

## BRIEF REPORT

## Recombinant Osteoprotegerin for Juvenile Paget's Disease

Tim Cundy, M.D., James Davidson, Ph.D., Michael D. Rutland, M.B., B.S., Carolyn Stewart, M.M.Sc., and Alex M. DePaoli, M.D.

## SUMMARY

Juvenile Paget's disease, a genetic bone disease characterized by accelerated bone turnover, results from inactivating mutations in the gene encoding osteoprotegerin — a key regulator of osteoclastogenesis. The effects of recombinant osteoprotegerin were investigated in two adult siblings with juvenile Paget's disease. Bone resorption (assessed by N-telopeptide excretion) was suppressed by once-weekly subcutaneous doses of 0.3 to 0.4 mg per kilogram of body weight. After 15 months of treatment, radial bone mass increased in one patient by 9 percent and in the other by 30 percent, skeletal bisphosphonate retention decreased by 37 percent and 55 percent, respectively, and there was radiographic improvement. Apart from mild hypocalcemia and hypophosphatemia, no apparent adverse events occurred.

From the Department of Medicine, Faculty of Medical and Health Sciences, University of Auckland (T.C.); the Department of Biochemistry, LabPlus (J.D.); and the Department of Nuclear Medicine, Auckland City Hospital (M.D.R.) — all in Auckland, New Zealand; and Amgen, Thousand Oaks, Calif. (C.S., A.M.D.).

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**T**HE CHANGE IN THE SIZE AND SHAPE OF THE SKELETON DURING growth and its renewal during adult life depend on the closely coordinated activity of bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts). The receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL)—osteoprotegerin—RANK system constitutes a critical mechanism for signaling between osteoblasts and osteoclasts.<sup>1</sup> In response to factors such as parathyroid hormone that stimulate the remodeling cycle, the cytokine RANKL is expressed and secreted by osteoblasts. The interaction of RANKL with RANK, located on preosteoclasts, stimulates the differentiation of these cells into active, bone-resorbing osteoclasts. In parallel with the production of RANKL, osteoblasts secrete another molecule, osteoprotegerin, which binds to RANKL and inhibits the RANKL–RANK interaction. Osteoprotegerin is thus an endogenous inhibitor of osteoclast activity.<sup>2,3</sup>

Juvenile Paget's disease (Mendelian Inheritance in Man number 239000) is a rare, autosomal recessive bone disease characterized by greatly accelerated bone turnover. It presents in infancy or childhood with progressive deformity, growth retardation, and deafness (due to cochlear involvement). Most cases of juvenile Paget's disease arise from inactivating mutations in the gene that encodes osteoprotegerin, member 11B of the superfamily of tumor-necrosis-factor receptors (*TNFRSF11B*).<sup>4-6</sup> The osteoprotegerin-knockout mouse recapitulates the juvenile Paget's disease phenotype in humans<sup>7,8</sup> — thus, the disease appears to result from osteoprotegerin deficiency. We report the effects of treatment with recombinant osteoprotegerin in two adult siblings with juvenile Paget's disease.

## METHODS

**PATIENTS**

We studied a 31-year-old woman and her 24-year-old brother, both of whom had juvenile Paget's disease. In both patients, bony deformity had started to develop at around 5 years of age, and by the age of 15 years both were wheelchair-bound. Both had severe kyphosis, as a result of widespread vertebral deformity, as well as severe acetabular protrusion, short stature, macrocephaly, and deafness. Both were homozygous for an in-frame deletion of nucleotides 638 to 640 (GAC) in exon 3 of *TNFRSF11B*, causing the loss of an aspartate residue at position 182.<sup>5</sup> Both patients had briefly received treatment with intravenous pamidronate (at a dose of 1.25 to 1.75 mg per kilogram) three years earlier, with incomplete and short-lived suppression of plasma alkaline phosphatase activity. Because of prominent side effects from the first dose of the bisphosphonate and difficulty traveling, the patients had declined further treatment. Both took supplements of calcium (0.5 g daily) and cholecalciferol (50,000 IU weekly) throughout the study. The study protocol was approved by the Auckland regional ethics committee, and informed consent was obtained from both subjects.

**BIOCHEMISTRY**

Plasma concentrations of calcium, phosphate, and albumin and alkaline phosphatase activity were measured with the use of an autoanalyzer. The values for plasma total calcium concentrations were corrected for variations in the plasma albumin concentration. The normal range for calcium is 9.0 to 10.6 mg per deciliter (2.25 to 2.65 mmol per liter), and that for plasma phosphate is 2.5 to 4.3 mg per deciliter (0.8 to 1.4 mmol per liter). Parathyroid hormone was measured by a chemiluminescence immunoassay (E170, Roche). Bone resorption was assessed in terms of N-telopeptide (bone collagen equivalent), as measured by enzyme-linked immunosorbent assay (Osteomark NTx, Ostex), in relation to creatinine in randomly collected urine specimens; and deoxypyridinoline, as measured by competitive chemiluminescence immunoassay (Immulite 2000, Diagnostic Products), in relation to creatinine in randomly collected urine specimens. Bone formation was assessed in terms of plasma total alkaline phosphatase activity, bone-specific alkaline phosphatase activity (Ostase, Beckman Coulter), and plasma concentrations of osteocal-

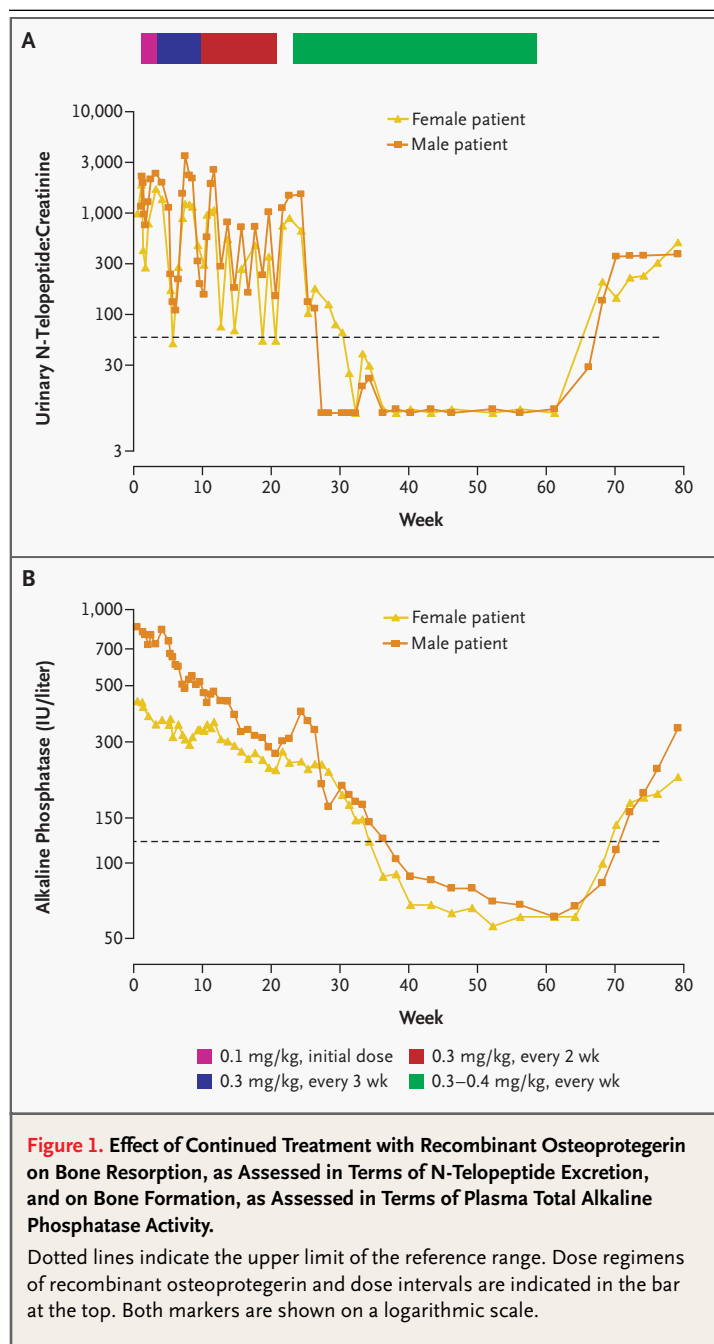
cin and procollagen I N-terminal peptide (E170, Roche).

**BONE DENSITOMETRY, RADIOGRAPHY, AND QUANTITATIVE SCINTIGRAPHY**

Bone density was measured in the forearm by dual-energy x-ray absorptiometry with the use of the DPX-L densitometer (Lunar) at two sites, the ultradistal radius (predominantly trabecular bone) and at one third the length of the radius from its distal end (predominantly cortical bone). Because of the deformity and immobility of the patients, it was not possible to measure other sites. Bone densitometry and radiography were performed before and after 15 months of treatment. Quantitative scintigraphy, to determine the skeletal clearance of bisphosphonate, was undertaken before and after the treatment period. After injection of a standard imaging dose of technetium-99m-labeled methylene diphosphonate, blood samples were collected at one, two, and three hours, and plasma concentrations of technetium-99m-labeled methylene diphosphonate were used to measure the total (renal and skeletal) plasma clearance. Urine was collected for three hours after the injection, and the renal-only clearance of technetium-99m-labeled methylene diphosphonate was calculated. Skeletal clearance was calculated from the difference between total clearance and renal-only clearance.

**TREATMENT**

Recombinant osteoprotegerin was synthesized in a manner consistent with good manufacturing practice as a molecule fused with the Fc-fragment of IgG1 (AMG 0007, Amgen). The aqueous solution of 30 mg per milliliter was stored at  $-70^{\circ}\text{C}$  until use and, after thawing, was administered as a subcutaneous injection. Initially, a dose-finding exercise was planned, following the protocol used by Bekker et al.<sup>9</sup> of injections at three-week intervals with the use of escalating doses (0.1 mg per kilogram of body weight, followed by 0.3 mg, 1.0 mg, and 3.0 mg per kilogram). After each injection, measurements of serum calcium and phosphate and of urine markers of bone resorption were performed daily for 5 days, and then two to three times weekly for another 15 days. After a dose was established that inhibited bone turnover effectively (as judged on the basis of the nadir in bone-resorption markers), the dose was continued at three-week intervals, provided that bone resorption remained suppressed during the three-week period.



#### IMMUNOASSAY FOR ANTI-RECOMBINANT OSTEOPROTEGERIN ANTIBODIES

Because recombinant osteoprotegerin is potentially antigenic, plasma samples were examined at baseline and after months 1 through 4 of the 15-month study period to detect antibodies to recombinant osteoprotegerin. The assay can detect 50 ng per milliliter of antibody to recombinant osteoprotegerin in human serum.

## RESULTS

### RESPONSE TO INITIAL DOSES

Three to eight days after a single dose of 0.1 mg per kilogram of recombinant osteoprotegerin, the greatest reduction in urine deoxypyridinoline occurred; the nadir value averaged 38 percent below the baseline level. For N-telopeptide excretion, the nadir value averaged 65 percent below baseline. The dose of 0.1 mg per kilogram was associated with a reduction in the levels of plasma calcium and phosphate to 8.3 to 8.4 mg per deciliter (2.07 to 2.11 mmol per liter) and 1.9 to 2.2 mg per deciliter (0.6 to 0.7 mmol per liter), respectively. In response to the first dose of 0.3 mg per kilogram, the nadir value for urine deoxypyridinoline averaged 72 percent below baseline, and the nadir value for N-telopeptide excretion averaged 95 percent below baseline. The dose of 0.3 mg per kilogram was associated with a reduction in plasma calcium and phosphate levels to 7.4 to 7.6 mg per deciliter (1.85 to 1.90 mmol per liter) and 1.5 to 1.9 mg per deciliter (0.5 to 0.6 mmol per liter), respectively.

### CONTINUED TREATMENT

Because hypocalcemia was found, the decision was made not to escalate the dose of recombinant osteoprotegerin above 0.3 mg per kilogram. Injections every three weeks resulted in only transient suppression of N-telopeptide excretion, so the dose interval was shortened, initially to once every two weeks and then to once weekly. After the male patient had received 25 weeks of treatment and the female patient had received 31 weeks of treatment, the weekly dose was increased to 0.4 mg per kilogram, in an effort to maintain suppression of bone resorption at the lower end of the reference range. With this regimen, N-telopeptide excretion (measured six days after a dose) remained suppressed for eight months, with no attenuation of effect (Fig. 1). Plasma calcium levels ranged between 8.2 and 9.4 mg per deciliter (2.05 and 2.35 mmol per liter) and plasma phosphate concentrations between 1.5 and 2.8 mg per deciliter (0.5 and 0.9 mmol per liter). During the early weeks of treatment, transient mild symptoms of hypocalcemia were reported by both patients. The hypocalcemia was accompanied by mild, transient elevations in plasma levels of parathyroid hormone. Plasma alkaline phosphatase activity and the other markers of bone formation were suppressed into the normal range (Table 1). No antibodies to osteoprotegerin were detected. Both patients reported a subjective decrease in bone pain.

**MEASUREMENTS AFTER TREATMENT**

At the ultradistal-radius site, there was no change in bone density in either patient. At the one-third forearm site, there was an increase of 9 percent in the female patient and an increase of 30 percent in the male patient.

The skeletal clearance of technetium-99m-labeled methylene diphosphonate decreased by 37 percent in the female patient and by 55 percent in the male patient, indicating markedly reduced bone turnover (Table 1). Skeletal radiographs showed significant improvement, with healing of lytic lesions and improved radiographic density (Fig. 2).

**ADVERSE EVENTS**

Treatment with recombinant osteoprotegerin was not associated with local reactions. Transient mild symptoms of hypocalcemia were noted, and the female patient was admitted to the hospital for one night because of symptoms of asthma (a preexisting condition) three days after her second injection. Regular monitoring of renal, hepatic, hematologic, and thyroid function showed no abnormalities. No fractures were sustained by either patient during the treatment period.

**DISCONTINUATION OF TREATMENT**

After 15 months, the treatment was withdrawn, and biochemical indexes of bone turnover were followed for a further 5 months. Bone turnover revert-

ed to an accelerated pattern after the cessation of treatment (Fig. 1).

**DISCUSSION**

In this study of two adult siblings with juvenile Paget's disease resulting from homozygous inactivating mutations in the gene encoding osteoprotegerin, we observed remarkable responses to experimental treatment with recombinant osteoprotegerin. A subcutaneous injection once weekly could suppress completely the high rate of bone resorption, and there was a secondary reduction in bone formation. With continued treatment, there were decreases in the rate of bone turnover, as shown by reductions in the skeletal clearance of technetium-99m-labeled methylene diphosphonate, increases in cortical bone mass, and improved radiographic appearances. The only side effect of note was not unexpected: hypophosphatemia and hypocalcemia with secondary hyperparathyroidism, which occurred as bone resorption was abruptly inhibited (the so-called hungry bone syndrome).

These patients with juvenile Paget's disease appeared to be more sensitive to exogenous osteoprotegerin than are normal subjects and women with breast cancer metastatic to bone. For example, in studies in normal postmenopausal women, Bekker et al.<sup>9</sup> found that three to seven days after a single injection of recombinant osteoprotegerin at

**Table 1. Effects of Long-Term Treatment with Osteoprotegerin on Bone Turnover and Bone Density.\***

Variable	Normal Values	Woman, Age 31 Yr		Man, Age 24 Yr	
		Week 0	Week 64	Week 0	Week 64
Osteocalcin ( $\mu\text{g}/\text{liter}$ )	<45	155	13	178	20
Procollagen I N-terminal peptide ( $\mu\text{g}/\text{liter}$ )	<60	935	20	1972	20
Bone alkaline phosphatase ( $\mu\text{g}/\text{liter}$ )	<20	112	15	273	21
Total alkaline phosphatase (U/liter)	30 to 120	352	66	727	79
Parathyroid hormone (pmol/liter)	1 to 6	6.1	7.5	6.2	1.5
N-telopeptide:creatinine	<51	1743	11	2452	11
Bone mineral density (T score) <sup>†</sup>					
Ultradistal radius	-2 to +2	-4.0	-4.1	-3.6	-3.6
One-third radius site	-2 to +2	-3.8	-3.2	-4.9	-3.4
Skeletal clearance of methylene diphosphonate (ml/min/1.73 m <sup>2</sup> of body-surface area)	25 to 70	79	50	96	43

\* All biochemical measurements were made in plasma, except those for N-telopeptide, which was measured in urine. To convert values for alkaline phosphatase to nanokatal per liter, multiply by 16.67.

<sup>†</sup> T scores are the number of standard deviations the bone-mineral-density measurement is above or below the young-normal mean bone mineral density.



**Figure 2.** Radiographs of the Right Humerus in the Male Patient (Panel A) and the Female Patient (Panel B), before (Left) and after (Right) 15 Months of Treatment with Recombinant Osteoprotegerin.

In the right humerus of the male patient there was deformity (probably the result of a fracture), and there were numerous lucent areas within which mineral was deposited during the study treatment. In the right humerus of the female patient, the radiographic density of cortical bone improved after treatment.

a dose of 0.1 mg per kilogram, the excretion of the bone-resorption markers deoxypyridinoline and N-telopeptide were reduced by an average of 12 percent and 15 percent, respectively. In our subjects, the same dose reduced these markers by an average of 38 percent and 65 percent, respectively. Similarly, in patients with metastatic bone disease from breast cancer,<sup>10</sup> levels of N-telopeptide fell by an average of only 18 percent after a dose of 0.1 mg per kilogram and by 30 percent after a dose of 0.3 mg per kilogram.

One concern was that the recombinant osteoprotegerin might be immunogenic and induce antibody formation that could be detrimental. We found no serologic evidence of antibody formation, and the suppression of bone turnover was maintained, with no attenuation of effect, suggesting that with up to 15 months of treatment, at least, the production of neutralizing antibodies was not a problem. The effects on bone resorption were readily reversible, with increases in N-telopeptide excretion clearly evident three weeks after the final injection.

Calcitonin and bisphosphonates, which act directly on osteoclasts, have been used to treat juvenile Paget's disease,<sup>11,12</sup> but until recently there have been no data on their long-term effectiveness. Two recent studies have suggested that intensive intravenous bisphosphonate treatment can suppress bone turnover and prevent deformity in affected children.<sup>13,14</sup> Deformity in juvenile Paget's disease arises when abnormally high bone turnover is present during growth. Because the patients in this study were adults, one would not expect to see improvement in deformity with treatment. Recombinant osteoprotegerin, given as a once-weekly, self-administered subcutaneous injection, could be a valuable

treatment for this serious disorder, as perhaps could be the recently developed monoclonal antibody that binds with high affinity and specificity to RANKL, preventing the binding of RANKL to RANK.<sup>15</sup> Our study provides corroborative evidence that the features of juvenile Paget's disease are indeed the result of osteoprotegerin deficiency and confirms the critical role of osteoprotegerin in regulating bone turnover in humans. It constitutes a practical example of

a genetic bone disease treated with replacement of an abnormal or missing protein.

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Dr. DePaoli reports having equity in Amgen and is employed by the company.

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