Novel Method to Enhance Sternal Healing After Harvesting Bilateral Internal Thoracic Arteries With Use of Basic Fibroblast Growth Factor

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Background—Poor healing of the sternum often limits the use of bilateral internal thoracic arteries (BITAs) in coronary bypass surgery, especially for diabetic patients. We have reported that basic fibroblast growth factor (bFGF) enhanced regeneration of the skull. This study was designed to evaluate the effects of topical use of bFGF on sternal healing after removing the BITAs.

Methods and Results—Forty-five Wistar rats were subjected to median sternotomy and were divided into 3 groups: 15 had the BITAs removed and had a bFGF sheet applied on the posterior table of the sternum (group A), 15 had just the BITAs removed (group B), and 15 had intact BITAs (group C). Five and 10 rats were euthanized 2 and 4 weeks after surgery, respectively, in all 3 groups. Peristernal blood flow, measured with use of a noncontact laser flowmeter, decreased after removal of the BITAs ($P<0.001$). Four weeks after the surgery, PBF markedly increased only in group A (9.7±1.2, 6.5±0.6, and 8.2±0.5 mL·min$^{-1}$·100 g$^{-1}$ for groups A, B, and C, respectively; $P<0.01$ by ANOVA). Four weeks after surgery, the following findings were obtained only in group A: (1) nearly completely healed sternum filled with regenerated bone tissue, (2) marked angiogenesis around the sternum, and (3) osteoblasts in an active form around the edge of the sternum.

Conclusions—The results suggest that use of the bFGF sheet offset the sternal ischemia and accelerated sternal healing. This method may help to decrease sternal necrosis in high-risk patients or allow extended use of BITAs in coronary bypass surgery. (Circulation. 2000;102[suppl III]:III-307-III-311.)

Key Words: angiogenesis ▪ blood flow ▪ growth substances

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low or poor healing of the sternum is one of the potential problems encountered after sternotomy and is therefore one of the potential problems associated with heart surgery. Slow healing prolongs the patients' hospital stay, increases health care costs considerably, and delays the patients' return to work or social activities. Poor healing of the sternum often leads to deep sternal wound infection, which is serious.1-3 Previous studies have identified risk factors for sternal wound complications, such as obesity, chronic obstructive pulmonary disease, elderly age, peripheral vascular disease, reoperation, diabetes mellitus, use of internal thoracic artery (ITA) conduits, prolonged operation time, low cardiac output, prolonged ventilation time, and reexploration for bleeding.4-8 The number of patients with some of the above risk factors is increasing, and slow/poor sternal healing will become even more problematic in this population. Slow/poor healing often limits the use of bilateral internal thoracic arteries (BITAs) in coronary bypass surgery, especially in diabetic patients whose hearts are shown to benefit from BITA grafting,7 because diabetic patients are more prone to sternal necrosis, particularly after removal of the BITAs because of the lack of blood supply.

It has been reported that basic fibroblast growth factor (bFGF) is not only a potent angiogenic mitogen but also an effector that can stimulate bone formation.8 However, when bFGF is injected in free form, it does not stay at the injection site for a period long enough for its effective biological activity to produce the expected results. In an attempt to solve this problem, we developed a biodegradable hydrogel composed of acidic gelatin to enable bFGF to be released at the site of action for an extended time period. We have demonstrated that gelatin hydrogels, with incorporated bFGF, enhanced in vivo bone regeneration in the skull. The purpose of the present study was to evaluate the effect of topical use of bFGF on sternal healing after the removal of BITAs in rats.

Methods

Preparation of bFGF-Incorporated Gelatin Hydrogel Sheets

Gelatin with an isoelectric point of 4.9 was isolated from bovine bone collagen by an alkaline process using Ca(OH)$_2$ (Nitta Gelatin Co). The weight-average molecular weight of the gelatin was 99 000
when measured by gel filtration chromatography relative to standard polyethylene glycol samples. Human recombinant bFGF with an isoelectric point of 9.6 was obtained from Kaken Pharmaceutical Co. Gelatin in 10 wt% aqueous solution was chemically cross-linked with various amounts of glutaraldehyde at 25°C to prepare sheets with different extents of cross-linking. Briefly, 4.5 mL of an aqueous gelatin solution containing glutaraldehyde was cast into a polytetrafluoroethylene mold (5×5 cm², 1.8-mm depth). After the cross-linking reaction, which lasted for 12 hours at 25°C, the resulting hydrogel sheets were immersed in 50 mmol/L of glycine aqueous solution at 37°C for 1 hour to block residual aldehyde groups of glutaraldehyde, then rinsed with double-distilled water and 100% ethanol, and autoclaved while immersed in double-distilled water to obtain sterilized sheets. These were freeze-dried, followed by impregnation with an aqueous solution containing 100 μg of bFGF, to obtain gelatin hydrogels with incorporated bFGF. The prepared hydrogel sheets were rectangle-shaped (1×10 mm) and 0.7-mm thick. All experimental processes were conducted under sterile conditions.

Animal Experiments
Forty-five male Wistar rats weighing between 300 and 400 g were orally intubated after anesthesia with use of a small amount of 99.5% ether and were ventilated on a small volume-cycled animal ventilator (rodent ventilator model 683, Harvard Apparatus). Anesthesia was maintained during the operation with 1% to 2% isoflurane. After a midline skin incision, with the animal in the supine position, the bilateral major pectoral muscles were divided from the junction of the sternum, and intercostal muscles on both sides of the sternum were exposed. Median sternotomy was carefully performed by using a rotating saw (D-7200, AESCULAP), leaving part of the narrow sternum on both sides. The bleeding from the bone marrow was stopped through the use of bone wax (Nestor, Nippon Shoji). The 45 rats were randomly divided into 3 groups: group A (n = 15) had the BITAs removed, and a gelatin hydrogel sheet with incorporated bFGF was placed on the posterior table of the sternum before closing the sternum; group B (n = 15) had the BITAs removed, and the sternum was closed without using the sheet; and group C (n = 15) had the BITAs left intact, and the sternum was closed without the sheet. For removal of the BITAs (ie, in groups A and B), they were ligated using 6-0 polypropylene sutures near the origin and at the distal bifurcation, and the BITAs, with their beds, were destroyed by use of an electrical coagulator. When the gelatin hydrogel sheets with incorporated bFGF (100 μg per sheet) were placed in the animals in group A, the ITA beds were also covered by the sheet, and the sheet was stabilized with 6-0 polypropylene sutures. After positive end-expiratory pressure was applied to fully inflate the lung, the sternum was parasternally closed with 4 interrupted braided polyester sutures. The muscle layer and the skin were carefully sutured with 4-0 nylon monofilaments. Streptomycin was administered intramuscularly just after skin closure (50 mg per rat). Rats were euthanized by intravenous administration of a lethal dose of sodium pentobarbital for 2 weeks (5 animals in each group) and 4 weeks after the surgery. The sternum was stabilized with 6-0 polypropylene sutures. After positive end-expiratory pressure was applied to fully inflate the lung, the sternum was parasternally closed with 4 interrupted braided polyester sutures. The muscle layer and the skin were carefully sutured with 4-0 nylon monofilaments. Streptomycin was administered intramuscularly just after skin closure (50 mg per rat). Rats were euthanized by intravenous administration of a lethal dose of sodium pentobarbital for 2 weeks (5 animals in each group) and 4 weeks after the surgery. The sternum was excised and fixed in 10 wt% EDTA solution at 4°C for 3 days, embedded in paraffin, and sectioned at 10-μm thickness. The sections were obtained at the third, fourth, and fifth intercostal spaces of the sternum and stained with hematoxylin-eosin 2 and 4 weeks after the surgery. The histological sections were analyzed by use of a microscope equipped with a video camera connected to an image analysis system (SP-1000, Olympus). Photographs of formalin-fixed bone specimens from different experimental groups were taken with the use of the same type of x-ray film. Bone specimens were demineralized in 10 wt% EDTA solution at

Histological Assessment of Angiogenesis
Arterioles (>25 and <100 μm in external diameter) and capillaries (<25 μm in external diameter) were counted in preparations stained with hematoxylin-eosin. Five fields were randomly chosen from the connective tissue around the sternum. Two pathologists without knowledge of the treatments counted the number of vessels per unit area (200×200 μm²) by using a grid method where the density of arterioles in each 5-mm×5-mm field was assessed by determining the mean number of vessels in 5 randomly chosen unit areas (200 μm×200 μm) with use of a section ocular micrometer (Olympus) at ×400 magnification. The total number of vessels in the 25 unit areas (5 fields with 5 unit areas per field) was counted and averaged. To maintain randomness, an optic lens containing a protractor and micrometer was used for selection of the 5 portions.

Assessment of Bone Formation
Bone regeneration in the sternum was assessed by soft x-ray analysis and histological examination. Soft (high-contrast) x-ray pictures of the sternum were taken at 46 kV and 2 mA for 45 seconds by use of an x-ray apparatus (type CMB, Koizumi X-Sepkousha). Photographs of formalin-fixed bone specimens from different experimental groups were taken with the use of the same type of x-ray film. Bone specimens were demineralized in 10 wt% EDTA solution at 4°C for 3 days, embedded in paraffin, and sectioned at 10-μm thickness. The sections were obtained at the third, fourth, and fifth intercostal spaces of the sternum and stained with hematoxylin-eosin 2 and 4 weeks after the surgery. The histological sections were analyzed by use of a microscope equipped with a video camera connected to an image analysis system (SP-1000, Olympus). The area of new bone in each section prepared from the sternum was measured at ×2 magnification. To observe the osteoblasts in active form, which are characterized by a basophilic cuboid cytoplasm located adjacent to the bone surface, and osteoclasts that have a basophilic cytoplasm and plural nuclei, the histological sections were viewed at high magnification (×400) with a light microscope.

Statistical Analysis
All of the data were analyzed by 1-way ANOVA to assess the statistical significance among experimental groups. Experimental results were expressed as mean±SD. Results of the statistical analyses were regarded as significant at a value of P<0.05.

Results

Peristernal Blood Flow
Preoperative PBF was 8.6±0.6 (mean±SD) mL·min⁻¹·100 g⁻¹. Although the PBF did not change after the median sternotomy alone (ie, intact BITAs), it was significantly reduced to 4.2±0.6 mL·min⁻¹·100 g⁻¹ after removal of the BITAs (P<0.001). PBF 2 weeks after the surgery in group A and group B was 6.5±0.7 and 5.9±0.55 mL·min⁻¹·100 g⁻¹, respectively, both of which were significantly lower than that in group C (8.0±0.7 mL·min⁻¹·100 g⁻¹). However, 4 weeks after surgery in groups A, B, and C, PBF was 9.7±1.2, 6.5±0.6, and 8.2±0.5 mL·min⁻¹·100 g⁻¹, respectively. Significant differences were noted among the 3 groups (P<0.01). Results for PBF before and after median sternotomy with different surgeries in each group are summarized in Figure 1.

Histological Assessment of Angiogenesis
Histological examination of the tissue around the sternum confirmed that there was an increase in the number of vessels.
Figure 1. Changes in PBF in the 3 groups. PBF was measured by means of noncontact laser Doppler flowmeter before median sternotomy and 2 and 4 weeks after surgical treatment as follows: a gelatin sheet containing 100 μg of bFGF was applied after removal of BITAs (group A); BITAs were removed with no gelatin sheet applied (group B); and BITAs were left intact (group C).

Photomicrographs of the connective tissue around the sternum 4 weeks after surgery are shown in Figure 2. There were more capillaries and arterioles (10 to 50 μm in diameter) around the sternum in group A than in group B or C (Figure 2). Figure 3 shows the number of arterioles and capillaries per unit area around the sternum 4 weeks after surgery in each of the 3 groups. In group A, a larger number of vessels was seen in the connective tissue around the sternum. On the other hand, in groups B and C, significantly lower numbers of vessels were noted. The number of the arterioles and the number of capillaries per unit area around the sternum increased to a greater extent in group A than in the other 2 groups (30.5±3.2, 15.8±2.7, and 12.3±1.5 vessels per unit area for groups A, B, and C, respectively; P<0.01).

Assessment of Bone Formation
Soft x-ray photographs of the sternum 4 weeks after the different surgeries are shown in Figure 4. Dehiscence of the separated original sternum was observed in group B. In contrast, groups A and C had no sternal dehiscence. Almost complete bone regeneration was seen in group A only.

Figure 2. Effects of bFGF on angiogenesis around sternum. Micrographs show new vessels in connective tissue around sternum after surgical treatment as follows: a gelatin sheet containing 100 μg of bFGF was applied after removal of BITAs (A); BITAs were removed with no gelatin sheet applied (B); and BITAs were left intact (C). Increased numbers of capillaries and arterioles were noted (arrows). Original magnification×400 (hematoxylin-eosin).

Figure 3. Effects of bFGF on number of vessels in connective tissue around sternum. Five fields were randomly chosen in connective tissue from around sternum, and number of vessels per unit area (200×200 μm²) was counted by grid method for each of the groups: group A, treatment with bFGF after removal of BITAs; group B, BITA removal; and group C, intact BITAs.

Figure 4. Effects of bFGF on bone regeneration as shown by x-ray analysis. Soft x-ray photographs of sternum were taken 4 weeks after surgical treatment as follows: a gelatin sheet containing 100 μg of bFGF was applied after removal of BITAs (A); BITAs were removed with no gelatin sheet applied (B); and BITAs were left intact (C). Sternal dehiscence (arrows) was observed. Bar=5 mm.
3 groups were not statistically significant (1.79 ± 0.61, 0.87 ± 0.70, and 1.37 ± 0.92 mm² for groups A, B, and C, respectively). Four weeks after the surgery, however, group A had a significantly larger area of new bone formation than did the other 2 groups (5.13 ± 2.82, 2.17 ± 0.91, and 2.01 ± 0.89 mm² for groups A, B, and C, respectively).

Discussion

Several clinical and experimental studies have demonstrated a decrease in sternal blood flow after ITA harvesting. Arnold¹¹ reported that BITA mobilization may render the sternum avascular; he studied 52 human sternal specimens by postoperative radioisotope angiography with use of a radioactive microsphere technique. Results of the study by Arnold were supported by the results of Seyfer et al,¹² who measured sternal blood flow in rhesus monkeys with the use of microspheres. The blood flow was unchanged after median sternotomy alone, but mobilization of the unilateral ITA decreased sternal blood flow by 90%. Recently, using radioactive microspheres, Parish et al¹³ demonstrated that chest wall blood flow was significantly decreased compared with the preharvest level after ITA mobilization in a canine model. Our results of PBF as measured by means of a noncontact laser Doppler flowmeter were consistent with the foregoing experimental studies, suggesting that the small animal model examined in the present study is compatible with the large animal model.

Our histological study of the blood vessels around the sternum showed an increase in the number of vessels, suggesting that increased PBF in group A (ie, in which a bFGF-containing gelatin hydrogel sheet was applied) is associated with the angiogenic effect of bFGF.¹⁴ Some clinical trials have suggested that harvesting the ITA conduits in a skeletonized compared with a pedicled fashion could reduce the sternal devascularization.¹⁵,¹⁶ However, there are wide variations of the anatomy of the collateral vessels around the ITA; Jesus and Acland¹⁷ classified the collaterals into 6 types in a human anatomic study. In some types in which collaterals from the lateral chest wall to the ITA connect to the anterior wall of the ITA via a common vertical channel, the collaterals from the chest wall to the sternum can be potentially preserved by careful skeletonization of the ITA (ie, if the common vertical channel alone served). However, if the collaterals connect to the side wall of the ITA (ie, no common vertical channel), even careful skeletonization can destroy the collaterals from the lateral chest wall to the sternum.

Among the many growth factors recently reported to regulate bone metabolism, bFGF is recognized as a potent mitogen for a variety of mesenchymal cells.¹⁸ In skeletal tissues, bFGF is produced by cells of the osteoblastic lineage, accumulating in the bone matrix, and it acts as an autocrine/paracrine factor for bone cells. bFGF variably regulates the proliferation and differentiation of cells of the osteoblastic lineage and thereby modulates the formation of bone. Although bFGF has both angiogenic and osteogenic effects as described above, its activity does not last long enough to show an effect in vivo, in terms of enhancing bone regeneration, if bFGF is given in a free form. Thus, we prepared a biodegradable hydrogel composed of alkaline-processed “acidic” gelatin, which could ionically interact with bFGF. Previously, we reported that such hydrogels enabled the sustained release of biologically active bFGF through hydrogel degradation.¹⁹ The residual radioactivity of ¹²⁵I-labeled bFGF-incorporated gelatin hydrogels inserted into the mouse...
back decreased with time and remained at the therapeutic level for ≈30 days after implantation.

Results of the histological examination suggested that the gelatin hydrogel sheet with incorporated bFGF helped to facilitate the bone regeneration seen 2 and 4 weeks after the surgery and that the sheet helped to increase the number of osteoblasts in active form around the sternal perimeter. The results are basically compatible with those of our previous study involving treatment of a defect of the skull in rabbits; the number of osteoblasts in active form increased during the initial 2 weeks regardless of bFGF treatment, but 12 weeks after the surgery, the increase was maintained only in the bFGF-treated group.20 In our previous study, we evaluated after the surgery, the increase was maintained only in the study involving treatment of a defect of the skull in rabbits; surgery and that the sheet helped to increase the number of bone regeneration seen 2 and 4 weeks after the gelatin hydrogel sheet with incorporated bFGF helped to facilitate the bone regeneration seen 2 and 4 weeks after the surgery and that the sheet helped to increase the number of osteoblasts in active form around the sternal perimeter. The results are basically compatible with those of our previous study involving treatment of a defect of the skull in rabbits; the number of osteoblasts in active form increased during the initial 2 weeks regardless of bFGF treatment, but 12 weeks after the surgery, the increase was maintained only in the bFGF-treated group.20 In our previous study, we evaluated after the surgery, the increase was maintained only in the study involving treatment of a defect of the skull in rabbits; surgery and that the sheet helped to increase the number of

bFGF offset sternal ischemia and facilitated its healing, probably because of the angiogenic and osteogenic effects of bFGF. This method may help to decrease the chance of sternal necrosis in high-risk patients and thus can potentially extend the use of BITAs in coronary bypass surgery.

References

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