Novel Insights from Clinical Practice

HORMONE RESEARCH IN PÆDIATRICS

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Insulin-Like Growth Factor-I Treatment in Primary Growth Hormone Insensitivity: Effect of Recombinant Human IGF-I (rhIGF-I) and rhIGF-I/rhIGF-Binding Protein-3 Complex

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Established Facts

- Recombinant human insulin-like growth factor I (rhIGF-I) replacement therapy in growth hormone insensitivity syndrome (GHIS), unlike GH replacement therapy in GH deficiency, does not adequately restore growth.
- Pharmacological studies of a IGF-I compound containing a 1:1 molar complex of rhIGF-I and rhIGFBP-3 (rhIGF-binding protein-3) demonstrated that the complex was effective in increasing levels of circulating total and free IGF-I and that the administration in patients with GHIS should be safe, well-tolerated and more effective than rhIGF-I on its own.

Novel Insights

- We describe in this report to our knowledge the first clinical follow-up of a patient treated with various forms of IGF-I over a period of 14 years.
- The rhIGF-I/rhIGFBP-3 compound therapy seems to be not necessarily efficient in treating patients with GHIS when compared with rhIGF-I alone as
  - IGF-I concentration in the serum did not increase sufficiently as it did on isolated rhIGF-I replacement therapy;
  - acid-labile subunit (ALS) seems to be more important as previously suggested for the stability in the ternary complex of IGF-I, IGFBP-3 and ALS;
  - rhIGFBP-3 is not glycosylated and therefore nonphysiologic in its structure, and finally
  - positive pharmacological studies may not equally represent clinical effectiveness.

Key Words
Growth hormone insensitivity syndrome · Growth hormone receptor · Insulin-like growth factor-I · Growth · Childhood

Abstract
Background/Aims: Growth hormone insensitivity syndrome (GHIS) is a rare cause of growth retardation characterized by high serum GH levels, and low serum insulin-like growth factor I (IGF-I) levels associated with a genetic defect of the GH receptor (GHR) as well post-GHR signaling pathway. Based on clinical, as well as biochemical characteristics, GHIS can be genetically classified as classical/Laron's syn-

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drome and nonclassical/atypical GHIS. Recombinant human IGF-I (rhIGF-I) treatment is effective in promoting growth in subjects who have GHIS. Further, pharmacological studies of a IGF-I compound containing a 1:1 molar complex of rhIGF-I and rhIGF-binding protein-3 (BP-3) demonstrated that the complex was effective in increasing levels of circulating total and free IGF-I and that the administration in patients with GHIS should be safe, well-tolerated and more effective than rhIGF-I on its own. ***Patient/Methods:*** We describe the long-term effect of various IGF-I preparations (rhIGF; rhIGF-I/rhIGFBP-3) in a single subject treated for more than 14 years while focusing on height, height velocity as well as on additional auxological and laboratory data. ***Results:*** This study confirms that rhIGF-I is effective in promoting growth in children with GHIS. However, on the combined rhIGF-I/rhIGFBP-3 treatment as well as off rhIGF-I therapy the height velocity decreased drastically (2 and 1.8 cm vs. overall 6.5 cm/year on rhIGF-I, respectively). On rhIGF-I treatment, serum IGF-I was found to be well within the normal range, whereas serum IGFBP-3 remained low. On the rhIGF-I/rhIGFBP-3 compound therapy, however, serum IGFBP-3 increased into the normal range, which was not the case for serum IGF-I. Importantly, the increase of the serum IGFBP-3 level excludes noncompliance. In addition, body mass index as well as dual-energy X-ray absorptiometry analysis underlined the positive effect of rhIGF-I treatment on body composition. ***Conclusions:*** The rhIGF-I/rhIGFBP-3 compound therapy seems to be not efficient in treating this individual patient with GHIS when compared with rhIGF-I alone.

Introduction

Growth hormone insensitivity syndrome (GHIS) is a rare cause of growth retardation with only about 300 subjects worldwide described so far. It is characterized by high serum GH and low serum insulin-like growth factor I (IGF-I) levels mainly associated with a genetic defect of the GH receptor (GHR) as well post-GHR signaling pathway [1, 2]. Based on clinical, as well as biochemical characteristics, GHIS can be genetically classified as classical/Laron's syndrome and non-classical/atypical GHIS [1, 2]. As determined in short- and long-term studies, recombinant human IGF-I (rhIGF-I) treatment is effective in promoting growth in these subjects [3]. Moreover, pharmacological studies of an IGF-I compound containing a 1:1 molar complex of rhIGF-I and rhIGFBP-3 (IGF-binding protein; Insmed, Inc., Glen Allen, Va., USA) demonstrated that the complex was effective in increasing levels of circulating total and free IGF-I and that the administration in patients with GHIS should be safe and well-tolerated [4]. In addition, it was even hypothesized that this complex mimics the physiological effects of IGF-I with a more favorable pharmacokinetic profile [4].

In this report we describe the long-term effect of various IGF-I forms (rhIGF, rhIGF-I and rhIGFBP-3) in a previously reported Tamil family [5].

Subjects and Methods

Subjects

The family history as well as the effect of the first intermittent rhIGF-I therapy (Igef®; Pharmacia & Upjohn, Dübendorf, Switzerland) has been previously described in detail [5].

In summary, the parents and the first child (girl) were of normal phenotype, whereas the second child (boy) was presented at the age of 7 months with severe growth retardation [58 cm, –6.1 standard deviation score (SDS); 5.7 kg, –3.4 SDS] and the clinical features of isolated GH deficiency to the outpatient clinic of the University Children's Hospital in Bern, Switzerland. Based on the normative data of the First Zurich Longitudinal Study of Growth and Development [6], height and weight data are expressed as SDS [6]. Detailed assessment revealed GHIS with absent GH-binding protein (GHBP). Of note, birth weight (37th week of pregnancy: 2.9 kg, –0.2 SDS) as well as length (48 cm, –0.1 SDS) were in the normal range [6], but growth failure became obvious during the first 6 months of life. After the diagnosis, rhIGF-I (Igef®; 80 μg/kg) was subcutaneously injected twice daily after meals. Because of supply problems with the rhIGF-I, the treatment was interrupted several times giving the opportunity to analyze the impact of this intermittent long-term replacement therapy (5.5 years of therapy within a follow-up period of 8 years) before puberty.

However, as one of the most limiting factors for the clinical use of IGF-I is the shortage of the drug, a new rhIGF-I compound was developed which contained a 1:1 molar complex of rhIGF-I and rhIGFBP-3 (SomatoKine®, Insmed, Inc.; 1 mg/kg/day, equivalent to 200 μg/kg/day). This new form was used in two doses (200 and 400 μg/kg/day) in this patient, which was replaced thereafter by another rhIGF-I (Increlex®, Tercica, Ipsen, Germany; 120 μg/kg twice daily). Following the calculation of the body mass index (BMI; kg/m^2), the values were transformed into SDS according to the normative values obtained from the First Zurich Longitudinal Study of Growth and Development [6]. Height velocity was calculated if height measurements were available in at least a 6-month interval. Radiographs were taken of the left hand and wrist for assessment of skeletal age according to Greulich and Pyle [7]. The experimental protocol was approved by the Ethical Committee of the University Children's Hospital as well as by the equivalent of Swissmedic (governmental office in charge to supervise the drugs used not registered in Switzerland). Informed consent was obtained from the parents.

Hormonal Measurement, Laboratory Methods

The serum GH was measured by the DSL-10-1900 Active® human GH ELISA kit (Diagnostic Systems Laboratories, Webster, Tex., USA), IGF-I and IGFBP-3 measurements were performed

using the IGF-I solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 1000® IGF-I; DPC, Los Angeles, Calif., USA). Serum GHBP was determined by the DSL-10-48100 Active® human GHBP ELISA kit (Diagnostic Systems Laboratories). Serum acid-labile subunit (ALS) levels were measured with a commercial ELISA (Diagnostic System Laboratories). Detection limit: 0.7 ng/ml; interassay coefficient of variation was 8.2%. All tests were standardized for our laboratory, and own normative values were obtained.

To analyze the appropriate peak serum concentrations of IGF-I and IGFBP-3, the blood samples were obtained 1, 3 and 12 h following rhIGF-I and/or rhIGF-I/rhIGFBP-3 injections [4, 8].

Complete blood count, glucose, electrolytes, urea nitrogen, creatinine, calcium, phosphorus, liver enzymes, TSH, fT4, fT3, lipids and glycated hemoglobin were measured using standard automated techniques and determined at the central facility of the University Hospital.

Body Composition

Whole-body and lumbar spine bone mineral density (BMD), bone mineral content, fat and lean body mass were measured using dual-energy X-ray ubsorptiometry (DEXA) (Hologic QDR 4500, pediatric software version 5.64; Hologic, Waltham, Mass., USA) as previously described [9, 10]. To take into account the influence of vertebral growth between each measurement, the lumbar spine BMD (g/cm^2) was corrected using a simple mathematical method [11]. Briefly, volume density (d) of the lumbar spine, approximately a cylinder of height (h), and diameter (A; A and h are obtained from DEXA analysis), was expressed in g/cm^3. The equation we used was: $d = 4/\{pi\} \times h \times BMD/A$ [11]. The volume-related BMD (d) was a constant value of 0.255 ± 0.015 g/cm^3 [11].

Statistics

When appropriate, data are expressed as the mean ± SD. Only descriptive statistics are presented, as it was not appropriate to perform statistical hypothesis testing due to small sample size.

Results

Follow-Up on rhIGF-I Treatment; Effects of IGF-I Treatment on Growth

The clinical diagnosis and molecular analysis has been previously reported [5]. Briefly, separate PCRs of each of the exons of the GHR gene revealed that exon 5 in the patient was missing. Thereafter, 'Long PCR' from exons 4 to 6 revealed a 4,097-bp deletion encompassing exon 5, in a homozygous state in the patient and in a heterozygous state in both parents. RT-PCR analysis revealed an exact absence of exon 5 resulting in a frame shift, leading to a stop codon in exon 6, which predicts a truncated, non-functional GHR protein. For IGF-I supply reasons this affected boy was intermittently treated with various IGF-I's. Most interestingly, as depicted, the growth pattern in relation to the rhIGF-I therapy could be followed over a period of more than 14 years (fig. 1). The details of the overall auxological evaluations are shown in table 1. It is important to stress that except for the period where the rhIGF-I/rhIGFBP-3 compound was used, the rhIGF treatment resulted in a significant increase of height velocity (Δ height SDS; table 1; fig. 1). However, on the rhIGF-I/IGFBP-3 treatment as well as off IGF-I, the height velocity decreased drastically and height did not increase adequately [2 vs. 5.6 cm (Igef®) and 7.2 cm/year (Increlex®), respectively]. On isolated rhIGF-I treatment, IGF-I in the circulation was well within the normal range, whereas the IGFBP-3 remained low (table 2). On the rhIGF-I/rhIGFBP-3 compound therapy however, IGFBP-3 could be increased into the normal range, which was not the case for IGF-I concentration in circulation (table 2). Further, the increase of rhIGF-I/rhIGFBP-3 to the higher dose of 400 μg/kg/day (2 mg/kg/day) for 1 month did not result worthy of mention in any serum IGF-I change, whereas IGFBP-3 further increased (table 2). It is of importance that IGF-I as well as IGFBP-3 values were taken 1, 3, and 12 h after the injection of either rhIGF-I or rhIGF-I/rhIGFBP-3. For rhIGF-I only the peak serum concentration was obtained at 1–3 h, and at 12 h around 65% of the peak values were measured whereas on the combination rhIGF-I/rhIGFBP-3 the concentration remained constantly low. Therefore, in the table 2, as stated in the legends, the mean values are given. Whenever the IGF-I 24-hour area under the curve was compared, the IGF-I levels obtained on both 200 μg/kg/day as well 400 μg/kg/day were far below (50–60%) the concentrations found, when rhIGF-I alone was used.

Importantly, as far as the combination therapy is concerned, the increase of IGFBP-3 level underlines the fact that this patient as well as his family was compliant. Further, during puberty serum IGF-I and IGFBP-3 data are given in puberty-adjusted normative values as well (table 2).

BMI, Body Composition and Circulating Levels of ALS and Lipids, Glucose, Glycated Hemoglobin and Homeostasis Model Assessment

As on all the rhIGF-I treatment regimens the BMI increased (table 1), DEXA was performed in order to compare total body fat content (%) and BMD (g/cm^2; Z-scores). The increase of BMI was mainly based on an increase of total body fat content as well as bone density (table 1). The point is that body weight, mainly fat, increases out of proportion to linear growth; that is not the case with GH deficiency (GHD) children receiving GH where BMI (and SDS) may actually decrease. Plasma cholesterol was in the normal range and increased modestly over time (3.92

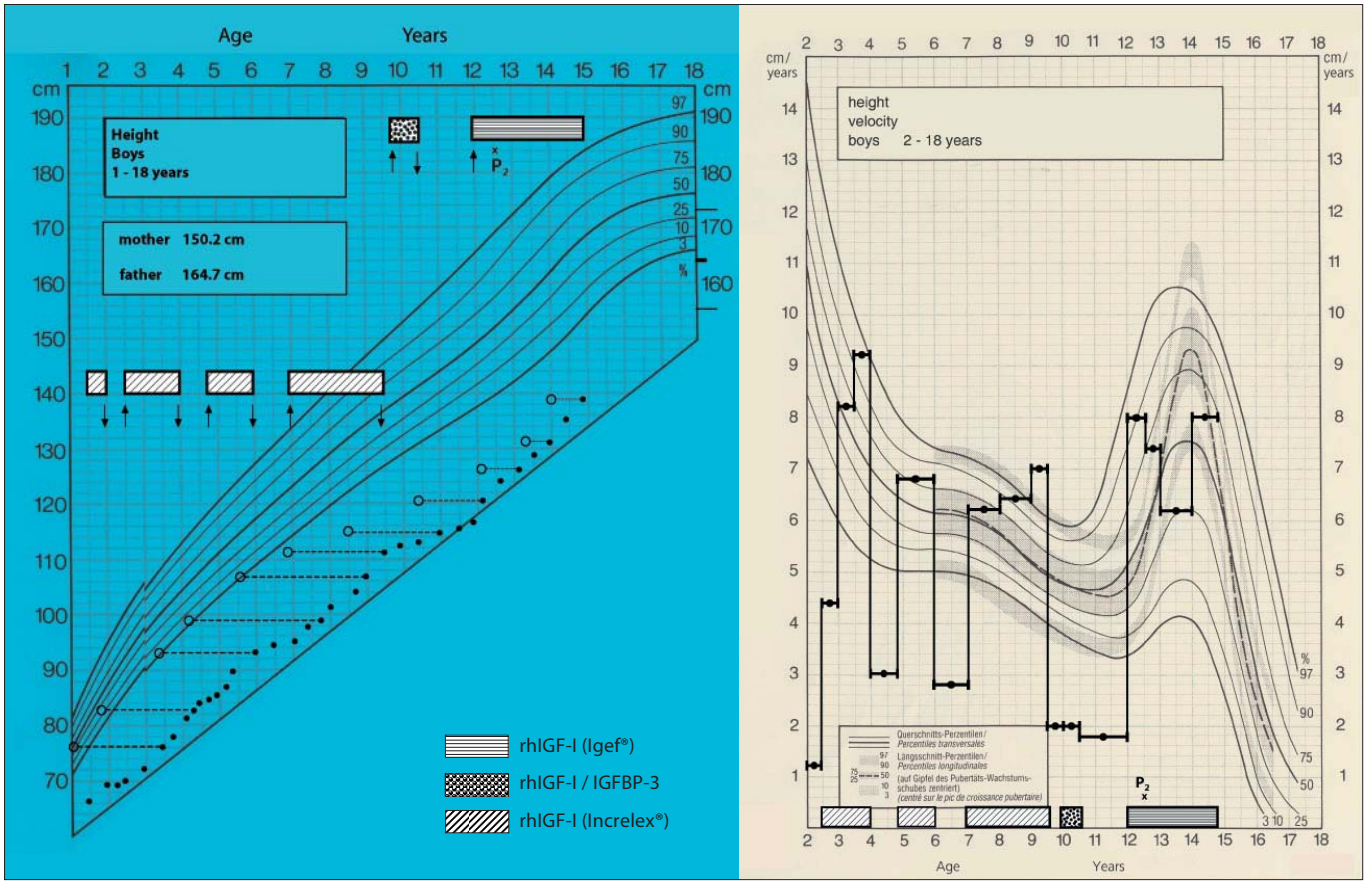

Fig. 1. a Growth chart of the patient. Percentiles are shown on the extreme right. ● = Height measurements, ○ = bone ages. The arrow pointing up marks the beginning of either rhIGF-I or rhIGF-I/rhIGFBP-3 therapy, whereas the arrow pointing down the end of the therapy. Parental target height is indicated at the right side of the chart. The various rhIGF-I are also indicated. * P2 = Pubertal stage 2, testicles' size of 4 ml. **b** Height velocity chart of the patient. The various height velocities are presented according the treatment. * P2 = Pubertal stage 2, testicles' size of 4 ml.

± 0.6 vs. 4.35 ± 0.7 mmol/l, respectively; normal <5 mmol/l). Triglyceride concentrations increased on rhIGF-I (1.13 ± 0.15 vs. 1.56 ± 0.2 mmol/l; normal <2 mmol/l). No changes in serum ALS were observed following the administration of any rhIGF-I form.

Further no consistent effects of any IGF-I therapy were observed on white cell blood count, red cell indexes, platelets, reticulocytes, total serum protein, albumin, calcium, phosphorus, liver enzymes, serum electrolytes, TSH, fT4 and fT3 analyses.

In addition, no apparent effect on insulin sensitivity as estimated by homeostasis model assessment (HOMA 1.3 ± 0.3) [12] was noticed, and glycated hemoglobin concentrations were normal and unchanged throughout all the years (4.6 ± 0.6%; normal 4.1–5.7%).

Adverse Events

After the diagnosis, rhIGF-I and rhIGF-I/rhIGFBP-3 were subcutaneously injected twice and once daily after meals, respectively. Besides the impressive lipohypertrophy [5], no side effect which could be attributed to this therapy was noted. In particular, no hypoglycemic episodes were observed as the injection was given following a meal and the blood sugar was closely followed. Further, no signs or symptoms of anaphylaxis, allergic, or local reactions at injection site were observed. The boy experienced some pain with injections of either rhIGF-I only or the combined therapy. Overall, no difference was noted focusing on any adverse event.

Table 1. Auxological characteristics of the patient according to the treatment

Age years	IGF-I treatment	Duration years	Height SDS start end	Δ height SDS	Overall height velocity, cm/year	BMI start/end	Δ BMI	BMI SDS start/end	Δ BMI SDS	Total body fat, %	BMD g/cm^2 Z-score
1.5–9.5	On/off[a] Igef® rhIGF-I Pharmacia & Upjohn 2 × 80 μg/kg/day	9.5	–7.1/–5.6	1.5	5.6	16.1/19.2	3.1	–0.26/0.96	1.22	20.8[b] 25.3 ± 2.1[c]	–2.1 SD[b] –1.6 ± 0.7 SD[c]
9.5–9.7	Off treatment	0.2	–5.6/–5.2	0.5	2	19.2/18.9	–0.3	0.96/0.74	–0.22	21.5	–1.5 SD
9.7–10.5	SomatoKine® IGF-I/IGFBP3 Insmed 1 mg/kg/day	0.8	–5.2/–5.5	–0.3	2	18.9/20.5	1.6	0.74/1.18	0.44	24.3	–1.2 SD
10.5–12	Off treatment	1.5	–5.5/–5.3	0.2	1.8	20.5/21.6	1.1	1.18/0.84	–0.34	22.3	–1.4 SD
12–14.8	Increlex® rhIGF-I Tercica/Ipsen 2 × 120 μg/kg/day	2.8	–5.3/–3.6	1.7	7.2	21.6/24.1	2.5	0.84/1.4	0.56	26.7 ± 2.8	0.9 ± 0.4 SD

[a] Data partially published [5]; [b] at the beginning of IGF-I replacement therapy; [c] on rhIGF-I replacement therapy.

Discussion

GH insensitivity describes a range of disorders with demonstrable resistance to the action of GH [13]. There are two main etiological categories: the first comprises genetic disorders caused by mutations in genes that play a role in the GH-IGF-I axis, and the second category consists of disorders with acquired GHI. However, classical GHIS caused by a genetic abnormality of the GHR and/or GHR signaling is rare. Its phenotype resembles that of GHD, but with high levels of circulating GH, where also deficiencies of GH-dependent proteins, including IGF-I, IGF-BP3, and ALS are seen.

Before the development of rhIGF-I, no effective treatment could be offered to patients with GHIS. But over the last 20 years, many studies have been performed demonstrating the benefit of rhIGF-I [14–20]. In a recent study, Chernausek et al. [21] report that rhIGF-I treatment has a prolonged effect up to 12 years on growth in children with GHIS. Although most subjects did not experience sufficient catch-up growth in order to reach their target height, it seems that they achieve adult heights significantly taller than expected in the absence of rhIGF-I therapy [21]. A possible explanation for the reduced biological effect of rhIGF-I is the pharmacokinetics of this compound [22]. Grahnen et al. [22] analyzed clearance and half-life values in both healthy controls and patients with classical GHIS. In patients with GHIS there was a more rapid turnover of IGF-I, with clearance and half-life values calculated to be 0.6 mg/min/kg body weight and 6 h, respectively; whereas the values in healthy controls were found to be 0.2 mg/min/kg and 20 h. The increased clearance and decreased half-life are likely to be caused by the low serum IGFBP-3 and ALS production/concentration in subjects with GHIS [20]. Therefore, to prolong the half-life and to decrease the clearance rate, the IGF-I/IGFBP-3 combination (SomatoKine®) was developed. It was produced by the expression of recombinant IGF-I and IGFBP-3, which were separately partially purified and combined as a 1:1 molar complex due to the very high (nanomolar) dissociation constant (K_d) [23].

After extensive in vitro as well as animal studies, it was also reported in patients with GHIS that rhIGF-I/rhIGFBP-3 mimics the physiological effect of IGF-I with a far better pharmacokinetic profile [4, 24, 25]. Upon entering the circulation, rhIGF-I/rhIGFBP-3 binds to ALS to form the ternary complex representing the natural form of IGF-I in circulation [26]. In a pharmacokinetic study, Camacho-Hübner et al. [4] concluded that the rhIGF-I/rhIGFBP-3 complex was effective in increasing levels of circulating total and free IGF-I into the normal range for a prolonged period after a single subcutaneous administration. In this study the drug was well-tolerated and did not cause acute side effects. Therefore, rhIGF-I/rhIGFBP-3 warrants additional evaluation in clinical studies for the treatment of IGF-I deficiency caused by

genetic defects of the GHR or to acquired severe GH resistance and in syndromes of severe insulin resistance [4].

As far as rhIGF-I treatment is concerned, our findings come without surprise and confirm previous reports showing that rhIGF-I is effective in promoting growth in children with GHIS with a growth response being less than observed when GH replacement is given to severely GH-deficient (GHD) children. Children who have GHD normally achieve adult heights within normal range; this does not hold true in those with GHIS [21, 27]. Alleged reasons may be that actions of IGF-I involve both endocrine and paracrine/autocrine mechanisms, but by injecting rhIGF-I only the endocrine compartment has been partially restored [28]. Furthermore, blood-borne IGF-I complexes normally with IGFBP-3 as well as ALS, which all depends on sufficient GH secretion definitively not being corrected by rhIGF-I application only. Therefore, as an additional consequence following rhIGF injections, IGF-I clearance and half-life may be negatively altered [22]. Along that avenue it is easy to follow that a combined treatment with rhIGF-I and rhIGFBP-3 may improve the therapeutic efficacy [4]. However, as it has been shown that ALS is important for stabilizing the IGF-I/IGFBP-3 complex and, in addition, extends its half-life, the lack of increase of ALS in these circumstance of GHIS may question the rationale for the rhIGF-I/rhIGFBP-3 complex [29]. In addition, rhIGFBP-3 is not glycosylated and therefore nonphysiologically present in its compound form.

Unfortunately, in our patient the appropriate replacement therapy of SomatoKine, 1 mg/kg/day, equivalent to 200 μg/kg/day, did not result in any effect and, therefore after nearly 1 year the treatment was discontinued (tables 1, 2; fig. 1). Since the injection volumes using the rhIGF-I/rhIGFBP-3 compound are much larger, compliance may have been a factor, however the patient and family were very keen on this therapy, which is also mirrored in the increased values of serum IGFBP-3 (table 2). Of interest, however, is that serum IGF-I did not increase adequately, neither after 3 nor 12 h following the injection. The same finding holds true when the rhIGF-I/rhIGFBP-3 compound was increased for 1 month up to 400 μg/kg/day, a dose which was reported to be more effective in restoring height velocity [30]. However, while studying the data reporting the improved effect, whenever the higher rhIGF-I/rhIGFBP-3 dose (200 vs. 400 μg/kg/day) was used, in detail (height SDS, pretreatment height velocity, etc.) the 1-year results have to be taken with caution [30, 31].

Table 2. Clinical and laboratory findings

	rhIGF-I (Iget®; Pharmacia & Upjohn) before and on 80 μg/kg twice daily					rhIGF-I/rhIGFBP-3 (SomatoKine®; Insmed Inc.) before and on 1 and 2 mg/kg/day			rhIGF-I (Increlex®, Tercica, Ipsen) before and on 120 μg/kg twice daily	
	before	on	before	on	before	on	before	on 1 mg/kg/day	on 2 mg/kg/day	before	on
Age, years	2.0	2.5–4	4.5	4.8–6	6.5	7–9.5	9.0	9.7–10.2 10.4–10.5	10.3–10.4	12	12.6–14.8
GH basal, ng/ml	125	ND	134	ND	129	ND	135	ND	ND	137	ND
IGF-I, ng/ml (SDS)[a]	<25 (<–2.8)	127 (0.1)	<25 (<–2.7)	196 (0.8)	<25 (<–2.8)	204 (0.6)	<25 (<–3)	87 (–2.0)	95 (–1.8)	<25 (<–3.5)	505 (0.5) [+3.7]
Normal mean; range	125; 51–303	118; 49–289	119; 50–286	124; 52–297	134; 57–316	148; 64–345	169; 74–388	200; 88–452	200; 88–452	395; 183–850	395;183–850 [159; 49–342]
Mean SDS ± SD[b]		0.4 ± 0.2		1.0 ± 0.3		1.2 ± 0.3		–1.6 ± 0.4	–1.6 ± 0.4		0.7 ± 0.2 [3.2 ± 0.4]
IGFBP-3, mg/l (SDS)[a]	0.48 (–2.6)	0.93 (–1.8)	0.41 (–2.8)	0.95 (–2.5)	0.39 (–3.1)	0.89 (–2.8)	0.45 (–3.5)	4.4 (0.2)	5.7 (0.7)	<0.5 (<–4.1)	1.0 (–3.6) [–2.3]
Normal mean; range	1.8; 0.8–3.9	2.0; 0.9–4.3	2.4; 1.1 ± 5.2	2.7; 1.3 ± 5.6	2.9; 1.4–6.1	3.2; 1.6–6.5	3.6; 1.8–7.1	4.1; 2.1–7.7	4.1; 2.1–7.7	5.4; 3.1–9.5	5.4; 3.1–9.5 [3.6; 1.4–5.2]
Mean SDS ± SD[b]		–1.8 ± 0.2		–2.3 ± 0.3		–2.3 (0.4)		–0.3 ± 0.5			–3.2 ± 0.8 [–2.2 ± 0.3]

ND = Not done; [a] at 3 months after beginning; [b] during treatment. As IGF-I and IGFBP-3 were measured 1, 3, and 12 h following the injections, the values on treatment are given as mean. IGF-I and IGFBP-3 Immulite® measurements; range: 5–95 percentile [pubertal adjustments; Tanner-specific normative values].

A faulty rhIGF-I/rhIGFBP-3 complex leading to an increase of serum IGFBP-3 but not IGF-I can be hypothesized, but it is more likely that the rhIGF-I did not effectively leave the subcutaneous depot. Although it cannot be an enzyme, like insulinase eliminating insulin, which destroys rhIGF-I in the subcutaneous tissue, the rhIGF-I component is very likely to be less resistant to degradation than rhIGFBP-3. Other causes to be discussed that were excluded: (a) lack of rhIGF-I in the batch used, however we measured the concentration of rhIGF-I and rhIGFBP-3 in one vial of the batches used and were able to confirm the given concentration but no functional tests in cell lines were performed, and (b) insufficient dose: we increased the dose for 1 month up to 2 mg/kg/day, this also without any effect. In that situation the isolated increase of serum IGFBP-3 may have even had a negative impact.

Although IGFBP-3 is the major carrier for circulating IGFs in postnatal life, it has been shown to have not only IGF-I-dependent but also IGF-independent effects on proliferation of several cancer cell lines, but also to affect chondrocyte proliferation and differentiation [32–34]. Further, overexpression of IGFBP-3 in transgenic mice exhibited in the presence of increased IGF-I levels a significant reduction in both birth weight and litter size, so was the early postnatal growth [35]. Taken all together, these data may highlight that the IGF-I-independent effect of IGFBP-3 has to be taken into account and cannot be neglected when patients are treated with a rhIGF-I/rhIGFBP-3 combination. However, another explanation would be that if IGFBP-3 rises, IGF-I should also increase, but the IGFBP-3 measured in circulation is somehow degraded, so it may still be measured in the assay, but does not retain IGF-I. However, in this circumstance an increase of serum IGF-I should be found most likely 1 and/or 3 h after the rhIGF-I/rhIGFBP-3 injection, what was not the case at all.

To summarize, we describe in this report to our knowledge the first clinical follow-up of a patient treated with various forms of IGF-I, emphasizing the ineffectiveness of the compound rhIGF-I/rhIGFBP-3 treatment in this patient suffering from GHIS when compared with the isolated rhIGF-I therapy. Although actually the rhIGF-I/rhIGFBP-3 compound is off market, the previous off-label promotion of this replacement therapy may underline the restricted impact of limited pharmacological studies and that in these circumstances only rigorously controlled investigative protocols may provide more detailed information. This is of high interest as well as of scientific importance, as manufacturers repeatedly state that the necessity of rhIGF-I replacement is to expand to any GHIS in the broadest sense such as idiopathic short stature, and Noonan syndrome [36, 37].

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