Long-term effects of insulin-like growth factor (IGF)-I on serum IGF-I, IGF-binding protein-3 and acid labile subunit in Laron syndrome patients with normal growth hormone binding protein

Hannah Kanety, Aviva Silbergeld1, Beatrice Klinger2, Avraham Karasik, Robert C Baxter1 and Zvi Laron1,2

Institute of Endocrinology, Chaim Sheba Medical Center, Tel Hashomer, Israel 52621, 1Felsenstein Medical Research Center, 2Endocrinology and Diabetes Research Unit, Schneider Children's Medical Center, Petah Tikva, Tel-Aviv University Sackler School of Medicine, Israel 49202 and

Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, New South Wales 2065, Australia

(Correspondence should be addressed to Z Laron, Endocrinology and Diabetes Research Unit, Schneider Children's Medical Center, Petah Tikva, Israel 49202)

Abstract

A minority of patients with Laron syndrome have normal serum GH binding protein (GHBP), indicating that the defect is elsewhere than in the extracellular domain of the GH receptor. We have evaluated the effect of long-term IGF-I treatment on serum IGF-binding protein (IGFBP)-3 and the acid-labile subunit (ALS) in three siblings with Laron syndrome caused by a GH post-receptor defect and with normal GHBP. The children (a boy aged 3 years, a girl aged 4 years and a boy aged 10 years) were treated by daily s.c. injection of IGF-I in a dose of 150 μg/kg. IGFBP-3 was measured by RIA and Western ligand blotting, ALS by RIA. Basal values of IGFBP-3 and ALS were low. During IGF-I treatment, the IGFBP-3 concentrations in the girl gradually increased, whereas in the boys there was a 60% decrease during the first week, followed by gradual increase towards baseline. The ALS concentrations followed a similar pattern. We conclude that IGF-I treatment induces an initial suppression and then an increase in the IGFBP-3 and ALS concentrations, confirming data from animal experiments that IGFBP-3 synthesis is not solely under GH control. The differences in responsiveness between the female and male siblings may reflect genetic differences, or lower circulating concentrations of IGF-I in the boys compared with the girl.

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Introduction

Laron syndrome is a hereditary form of dwarfism characterized by extremely low serum concentrations of insulin-like growth factor (IGF)-I, despite high concentrations of biologically active growth hormone (GH), and by resistance to exogenous GH (1–4). In most of the patients, the disease is caused by defects in the GH receptor gene, leading to absence of a functional GH receptor (3, 4), or by defects in post-receptor mechanisms (5). Serum GH-binding protein (GHBP), which is identical to the extracellular domain of the GH receptor (6), was reported to be absent in patients with Laron syndrome (7, 8) if the deletions or mutations are in the extracellular domain of the GH receptor (3). Several patients with Laron syndrome with detectable GH-binding activity have been reported (5, 9–11) suggesting that these patients have defects in either the transmembrane or intracellular domains of the GH receptor, or a post-receptor defect, all leading to deficiency in the biosynthesis of IGF-I.

We have previously described three siblings with Laron syndrome and normal serum GHBP, and presented evidence that their disease is caused by a GH post-receptor defect (5). Analysis of their GH receptor revealed a normal structure (J S Parks et al., unpublished data), supporting this conclusion. In the present study, we evaluated the effect of long-term IGF-I treatment of these three children on their serum IGFBP-3 and acid-labile subunit (ALS) concentrations, which have an important role in prolonging the metabolic half-life of the circulating IGF-I, affecting its biological activity (12).

Patients and methods

Patients

The pertinent clinical data of the three siblings studied are shown in Table 1. All three were very short (−4 to −5 height standard deviation score) and displayed...
characteristics typical of Laron syndrome (5). They were treated with recombinant human IGF-I (FK 780, Fujisawa Pharmaceutical Co., Osaka, Japan), given s.c. once daily in a dose of 150 \( \text{mg/kg} \). Blood was sampled after an overnight fast, before the first injection. The following blood samples were taken 24 h after the previous s.c. administration of IGF-I, at specified intervals. Serum and plasma samples were frozen until required for assay for IGF-I, GH, GHBP, IGFBPs and ALS. The study was approved by the Hospital’s Ethics Committee and informed consent was obtained from the parents.

**Hormone assays**

Sera were assayed for immunoreactive IGFBP-3 and ALS as described previously (13,14). The lower limit of detection of IGFBP-3 was 0.1 mg/l and that of ALS was 0.25 mg/l; the intra- and interassay coefficients of variation (CV) for IGFBP-3 were 6.2% and 11.9% (13) respectively, and those for ALS were 3.4% and 10.5% respectively (14). Serum IGF-I was measured by specific RIA as described previously (15). The lower limit of detection of IGF-I was 1 nmol/l; the intra-assay CV was 4.7% and the interassay CV was 8%. Serum GHBP and plasma GH were determined as reported previously (5, 15).

**Characterization of IGFBPs**

Serum IGFBPs were analysed by Western ligand blotting, using the procedure of Hossenlopp et al. (16). Samples were analysed by electrophoresis on 10% SDS–polyacrylamide gels under non-reducing conditions. Size-fractionated proteins, electroblotted onto nitrocellulose paper, were detected by incubation with \( 1 \times 10^6 \) c.p.m. \( ^{125}\text{I} \)-labelled IGF-I (Amersham, UK) and the radiolabelled IGFBPs were visualized by autoradiography. Analysis of specific IGFBPs was performed by immunoblotting as described previously (5, 15).

**Results**

Table 1 summarizes the hormone data of these patients with Laron syndrome before the long-term treatment with IGF-I. Serum GHBP activity was normal for age in all three children, whereas GH concentrations were high. Serum IGF-I concentrations were subnormal for age (< 3 nmol/l), and did not increase in response to 7 days administration of exogenous hGH. In the female sibling, IGF-I treatment resulted in a progressive increase in the concentration of IGF-I, with a twofold increase after 1 week of IGF-I administration and a sixfold increase after 6 months (Fig. 1, upper panel). In the male siblings, only a small increase in IGF-I

<table>
<thead>
<tr>
<th>Sibling no.</th>
<th>Sex</th>
<th>Age(^a) (years)</th>
<th>GHBP (% RSB)</th>
<th>GH(^b) (µg/l)</th>
<th>IGF-I (nmol/l)</th>
<th>IGFBP-3(^c) (mg/l)</th>
<th>ALS(^c) (mg/l)</th>
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<td>1</td>
<td>M</td>
<td>3</td>
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<td>F</td>
<td>4</td>
<td>57</td>
<td>10.5</td>
<td>1.2</td>
<td>0.35 (2.0)</td>
<td>1.6 (20.8)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>10</td>
<td>66</td>
<td>3.0</td>
<td>2.7</td>
<td>1.25 (2.9)</td>
<td>8.9 (20.9)</td>
</tr>
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</table>

\(^a\)Chronological age. \(^b\)Serum GH concentrations at the time of this investigation were lower than at diagnosis (5). \(^c\)Values in parentheses are from age- and sex-matched controls measured in this study. RSB, relative specific binding.

Normal values for IGFBP-3: 3–4 years – 2.4 ± 0.6 mg/l, range 1.2–3.5 mg/l; 10 years – 3.6 ± 0.7 mg/l, range 2.1–4.6 mg/l (13, 17). Normal values for ALS: 3–4 years – 14.2 ± 6.0 mg/l, range 4–22 mg/l; 10 years – 19.3 ± 5.6 mg/l, range 9–28 mg/l (14).
concentration was evident during long-term IGF-I treatment, with a twofold increase after 6 months of treatment (Fig. 1, upper panel). Serum concentrations of IGFBP-3 in these children with Laron syndrome were measured by RIA and found to be 17–45% of normal values (Table 1). In the female sibling, IGF-I treatment led to a gradual increase in IGFBP-3 concentrations (Fig. 1, middle panel). In the male siblings, 1 week of IGF-I treatment resulted in a 60% decrease in IGFBP-3 concentrations, followed by an increase towards baseline values in the following months (Fig. 1, middle panel).

The changes in IGFBPs were also evident in Western ligand blotting. Figure 2 shows the IGFBP profiles in serum samples of the female sibling during the 24 weeks of IGF-I treatment. Western ligand blotting confirmed the trend in IGFBP-3 concentrations indicated by RIA, with a striking increase in the intensity of the 40–43 kDa doublet representing IGFBP-3. Similar results were obtained by immunoblotting with a specific IGFBP-3 antibody (not shown). Western ligand blotting of serum samples from the male siblings also confirmed the trend in IGFBP-3 concentrations indicated by RIA, with a dramatic decrease after 1 week of IGF-I treatment (Fig. 3, lower panel), followed by a return to pretreatment values as described previously (5). In contrast, 1 week of treatment with exogenous hGH (0.1 U/kg per day) resulted in a small increase in IGFBP-3 in the male siblings and a scarcely detectable increase in IGFBP-3 in the female sibling (Fig. 3, upper panel). No consistent change in IGFBPs-1, -2 and -4 was observed during the 24 weeks of IGF-I treatment in all three siblings (Fig. 2 and (5)), whereas GH administration led to an increase in IGFBP-2 (Fig. 3, upper panel). As depicted in Fig. 4 (upper panel), IGF-I treatment of these patients with Laron syndrome led to an increase in the IGF-I/IGFBP-3 molar ratio, as reported in normal growth (17).

Serum concentrations of ALS in these children, measured by RIA, were low (8–40% of normal, Table 1). The changes in ALS during IGF-I treatment were similar to those found for IGFBP-3 (Fig. 1, middle and lower panels). In contrast to the response to IGF-I treatment, ALS concentrations in the male siblings were not suppressed after 1 week of administration of exogenous hGH (4.2 compared with 4.1 mg/l for sibling 1 before and after exogenous hGH).

In normal individuals, ALS is present in excess, in molar terms, compared with IGFBP-3 (14).
ALS:IGFBP-3 molar ratio showed a marked decrease in the male siblings and an increase in the female, after 1 week of IGF-I treatment, becoming stable over the following weeks of treatment (Fig. 4).

Discussion

Circulating IGFs are bound to specific IGF-binding proteins, of which IGFBP-3 binds the majority of IGFs. IGF-I and IGFBP-3 form a 150 kDa stable ternary complex by binding the ALS, which is present in excess in the circulation (12). Formation of the ternary complex stabilizes IGF-I in the plasma and prolongs its metabolic half-life, assuring a steady supply of IGF-I to the tissues. The components of the ternary complex are synthesized by different cell populations in the liver: IGF-I and ALS in hepatocytes; IGFBP-3 in non-parenchymal Kupffer cells and sinusoidal endothelial cells (18, 19).

Until recently, it was assumed that IGFBP-3 production in humans is regulated only by GH (12, 13). This assumption was challenged by our previous findings that continuous administration of IGF-I to two children with Laron syndrome, with undetectable GHBP, induced a progressive increase in IGFBP-3 as revealed by Western ligand blotting (15). In this paper, we extended this observation, demonstrating by RIA that serum concentrations of both IGFBP-3 and ALS are influenced in vivo by IGF-I. The IGFBP-3 and ALS responses to IGF-I treatment differed between the male and female siblings, with both analytes showing a marked initial decline in the boys, but an increase in the girl. As there is no known sexual dimorphism in the concentrations or responses of these proteins in any condition, this difference may suggest a difference in the GH resistance between the male and female siblings. The responses of the males – a decline in both ALS and IGFBP-3 over the first week of IGF-I treatment – is in accord with previously reported responses to IGF-I in healthy (20) and calorically restricted (21) adults, and in adult patients with Laron syndrome (22). As IGF-I is not known to suppress the cellular production of either protein, this response might be secondary to a suppression of GH secretion and serum GHBP by IGF-I (23). A similar suppression of ALS (but not IGFBP-3) by IGF-I treatment has previously been described in another GH resistant boy (24).

In contrast, in the present study the increase in IGFBP-3 in response to IGF-I seen in the female sibling, which was similar to that reported in other children with Laron syndrome (15), might reflect a stimulation of IGFBP-3 synthesis or release by IGF-I, in the absence of any overriding influence of GH. This observation is also supported by experimental studies showing that IGFBP-3 production (19, 25) and release (26) is IGF-I dependent. In vivo, studies of hypophysectomized rats have indicated that, whereas GH regulates ALS concentrations, IGF-I is the primary regulator of circulating IGFBP-3 (27–29). The ALS response to IGF-I in the female sibling may have been an indirect effect, via the insulin receptor, as it has been demonstrated that low concentrations of insulin and high concentrations of IGF-I stimulated the ALS mRNA expression in hepatocytes (19). In addition, the induction of IGFBP-3 by IGF-I in patients with Laron syndrome could serve to stabilize the ALS by formation of the ternary complex, thus influencing the turnover of both ALS and IGFBP-3.

The different responses of the male and female patients observed in this study may reflect genuine differences between them with regard to the synthesis or turnover of ALS or IGFBP-3, or both. These differences may indicate that the girl is homozygous for the so far unidentified post-receptor defect, whereas the boys are double heterozygous. In favour of such an hypothesis is the more typical clinical appearance of Laron syndrome in the girl than in the boys. Alternatively, the difference may reflect the lower concentrations of circulating IGF-I registered in the boys during the replacement treatment.

In conclusion, in this study we have demonstrated that, in patients with Laron syndrome caused by a GH post-receptor defect, changes in IGFBP-3, induced by IGF-I treatment, were in parallel with changes in ALS.

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