Published in final edited form as:
Cell Tissue Res. 2012 March ; 347(3): 545–552. doi:10.1007/s00441-011-1188-4.

Endogenous Tissue Engineering: PTH Therapy for Skeletal Repair

Masahiko Takahata[1], **Hani A. Awad**[1,2,3], **Regis J. O'Keefe**[1,3], **Susan V. Bukata**[1,3], and **Edward M. Schwarz**[1,2,3]

[1]University of Rochester, The Center for Musculoskeletal Research, Rochester, NY

[2]University of Rochester, Department of Biomedical Engineering, Rochester, NY

[3]University of Rochester, Department of Orthopaedics, Rochester, NY

Abstract

Based on its proven anabolic effects on bone in osteoporosis patients, recombinant parathyroid hormone (PTH_{1-34}) has been evaluated as a potential therapy for skeletal repair. Research in animals has investigated the effect of PTH_{1-34} in various skeletal repair models such as fractures, allografting, spinal arthrodesis, and distraction osteogenesis. These studies demonstrated that intermittent PTH_{1-34} treatment enhances and accelerates the skeletal repair process via a number of mechanisms, which includes effects on mesenchymal stem cells (MSC), angiogenesis, chondrogenesis, bone formation and resorption. Furthermore, PTH_{1-34} was demonstrated to enhance bone repair in challenging animal models of aging, inflammatory arthritis and glucocorticoid-induced bone loss. This pre-clinical success has led to off-label clinical use, and a number of case reports documenting PTH_{1-34} treatment of delayed-unions and non-unions have been publish. Moreover, a phase 2 clinical trial of PTH_{1-34} treatment of patient with a radius fracture has now been completed. Although this trial failed to achieve its primary outcome, largely due to effective healing in the placebo group, several secondary outcomes were statistically significant, highlighted several important issues about the appropriate patient population for PTH_{1-34} therapy for skeletal repair. Here we review our current knowledge of the effects of PTH_{1-34} therapy for bone healing, enumerate several critical unresolved issues (e.g. appropriate dosing regimen and indications), and discuss this drug's long term potential as an adjuvant for endogenous tissue engineering.

Keywords

Parathyroid Hormone (PTH); skeletal repair; fracture insufficiency; allograft

Introduction

Parathyroid hormone (PTH) is a major systemic regulator of calcium homeostatsis (Harada and Rodan 2003). It is released from the parathyroid gland in response to hypocalcaemia, and increases serum calcium concentration by promoting osteoclast-mediated bone resorption, calcium reabsorption in the kidneys, and intestinal absorption of calcium through production of the active vitamin D metabolite (1,25-dihydroxy vitamin D). Therefore, continuous exposure to PTH leads to hypercalcaemia and a decrease in bone volume, which is referred to as its catabolic effect. However, it has long been known that intermittent (once

Corresponding Author Edward M. Schwarz, PhD The Center for Musculoskeletal Research University of Rochester – Box 665 601 Elmwood Avenue Rochester, NY 14642 Phone (585) 275-3063; Fax (585) 275-1121 edward_schwarz@urmc.rochester.edu.

daily) exogenous PTH administered leads to an anabolic effect on bone, and the cellular and molecular mechanisms involved have been reviewed (Jilka 2007). As a result of this unique mechanism of action on bone, PTH has been approved as the only anabolic therapy for postmenopausal osteoporosis (Neer et al. 2001). Considering that bone formation is critical for fracture healing, there is also a strong rationale for PTH therapy for skeletal repair.

To date, several forms of PTH, including full length human PTH (PTH_{1-84}) and the N-terminal 1-34 amino acid peptide of human PTH (PTH_{1-34}), have been developed for the treatment of metabolic bone diseases. Among them, PTH_{1-34}, generically referred to as teriparatide, has been extensively studied based on investigations of PTH fragment analogs that assigned the major determinants of receptor-binding affinity, and intracellular signaling through cAMP to this peptide. Although there have not been any head-to-head comparison studies between PTH_{1-34} and full-length native PTH_{1-84}, in terms of their effects on bone, the activity of PTH_{1-34} is considered to be equivalent to that of PTH_{1-84}. Another major consideration that led to the clinical development of PTH_{1-34} vs. PTH_{1-84} is that industrial scale production of the recombinant peptide can be most cost-effectively done by fermentation in *E. coli*, followed by standard protein purification. As such, PTH_{1-34} was the first molecule to be approved for osteoporosis therapy, and is currently used worldwide. Therefore, most of the studies referred to in this review involve data on PTH_{1-34}. Since Andreassen et al first reported the efficacy of intermittent PTH_{1-34} therapy on rat tibial fracture healing in 1999 (Andreassen et al. 1999), a number of studies have shown that this treatment enhances skeletal repair regardless of the skeletal site and mode of bone healing (Bukata and Puzas 2010). These studies suggest that PTH_{1-34} enhances not only bone remodeling, but also osteogenesis and chondrogenesis during skeletal repair, thereby leading to dramatic effects on bone healing. Although local therapy using growth factors such as bone morphogenetic protein (BMP)-2 and BMP-7 is another attractive option as an adjuvant therapy for skeletal repair based on a success of these use in spine fusion surgery (Einhorn 2003), PTH_{1-34} therapy has some advantages over the local growth factor therapy. Local growth factor therapy requires surgical implantation with a carrier material at the lesion site, and is only effective for maximum of a few days. In contrast, PTH_{1-34} therapy can be applied to any type of skeletal disorders including the cases that would be treated non-surgically (i.e. cervical spine fractures), and can be commenced at any time. In addition, PTH_{1-34} therapy can be continued through the entire healing period.

The aim of the present article is to provide an overview of the published studies that demonstrated the efficacy of PTH_{1-34} therapy in a variety of skeletal repair models, and to review our current knowledge of the mechanism of PTH_{1-34} action on bone healing. In addition, we will discuss the potential clinical application of the PTH_{1-34} therapy for skeletal repair, and highlight the remaining issues that must be resolved to achieve clinical success.

Effects of PTH_{1-34} therapy on skeletal repair in animal models

While a variety of skeletal repair models have been investigated to examine the efficacy of systemic intermittent PTH_{1-34} therapy, the vast majority of this research has been on long bone fracture healing. However, pre-clinical studies of PTH_{1-34} therapy on membranous bone formation, critical sized bone defects, spinal arthrodesis, and distraction osteogenesis have also been published. Additionally, efficacy of PTH_{1-34} therapy has been tested in impaired bone healing models such as aging, estrogen deficiency, inflammatory-erosive arthritis and steroid-use, has also been demonstrated as described below.

1. Long bone fracture/osteotomy models

The efficacy of PTH_{1-34} therapy (60 or 200 μg/kg/day) on long bone healing at 20 and 40 days was first reported by Andreassen et al in rat tibial fracture model (Andreassen et al.

1999). The high dose increased the ultimate load and the external callus volume by 75% and 99% respectively on day 20, and by 175% and 72% respectively on day 40, while the low dose had no effects on day 20, but demonstrated 132% and 42% increases respectively 40 day after fracture. These treatments also increased the bone mineral content (BMC) of the callus. One concern with this study, and several others that replicated the results, is that the doses of PTH_{1-34} were suprapharmacological. To address this, Alkhiary et al tested the efficacy of 5 and 30μg/kg/day in a rat closed femur fracture model (Alkhiary et al. 2005). The results showed that high dose PTH_{1-34} markedly increases the torsional strength, stiffness, BMC, bone mineral density (BMD), and cartilage volume, while the low dose had no significant effects by day 21. However, on day 35 both groups showed significant increases in BMC and BMC and total bone volume. While biomechanical properties significantly increase in the high dose group, the low dose treatment was similar to placebo at this time.

Towards clinical translation of this therapy, Manabe et al investigated the effect of PTH_{1-34} on fracture healing in a primate femoral osteotomy model (Manabe et al. 2007). The animals received vehicle, low dose (0.75μg/kg) or high dose (7.5 μg/kg) twice a week, from 3 weeks before surgery to 26 weeks after surgery. All the animals achieved complete union by 26 weeks after surgery; however, PTH_{1-34} treated animals showed superior biomechanical and matrix properties at the fracture site, indicating that PTH_{1-34} accelerated the restoration of structural and mechanical properties in fractured femur.

2. Cortical bone defect (membranous bone formation) models

Different from long bone fracture, which heals by the combination of endochondral bone formation and membranous bone formation, primary mode of bone healing in cortical bone defects is membranous ossification. Thus, to assess PTH_{1-34} effects on membranous bone healing, Komatsu et al conducted a longitudinal analysis of bone regeneration in a rat femoral cortical bone defect model (Komatsu et al. 2009). After making 2-mm circular defects in the femoral diaphyses, rats were treated for 5 weeks with PTH_{1-34} (0, 3, 10, or 30μg /kg/day). Longitudinal microCT analyses demonstrated dose-dependent PTH_{1-34} enhanced cortical bone healing as evidenced by increased BMD. Similarly, results were also obtained in a rat calvarial defect model (5 mm diameter) using 60μg /kg/day PTH_{1-34} (Andreassen and Cacciafesta 2004). Thus, PTH_{1-34} is equally effective for endochondral and intramembranous bone healing.

3. Massive structural allografting for critical sized bone defect model

Perhaps the greatest opportunity for PTH_{1-34} adjuvant therapy is for critical bone defects (>3cm), which remain a major challenge for reconstructive surgery. Although massive structural allografts are the gold standard, their limited osteogenesis and lack of remodeling are directly associated with the 23-43% clinical failure rate (Awad et al. 2007). To address this, Reynolds et al evaluated delayed 40μg/kg/day PTH_{1-34} from 1 to 5 weeks post-op, in a mouse model of 4 mm-long femoral allografting (Reynolds et al. 2010). The results demonstrated significantly increased graft-host integration, as well as callus and trabecular bone formation, as measured by microCT. In similar studies, Jacobson et al demonstrated these PTH_{1-34} using an inorganic scaffold instead of an allograft (Jacobson et al. 2010).

4. Spinal arthrodesis models

Spinal arthrodesis (fusion) surgery is the standard treatment for degenerative and traumatic spine diseases that are associated with severe neck or back pain, and sometimes neurologic problems. The lengthy healing period, and the fact that bony fusion is never achieved in many cases, remains a serious problem. Consequently, PTH_{1-34} has potential to be an efficient adjuvant therapy for spinal fusion, which has been studied in animal models (Abe

et al. 2007; Lawrence et al. 2006; Lehman et al. 2010; O'Loughlin et al. 2009). All these studies reached a similar conclusion that PTH_{1-34} therapy improves fusion success rate as well as accelerates the time to achieve fusion.

5. Distraction osteogenesis models

Distraction osteogenesis is used for leg, mandible, and maxilla lengthening in the treatment of congenital disorders, trauma, and tumors. The main problem with this method is that the time until full recovery may be up to a year, partly because of the time needed for the newly formed bone to consolidate and restore mechanical strength. Consistent with the aforementioned skeletal repair models, PTH_{1-34} therapy was demonstrated to increase mechanical strength and callus mineralization in rat and rabbit limb lengthening models (Aleksyniene et al. 2009; Seebach et al. 2004). Of note is that the significant effects of PTH_{1-34} therapy in the rabbit model was limited to the consolidation period suggesting that only short-course treatment is needed.

6. Impaired bone healing models

Another important potential application for PTH_{1-34} therapy is in the setting of impaired bone healing from senescence, glucocorticoid-use, inflammatory-erosive arthritis and the menopause. To assess the effects of PTH_{1-34} on aged animals, Andreassen et al gave 27-month-old rats high dose (200μg/kg/day) therapy and analyzed tibial fracture healing versus young rats (Andreassen et al. 1999; Andreassen et al. 2001). The results demonstrated that despite marked differences in the healing patterns between young and aged animals, PTH_{1-34} therapy significant enhances callus formation in both groups. Moreover, the drug effects were more robust on callus bone formation in aged rats, while callus volume remained unchanged from day 21 to day 56 in aged rats, indicating that PTH_{1-34} had limited effects on callus remodeling in aged rats.

Glucocorticoids (GC) are known to induce osteopenia via apoptosis of osteoblasts and osteocytes, as well as decrease osteoblast development and bone formation. Therefore, it is considered that chronic GC administration impairs bone healing. Consistently, GC is also detrimental to fracture healing. Bostrom et al tested whether PTH related peptide (PTHrP) treatment enhances fracture healing in rabbits receiving GC and found that only 15% of the osteotomies achieved radiographic union at 6 weeks in the GC-treated group, whereas over 80% of the osteotomies achieved union at 6 weeks in untreated control group (Bostrom et al. 2000). PTHrP improved union rate as well as increase radiographic intensity and mechanical strength of osteotomy site. Although the mechanism by which PTH and PTHrP improves bone healing in GC-treated animals is not completely understood, studies suggest that it suppresses osteoblast apoptosis and osteoblast dysfunction (Jilka et al. 1999; Yao et al. 2008).

Inflammatory-erosive arthropathies such as rheumatoid arthritis (RA), cause focal bone loss that leads to joint damage that is considered to be irreversible. However, the potential to repair these osteolytic lesions with PTH_{1-34} has been demonstrated in a mouse model of RA (Redlich et al. 2004). As many RA patients are postmenopausal women with osteoporosis, data the effects of PTH_{1-34} on focal erosions should be forth coming from clinical studies of on-label use.

Estrogen deficiency is also considered as a risk factor for impaired fracture healing. Kim et al demonstrated that fracture healing in ovariectomized rats was impaired compared to sham operated controls, and that PTH_{1-34} treatment augmented mechanical strength as well as callus bone formation during fracture healing (Kim and Jahng 1999). Nozaka et al demonstrated that this PTH_{1-34} effect could be due to proliferation of osteoprogenitor cells

in the early phase of healing (Nozaka et al. 2008). They also suggested the possibility that PTH_{1-34} increases osteoblastogenesis by decreasing adipogenesis, which is known to occur during estrogen deficiency.

7. Tissue-engineered bone regeneration models with local administration of PTH_{1-34}

Some researchers propose local PTH_{1-34} therapy combined with scaffold materials for bone regeneration. Jung et al demonstrated that an arginine–glycine–aspartic acid modified polyethylene glycol-based matrix containing covalently bound peptides of the PTH_{1-34} enhanced bone regeneration in a canine mandible bone defect simulating an alveolar defect with a circular gap of 1.5mm (Jung et al. 2007). Arrighi et al developed a transglutaminase substrate for binding to fibrin as a delivery and cell-invasion matrix with an intervening plasmin-sensitive link to PTH_{1-34} fragment (Arrighi et al. 2009). They demonstrated that this scaffold material with PTH_{1-34} enhanced the bone regeneration of cylindrical drill defects (8 mm diameter, 13 mm depth) in the femur and humerus of female sheep. Although an explanation as to why local therapy (continuous release of PTH_{1-34} to the lesion) is as effective as intermittent systemic therapy remains elusive, local therapy seems to have advantages over systemic therapy in that it is possible to decrease the cost and avoid painful daily injection.

Mechanism of PTH action on bone repair

Based on the studies of various skeletal repair models, it is evident that PTH_{1-34} has multiple mechanisms of action. Cellular mechanisms involved in PTH_{1-34} stimulated bone repair include proliferation and differentiation of MSC, chondroprogenitors and osteoprogenitors, chondrocyte maturation, production of bone matrix proteins, and osteoclastogenesis.

Mesenchymal cells proliferation and differentiation

One of the most prominent effects of PTH_{1-34} on bone repair is to enhance proliferation and differentiation of MSC in the early phase of bone healing, as has been demonstrated in a rat model (Nakajima et al. 2002). These investigators also demonstrated PTH_{1-34} induced proliferation of chondroprogenitors at the fracture site using the same rat fracture model (Nakazawa et al. 2005). These studies indicate that increases in both osteoprogenitors and chondroprogenitors due to PTH_{1-34} induced proliferation and differentiation contributes to increase callus formation during fracture healing.

At the molecular level, Kaback et al showed that PTH_{1-34} enhances MSC differentiation into osteoblasts via induction of osterix and Runx2 expression *in vitro* (Kaback et al. 2008). Subsequently, they demonstrated that 40μg/kg/day PTH_{1-34} increases *osterix* expression at the fracture site *in vivo*. This treatment also increased expression of osteoblastic marker genes including *type 1 collagen* and *osteocalcin*. Thus, systemic PTH_{1-34} has direct effects on MSC and osteoblast gene expression.

Endochondral bone formation

A hallmark of PTH_{1-34} therapy during fracture healing is increased cartilaginous callus formation. It appears that this occurs through a complicated mechanism that involves chondrogenesis, osteogenesis and delayed chondrocyte hypertrophy and remodeling. Okazaki et al showed that the PTH/PTHrP receptor (PPR) is expressed in MSC, proliferating chondrocytes, and early hypertrophic chondrocytes, but not in hypertrophic chondrocytes in cartilaginous callus during fracture repair (Okazaki et al. 2003). They also showed that PPR is expressed in osteoblasts and osteocytes in bony callus. These results indicate that PTH_{1-34} targets MSC, chondrocytes and osteoblasts during endochondral bone healing. Kakar et al performed a comprehensive investigation of PTH_{1-34} actions on

endochondral bone formation using a mouse closed femoral fracture model (Kakar et al. 2007). Mice were treated with 30 μg/kg/day or placebo for 14 days after fracture, and the results showed that PTH_{1-34} preferentially enhanced chondrogenesis over osteogenesis (3-fold). This enhanced chondrogenesis leads to increased cartilaginous callus formation in the early phase of fracture repair, but thereafter PTH_{1-34} enhances chondrocyte maturation and mineralization in the fracture callus, as evidenced by both an earlier peak in *Sox5* expression and the corresponding earlier induction of *type X collagen*. To understand the counterintuitive actions of PTH_{1-34} on chondrocyte proliferation and maturation, the knowledge about the PTHrP/Indian hedge-hog (IHH) regulatory system in the growth plate during bone development may be helpful. Kakar et al showed that PTH-treated bones initially increase PTHrP expression during the earliest time-points (days 2-3) and concomitantly decrease IHH expression. Since PTHrP primarily maintains chondrocytes in a proliferative phase, PTH treatment presumably increases their proliferation through PTHrP in this phase. Following this phase, PTHrP expression was found to be transiently decreased in PTH treated bones, which allows chondrocytes to initiate maturation. Further molecular analyses revealed that PPR signaling leads to the induction of the wnt/β-catenin pathways. Immunohistochemistry and Western blot analyses showed that nuclear β-catenin levels in the PTH_{1-34} treated bones were transiently decreased on day 5, which corresponds with chondrogenic cell recruitment into the callus. Thereafter, nuclear β-catenin levels increased again to support osteoblast differentiation and activation. These studies highlight the pleiotropic effects of PTH_{1-34} on endochondral bone formation, and the need for additional research to clarify its exact mechanisms of action.

Membranous bone formation

PTH_{1-34} also has important effects on membranous ossification as has been demonstrated in bone defect and bone-chamber models (Andreassen et al. 2004; Komatsu et al. 2009; Skripitz et al. 2000). These studies provided histological evidence that PTH dose dependently induces osteogenesis within defects, especially at endocortical surfaces and in intramedullary space. In the bone chamber studies, it was shown that PTH increased the trabecular bone density, but did not affect the bone-ingrowth distance, indicating that the drug effects are more robust at the endosteal surface and on newly formed trabecular bone, versus periosteal surfaces. It was also shown that systemic and local bone formation markers including serum procollagen type I N-terminal propeptide and mRNA expression of *collagen1a2* and *osteocalcin* were all increased by PTH treatment in their bone defect model, suggesting that PTH treatment increases osteoblastic activity as well as increases proliferation and differentiation of osteoprogenitor cells during membranous bone formation. Modulation of insulin like growth factor I (IGF-I) may also be an important mechanism by which PTH_{1-34} enhances osteoblastic activity. Nakajima et al demonstrated that the increased expression of bone matrix proteins by PTH_{1-34} therapy is accompanied by enhanced expression of IGF-I during the early stage of rat fracture healing (Nakajima et al. 2002). In addition, other studies have demonstrated that the anabolic actions of PTH_{1-34} on bone formation are mediated by IGF-I. Neutralizing antibody against IGF-I prevents PTH induced collagen synthesis, but not its mitogenic effect *in vitro* (Canalis et al. 1989). Moreover, IGF-I knockout mice fail to show increased bone formation in response to PTH (Bikle et al. 2002; Miyakoshi et al. 2001).

Callus remodeling

After the fractured bone is initially stabilized by cartilage and woven bone, the fracture callus is gradually remodeled to lamellar bone, and forms a new cortical shell. Through this osteoclastic and osteoblastic remodeling, the fracture site restores it geometrical and biomechanical environment. Since long-term observation (at least 12 w in rodent models) is required to investigate the effects of PTH_{1-34} on the entire process of fracture healing

including callus remodeling, only a few studies have addressed this question. Komatsubara et al investigated the effects of PTH_{1-34} therapy on the late phase of fracture healing, focusing on callus remodeling and geometrical changes in a rat femoral osteotomy model (Komatsubara et al. 2005). In this study, 10 or 30 μg/kg PTH_{1-34} was administered to rats three times a week, and healing was observed 3, 6, and 12 weeks after surgery. Histomorphometric analysis showed that PTH_{1-34} accelerates remodeling of woven bone to lamellar bone in the callus, as evidenced by increased lamellar bone/callus area in PTH_{1-34} treatment groups at 3 weeks after surgery. Additionally, the percent new cortical shell area was demonstrated to be significantly higher in the PTH_{1-34} treatment groups compared to the control, indicating that PTH_{1-34} enhances cortical shell formation of the fracture callus. Furthermore, they confirmed these finding by the study of monkey femur fracture model (Manabe et al. 2007). Interestingly, total area and percent bone area of fracture callus were significantly lower in both low-dose (0.75μg/kg) and high-dose PTH_{1-34} (7.5μg/kg) treatment groups than in control group at 26 weeks after surgery, while callus porosity decreased dose-dependently following PTH_{1-34} treatment, and the mean degree of callus mineralization was significantly higher in the high-dose group versus placebo control. The ultimate stress and elastic modulus in fractured femur were also significantly higher in the high-dose PTH_{1-34} group versus control. These results suggest that PTH_{1-34} accelerates the remodeling process of fracture callus by shrinking callus size and increasing degree of mineralization of the fracture callus, thereby restoring intrinsic material properties.

The cellular mechanisms of PTH_{1-34} stimulated callus remodeling and maturation are largely explained by its effects on osteoblast and osteoclast function. PTH_{1-34} is known to increase the bone formation rate via direct stimulation of osteoblast function, and inhibition of apoptosis that extends the life of osteoblasts (Jilka et al. 1999). Given that the robust fracture induction of osteoblasts is coupled to their subsequent apoptosis during bone repair (Li et al. 2002), the effect of PTH_{1-34} on osteoblasts is amplified during this process. Also, PTH_{1-34} sustains osteoblast activity during the remodeling period, which enhances callus remodeling. While osteoclasts are also considered to play an important role in callus remodeling, opinions on how they are affected by PTH_{1-34} are divided. Komatsubara et al reported that PTH_{1-34} increases osteoclast density at fracture callus at 3, 6, and 12 weeks after fracture in rat (Komatsubara et al. 2005; Nakajima et al. 2002), while others showed that osteoclast activity does not change with PTH_{1-34} treatment as evidenced by the number of osteoclasts at fracture callus in rat (Alkhiary et al. 2005) and serum TRAP activity in rat fracture model (Nakajima et al. 2002). Only one study reported that PTH_{1-34} down-regulates osteoclast activity, as measured by serum TRAP5b levels (Komatsu et al. 2009). Meanwhile, studies of osteoporosis treatment suggest that anabolic actions of PTH involve increased osteoclast activity. McClung et al reported that PTH_{1-34} therapy significantly increased both bone formation and resorption markers (serum procollagen type I N-terminal propeptide, and urinary N-telopeptide corrected for creatinine) in postmenopausal osteoporosis women (McClung et al. 2005). Chavassieux et al reported that PTH_{1-34} therapy was ineffective against the patient with Pycnodysostosis, which is cathepsin K deficiency (Chavassieux et al. 2008). These controversial results suggest that PTH_{1-34} induces transient effect on osteoclasts behavior during skeletal repair, and the subject warrants further investigation.

Clinical perspectives of PTH therapy for skeletal repair

There are two potential benefits of PTH_{1-34} therapy for bone repair. The first is accelerated healing, which would allow patients to return to normal daily-life and work faster. Additionally, this therapy could reduce the financial costs and chronic morbidity associated with long-term disability. To test this, a randomized, double-blind, placebo-controlled study (phase 2 clinical trial) of PTH_{1-34} was performed with 102 postmenopausal women that sustained a distal radius fracture (Aspenberg et al. 2009; Aspenberg and Johansson 2010).

Unfortunately, this study failed to meet its primary prospective endpoint that 40μg/day teriparatide (double the dose for osteoporosis treatment dose) would shorten the time to cortical bridging. Although this study did show that 20 μg/day PTH_{1-34} accelerated the time to radiographic healing from 9.1 to 7.4 weeks versus placebo ($p = 0.006$), the results of this study raise a question of clinical significance, and how much benefits can be obtained from this treatment when used for fractures in which a normal healing process can be expected.

Another potential benefit of PTH_{1-34} therapy is enhanced healing in the patients who suffer from delayed or non-unions. Since available adjuvant therapies against impaired bone healing are limited, PTH_{1-34} therapy could be a reliable and safe treatment option for these conditions. Clinical case reports of off-label use of PTH_{1-34} to heal delayed-unions support this idea that the drug could be effective as an adjuvant for impaired bone healing. Reynolds et al. reported a case that teriparatide effectively improved the bonny union between the fractured tibial segments that showed no sign of healing 4.5 months after the injury (Reynolds et al. 2009). Another case reported documented the use of teriparatide in a patient with a delayed union of a humeral shaft fracture, which achieved healing with no other intervention to which the outcome may be attributed (Oteo-Alvaro and Moreno 2010). There have also been 3 case reports painful delayed unions of odontoid fractures whose successfully outcome have been attributed to teriparatide therapy (Rubery and Bukata 2010). Although the data from this report are only considered to be anecdotal level IV (case series) evidence, it is important to note that these were elderly patients (over 80 years-old) with concomitant medical problems such as postmenopausal osteoporosis, vitamin D deficiency, and diabetes, while the patients in the first two case reports were healthy adult men who had no specific risk factors for impaired union other than the high energy trauma.

Unresolved issues and future directions

Several critical questions must be addressed toward clinical application of PTH_{1-34}. The first is whether osteoporosis treatment dose (20μg/day) is sufficient for enhancing skeletal repair. Fracture healing studies in animal models used doses of that would represent supraphysiologic doses of teriparatide in humans, and it is unclear whether these same effects would be seen in human at lower doses. Rodent models report the use of 5-200 (typically 40) μg/kg BW/day for anabolic effects on skeletal repair, while the clinical dose for osteoporosis treatment is 20μg per day total (which in a 50 to 80kg person translates to 0.25 to 0.4μg /kg/day). This difference in effective dose is considered to be derived from species difference in terms of response and metabolism of PTH_{1-34}. However, it is possible that more than osteoporosis dose of PTH may be necessary to enhance skeletal repair in humans. However, the effective dose of PTH_{1-34} for skeletal repair should be validated by clinical trials because of difficulty in translating the animal dose to human.

Appropriate dosing regimen and indication of PTH_{1-34} therapy are also critical issues to be addressed. Although several months of PTH_{1-34} treatment for skeletal repair may seem ideal, the potential risk of osteosarcoma needs to be considered with the high cost of the drug and the inconvenience of daily subcutaneous injections. Thus, elucidating the critical period in which PTH_{1-34} therapy mediates its significant effect on healing is critical. In the case of trauma, it may be difficult to commence PTH_{1-34} treatment immediately after injury, and it is unknown whether delayed PTH treatment is as effective as immediate treatment. Furthermore, there is no clinical information on how late PTH_{1-34} therapy can be initiated to achieve effective results on delayed union or non-union. PTH_{1-34} may be effective for delayed-union as long as bone formation is still in active at fracture site, but not for non-union, in which bone formation is not active any more. As shown in the study of distraction osteogenesis that showed PTH_{1-34} therapy during the consolidation period is sufficient to show maximum effect on its healing (Aleksyniene et al. 2009), there may be an ideal

treatment window that exert maximum benefit according to the type of skeletal repair. Thus, prospective clinical studies are needed to determine the value of PTH_{1-34} therapy, which holds great promise for skeletal repair.

Acknowledgments

This work was funded in part by grants from the Aircast Foundation and grants from the National Institutes of Health (AR056696, AR054041, DE019902).

References

Abe Y, Takahata M, Ito M, Irie K, Abumi K, Minami A. Enhancement of graft bone healing by intermittent administration of human parathyroid hormone (1-34) in a rat spinal arthrodesis model. Bone. 2007; 41:775–785. [PubMed: 17707711]

Aleksyniene R, Thomsen JS, Eckardt H, Bundgaard KG, Lind M, Hvid I. Parathyroid hormone PTH(1-34) increases the volume, mineral content, and mechanical properties of regenerated mineralizing tissue after distraction osteogenesis in rabbits. Acta Orthop. 2009; 80:716–723. [PubMed: 19995322]

Alkhiary YM, Gerstenfeld LC, Krall E, Westmore M, Sato M, Mitlak BH, Einhorn TA. Enhancement of experimental fracture-healing by systemic administration of recombinant human parathyroid hormone (PTH 1-34). J Bone Joint Surg Am. 2005; 87:731–741. [PubMed: 15805200]

Andreassen TT, Cacciafesta V. Intermittent parathyroid hormone treatment enhances guided bone regeneration in rat calvarial bone defects. J Craniofac Surg. 2004; 15:424–427. discussion 428-429. [PubMed: 15111801]

Andreassen TT, Ejersted C, Oxlund H. Intermittent parathyroid hormone (1-34) treatment increases callus formation and mechanical strength of healing rat fractures. J Bone Miner Res. 1999; 14:960–968. [PubMed: 10352105]

Andreassen TT, Fledelius C, Ejersted C, Oxlund H. Increases in callus formation and mechanical strength of healing fractures in old rats treated with parathyroid hormone. Acta Orthop Scand. 2001; 72:304–307. [PubMed: 11480610]

Andreassen TT, Willick GE, Morley P, Whitfield JF. Treatment with parathyroid hormone hPTH(1-34), hPTH(1-31), and monocyclic hPTH(1-31) enhances fracture strength and callus amount after withdrawal fracture strength and callus mechanical quality continue to increase. Calcif Tissue Int. 2004; 74:351–356. [PubMed: 15255072]

Arrighi I, Mark S, Alvisi M, von Rechenberg B, Hubbell JA, Schense JC. Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. Biomaterials. 2009; 30:1763–1771. [PubMed: 19124152]

Aspenberg P, Genant HK, Johansson T, Nino AJ, See K, Krohn K, Garcia-Hernandez PA, Recknor CP, Einhorn TA, Dalsky GP, Mitlak BH, Fierlinger A, Lakshmanan MC. Teriparatide for Acceleration of Fracture Repair in Humans: A Prospective, Randomized, Double-blind Study of 102 Postmenopausal Women with Distal Radial Fractures. J Bone Miner Res. 2009

Aspenberg P, Johansson T. Teriparatide improves early callus formation in distal radial fractures. Acta Orthop. 2010; 81:234–236. [PubMed: 20367417]

Awad HA, Zhang X, Reynolds DG, Guldberg RE, O'Keefe RJ, Schwarz EM. Recent advances in gene delivery for structural bone allografts. Tissue Eng. 2007; 13:1973–1985. [PubMed: 17518728]

Bikle DD, Sakata T, Leary C, Elalieh H, Ginzinger D, Rosen CJ, Beamer W, Majumdar S, Halloran BP. Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. J Bone Miner Res. 2002; 17:1570–1578. [PubMed: 12211426]

Bostrom MP, Gamradt SC, Asnis P, Vickery BH, Hill E, Avnur Z, Waters RV. Parathyroid hormone-related protein analog RS-66271 is an effective therapy for impaired bone healing in rabbits on corticosteroid therapy. Bone. 2000; 26:437–442. [PubMed: 10773582]

Bukata SV, Puzas JE. Orthopedic uses of teriparatide. Curr Osteoporos Rep. 2010; 8:28–33. [PubMed: 20425088]

Canalis E, Centrella M, Burch W, McCarthy TL. Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone cultures. J Clin Invest. 1989; 83:60–65. [PubMed: 2910920]

Chavassieux P, Karsdal M Asser, Segovia-Silvestre T, Neutzsky-Wulff AV, Chapurlat R, Boivin G, Delmas PD. Mechanisms of the anabolic effects of teriparatide on bone: insight from the treatment of a patient with pycnodysostosis. J Bone Miner Res. 2008; 23:1076–1083. [PubMed: 18302508]

Einhorn TA. Clinical applications of recombinant human BMPs: early experience and future development. J Bone Joint Surg Am. 2003; 85-A(Suppl 3):82–88. [PubMed: 12925614]

Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature. 2003; 423:349–355. [PubMed: 12748654]

Jacobson JA, Yanoso-Scholl L, Reynolds DG, Dadali T, Bradica G, Bukata S, Puzas EJ, Zuscik MJ, Rosier R, O'Keefe RJ, Schwarz EM, Awad HA. Teriparatide therapy and Beta-tricalcium phosphate enhance scaffold reconstruction of mouse femoral defects. Tissue Eng Part A. 2010; 17:389–398. [PubMed: 20807012]

Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. Bone. 2007; 40:1434–1446. [PubMed: 17517365]

Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. J Clin Invest. 1999; 104:439–446. [PubMed: 10449436]

Jung RE, Cochran DL, Domken O, Seibl R, Jones AA, Buser D, Hammerle CH. The effect of matrix bound parathyroid hormone on bone regeneration. Clin Oral Implants Res. 2007; 18:319–325. [PubMed: 17386063]

Kaback LA, Soung do Y, Naik A, Geneau G, Schwarz EM, Rosier RN, O'Keefe RJ, Drissi H. Teriparatide (1-34 human PTH) regulation of osterix during fracture repair. J Cell Biochem. 2008; 105:219–226. [PubMed: 18494002]

Kakar S, Einhorn TA, Vora S, Miara LJ, Hon G, Wigner NA, Toben D, Jacobsen KA, Al-Sebaei MO, Song M, Trackman PC, Morgan EF, Gerstenfeld LC, Barnes GL. Enhanced chondrogenesis and Wnt signaling in PTH-treated fractures. J Bone Miner Res. 2007; 22:1903–1912. [PubMed: 17680724]

Kim HW, Jahng JS. Effect of intermittent administration of parathyroid hormone on fracture healing in ovariectomized rats. Iowa Orthop J. 1999; 19:71–77. [PubMed: 10847519]

Komatsu DE, Brune KA, Liu H, Schmidt AL, Han B, Zeng QQ, Yang X, Nunes JS, Lu Y, Geiser AG, Ma YL, Wolos JA, Westmore MS, Sato M. Longitudinal in vivo analysis of the region-specific efficacy of parathyroid hormone in a rat cortical defect model. Endocrinology. 2009; 150:1570–1579. [PubMed: 19022894]

Komatsubara S, Mori S, Mashiba T, Nonaka K, Seki A, Akiyama T, Miyamoto K, Cao Y, Manabe T, Norimatsu H. Human parathyroid hormone (1-34) accelerates the fracture healing process of woven to lamellar bone replacement and new cortical shell formation in rat femora. Bone. 2005; 36:678–687. [PubMed: 15781006]

Lawrence JP, Ennis F, White AP, Magit D, Polzhofer G, Drespe I, Troiano NW, Grauer JN. Effect of daily parathyroid hormone (1-34) on lumbar fusion in a rat model. Spine J. 2006; 6:385–390. [PubMed: 16825043]

Lehman RA Jr. Dmitriev AE, Cardoso MJ, Helgeson MD, Christensen CL, Raymond JW, Eckel TT, Riew KD. Effect of teriparatide [rhPTH(1,34)] and calcitonin on intertransverse process fusion in a rabbit model. Spine (Phila Pa. 2010; 35:146–152. 1976.

Li G, White G, Connolly C, Marsh D. Cell proliferation and apoptosis during fracture healing. J Bone Miner Res. 2002; 17:791–799. [PubMed: 12009009]

Manabe T, Mori S, Mashiba T, Kaji Y, Iwata K, Komatsubara S, Seki A, Sun YX, Yamamoto T. Human parathyroid hormone (1-34) accelerates natural fracture healing process in the femoral osteotomy model of cynomolgus monkeys. Bone. 2007; 40:1475–1482. [PubMed: 17369013]

McClung MR, San Martin J, Miller PD, Civitelli R, Bandeira F, Omizo M, Donley DW, Dalsky GP, Eriksen EF. Opposite bone remodeling effects of teriparatide and alendronate in increasing bone mass. Arch Intern Med. 2005; 165:1762–1768. [PubMed: 16087825]

Miyakoshi N, Kasukawa Y, Linkhart TA, Baylink DJ, Mohan S. Evidence that anabolic effects of PTH on bone require IGF-I in growing mice. Endocrinology. 2001; 142:4349–4356. [PubMed: 11564695]

Nakajima A, Shimoji N, Shiomi K, Shimizu S, Moriya H, Einhorn TA, Yamazaki M. Mechanisms for the enhancement of fracture healing in rats treated with intermittent low-dose human parathyroid hormone (1-34). J Bone Miner Res. 2002; 17:2038–2047. [PubMed: 12412812]

Nakazawa T, Nakajima A, Shiomi K, Moriya H, Einhorn TA, Yamazaki M. Effects of low-dose, intermittent treatment with recombinant human parathyroid hormone (1-34) on chondrogenesis in a model of experimental fracture healing. Bone. 2005; 37:711–719. [PubMed: 16143574]

Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med. 2001; 344:1434–1441. [PubMed: 11346808]

Nozaka K, Miyakoshi N, Kasukawa Y, Maekawa S, Noguchi H, Shimada Y. Intermittent administration of human parathyroid hormone enhances bone formation and union at the site of cancellous bone osteotomy in normal and ovariectomized rats. Bone. 2008; 42:90–97. [PubMed: 17997377]

O'Loughlin PF, Cunningham ME, Bukata SV, Tomin E, Poynton AR, Doty SB, Sama AA, Lane JM. Parathyroid hormone (1-34) augments spinal fusion, fusion mass volume, and fusion mass quality in a rabbit spinal fusion model. Spine (Phila Pa. 2009; 34:121–130. 1976.

Okazaki K, Jingushi S, Ikenoue T, Urabe K, Sakai H, Iwamoto Y. Expression of parathyroid hormone-related peptide and insulin-like growth factor I during rat fracture healing. J Orthop Res. 2003; 21:511–520. [PubMed: 12706025]

Oteo-Alvaro A, Moreno E. Atrophic humeral shaft nonunion treated with teriparatide (rhe PTH 1-34): a case report. J Shoulder Elbow Surg. 2010; 19:e22–28. [PubMed: 20846618]

Redlich K, Gortz B, Hayer S, Zwerina J, Doerr N, Kostenuik P, Bergmeister H, Kollias G, Steiner G, Smolen JS, Schett G. Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. Am J Pathol. 2004; 164:543–555. [PubMed: 14742260]

Reynolds DG, Shaikh S, Papuga MO, Lerner AL, O'Keefe RJ, Schwarz EM, Awad HA. muCT-based measurement of cortical bone graft-to-host union. J Bone Miner Res. 2009; 24:899–907. [PubMed: 19063685]

Reynolds DG, Takahata M, Lerner AL, O'Keefe RJ, Schwarz EM, Awad HA. Teriparatide therapy enhances devitalized femoral allograft osseointegration and biomechanics in a murine model. Bone. 2010

Rubery PT, Bukata SV. Teriparatide may accelerate healing in delayed unions of type III odontoid fractures: a report of 3 cases. J Spinal Disord Tech. 2010; 23:151–155. [PubMed: 20051918]

Seebach C, Skripitz R, Andreassen TT, Aspenberg P. Intermittent parathyroid hormone (1-34) enhances mechanical strength and density of new bone after distraction osteogenesis in rats. J Orthop Res. 2004; 22:472–478. [PubMed: 15099623]

Skripitz R, Andreassen TT, Aspenberg P. Parathyroid hormone (1-34) increases the density of rat cancellous bone in a bone chamber. A dose-response study. J Bone Joint Surg Br. 2000; 82:138–141. [PubMed: 10697330]

Yao W, Cheng Z, Pham A, Busse C, Zimmermann EA, Ritchie RO, Lane NE. Glucocorticoid-induced bone loss in mice can be reversed by the actions of parathyroid hormone and risedronate on different pathways for bone formation and mineralization. Arthritis Rheum. 2008; 58:3485–3497. [PubMed: 18975341]