Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 341 (2006) 989–994
BBRC
www.elsevier.com/locate/ybbrc

Impaired bone anabolic response to parathyroid hormone in Fgf2−/− and Fgf2+/− mice

M.M. Hurley [a,*], Y. Okada [b], L. Xiao [a], Y. Tanaka [b], M. Ito [c], N. Okimoto [b], T. Nakamura [b], C.J. Rosen [d], T. Doetschman [e], J.D. Coffin [f]

[a] University of CT Health Center, Farmington, CT, USA
[b] University of Occupational and Environmental Health, Kitakyushu, Japan
[c] University of Nagasaki, Nagasaki, Japan
[d] Jackson Laboratory, Bangor, ME, USA
[e] University of Cincinnati, Cincinnati, OH, USA
[f] University of Montana, Missoula, MT, USA

Received 9 January 2006
Available online 24 January 2006

Abstract

Since parathyroid hormone (PTH) increased FGF2 mRNA and protein expression in osteoblasts, and serum FGF-2 was increased in osteoporotic patients treated with PTH, we assessed whether the anabolic effect of PTH was impaired in Fgf2−/− mice. Eight-week-old Fgf2+/+ and Fgf2−/− male mice were treated with rhPTH 1–34 (80 μg/kg) for 4 weeks. Micro-CT and histomorphometry demonstrated that PTH significantly increased parameters of bone formation in femurs from Fgf2+/+ mice but the changes were smaller and not significant in Fgf2−/− mice. IGF-1 was significantly reduced in serum from PTH-treated Fgf2−/− mice. DEXA analysis of femurs from Fgf2+/+, Fgf2+/−, and Fgf2−/− mice treated with rhPTH (160 μg/kg) for 10 days showed that PTH significantly increased femoral BMD in Fgf2+/+ by 18%; by only 3% in Fgf2+/− mice and reduced by 3% in Fgf2−/− mice. We conclude that endogenous Fgf2 is important for maximum bone anabolic effect of PTH in mice.

Keywords: Fgf2 null; Fgf2 haploinsufficient; Mice; PTH; Bone formation; DEXA; Micro-CT

Parathyroid hormone (PTH) functions to maintain normal serum calcium and is a major regulator of bone cell function [1]. PTH has been shown to increase bone formation in vivo [1], decrease bone formation in vitro [2], and stimulate bone resorption [3]. Although PTH is a potent bone resorber [4,5], it is currently utilized for the treatment of osteoporosis, since studies have shown that intermittent PTH treatment increases bone mass and strength in osteopenic rats and improves vertebral bone mass in humans [6,7]. Several mechanisms are postulated to mediate the anabolic effects of PTH. They include increased production of local growth factors [8–10], activation of bone lining cells [11], recruitment of osteoblast precursors [12], and reduced apoptosis [13–15]. Although the local factors that mediate the anabolic effects of PTH have not been fully defined, several candidates have been identified. Since PTH treatment increases insulin-like growth factor 1 (IGF-1) and transforming growth factor beta (TGFβ) expression in bones cells [8–10], they are considered as potential mediators of the anabolic effect of PTH in bone. The anabolic effect of PTH appears to be mediated in part by IGF-1 [9] since studies in Igf1 null mice demonstrated impaired anabolic response to PTH [16]. However, the role of other anabolic growth factors such as fibroblast growth factor 2 (FGF-2) [17] in the anabolic response to PTH has not been characterized.

FGF-2 regulates the proliferation and differentiation of osteoblasts in vitro [17] is stored in the extracellular matrix

* Corresponding author. Fax: +1 860 679 1258.
E-mail address: Hurley@nso1.uchc.edu (M.M. Hurley).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.01.044

(ECM) [17], and is synthesized by osteoblast/stromal cells [17]. FGF-2 is an important modulator of cartilage and bone growth and differentiation [17]. Fibroblast growth factor receptors (FGFR) are also important regulators of bone growth and development and are differentially expressed in bone and cartilage [17]. In contrast to the inhibitory effect of chronic treatment [17–19], in animal models, intermittent FGF-2 treatment stimulated bone formation in vitro [20], as well as in vivo [21]. In addition, several investigators reported that FGF-2 enhanced the growth and expression of the osteogenic phenotype of dexamethasone-treated human bone marrow-derived bone-like cells [22,23] resulting in the deposition of greater mineralized matrix than in control cultures [24]. In previous studies, we reported that the expression of FGF-2 mRNA and protein in murine osteoblasts was increased by PTH [25]. In vitro [26] and in vivo [27] studies have shown that FGF-2 also up-regulated the expression of IGF-1 in rodent osteoblasts that is implicated in the anabolic response to PTH [28]. We previously reported that Fgf2 null mice [29] developed decreased bone mass and bone formation rates with age suggesting a role for endogenous FGF-2 in maintaining bone mass [30]. In other studies we reported that Fgf2 null mice form fewer osteoclasts in culture and have an impaired hypercalcemic response to high dose acute PTH in vivo [31]. Since we recently observed that the anabolic response to PTH in humans was associated with increased serum levels of FGF-2 [32], we utilized the Fgf2 null and haploinsufficient mice to assess whether the anabolic response to PTH was impaired in these mutant mice.

Materials and methods

Animals. Development of Fgf2 null mice was previously described [29]. Heterozygote Fgf2+/− mice that are maintained on a Black/Swiss/129 Sv background were bred and housed in the transgenic facility in the Center for Laboratory Animal Care at the University of Connecticut Health Center. Genotyping of mice was performed using primers as previously described [29,30]. Animal protocols were approved by the University of Connecticut Health Center's Animal Care Committee.

In vivo bone formation assay. Eight-week-old male mice or 15-month-old female Fgf2+/+ and Fgf2−/− mice were weighed and injected s.c. once daily with vehicle or PTH 1–34 (80 μg/kg body wt) for 4 weeks. Human recombinant PTH 1–34 was purchased from Bachem, Torrence, CA. Mice were injected with calcein (0.6 mg/kg) on day 18 and day 24 to assess new bone formation. In shorter studies, 8-week-old male Fgf2+/+, Fgf2+/−, and Fgf2−/− mice were weighed and injected s.c. once daily with vehicle or PTH 1–34 (160 μg/kg body wt) for 10 days and femoral bones were harvested for measurement of bone mineral density.

Measurement of bone mineral density. Femoral bones were harvested and stored in 70% ethanol. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DEXA; PIXimus Mouse 11 densitometer (GE Medical System, Madison WI).

Micro-computerized tomography. Quantitative micro-computed tomography (Micro-CT) analysis of the metaphyseal cancellous bones of the distal femurs was performed as previously described [30] with Micro-CT instrumentation (μCT20, Scanco Medical AG, Bassersdorf, Switzerland). Using two-dimensional data from scanned slices, three-dimensional analysis was conducted to calculate morphometric parameters defining trabecular bone mass and micro-architecture including bone volume density (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and the structure model index (SMI), an indicator of plate-like versus rod-like trabecular architecture.

Bone histomorphometry. Trabecular bone architecture of the femoral metaphysis of mice was determined by dynamic histomorphometry. Bones were dehydrated in a progressive ethanol series for histological analysis to determine dynamic parameters of bone formation. The Bioquant program for histomorphometry (Bioquant-True Color Windows, Advanced Image Analysis Software, sVGA Frame Grabber Image Processing Board, Optronics DEI-470 Video Camera and Specialized Computer) uses a light microscope for histomorphometric measurements. Bone histomorphometry [33,34] was performed on femoral bones. For static histomorphometry, bones were isolated fixed in 4% paraformaldehyde at 4 °C, dehydrated in progressive concentrations of ethanol, cleared in xylene, and embedded in paraffin. Five micron sections were cut, deparaffinized, and stained for osteoclast using tartrate-resistant acid phosphatase kit (TRAP) according to the manufacturer's instructions. Statistical analysis was performed using paired Student's *t* test or analysis of variance (ANOVA) to determine differences between groups.

IGF-1 radioimmunoassay. IGF-protein was measured using a commercially available kit (ALPCO, Windham, NH) as previously described [35]. IGFBPs were removed by an acid dissociation step, followed by the addition of a neutralization buffer containing excess recombinant human IGF-II that bound IGFBPs prior to immunoassay with a human anti-IGF-I polyclonal antibody. The assay sensitivity is 0.01 ng/ml IGF-I. There is no cross-reactivity with IGF-II.

Statistic analysis. The significance of difference between two groups was evaluated with an unpaired two-tailed Student's *t* test. For the comparison among multiple groups analysis of variance (ANOVA) was used.

Results

We assessed whether PTH induced trabecular bone formation was impaired in Fgf2−/− mice. We first examined the effect of PTH in growing Fgf2 knockout and WT male mice. Knockout mice were healthy with normal fertility. The mice (8-week-old male mice) were given daily subcutaneous injections of bPTH (1–34) (80 μg/kg) or vehicle for 4 weeks after which bones were harvested for Micro-CT and histological analyses. Although Micro-CT (Fig. 1 and Table 1), analysis of femoral bones from vehicle-treated Fgf2−/− mice revealed a small reduction in trabecular

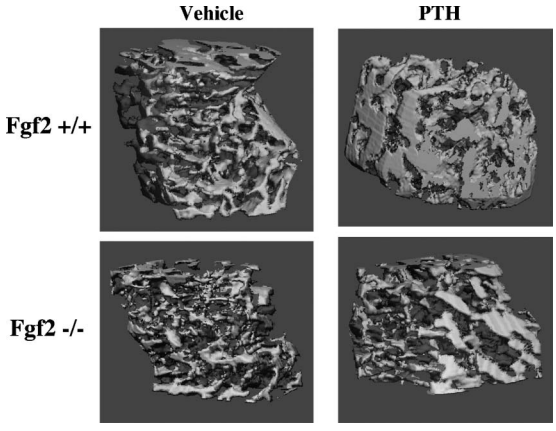

Fig. 1. Micro-CT of the effect of administration of rhPTH (80 μg/kg body wt) on bone formation in Fgf2+/+ and Fgf2−/− mice. Eight-week-old male Fgf2+/+ and Fgf2−/− mice were injected subcutaneously once daily for 4 weeks with either vehicle or RhPTH as described under experimental procedures. Representative Micro-CT 3-dimensional image of femurs harvested from each treatment group and genotype are shown.