

Additional Beneficial Effects of Recombinant Growth Hormone in Alendronate-treated Patients with Idiopathic Osteoporosis

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Abstract. In order to study the benefit of adding recombinant human growth hormone (rhGH) to antiresorptive therapy, six patients with idiopathic osteoporosis (IO) receiving alendronate plus calcium and vitamin D were started on daily subcutaneous injections of rhGH 2.0 IU for one year. Fasting morning urine and serum samples were collected for N-telopeptide of type-1 collagen (NTX), serum bone-specific alkaline phosphatase (BSAP) and insulin-like growth factor 1 (IGF-1) during the study. Bone mineral density (BMD) was determined by dual-energy x-ray absorptiometry at baseline and 01 year. The effect of rhGH was evaluated comparing the percentage changes in BMD during the last year on ALN with the results obtained with the combined therapy. Serum IGF-1 increased in all patients but variations were not significant ($p=0.266$). Serum BSAP did not significantly change ($p=0.078$) but median NTX increased at 45 days from 12.3 to 19.8 nMBCE/mMCR ($p=0.012$) and tended to return to baseline values at 12 months (15.2 nMBCE/mMCR). Comparing with isolated ALN therapy, a beneficial effect on bone density was observed in 2/3 of the patients at lumbar spine, and percentage change (median and quartiles) varied from -0.65% (-2.33 and 2.23) on ALN to 0.70% (-0.35 and 3.03) on ALN+GH. Although no bone gain occurred at the femoral neck, our data point to a positive effect of rhGH in patients with idiopathic osteoporosis.

Key words: Growth hormone, IGF-1, Alendronate, Bone mineral density, Idiopathic osteoporosis

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IDIOPATHIC OSTEOPOROSIS (IO) is a rare condition that affects both sexes, women before menopause and men up to sixty-five years. The diagnosis precludes the exclusion of all potential causes of secondary osteoporosis. Clinical presentation is variable ranging from a low bone density in asymptomatic patients to very symptomatic osteoporosis presenting as multiple fragility fractures.

Inadequate peak bone mass [1] and increased bone loss are probably involved in the pathogenesis of idiopathic osteoporosis [2]. Both phenomena have been

related to low concentrations of insulin-like growth factor 1 (IGF-1) in serum [3, 4] and bone [5], and histomorphometric analysis show decreased osteoblastic surface and function [5, 6]. Rosen *et al.* found an association of low serum IGF-1 and homozygosity for a specific allele of the IGF-1 microsatellite (192/192) in men with idiopathic osteoporosis [7].

It is likely that skeletal IGF-1 is one of several coupling factors in the bone remodeling process [6, 7] and IGF-1 gene promoter polymorphism are correlated to risk of fracture in the elderly [8]. Growth hormone is the major physiologic regulator of IGF-1 production by the liver, and impaired GH secretion results in low circulating IGF-1 levels. GH-deficient children have low serum IGF-1, and never attain adequate peak bone mass at adulthood unless they receive growth hormone; this is probably due to deficient bone accretion during childhood and/or early adulthood [1]. Patients with adult-onset GH deficiency also have low bone mass as compared to age-matched healthy subjects, mostly due to decreased bone formation [9].

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Abbreviations: alendronate (ALN), bone mineral density (BMD), bone-specific alkaline phosphatase (BSAP), insulin-like growth factor-1 (IGF-1), N-telopeptide of type-1 collagen (NTX), recombinant human growth hormone (rhGH)

Table 1. Summary of clinical history and first densitometry data of the six patients with idiopathic osteoporosis

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
sex and age at study (yrs)	Male, 58	Male, 20	Male, 23	Female, 55	Female, 40	Female, 47
fragility fractures	right humerus at 48 yrs, vertebral bodies	since age 10: right tibia and ankle, left humerus	since age 8: both legs and arms, vertebral bodies	left hip during delivery of one child at age 37, vertebral bodies	no	no
age (yrs) at first densitometry	53	14	21	37	39	41
LS BMD (g/cm ²)	0.846	0.473	0.579	0.620	0.884	0.861
LS T-score	-3.3	ND	-5.5	-4.8	-2.6	-1.7
LS Z-score	-2.5	-5.1	-4.5	-3.6	-2.1	-1.4
FN BMD (g/cm ²) (#total body)	0.651	0.698 (total body)	0.457	0.328	0.651	0.568
FN T-score	-3.5	ND	-4.7	-5.4	-2.7	-3.3
FN Z-score (#total body Z-score)	-2.4	-4.0 (total body)	-3.9	-4.3	-2.2	-2.7
markers of turnover (N range)	Dpyr: 4.1 (2.3-5.4)	Pyr: 30 (15-40)	ND	ND	NTX: 17.6 (14-73)	NTX: 13 (14-73)
bone histomorphometry	ND	ND	low turnover	low turnover	ND	ND

LS = lumbar spine; FN = femoral neck; BMD = bone mineral density; DPyr = D-pyridinolin and Pyr = Pyridinolin (nmol/mMCR); NTX = N-telopeptide of type I collagen (nMBCE/mMCR); ND : not done.

The reasons for the low circulating IGF-1 levels in a subset of patients with idiopathic osteoporosis remain unclear as previous studies of GH secretion in these patients have failed to identify any significant abnormalities [3, 4].

Antiresorptive drugs, mostly bisphosphonates, are effective in reducing fracture rate, but never normalize bone mass and quality. Anabolic drugs, which directly stimulate osteoblastic bone formation, are more promising in reverting osteoporosis [10].

Recombinant growth hormone (rhGH) promotes a transient increase in bone turnover, which may last for more than six months and delay the expected increase in bone density [11]. In order to prevent such effect, bisphosphonates have been successfully associated with growth hormone in IGF-1-deficient mice [12] and GH-deficient patients [13].

The aim of this study is to investigate the benefits of adding continuous rhGH during one year to chronic alendronate therapy in patients with IO, evaluating bone turnover and bone density.

Patients

Six patients (3 men and 3 women, 20-50 yrs old) with idiopathic osteoporosis were included in this study, selected from the Osteoporosis Research Center of the Division of Endocrinology, University Hospital, Rio de Janeiro, Brazil. The osteoporosis was consid-

ered idiopathic after extensive clinical and laboratory investigation that excluded endocrine disorders (hyperthyroidism, hypercortisolism, hyperparathyroidism, hyperprolactinemia, hypogonadism or growth hormone deficiency), other chronic diseases such as hepatic, cardiac or renal insufficiency, intestinal malabsorption or use of medications that could cause bone loss. All men and one woman had previous fragility fractures at the time of diagnosis, while the others were considered osteoporotic on bone densitometry, i.e. bone density at lumbar spine and/or femoral neck < -2.5 SD from peak bone mass. Two women had the diagnosis of idiopathic osteoporosis several years preceding menopause, and were on hormone replacement therapy for at least two years.

All patients were receiving alendronate 70mg/week plus calcium carbonate 1000 mg/day and vitamin D 400 U/day for several years before being enrolled in the protocol, and these medications were maintained during the whole period of the study. The ethics committee of the University Hospital approved the protocol and all patients signed an informed consent.

A summary of patients' clinical history is shown below, and the results of the first bone density are shown in table 1.

1. AP, white 58 years, male, diagnosis of osteoporosis after fall and fractures of the right humerus and vertebral bodies at age forty-eight. Despite normal gonadal function, received testosterone for two years with-

out significant increase in BMD. On alendronate plus calcium and vitamin D for two years, with modest bone gain.

2. CFS, white 20 years, male, diagnosis of osteoporosis at eleven years due to pain and microfractures in right tibia, followed by spontaneous fracture in left humerus. Received calcium, calcitriol and phosphate solution with no significant increase in BMD. On alendronate plus calcium and vitamin D for seven years, but lumbar spine BMD decreased in the last year.

3. SCR, white 23 years, male. Late diagnosis of osteoporosis at age fourteen when already had leg deformities and thoracic kiphosis due to crush fractures of several vertebral bodies. Previous history of fragility fractures with minimum trauma of both legs and arms since eight years old, but without diagnosis or treatment. Spontaneous puberty at fifteen, and few fractures since then. Bone histomorphometry after double tetracycline cycles at sixteen years detected low turnover osteoporosis. Received testosterone for two years, followed by alendronate for the last three years, with calcium and vitamin D. After initial bone gain, had decrease in LS and FN BMD in the last year on alendronate.

4. CMSB, white 55 years, female. Pain in the feet since age 37 and left hip fracture during delivery of a healthy child at age thirty-nine. Three years later she complained of muscle weakness and X-rays showed diffuse osteopenia with collapse of several vertebral bodies. Fractured right tibia and fibula with minimum trauma at 45 yrs despite salmon calcitonin plus calcium and vitamin D for 16 months. Bone histomorphometric analyses detected severe osteoporosis of low turnover after six months off medication. On alendronate plus calcium and vitamin D for the last four years, during which fractured left tibia and arm. Spontaneous menopause at 53 yrs, started hormonal replacement with percutaneous estradiol plus oral medroxyprogesterone since then. In the last year on alendronate no significant increase in BMD.

5. MCSA, white 40 years, female, diagnosis of osteoporosis by casual bone densitometry for check-up when 39 years. Asymptomatic and with regular menses. On alendronate plus calcium and vitamin D during the last year, increased LS but not FN BMD.

6. MTCM, white 47 years, female, asymptomatic, diagnosis of osteoporosis by bone densitometry at 41 years due to a positive familial history. On alendronate with calcium and vitamin D for five years. Spontaneous menopause at age 45, started hormonal replacement with percutaneous estradiol plus oral medroxyprogesterone since then. Significant decrease in LS BMD in the last year on alendronate, with no change in FN BMD.

Methods

Recombinant human rhGH (Novotropin, Novo Nordisk, Inc. Denmark) in a daily doses of 02 U (0.65 mg) given as SC injections at bedtime was added for 12 months to chronic alendronate (Endrox, Merck, Brazil), calcium and vitamin D. Doses of all medications including hormone replacement for menopause remained unchanged during the study. Patients returned monthly for clinical examination that included blood pressure and body weight measurement. The adherence to treatment was determined by the number of remaining doses in the returned vials of rhGH and all other medications, which could not exceed 10%.

At baseline and also at 3 months, 6 months and 12 months blood was drawn after overnight fasting for routine tests. Serum samples were kept frozen at -700°C until evaluation of insulin-like growth factor (IGF-1) and bone-specific alkaline phosphatase (BSAP). At baseline, 45 days and 12 months a sample of the second voided urine was collected and kept frozen at -400°C until evaluation of the N-telopeptide of type-I collagen (NTX), and results were related to creatinine in the same urine sample. Serum calcium, phosphate, parameters of liver function, lipids and blood glucose were measured by automated techniques. Serum IGF-1 concentration was measured by immunoradiometric assay using a commercial kit DSL 5600 ACTIVE, Diagnostic Systems, Texas, EUA; reference range for adults of both sexes are 80-500ng/mL. BASP was measured by enzyme-linked immunosorbent assay using the commercial kit ALKPHASE B, Metra Biosystems, California, USA; reference range for males are 15-41.3 U/L and for premenopausal women 11.6-29.6 U/L. Urinary NTX was measured by enzyme-linked immunosorbent assay using the commercial kit Osteomark, Ostex International Seattle, WA, USA); normal values ranged from 14-76

Table 2. Individual Values of IGF-1 (ng/mL), BSAP (U/L) and NTX (nMBCE/mMCr) along combined therapy with rhGH and Alendronate in six patients with idiopathic osteoporosis.

	Baseline	45days	3months	6months	9months	12months
IGF-1 (ng/ml)						
Patient 1 (M)	540	680	560	420	645	703
Patient 2 (M)	205	690	690	450	416	338
Patient 3 (M)	520	750	640	580	572	364
Patient 4 (F)	38	168	230	350	302	120
Patient 5 (F)	97.6	96	356	208	394	207
Patient 6 (F)	448	426	334	337	453	393
BSAP (U/L)						
Patient 1 (M)	20	14	16	15.3	12.7	13.5
Patient 2 (M)	16	17	19	19	11	15.4
Patient 3 (M)	35	37	31	28	21.1	20.6
Patient 4 (F)	12	16	15	12	14.8	12.4
Patient 5 (F)	17.2	11	16.4	13	11	10
Patient 6 (F)	14.8	19	24.4	15.6	24.6	14
NTX (nMBCE/mMCr) *						
Patient 1 (M)	14	19				5.9
Patient 2 (M)	14	33				20.8
Patient 3 (M)	30	46				38.6
Patient 4 (F)	10	11				11.3
Patient 5 (F)	9	14.2				9.9
Patient 6 (F)	10.5	20.6				19

* $p=0.012$ comparing 45 days to baseline. P: patient, (M): male, (F): female.

nMBCE/mMCr in premenopausal females and up to 85 nMBCE/mMCr in males.

Before starting rhGH and after one year bone mineral density was measured at the lumbar spine (LS) and femoral neck (FN) using dual X-ray absorptiometry (Expert, Lunar Corp. Madison, WI, USA). Percent variations of BMD $\geq 1.5\%$ at LS and $\geq 1.8\%$ at FN were considered significant.

Statistics analysis was performed using the software StatView version 4.5 from Abacus Concepts, Inc. (Berkeley, CA). One-way anova for repeated measures, Wilcoxon test and Friedman test were used to evaluate the variations in IGF-1 and bone markers. Paired two-tail Student's *t* test was used to assess changes of BMD. *P* values of less than 0.05 were considered significant.

Results

Treatment was well tolerated and none of the patients had to be withdrawn from the study. Two pa-

tients (CMSB and MTCM) experienced mild and transient side effects such as arthralgia and fluid retention probably related to rhGH. Blood pressure and body mass index did not change, as well as parameters of renal and hepatic functions, serum calcium, phosphate and lipids. Mean blood glucose increased during treatment ($p=0.05$) but individual values remained within normal range.

Serum IGF-1 increased in all patients and remained above baseline values during treatment. The mean IGF-1 calculated along the combined therapy (429.1 ± 162.2 ng/mL) tended to be higher than baseline values (308.1 ± 221.9 ng/mL, $p=0.070$), but variations were not significant ($p=0.266$). Median urinary NTX increased at 45 days from 12.3 to 19.8 nMBCE/mMCr ($p=0.012$) and tended to return to baseline values at 12 months (15.2 nMBCE/mMCr). The variations in serum BSAP were not significant ($p=0.078$). Individual values are shown in table 2.

Absolute values of bone mineral density, T scores and Z scores immediately before and one year after combined therapy with rhGH plus alendronate are

Table 3. Variation in bone mineral density (g/cm²) and percent changes in lumbar spine and femoral neck BMD in the last year on ALN and after 1 year of ALN plus rhGH in six patients with idiopathic osteoporosis

	Patient 1 (M)	Patient 2 (M)	Patient 3 (M)	Patient 4 (F)	Patient 5 (F)	Patient 6 (F)
LS BMD (g/cm ²) before adding rhGH	1.011	0.822	0.554	0.609	0.911	0.955
LS BMD (g/cm ²) after 1yr ALN+rhGH	1.062	0.850	0.552	0.608	0.890	0.970
% change LS BMD last yr on ALN	2.8	-1.8	-9.2	0.5	3.1	-2.5
% change LS BMD 1yr ALN+rhGH	5.0	3.4	-0.4	-0.2	-2.3	1.6
net effect on LS BMD	positive	positive	positive	no effect	negative	positive
FN BMD (g/cm ²) before adding rhGH	0.729	0.788	0.483	0.459	0.644	0.623
FN BMD (g/cm ²) after 1yr ALN+rhGH	0.704	0.833	0.498	0.454	0.640	0.616
% change FNBMD last yr on ALN	2.2	*	-5.8	0.2	-1.1	0.2
% change FNBMD 1yr ALN+rhGH	-3.4	5.7	3.1	-1.1	-0.6	-1.1
net effect on FN BMD	negative	positive	positive	no effect	no effect	no effect

* Previous analysis considered total body BMD instead of FN BMD, limiting evaluation of % change during last year on ALN alone. (M): male, (F): female.

The effect of adding rhGH was considered as positive when the increase in BMD was bigger than 1.5% at LS and bigger than 1.8% at FN, or when patients stopped losing bone.

shown in table 3. Comparing with isolated ALN therapy, a beneficial effect on bone density was observed in 2/3 of the patients at lumbar spine, and percentage change (median and quartiles) varied from -0.65% (-2.33 and 2.23) on ALN to 0.70% (-0.35 and 3.03) on ALN+GH. To estimate the effect of adding rhGH, the variation of bone density after one year of combined therapy was compared to the variation of BMD in the last year of treatment with alendronate (table 3). The effect of adding rhGH was positive in four and negative in one of the patients at lumbar spine, as they showed a net variation of more than 1.5%. The response at the femoral neck was positive in one man and negative in another. No female patient changed femoral neck BMD significantly during the study period.

Discussion

Osteoporosis is a disease in which low bone mass originates from an imbalance between bone resorption and bone formation. Antiresorptive drugs are more effective in situations of increased bone turnover, commonly seen in early postmenopausal [14, 15], other causes of hypogonadism [16] and post-transplantation osteoporosis [17], but are also used in situations of decreased bone formation such as glucocorticoid-induced osteoporosis [18]. Bisphosphonates, the most widely used antiresorptive drugs, induce a limited in-

crease in bone density and decrease fracture rate, but never allow a full recovery of bone architecture [10].

Idiopathic osteoporosis has been related to low insulin-like growth factor 1 (IGF-1) concentrations in blood [3, 6] and bone [2], and to osteoblastic dysfunction, leading to limited bone formation whatever the rate of bone resorption [19]. Thus, even the patients with IO and normal serum IGF-1 could benefit from bone anabolic drugs.

Growth hormone has been used as a bone anabolic agent in GH-deficient patients [9, 20-23] and in some situations where the main factor causing osteoporosis was the decrease in osteoblastic function, such as anorexia nervosa [24], age [25] and idiopathic osteoporosis [26, 27].

Most studies of the therapeutic effect of rhGH on bone were conducted in GH deficient adults, and show an early increase in biochemical markers of bone turnover. With the maintenance of rhGH replacement these markers decrease, but remain above baseline values [28], pointing to a continuing increased bone turnover [9]. This may lead to an initial decline in BMD within the first year of rhGH treatment, although a net bone gain is well recognized with long term treatment [9, 21, 22, 26].

A randomized controlled trial in osteoporotic GH-deficient adult patients receiving stable doses of rhGH for 4 years reported the beneficial effects of adding bisphosphonates [13]. The increase in lumbar spine

bone density was significantly greater in alendronate treated patients (4.4%) than in patients maintained on rhGH alone (0.7%), $p=0.006$. This increase in LS BMD in the ALN treated patients was associated with a significant decrease after 6 months (with no further decrease thereafter) in the measured markers of bone turnover, i.e. bone-specific alkaline phosphatase, osteocalcin, and urinary Ntelopeptide/creatinine ratio, whereas bone markers did not change in the group maintained with GH alone.

Stabnov *et al.*, using an IGF1 MIDI mouse model (which had more than 60% reduction in circulating IGF-1 levels), confirmed the hypothesis that treatment with alendronate along with IGF-1 during the pubertal growth phase was more effective than IGF-1 alone in increasing the total body bone mineral content (BMC) and periosteal circumference, increasing peak bone mass [12].

The only group that studied the effect of rhGH treatment in idiopathic osteoporosis did not use bisphosphonates [26]. They investigated the effects of growth hormone treatment on bone turnover, bone size, bone mineral density (BMD), and bone mineral content (BMC) in 29 men, 27-62yr old, with idiopathic osteoporosis. All patients were treated with rhGH (either as continuous treatment with daily injections of 0.4mg GH/d or as intermittent treatment with 0.8mg/d for 14 days every 3 months) for 24 months with a follow-up period of 12 months. Patients also received 500mg of calcium and 400 U of vitamin D3 daily during the whole period of 36 months. A positive effect on lumbar spine bone density was seen with either therapeutic schedules of rhGH after 24 months, and a further increase occurred at 36 months. No significant variation occurred at the femoral neck after the same period.

In the present study all patients with idiopathic osteoporosis were on chronic alendronate before adding rhGH, based upon the well recognized benefits of bisphosphonate in the management of primary and secondary osteoporosis in men and women, and also in patients with idiopathic osteoporosis, specially those with high bone turnover. We decided to maintain alendronate during the 12 months of rhGH therapy to test the hypothesis that the bisphosphonate would prevent the initial transient deleterious effect of increasing bone turnover induced by rhGH treatment. As a consequence, the expected anabolic effect of rhGH on bone density could already be evident after the first year.

One of the female patients had a low serum IGF-1

at baseline, but GH response to provocative tests was normal. During the study the standard daily dose of 02 U of rhGH (0.65mg) increased IGF-1 levels in all patients, and eventual supernormal levels were not sustained after six months.

Biochemical markers of turnover were in the low-normal range at baseline as expected in chronic alendronate users. Alendronate did not prevent the initial rise in bone turnover induced by rhGH as shown by the significant increase in urinary N telopeptide/creatinine ratio at 45 days, but probably contributed to the normalization of bone resorption rate after 01 year of combined treatment, thus favoring bone formation.

The effect of adding rhGH was considered positive at lumbar spine in two thirds of the patients, as they stopped losing bone or had a percentage increase of more than 1.5%. Previous study of the use of rhGH in men with idiopathic osteoporosis also showed an increase in BMD only at lumbar spine [26]. These findings are in accordance with the more precocious response of trabecular bone to any therapeutic regimen for osteoporosis as compared to cortical bone.

All male patients but only one of the three female patients had a net bone gain at lumbar spine with the combined therapy. A gender difference in the skeletal response to GH has been described in GH-deficient patients, with women being less GH responsive than men, although the mechanism remains unclear [29, 30]. Probably the gender difference in the skeletal response to GH can partly be explained too by the effects of sex steroid hormone [31]. In this group, the pre-existing sex hormone supplement may affected the observed GH effects on bone. One data suggest that short-term administration of hormone replacement therapy (HRT) exerts beneficial effects on bone metabolism and BMD in postmenopausal women, which are not significantly altered by the coadministration of GH; although in andropausal men, testosterone (T) administration to achieve physiologic levels did not result in significant effects on bone metabolism or BMD, whereas GH + T increased one marker of bone formation and decreased one marker of bone resorption [32]. Recent studies shows that oral estrogen reduces hepatic production of IGF-1, while testosterone increases it [33, 34]. Indeed two of three male patients had been taking testosterone, but before starting GH therapy; while two of three female patients were on estrogen replacement before and during GH therapy, but used percutaneous estrogen.

Bone histomorphometric analyses performed on GH-deficient men treated with rhGH for 1 year indicated that this hormone initializes bone remodeling cycles with a relatively greater enhancement of bone formation [35], probably explaining the further increase in bone density observed by Gillberg *et al.* after stopping rhGH in men with idiopathic osteoporosis [26]. Thus, it is possible that our patients will continue to increase bone density in the following year after stopping rhGH.

One limitation of this study is the small sample size, but idiopathic osteoporosis is an uncommon disease. The other limitation is the absence of a placebo-treated control group. However, the focus of this investigation was to evaluate the additive effect of an anabolic drug to a chronic antiresorptive regimen, and for this purpose, each patient could be considered as his own control.

Conclusion

The importance of this study is that it is the first to investigate the effects of rhGH plus alendronate on bone turnover and bone mineral density in patients with idiopathic osteoporosis, and the early positive effect is encouraging.

More studies using this regimen for longer periods are needed to further define the effects of rhGH plus alendronate on bone density and fracture risk in patients with idiopathic osteoporosis.

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