Comparison of the Therapeutic Effects Recombinant Human Acidic and Basic Fibroblast Growth Factors in Wound Healing in Diabetic Patients

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To explore an optimal improvement of diabetic wound healing we have comparatively studied the effects of recombinant human acidic and basic fibroblast growth factors (rhaFGF and rhbFGF) on the healing impaired skin wounds in diabetic patients. Before using rhaFGF in diabetic patients, its pharmacokinetic features and possible toxic effects were evaluated using a rabbit model. For the 139 diabetic patients, 25% of them were divided into the group to receive topical rhbFGF daily at the dose of 100 U/0.1 ml/cm^2 as positive control (n = 35), and the remainder to receive topical rhaFGF at the same dose as rhbFGF (n = 104). Pharmacokinetic studies showed that plasma concentration of rhaFGF rapidly increased and reached to peak levels at 0.5 hr and then quickly decreased to normal levels at 3 hr after topical application on the wounds. No detectable toxic effects were found by examining multiple organs of the rabbits at 28 days after applying rhaFGF at 900 U/cm^2. Topical application of either rhbFGF or rhaFGF at 100 U/cm^2 effectively cured the skin wounds in the diabetic patients. Although there was no remarkable difference between groups, rhaFGF provided a slightly better healing rate and efficiency than rhbFGF did. Therefore rhaFGF will become an alternative candidate for diabetic wounds, especially when diabetic wounds were in acidic environment due to bacterial infection.

Key words —— diabetic wound healing, acidic fibroblast growth factor, clinical observation

INTRODUCTION

The prevalence of diabetes has increased tremendously world wide, and diabetic complications have become a serious public health issue. One of these complications is the impairment of wound healing in diabetic patients. The absence of the cellular and molecular signals required for the normal wound-healing process may be a major contributing factor to the poor healing of diabetic wounds.[1–4] Cytokines, especially various growth factors, provide the cellular and molecular signals required for the normal healing process but are deficient in diabetic wounds.[1–4] Topical application of several growth factors to the healing impaired wounds could stimulate fibroblast and endothelial cell proliferation, increase the rate and degree of granulating tissue and capillary formation, and thus accelerate wound healing.[5–7]

Both acidic and basic fibroblast growth factor (aFGF and bFGF or FGF-1 and FGF-2) have many biologic activities including stimulating the proliferation of fibroblasts and capillary endothelial cells, thus promoting angiogenesis and wound healing.[8–11] We have demonstrated that accelerated healing time was achieved with the topical application of recombinant bovine bFGF (rbbFGF) in patients with second-degree skin burns.[12] Recombinant human bFGF (rhbFGF) was subsequently approved in China for the second-degree skin burns

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and diabetic wounds.[13–16)]

Although the application of aFGF in diabetic animal models was reported,[17, 18)] no documentation indicating successful therapy with aFGF for skin-wound healing in diabetic patients is available. Given that bFGF is unstable in the acidic condition if the wound area is infected by bacteria, especially under diabetic conditions, recombinant human aFGF (rhaFGF) was developed in our laboratory.[19, 20)] aFGF has also been shown to be effective in treating deep partial-thickness burns and skin-graft donor sites.[21)] However, there was no comparative study between bFGF and aFGF. In this report, therefore, we aimed at examining the pharmacokinetic features and toxicologic effects of rhaFGF used topically in rabbits and comparing the therapeutic effects of rhaFGF and current clinical used rhbFGF on diabetic wound healing.

MATERIALS AND METHODS

Reagents —— rhaFGF was obtained from the Center of Medicine and Biotechnology R&D (Jinan University, Guangzhou, China; New Drug Clinical Trial License Number: 2002SL0024) and rhbFGF [New Drug License Number: (2000)S-06] was purchased from Torita Bio-Pharma, Co. (Beijing, China). For pharmacokinetic study, ^{125}I-rhaFGF was prepared based on the Iodogen method.[22)] Briefly, incorporation of ^{125}I into rhaFGF was achieved by incubating 18.5 MBq (0.5 mCi) of Na^{125}I and 1 mg of rhaFGF in Iodogen (Sigma)-coated plastic tubes (16 μg/tube) for 60 min in a water-ice bath with occasional vortex. The radiolabelled rhaFGF was purified using cation-exchange resin chromatography.

Experimental Design for Animal Studies —— The animal study protocol was approved by the Ethics Committees of Experimental Animal Use and Care of Wenzhou Medical College and Jinan University. Two sets of animal studies were performed. For the first, 2.25–2.75-kg male New Zealand rabbits were divided into groups with and without superficial scraping injury. Both groups of rabbits were etherized for anesthesia and shaved with a razor. The paravertebral skins were cleansed with 70% alcohol and abrasion-type superficial wounds were made on the paravertebral dorsal skin by application of 100% acetone to the skin, followed by rubbing with a coarse emery board.[23)] The superficial wounds were designed to mimic the type of wound typically seen in human skin after minor scrapes. A total of 67.2 μg ^{125}I-rhaFGF at 180 U/cm^2 was topically applied to the entire wound area (skin wound group) and the same area of normal skin (normal skin group). After a few minutes to allow the FGF solution diffuse into the wound or normal skin, a sterilized plastic membrane larger than the area to which FGF solution was applied was placed over the wound for 6 hr to avoid solution evaporation and then changed with wound dressing. Whole blood, urine, and feces were collected at different times to measure total radioactivity. The blood concentrations of intact ^{125}I-rhaFGF at different time points were evaluated using radiopaper chromatography. At the end of the experiments (96 hr after topical application of ^{125}I-rhaFGF), the rabbits were killed and various organs (listed in Table 1) were collected to measure the total radioactivity in each organ.

To investigate the possible toxic effects of rhaFGF, 2.25–2.75-kg male New Zealand rabbits were used. Three doses of rhaFGF (3600, 1800, and 900 U/cm^2 body surface) dissolved in 1% albumin solution were applied on the superficial scraping wound. rhaFGF was applied topically daily for 4 weeks. The procedures for producing superficial scraping wounds and topical application of rhaFGF were the same as the above. Possible toxic effects were systemically examined by monitoring hematologic, cardiologic, urologic, and histopathologic parameters as previous described.[24)] Monitoring was performed once before making the wound (day 0), once immediately (day 29) and once at two weeks (day 42) after 4-week topic application of rhaFGF.

Clinical Observations —— One hundred thirty-nine type 2 diabetic men and women, ranging in age from 18 to 75 (mean 52.2 ± 17.31) years, with chronic skin wounds (without other growth factor treatments) were included. They all had skin wounds at least 2 cm in diameter through the full skin thickness which remained unhealed after at least 8 weeks of routine treatments. For the best clinical trial design, patients should be equally randomized to control and treatment groups. However, rhbFGF as the comparative control in this study has been used routinely for diabetic patients in most clinics in China. We performed this prospective study by randomly, but unequally, dividing 75% of these diabetic patients into the rhaFGF group (n = 104) to receive topical rhaFGF on the skin wound and the remainder into the rhbFGF group to receive topical application of rhbFGF as a positive control

Table 1. Biochemical Measurements of General Hematologic Indices (n = 10)

Tested index	Tested time	Control	Low dose (900 U/cm^2)	Middle dose (1800 U/cm^2)	High dose (3600 U/cm^2)
Erythrocyte ($\times 10^{12}$/l)	d0	6.55 ± 0.65	6.01 ± 0.63	6.05 ± 0.39	6.41 ± 0.93
	d29	6.05 ± 0.59	5.99 ± 0.62	5.95 ± 0.48	5.58 ± 0.36
	d42	6.60 ± 0.67	7.09 ± 0.95	6.58 ± 0.41	6.6 ± 0.89
HB (g/l)	d0	139 ± 12	134 ± 11	133 ± 8	139 ± 18
	d29	128 ± 10	130 ± 11	125 ± 13	120 ± 8
	d42	101 ± 4	108 ± 9	105 ± 6	97 ± 6
Blood platelets ($\times 10^9$/l)	d0	16 ± 3.7	15 ± 2.1	15 ± 4.2	18 ± 2.4
	d42	20 ± 3.2	17 ± 6.3	21 ± 6.9	15 ± 5
Blood coagulation time (s)	d0	351 ± 66	496 ± 159	582 ± 126	438 ± 178
	d29	297 ± 25	297.58 ± 0.0	306 ± 42	291 ± 30
	d42	345 ± 54	375 ± 62	383 ± 86	278 ± 33
Leukocytes ($\times 10^9$/l)	d0	9.3 ± 2.5	8.9 ± 2.5	9.4 ± 3.7	9.5 ± 2.9
	d29	7.6 ± 1.5	8.1 ± 2.0	8.0 ± 1.7	7.3 ± 1.0
	d42	7.2 ± 0.4	6.3 ± 1.7	6.0 ± 1.3	7.1 ± 2.2
Leukocyte class (%) Lymphocytes	d0	48.6 ± 13.6	47.0 ± 10.0	47.0 ± 12.3	45.5 ± 13.2
	d29	52.5 ± 5.9	54.9 ± 10.5	45.8 ± 12.6	52.6 ± 5.9
	d42	49.8 ± 6.1	51.8 ± 5.8	48.3 ± 8.1	48.3 ± 9.0
Neutrophils	d0	49.2 ± 12.5	50.1 ± 10.1	50.0 ± 12.0	51.2 ± 13.1
	d29	42.4 ± 6.1	39.5 ± 9.6	49.1 ± 12.7	42.9 ± 6.1
	d42	46.5 ± 5.9	42.0 ± 5.1	46.5 ± 8.4	48.0 ± 8.1
Eosinophils	d0	1.2 ± 1.2	1.4 ± 1.6	1.2 ± 1.2	1.1 ± 0.9
	d29	1.4 ± 1.3	1.4 ± 1.1	0.9 ± 0.8	1.4 ± 1.1
	d42	1.3 ± 1.3	1.3 ± 1.1	2.5 ± 1.1	1.8 ± 1.1
Monocytes	d0	1.7 ± 1.1	1.7 ± 1.0	1.9 ± 1.0	2.5 ± 1.0
	d29	3.7 ± 2.0	4.4 ± 1.0	4.3 ± 1.0	3.2 ± 1.0
	d42	3.3 ± 2.3	5.0 ± 1.9	3.3 ± 1.3	2.0 ± 0.7

(n = 35). We selected rhbFGF as the positive control because of the following three reasons: patients accept rhbFGF, since it has been extensively used in clinics in China; more importantly, we wanted to compare the therapeutic effects of rhaFGF and rhbFGF directly to determine whether rhaFGF has superior therapeutic effects; and there was no previous study directly comparing the effects of aFGF and bFGF in human skin wounds, especially under diabetic conditions.

The clinical trail was licensed by the State Food and Drug Administration (SFDA), License Number: 2002SL0024, and approved by the Ethics Committees of Wenzhou Medical College and Jinan University. All patients gave written informed consent for study participation. The growth factors were dissolved in normal saline solution. The patients were given topical rhaFGF and rhbFGF at a dose of 100 U/0.1 ml/cm^2 in the rhaFGF group (n = 104) and rhbFGF group (n = 35), respectively. After treatment, all wounds were covered with sterile cotton dressings without antibiotics which were removed with saline irrigation when new FGF solution was applied. Subsequent doses of either rhaFGF or rhbFGF were given daily at approximately the same time until the wound was healed or 6 weeks of treatment. The chronic cutaneous wounds were photographed weekly with a Minolta XD7 camera with a MD 50 nm lens and range flash (Osaka, Japan). At the end of the 6-week treatment, the wound were divided into four categories: complete healing; significant healing if more than 50% of the wound area had healed; effective healing if 20–50% of the wound area had healed; ineffective healing if less than 20% of the wound area had healed. The total healing rate equals: (complete healing + significant healing)/total number *100.

Statistical Analysis —— All data are presented as mean ± S.D. Statistical analysis was performed using SPSS software (10.00) with one-way analysis of variance (ANOVA), and then in multiple comparisons with the Bonferroni test. Statistical significance was set at $p < 0.05$.

RESULTS

Animal Studies

Dynamic analysis showed that the blood concentration of ^{125}I-rhaFGF increased sharply and reached its peak value of 73.03 pg/ml 0.5 hr after topical application of the growth factor to the wound and then decreased quickly in 3 hr. The theoretical maximum blood concentration was C_{max} = 82 ± 20 pg/ml, the time for reaching C_{max} was T_{max} = 0.80 ± 0.27 hr, and the half-life was $T_{1/2}$ = 0.9 ± 0.6 hr (Fig. 1A). The blood radioactivity derived from ^{125}I-rhaFGF and its metabolites increased relatively slower and reached its peak in 3 hr and then decreased slowly within 96 hr (Fig. 1B). Distributing profiles of radioactivity derived from the administered ^{125}I-rhaFGF and its metabolites in different organs are shown in Fig. 1C, which indicates that they accumulated predominantly in the thigh skin and kidney, and the least in the brain 96 hr after topical application of growth factor to the wound skin. rhaFGF and its metabolites were mainly secreted in the urine (Fig. 1D). Evaluation of the accumulated radioactivity showed that 10.5% of ^{125}I-rhaFGF and its metabolites were eliminated in the urine and 1.4% in the feces.

Several assessments were performed to determine whether there were any toxic effects. General behavior and health, including body weight, body temperature, breath rate, and color and states of feces and urine showed no significant difference between before (day 0) and the next day (day 29) or 2 weeks (day 42) after the 4-week topical application of rhaFGF at concentrations of 900, 1800, and 3600 U/cm^2 (data not shown). Electrocardiography did not show any significant difference in cardiac

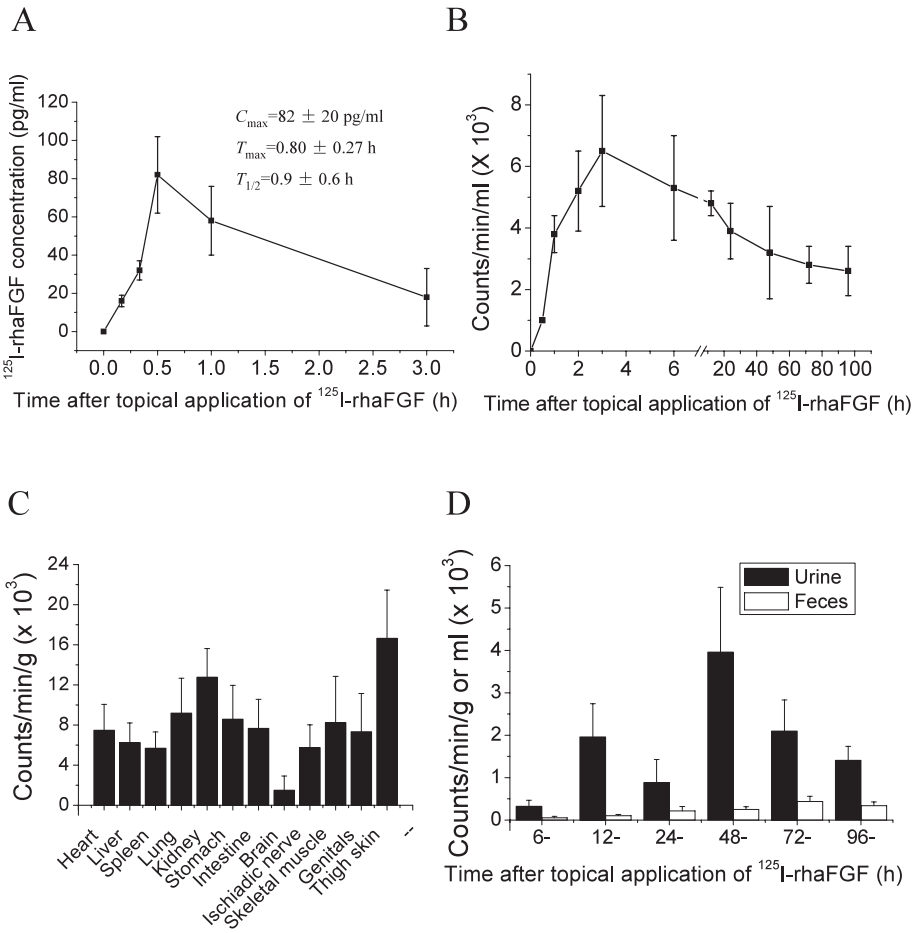

Fig. 1. Pharmacokinetic Features of Topical Application of rhaFGF on Skin Wounds

Traumatic cutaneous wounds were made using the rabbit model as described in materials and methods, and rhaFGF was applied. Dynamic analysis of blood concentrations of ^{125}I-rhaFGF was measured with paper chromatography at different times after rhaFGF topical application to wounds (A). Distribution profiles of radioactivity derived from ^{125}I-rhaFGF and its metabolites in blood (B), urine and feces (D), and different organs (C) were detected at the indicated time points or endpoint of the experiment (n = 10).

Table 2. Biochemical Measurements for Clinical Routine Blood Tests (n = 10)

Tested index	Tested time	Control	Low dose (900 U/cm^2)	Middle dose (1800 U/cm^2)	High dose (3600 U/cm^2)
ALT (U/l)	d0	92 ± 48	70 ± 23	92 ± 34	89 ± 33
	d29	92 ± 35	93 ± 64	73 ± 18	91 ± 39
	d42	65 ± 26	102 ± 26	75 ± 10	76 ± 23
AST (U/l)	d0	58 ± 35	46 ± 14	79 ± 45	47 ± 14
	d29	65 ± 36	78 ± 46	71 ± 25	68 ± 25
	d42	55 ± 40	80 ± 50	72 ± 53	43 ± 11
ALP (U/l)	d0	85 ± 30	108 ± 31	102 ± 30	87 ± 35
	d29	124 ± 51	114 ± 51	94 ± 37	86 ± 31
	d42	116 ± 16	82 ± 12*	98 ± 19	86 ± 31
CK (U/l)	d29	4693 ± 2098	3906 ± 2503	3533 ± 2073	2826 ± 2403
	d42	2106 ± 657	2873 ± 1915	2130 ± 1546	2130 ± 2412
ALB (g/l)	d0	43.6 ± 2.9	42.7 ± 2.5	42.5 ± 3.7	44.2 ± 3.4
	d29	40.1 ± 1.5	42.4 ± 4.2	41.6 ± 2.2	42.4 ± 2.6
	d42	40.8 ± 0.7	42.0 ± 4.4	42.7 ± 2.4	41.5 ± 3.6
BUN (mmol/l)	d0	9.05 ± 1.39	7.97 ± 1.24	8.12 ± 1.08	9.09 ± 2.05
	d29	8.25 ± 1.29	7.73 ± 1.04	8.45 ± 0.96	8.07 ± 0.79
	d42	7.91 ± 0.38	7.00 ± 0.61	7.14 ± 0.68	7.21 ± 0.66
Creatinine (umol/l)	d0	111.7 ± 22.2	100.0 ± 12.1	106.4 ± 13.6	114.4 ± 21.2
	d29	99.0 ± 10.7	106.1 ± 25.0	106.0 ± 18.3	100.9 ± 19.7
	d42	110.3 ± 13.5	109.6 ± 22.7	116.6 ± 10.5	85.8 ± 16.4
GLU (mmol/l)	d0	7.16 ± 1.46	7.60 ± 2.02	7.67 ± 2.26	8.16 ± 1.89
	d29	6.87 ± 0.71	7.08 ± 1.96	6.47 ± 1.49	6.85 ± 1.47
	d42	7.78 ± 0.44	9.89 ± 2.00	9.24 ± 3.11	7.47 ± 0.38
TCHO (mmol/l)	d0	1.32 ± 0.29	1.43 ± 0.79	1.21 ± 0.30	1.42 ± 0.50
	d29	1.55 ± 0.35	1.93 ± 1.09	1.36 ± 0.35	1.44 ± 0.52
	d42	1.96 ± 0.25	2.31 ± 1.76	1.34 ± 0.56	1.37 ± 0.38
TBIL (umol/l)	d0	6.9 ± 2.8	4.7 ± 0.8	5.0 ± 2.4	5.4 ± 1.5
	d29	2.2 ± 0.8	1.9 ± 0.6	1.9 ± 0.3	2.0 ± 0.8
	d42	2.3 ± 0.4	2.5 ± 0.4	2.0 ± 0.1	1.7 ± 0.4

*$p < 0.05$ *vs.* corresponding control.

function before and 4 weeks after growth factor application (data not shown). There was no significant change in hematologic examinations (Table 1). Functional evaluation of the liver, heart, and kidney was performed by biochemical measurements as listed in Table 2, which showed that alkaline phosphatase (ALP) significantly decreased only in the group treated with low-dose rhaFGF (900 U/cm^2) at the recovery stage (2 weeks after the 4-week application of rhaFGF to wound skin) (Table 2). In addition, since there was no significant change in other measure of liver function, we assumed that the ALP changed for some unknown reasons and might not have clinical relevance, which should be confirmed. Two weeks after the 4-week topical application of rhaFGF to the wound skin, the animals were killed to examine organ weights. No differences in organ weight (data not shown) or ratio of organ weight/body weight were found (Table 3).

Clinical Studies

Fever, decreases in hepatic and renal function, local pain, and infections are commonly defined as clinical side effects and therefore these endpoints were also examined in the 139 patients with diabetes, before and after treatment in this study. There was no significant discomfort response, including fever, headache, vomiting, breathing difficulty and skin rash and allergy in the rhaFGF or rhbFGF group (data not shown). Temporally local wound pain occurred in both groups from a slight to mild extent, but disappeared without special care. Six patients in the rhaFGF group (1.86%) and two in the rhbFGF group (1.82%) experienced local temporally pain ($p > 0.05$). These general observations are consistent with the finding from the animal studies that topical application of rhaFGF to the wound skin did not cause any significant toxic effects (Fig. 1 and Table 3).

Table 3. Organ Weight to Body Weight Ratios (n = 10)

Examined index	Examined time	Control	Low dose (900 U/cm^2)	Middle dose (1800 U/cm^2)	High dose (3600 U/cm^2)
Heart	d29	0.23 ± 0.03	0.24 ± 0.04	0.24 ± 0.01	0.24 ± 0.02
Liver	d29	2.14 ± 0.42	2.83 ± 0.40	2.86 ± 0.27	2.40 ± 0.24
Spleen	d29	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
Lung	d29	0.46 ± 0.10	0.46 ± 0.10	0.56 ± 0.19	0.44 ± 0.04
Brain	d29	0.31 ± 0.03	0.33 ± 0.03	0.38 ± 0.06	0.33 ± 0.02
Left kidney	d29	0.25 ± 0.01	0.25 ± 0.03	0.26 ± 0.03	0.24 ± 0.02
Right kidney	d29	0.24 ± 0.03	0.26 ± 0.04	0.27 ± 0.03	0.24 ± 0.02
Adrenal gland	d29	0.011 ± 0.002	0.014 ± 0.004	0.014 ± 0.004	0.011 ± 0.002
Thymus	d29	0.15 ± 0.05	0.13 ± 0.03	0.10 ± 0.04	0.12 ± 0.03
Thyroid gland	d29	0.005 ± 0.001	0.004 ± 0.002	0.007 ± 0.003	0.006 ± 0.001
Testis	d29	0.18 ± 0.01	0.28 ± 0.04	0.27 ± 0.07	0.23 ± 0.07
Prostate	d29	0.07 ± 0.03	0.04 ± 0.01	0.04 ± 0.00	0.06 ± 0.01
Uterus	d29	0.12 ± 0.06	0.20 ± 0.05	0.28 ± 0.17	0.22 ± 0.02
Heart	d42	0.23 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.03
Liver	d42	2.50 ± 0.35	2.68 ± 0.14	2.72 ± 0.18	2.93 ± 0.27
Spleen	d42	0.06 ± 0.01	0.08 ± 0.05	0.04 ± 0.01	0.06 ± 0.02
Lung	d42	0.36 ± 0.04	0.33 ± 0.01	0.46 ± 0.10	0.46 ± 0.11
Brain	d42	0.31 ± 0.02	0.34 ± 0.04	0.34 ± 0.03	0.34 ± 0.03
Left kidney	d42	0.21 ± 0.02	0.27 ± 0.03	0.24 ± 0.02	0.27 ± 0.02
Right kidney	d42	0.21 ± 0.02	0.26 ± 0.02	0.24 ± 0.03	0.27 ± 0.03
Adrenal gland	d42	0.011 ± 0.003	0.014 ± 0.003	0.016 ± 0.003	0.015 ± 0.004
Thymus	d42	0.14 ± 0.03	0.12 ± 0.02	0.13 ± 0.03	0.21 ± 0.04
Thyroid gland	d42	0.004 ± 0.002	0.004 ± 0.001	0.005 ± 0.002	0.006 ± 0.002
Testis	d42	0.20 ± 0.04	0.25 ± 0.03	0.23 ± 0.05	0.24 ± 0.02
Prostate	d42	0.05 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Uterus	d42	0.19 ± 0.12	0.22 ± 0.03	0.13 ± 0.07	0.24 ± 0.14

After treatment with rhaFGF and rhbFGF, wound sizes were photographed daily. The wound healing states were compared by three measurements, complete healing (Fig. 2A), effective healing (healed > 50%, Fig. 2B), and total effectiveness (Fig. 2C). These measurements clearly revealed that the therapeutic effects of rhaFGF are slightly better than those of rhbFGF.

DISCUSSION

In the present study, we systemically evaluated the pharmacokinetic features and potential toxicities of topically applied rhaFGF to skin wounds using a rabbit cutaneous wound model. No detectable toxic effects were observed in the animal model. We directly compared for the first time the therapeutic effects of rhaFGF and rhbFGF on the healing impaired cutaneous wounds of diabetic patients. We demonstrated that the therapeutic effects of rhaFGF on diabetic wound healing are slightly better than those of rhbFGF, without detectable side effects.

Patients with diabetes mellitus experience impaired wound healing, often resulting in chronic foot ulcers. Hospital discharge data indicate that 6–20% of all diabetic individuals hospitalized (mostly with type 2 diabetes) have a lower extremity ulcer. Diabetes is accompanied by delayed wound healing and insufficient granulation tissue formation, most likely due to the lack of signaling required for wound healing.[1–4] Several experimental studies demonstrated that fibroblasts derived from animal or human chronic diabetic wounds have a decreased proliferation rate and abnormal morphology.[25,26]

The human derm is expresses multiple FGFs and predominantly FGF receptor 1, suggesting their important roles in maintaining normal dermal formation and function.[27,28] So far, although a total of 22 different FGF molecules have been described, aFGF and bFGF differ from most other FGFs in several important aspects:[27,28] aFGF and bFGF show strong homology (55%) in their amino acid sequences; mRNA expression of both aFGF and bFGF is detectable in a variety of tissues

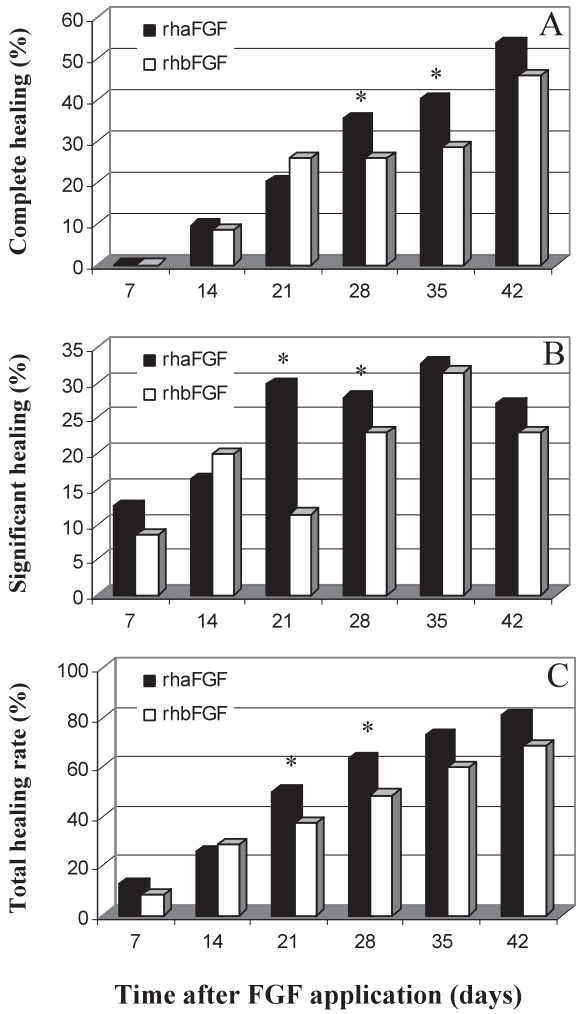

Fig. 2. Comparison of the Therapeutic Effects of rhaFGF and rhaFGF in Healing Impaired Skin Wounds of Diabetic Patients

Diabetic patients with chronic skin ulcers were divided into two groups to receive topical application of either rhaFGF or rhbFGF at the same dose of 100 U/0.1 ml/cm^2. After treatment, wound sites were photographed daily. The wound healing states were compared by three measurements: complete healing (A), significant healing (B), and total healing rate (C). $^*p < 0.05$ *vs.* rhbFGF.

during development and in adulthood; both aFGF and bFGF lack signal peptides at their N-terminal ends and are found in the cytosol; both factors appear to be released from cells through a nonclassical secretory pathway; and both aFGF and bFGF are found in the cell nucleus, suggesting a role in cell proliferation. aFGF and bFGF have been extensively shown to promote angiogenesis as well as stimulate proliferation of many cell types involved in wound healing, including endothelial cells, fibroblasts, and keratinocytes.[4,5,29,30] Therefore it is not surprising to find similar therapeutic effects of rhaFGF and rhbFGF in diabetic wound healing.

Although there are several common features between aFGF and bFGF, there are also some differences.[31] Both aFGF and bFGF play important roles in the development and maintenance of neuronal tissue, but they are distributed into different types of neuronal cell and play different roles.[32] Heparin sulfate (HS)-like and heparin-like glucosaminoglycans were isolated from diabetic rat skin showing high affinity for aFGF and bFGF. The fractions purified from the control rats and the heparin-like glucosaminoglycan isolated from the diabetics mediated the biological activity of both FGFs in a dose-dependent manner. In contrast, the diabetic HS-like fractions promoted the biological activity of bFGF but not of aFGF. The results support the concept that the structural domains in HS required for aFGF- and bFGF-mediated receptor signaling are different. Thus the inability of the major HS species isolated from diabetic rat skin to mediate the biological activity of aFGF may be an important factor in the impaired wound healing in diabetic skin.[29] In humans, the present study is the first to compare directly the therapeutic effects of aFGF and bFGF in diabetic wound healing. Although there was no remarkable difference between the groups treated with these two growth factors, a slightly better effect of aFGF was noted.

Similar findings of the superiority of rhaFGF using other models were documented previously. Alberts demonstrated a different regulation of aFGF and bFGF in rat aortic smooth muscle cells.[33] In a model of the debridement of the entire corneal epithelium of the rabbit eye,[34] both aFGF and bFGF were administered topically. Although a dose-response effect was observed in each case, aFGF was found to be more potent than bFGF in increasing the rate of wound healing of the cornea.[34] This may be due to the higher sensitivity of corneal stromal fibroblasts to aFGF than to bFGF.[30] However, whether that difference affects chronic wound healing remains to be investigated. In addition, studies have shown that aFGF had better biological activity and stability in an acidic environment.[35,36] Although we did not observe significant infection of diabetic ulcers in the absence of antibiotic treatment or directly measure pH levels in the ulcer sites, we could not eliminate the existence of mild, unrecognizable infection in the ulcer sites, which might cause a relatively acidic environment.

In summary, this is the first study to show that the therapeutic effects of rhaFGF on diabetic wound healing is similar to or better than those of rhbFGF in type 2 diabetic patients. Given that acidification

is present in normal skin and frequent in burns, chronic ulcers, and infected wounds under diabetic and nondiabetic conditions, rhaFGF can be considered as an alternative approach to diabetic wound healing in addition to rhbFGF. A few limitations in the present studies include an unequal distribution to the two types of FGF treatment, and no examination of the molecular and histopathologic differences between the two groups. These limitations will be compensated for in future studies.

Declaration of Competing Interests: None to declare.

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