

## Effect of teriparatide on bone regenerate after distraction osteogenesis

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### Abstract

**Objective:** To determine the effect of teriparatide on new bone formation in a rat model of distraction osteogenesis.

**Methods:** The experimental study was conducted at the Aga Khan University Hospital, Karachi, in November-December 2010, and comprised male Sprague-Dawley rats weighing 250gm each who were allocated to two treatment groups, teriparatide and saline, both given subcutaneously for 7 weeks. Femoral distraction was done for 3 weeks at the rate of 0.4mm/day, followed by a further 4 weeks for consolidation. New bone formation was assessed using X-ray scoring system, bone densitometry and histology.

**Results:** The 12 rats in the study were divided into two groups of 6(50%) each. All rats in the teriparatide group showed new bone formation whereas bone formation was present only in 2(33.3%) rats in the saline group. Bone densitometry showed that area (size) of the new bone formed adjacent to the margins of the osteotomy site as well as the total bone mineral content of the new bone was significantly higher ( $p<0.05$ ) in the teriparatide group. Histological analysis showed larger but statistically insignificant ( $p>0.05$ ) area of woven and trabecular new bone in the teriparatide group.

**Conclusion:** The results suggested a promising role of parathyroid analogue therapy in distraction osteogenesis for promoting bone formation and consolidation. This may have strong clinical implications in cases of limb lengthening and bone transport.

**Keywords:** Teriparatide, Parathyroid hormone, Distraction osteogenesis, Limb lengthening, Bone formation, Fracture healing. (JPMA 64: S-3 (Suppl. 2); 2014)

### Introduction

Reconstruction of skeletal defects and limb lengthening by distraction osteogenesis is a master technique providing solution to many complex orthopaedic problems. Woven bone formed initially between the distracted ends later consolidates into mature bone. Consolidation time is usually three times longer than the distraction time or sometimes even longer. This may require the patient to wear the external fixator for long duration, sometimes even up to a year. A prolonged fixator time may produce non-compliance to this treatment method and predisposes to pin tract infection and wire loosening. Efforts to minimise consolidation time represent an active area of interest for research. Both pharmacological and mechanical solutions are being studied.

Teriparatide is the synthetic form of naturally occurring human parathyroid hormone (PTH), amino acid sequence 1 through 34, of the complete molecule (containing 84 amino acids). It is a medication to treat osteoporosis and works in a different way than other drugs for osteoporosis. Teriparatide causes new bone formation

and has been approved by US Food and Drug Administration (FDA) to treat osteoporosis in postmenopausal women and in men with hypogonadal or idiopathic osteoporosis who are at high risk for fracture. Teriparatide has been shown to increase the density and mechanical strength of the callus after fracture healing in experimental models.<sup>1,2</sup>

To date, only few studies have addressed the role of teriparatide in distraction osteogenesis. High doses of 60 $\mu$ g/kg and 25 $\mu$ g/kg have demonstrated to cause an anabolic effect on new bone formation.<sup>3,4</sup> The role of teriparatide in a low dose on bone formation in distraction osteogenesis has not been observed previously. The current study was planned to see the effects of a much lower dose of teriparatide (10  $\mu$ g/kg) to investigate its role in new bone formation in a rat model of distraction osteogenesis.

### Subjects and Methods

The experimental study was conducted at the Aga Khan University Hospital (AKUH), Karachi, in November-December 2010, and comprised male Sprague-Dawley rats weighing 250gm each. The rats were obtained from the institutional animal breeding facility and housed in the cages containing 3 rats each. They were fed standard rat chow and water ad libitum. The animal rooms had a 12-hour light-dark cycle, and

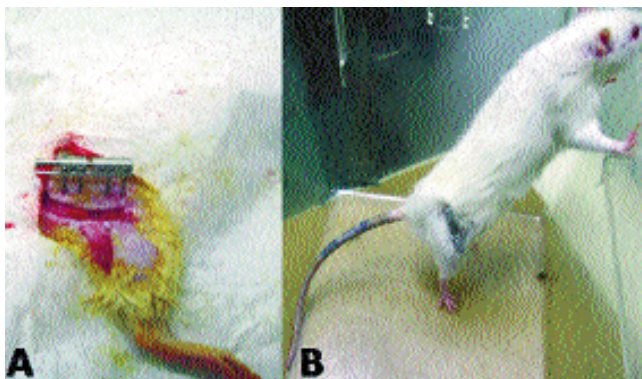
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controlled temperature and humidity.

Uniplanar external fixators with threaded rods for distraction, drill, drill guide (jig) and fixator pins were obtained from Karolinska University Hospital, Stockholm, Sweden.

All experiments were performed in accordance with international guidelines for the care and use of experimental animals, and after approval from the institutional ethics committee. Rats were anaesthetised using intraperitoneal injection of a combination of pentobarbital (500mg/10ml), ketamine (1: 1.333; Gedeon Richter) and valium (10mg/2ml, 1:10; Roche) after short exposure to ether in a glass jar. Skin of the lower abdomen and right lower limb was shaved, scrubbed with Hibiscrub™ and painted with Pyodine™. After sterile draping, incision was made over the right femur. Vastus lateralis muscle was reflected from lateral inter-muscular septum anteriorly to expose shaft of the femur. Using drill guide, four holes were drilled through lateral cortex, and Schanz screws were placed. The Schanz screw ends were passed through stab incisions on the skin and attached to the external fixator. Mid-shaft corticotomy was performed using multiple drill holes and a curved hand-saw. Using the fixator's threaded rod mechanism, both bone ends were brought in close apposition. The vastus muscle was replaced over the bone, anchored to the septum with 4/0 vicryl, and skin was sutured with 4/0 silk (Figure-1A). Postoperatively, all animals were given antibiotic Cefazolin (Inj. Kefzol, 100mg/kg, AGP Pharma) twice a day and analgesia with Paracetamol (60mg/kg, Glaxo Smith Kline) and Ibuprofen (80mg/kg, Abbot) mixed in drinking water for the first three days. Rats were allowed full activity within their cages (Figure-1B). After a latent period of 7 days, distraction was started at 0.4mm a day



**Figure 1 (A,B):** Photographs showing (A) application of the external fixator with two pins proximal and two pins distal to the femoral osteotomy just before skin closure, and (B) rat with full weight bearing on the operated leg.

(0.2mm twice a day), and continued for 3 weeks. A further 4 weeks were given for consolidation of the regenerated bone; rats were then euthanised with ketamine overdose and both lower limbs were dissected.

Rats were allocated to two treatment groups, teriparatide and saline. Teriparatide was obtained in the form of pre-filled pens (Forteo™; Eli Lilly Pakistan Pvt Ltd). Drug was diluted in 720µl sterile water to a concentration of 20µg/ml, and administered thrice a week by subcutaneous injection at a dose of 10µg/kg body weight<sup>5</sup> per dose for 7 weeks.

Samples were kept in 4% Zamboni's fixative for 24 hours, and then demineralised in 20% ethylenediaminetetraacetic acid (EDTA) (pH 7.3).<sup>6</sup>

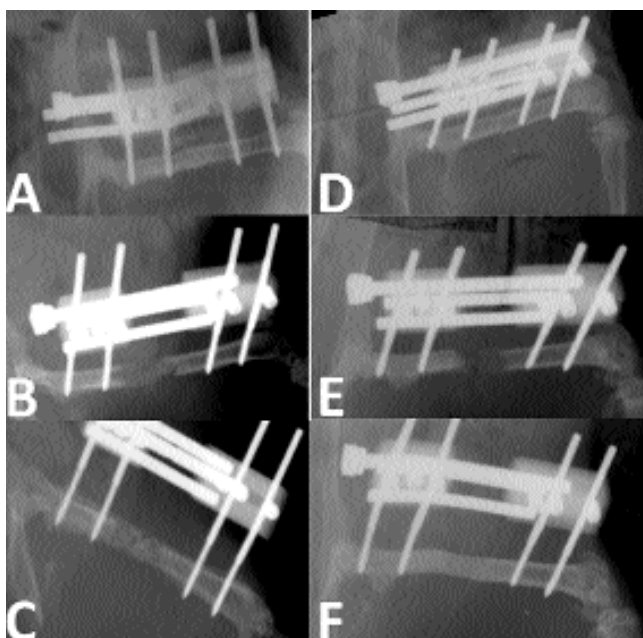
X-rays were performed at 3 time points; immediately after surgery, at the end of distraction, and at the end of another 4 weeks of consolidation phase. X-rays were scored for the quality of regenerated bone according to the method of Lane and Sandhu.<sup>7</sup> Three blinded observers performed scoring of scanned X-ray images independently, and the mean was used for statistical comparisons using Mann-Whitney test.

Bone densitometry (DEXA) scan was performed after the limbs were dissected. The corticotomy gap was analysed for bone mineral content, density and new bone area. Separate analysis was performed at centre of bone regenerate and at two ends adjacent to the original bone ends. The data obtained included ossified area (cm<sup>2</sup>), bone mineral content (mg) and bone mineral density (mg/cm<sup>2</sup>). Data was described as mean  $\pm$  standard deviation (SD), and group comparisons were made using student's t-test. Histological analysis entailed image analysis of area fraction per high power field (HPF) for intramembranous ossification, enchondral ossification, woven bone and mature trabecular bone. A Nikon light/fluorescence microscope (Eclipse E800; Nikon Inc, Yokohama, Japan) with a 20X objective and a DXM-1200 digital camera with the supplied ACT-1 software was used. Digital image analysis was performed using Image Tools public domain software (Version 3.0, University of Texas, San Antonio, USA). The observer demarcated on computer the areas on each image according to the above four tissue types, and the area was measured in square microns. The whole tissue sections were analysed, and mean area per HPF was calculated. Area fraction for each tissue type was then calculated. Data was described as mean  $\pm$  standard error of mean (SEM), and group comparisons were made using Mann-Whitney non-parametric test.

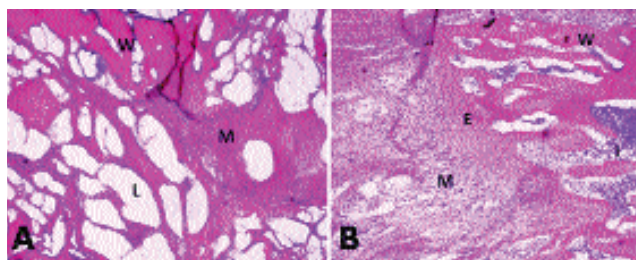
**Results**

The 12 rats in the study were divided into two groups of 6(50%) each. All rats in the teriparatide group showed new bone formation whereas bone formation was present only in 2(33.3%) rats in the saline group. All rats started ambulation after recovery from anaesthesia, and exhibited full weight bearing within 1-2 days (Figure-1B).

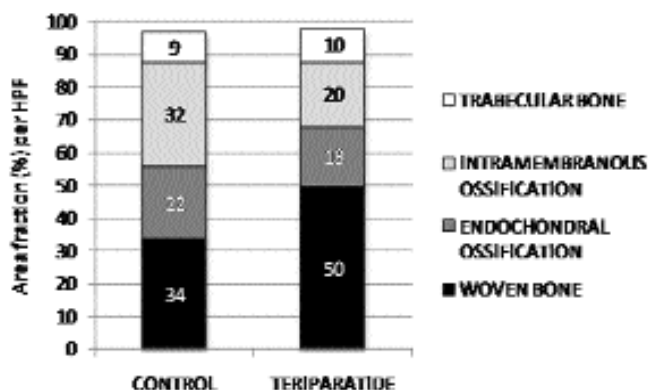
In 2(33.3%) rats in the saline group, new bone formation was observed, while in all the 6(100%) rats in teriparatide group there was new bone formation Figure-2). Mean



**Figure 2 (A-F):** Radiographs showing the femur after osteotomy (A,D), at completion of distraction (B,E) and consolidation (C,F) belonging to control (A-C) and teriparatide (D-F) groups.

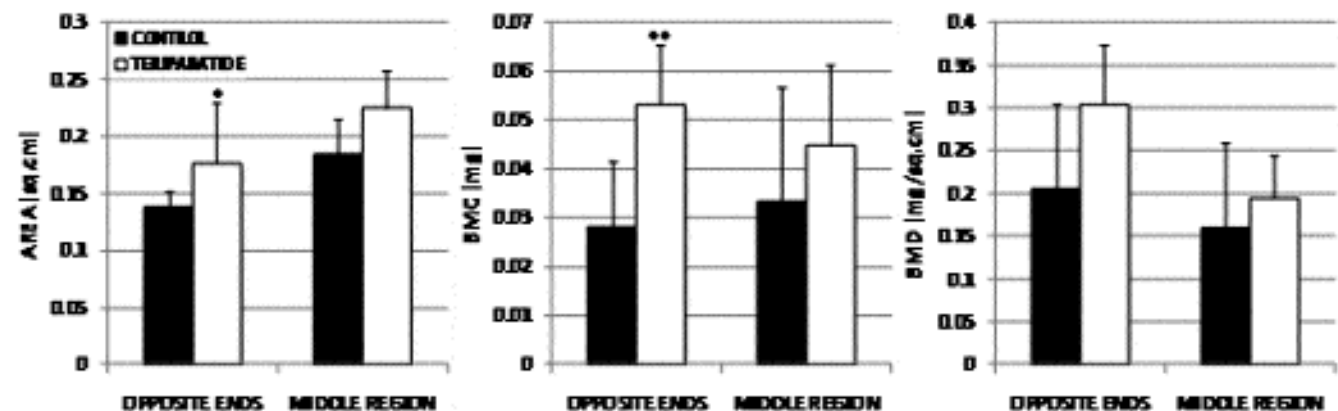


**Figure 4(A,B):** Photomicrograph of a sample of regenerate tissue from a rat in the control group (A) showing mesenchymal tissue [M] and some woven bone [W] amidst large lacunar spaces [L], and from the teriparatide group showing intramembranous [M] and enchondral [E] ossification, woven bone [W] and inflammatory infiltrate [I].



**Figure 5:** Chart showing results of histological analysis. The teriparatide group has higher mean area fraction for woven bone and lower intramembranous ossification as compared to the saline group, but the difference was not statistically significant.

radiological score was  $2.2 \pm 0.5$  for the saline group compared to  $3.2 \pm 0.3$  for teriparatide group, but the difference was not statistically significant ( $p > 0.05$ ).



**Figure 3:** Charts showing results of bone mineral analysis of the regenerate bone region in control and teriparatide groups. The bone area as well as mineral content of the opposite ends, i.e. the peripheral regions of the distraction region, were significantly higher in teriparatide compared to saline group. \* $p < 0.05$ , \*\* $p < 0.01$ .

The average area (size) of new bone formed adjacent to margins of the osteotomy site was 28% greater in the teriparatide group compared to the saline group ( $p=0.025$ ) (Figure-3). Moreover, the average mineral content of the same area was 88% higher in the teriparatide group ( $p=0.007$ ). DEXA was also 48% higher in the teriparatide group ( $p=0.076$ ).

On histological analysis of the area fraction among the different tissue types, the teriparatide group exhibited a greater proportion of woven bone (+47%) and lesser of intra-membranous ossification (-38%) ( $p>0.05$ ). When comparing the total area ( $\mu\text{m}^2/\text{HPF}$ ), the teriparatide group appeared to have larger area of woven bone (+153%) and trabecular new bone (+225%) ( $p>0.05$ ) (Figures-4,5).

## Discussion

This pilot study shows that treatment with parathyroid hormone analogue teriparatide at a dose of  $10\mu\text{g}/\text{kg}$  thrice weekly results in significant increase in size of new bone formation and total mineral content in rat model of distraction osteogenesis. Our results confirm the known anabolic effects of teriparatide therapy on new bone formation in distraction osteogenesis.

To our knowledge only few experimental studies have investigated the effects of teriparatide on bone regeneration in distraction osteogenesis.<sup>3,4</sup> The studies used much higher doses of  $60\mu\text{g}/\text{kg}$  and  $25\mu\text{g}/\text{kg}$  respectively. We tested a much smaller dose ( $10\mu\text{g}/\text{kg}$ ), which also showed beneficial effect of teriparatide in distraction osteogenesis. This dose is closer to the human dose of  $0.3\mu\text{g}/\text{kg}/\text{day}$ . One study<sup>5</sup> showed that teriparatide at a dose of  $10\mu\text{g}/\text{kg}$  administered to rats with fractured femora resulted in increased bone mineral density (BMD) compared to vehicle treated controls. We think that using low-dose teriparatide is cost-effective, ensures better patient compliance and reduces undesirable side effects etc.

A study<sup>3</sup> showed that the total regenerate callus volume was 58% higher and the bone mineral content (BMC) and BMD were 24% higher in teriparatide-treated rats than in controls. Another study<sup>4</sup> on rabbits showed a 60% higher BMC in teriparatide-treated group than in controls. Our study showed that average area (volume) of new bone formed adjacent to the margins of the osteotomy site was 28% greater in the teriparatide group compared to the saline group. The average BMC of the same area was 88% higher in the teriparatide group ( $p<0.01$ ) and BMD was 48% higher in the teriparatide group, though not significant. Our results are quite comparable to the other two studies despite

using a much lower dosage schedule. In addition, our findings confirm the results of other studies showing anabolic effects on newly forming bone in fracture healing, bone chamber studies, or titanium implant anchorage in low-density trabecular bone.<sup>1,2,8-10</sup> The anabolic effect of teriparatide is also visible on the intact contralateral tibia as was shown in one study.<sup>3</sup> We did not check this outcome parameter.

One study<sup>11</sup> suggested that bone formation in the distracted gap was most active between 18 and 24 days after the operation. It suggested that interventions to enhance bone formation should focus on this time period. Osteoblasts are the target cells for teriparatide; their presence in the immature tissue containing pluripotent cells during distraction phase is not known. A study<sup>4</sup> showed that giving teriparatide during both distraction and consolidation phases is not superior to giving it during consolidation phase only. Giving the drug throughout the treatment period may not be very cost-effective. As such, the effect of low-dose teriparatide in different phases of distraction and consolidation needs to be studied further.

## Conclusion

The results suggest a positive role of teriparatide on new bone formation during distraction and consolidation phases; a similar anabolic response can be expected in humans undergoing distraction osteogenesis. There is sufficient evidence to warrant clinical studies on the possible utility of teriparatide for improving bone regenerate formation in distraction osteogenesis. Trials may employ low doses, which are likely to cause fewer untoward effects and result in better patient compliance in addition to being more cost-effective than the doses conventionally used for osteoporosis.

## Acknowledgements

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## References

1. 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;14:960-8.
2. Holzer G, Majeska RJ, Lundy MW, Hartke JR, Einhorn TA. Parathyroid hormone enhances fracture healing. A preliminary report. *Clin Orthop Relat Res* 1999;366:258-63.
3. 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;22:472-8.
4. Alekysniene 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;80:716-23.
5. Komatsubara S, Mori S, Mashiba T, Nonaka K, Seki A, Akiyama T, et al. 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;36:678-87.
  6. Bjurholm A, Kreicbergs A, Schultzberg M. Fixation and demineralization of bone tissue for immunohistochemical staining of neuropeptides. *Calcif Tissue Int* 1989;45:227-31.
  7. Lane JM, Sandhu HS. Current approaches to experimental bone grafting. *Orthop Clin North Am*, 1987; 18: pp 213-25.
  8. Skripitz R, Aspenberg P. Implant fixation enhanced by intermittent treatment with parathyroid hormone. *J Bone Joint Surg Br* 2001;83:437-40.
  9. 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; 82:138-41.
  10. Gabet Y, Muller R, Levy J, Dimarchi R, Chorev M, Bab I. Parathyroid hormone 1-34 enhances titanium implant anchorage in low-density trabecular bone: a correlative micro-computed tomographic and biomechanical analysis. *Bone* 2006;39:276-82.
  11. Richards M, Goulet JA, Schaffler MB, Goldstein SA. Temporal and spatial characterization of regenerate bone in the lengthened rabbit tibia. *J Bone Miner Res* 1999;14:1978-86.
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