Abstract  Fibroblast growth factor-2 (FGF-2) has been found to have stimulatory effects on fracture repair at diaphysis, while its effect on metaphyseal fracture repair, where spongiosal bone is dominant, has not been studied. This study was conducted to investigate the effect of FGF-2 on metaphyseal fracture healing in a rabbit proximal tibial metaphyseal model. The proximal tibial metaphysis of 6-month-old Japanese white rabbits was osteotomized bilaterally. Then 400 µg of FGF-2, mixed with gelatin hydrogel, and gelatin hydrogel alone (the control) were injected to each osteotomy site of the rabbit proximal tibiae, and the osteotomies were fixed with staples. One and 2 weeks after surgery, the osteoid area in the repairing spongiosal bone at the fracture site was significantly larger in the FGF-2 group than in the control group ($P < 0.05$). On immunohistochemistry, proliferating-cell nuclear antigen-positive cells had a tendency to show greater numbers in the FGF-2 group. After 4 and 8 weeks, values for bone mineral density and the cancellous bone area in the healing region of the fracture site were significantly larger in the FGF-2 group ($P < 0.05$). These data suggest that local application of FGF-2 may have an accelerating effect on the repair of metaphyseal fractures. Exogenous recombinant human rhFGF-2 may have potential clinical applications in metaphyseal fracture treatment.

Keywords  fibroblast growth factors (FGFs) · metaphysis · fracture repair · spongiosal bone · bone induction

Introduction

The healing of fractures is a complex multistep process, involving periosteal and endosteal reactions. In the healing of diaphyseal fractures, the most important response is that of the periosteum, where intramembranous and endochondral ossifications combine to complete the healing process. However, the repair process of metaphyseal fractures is different from that of diaphyseal fractures. At the metaphysis or epiphysis, fracture healing is mainly medullary, and new appositional bone formation occurs in the existing trabecular bone tissue. External callus formation either does not occur or only plays a subsidiary role [1]. Clinically, metaphyseal fractures have several unique problems when compared with diaphyseal fractures. For example, because the fracture site is close to the end of the long bone or joint, internal fixation is difficult. In addition, bone defects are commonplace, and thus bone grafts are often necessary. Furthermore, the resultant irregularity of the articular surface causes secondary osteoarthritis. Therefore, treatments to enhance fracture healing may be even more beneficial and needed for metaphyseal fractures than for diaphyseal fractures. Although numerous studies using various animal models have been performed on fracture healing and treatment, most address the diaphyseal fractures of long bones. Metaphyseal fractures of long bones have not been well investigated [2].

Treatments to enhance fracture healing and bone formation have been developed using physical and biological methods, such as mechanical stimulation, low-intensity pulsed ultrasound; tissue engineering techniques; and the use of osteoconductive biomaterials [24]. Fracture healing consists of a series of cellular responses, which are under the control of specific paracrine and autocrine intercellular signaling pathways. Growth factors, which are now known to play roles in cell division, migration, differentiation, and enzyme production, have been found to appear at different stages to regulate the proliferation, maturation, and differentiation of mesenchymal cells, osteogenic cells, and osteoblasts. The application of growth factors to stimulate fracture healing has aroused considerable
interest recently. For example, the application of fibroblast growth factor (FGF)-1 was reported to be successful in enhancing fracture repair in vivo [3]. Recently, treatment of open tibial fractures in 450 patients, using recombinant human bone morphogenetic protein (BMP)-2, has also been documented [4]. Other growth factors, such as transforming growth factor-β [5], other FGFs [6,7], other BMPs [8,9], insulin-like growth factor [10,11], and parathyroid-related protein [12], have been reported to regulate bone metabolism.

Fibroblast growth factor-2 (basic FGF; FGF-2) is one of the 23 members of the FGF family, which together participate in many different cell and organ systems. FGF-2 stimulates smooth muscle cell growth, wound healing, and tissue repair. FGF-2 also plays an important role in the differentiation and function of the skeletal and nervous systems [13–15]. Recent reports have shown that the addition of exogenous FGF-2 to a fracture site or bone defect during the early healing stage accelerates fracture repair and bone formation [7,16,17]. However, these reports were based on a diaphyseal fracture model. Noting the anatomic differences, there is the possibility that response to the exogenous application of biotic factors may be different at the diaphysis and at the metaphysis. Nakamura et al. [16] reported that a single local injection of FGF-2 into the distal femoral spongiosa stimulated bone formation.

To date, no experimental study has been performed to investigate the effect of FGF-2 on metaphyseal fractures.

In this study, we established a metaphyseal fracture model at the proximal bilateral tibia of the rabbit, and attempted to evaluate the effects of FGF-2 on metaphyseal fracture healing by a single administration of FGF-2 into the osteotomy site.

Materials and methods

Twenty-one 6-month-old Japanese white rabbits were used in the study. The study was reviewed by the Committee on the Ethics of Animal Experiments of the Faculty of Medicine, Kyushu University, and was carried out under the control of the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and the Laws (no. 105) and Notification (no. 6) of the Government of Japan. The animals were anesthetized with sodium pentobarbital (25 mg/kg body weight; Abbott, Chicago, IL, USA) intramuscularly. Recombinant human FGF-2 was obtained from Kaken Pharmaceutical (Tokyo, Japan). Biodegradable gelatin hydrogel, used as the carrier for FGF-2, was prepared through the glutaraldehyde crosslinking of acidic gelatin with a pI (isoelectric point) of 5.0, as previously reported [18].

Fracture model and FGF-2 injection

A 4-cm longitudinal skin incision was made medial to the patella and patellar ligament. A 23-gauge needle was inserted into the joint to confirm the tibia joint level. An osteotomy using a sagittal blade (Striker, Kalamazoo, MI, USA) was then performed on the bilateral tibiae at the proximal metaphysis. FGF-2, at a dose of 400 µg in 100 µl gelatin hydrogel, was injected into the osteotomy site of the left tibia (FGF-2 group) immediately after osteotomy, while 100 µl gelatin hydrogel alone was injected to the right tibia as the control group. The osteotomy site was then fixed with a staple. The staple was made from Kirschner wire 1.0 mm in diameter, 10 mm in width, and 7 mm in depth.

After surgery, the animals were returned to their individual cages for recovery. At 1 (n = 4), 2 (n = 5), 4 (n = 5), 6 (n = 4), and 8 weeks (n = 3) postoperatively, the animals were killed with an overdose of sodium pentobarbital. The bilateral tibiae were then harvested and fixed with 10% formalin for histologic and immunohistochromic studies.

Radiological analysis and measurement of bone mineral content and density

Soft X-ray radiograms (Softex C-SM, Softex, Tokyo, Japan) of the bilateral tibiae were taken after the animals had been killed. Bone mineral density in the healing region of the fracture site was measured transversely with a bone mineral analyzer, using dual-energy X-ray absorptiometry (DEXA; Dichroma Scan DCS-600R; Aloka, Tokyo, Japan). The measurement was done 4 mm from each of the proximal and distal sides of the fracture line, and was one-third of the medial part in width.

Morphological analysis

The tibiae were cut coronally in to anterior and posterior parts. Both parts were demineralized in ethylenediamine tetraacetic acid (EDTA) for 2 weeks, embedded in paraffin, and cut into 4-µm-thick sections. The sections of the anterior part were stained with hematoxylin-eosin and Masson’s trichrome. Serial sections were used for immunohistochemistry. Osteoid staining was performed on the posterior part according to the Goland-Yoshiki method [34].

The total area of cancellous bone in the Masson-trichrome-stained sections was measured using an image analysis computer system (Olympus Image Analysis System, SP-1000; Olympus, Tokyo, Japan). The areas measured were 6 × 3 mm² in size, which was 3 mm from both the proximal and distal sides of the osteotomy line, and 3 mm from inner margin of cortical