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Immunogenicity Study of Recombinant Human Basic Fibroblast Growth Factor

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Abstracts – The immunogenicity of the recombinant human basic fibroblast growth factor (rh-bFGF) was investigated by tests for active systemic anaphylaxis (ASA), passive cutaneous anaphylaxis (PCA), passive hemagglutination (PHA) and guinea pig maximization test (GPMT) in mice or guinea pigs. Guinea pigs were sensitized with rh-bFGF (100-1000 μg/kg) or rh-bFGF-CFA mixture (1000 μg/kg). All animals sensitized with rh-bFGF alone or mixture with CFA showed symptoms of anaphylactic shock. IgE antibodies to rh-bFGF were detected in sera obtained from rh-bFGF and rh-bFGF-Alum (1000 μg/kg) sensitized mice, indicating that rh-bFGF has immunogenicity eliciting potential. IgG and/or IgM antibodies to rh-bFGF were also detected in all the sera obtained from sensitized mice by PHA. In the GPMT for delayed type skin reaction, no skin reaction was observed in sensitized guinea pigs after intradermal injection and dermal application of 0.01% rh-bFGF. However, these positive reactions were consistent with the results of another rh-bFGF, showing that rh-bFGF is a heterogenous protein to rodents. Considering the fact that rh-bFGF is a genuine human protein of which structure is identical to the endogenous human bFGF, it is thought that rh-bFGF is rarely associated with immunological problems in clinical use.

Keywords□ Recombinant Human basic Fibroblast Growth Factor, Immunogenicity, Guinea Pig Maximization Test.

In the past decade there has been an enormous increase in research involving fibroblast growth factor (FGF) as a result of the development of effective methods for the isolation of the potential and availability of characterization nucleic acid probes (Abraham et al., 1986; Rifkin and Moscatelli, 1989). FGF was originally identified in extracts of pituitary and brain that stimulated the growth of 3T3 cells (Armelin, 1973; Gospodarowicz, 1974). The activity was shown to be due to two proteins, acidic FGF (aFGF) and basic FGF (bFGF) (Thomas, et. al., 1984, Esch et. al., 1985). According to the nomenclature accepted by a conference organized by the New York Academy of Science in 1991 (Baird and Klagsburn, 1991), the FGF family has seven members: FGF-1 or aFGF, FGF-2 or bFGF, FGF-3, FGF-4, FGF-5, FGF-6 and FGF-7.

One of the most well-characterized members of the group is bFGF, and since successful cloning of the gene, large amounts of bFGF can be manufactured by mass-cultivation of bacteria by gene-recombination procedures. Basic FGF exhibits a wide range of *in vitro* biological activities, such as stimulation of cell mitogenesis and chemotaxis (Baird and Walicke, 1989; Gospodarowicz, et. al., 1987; Rifkin and Moscatelli, 1989). The mitogenic effects of bFGF are directed primarily toward cells of mesodermal or neuroectodermal origin. It is now well-known that bFGF induces proliferation of vascular endothelial and smooth muscle cells, corneal endothelial cells, osteoblasts, certain neural cells and chondrocytes as well as fibroblasts (Gospodarowicz, et. al, 1987). In addition, bFGF has been shown *in vivo* to be an effective stimulant of neovascularization (Folkman and Klagsbrun, 1987) and a strong angiogenic action (Sato, 1993). Based on the fact that wide range of cell types are bFGF sensitive, it has been hypothesized that bFGF has a diverse role in wound healing, tissue regeneration and embryonic development (Rifkin and Moscatelli, 1989; Szabo and Sandor, 1996). By far the most important,

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from a therapeutic standpoint, are the diseases associated with cardiovascular ischemia due to progressive artherosclerosis (Lobb, 1988). Currently bFGF is the subject of phase I trials in patients with ischemic heart disease in the USA (Shou et. al., 1996), and phase II trials in patients with peptic ulcers in USA and Europe (Rabasseda and Mealy, 1995).

Recently, Dong-A Pharmaceutical Co. has developed the process to produce large quantities of recombinant human bFGF(rh-bFGF) in *Escherichia coli*. And it is now under preclinical development for the treatment of poorly or non-healing wounds such as intractable dermal ulcer, venous ulcer, diabetic ulcer and traumatic wound, and neurological and cardiovascular disorders such as stroke and coronary artery disease. The purpose of the present study was to evaluate the antigenicity of rh-bFGF in guinea pigs and mice, and the contact allergenicity using guinea pigs.

MATERIALS AND METHODS

Test substance

The test substance, a recomnbinant human bFGF (DA-3050, Lot No.; B-005, Purity; >98% by HPLC method, molecular weight; 17000 dalton by SDS-PAGE method), was produced by Dong-A Pharmaceutical Co. (Kyunggi, Korea). It was an aqueous solution containing 10 mg/ml of rh-bFGF in vehicle (citrate buffer of pH 6.0). The substance was stored at -4℃ before use. As positive controls, bovine serum albumin (BSA, Sigma) in the antigenicity test and 2, 4-dinitrochlorobenzene (DNCB, Sigma) in GPMT were used. The vehicle was used as a negative control. BSA and DNCB were dissolved in physiological saline and corn oil, repectively.

Animals

Male Hartley guinea pigs (350-450 g), Sprague-Dawely rats (12 weeks of age) and C57BL/6 mice (6 weeks of age) were purchased from animal supplier and Charles River Japan (Kanagawa, Japan) and used in the experiments. Animals were housed in each cage maintained at a temperature of 23±2℃, with a relative humidity of 55±15%, 12-h-lighting period and an air exchange of 12 times/hour. They were fed commercial pellet-form food (Cheil) and tap water *ad libitum*.

Sensitization procedure

Guinea pigs

Six male Hartley guinea pigs in each group were sensitized by subcutaneous injection of the test or control substance with or without complete Freund's adjuvant (CFA, Difco). Vehicle (negative control) or rh-bFGF at doses of 100 or 1000 μg/kg were sensitized with two times per week for 3 weeks. A mixture of rh-bFGF and CFA (bFGF-CFA 1000 μg/kg) or a mixture of BSA and CFA (BSA-CFA 10 μg/head) were also sensitized by s.c. injection for 3 times at one-week intervals. The administration volume was 0.5 ml/head.

For the maximization test in guinea pigs, the sensitization groups were comprised of a rh-bFGF administration group, a DNCB administration group and a non-treatment group. Five male and female guinea pigs in each group were used. Hairs on the shoulder region were removed and an area of 2×4 cm was marked with an oil pen. In the marked area 3 pairs of symmetrical intradermal injections were given simutaneously. A mixture of distilled water and CFA (1 : 1) was injected at either side of the upper part, rh-bFGF (0.01%) or DNCB (0.1%) at either side of the middle part and a mixture of rh-bFGF and CFA or a mixture of DNCB and CFA at either side of the lower part. The concentration of rh-bFGF used in the present study was 0.01% which is the anticipated concentration for clinical use. The volume administered was 0.05 ml per site. On day 6, since rh-bFGF has not shown the primary irritative effects, 0.5 g of vaseline ointment containing 10% sodium lauryl sulfate (SDS, Sigma) was applied non-occulsively at the intradermal treatment site for the purpose of enhancing the test substance penetration. The shoulders at the intradermal injection site of the guinea pigs were removed again 7 days after the first immunization. Whatman paper (No 3, 2×4 cm) incorporating 0.2 ml of rh-bFGF or 0.2g vaseline ointment containing 1% DNCB was applied at the intradermal treatment site, and fixed occulsively for 48 hours.

Mice

Six male C57BL/6 mice in each group were used in this study. As the sensitization groups, three rh-bFGF dosage groups, a BSA group and a negative control group were prepared. The sensitization was performed by injecting subcutaneously vehicle or rh-bFGF alone (100 or 1000 μg/kg) two times per week for three weeks, and intraperitoneally a mixture of rh-bFGF and aluminium hydroxide gel (Alum, Serva; bFGF-Alum 1000 μg/kg) or

a mixture of BSA and Alum (BSA-Alum 1 μg/head) 3 times at one-week intervals. The volume injected was 10 ml/kg.

Acute Systemic Anaphylaxis in guinea pigs

3-week after the last sensitization, the challenge antigen was injected (1 ml/head) into a cephalic vein and animals were observed for 2 hours for the occurrence of an active systemic anaphylaxis reaction. The signs observed were graded according to the followings.

Grade 0 (−) : Asymptomatic
I(±) : Mild : Symptomes with 1~4
II(+) : Moderate : Symptomes with 1~10
III(++) : Severe : Symptomes with 1~19
IV(+++) : Death : death

0. No sign, 1. restless, 2. piloerection, 3. tremor, 4. rubbing or licking nose, 5. sneezing, 6. coughing, 7. hyperpnea, 8. urination, 9. evacuation, 10. lacrimation, 11. dyspnea, 12. rhonchus, 13. cyanosis, 14. staggering, 15. jumping, 16. gasping and writhing, 17. convulsion, 18. side position, 19. Cheyne-Stokes respiration, 20. death.

Passive Cutaneously Anaphylaxis reaction in mouse-rat

Mouse IgE antibodies were measured according to the method of Mota and Wong (1969) from 24-h PCA with rats as recipients. The blood was obtained from the retro-orbital venous plexus of the sensitized mice two weeks after the last sensitization, and was centrifuged to obtain the sensitized sera. The sera obtained were used after 2-fold serial dilutions with physiological saline starting from a 4-fold dilution. The hair was removed from the dorsal skin of the non-treated rats (approximately 14 weeks), and the diluted sensitized serum was injected intradermally at 0.01 ml at each site for passive immunization. Approximately 24 hours later, the challenge antigen was injected into the tail vein at 1 ml/head. Mixture of equal volumes of Evan's blue (Sigma, 0.5%) and rh-bFGF or BSA were used as the challenge antigen. Thirty min after the challenge, all recipient animals were sacrificed. The dorsal skin was removed and the diameter of the blue region at the intradermal injection sites was measured. When the macular cerrulean showed more than 5 mm in diameter {(longest+shortest)/2}, the reaction was judged to be positive. PCA titer was expressed as the reciprocal of the highest serum dilution producing a positive reaction.

Passive Hemoagglutination reaction

The sera obtained from the mice (PCA in mouse-rat) were used in this test. The sensitized sera were diluted from 2^3 to 2^{10} with a microtiter method. The erythrocytes coated with antigen were performed according to the method of Avrameas et al (1969). Each antigen solution (15 ml) either rh-bFGF or BSA was mixed by adding slowly 0.4 ml of sRBC (sheep RBC, Korea Media Corp.) sediments and 1.0 ml of 2.5% glutaraldehyde (Sigma), and the mixture was stirred for 1 hour at room temperature. After washing coated erythrocytes with PBS, the coated erythrocytes were suspended in PBS containing 1% normal mouse serum to obtain a 1% erythrocyte concentration. To each diluted serum, same volume (50 μl) of the antigen-coated erythrocyte suspension was added, and the mixture was well mixed. The mixture was then incubated at 37℃ for 2 hours, and placed at room temperature for 2~3 hours. The hemoagglutination condition was examined at the bottom of the plate. PHA titer was expressed as described previously for PCA.

Maximization Test in guinea pigs

Maximization test in guinea pigs was performed according to the method of Magnusson and Kligman (1969). Two weeks after sensitization by application of each antigen, the right flank of the animals were shaved. Whatman paper (No 3) incorporating 0.1 ml of rh-bFGF or 0.1 g vaseline ointment containing DNCB (0.1%) was applied to the shaved site (2×2 cm) and fixed occulsively for 24 hours. At 24, 48 and 72 hours following the commencement of the application, the skin was observed for the occurrence of allergic erythema, crust formation, and edema. The degree of the changes was recorded according to Magnusson and Kligman (Table I, II).

RESULTS

Acute Systemic Anaphylaxis in guinea pigs

In the rh-bFGF 100 μg/kg sensitized group, licking or rubbing nose, piloerection, sneezing, coughing, labored

Table I. Evaluation of skin reactions

Skin reaction	Score
No reaction	0
Scattered mild redness	1
Moderate and diffuse redness	2
Intense redness and swelling	3

Table II. Maximization grading

Sensitization rate	Grade	Classification
0-8	I	weak
9-28	II	mild
29-64	III	moderate
65-80	IV	strong
81-100	V	extreme

breathing, evacuation of feces and micturition, staggering gait, convulsion and side position were observed following challenge with rh-bFGF 1000 μg/kg. But, no animal was dead. In the rh-bFGF 1000 μg/kg group, all animals showed the symptoms described above after challenging with antigen. Five out of six animals died within 4-5 min and one survived for 2 hours. On the other hand, all the guinea pigs sensitized with rh-bFGF-CFA or BSA-CFA exhibited the anaphylactic symptoms, and died of an anaphylaxis within 5 min after the injection of challenge antigen (Table III).

Passive Cutaneously Anaphylaxis reaction in mouse-rat

The rats received intradermal injection of immunized mouse sera were challenged intravenously with rh-bFGF or BSA. As shown in Table IV, IgE antibodies to rh-bFGF were detected in all sera obtained from mice sensitized with rh-bFGF 100, 1000 μg/kg or bFGF-Alum. PCA titer was therefore more than 8 (8->64) in all rh-bFGF sensitized groups. The sera obtained from BSA-Alum sensitized mice showed positive reactions when challenged with BSA, resulting in a more than 64 of PCA titers. In negative control, sera sample obtained from the vehicle-treated group showed no positive reactions on challenge with vehicle, rh-bFGF or BSA.

Passive Hemoagglutination reaction

Table IV. Results of passive cutaneous anaphylaxis reaction in mouse-rat

Test substance	Dose (μg/kg)	Routes	No. of serum	Challenging Antigen		
				Vehicle	rh-bFGF	BSA
Vehicle[a]	-	s.c.	1	<4[b]	-[c]	-
			2	<4	-	-
			3	-	<4	-
			4	-	<4	-
			5	-	-	<4
			6	-	-	<4
rh-bFGF	100	s.c.	1	-	8	-
			2	-	8	-
			3	-	8	-
			4	-	32	-
			5	-	32	-
			6	-	8	-
	1000	s.c.	1	-	32	-
			2	-	16	-
			3	-	64	-
			4	-	32	-
			5	-	32	-
			6	-	32	-
rh-bFGF+ Alum	1000	i.p.	1	-	>64	-
			2	-	>64	-
			3	-	<64	-
			4	-	>64	-
			5	-	>64	-
			6	-	>64	-
BSA+ Alum	5 μg/ head	i.p.	1	-	-	>64
			2	-	-	>64
			3	-	-	>64
			4	-	-	>64
			5	-	-	>64
			6	-	-	>64

a; Buffer b; PCA titer c; Not tested.

When the rh-bFGF 100 or 1000 μg/kg sensitized serum was mixed with erythrocytes coated with rh-bFGF, one

Table III. Results of active systemic anaphylaxis in guinea pigs

Group	Sensitization		Challenge antigen (μg/kg)	No. of animals	Severity[a]				
	Antigen	Dose (μg/kg)			0	I	II	III	IV
1	Vehicle	-	Vehicle	2	2	0	0	0	0
			rh-bFGF 1000	2	2	0	0	0	0
			BSA 10 μg/head	2	2	0	0	0	0
2	rh-bFGF	100	rh-bFGF 1000	6	0	0	0	6	0
3	rh-bFGF	1000	rh-bFGF 1000	6	0	0	0	1	5
4	rh-bFGF+CFA	1000	rh-bFGF 1000	6	0	0	0	0	6
5	BSA+CFA	10 μg/head	BSA 10 μg/head	6	0	0	0	0	6

a; Grade 0 : No symptome, I : Mild, II : Moderate, III : Severe, IV : Death.

Table V. Results of passive hemagglutination reaction

Test substance	Dose (μg/kg)	Coated Ag[b]	No. of serum	Response[c]						
				1	2	3	4	5	6	Mean
Vehicle[a]	-	rh-bFGF	6	<8	<8	<8	<8	<8	8	1.3
rh-bFGF	100	rh-bFGF	6	<8	<8	8	<8	<8	<8	1.3
	1000	rh-bFGF	6	<8	<8	32	64	<8	16	18.6
rh-bFGF + Alum	1000	rh-bFGF	6	32	32	64	128	32	64	58.7
BSA+Alum	5 μg/head	BSA	6	128	256	512	128	128	512	277.3

a; Buffer b; Antigen c; PHA titer.

out of six sera in the rh-bFGF 100 μg/kg group showed hemoagglutination a PHA titer of 8, and three out of six sera in the rh-bFGF 1000 μg/kg group showed hemagglutination a PHA titer between 8 and 64. In the rh-bFGF-Alum group, hemagglutination was observed in all the animals with the PHA titer of 32, 32, 64, 128, 32 and 64, respectively. When the BSA-Alum sensitized serum was mixed with erythrocytes coated with BSA, hemagglutination was observed in all animals indicating a hemagglutination titer of between 128 and 512 (mean 277) (Table V).

Maximization Test in guinea pigs

In the 0.01% rh-bFGF sensitized group, no skin reaction was observed in any animals at any observation times following challenge with rh-bFGF. In the DNCB sensitized group, moderate to severe erythema and swelling were observed in all animals at all observation times after DNCB challenge (Table VI).

DISCUSSION

Basic FGF is a new type of therapeutic agent for wound healing which stimulates the responses important for wound healing processes such as cell proliferation, cell migration, angiogenesis, granulation tissue formation and re-epithelialization (Folkman and Klagsbrun, 1987; Norrby, 1994; Presta et al, 1989; Sprugel et al, 1987; Tsuboi and Rifkin, 1990). As the factor has multiple actions, it would possibly be used not only topically but also systemically for various diseases. As a part of the study to foresee the safety of rh-bFGF in clinical use, the antigenicity and the contact allergenicity study of the drug administered via subcutaneously or topically route were performed.

In the ASA test in guinea pigs, anaphylactic shock was observed following administration of the challenge antigen in all animals sensitized with rh-bFGF. The same symptoms were also observed in the BSA-treated, positive control, guinea pigs. These symptoms therefore are considered to be attributable to anaphylactic reaction initiated by interaction of antigen and antibody. This result demonstrates that rh-bFGF is positive in ASA reactions in guinea pigs. Levine and Vaz (Levine and Vaz, 1970) and other investigators reported IgE type antibody formation in a certain inbred strain mice injected intraperitoneally with antigen together with Alum in a similiar fashion to human IgE-type antibody formation. Furthermore, Mota and Wong (1969) have found that murine IgE type antibody is different from IgG_1 type antibody in ability to sensitize rat skin, and that only IgE type antibody participates in the PCA reaction in the rat. Therefore, to investigate whether or not rh-bFGF can elicit IgE type antibody formation, C57BL/6 strain mice were immunized with rh-bFGF as an Alum adjuvant. In the results, blue regions were observed at all site treated with sera from animals sensitized with rh-bFGF 100, 1000 μg/kg or rh-bFGF-Alum after challenge with rh-bFGF. The PCA titer was more than 8 in all the sensitized sera. From these results, it was thought that mouse IgE type antibody to rh-bFGF was also produced in mice. In the PHA, antigen is physically or covalently bound to the surface of passive carriers, erythrocytes, of antigens, and named as a passive hemagglutination. In general, agglutination assay is used to detect the presence of IgM and/or IgG type antibody, and IgM is said to be 750 times more efficient than IgG in agglutination reactions. In this study, the erythrocytes coated with rh-bFGF were examined according to the method of Avrameas et al (1969) and PHA assay was performed following the guidelines of the KFDA (Standards for toxicity of drugs, 1998). When the rh-bFGF sensitized serum was mixed with erythrocytes coated with rh-bFGF, hemagglutination titer was more than 8 (1/6 sera in bFGF

Table VI. Evaluation of skin reaction in guinea pig maximization test

Sensitization	Challenge	Time (hr)	Sex	No. of animals	Score of skin reaction				Mean	Sensitization rate (%)	Grade
					0	1	2	3			
Untreatment	Vehicle	24	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
	rh-bFGF	24	M	5	5	0	0	0	0	0	II
			F	5	5	0	0	0	0		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
rh-bFGF	vehicle	24	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
	rh-bFGF	24	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
Untreatment	vaseline	24	M	5	5	0	0	0	0	10	II
			F	5	4	1	0	0	0.2		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
	DNCB	24	M	5	4	1	0	0	0.2	10	II
			F	5	5	0	0	0	0		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
DNCB	vaseline	24	M	5	4	1	0	0	0.2	10	II
			F	5	5	0	0	0	0		
		48	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
		72	M	5	5	0	0	0	0	0	I
			F	5	5	0	0	0	0		
	DNCB	24	M	5	0	0	4	1	2.2	100	V
			F	5	0	0	3	2	2.4		
		48	M	5	0	2	2	1	1.8	100	V
			F	5	0	0	4	1	2.2		
		72	M	5	0	3	2	0	1.4	100	V
			F	5	0	2	3	0	1.6		

100 μg/kg group, 3/6 in bFGF 1000 μg/kg group and 6/6 in bFGF-Alum group, respectively), indicating that rh-bFGF is positive in PHA.

As described above, rh-bFGF has the potential for immediate type allergic reaction in ASA (guinea pig), PCA (mouse-rat) and PHA. But, these positive results were thought to be caused due to the fact that rh-bFGF is a heterogenous protein to guinea pigs and mice. In fact, the other rh-bFGF, KCB-1, developed by Kaken Pharmaceutical Co. Ltd. (Japan), showed positive reactions in ASA and PCA in guinea pigs, Arthus reaction in guinea pigs and PHA, and the authors analysed the results in the same way (Nagatahira et al, 1996b). Nakamura et al (1996a, b, c) also reported that IgG and/or IgM type antibody titers were elevated dose dependently in rats and dogs subcutaneously treated with rh-bFGF for one-month or more, and said that it was attributable to the fact that rh-bFGF is a heterogenous protein to rats or dogs. In clinical trials, however, antibody to KCB-1 was not produced in patients and normal volunteers (Ishibashi et al, 1996; Itoh and Uji, 1992). Therefore, considering the fact that rh-bFGF is a genuine human fibroblast growth factor of which structure is identical with endogenous human protein, it is thought that rh-bFGF is rarely associated with immediate type allergic reactions in clinical use.

New drugs, chemicals, including a variety of cosmetics, and industrial products are assessed for any risk of contact sensitization in the skin by substances used as drugs for topical application. As the rh-bFGF has multiple actions, it would possibly be used not only systemically but also topically for various disease. In this study, the maximization test in guinea pigs were performed to assess the contact sensitization according to Magnusson and Kligman (1969). In results, no positive skin reactions interpreted as being delayed hypersensitivity reactions were observed in any animals sensitized with rh-bFGF following challenged with antigen, indicating that rh-bFGF is negative in the delayed type skin reaction. These findings are comparable to the results of a previous study that KCB-1, another rh-bFGF, had no positive reactions in the contact allergenicity test except for the cutaneous reactions represented an allergic reaction related to humoral antibodies (Nagatahira et al, 1996a). In the current study, however, no allergic reactions related to humoral antibodies were observed and the discrepant results were thought to be attributable to the dose of rh-bFGF used. The dose of rh-bFGF, in this study, intradermally delivered on the guinea pig was 0.1 mg/ml, 48.4 times lower than that used in the KCB-1 study.

In summary, the results from these immunogenicity studies demonstrate that rh-bFGF has the potential for immediate type allergic reaction, but not for delayed type skin reaction in animals. However, the positive reactions were, as discribed above, thought to be caused due to the fact that rh-bFGF is a heterogenous protein to rodents. Therefore, considering the fact that rh-bFGF is a genuine human endogenous protein, it is thought that rh-bFGF is rarely associated with immunological problems in clinical use.

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