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Accelerated Wound Healing by Recombinant Human Basic Fibroblast Growth Factor in Healing-impaired Animal Models

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Abstracts – The stimulatory effect of recombinant human basic fibroblast growth factor (bFGF) on wound healing was evaluated in healing-impaired animal models. Full-thickness wounds were made in prednisolone-treated mice, streptozotocin (STZ)-induced diabetic rats and mitomycin C (MMC)-treated rats. Saline or bFGF at a dose of 1, 5, or 25 μg per wound was applied to the open wound once a day for three to five days. The degree of wound healing was assessed using wound size and histological parameters such as degree of epidermal and dermal regeneration. Local application of bFGF accelerated wound closure significantly in a dose-dependent manner in all healing-impaired wounds ($p<0.05$). The wound healing effect of bFGF was further confirmed by histological examination in MMC-treated rats. Epidermal and dermal regeneration were enhanced in bFGF-treated wounds with a dose-related response. Dermal regeneration parameters such as collagen matrix formation and angiogenesis were significantly increased in 5 μg, or 25 μg of bFGF-treated wounds when compared to saline-treated wounds ($p<0.05$). Lectin immunostaining on day 8 for vascular endothelium showed an increased number of neovessels in bFGF-treated wounds. These results suggest that topical application of bFGF has beneficial effects on wound healing by angiogenesis and granulation tissue formation in healing-impaired wounds.

Keywords□ basic Fibroblast Growth Factor, bFGF, Local irritation, Rabbit

While normal healthy people rarely have problems with wound healing, many medical and surgical complications can be attributed to impairment or deficiencies in wound repair. Because open wounds have lost the skin barrier that protects tissues from microbial invasion and from the loss of tissue fluid. Without immediate wound closure, infections become more frequent. Most wound complications are associated with host impairment such as malnutrition, diabetes, or treatment with chemotherapy, steroids, or radiation (Poole, 1985; Irvin et al, 1985). Recently, attempts have been made to improve healing using growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (Greenhalgh et al, 1990; Mustoe et al, 1991; Servold, 1991; Szabo et al, 1995). Of these, basic fibrobalst growth factor (bFGF) has shown promising results as a new wound healing agent (Okumura et al, 1996a; Ishibashi et al, 1996a, b). bFGF has been known to proliferate mesenchymal cells such as endothelial cells, fibroblasts and vascular smooth muscle cells, and keratinocytes (Ledoux et al, 1992; DeLapp et al, 1990). Dong-A pharmaceutical company (Seoul, Korea) has developed a recombinant human bFGF (rhbFGF) as a wound healing agent using rDNA technology. In the present study we examined the wound healing effect of rhbFGF in healing-impaired animals.

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MATERIALS AND METHODS

Test material

Recombinant human basic fibroblast growth factor (bFGF, Code : DA-3050) was obtained from the Research Laboratories of Dong-A Pharm. Co. (Kyunggido, Korea). Human bFGF gene was expressed in *E. coli* and the recombinant protein was purified to homogeneity by a sequential procedure of column chromatographies. For administration bFGF was diluted in an adequate volume of saline. bFGF was stable in saline at 4℃ for at least 4 weeks. Other chemicals used in the current study was purchased from Sigma (USA).

Animals

Animal use followed institutional guidelines of Research Laboratories of Dong-A Pharm. Co. Male Sprague-Dawley (IGS CD®) rats (230-250 g), and hairless mice (26-30 g) were purchased from Charles River Japan (Japan). Animals were acclimated for at least 1 week prior to use for the studies and maintained in individual standard polycarbonate cages throughout the experiment. The animals were fed standard rat food and tap water *ad libitum*. At least 6 animals were used in each test condition for the experiments.

Full-thickness excisional wound in steroid-treated mice

The procedure was essentially identical to that reported by Okumura et al. (1996b). Hairless mice were injected with 25 mg/kg of prednisolone intraperitoneally. Five hours later a circular excisional wound (1.4 diameter) on each animal were made under light ether anesthesia by cutting out the full-thickness skin with surgical scissors. The wounds were left open, and each mice wore a collar to prevent licking or biting of the wound during the experiment. bFGF was applied once a day for three days at the doses of 5 or 25 μg per wound. The same volume of saline (0.2 ml) was applied to control mice.

Bacteria-contaminated wound in streptozotocin-induced diabetic rats

Rats were pretreated with streptozotocin (60 mg/kg, ip) 7 days before experiment. Blood was sampled from the tail vein and blood glucose level was determined by Glucostar-Glucostix (Miles-Sankyo Co., Tokyo, Japan). Animals with blood glucose level of less than 300 mg/dl under non fasting conditions were excluded from the study. Under light ether anesthesia, a 1.6 cm diameter template was used to mark the shaven back and a full-thickness excision was made by scissors. Then wound surface was contaminated with bacteria by inoculating 1×10^8 organisms of *Staphylococcus aureus* Smith strain. Either saline as a control or bFGF at doses of 1, 5, or 25 μg in saline (0.2 ml) was applied evenly on the surface of the wound once daily for 5 days from day 0.

Full-thickness excisional wound in mitomycin C-treated rats

Thirty minutes after i.p. injection of mitomycin C (2 mg/kg), a 1.6 cm diameter excisional wound was made on the previously shaven back of each rat under ether anesthesia. Either saline as a control or bFGF at doses of 1, 5, or 25 μg in saline (0.2 ml) was applied on the wound once daily for 3 days from day 0. Two out of 9 rats of each group were sacrificed on day 8 for histological examination and lectin histochemistry. The rest animals were sacrificed at the end of the experiment and histologic examination was performed.

Measurement of the wound area

The wound area was examined daily until wound closure with regenerated epidermis. Wound closure day for each animal was recorded. Twice weekly the wound area was traced, and the square size was measured under a dissecting microscope with a square grid ($\times10$) by an examiner unaware of the treatment condition. Data are expressed as % of the initial wound area which was measured on the day following wound. In each experiment, wound closure on day 14 after excision was calculated as: % closed=[(Area on Day 0-Open Area on Day 14)/Area on Day 0]$\times100$ (Greenhalgh et al, 1990). On the final day of study, animals were euthanized by deep ether anesthesia and the wound site and surrounding skin were prepared by routine procedure for microscopic examination.

Histologic examination

In the experiment to investigate the wound healing effect of bFGF in MMC-treated rats, two and seven animals from each group were sacrificed on day 8 and day 18, respectively, for histologic examination. Wounded skin samples were removed and fixed in 10% neutral formalin solution. Fixed skin tissue was processed by routine procedures to obtain paraffin-embedded histological sections. After Masson trichrome staining or hematoxylin and eosin staining, skin samples obtained on day 18 were examined by a veterinary pathologist under the light microscope (BHL-2, Olympus, Japan) according to the criteria described in Table I (Niwano et al, 1996). Lectin histochemistry was conducted to evaluate the neovascularization in the wound area on day 8 by the method of Alroy et al (1987). In brief, formalin-fixed, paraffin-embedded skin tissues were sectioned at a thickness of 5 μm, deparaffinized and hydrated. After blocking endogenous peroxidase with 2% H_2O_2, the sections were incubated with biotinylated lectin (Vector Laboratories, Burlingame, CA) for 30 min and then washed three times with PBS, followed by incubation with avidin-biotin-peroxidase complex (ABC) (Vector Laboratories) for 30 min and washed again three times with PBS. The visualant, horse-radish peroxidase, was activated by incubation for 10 min in PBS solution containing diami-

Table I. Scores for histological findings of wound areas produced on the back of rats

Scores	Epidermal regeneration		Dermal regeneration	
	Elongation	Differentiation and keratinization	Collagen matrix formation	Angiogenesis
±	Little epidermal elongation under the scab	Only stratum basale observed	Wound area filled with infiltrated inflammatory cells, migrated fibroblasts and tissue fluid	No newly formed capillary vessels observed
1	Partly epidermal elongation in the margin of the wound area	Stratum spinosum observed on the stratum basale	A few/ minimal collagen fibrils observed in the superficial wound area under the scab	Newly formed capillary vessels observed in the margin of the wound area
2	Epidermal elongation spreading over 2/3 of the wound surface	Stratified epithelium observed with the scab	Collagen fibrils observed in the deep wound area under the scab	Newly formed capillary vessels observed in moderate numbers in the entire wound area
3	Epidermal elongation spreading over the entire surface	Complete remodeling of epithelium observed with no scab	Wound area filled with collagen bundles observed in parts of/ portions of the margin of the wound area	Newly formed capillary vessels observed frequently in the entire wound area
4			Collagen bundles observed in the entire wound area	Thick capillary vessels disposed vertically toward the wound surface
5			Abundant collagen bundles in the entire wound area	
6			Complete remodeling of dermis	

nobenzidine and H_2O_2. The sections were then washed in tap water, counterstained with methyl-green, dehydrated and coverslipped.

Statistical analysis

Values are expressed as the mean ± standard error of the mean. Significance of the difference in wound closure parameters among groups were compared by analysis of variance (ANOVA) and assessed by Turkey's multiple comparison test. For histologic score, after rank transformation, Mann-Whitney rank sum test and t-test were used. A significance level of $p \leq 0.05$ was used for all comparisons. The analyses were performed using the SigmaStat statistical software (Jandel Scientific Software, ver. 2.0, USA).

RESULTS

Effect of bFGF on wound healing in steroid-treated mice

Wounds treated with bFGF (5 or 25 μg /day) or saline for the first 3 days after surgery were examined for 21 days. The course of wound closure in each group was shown in Fig. 1. Topical application of bFGF accelerated wound closure in steroid-treated mice in a dose-dependent manner and the wound area in bFGF-treated animals

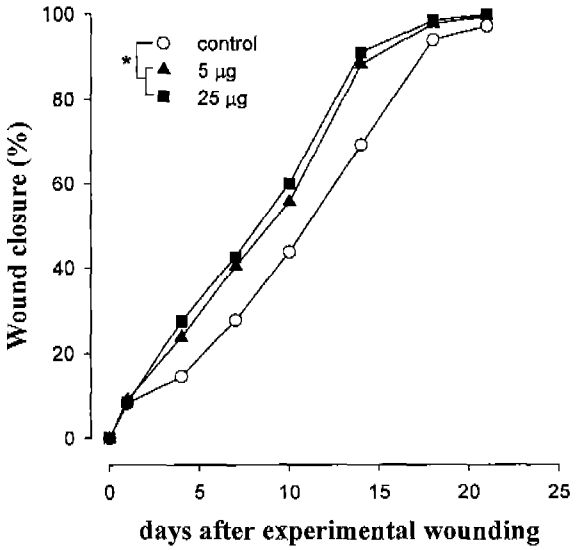

Fig. 1. Effect of local application of bFGF on the course of wound closure in prednisolone-treated mice. After steroid treatment hairless mice underwent a full-thikness excisional wound on day 0, and saline (○) or bFGF at doses of 5 μg (▲) or 25 μg (■) was applied to the wound site for 3 consecutive days. Each point shows mean of at least 6 animals. *p<0.05, compared with saline application.

was significantly reduced than vehicle-treated mice from day 4 ($p<0.05$). Grossly bFGF-applied wound showed

Table II. Effect of bFGF on the wound closure on day 14 in healing-impaired animal models

Wound model		Steroid-treated mice	Contaminated wound in diabetic rats	Mitomycin-treated rats
Saline control		69.2±4.12	57.7±6.41	80.33±5.06
bFGF	1 μg	-	73.1±2.25*	89.51±2.24*
	5 μg	87.9±2.98*	75.6±2.27*	92.57±2.00*
	25 μg	90.8±2.30*	77.9±2.99*	92.40±2.31*

Each value of wound closure (%) shows mean±S.E. (n=6~9). *p<0.05, compared with the saline control. One-way ANOVA followed by Turkey's multiple comparison.

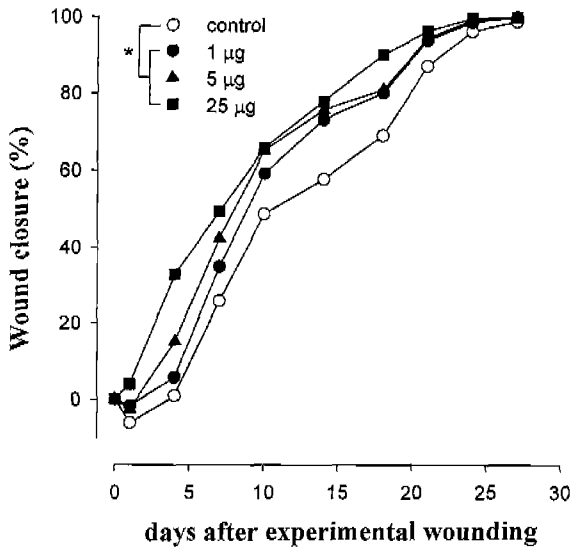

Fig. 2. Acceleration of wound closure by local application of bFGF in STZ-induced diabetic rats. After induction of diabetes with STZ, rats underwent a full-thikness excisional wound on day 0 and 10^8 Staphylococcus aureus was inoculated, and saline (○) or bFGF at doses of 1 μg (●), 5 μg (▲) or 25 μg (■) was applied to the wound site for 5 consecutive days. Each point shows mean of at least 6 animals. *p<0.05, compared with saline application.

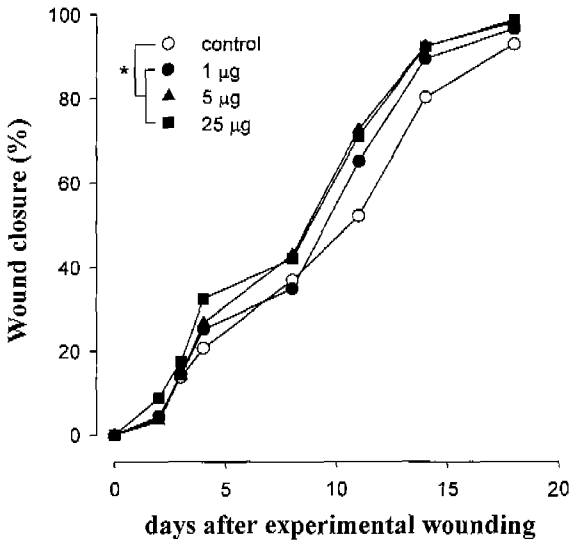

Fig. 3. Acceleration of wound closure by local application of bFGF in MMC-treated rats. MMC-treated rats took a full-thikness excisional wound on day 0, and saline (○) or bFGF at doses of 1 μg (●), 5 μg (▲) or 25 μg (■) was applied to the wound site for 3 consecutive days. Each point shows mean of at least 7 animals. *p<0.05, compared with saline application.

elevated wound bed and more erythematous feature than the vehicle control animals. The percent of wound closure on day 14 in control, 5 μg and 25 μg of bFGF groups was 69.2±4.12 %, 87.9±2.98 % and 90.8±2.30 %, respectively (Table II).

Effect of bFGF on healing of contaminated wound in diabetic rats

The effect of bFGF on a contaminated wound was studied in streptozotocin-induced diabetic rats (Fig. 2). Repeated topical application of bFGF for 5 days accelerated wound closure with a dose-related response. On day 14 after the excision, wound size in all the bFGF-treated groups was significantly reduced when compared to saline-treated control (p<0.05). Wound closure in control and 1, 5 and 25 μg of bFGF group was 57.7±6.41, 73.1±2.25, 75.6±2.27 and 77.9±2.99 %, respectively (Table II).

Effect of bFGF on wound healing in mitomycin C-treated rats

As shown in Fig. 3, bFGF application to mitomycin C (MMC)-treated rats induced an acceleration of wound healing. Statistical analysis revealed the difference in wound closure between bFGF and saline treated groups (p<0.05). Wound closure on day 14 in all bFGF groups was greater than control group (Table II), though statistical difference was not found among the bFGF-treated groups. At the end of the experiment, histologic examination revealed that topical application with bFGF increased dose-dependently all the histologic parameters of the wound healing such as elongation of regenerated epithelia, differentiation, keratinization, collagen matrix formation, and angiogenesis (Table III). In the case of collagen matrix formation and angiogenesis, middle (5 μg) and high dose (25 μg) of bFGF-treated wounds showed

Table 3. Effect of bFGF on the histologic changes of wounds in MMC-treated rats

Group		n	Elongation	Differentiation & keratinization	Collagen matrix formation	Angiogenesis
Saline control		7	2.29±0.31	2.14±0.28	1.86±0.37	1.86±0.37
bFGF	1 μg	7	2.57±0.22	2.14±0.37	2.14±0.15	2.00±0.34
	5 μg	7	2.71±0.27	2.43±0.22	3.71±0.39*	3.00±0.33*
	25 μg	7	2.86±0.29	2.71±0.20	3.86±0.37*	3.29±0.31*

Each value shows mean±S.E. *p<0.05, compared with the saline control. Mann-Whitney rank sum test followed by t-test.

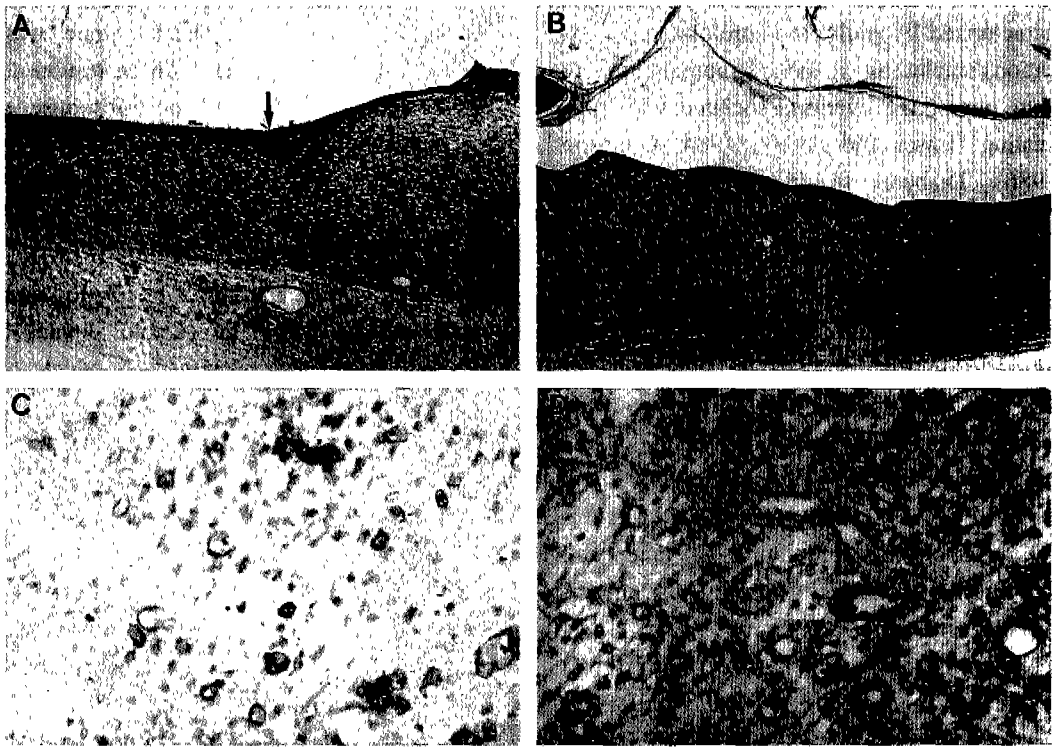

Fig. 4. Light microphotographs of the wound site treated with saline (A and C), or bFGF at 25 μg (B and D) per wound. MMC-treated rats took a full-thickness wound on day 0, and tissues were taken on day 8. Saline-treated wound showed regenerated epidermis (RE) (arrow) at the margin of the wound (A), whereas bFGF-treated wound exhibit full covering of the wound by RE (B). Lectin histochemistry showed an increased number of neovessels in granulation tissue of bFGF-treated wound (D) when compared to that of saline-treated wound (C). A and B : Hematoxylin and eosin staining, ×40, C and D : Lectin immunostaining, ×400.

a significant increase when compared to saline control group ($p<0.05$). On day 8 after wounding, bFGF-treated wounds showed elongated regenerated epidermis, elevated wound base, and compact granulation tissue under regenerated epidermis (Fig. 4). These changes were more prominent in middle and high dose of bFGF-treated groups. Lectin immunostaining showed increased number of newly formed capillaries of the granulation tissue in bFGF-treated wounds when compared to control (Fig. 4).

DISCUSSION

Wound repair results from a series of highly orchestrated cellular and biochemical events. In the healing process, angiogenesis, granulation tissue formation and rapid epidermal regeneration are essential for prompt wound healing (Clark, 1985). However, clinical conditions such as diabetes, obesity, corticosteroid treatment, infection, malnutrition and chemotherapy, are known to impair the events associated with normal wound healing (Falanga, 1993). Non-healing wound or skin ulcer is commonly encountered in the clinical practice and regarded as one of the important medical problems. Because a lack of cytokines and growth factors has been suggested as an important pathogenic factor in chronic wounds, attempts have been made to improve healing

using growth factors. In this study we reproduced the healing effect of bFGF in healing-impaired wound models such as diabetes, steroid-treated and antineoplastic-treated animal models (Okumura et al, 1996b). In the results, repeated local application of bFGF showed a dose-related acceleration of the wound closure in all the three models.

To mimic impaired wound healing in diabetic patients, we used streptozotocin (STZ)-induced diabetes in rats. Diabetes is characterized by multiple neurologic and vascular complications including diabetic microangiopathy and is known to impair the events associated with normal wound healing (Enser and Avery, 1984; Goodson and Hunt, 1986). Diabetes results in alterations in mRNA levels of bFGF in various tissues of rats with STZ treatment, and insulin treatment partially normalized these levels (Karpen et al., 1992). These findings suggest that dysregulation of bFGF may contribute to the development of microangiopathy as well as the impaired wound healing. Indeed, Klingbeil et al (1991) showed that wound healing is markedly retarded in STZ-diabetic animals and that this retardation is antagonized by treatment with bFGF. bFGF is a potent endothelial cell mitogen that also affects the proliferation of other cell types, including fibroblasts, smooth muscle cells and epithelial cells (Folkman and Klagsbrun, 1987). Thus, many of the cell types that are required for tissue repair at the wound site are modulated by bFGF. And these findings suggest a rationale of the use of bFGF to enhance impaired wound healing.

In the present study, staphylococcal infection caused a wound expansion in early stages of healing, which is consistent with the results reported elsewhere (Okumura et al, 1996b). Bacterial products such as toxins and proteolytic enzymes might interfere with influx of fibroblast and endothelial cells by destruction of the scaffolds (Stenberg et al, 1991). This phenomenon can be recovered, at least in part, by bFGF through its stimulation of extracellular matrix formation and neovascularization (McGee et al, 1988; Okumura et al, 1996c). It is thought that bFGF accelerates healing of infected wound not by antibacterial effect but by chemoattraction and angiogenesis because bFGF lacks antibacterial action (Hayward et al, 1992).

Lectin histochemistry has been widely used to identify the vascular endothelia. Pierce et al (1992) reported that various recombinant growth factors such as bFGF and PDGF increase single endothelial cells and neovessels in wound tissue. We reproduced this effect in the present study as lectin-positive endothelia were more predominant in the bFGF-treated wounds than in control wounds.

Okumura et al (1996a) reported that the maximal wound healing effect of bFGF was obtained at 20 μg per wound as a single application and 0.2 to 2 μg per wound in repeated application for 3 to 5 d. They found that high doses of 20 μg or more per wound showed reduced effects, though healing rate was significantly faster than control animals. These results demonstrate the bFGF shows bell-shaped dose-response curve. In the present study, however, high dose of bFGF at 25 μg exerted maximum effect, which was better than middle (5 μg) and low dose (1 μg). It is likely that the concentration of bFGF in wound environment is also important as well as applied amount of bFGF. Researchers of Kaken Pharmaceutical company (Kyoto, Japan) applied bFGF to experimental wound with total volume of 20 μl, while we applied 100 μl (Okumura et al, 1996a; Okumura et al, 1996b). In this regard, bFGF concentration in the present study was 5 times diluted when compared to that used by Okumura et al (1996b), which is thought to render high dose of bFGF (25 μg) was more effective than middle and low doses. Though the dose-dependent wound healing effect of bFGF was obtained, no statistically significant difference between low and high dose of bFGF was found in the current study. Because wound repair is a series of highly orchestrated complex events in which various factors participate, excessive supply of a single growth factor is thought to possess a limited influence in the whole process.

In conclusion, our results demonstrate that locally applied rhbFGF has beneficial effects on delayed wound healing in diabetic patients or people undergoing chemotherapy and steroids treatment.

REFERENCES

Alroy, J., Goyal, V. and Skutelsky, E. (1987). Lectin histochemistry of mammalian endothelium. *Histochemistry* **86**, 603-607.

Clark, R. A. F. (1985). Cutaneous tissue repair: basic biologic considerations. I. *J. Am. Acad. Dermatol.* **13**, 701-770.

DeLapp, N. W. and Dieckman, D. K. (1990). Effect of basic fibroblast growth factor (bFGF) and insulin-like growth factors type I (IGF-I) and type II (IGF-II) on adult human keratinocyte growth and fibronectin secretion. *J. Invest. Dermatol.* **94**, 777-780.

Enser, M. and Avery, N. C. (1984). Mechanical and chemical

properties of the skin and its collagen from lean and obese-hyperglycaemic (ob/ob) mice. *Diabetologia* **27**, 44-49.

Falanga, V. (1993). The "wrinkle test": clinical use for detecting early epidermal resurfacing. *J. Dermatol. Surg. Oncol.* **19**, 172-173.

Folkman, J. and Klagsbrun, M. (1987). Angiogenesis factors. *Science* **235**, 442-447.

Goodson, W. H. III. and Hunt, T. K. (1986). Wound collagen accumulation in obese hyperglycemic mice. *Diabetes* **35**, 491-495.

Greenhalgh, D. G., Sprugel, K. H., Murray, M. J. and Ross, R. (1990). PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am. J. Pathol.* **136**, 1235-1246.

Hayward, P., Hokanson, J. A., Heggers. J., Fiddes, J., Klingbeil, C., Geoger, M. and Robson, M. (1992). Fibroblast growth factor reserves the bacterial retardation of wound contraction. *Am. J. Surg.* **163**, 288-293.

Irvin, G. L., Robinson, D. S. and Hubbard, S. (1985). Operative risks in patients with colorectal cancer. *Am. Surg.* **51**, 418-822.

Ishibashi, Y., Soeda, S., Ohura, T., Nishikawa, T., Niimura, M., Nakajima, H., Mizoguchi, M., Shioya, N., Tsukada, S., Hori, Y. and Ogawa, N. (1996a). Clinical effects of KCB-1, a solution of recombinant human basic fibroblast growth factor, on skin ulcers. – A phase III study comparing with sugar and povidone iodine ointment –. *Iyaku Rinsho* **12**, 2159-2187.

Ishibashi, Y., Soeda, S., Ohura, T., Nishikawa, T., Niimura, M., Nakajima, H., Mizoguchi, M., Shioya, N., Tsukada, S., Hori, Y., Furue, M. and Ogawa, N. (1996b). The clinical effects of KCB-1 on skin ulcers. – A double blind trial to investigate an optimal dose –. *Iyaku Rinsho* **12**, 1809-1834.

Karpen, C. W., Spanheimer, R. G., Randolph, A. L. and Lowe, W. L. Jr. (1992). Tissue-specific regulation of basic fibroblast growth factor mRNA levels by diabetes. *Diabetes* **41**, 222-226.

Klingbeli, C. K., Cesar, L. B. and Fiddes, J. C. (1991). In Clinical and Experimental Approaches to Dermal and Epidermal Repair (Barbul, A., Caidwell, M., Eaglestein, W., Hunt, T. K., Marshall, D., Pines, E. and Skover, G.), pp. 443-458. John Wiley and Sons, New Jersey.

Ledoux, D., Gannoun-Zaki, L. and Barritault, D. (1992). Interactions of FGFs with target cells. *Prog. Growth Factor Res.* **4**, 107-120.

McGee, G. S., Davidson, J. M., Buckley, A., Sommer, A., Woodward, S. C., Aquino, A. M., Barbour, R. and Demetriou, A. A. (1988). Recombinant basic fibroblast growth factor accelerates wound healing. *J. Surg. Res.* **45**, 145-153.

Mustoe, T. A., Pierce, G. F., Morishima, C. and Deuel, T. F. (1991). Growth factor-induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. *J. Clin. Invest.* **87**, 694-703.

Niwano, Y., Koga, H., Kanai, K., Hamaguchi, H. and Yamaguchi, H. (1996). Wound healing effect of the new imidazole antimycotic lanoconazole in rats. *Arzneim.-Forsch./Drug Res.* **46**, 218-223.

Okumura, M., Yajima, M., Nishimura, T., Ikeda, H. and Nishimori, T. (1996a). General pharmacology of recombinant human basic fibroblast growth factor. *Arzneim.-Forsch./Drug Res.* **46**, 727-739.

Okumura, M., Okuda, T., Nakamura, T. and Yajima, M. (1996b). Effect of basic fibroblast growth factor on wound healing in healing-impaired animal models. *Arzneim.-Forsch./Drug Res.* **46**, 547-551.

Okumura, M., Okuda, T., Okamoto, T., Nakamura, T. and Yajima, M. (1996c). Enhanced angiogenesis and granulation tissue formation by basic fibroblast growth factor in healing-impaired animals. *Arzneim.-Forsch./Drug Res.* **46**, 1021-1026.

Pierce, G. F., Tarpley, J. Yanagihara, D., Mustoe T. A., Fox, G. M. and Thomason, A. (1992). PDGF-BB, TGF-β1 and basic FGF in dermal wound healing: neovessel and matrix formation and cessation of repair. *Am. J. Pathol.* **140**, 1375-1388.

Poole, G. U. Jr. (1985). Mechanical factors of abdominal closure. The prevention of fascial dehiscence. *Surgery* **97**, 631-639.

Servold, S. A. (1991). Growth factor impact on wound healing. *Clin. Podiatr. Med. Surg.* **8**, 937-953.

Stenberg, B. D., Phillips, L. G., Hokanson, J. A., Heggers, J. P. and Robson, M. C. (1991). Effect of bFGF on the inhibition of contraction caused by bacteria. *J. Surg. Res.* **50**, 47-50.

Szabo, S., Kusstatscher, S., Sakoulas, G., Sandor, Z., Vincze, A. and Jadus, M. (1995). Growth factors: new 'endogenous drugs' for ulcer healing. *Scand. J. Gastroenterol.* **210** (Suppl.), 15-18.