CLINICAL STUDY

GH in combination with bisphosphonate treatment in osteogenesis imperfecta

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Abstract

Objective: To verify the effects of bisphosphonates (Bps) in combination with recombinant human GH (rGH) in pediatric osteogenesis imperfecta (OI) patients; we focused on possible improvement of bone mineral density (BMD), projected bone areas, growth velocity, and fractures risk.

Design: A randomized controlled 1-year clinical trial on 30 prepubertal children (M:F = 14:16) affected by OI (type I, IV, and III) being treated with neridronate.

Methods: Following an observational period of 12 months during ongoing neridronate treatment, the patients were randomly divided into two groups: 15 were treated for 12 months with rGH and neridronate (group Bp+rGH) and 15 continued neridronate alone (group Bp). We evaluated auxological parameters, number of fractures, bone age (BA), bone metabolic parameters, and bone mass measurements (at lumbar spine and radius by dual-energy X-ray absorptiometry).

Results: The mean variation in percentage of BMD ($\Delta\%$ BMD) – at lumbar spine (L2–L4), at distal and ultradistal radius – and the projected area of lumbar spine increased significantly in group Bp+rGH ($P < 0.05$). Growth velocity was significantly higher during rGH treatment in group Bp+rGH versus group Bp and versus pretreatment ($P < 0.05$), with no difference in increase in BA or fracture risk rate. Patients with quantitative (–qt) collagen synthesis defects had a higher, although not significant, response to rGH in terms of growth velocity and BMD.

Conclusions: In OI patients, the combined rGH–Bp treatment may give better results than Bp treatment alone, in terms of BMD, lumbar spine projected area and growth velocity, particularly in patients with quantitative defects.

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Introduction

Osteogenesis imperfecta (OI) is a heterogeneous group of inherited disorders of connective tissue characterized by evidences of connective tissue malfunction (1). Clinical severity varies widely, and patients are characterized by a heterogeneous degree of bone fragility (from mild osteopenia to progressive skeletal deformities due to recurrent fractures), various degrees of short stature (from normal height to highly compromised height), blue sclerae, and dental abnormalities (from recurrent caries to dentinogenesis imperfecta). The types initially delineated by Silience were mild nondeforming type I, perinatal lethal type II, severely deforming type III, and moderately deforming type IV, but four additional groups of moderately severe conditions have now been defined (types V–VIII) (2). Prognosis of OI is mainly influenced by the degree of bone fragility, the severity of which increases in the order: type I < types IV, V, VI, VII, VIII < type III < type II. Most OI cases are caused by

heterozygosity of dominant mutations in one of the two genes encoding the chains of type I collagen (*COL1A1* and *COL1A2*) (3), with quantitative (–qt; mild or moderate forms) or qualitative (–ql; severe or lethal forms) defects of the synthesis of type I collagen (4). Cartilage-associated protein (*CRTAP*), prolyl 3-hydroxylase 1 (*P3H1/LEPRE1*), and cyclophilin B (*CyPB/PIIB*) form an intracellular collagen-modifying complex that 3-hydroxylates proline at position 986 (P986) in the $\alpha 1$ chains of collagen (types I, II, and V); mutations in these essential cofactors cause alterations in the posttranslational chain modification and collagen folding, which are responsible for autosomal-recessive lethal or severe OI (5–12). However, other genes may cause OI since the etiologies of types V and VI are still unknown.

Treatment strategy by a multidisciplinary team approach (13) should aim for maximum long-term function and autonomy. Regarding the pharmacological approach, bisphosphonates (Bps) are now considered a 'standard care' for children with OI (14, 15).

When administered either orally or parenterally, these analogs of pyrophosphate form a strong, rapid bond to hydroxyapatite crystals in bone and, by decreasing osteoclast activity and number, inhibit bone resorption and reduce bone turnover, although effects on bone formation occur as well (16). These effects are improvements in vertebral mass and shape, an increment in cortical width and cancellous bone volume and suppression of bone turnover as has been shown by histomorphometric studies. The positive effects of Bps on pain and mobility and on decreasing long bone fractures have been suggested by observational trials but never by controlled trials (17). Several compounds with different relative potencies for inhibiting bone resorption are used in humans, but Bp derivatives with an amino group at the end of the side chain are very active. Pamidronate was the first nitrogen containing Bp described, and neridronate, a 6-amino-1-idrossiesilidene-1,1-bisphosphonate, is the only Bp authorized in Italy for use in OI children. The benefits from cyclic pamidronate treatment seem to be largely achieved in the first 2–4 years (18), probably due to the marked suppression of bone turnover that these substances produce. At any rate, in our opinion, it does not seem advantageous to stop Bp treatment in growing children. Strict rehabilitative therapy together with adequate Ca and P intake during treatment could be a way to prevent fractures caused by localized bone fragility, both in the new added metaphyseal bone tissue (19) and in the treated portion (some authors found more osteopetrotic bone ceased after treatment) (20).

On the other hand, GH has a positive effect on bone growth and bone turnover by stimulating osteoblasts, collagen synthesis, and longitudinal bone growth (21); even though during the first 6 months of GH therapy in GH deficiency (GHD) patients, bone resorption is usually greater than bone formation, and there are more resorption markers (22). Besides these actions on bone, in osteoblast cultures, GH has also shown a positive action on collagen metabolism (23, 24), that is, GH stimulates the expression of insulin-like growth factor 1 (IGF1) and IGF-binding protein 3 (IGFBP-3), which in turn regulates the synthesis of type I collagen (25, 26). There is limited literature regarding recombinant human GH (rGH) experience in OI; two types of approach have been attempted – one is an extensive, general stimulus of bone metabolism increasing bone apposition and the other increases growth, stimulating collagen synthesis, in cases of ascertained GHD. One of the first attempts to treat OI patients with rGH was made more than 30 years ago by Kruse & Kuhlencordt (27); both their patients had an increase in periosteal new bone formation and intracortical bone resorption (assessed using histomorphometry of bone biopsies), with relative enhanced osteoblastic activity (27). Following these results, no further study was reported in the literature until 1993 when GH–somatomedin axis activity was studied in a limited number of

nonselected OI patients (28). Even if other studies had shown that IGF1 serum levels are mostly in the low normal physiological range (29), Marini *et al.* (28) found hypoactivity of this axis (without a true GH deficit) in approximately half of their OI patients and treated them with rGH or clonidine (a pituitary GH secretagogue). In a further study by this group, the authors concluded that there is also a group of type IV OI children who would benefit from rGH treatment in terms of linear growth, bone matrix synthesis, and bone histomorphometric parameters (30). Sillence *et al.* showed positive height growth, an increase in skeletal volume and bone mineral density (BMD), and infer that the subsequent reduction in fracture frequency appears to confer a significant therapeutic benefit. Animal studies, in a mouse model of OI with bone phenotype comparable to a mild form of OI in humans (*oim/+*), showed that systemic rGH injections (31) or GH transgene expression in erythroid marrow (32) increased spine and femur length, produced significant changes in densitometry parameters and ameliorated the biomechanical structural properties of bone.

In spite of rGH action on bone turnover and bone mineralization resulting in an increase in BMD and on the growth velocity rate, few human studies have been performed using rGH in patients with OI (28, 33–35). Since, to our knowledge, there are no focused controlled trials on combined rGH and Bp treatment in OI patients, following our past positive experience with rGH therapy in mild forms of OI with quantitative defects of type I collagen synthesis (33), we decided to continue rGH treatment in a second group of patients with mild and moderate forms of OI (the majority with type I and a good proportion with type IV) in order to couple the prevalent inhibition of bone resorption due to Bps with the stimulation of bone apposition obtained by rGH. Thus, the purpose of this randomized controlled 1-year clinical trial was to investigate whether the combination of Bps with rGH can further ameliorate bone metabolism in prepubertal children with OI, who are already receiving treatment with neridronate. The main outcomes of the study were an improvement in BMD and projected bone areas, as measured by dual-energy X-ray absorptiometry (DXA) at the spine and wrist, an increase in growth velocity and a reduction in the incidence of peripheral fractures.

Methods

Patients

Of the patients affected by OI (types I, IV, and III; $n=210$) followed up in our pediatric clinic between February 2004 and March 2006, we enrolled 30 prepubertal children (M:F=14:16; mean age: 7.3 ± 1.3 years; range 5.1–10.8 years for boys and 5.2–8.8 years for girls) already being treated with neridronate (mean period: 2.9 ± 1.4 years; range

1.2–4.6 years). Informed written consent was obtained from all parents of the patients before beginning the study protocol, which was approved and constantly monitored by our institution's ethics committee. All patients had complete molecular characterization in type I collagen genes as described previously (4). The patients were stratified (by FA) into mild (type I: 18) and severe–moderate (types III–IV: 9+3) forms, and then randomized into two comparable groups regarding sex (both composed of seven boys and eight girls), age, height, and clinical severity of OI, using a computer pseudorandom number generator: group Bp+rGH (OI type I:V:III=9:4:2) and group Bp (OI type I:V:III=9:5:1). Over a period of 12 months, the patients from both groups were carefully observed while they continued their Bp treatment; then, patients in the Bp+rGH group started the 12-month rGH and neridronate treatment period, whereas the Bp group of patients continued neridronate alone. All the children had their dietary vitamin D (25-hydroxyvitamin D, 25OHD) and Ca intake measured regularly. With an ordinary diet or by using Ca supplements, Ca consumption was maintained above 800 or 1000 mg daily according to the patient's age (<7 or >7 years of age respectively), and 25OHD serum levels were kept above 20 ng/ml.

Treatment

Neridronate (Nerixia, Abiogen Pharma, Pisa, Italy) was given at a dose of 2 mg/kg (diluted in 250 ml of saline solution and infused i.v. over 3 h) every 3 months; rGH (Genotropin, Pfizer Italia, Rome, Italy) was administered s.c. at a dose of 0.05 mg/kg per day, 6 days a week (~0.3 mg/week per kg). Since rGH was given at home, in order to monitor rGH treatment compliance, the patients had to bring back their empty vials every 3 months.

Study design

We assessed the clinical, laboratory, and densitometry parameters of the 30 patients between February 2004 and February 2008; every 3 months, all patients were evaluated for their auxological and clinical parameters (weight, height, body mass index (BMI), growth velocity, and Tanner stages), number of peripheral or extravertebral fractures, Ca and other nutrient intake, physical activity, and clinical symptoms related to the disease (skeletal, respiratory, etc.). Bone metabolic parameters and bone mass measurements were determined at the time of enrollment (T–1), after 12 months (T0), or when the Bp+rGH group started rGH treatment, after 18 months (T1), or after exactly 6 months of rGH treatment, and finally at the end of the trial (T2), 24 months from enrollment or 12 months after starting rGH treatment. Bone metabolic parameters were obtained during fasting in the morning (between 0800 and 1000 h) and before the infusion of

neridronate. We evaluated serum Ca, P, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25OHD, osteocalcin (Oc), C-terminal cross linking telopeptides of type I collagen (β -CTX), and IGF1; we also measured urinary (second morning voiding) Ca, P, and creatinine. Routine biochemistry, including creatinine, was carried out on the same day of sample collection. Aliquots of both serum and plasma were stored at -20°C until determination. Bone mass measurements were performed at lumbar spine, hip, and radius by DXA. All patients underwent bone age (BA) determination only at the beginning of the rGH therapy (T0) and after 12 months (T2).

Clinical and laboratory studies

The children were weighed on standard clinical scales while wearing minimal clothing and no shoes. Standing height and lying length were measured by a Harpenden stadiometer. Height and weight were normalized using Italian reference data (36) and expressed in s.d.s. Growth velocity is expressed in cm/year. Peripheral fractures from low trauma were all self-reported by the patients and were confirmed by X-ray. Serum intact 1–84 PTH levels were determined by immunometric chemiluminescence assay (Nichols Institute Diagnostics, San Clemente, CA, USA). The intra- and interassay coefficients of variations (CV) were below 6.7 and 9.2% respectively. The analytical sensitivity was 0.1 pmol/l. Plasma 25OHD was measured by HPLC (Eureka srl, Chiaravalle, Ancona, Italy). The intra- and interassay CV were below 5.2 and 7.8% respectively. The analytical sensitivity was 5 nmol/l. Serum Oc was measured by immunometric chemiluminescence assay (Diagnostic Products Corp., Los Angeles, CA, USA). The intra- and inter-assay CV were below 4.5 and 7.1% respectively. The analytical sensitivity was 0.02 nmol/l. Serum β -CTX was determined by Elecsys β -Cross Laps electro chemiluminescence immunoassay (Roche Diagnostics). The intra- and inter-assay CV were below 4.7 and 7.6% respectively. The analytical sensitivity was 0.01 ng/ml. Serum IGF1 concentrations were measured by immunometric chemiluminescence assay (Immulite 2000 IGF1; Diagnostic Products Corp.); the intra- and inter-assay CV were 3.9 and 5.4% respectively. The analytical sensitivity was 20 ng/ml. Serum Ca, P, and ALP values and urinary Ca, P, and creatinine values were determined by standard techniques. BA was determined according to the method of Greulich & Pyle (37), and expressed in years. Bone mineral parameters and projected area at the lumbar spine and total hip were measured by DXA (QDR 4000; Hologic, Waltham, MA, USA). The second, third, and fourth lumbar vertebrae were scanned by anteroposterior projection. Bone mass was expressed as areal BMD in milligrams per square centimeter, and the values were normalized to age-matched group and expressed in s.d. (38). The precision error for BMD at different skeletal

sites was assessed by double measurements at one of the time points of the study in all participants. The CV are somewhat greater than those observed in adults, ranging from 1.4 to 2.9% respectively at spine and hip (38). DXA evaluations at the hip and vertebral lateral projections were excluded from the statistical analysis since appropriate measurements in these sites could not be obtained in 20% of the patients, mainly due to problems in obtaining correct positioning. Bone mineral content (BMC) and BMD at the forearm were measured by the Osteoplan system (NIM, Verona, Italy) as reported previously (39). The CV for duplicate measurements were fewer than 1.7% in normal children. Both BA and DXA observations were determined by the same expert (FA), blinded to the treatment group. Molecular characterization was performed by direct analysis of genomic DNA as reported previously (4). Based on the effects of mutations on collagen synthesis, from molecular study results, we could differentiate between qualitative and quantitative defects of type I collagen synthesis, subdividing both groups (Bp+rGH and Bp) into quantitative and qualitative subgroups.

Statistical analysis

Descriptive analyses were expressed as mean $\pm$ s.d. The percentage variation (%) in the measured parameters was calculated as follows: ((measured value – initial value)/initial value) $\times$ 100. Once tested for normality in the OI population, statistical analyses were performed by unpaired *t*-test and repeated measures ANOVA. All statistical analyses were performed using a data analysis system (Kaleidagraph 4.03, Synergy Software, Reading, PA, USA) run on an Apple MacBook Pro Computer. Statistical significance was set at $P < 0.05$.

Results

At the beginning of the study protocol, the patients in the Bp+rGH group and those in the Bp group were comparable for sex, chronological age (CA), height, growth velocity, weight, BMI, and duration of previous

neridronate treatment. The children showed both reduced height and a low growth velocity as compared with children of the same age and sex (s.d.s). All patients remained prepubertal until the end of the study. rGH treatment compliance was above 95%.

At the beginning of the study, the mean lumbar BMD, expressed in s.d., in both groups was lower than that of the normal population (T–1: group Bp+rGH: mean -3.5 ± 1.2 ; range $-6.5/-1.8$; group Bp: -3.4 ± 1.4 s.d.; $-6.8/-1.7$). The comparison was done between different DXAs of the same patients. In the first year of follow-up, during neridronate therapy alone, BMD improved and was rather equal in two groups (T0: group Bp+rGH: -3.0 ± 1.3 s.d.; $-5.4/-1.6$; group Bp: -2.9 ± 1.2 s.d.; $-6.2/-1.5$), but it increased more, though not significantly, in the Bp+rGH group than in the Bp group during the subsequent year, when the first group received the combination of neridronate and rGH (T2: group Bp+rGH: -2.3 ± 1.1 s.d.; $-5.0/-1.0$; group Bp: -2.7 ± 1.3 s.d.; $-5.5/-1.4$). The patients' mean percentage variations of BMD ($\Delta\%$ BMD) are reported in Table 1. The $\Delta\%$ BMD at the lumbar spine (L2–L4) had a significant increase in the Bp+rGH group in the second year of follow-up (after 12 months of rGH treatment) compared with the Bp group ($P < 0.05$). In the first year of treatment (during neridronate treatment alone), the $\Delta\%$ BMD was similar to that expected for growth in the same patients (estimated around 6–8%/year) (38), but it increased significantly during rGH treatment in the Bp+rGH group, compared with the pretreatment and the Bp group ($P < 0.05$). The projected area of lumbar spine increased significantly in the Bp+rGH group during rGH treatment compared with the Bp group ($P < 0.05$). The $\Delta\%$ BMD at the distal and ultradistal radius showed a significant increase in the Bp+rGH group, after both 6 and 12 months of rGH treatment ($P < 0.05$ in each case versus Bp group). When analyzing a possible correlation between response to rGH and duration of prior Bp therapy, we found that the trend of DXA values was better in patients who had started neridronate more recently, but the difference was not statistically significant.

Table 1 Bone densitometry data expressed as $\Delta\%$ bone mineral density (BMD) and lumbar spine projected area expressed as $\Delta\%$ cm² of the two groups of patients (group bisphosphonate (Bp) + recombinant human GH (rGH) and group Bp) in the intervals between time points: 12 months before the start of rGH (T–1), at the start of rGH in group Bp+rGH (T0), at 6 and 12 months of rGH treatment (T1 and T2 respectively).

Intervals	DXA lumbar spine (L2–L4) ($\Delta\%$ BMD)		Lumbar spine projected area (L2–L4) ($\Delta\%$ cm ²)		DXA distal radius ($\Delta\%$ BMD)		DXA ultradistal radius ($\Delta\%$ BMD)	
	Group Bp+rGH	Group Bp	Group Bp+rGH	Group Bp	Group Bp+rGH	Group Bp	Group Bp+rGH	Group Bp
T–1 $\rightarrow$ T0	7.8 $\pm$ 3.3	8.3 $\pm$ 1.5	5.1 $\pm$ 0.9	4.8 $\pm$ 1.0	4.7 $\pm$ 2.2	4.0 $\pm$ 3.3	7.6 $\pm$ 3.9	8.5 $\pm$ 4.9
T0 $\rightarrow$ T1	8.0 $\pm$ 5.7	4.0 $\pm$ 2.9	4.0 $\pm$ 0.7	3.2 $\pm$ 0.8	5.9 $\pm$ 4.1*	1.7 $\pm$ 2.9	15.2 $\pm$ 3.8*	5.1 $\pm$ 4.2
T1 $\rightarrow$ T2	9.6 $\pm$ 5.6	5.2 $\pm$ 3.0	5.1 $\pm$ 1.0	2.7 $\pm$ 0.9	3.8 $\pm$ 1.8	2.5 $\pm$ 2.2	8.3 $\pm$ 2.9	4.3 $\pm$ 3.1
T0 $\rightarrow$ T2	19.1 $\pm$ 4.9†	9.2 $\pm$ 3.1	9.1 $\pm$ 2.2†	5.9 $\pm$ 1.5	9.8 $\pm$ 4.0†	4.2 $\pm$ 3.3	23.5 $\pm$ 4.0†	9.6 $\pm$ 4.5

*Significant increase in group Bp+rGH at 6 months of rGH treatment: $P < 0.05$ versus group Bp. †Significant increase in group Bp+rGH at 12 months: $P < 0.05$ versus group Bp.

Growth velocity (cm/year) increased in the Bp+rGH group compared with the pretreatment period, and by the end of rGH treatment, it was even significantly higher in the Bp+rGH group than in the Bp group ($P<0.05$ versus pretreatment and versus group Bp). There was no difference in the increase in BA, and the BA to CA ratio (BA/CA) remained similar between the two groups (BA/CA: group Bp+rGH: 0.98 ± 0.09 ; group Bp: 0.97 ± 0.10). The total number of fractures was small in both groups in the period from T-1 to T0 (four in the Bp+rGH group and three in the Bp group), and did not increase in the period from T0 to T2 (three in Bp+rGH group and three in Bp group). Table 2 summarizes the clinical data results of both groups (Bp+rGH and Bp).

PTH and 25OHD levels were in the normal range with no significant variations found during the study. CTx levels were above normal in both groups, and the values did not change significantly during the study. Oc levels were in the low normal range in both groups at the beginning of the study, and remained at the same level in the Bp group, whereas they increased, but not significantly, during rGH treatment in the Bp+rGH group. IGF1 levels were in the low normal range before therapy, and increased significantly after 12 months of rGH (T2) in the Bp+rGH group, both in comparison to pretreatment (T-1) and to the Bp group ($P<0.05$ versus T-1 and versus group Bp). Biological data are shown in Table 3.

We also subdivided patients based on the quantitative or qualitative molecular collagen defects into subgroup Bp+rGH-qt (9), subgroup Bp-qt (9), and subgroup Bp+rGH-ql (6) or subgroup Bp-ql (6). At T2 (after 12 months of rGH in the treated group), the $\Delta\%$ BMD at the lumbar spine (L2-L4) had increased significantly in subgroup Bp+rGH-qt versus subgroup Bp-qt (19.6 ± 4.8 vs $9.0\pm3.0\%$; $P<0.05$) and in Bp+rGH-ql versus Bp-ql (17.1 ± 4.6 vs 9.3 ± 3.5 ; $P<0.05$). The $\Delta\%$ BMD at the lumbar spine had increased more in subgroup Bp+rGH-qt in comparison with subgroup Bp+rGH-ql, but not significantly. The $\Delta\%$ BMD at the distal radius (% BMD) showed a significant increase in subgroup Bp+rGH-qt versus subgroup Bp-qt (6.0 ± 4.2 vs 1.8 ± 2.8 ; $P<0.05$) and in subgroup Bp+rGH-ql versus subgroup Bp-ql (5.7 ± 4.0 vs 1.7 ± 2.7 ; $P<0.05$) already at T1, and it increased further at T2 (9.9 ± 2.1 vs 4.4 ± 3.0 and 9.2 ± 2.3 vs 4.1 ± 3.2 ; $P<0.05$ in both cases). The $\Delta\%$ BMD at ultradistal radius showed the same significant increase in subgroup Bp+rGH-qt versus subgroup Bp-qt (15.4 ± 3.7 vs 5.1 ± 4.3 ; $P<0.05$) and in subgroup Bp+rGH-ql versus subgroup Bp-ql (14.8 ± 3.8 vs 4.9 ± 4.1 ; $P<0.05$), both at T1 and at T2 (23.9 ± 4.7 vs 9.7 ± 4.7 and 22.6 ± 4.2 vs 9.1 ± 4.4 ; $P<0.05$ in both cases). Furthermore, subgroup Bp+rGH-qt showed a greater, but not significantly, increase in densitometry values (distal and ultradistal radius) compared with subgroup Bp+rGH-ql (Fig. 1).

Table 2 Clinical data for the two groups of patients (group bisphosphonate (Bp) + recombinant human GH (rGH) and group Bp): 12 months before the start of rGH (T-1), at the start of rGH in group Bp+rGH (T0), at 6 and 12 months of rGH treatment (T1 and T2 respectively) and in the intervals between time points (growth velocity and number of fractures). Data are expressed as mean $\pm$ s.d.

Time	Height (cm)		Height (SDS)		Weight (kg)		Bone age (years)		Growth velocity (cm/year)		Fractures/year (no.)	
	Group		Group		Group		Group		Group		Group	
	Bp+rGH	Group Bp	Bp+rGH	Group Bp	Bp+rGH	Group Bp	Bp+rGH	Group Bp	Bp+rGH	Group Bp	Bp+rGH	Group Bp
T-1	112.5 $\pm$ 8.1	111.7 $\pm$ 7.8	-1.5 $\pm$ 1.5	-1.7 $\pm$ 1.6	18.5 $\pm$ 2.1	19.0 $\pm$ 1.9	7.6 $\pm$ 1.7	7.5 $\pm$ 1.8	4.4 $\pm$ 1.3	5.0 $\pm$ 1.2	4	3
T0	117.0 $\pm$ 7.9	116.8 $\pm$ 6.7	-1.4 $\pm$ 1.4	-1.5 $\pm$ 1.4	20.2 $\pm$ 1.8	20.5 $\pm$ 1.7	7.2 $\pm$ 1.4 [†]	7.2 $\pm$ 1.4 [†]	7.2 $\pm$ 1.4 [†]	5.1 $\pm$ 1.0	1	2
T1	120.6 $\pm$ 6.9	119.3 $\pm$ 7.2	-1.0 $\pm$ 1.5	-1.4 $\pm$ 1.5	21.1 $\pm$ 1.6	21.7 $\pm$ 1.8	8.6 $\pm$ 1.6	8.5 $\pm$ 1.8	6.9 $\pm$ 1.5	5.2 $\pm$ 1.2	2	1
T2	124.1 $\pm$ 7.5	122.0 $\pm$ 7.4	-0.9 $\pm$ 1.4	-1.4 $\pm$ 1.3	22.0 $\pm$ 2.2	22.7 $\pm$ 2.3	8.6 $\pm$ 1.6	8.5 $\pm$ 1.8	7.1 $\pm$ 1.4 [†]	5.1 $\pm$ 1.3	3	3

* $P<0.05$ versus group Bp; [†] $P<0.05$ versus T-1 $\rightarrow$ T0. Group Bp+rGH: n 15, 7 males; Ol type I, IV, III: 9, 4, 2; molecular characterization: quantitative – qualitative: 9 (Bp+rGH 1) – 6 (Bp+rGH 2). Group Bp: n 15, 7 males; Ol type I, IV, III: 9, 5, 1; molecular characterization: quantitative – qualitative: 9 (Bp1) – 6 (Bp2).

Table 3 Biological data for the two groups of patients (group bisphosphonate (Bp) + recombinant human GH (rGH) and group Bp): 12 months before the start of rGH (T-1), at the start of rGH in group Bp+rGH (T0), at 6 and 12 months of rGH treatment (T1 and T2 respectively).

Time	PTH (normal value 10–65 pg/ml)		Oc (normal value 2.1–8.9 nmol/l)		CTx (normal value 0.150–0.450 nmol/l)		IGF1 (normal value 20–65 nmol/l)	
	Group Bp+rGH	Group Bp	Group Bp+rGH	Group Bp	Group Bp+rGH	Group Bp	Group Bp+rGH	Group Bp
T-1	27.8±6.5	31.3±6.1	3.8±1.6	4.1±2.3	0.63±0.10	0.72±0.22	14.5±5.5	15.9±6.8
T0	31.4±7.9	35.9±6.8	3.9±1.5	3.7±2.2	0.65±0.12	0.69±0.43	17.4±6.7	18.4±5.2
T1	32.1±7.6	37.6±8.0	6.6±3.0	4.3±2.1	0.64±0.10	0.63±0.14	24.2±5.6	16.9±6.0
T2	40.4±8.2	36.4±7.2	7.4±4.6	4.4±2.9	0.71±0.22	0.80±0.38	26.2±6.3 ^{*,†}	17.3±2.3

**P*<0.05 versus group Bp; [†]*P*<0.05 versus T-1. CTx: normal values: 0.142–0.517 nmol/l.

During rGH treatment (both at 6 and 12 months), growth velocity was significantly higher in the treated subgroups (Bp+rGH-qt = 7.6 ± 1.4 cm versus Bp-qt = 5.4 ± 1.6 ; Bp+rGH-ql = 6.4 ± 1.6 versus Bp-ql = 4.3 ± 1.5 ; *P*<0.05). In the same period, growth velocity was higher in subgroup Bp+rGH-qt than in subgroup Bp+rGH-ql and in subgroup Bp+rGH-ql than in group Bp (both Bp-qt and Bp-ql), but not significantly. There were no differences in the number of fractures among the subgroups. IGF1 was the only biological data that showed a significant increase in subgroup Bp+rGH-qt compared with subgroup Bp-qt at T1 and at T2 (24.9 ± 5.4 vs 16.7 ± 4.0 and 26.8 ± 6.1 vs 17.1 ± 2.4 nmol/l; *P*<0.05 in both cases), and its levels were in the high normal range. In subgroup Bp+rGH-ql, IGF1 did not increase significantly compared with Bp-ql at T1, but it did at T2 (25.6 ± 6.0 vs 17.5 ± 2.3 nmol/l; *P*<0.05).

Discussion

The purpose of this study was to clarify whether rGH can ameliorate bone metabolism in patients with OI who had already benefited from ongoing Bp treatment. We designed an open-label randomized clinical trial (RCT) over 1 year, selecting 30 patients affected by OI types I, III, or IV in treatment with neridronate. A limitation of the study could be the fact that we did not use a placebo in the Bp group, but this decision was necessary because placebos administered subcutaneously are not acceptable according to our ethics committee standards. But on the other hand, a strength of the study is that only prepubertal children were assessed, thus removing possible sex steroid effect as confounding factors.

Histomorphometric studies in patients with OI have shown a high bone turnover rate and the frequent loss of superimposed bone, due to non-use. However, these characteristics are usually caused by impaired ambulation due to frequent fractures, deformities, and chronic pain (14). The rationale behind Bp treatment in OI is founded on various bone metabolic data (40, 41), and on the fact that Bps have achieved significant

clinical improvements in infants (21), children, and adolescents (38) suffering from moderate and severe forms of OI. In particular, their results in BMD gain and reduced fracture incidence have been proven by clinical trials (42), whereas their action in decreasing pain, increasing mobility (30), and even their positive impact on growth (43) has been seen in observational trials. Albeit there are few studies on its use in OI (27–30), it is known that rGH stimulates bone apposition obtaining an improvement in well-being, especially concerning skeletal integrity, and that it has positive effects on BMD and growth velocity (21, 26, 31).

Since in our past experience BMD and growth improved with neridronate treatment in our OI patients, compared with pretreatment and controls (38, 44), we decided to couple the prevalent inhibition of bone resorption by Bps with the anabolic effects of rGH in order to study the possible advantages of the association on BMD, projected bone areas, growth, and peripheral fracture risks. The dosage we used was the highest recommended dose for GH-deficient children (0.3 mg/week per kg). To our knowledge, this is the first controlled study using both Bps and rGH in the treatment of OI patients.

Bone mass increases throughout childhood, and adult bone mass is usually attained during the first two decades of life; however, an analysis of BMC (g) and BMD (g/cm²) relating to age, height, and weight is important since it is known that smaller bones could have a lower BMD compared with larger ones, even if the volumetric BMD (g/cm³) is eventually the same (45). A limitation of the study is that it was not possible to calculate the bone mineral apparent density and to do bone geometry, but in our study, we consider the BMD data to be reliable because, not only was our patients' increase in BMD much greater than that expected with growth, but their increase in height was also not as important as their increase in BMD. As expected, Bp treatment improved BMD in our patients in the first year of the study when treatment was based on neridronate alone. During combined Bp-rGH therapy, densitometry results showed a significant, further positive increase in DXA lumbar spine (L2–L4) at 12 months, and in DXA distal and ultradistal radius after 6 and 12 months.

Figure 1 Mean percent changes (% BMD) in relation to Time - 1 (start of study) in the BMD of the lumbar spine (A), distal radius (B), and ultradistal radius (C) of the four subgroups of patients. Point 0 (T - 1): 12 months before starting rGH; point I (T0 - T - 1): first observational year; point II (T1 - T0): first 6 months of rGH in groups Bp + rGH-qt and Bp + rGH-ql; point III (T2 - T1): last 6 months of rGH treatment. Group Bp + rGH: neridronate and rGH, *n* 15, squares; Bp + rGH-qt: quantitative: *n* 9, white squares; Bp + rGH-ql: qualitative: *n* 6, black squares; group Bp: neridronate, *n* 15, circles; Bp-qt quantitative: *n* 9, white circles, Bp-ql qualitative: *n* 6, black circles. **P* < 0.05: rGH plus neridronate treatment versus neridronate treatment alone at 12 months in both subgroups (Bp + rGH-qt versus Bp-qt, Bp + rGH-ql versus Bp-ql). ***P* < 0.05: rGH plus neridronate treatment versus neridronate treatment alone at 6 months in both subgroups.

In this period, our patients had a significant increase in projected area at the lumbar spine, which may testify a direct action on bone growth or better bone repair of vertebral shape and morphology.

In terms of growth velocity, the response was better during combined rGH-neridronate therapy than in the pretreatment period or than in the Bp group. BA did not

increase faster than CA in treated patients; thus, the improvement in growth rate may eventually ameliorate final predicted height. Some studies have reported a potential increased fracture risk in patients with OI treated with rGH, which has probably limited the use of rGH in OI to date (46). In other cases, the use of depot rGH in an OI patient with poor linear growth appeared to be effective and well tolerated, and the number of fractures did not increase after initiation of rGH therapy (47, 48). Previous treatment with Bp is probably helpful in preventing an increase in the fracture rate due to administration of rGH. In our patients, this increase in growth velocity occurred without any apparent increase in skeletal deformity, and the fracture index per year did not change at all. Certainly, the main goal of OI therapy is to improve the biomechanical quality of bone and BMD without increasing bone fragility, but the extra linear growth stimulated by rGH may be accepted as an additional beneficial effect.

As for biological data, the only significant change in metabolic and bone markers during combined treatment was the serum concentrations of IGF1. They were in the low-normal physiological range before therapy, as reported by others (49), but after 12 months of rGH treatment they increased significantly in the Bp + rGH group compared with pretreatment and to the control group. Oc levels were in the low normal range at the beginning of the study and increased, but not significantly, during rGH treatment; CTx levels were higher than normal, but did not change significantly during the study. We cannot say if the behavior of these bone metabolic markers indicates a slight reactivation of bone metabolism toward anabolic behavior, but the small variations in bone metabolic markers, compared with what we found in a previous study (33), are probably due to the fact that Bp treatment maintains a certain suppression of bone turnover.

Finally, we analyzed data on the basis of quantitative and qualitative molecular collagen synthesis defects. Both $\Delta\%$ BMD at the lumbar spine and growth velocity had a significantly higher increase in rGH-treated subgroups compared with the corresponding untreated subgroups. Nevertheless, the positive effects of rGH were not as great as necessary to completely recover the negative effects caused by a basic molecular synthesis defect (for instance, to make a moderate OI patient milder). Growth increased with rGH treatment; in quantitative defects, the increase was quite significant, whereas in qualitative defects it was still significant, though to a lesser extent. In the subgroups, there were no differences regarding the number of extra-vertebral fractures and biological data.

In conclusion, combined rGH and Bp treatment i) positively increases BMD at the lumbar spine and wrist and in the lumbar spine projected area; ii) significantly increases the rate of linear growth velocity, with no BA advancement; and iii) does not influence the peripheral fracture rate. Because of the

inadequate sample size, our study was not able to answer the question of whether rGH has an effect on the fracture rate or whether there is a risk of negative interference with bone metabolism due to long-term treatment with BPs. At any rate, the combined rGH-Bp therapy could be beneficial. It is important that further studies be carried out to investigate the interrelationships between the drugs, dosage, schedule, and duration of administration and the influence of other factors (puberty, for instance) in the therapeutic response in order to obtain definitive results. Furthermore, long-term studies are ongoing and necessary to better understand whether rGH plays a role in the treatment of children with OI. Until the efficacy and safety of these drugs have been confirmed in larger long-term studies, the use of rGH in patients with OI can only be recommended within clinical trials.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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