Long-term treatment of Laron type dwarfs with insulin-like growth factor-1 increases serum insulin-like growth factor-binding protein-3 in the absence of growth hormone activity

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Insulin-like growth factor binding protein-3 (IGFBP-3) is the major carrier of insulin-like growth factor I (IGF-I) in serum, and its production is growth hormone (GH) dependent. It is unclear whether in humans IGFBP-3 production is directly regulated by GH or mediated via IGF-I. We addressed this question in six patients with Laron-type dwarfism, a syndrome characterized by the absence of GH receptor activity (LTD), who were chronically treated with recombinant IGF-I. Analysis of the electrophoretic profiles of serum IGFBPs in these patients by Western ligand blotting revealed an extremely low IGFBP-3 level. A striking progressive increase in serum IGFBP-3 was observed with continuous treatment, despite the absence of GH action. In LTD children, serum IGFBP-3 increased up to 19-fold after six months of therapy and equalled levels observed in controls, whereas in adult LTD patients the increase was smaller. A rise in serum levels of 34, 30 and 24 kDa BPs (presumably IGFBP-2, -1 and -4, respectively) was also noted with chronic IGF-I therapy. This proof of GH-independent induction of IGFBP-3 by IGF-I may be a major advantage in the therapeutic use of biosynthetic IGF-I in several types of short stature children.

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IGF-I is carried in body fluids by specific high-affinity binding proteins that modulate its metabolic and proliferative actions (1–5). While the precise mechanism by which IGFBPs modulate the interaction of IGF-I with its receptor is unclear, the regulation of IGFBPs seems crucial to the availability of free IGF-I, its activity and clearance. To date, six IGFBPs have been identified, sequenced and termed IGFBP-1 through IGFBP-6 (6, 7). Understanding of the regulation of IGFBP synthesis is incomplete. IGFBP-1 and IGFBP-2 are inversely related to plasma GH and insulin levels (8–10), while IGFBP-3, the predominant binding protein in serum, is GH dependent (1, 11). Administration of IGF-I to cell cultures and animal models has been shown to induce an increase in IGFBP-3 (12–15). However, it is unclear whether in humans an elevation in IGFBP-3 can be achieved by IGF-I in the absence of GH activity.

Laron-type dwarfism (LTD) is an inherited syndrome characterized by extremely low serum IGF-I levels, despite high levels of biologically active GH and by resistance to exogenous GH (16–18). The disease is caused by defects in the GH receptor gene, leading to the absence of functional GH receptors (19, 20). Therefore, LTD is a human model to study the hormonal regulation of growth, in the complete absence of GH action. To explore the isolated effect of IGF-I on its binding proteins, we analyzed the electrophoretic profiles of serum IGFBPs in LTD patients chronically treated with recombinant IGF-I.

Materials and methods

Subjects

Six proven LTD patients (two children and four adults) whose pertinent data are given in Table 1 were investigated. All had extreme short stature, looked like GH-deficient patients, had low IGF-I levels which did not rise upon five daily hGH injections and had undetectable serum GH binding protein (21). Patients were treated with recombinant human IGF-I (FK 780 Lot 115707K, Fujisawa Pharmaceutical Co, Osaka, Japan) (22), given subcutaneously once daily in a dose of 120–150 μg/kg for a six-month period. Blood was sampled after an overnight fast before the first injection. The following blood samples were taken 24 h after the last subcutaneous IGF-I administration, at the specified intervals during the chronic therapy with recombinant IGF-I. Plasma and serum samples were frozen until assayed for
IGF-I, GH, insulin and IGFBPs. The study was approved by the Hospital’s Ethics Committee and informed consent obtained from all parents and/or adult patients.

Hormone assays

Serum IGF-I was determined by RIA after acid-ethanol extraction followed by cryoprecipitation. The RIA incorporated rabbit anti-IGF-I antiserum B01066S and recombinant IGF-I (Fujisawa Pharmaceutical Co, Osaka, Japan). The sensitivity of the method was 25 pmol/l, the interassay coefficient of variation (cv) was 4.7% and the intraassay cv was 8%. Plasma GH was measured by a double antibody RIA using HGH-RP (NPA, MD) as a standard. The sensitivity of the assay was 0.5 µg/l. Interassay cv was 12.5% and intraassay cv 16.5%. Plasma insulin was measured using a commercial RIA (DPC, CA). Serum GH binding protein was determined as previously reported (21).

Western ligand blots

Serum samples of all LTD patients before and during IGF-I therapy were analyzed. For each LTD patient, serum of an age- and sex-matched control was examined in parallel. Recombinant IGFBP-1 (gift from Professor M Seppala, Helsinki, Finland) was analyzed in parallel and served as an internal control. The procedure for IGFBP identification was essentially that of Hossenlopp et al. (23). Serum samples (3 µl) were analyzed by electrophoresis on 12% SDS-polyacrylamide gels under non-reducing conditions. Molecular weight standards (BioRad, CA) were run in parallel lanes. The size fractionated proteins were electroblotted onto nitrocellulose paper. After extensive washings, the filter was incubated overnight with $2 \times 10^6$ cpm [$^{125}$I]IGF-I (Amersham, UK) at 4°C. Radiolabelled IGFBPs were visualized by autoradiography. Autoradiographies were quantitated by computing laser densitometry (Densitometer 300A, Molecular Dynamics, CA).

Results

Table 1 summarizes the hormone data of LTD patients before and during chronic treatment with exogenous recombinant IGF-I. Prior to treatment all the LTDs had extremely low serum IGF-I levels. A steady increase in serum IGF-I levels was observed in treated patients. This increase was fivefold in the adult LTD patients and up to 18-fold in one of the children. No change in plasma GH levels was observed in adults, while in children a decrease of around 50% was observed. A similar decline in fasting plasma insulin levels was noticed in the adult LTD patients.

To determine the changes in IGFBPs during the chronic treatment of LTD patients with exogenous recombinant IGF-I, serum samples were analyzed by Western ligand blotting. Fig. 1 shows the Western ligand blot profile of IGFBPs in serum samples from LTD patients of three different ages along the 24 weeks of IGF-I therapy. For each LTD patient, serum of an age- and sex-matched control was analyzed in parallel. In this blot IGFBP-3 appears as a doublet migrating at a Mr of $\sim 40-43$ kDa (1, 9) and IGFBP-2 as a single band at $\sim 34$ kDa (9). Though not identified by a specific antibody, the band at 30 kDa is most probably IGFBP-1 (23), as it co-migrated with recombinant IGFBP-1 analyzed in parallel (not shown), whereas the band at 24 kDa presumably represents IGFBP-4 (24, 25). The autoradiographs from all patients were quantitated by densitometry and levels
of each binding protein were evaluated. The alterations in IGFBP levels during IGF-I treatment are summarized in Figs. 2 and 3.

Serum IGFBP-3 levels were extremely low in LTD patients compared with controls in both children and adults (Figs. 1, 2), as previously reported (9, 26, 27). The relative amount of serum IGFBP-3 in LTD children was only 5% of age-matched controls (Fig. 1, left panel), while mean serum IGFBP-3 in the adult LTDs was 16% of adult control levels (Fig. 2, right panel). Chronic treatment with exogenous recombinant IGF-I induced a progressive rise of serum IGFBP-3 in all patients. In children, the increase in serum IGFBP-3 was striking and after six months of therapy IGFBP-3 levels were almost as high as in age-matched controls (Fig. 2, left panel). In adult LTDs, the increase in IGFBP-3 was less dramatic and serum levels remained lower than in controls (Fig. 2, right panel). The increase in IGFBP-3 was a slowly progressive process, and closely paralleled the increase in serum IGF-I. After one week of therapy, IGFBP-3 was only slightly increased in most patients, with a reduction in IGFBP-3 in two patients as previously described (27). The increase in IGFBP-3 was evident in all patients after four weeks of therapy.

IGFBP-2 was the most prominent IGFBP in serum of LTD children (Fig. 1) and its levels were 200–380% higher than in controls, while in adult LTD patients IGFBP-2 levels resembled that found in controls (Fig. 3). Chronic administration of IGF-I provoked a steady rise in serum IGFBP-2 which was more evident in adults whose serum IGFBP-2 levels were relatively lower before therapy (Fig. 3).

The 30 kDa IGFBP, most probably representing IGFBP-1, was higher in LTD children compared with controls, while in adult LTD patients its level resembled controls (Fig. 3). An early consistent rise in serum IGFBP-1 was noted in most LTD patients with chronic IGF-1 treatment (Fig. 3). This increase was inversely correlated to the decrease in fasting plasma insulin levels seen with IGF-I therapy.

Serum levels of the 24 kDa binding protein, presumably representing IGFBP-4, were not consistently different from those in controls. Nevertheless, a steady increase was observed in all patients upon exposure to IGF-I therapy (Fig. 3). It is noteworthy that despite the increase in IGFBP-1 and IGFBP-4 levels during IGF-I therapy they remained a relatively small percentage of components of the total IGFBPs (Fig. 1).
Discussion

Our present investigation establishes that IGF-I can induce IGFBP-3, its principal serum carrier protein, in the complete absence of GH activity. This finding provides the first definite proof that IGFBP-3 in humans is regulated directly by IGF-I and is GH independent. This observation is supported by several recent studies which have implicated that IGFBP-3 production is IGF-I dependent (9, 10, 12-15, 28); in vitro, IGF-I induced an increase in IGFBP-3 in several cell lines in culture (12). Hypophysectomized rats treated by subcutaneous administration of IGF-I demonstrated an increase in IGFBP-3 levels (13, 14). In GH-deficient patients treated by exogenous GH, the rise in IGF-I after GH treatment preceded the rise in IGFBP-3 (28).

We can therefore state that GH effect on IGFBP-3 levels is mediated via IGF-I as are most of the growth promoting effects of GH. Though this rise is most probably due to increased synthesis it could also stem from an alteration in IGFBP-3 degradation. Several recent reports point to the presence of an IGFBP-3 specific protease in serum of some LTDs and suggested that in the absence of GH-GH receptor-IGF axis, serum IGFBP-3 levels are in part regulated by this protease (26, 29). The question arises whether this protease, which may be negatively regulated by IGF-I and thus inhibited by IGF-I therapy, may contribute to the higher serum levels of IGFBP-3. IGFBP-3 forms a complex of Mr 150 kDa in serum once it binds IGF-I (30) which is less prone to clearance from the circulation. Increase in IGF-I availability may allow IGFBP-3 to form the high molecular weight complex thus effecting its degradation. It is feasible that these effects, combined with an increase in IGFBP-3 synthesis, may result in the rise in IGFBP-3 levels upon IGF-I therapy of children with LTD. Why this effect is less prominent in the adults LTDs is yet unexplained.

An increase in serum levels of other IGFBPs was also detected upon IGF-I therapy. The increase in serum IGFBP-1 levels paralleled the decrease in insulin levels achieved with therapy. As insulin is the major regulator of IGFBP-1 (8), the rise in the latter is most probably due to this observed decrease in insulin. IGFBP-2 was prominent in serum of LTD patients prior to therapy, suggesting that it is not dependent on availability of IGF-I. The increase in IGFBP-2 with IGF-I therapy was somewhat surprising as in conditions of high serum IGF-I levels, i.e. acromegaly, serum IGFBP-2 is low (1). In accordance with our data, IGFBP-2 was shown to rise in normal human subjects upon infusion of IGF-I, while GH therapy suppressed this elevation (9). Thus the exact role of GH/IGF-I in the regulation of IGFBP-2 is complex and may be modulated by changes in insulin levels (9).

Recently, we have shown that chronic IGF-I therapy led to a striking stimulation of growth in children with
Laron dwarfism (31). Of great interest is the question whether the increase in IGFBP-3 is a prerequisite to the observed growth-promoting effects of IGF-I. The progressive increase in IGFBP-3 levels alters the pharmacokinetics of exogenous IGF-I and enables the achievement of higher serum IGF-I levels and a slower clearance (32). Consequently, the prolonged duration of IGF-I in the circulation may allow the manifestation of its full effect on growth promotion, thus leading to the striking catch-up growth in LTD children during IGF-I therapy (31). The GH-independent production of IGFBP-3, which seems essential to permit growth, implies that IGF-I therapy may be more appropriate than GH therapy in certain types of short stature children, such as GH resistant states and in cases of undesirable effects of GH. Measurement of serum IGFBP-3 levels is recommended as a marker for IGF-I activity in GH and IGF-I treated patients (28).

Acknowledgments. We thank S Moshe for her assistance. ZL is incumbent of the Irene and Nicholas Marsh Chair of Endocrinology and Diabetes, Sackler School of Medicine. This work was supported by a grant from the Reversion Foundation, Israel Academy of Science (to AK) and by the Fujisawa Pharmaceutical Co, Osaka, Japan (to ZL).

Addendum

Blots analyzing sera from LTD patients treated with chronic IGF-I were re-probed with specific IGFBP-2 and IGFBP-3 antibodies (UBI, Lake Placid, NY, USA). The identity of both BPs was confirmed by these Western blots. Moreover, the relationship between the intensity of bands representing the BPs in different sera was the same regardless of the probe used (radiolabelled IGF-I or IGFBP Ab).

References

28. Jørgensen JØL, Blum WF, Møller N, Ranke MB, Christiansen JS. Short-term changes in serum insulin-like growth factors (IGF) and IGF binding protein 3 after different modes of intravenous growth.

Received July 29th, 1992
Accepted October 6th, 1992