Basic fibroblast growth factor reduces scar formation in acute incisional wounds

Ichiro Ono, MD; Yoshikiyo Akasaka, MD; Risa Kikuchi, MD; Akiko Sakemoto, MD; Takafulmi Kamiya, MD; Toshiharu Yamashita, MD; Kowichi Jimbow, MD

1. Department of Dermatology, Sapporo Medical University School of Medicine Chuo-ku, Sapporo, Japan, and 2. Department of Pathology, Toho University School of Medicine, Tokyo, Japan

ABSTRACT

In order to identify a means to reduce scar formation of the skin after incision, this study examined the effect of local administration of basic fibroblast growth factor (bFGF) in humans. bFGF was administered to a sutured wound immediately after an operation. The drug was injected once into the dermis of the margins of wounds using a 27G needle or rinsing after performing dermocollae. The lengths of the treated wounds varied from 1 to 32 cm, and the subjects were 2–86 years old. Sutured wounds after excision of skin tumors from the face, trunk, and limbs and sutured wounds such as those at the donor sites of full-thickness skin grafts were treated with low-dose bFGF injections (0.1 µg/cm wound; Group 2), high-dose bFGF injections (1 µg/cm wound; Group 3), and rinsed with high-dose bFGF (1 µg/cm wound; Group 4). No patient treated with bFGF had hypertrophic scars, while some patients had hypertrophic or very wide scars in the control group (Group 1), and the ratios of minimum scarring of Group 2 (p < 0.001), Group 3 (p < 0.0001), and Group 4 (p < 0.0001) were statistically significantly higher than those of Group 1. Postoperative administration of bFGF inhibited hypertrophic scarring and widening of remaining scars without any serious side effects.

Many kinds of cells are involved in skin wound healing, which goes through phases of bleeding, coagulation, inflammation, proliferation, and remodeling through the interactions of cells involving the activities of cytokines and growth factors. On the other hand, it is possible to obtain recombinant human cytokines and growth factors in large amounts using genetic engineering techniques, making it feasible to use them clinically. Among them, basic fibroblast growth factor (bFGF, Fiblast Spray™, Kaken Pharmaceutical Co., Tokyo, Japan) has been used clinically for treating burn ulcers, diabetic ulcers, and decubitus in Japan following approval by the Japanese Ministry of Health, Labour and Welfare in 2002 (approval No. 21300AMZ00387000, 250 µg vial and 21300AMZ0038 8000, 500 µg vial), and it has clearly been effective in those situations where it has been sprayed onto wounds at a concentration of 1 µg/cm².

Originally, in 1974, Gospodarowicz isolated a protein that accelerated the proliferation of fibroblasts from bovine pituitary glands and called it the fibroblast growth factor (FGF). Thereafter, it was found that FGF could be separated into FGF-1, 2, and 7. In 1986, Abraham et al. analyzed human bFGF cDNA, and sequenced the human bFGF genes. Basic FGF is a single-chain polypeptide with a molecular weight of about 17 kDa, which has an affinity for phosphorus.

Surgically sutured wounds inevitably leave scars in humans, which could be considered to be "a normal process." The scars made by suture wounds include those that are narrow and fine, wide, atrophied, and hypertrophic. They may also become keloid, and the treatment and prevention of scars is not only a medical problem but also a social one.

Conventionally, topical administration of adrenocortical steroids and compression therapy has been used for treating hypertrophic scars (HS). It is now considered that treatment with growth factors immediately after surgery could influence the quality of the final scar. Previously, we examined the effect of bFGF on sutured incised wounds of full-thickness skin prepared in animals, and showed that the width of the resulting scar was narrower and flatter after a single treatment of bFGF than in controls. Based on this result, in this study, we directly administered bFGF to sutured wounds in clinical cases after surgery and observed healing, expecting cosmetically good results.

MATERIALS AND METHODS

Sutured wounds after surgical excision of skin tumors of the face, trunk, and limbs and those of donors of full-thickness skin grafting were examined. The lengths of the treated wounds were 1–32 cm, and the subjects were 2–86 years old and 230 in number. Because bFGF was administered at doses of 0.1 and 1 µg/cm of sutured wound in animal experiments, in the clinical cases bFGF
administration was started at a dose of 0.1 μg/cm (low-dose injection group: Group 2), and the drug was administered intradermally at a dose of 1 μg/cm (high-dose injection group: Group 3) immediately after the surgery. In addition, in the other group, wound margins after appropriate subdermal sutures were carefully rinsed with a solution containing bFGF (high-dose rinse group: Group 4) just before the completion of skin suturing and the results were compared with the patients without bFGF (control group: Group 1) (Table 1). This study was conducted with the informed consent of the subject or guardian after being approved by the ethics committee of Sapporo Medical University.

After the drug was carefully applied to the dermis of the wound, the sutured wound was covered with a hydrocolloid dressing (Absocure Surgical, Nitto Medical Corp., Ibaraki, Osaka, Japan), which was changed every 2–3 days, until the thread was removed on day 5–7 on the face and day 7–10 on the trunk and limbs. No other drugs such as ointment containing antibiotics were applied to the wounds. After removal of the thread, the patients were instructed to secure all wounds with an adhesive tape until 1–3 months after the operation. In addition, for judging therapeutic effects, inflammation and adhesion of wounds and adverse reactions such as pain and infection were evaluated at 3 months (primary end point), and the degree of scar formation was evaluated at 6–12 months (secondary end point) after the operation, or more in some cases (Figure 1).

### Table 1. Four groups compared at the sixth month after the operation

<table>
<thead>
<tr>
<th>Group</th>
<th>Control group: suturing without intradermal injection</th>
<th>Low bFGF injection group: intradermal injections of 0.1 μg bFGF per 1 cm of wound immediately after suturing</th>
<th>High bFGF injection group: intradermal injection of 1.0 μg bFGF per 1 cm wound after suturing</th>
<th>High bFGF rinse group: rinsed with 0.1 mL of 10 μg/mL bFGF solution per 1 cm wound after suturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative area</td>
<td>Control: 50.0%</td>
<td>Low bFGF: 63.3%</td>
<td>High bFGF: 56.0%</td>
<td>Rinse bFGF: 47.0%</td>
</tr>
<tr>
<td>Gender</td>
<td>Male: 38 (38%)</td>
<td>Female: 62 (62%)</td>
<td>Male: 35 (35%)</td>
<td>Female: 41 (41%)</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>44.3</td>
<td>44.1</td>
<td>44.2</td>
<td>43.7</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>19.7</td>
<td>17.6</td>
<td>20.5</td>
<td>18.4</td>
</tr>
</tbody>
</table>

*No statistical differences between groups.

bFGF, basic fibroblast growth factor.

Operations were performed as described previously and the subjects were treated intradermally with bFGF at doses of 0.1 μg/cm (low bFGF group; 30 subjects: Group 2) or 1 μg/cm (high bFGF group; 100 subjects: Group 3), while in the other group of 100 subjects the wound was rinsed with 0.1 mL of bFGF solution (100 μg/mL) per 1 cm of sutured wound (high-dose rinse group: Group 4), and the results were compared with those of 100 subjects not treated with bFGF (control group: Group 1) who were operated on by the same surgeons without bFGF injections (Table 1). The average ages of the four groups and areas of examined showed no statistical differences between groups (Table 2a–c). In this evaluation, patients with malignant melanoma and children under the age of 1 year were excluded.

Degree of scar formation was evaluated at 6–12 months after the operation (secondary end point) and was based on the following criteria mainly depending on scar width.22–24

I (Excellent): No or slight redness and almost no scar, below 0.25 mm in width.

II (Good): Slight redness of the scar and its surrounding, from 0.25 to 0.5 mm in width.

III (Fair): A flat reddish scar from 0.5 to 2.0 mm in width.

IV (Poor): HS above 2 mm in width, showing cosmetically and functionally unsatisfactory results of the operation (Figure 2).

This clinical trial began in April 2002. To evaluate the scar width, dermoscopic images were taken by a Dermo-Genius® (Licos Co., Munich, Germany) and recorded by a digital camera (Nikon Coolpix 5600, Nikon Corporation, Tokyo, Japan), and then the data were analyzed on an iMac G5 2G computer (Apple Computer Inc.,...
Cupertino, CA) using the software ImageJ version 1.35s (National Institute of Health, Bethesda, MD) to measure the scar width digitally. Measurements of the scar width were performed by two authors including the operator (I.O.), but not blindly. This measurement procedure was performed by us in other previous experiments using histopathological specimens.27

Statistical analysis
Statistical analysis of the differences of effects between groups was performed by the $\chi^2$ comparison test using Statview-J 4.5 (Abacus Concepts Inc., Berkeley, CA). Regarding the age of patient, the results are expressed as means ± standard deviation (SD) and statistical analysis was performed by analysis of variance (ANOVA), followed by Fisher’s comparison test using Statview-J 4.5. $p < 0.05$ was considered to be statistically significant.

RESULTS
Among the 100 subjects in the control group (Group 1), 14, 30, 36, and 20 were graded as I, II, III, and IV, respectively. On the other hand, among the 100 subjects treated with high-dose bFGF (Group 3), 46, 36, 15, and 3 were graded as I, II, III, and IV, respectively, showing that the proportions of subjects graded III and IV were significantly higher in the control group (Group 1) compared with the treatment groups. Scar formation was slight in the bFGF treatment groups (Groups 2–4) in which more than 70% of the subjects were left with only fine linear scars (graded as I and II), whereas only 44% of the subjects in the control group (Group 1) showed similar results.

The results of measuring the scar width using the pictures taken by a dermoscope revealed that more than 82.0% of the patients treated with high-dose bFGF injections (Group 3), like the rinsed group (Group 4) (77.0%) and the low-dose bFGF group (70.0%), had a scar width of < 0.5 mm, while 44.0% of the control group (Group 1) had a scar width of < 0.5 mm. In the bFGF treatment groups, no patient had HS, while some patients had hypertrophic or very wide scars in the control group (Group 1), and the ratios of grading of Group 2 ($p < 0.001$), Group 3 ($p < 0.0001$), and Group 4 ($p < 0.0001$) were statistically significantly higher than those of the control group (Group 1) (Tables 3 and 4, Figure 2).

In addition, no serious adverse reactions were observed in those treated with either dose of bFGF, and HS or scars of more than 3.0 mm in width were not seen. In some cases treated with bFGF, erythema around the wound persisted.

Table 3. Results of the clinical trial

<table>
<thead>
<tr>
<th></th>
<th>I Excellent</th>
<th>II Good</th>
<th>III Fair</th>
<th>IV Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (100 cases)</td>
<td>14 cases (14.0%)</td>
<td>30 cases (30.0%)</td>
<td>36 cases (36.0%)</td>
<td>20 cases (20.0%)</td>
</tr>
<tr>
<td>Low bFGF injected group (30 cases)</td>
<td>13 cases (43.3%)</td>
<td>8 cases (26.7%)</td>
<td>7 cases (23.3%)</td>
<td>2 cases (6.7%)</td>
</tr>
<tr>
<td>High bFGF injected group (100 cases)</td>
<td>46 cases (46.0%)</td>
<td>36 cases (36.0%)</td>
<td>15 cases (15.0%)</td>
<td>3 cases (3%)</td>
</tr>
<tr>
<td>High bFGF rinsed group (100 cases)</td>
<td>35 cases (35.0%)</td>
<td>42 cases (42.0%)</td>
<td>18 cases (18.0%)</td>
<td>5 cases (5.0%)</td>
</tr>
</tbody>
</table>

bFGF, basic fibroblast growth factor.
for longer, but had subsided by 1 year after surgery. Representa-
tive cases are described below.

Case 1
A 56-year-old man with basal cell carcinoma in the dor-
sum of his nose. The tumor including subcutaneous fat in
5 mm of free margin was excised under local anesthesia,
and then reconstruction was performed using a rhomboid
flap prepared laterally. Subcutaneous and dermal sutures
with 5-0 Vicryl (Ethicon Inc., Somerville, NJ) and 6-0
PDS-2 (Ethicon Inc., Somerville, NJ), respectively, and
skin suture with 7-0 black Nylon (BEAR Medical Corp.,
Ichikawa, Japan) were conducted. After suturing, 0.1 mL
of bFGF solution (1 mg/mL) per 1 cm of sutured wound
was injected into the dermis in the margin of the wound
(0.1 mg/cm). The postoperative course was very smooth.
Slight redness was observed 1 month after the operation,
but the scar became linear, and almost no scar was ob-
served 6 months after operation, which was classified as
“excellent” (Figure 3A–D).

Table 4. Results of statistical analysis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low bFGF</th>
<th>High bFGF</th>
<th>Rinse bFGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>( p &lt; 0.0001 )</td>
<td>( p &lt; 0.0001 )</td>
<td>( p &lt; 0.0001 )</td>
</tr>
<tr>
<td>Low bFGF</td>
<td>( p &lt; 0.0001 )</td>
<td>—</td>
<td>NS ( p = 0.1553 )</td>
<td>NS ( p = 0.4350 )</td>
</tr>
<tr>
<td>High bFGF</td>
<td>( p &lt; 0.0001 )</td>
<td>NS ( p = 0.1553 )</td>
<td>—</td>
<td>NS ( p = 0.3812 )</td>
</tr>
<tr>
<td>Rinse bFGF</td>
<td>( p &lt; 0.0001 )</td>
<td>NS ( p = 0.4350 )</td>
<td>NS ( p = 0.3812 )</td>
<td>—</td>
</tr>
</tbody>
</table>

bFGF, basic fibroblast growth factor; NS, nonsignificant.

Case 2
A 58-year-old woman with scar in her left elbow due to
fracture 10 years ago. The scar was excised under local

Figure 3. Case 1: A 56-year-old man with basal cell carcinoma
in the dorsum of his nose. (A) Before the operation. (B) Soon
after the operation. (C) Three months after the operation. (D)
Eighteen months after the operation.

Figure 4. Case 2: A 58-year-old woman with postoperative
scar. (A) Before the operation. (B) Soon after the operation. (C)
One month after the operation. (D) Sixteen months after the
operation.
anesthesia, and reconstruction was performed in a w-plasty. After sutured, 0.1 mL of bFGF solution (10 µg/mL) per 1 cm of sutured wound was injected into the dermis in the margin of the wound (1 µg/cm). After the treatment, the wound was covered with a hydrocolloid dressing, which was changed on the day after the operation. Slight redness was observed 1 month after the operation, but the scar became linear and decreased to < 0.5 mm in width 6 months after the operation in spite of slight redness. The result was classified as "good" (Figure 4A–D).

DISCUSSION

Although it has been established that growth factors play various important roles in wound healing, their use therapeutically has been considered mainly for accelerating the wound-healing process. However, administration of cytokines and growth factors to subjects with normal wound-healing ability may have little influence on the speed of wound healing, although using a drug that may stimulate the secretion of cytokines may improve the quality of wound healing including the prevention of scar formation.

Experimental studies have demonstrated that bFGF administration to the skin wound accelerates angiogenesis, granulation, and epithelialization, resulting in accelerated wound healing. Research on the clinical application of bFGF has progressed rapidly because of development of the recent elucidation of the structure of bFGF and of the genetic engineering techniques to produce it. Clinical studies with the use of recombinant bFGF have shown that bFGF is highly efficacious and safe for skin ulcers such as diabetic leg ulcers and decubitus as well as burns, and encouraging clinical results have been obtained in Japan. In the present study, the doses of bFGF treated in the wounds were 0.1 and 1 µg/cm, and the lengths of the sutured wounds treated did not exceed 32 cm, indicating that the total doses were not greater than 32 µg. Furthermore, bFGF was administered once immediately after the operation. Therefore, we concluded that the dose will not cause serious problems, as reported previously.

In addition, in the present clinical study, the subjects consisted mainly of those for whom the operation was performed for cosmetic reasons, but there were cases with malignant tumors including cases with completely resected basal cell carcinoma and Bowen’s disease. Because scar formation is also an issue in cases of reconstruction after resection of malignant tumors, the application of bFGF could be used widely if the safety of bFGF at the doses we used can be confirmed in other studies.

The scars made by suture wounds vary from being fine, wide, atrophied to hypertrophic. Shah et al. found that TGF-β1 and -β2 play important roles in scar formation, and that the antisera against TGF-β1 and TGF-β2 as well as TGF-β3 antagonized their effects, indicating that there is a possibility of preventing the formation of scar using mannose-6-phosphate as a TGF-β inhibitor. Their study demonstrated that isoform-specific differences in the role of TGF-β3 in wound healing and cutaneous scarring and the relative ratios of the three TGF-β isoforms are critical for controlling scar formation. The same study also suggested a novel therapeutic use of exogenous recombinant, TGF-β3, as an antiscarring agent. Wu et al. also reported that administration of TGF-β3 prevented skin and tendon scar formation, and that the use of other growth factors such as keratinocyte growth factor-2 immediately after an operation can influence the quality of the final scar. This is consistent with the results of our animal experiments, in which bFGF treatment indeed induced scarless healing, or at least, minimum scar formation.

Basic FGF is also attracting attention for its action as a morphogen. The epithelial–mesenchymal tissue interactions are important for the development of various organs, and soluble signaling molecules may be involved in this interaction. Basic FGF mesenchyme-derived factor, having mitogenic, motogenic, and morphogenic activities on various types of epithelial cells and morphogenetic activities on various types of epithelial cells, is considered to be a possible mediator of epithelial/mesenchymal interactions during not only organogenesis and organ regeneration but also wound healing.

Factors contributing to scar quality vary and are complicated. There are differences due to age, race, area of body, tension on the wound, inheritance, and so on. When trying to compare the scar quality of one treatment with that of another, those parameters should be the same. In this study, those parameters were not strictly controlled, but there was no statistical difference among age, gender, or area of body between the groups. With respect to the clinical application of substances like growth factors such as bFGF, defining the optimal dose is always important. Although we used only two concentrations for local injection in the present study, every group treated with bFGF showed better results than the control, and bFGF at a dose of about 1 µg/cm local injection as well as rinsing the wound with a solution containing bFGF (100 µg/mL) is considered to be practical. In the experimental results described previously, the sutured wounds in the groups treated with bFGF were not significantly different with respect to normal healing processes from the control group both macroscopically and histopathologically; but there were differences, in particular, less scar width and no HS was observed in groups treated with bFGF.

In 1988, McGee et al. were the first to present evidence that bFGF could improve wound breaking strength, and in 2002, Ono and Spyrou and Naylov each reported the inhibition of scar formation by bFGF in separate studies. In Spyrou’s experiments, multiple injections of 1 µg bFGF daily were performed. In contrast, in our experiment, bFGF injection was performed only once soon after suturing, leading to an inhibition of the differentiation of fibroblasts into myofibroblasts as well as to induction of apoptosis at days 10 and 15 after wounding. Finally, those treated wounds exhibited improved architecture of the neodermis, suggesting a possible antiscarring effect of bFGF in wound healing.

Based on these results, we conducted clinical trials. Basic FGF was administered at doses of 0.1 and 1 µg/cm of sutured wound in animal experiments, whereas clinical cases were treated initially with 0.1 µg/cm, and then the dose was increased to 1 µg/cm based on the results...
of initially treated clinical cases. In the clinical cases, the widths of the scars treated with bFGFs were <3 mm, whereas the width in the control scars were up to 8 mm at the same phase. There were only two subjects with scars wider than 2 mm among the 100 subjects treated intradermally with a dose of 1 μg/cm, and more than half of them had only linear fine scars. The results strongly suggest the utility of this therapy, because local injection of the growth factor soon after surgery only once has the potential to improve the final cosmetic outcome of surgery with a low cost ($1 US for 5 cm of wounds in Japan). Undesirable side effects including erythema and capillary dilatation were found in the area surrounding the scar, and capillary dilatation persisted for a relatively long time. Because the former may persist for 3–6 months after conventional therapy depending on the site, it may not be a characteristic of only this therapy. However, flat erythema of about 5 mm in width persisted for several months after the operation on both sides of the scar, which disappeared within 1 year after the operation.

The detailed mechanism by which bFGF reduces scar formation remains unknown. Considering that bFGF has angiogenic effects and that vigorous angiogenesis was observed 1–3 weeks after the operation in our animal experiments, these results suggest a strong association of angiogenesis with this symptom; and the cell proliferation, followed by apoptosis plays an important role.35

The scar-inhibiting effect of bFGF on sutured wounds was confirmed clinically by this research. The drug was injected into the dermis or topically applied by rinsing once immediately after the operation: there was no case of HS, and in many cases only linear fine scars remained without major side effects. Although more detailed studies will be required, including a double-blind control study concerning the time and frequency of administration to examine the effects of multiple and preoperative administration of the drug, this very simple technique of administering the growth factor topically may be applied to all kinds of sutured wounds to improve quality after healing. The results of the patients with so-called “HS,” such as the second patient, indicate that this strategy is also useful for the treatment of patients with scars that are a big social problem, especially among Asians.

In conclusion, in the present study, the administration of bFGF at the time of closure improved the quality of the scar to some extent. Postoperative administration of bFGF resulted in inhibition of scarring and may be considered to be useful clinically for any kind of sutured wounds. In this examination, a significant difference was observed between three bFGF-treated groups and the control group. However, a clear dose dependency was not observed in the two dosages used in this study. Moreover, local injection or rinsing with a high-bFGF solution after dermo-stitches produced an excellent result. From these results, if the wound is short in the head and neck region, then local dermal injection with 1 μg/cm is appropriate to obtain satisfactory results. For longer wounds when a large amount of bFGF needs to be administered, then a dermal injection of 0.1 μg/cm of bFGF solution is considered to be appropriate. In the limbs and trunk, however, treatment with a local injection of a high-bFGF solution, 1 μg/cm, is preferable. If it is difficult to administer into the dermis because the wounds are long or the dermal portion of the wound is thin, it may be better to rinse with a solution containing 100 μg/mL bFGF after completing the dermo-stitches. There have been no side effects except persistent telangiectasia around the scar in some patients, although this usually disappeared within 1 year after the operation. Finally, in some cases, bFGF was applied immediately after the excision of basal cell carcinoma or Bowen’s disease. Considering that bFGF has the potential to promote the proliferation of malignant tumor cells,13,14 bFGF treatment to reduce scar width and quality must be performed when the benefit of this strategy overwhelms the risk of side effects including tumor recurrence. Of course, the complete removal of the tumor lesion including enough free margin is essential to consider to improve the quality of the scar. Informed consent is also essential in this aspect. In addition, we excluded patients with malignant melanoma or advanced squamous cell carcinoma whose malignant potentials are very high, even if the tumor was removed completely.

REFERENCES

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