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Review Article

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Skeletal effects of systemic treatment with basic fibroblast growth factor

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Abstract

Systemic treatment of intact and ovariectomized rats with basic fibroblast growth factor (bFGF) has strong bone anabolic effects. These effects include marked increases in osteoblast number and activity along cancellous and endocortical bone surfaces, which result in accumulation of osteoid and augmentation of cancellous and cortical bone mass after only short-term treatment with bFGF. Osteoclast surface is markedly decreased in bFGF-treated rats, but this finding may be secondary to the extensive osteoid surface in these animals. Some undesirable skeletal effects of the growth factor include impaired bone mineralization and formation of structurally inferior woven bone. The lack of a bone anabolic response to bFGF at skeletal sites with fatty marrow and along the periosteal surface of cortical bone is also disappointing. Despite these disadvantages, bFGF stimulates cancellous bone formation to such a great extent that it may eventually be considered for use in patients with severe osteoporosis who are unresponsive to conventional therapies, provided that local delivery of the growth factor to bone can be achieved to avoid systemic side effects.

Keywords: Basic Fibroblast Growth Factor, *In Vivo*, Bone Formation, Osteoblasts, Osteoid.

Introduction

Basic fibroblast growth factor (bFGF) is a widely distributed peptide that affects the proliferation and differentiation of many types of mesoderm-derived cells[1]. In early studies, the detection of bFGF within bone matrix[2] and in cultured osteoblasts[3] strongly suggested that the growth factor is involved in the regulation of bone cell populations. This contention was supported by additional findings of increased proliferation of bovine and rat osteoprogenitor and osteoblast-like cells by the addition of bFGF to the culture medium[4-8]. Identification of receptors for bFGF in cultured osteoblasts and osteoclasts also provided firm evidence for a direct effect of the growth factor on these bone cells[9,10]. In cultures of bone marrow stromal cells isolated from rats and humans, bFGF has been shown to induce differentiation of these cells into osteoblasts and the formation of bone-like nodules[8,11-15]. These *in vitro* findings strongly suggested that bFGF would have a powerful bone anabolic effect *in vivo*.

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Skeletal effects of bFGF in intact rodents

The beneficial skeletal effects of bFGF and some disadvantages of its use are summarized in Tables 1 and 2, respectively. Mayahara et al.[16] first described the skeletal response to systemic treatment with bFGF. Young (6 weeks of age), rapidly growing, male and female rats were injected intravenously (IV) with bFGF at doses of 10, 30, 100, and 300 μg/kg daily for 2 weeks. The animals injected with the 2 highest doses of the growth factor were found qualitatively to have increased osteoblast numbers along cancellous and endocortical bone surfaces, which resulted in increased cancellous bone mass at several skeletal sites. In fact, cancellous bone filled the majority of the marrow cavity in the femur of rats treated with the highest dose of bFGF. In contrast, the periosteal surface did not respond to bFGF treatment and the growth factor did not induce an increase in cortical bone area in the tibial diaphysis. The investigators also briefly mentioned that bFGF stimulated osteoblast proliferation and increased cancellous bone mass in aged (71 weeks of age) female rats, but the sample size was not specified and supporting data were not presented.

These initial observations of the skeletal effects of bFGF were extended in a more quantitative manner by Nakamura et al.[17] and Nagai et al.[18]. Intravenous treatment of rapidly

growing, intact rats with a relatively low dose of bFGF (100 μg/kg) for only 7 days increased longitudinal bone growth in the proximal tibia, bone mineral density in the secondary spongiosa of the distal femur, and cancellous bone mass in the distal femoral and proximal tibial metaphyses. Cortical bone area in the femoral diaphysis and osteoid and medullary bone area within the tibial shaft were also increased by bFGF treatment. This augmentation of bone mass in bFGF-treated rats was associated with increases in the populations of both preosteoblasts and osteoblasts along cancellous and endocortical bone surfaces, which suggested that the growth factor stimulated not only the proliferation but also the differentiation of osteogenic cells. The observed increase in endocortical mineral apposition rate indicated that bFGF stimulated osteoblast activity as well. In agreement with Mayahara et al.[16], bFGF did not have a bone anabolic effect along the periosteal surface. In fact, Nagai et al.[18] reported that bFGF depressed periosteal bone formation in growing rats. Since the expression of TGF-β was increased in the endocortical preosteoblasts and osteoblasts of bFGF-treated rats, Nakamura et al.[17] suggested that the skeletal effects of bFGF may be mediated in part by TGF-β.

The above studies showed that a relatively low dose of bFGF (100 μg/kg) and a high dose (300 μg/kg) of the growth factor have strong bone anabolic effects. However, Nagai et al.[18] found that the high dose of bFGF also has some adverse skeletal effects in growing rats such as a widened growth plate, defective calcification at the growth plate-metaphyseal junction and along the endocortical surface, and decreased longitudinal bone growth. But these bone and cartilage defects returned to normal within 2 weeks after withdrawal of bFGF treatment.

Basic FGF was administered by IV injection in the rat studies described above. The findings of Kuroda et al.[19] suggest that this more difficult mode of administration may not be necessary as growing mice injected subcutaneously (SC) with full length and aminoterminally-truncated FGF-4 at doses of 100 and 300 μg/kg exhibited highly significant increases in cancellous bone mass, trabecular number, and indices of bone formation such as mineralizing surface and osteoid surface. However, in our own pilot study in ovariectomized (OVX) rats, daily SC injections of bFGF at a dose of 400 μg/kg for 2 weeks had only a slight, inconstant bone anabolic effect (data not shown). Nevertheless, the possibility that very high doses of bFGF may have a systemic bone anabolic effect when administered SC cannot be ruled out.

Subcutaneous injections of acidic and basic FGF have been shown to have a local bone anabolic effect along the periosteal surface of murine calvariae[20]. Both growth factors markedly increased calvarial width, and this response was associated with at least a two-fold increase in osteoblast surface. In contrast, osteoclast recruitment and, by inference, periosteal bone resorption was not affected by the local SC injection of acidic FGF.

In a related matter, local injection of bFGF promotes healing of segmental bone defects in rabbits[21] and bone fractures in rats and dogs[22,23]. The growth factor was found to stimulate not only callus formation, but also osteoclastic callus resorption[23]. If confirmed in clinical trials, these findings suggest that local application of bFGF may prove to be useful for improved healing of nonunions in humans.

Skeletal effects of bFGF in ovariectomized (OVX) rats

The ability of bFGF to stimulate bone formation and augment cancellous bone mass in intact rodents provided a rationale for preclinical testing of the growth factor as a potential osteoporosis therapy. Nakamura et al.[24] performed the first study with bFGF in OVX rats, a widely-used animal model for postmenopausal bone loss[25]. These animals were subjected to a single intraosseous injection of bFGF in the ilium at a dose of 400 μg at 16 weeks post-ovariectomy. The growth factor induced a marked accumulation of osteoid at 2 weeks post-injection, which eventually calcified to result in a peak increase in bone mineral density and cancellous bone volume at 8 weeks post-injection. These indices of bone mass in bFGF-treated OVX rats were far above those of vehicle-treated OVX rats as well as vehicle-treated control rats. Furthermore, bFGF was also found to dramatically increase the node to free end ratio, an index of trabecular connectivity, in the osteopenic ilium. The observed increase in cancellous mineral apposition rate during the first month post-injection indicated that bFGF stimulated osteoblast activity. Eroded surface, an index of bone resorption, was significantly decreased

↑	cancellous bone volume
↑	cortical bone area
↑	osteoid volume
↑	osteoblast surface
↑	osteoblast activity
↓	osteoclast surface*

*may be secondary to extensive osteoid surface in bFGF-treated rats.

Table 1. Summary of beneficial skeletal effects of bFGF

- Impaired bone mineralization
- Widened growth plates*
- Formation of structurally inferior woven bone
- Lack of bone anabolic effect at periosteal surface
- Lack of bone anabolic effect at fatty marrow sites

*Occurs only at high doses of bFGF

Table 2. Summary of skeletal disadvantages to treatment with bFGF.

by bFGF treatment at 2 and 4 weeks post-injection before increasing to a level above that of vehicle-treated OVX rats by 12 weeks post-injection. This increase in bone resorption at later times post-injection resulted in a steady decline in cancellous bone mass from the earlier peak at 8 weeks after the intraosseous bFGF injection. Nevertheless, the mean value for cancellous bone volume in bFGF-treated OVX rats was nearly identical to that of vehicle-treated control rats and still a factor of 2 greater than that of vehicle-treated OVX rats at 24 weeks post-injection. However, the fact that eroded surface remained increased at this last time point in bFGF-treated OVX rats compared with vehicle-treated control rats suggested that the increment in cancellous bone mass induced by treatment with bFGF would eventually be lost. These findings based on a single intraosseous injection of bFGF suggested that the growth factor is capable of restoring lost cancellous bone mass completely at an osteopenic skeletal site in OVX rats, but the newly-formed bone would not be maintained long after withdrawal of bFGF treatment.

The first study of the skeletal effects of systemic treatment with one of the fibroblast growth factors in OVX rats was performed by Dunstan et al.[20]. In their initial prevention study, these investigators found that daily IV injections of acidic FGF (FGF-1) for 28 days completely prevented cancellous bone loss in OVX rats as well as increased cancellous bone mass in sham-operated control rats. In older rats allowed to become osteopenic for 6 months after ovariectomy, daily IV injections of acidic FGF for 28 days at a dose of 200 μg/kg markedly increased cancellous bone and osteoid mass in the proximal tibial metaphysis to a level far above that of sham-operated control rats. Most of the abundant osteoid became calcified by 28 days after withdrawal of acidic FGF treatment. These findings indicate that acidic FGF was effective not only for the prevention of cancellous bone loss during the early stages of estrogen depletion but also for restoration of lost cancellous bone mass during the later stages of estrogen depletion.

The ability of bFGF (rather than aFGF) to reverse cancellous osteopenia in OVX rats was evaluated by Liang et al.[26]. At 2 months after surgery, OVX rats were injected IV for 14 days with bFGF at a daily dose of 200 μg/kg. Some bFGF-treated OVX rats were sacrificed at the end of this treatment period whereas others were sacrificed at 7 or 14 days after withdrawal of treatment with the growth factor. Osteoid volume, but not cancellous bone volume, was markedly increased in the proximal tibial metaphysis of bFGF-treated OVX rats at the end of the treatment period. However, during the withdrawal period, osteoid volume rapidly declined and cancellous bone volume increased as the osteoid calcified and became converted to bone. The end result was partial restoration of lost cancellous bone mass in bFGF-treated OVX rats after only 14 days of treatment with the growth factor. The observation of "osteoid bridges" between pre-existing bone spicules (Fig. 1A) suggested that bFGF also improves trabecular connectivity at an osteopenic skeletal site. Despite these positive effects, it is important to note that bFGF induces formation of woven bone, which is structurally inferior to lamellar bone. However, rapid formation of woven bone which extends into marrow spaces may be necessary to re-establish trabecular connectivity in the osteopenic skeleton. Addition of lamellar bone to pre-existing bone surfaces thickens isolated bone spicules but is unlikely to connect them.

Treatment of OVX rats with bFGF strongly stimulated bone formation, as indicated by marked increases of at least a factor of 10 in osteoblast and osteoid surfaces. In contrast, osteoclast surface was markedly decreased in bFGF-treated OVX rats, but this finding may be secondary to the extensive osteoid surface (>75%) as osteoclasts are rarely found adjacent

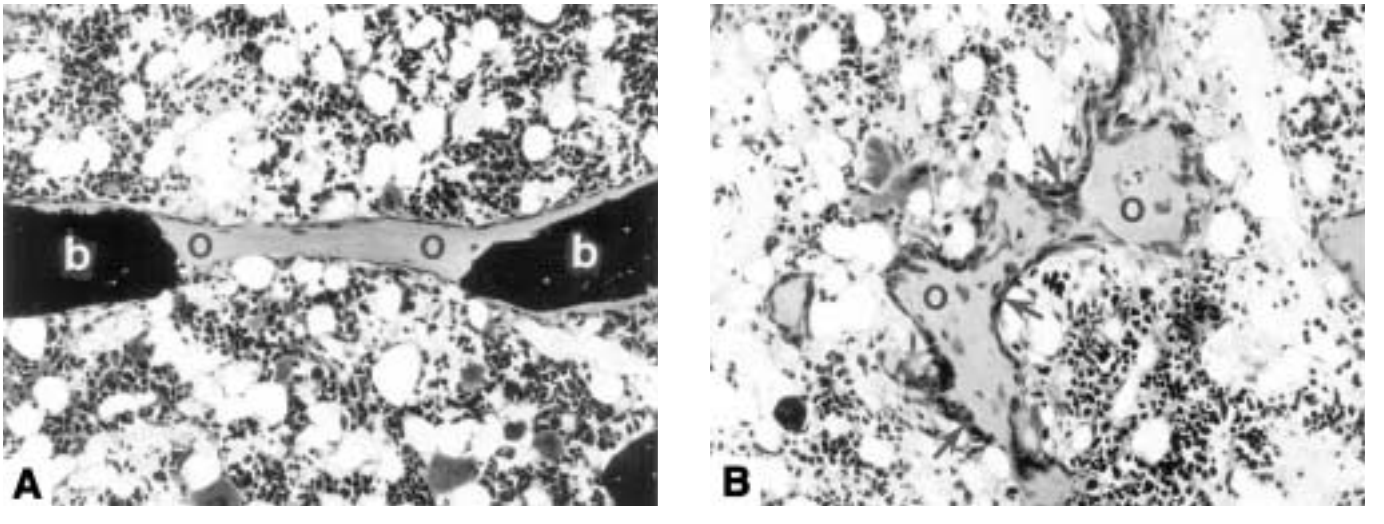

Figure 1. Cancellous bone tissue in the proximal tibial metaphysis of OVX rats treated with bFGF. Osteoid (o) bridges were observed extending between pre-existing bone (b) spicules (A), which suggests that bFGF may improve trabecular connectivity in the osteopenic skeleton. Treatment with bFGF also markedly increased osteoblast numbers (arrows) and induced formation of osteoid spicules without any apparent connections to pre-existing bone (B). However, it is not clear whether these osteoid spicules originate within bone marrow or are extensions from pre-existing cancellous and endocortical bone surfaces.

to unmineralized bone surfaces. Osteoid accumulation and an almost total lack of fluorochrome labeling in cancellous bone indicated that treatment with bFGF impaired bone mineralization. However, this skeletal process returned to normal soon after withdrawal of bFGF treatment as the osteoid rapidly calcified and mineralizing surface increased to at least the level of vehicle-treated OVX rats.

Longitudinal bone growth was not affected by bFGF treatment in OVX rats[26]. This finding is not consistent with the stimulatory effect of bFGF on cartilage cell proliferation *in vitro*[27,28] as well as reports of increased longitudinal bone growth in intact rats treated with the same dose of bFGF[18]. However, it is important to note that the OVX rats were 5 months of age at the beginning of bFGF treatment whereas the intact rats were only 8 weeks of age when first treated with the growth factor. Therefore, the inconsistent results may be due to an enhanced responsiveness of the cartilage of young, rapidly growing rats to bFGF.

Osteoid spicules (Fig. 1B) without any apparent connections to pre-existing bone were observed within the marrow cavity of bFGF-treated OVX rats. This finding is consistent with the results of *in vitro* studies which have shown bFGF to have the ability to induce formation of bone-like nodules in cultures of rat and human bone marrow stromal cells[8,11-15]. Although osteoid spicules in bFGF-treated rats have been described as new trabecular-like structures that formed *de novo* within bone marrow, the origin of these osteoid spicules has not been proven definitively. Amizuka et al.[29] reported that direct intra-osseous injection of bFGF in the proximal tibia of rats induced marrow stromal cells to differentiate into osteoblasts and secrete bone matrix proteins *in vivo* on the first day after injection of the growth factor. However, it is difficult to determine whether this effect was due primarily to bFGF or partial ablation of bone marrow by the needle. Therefore, the possibility that the bFGF-induced osteoid spicules in OVX rats originated from pre-existing bone surfaces and extended considerable distances into the bone marrow cavity cannot be ruled out.

The strong bone anabolic effects of bFGF in both intact and OVX rats were detected at skeletal sites with hematopoietic (red) marrow such as the ilium, distal femur, and proximal tibia[16-18,24,26]. Recently, Pun et al.[30] reported that the stimulatory effects of bFGF on bone formation in OVX rats are greatly attenuated at skeletal sites with fatty (yellow) marrow. In fact, not even a trend for increased osteoblast and osteoid surfaces was observed in the fatty caudal vertebra of bFGF-treated OVX rats whereas these indices of bone formation were increased by at least an order of magnitude in the lumbar vertebra, which is composed of red marrow. The reason for this site specificity in the bone anabolic effects of bFGF is unclear. The increased vascularity of red marrow may play a role in delivering higher local concentrations of the growth factor to the microenvironment, but the observation that another bone anabolic agent, parathyroid hormone, strongly stimulates bone formation regardless of marrow composition[31] argues against this possibility. The observed site specificity may be related to intraskeletal variations in the population of mesenchymal stem cells, which have receptors for bFGF[32] and are undoubtedly target cells for the growth factor. Mesenchymal stem cells are thought to be more common at red marrow sites than at fatty marrow sites, possibly due to their depletion at the latter skeletal sites by their commitment to the adipocytic rather than the osteoblastic phenotype[33,34]. It follows that bFGF may be expected to have a more pronounced bone anabolic effect at sites with red marrow due to a greater, uncommitted population of mesenchymal stem cells available for differentiation into osteoblasts in response to the growth factor. This may be the most likely explanation for the observations of Pun et al.[30].

All of the above skeletal effects of bFGF in OVX rats were detected in cancellous bone tissue. It is also important to determine the effects of bFGF on cortical bone in view of concerns that, in higher mammals and humans, another bone anabolic agent, parathyroid hormone, may augment cancellous bone at the expense of cortical bone. In the tibial diaphysis, treatment of OVX rats with the growth factor for only 7 to 14 days at doses of 100 or 200 μg/kg induced a small but statistically significant increase in cortical bone area[35]. Fluorochrome-based analyses of periosteal and endocortical bone formation were inconclusive due to the inhibitory effect of bFGF on bone mineralization. Therefore, the tissue-level mechanism for the observed augmentation of cortical bone mass in bFGF-treated OVX rats is unclear. In addition to increased cortical bone mass, abundant osteoid was observed within the marrow cavity of the tibial diaphysis in response to bFGF treatment[35]. The ability of the growth factor to markedly stimulate bone formation and increase both cancellous and cortical bone mass in OVX rats provides support for consideration of bFGF as a potential osteoporosis therapy.

Side effects of systemic treatment with bFGF

Since bFGF is a potent mitogen, systemic treatment with the growth factor has great potential for inducing side effects in various tissues and organs. Intact rats treated with a high dose of bFGF (300 μg/kg) exhibited a lower rate of weight gain and hypertrophy of glomerular epithelium in the kidney and alveolar epithelium in the lung[16]. Defective calcification was observed in the long bones of these animals along the endocortical surface and at the growth plate-metaphyseal junction, which resulted in a grossly widened growth plate[18]. Although these adverse effects were not observed in intact rats treated with a lower dose of the growth factor (100 μg/kg), these animals had mild anemia with extramedullary hematopoiesis in the liver and spleen[16]. Other investigators have reported impaired renal function and accompanying proteinuria in intact rats treated with the same dose of bFGF[36]. The retarded weight gain and skeletal side effects of bFGF treatment were no longer apparent by 2 weeks after withdrawal of treatment with the growth factor[18].

OVX rats treated with a moderate dose of bFGF (200 μg/kg) had normal rates of weight gain and were found to be

normocalcemic but hypophosphatemic[26]. An almost total lack of fluorochrome labeling in cancellous bone of these animals despite very high values for osteoblast surface indicated that bFGF treatment impaired bone mineralization. However, this skeletal process returned to normal soon after withdrawal of treatment with the growth factor. Aged OVX rats treated with a higher dose of bFGF (250 μg/kg) were found to be anemic as their mean hematocrit value was 23.0 ± 2.7 (SD) compared with a mean of 42.6 ± 1.1 in vehicle-treated OVX rats. These adverse side effects of relatively high doses of bFGF in rats make one concerned about the safety of long-term, systemic treatment with the growth factor in humans.

Basic FGF as a potential osteoporosis therapy

Many anti-resorptive agents are currently available for the treatment of osteoporosis, but a bone anabolic agent has yet to be approved by the FDA for use in osteoporotic patients. This therapeutic need generates interest in a strong stimulator of bone formation such as bFGF. Based on findings in the OVX rat, the strengths of the growth factor as a potential osteoporosis therapy are: 1) reversal of cancellous osteopenia after only a relatively short treatment period, 2) strong stimulation of bone formation at pre-existing cancellous and endocortical bone surfaces, 3) formation of osteoid bridges between bone spicules, which should improve trabecular connectivity in the osteopenic skeleton, and 4) formation of osteoid spicules within bone marrow, although it has not been proven conclusively that the origin of these osteoid spicules is bone marrow rather than a pre-existing bone surface.

Despite these strengths, there are many weaknesses that make it difficult to develop bFGF as an osteoporosis therapy. These weaknesses include: 1) the need to administer bFGF intravenously rather than orally, 2) the widespread mitogenic actions of bFGF and its high potential for adverse side effects in organs other than bone, 3) impaired bone mineralization, although this skeletal process returns to normal soon after withdrawal of bFGF treatment, 4) formation of woven bone, which is structurally inferior to lamellar bone, and 5) lack of a bone anabolic effect at skeletal sites with fatty marrow. Nevertheless, bFGF may eventually prove to be useful for treatment of patients with severe osteoporosis who are unresponsive to conventional therapies. In this situation, the proper therapeutic strategy may involve local delivery of bFGF to bone in order to induce mesenchymal stem cells to differentiate into osteoblasts and augment cancellous bone in the osteopenic skeleton.

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References

1. Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G. Structural characterization and biological functions of fibroblast growth factor. Endocrine Rev 1987; 8:95-114.
2. Hauschka PV, Mavrakas AE, Iafrati MD, Doleman SE, Klagsbrun N. Growth factors in bone matrix: Isolation of multiple types by affinity chromatography on heparin-sepharose. J Biol Chem 1986; 261:12665-12674.
3. Globus RK, Plouet J, Gospodarowicz D. Cultured bovine bone cells synthesize fibroblast growth factor and store it in their extracellular matrix. Endocrinology 1989; 124:1539-1547.
4. Globus RK, Patterson-Buckendahl P, Gospodarowicz D. Regulation of bovine bone cell proliferation by fibroblast growth factor and transforming growth factor β. Endocrinology 1988; 123:98-105.
5. Rodan SB, Wesolowski G, Thomas KA, Yoon K, Rodan GA. Effects of acidic and basic fibroblast growth factors on osteoblastic cells. Connect Tissue Res 1989; 20:283-288.
6. Canalis E, Centrella M, McCarthy T. Effects of basic fibroblast growth factor on bone formation *in vitro*. J Clin Invest 1988; 81:1572-1577.
7. Martin I, Muraglia A, Campanile G, Cancedda R, Quarto R. Fibroblast growth factor-2 supports *ex vivo* expansion and maintenance of osteogenic precursors from human bone marrow. Endocrinology 1997; 138:4456-4462.
8. Pri-Chen S, Pitaru S, Lokiec F, Savion N. Basic fibroblast growth factor enhances the growth and expression of the osteogenic phenotype of dexamethasone-treated human bone marrow-derived bone-like cells in culture. Bone 1998; 23:111-117.
9. Hurley MM, Tetradis S, Huang Y-F, Hock J, Kream BE, Raisz LG, Sabbieti MG. Parathyroid hormone regulates the expression of fibroblast growth factor-2 mRNA and fibroblast growth factor receptor mRNA in osteoblastic cells. J Bone Miner Res 1999; 14:776-783.
10. Chikazu D, Hakeda Y, Ogata N, Nemoto K, Itabashi A, Takato T, Kumegawa M, Nakamura K, Kawaguchi H. Fibroblast growth factor-2 directly stimulates mature osteoclast function through activation of FGF receptor 1 and p42/p44 MAP kinase. J Biol Chem 2000; 275: 31444-31450.
11. Noff D, Pitaru S, Savion N. Basic fibroblast growth factor enhances the capacity of bone marrow cells to form bone-like nodules *in vitro*. FEBS Lett 1989; 250:619-621.
12. Pitaru S, Kotev-Emeth S, Noff D, Kaffuler S, Savion N. Effect of basic fibroblast growth factor on the growth and differentiation of adult stromal bone marrow cells: Enhanced development of mineralized bone-like tissue in culture. J Bone Miner Res 1993; 8:919-929.
13. Hanada K, Dennis JE, Caplan AI. Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein-2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. J Bone Miner Res 1997; 12:1606-1614.

14. Scutt A, Bertram P. Basic fibroblast growth factor in the presence of dexamethasone stimulates colony formation, expansion, and osteoblastic differentiation by rat bone marrow stromal cells. Calcif Tissue Int 1999; 64:69-77.
15. Kotev-Emeth S, Savion N, Pri-Chen S, Pitaru S. Effect of maturation on the osteogenic response of cultured stromal bone marrow cells to basic fibroblast growth factor. Bone 2000; 27:777-783.
16. Mayahara H, Ito T, Nagai H, Miyajima H, Tsukuda R, Taketomi S, Mizoguchi J, Kato K. *In vivo* stimulation of endosteal bone formation by basic fibroblast growth factor in rats. Growth Factors 1993; 9:73-80.
17. Nakamura T, Hanada K, Tamura M, Shibanushi T, Nigi H, Tagawa M, Fukumoto S, Matsumoto T. Stimulation of endosteal bone formation by systemic injections of recombinant basic fibroblast growth factor in rats. Endocrinology 1995; 136:1276-1284.
18. Nagai H, Tsukuda R, Mayahara H. Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats. Bone 1995; 16:367-373.
19. Kuroda S, Kasugai S, Oida S, Iimura T, Ohya K, Ohyama T. Anabolic effect of aminoterminally truncated fibroblast growth factor 4 (FGF4) on bone. Bone 1999; 25:431-437.
20. Dunstan CR, Boyce R, Boyce BF, Garrett IR, Izbicka E, Burgess WH, Mundy GR. Systemic administration of acidic fibroblast growth factor (FGF-1) prevents bone loss and increases new bone formation in ovariectomized rats. J Bone Miner Res 1999; 14:953-959.
21. Kato T, Kawaguchi H, Hanada K, Aoyama I, Hiyama Y, Nakamura T, Kuzutani K, Tamura M, Kurokawa T, Nakamura K. Single local injection of recombinant fibroblast growth factor-2 stimulates healing of segmental bone defects in rabbits. J Orthop Res 1998; 16:654-659.
22. Kawaguchi H, Kurokawa T, Hanada K, Hiyama Y, Tamura M, Ogata E, Matsumoto T. Stimulation of fracture repair by recombinant human basic fibroblast growth factor in normal and streptozotocin-diabetic rats. Endocrinology 1994; 135:774-781.
23. Nakamura T, Hara Y, Tagawa M, Tamura M, Yuge T, Fukuda H, Nigi H. Recombinant human basic fibroblast growth factor accelerates fracture healing by enhancing callus remodeling in experimental dog tibial fracture. J Bone Miner Res 1998; 13:942-949.
24. Nakamura K, Kurokawa T, Aoyama I, Hanada K, Tamura M, Kawaguchi H. Stimulation of bone formation by intraosseous injection of basic fibroblast growth factor in ovariectomised rats. Int Orthop 1998; 22:49-54.
25. Kalu DN. The ovariectomized rat model of postmenopausal bone loss. Bone Miner 1991; 15:175-192.
26. Liang H, Pun S, Wronski TJ. Bone anabolic effects of basic fibroblast growth factor in ovariectomized rats. Endocrinology 1999; 140:5780-5788.
27. Froger-Gaillard B, Charrier AM, Thenet S, Ronot X, Adolphe M. Growth-promoting effects of acidic and basic fibroblast growth factor on rabbit articular chondrocytes aging in culture. Exp Cell Res 1989; 183:388-398.
28. Crabb ID, O'Keefe RJ, Puzas JE, Rosier RN. Synergistic effect of transforming growth factor β and fibroblast growth factor on DNA synthesis in chick growth plate chondrocytes. J Bone Miner Res 1990; 5:1105-1112.
29. Amizuka N, Yamada M, Watanabe J-I, Hoshi K, Fukushi M, Oda K, Ikehara Y, Ozawa H. Morphological examination of bone synthesis via direct administration of basic fibroblast growth factor in rat bone marrow. Microsc Res Tech 1998; 41:313-322.
30. Pun S, Dearden RL, Ratkus AM, Liang H, Wronski TJ. Decreased bone anabolic effect of basic fibroblast growth factor at fatty marrow sites in ovariectomized rats. Bone 2001; 28:220-226.
31. Li M, Liang H, Shen Y, Wronski TJ. Parathyroid hormone stimulates cancellous bone formation at skeletal sites regardless of marrow composition in ovariectomized rats. Bone 1999; 24:95-100.
32. van den Bos C, Mosca JD, Winkles J, Kerrigan L, Burgess WH, Marshak DR. Human mesenchymal stem cells respond to fibroblast growth factors. Hum Cell 1997; 10:45-50.
33. Horowitz MC, Raisz LG. Cytokines and prostaglandins in the aging skeleton. In: Rosen CJ, Glowacki J, Bilezikian JP (eds) The Aging Skeleton. Academic Press, San Diego, CA; 1999:195-207.
34. Robey PG, Bianco P. Cellular mechanisms of age-related bone loss. In: Rosen CJ, Glowacki J, Bilezikian JP (eds) The Aging Skeleton. Academic Press, San Diego, CA; 1999:145-157.
35. Pun S, Florio CL, Wronski TJ. Anabolic effects of basic fibroblast growth factor in the tibial diaphysis of ovariectomized rats. Bone 2000; 27:197-202.
36. Mazue G, Newman AJ, Scampini G, Della-Torre P, Hard GC, Iatropoulus MJ, Williams GM, Bagnasco SM. The histopathology of kidney changes in rats and monkeys following intravenous administration of massive doses of FCE 26184, human basic fibroblast growth factor. Toxicol Path 1993; 21:490-501.

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