had aprotinin therapy for several days. Positive RAST was observed in 6 out of the 14 patients immediately after the end of aprotinin therapy, in 9 out of the 12 patients at 2 weeks after the end of aprotinin therapy and in all 6 of the patients at 8 weeks after the end of aprotinin therapy (Table 4). The results obtained here indicated that the specific IgE antibody was highly detected in the sera taken from the patients insensitive to aprotinin as time passed after aprotinin administration. The RAST index attained its maximum (27.5)

Table 4 Immunological findings of patients with positive RAST who had no hypersensitivity reactions after aprotinin administration

No. of patients	Age	Sex	Total dose of aprotinin ($\times 10^4$ KIU)	Previous history of aprotinin therapy	Serum IgE ^{a)} (IU/ml)	Specific IgE (RAST index)				Specific IgG ^{b)} (Agar gel)
						A	B	C	D	
1	47	F	25	No	77*	NT	1.0	13.9	10.9	—
2	6	F	16.8	No	209	2.8	2.2	4.0	3.6	—
3	75	M	550	No	132	1.3	27.5	25.9	NT	—
4	48	M	390	No	143	1.1	0.6	1.7	2.1	—
5	48	M	300	No	77	1.6	2.4	16.5	NT	—
6	72	M	390	No	396	1.1	1.5	1.7	12.8	—
7	48	F	350	No	16	1.0	11.2	1.4	NT	—
8	55	M	210	No	165	1.1	1.2	14.9	NT	—
9	51	M	220	No	31	1.0	16.5	22.3	NT	—
10	69	M	210	No	187	1.3	1.2	NT	12.7	—
11	55	F	220	No	8	0.9	0.9	6.5	NT	—
12	70	M	1,200	No	48	1.0	0.8	2.4	NT	—
13	53	F	380	No	12	1.3	4.0	NT	NT	—
14	38	F	250	No	5	1.2	1.0	8.1	2.2	—

Time of sampling: A; Before administration, B; Immediately after the end of aprotinin therapy, C; 2 weeks after the end of aprotinin therapy, D; 8 weeks after the end of aprotinin therapy. —: Negative. NT: Not tested.

^{a)}: Serum IgE measured before aprotinin administration(*: measured immediately after the end of aprotinin therapy).

^{b)}: Specific IgG antibody tested within 8 weeks after the end of aprotinin therapy.

Table 5 Immunological findings of patients with negative RAST who had no hypersensitivity reactions after aprotinin administration

No. of patients	Age	Sex	Total dose of aprotinin ($\times 10^4$ KIU)	Previous history of aprotinin therapy	Serum IgE ^{a)} (IU/ml)	Specific IgE (RAST index)				Specific IgG ^{b)} (Agar gel)
						A	B	C	D	
15	28	M	25	No	99	1.2	1.1	0.9	1.1	—
16	38	M	25	No	30	1.1	1.3	1.3	NT	—
17	19	M	25	No	550	1.1	1.2	1.4	NT	—
18	18	F	25	No	165	1.6	1.1	1.4	NT	—
19	25	M	25	No	165	1.1	1.2	1.3	NT	—
20	33	M	59.5	No	198	1.9	1.2	1.5	1.9	—
21	28	M	58	No	27	1.3	0.9	1.1	1.0	—
22	4	M	16.5	No	23	1.1	1.3	1.2	1.0	—
23	36	M	48.5	No	19	1.0	1.0	0.8	1.3	—
24	4	F	14	No	14	1.4	0.8	0.9	1.1	—
25	12	M	49.5	No	22	1.3	1.0	1.0	1.0	—
26	28	F	38	No	110	1.0	1.1	1.3	0.9	—
27	3	M	12	No	33	1.1	0.9	NT	NT	—
28	6	M	15.3	No	5	0.9	0.8	0.9	1.0	—
29	26	F	46.5	No	26	1.0	1.0	1.3	NT	—
30	6	F	15	No	19	0.8	1.2	0.8	0.9	—
31	6	F	17	No	132	0.9	1.3	1.2	1.1	—
32	11	M	34	No	35	0.9	0.9	1.6	1.4	—
33	11	M	30.3	No	23	1.0	0.9	1.2	0.8	—
34	53	F	60	No	12	0.9	1.0	1.0	NT	—
35	50	M	60	Unknown	7	1.2	0.9	1.1	NT	—
36	52	M	55	No	77	1.3	0.9	1.3	NT	—
37	38	F	60	No	5	1.2	1.1	0.9	NT	—
38	56	M	55	No	132	1.2	0.9	1.1	NT	—
39	48	M	60	No	41	0.9	0.9	1.0	0.9	—
40	44	M	60	Unknown	176	0.9	1.0	NT	NT	—
41	61	F	60	No	14	1.0	1.5	NT	NT	—
42	57	M	65	No	121	0.9	1.2	NT	NT	—
43	39	M	280	No	77	0.9	0.9	NT	NT	—
44	43	M	540	No	101	0.9	0.9	1.2	NT	—

Time of sampling: A; Before administration, B; Immediately after the end of aprotinin therapy, C; 2 weeks after the end of aprotinin therapy, D; 8 weeks after the end of aprotinin therapy. —: Negative. NT: Not tested.

^{a)}: Serum IgE measured before aprotinin administration.

^{b)}: Specific IgG antibody tested within 8 weeks after the end of aprotinin therapy.

in the patients with no hypersensitivity reactions (group B). The frequency of positive RAST was totally 27.6% in the 29 male patients and 40.0% in the 15 female patients, although a significant difference was not observed between male and female patients. It is obvious from our findings that aprotinin is capable of producing the specific IgE antibody in some patients receiving aprotinin therapy. Indeed, Awaya et al.²¹⁾ have reported that aprotinin-induced lymphocyte proliferation is observed in 1 out of the 4 patients with a past history of aprotinin therapy, suggesting the antigenicity of aprotinin to human lymphocytes. Further study is needed to evaluate the clinical role of anti-aprotinin IgE antibody in hypersensitivity reactions, although anti-insulin antibody mainly responsible for resistance to insulin therapy in diabetic patients is known to be unconnected with the severity of hypersensitivity reactions to insulin²²⁾. By contrast, none of the patients produced the specific IgG antibody which was detected by agar-gel diffusion. From these results, we concluded that anti-aprotinin IgE antibody was transiently produced in some patients insensitive to aprotinin.

Direct evidence was not obtained in the present study that aprotinin played an important role in hypersensitivity reactions as a specific antigen rather than as a pseudoallergen. However, it should be emphasized that the frequency of hypersensitivity reactions is obviously higher in patients receiving a repeat administration of aprotinin than in those

receiving their initial administration. Our results, which are in agreement with the findings of some other authors¹⁰⁻¹³⁾, strongly suggest that immunological mechanisms might be involved in at least some of the hypersensitivity reactions to aprotinin. Thus, close attention should be paid when aprotinin is re-administered, because this agent is capable of producing the specific IgE antibody in humans.

SUMMARY

Sera obtained from patients who had received aprotinin therapy were tested for IgE and IgG antibodies specific to aprotinin. The specific IgE antibody was detected in 10 out of the 18 patients sensitive to aprotinin when assayed by radioallergosorbent test (RAST). In addition, 14 out of the 44 patients with no hypersensitivity reactions to aprotinin were positive to RAST. The frequency of positive RAST was higher in female patients as well as in patients receiving a repeat administration of aprotinin. No significant correlation was observed between the RAST value and the serum IgE level in patients receiving aprotinin therapy. In contrast, none of the sera from patients contained anti-aprotinin IgG antibody estimated by agar-gel diffusion and/or passive cutaneous anaphylaxis in guinea pigs. These results indicate that aprotinin is capable of producing the specific IgE antibody in humans.

Serum samples from patients who received aprotinin therapy were supplied by 21 different hospitals in Japan. We are grateful to the many medical doctors who supplied us with blood samples.

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