Serum Growth Hormone-Binding Protein (GHBP) Activity is Decreased by Administration of Insulin-Like Growth Factor I in Three Laron Syndrome Siblings with Normal GHBP (43769)

AVIVA SILBERGELD,[1] BEATRICE KLINGER, RUTH KERET, RINA ESHET, ANTONIO SELMAN ALMONTÉ, AND ZVI LARON

Endocrine and Diabetes Research Unit, Children's Medical Centre in Israel; Felsenstein Medical Research Center, Petah Tikva 49100, Israel; and Sackler Faculty of Medicine, Tel Aviv University, Israel

Abstract. **Three Laron Syndrome (LS) siblings with a post growth hormone (GH) receptor defect for insulin-like growth factor-I (IGF-I) synthesis were found to have serum GH-binding protein (GHBP) levels normal for age. Treatment with recombinant IGF-I (150 μg/kg/day) decreased serum GHBP activity to 62% of the basal value ($P < 0.001$) in two of the sibs in 1 week and in the third sib after 3 months of therapy. Scatchard analysis of the binding of [^{125}I]human GH (hGH) to GHBP in patients' sera before and during therapy revealed affinity constants $K_a = 1.55–1.80 \times 10^9\ M^{-1}$, similar to that of sera from healthy subjects. Variations in binding are due to changes in the binding capacity. IGF-I may be a regulatory factor for serum GHBP activity in man.**

[P.S.E.B.M. 1994, Vol 206]

Patients with Laron Syndrome (LS) have high circulating levels of growth hormone (GH), low levels of insulin-like growth factor-I (IGF-I), and are unable to generate IGF-I in response to exogenous GH because of defective GH receptors (1). The molecular defects identified so far in this disease are predominantly located in the extracellular domain of the GH receptor (2, 3).

High-affinity serum GH-binding protein (GHBP), which is structurally identical to the extracellular domain of the GH receptor (4), is indeed absent in LS patients (5). A comprehensive study demonstrated that 32 out of 35 LS patients studied by us had very low or undetectable levels of GHBP activity (6). However, in three prepubertal siblings with the LS phenotype, GHBP was present at levels which were normal for age (7).

Laron syndrome children with normal GHBP levels have also been described by others (8), and a defect in the transmembrane or intracellular domain of the mechanisms has been surmised. We report herein the effect of treatment with IGF-I on serum GHBP activity in three LS siblings with normal GHBP.

Subjects and Methods

Patients. Two males, aged 3 and 10.5 years (Sib 1 and 3) and a 4.5 year old female (Sib 2) were investigated. Upon referral there was severe growth retardation, height SDS being −5.2 (Sib 1) and −4.6 (Sib 2 and 3). They displayed typical LS characteristics: obesity, frontal bossing, saddle nose, and high-pitched voice. The diagnosis was made on the basis of high plasma GH levels and very low serum IGF-I levels which failed to rise during 7 days of GH administration (0.1 U/kg/day). A full description of the patients, and the detailed investigations which led to the conclusion that their disease was due to a post-GH-receptor defect appears elsewhere (7). The LS patients were

[1] To whom requests for reprints should be addressed at Endocrine and Diabetes Research Unit, Felsenstein Medical Research Center, Beilinson Campus, Petah Tikva 49100, Israel.

0037-9727/94/2063-0324$10.50/0

treated by recombinant IGF-I (FK 780) (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), administered sc (150 μg/kg/day). Blood was drawn after an overnight fast.

Assays. GHBP was assessed as previously published (9). Serum (100 μl) was incubated with [^{125}I]hGH (~25,000 cpm) (23°C, 16–20 hr) in the absence and in the presence of excess unlabeled recombinant human GH (hGH) (rhGH, Novo/Nordisk, Gentofte, Denmark) in a total volume of 250 μl. Bound and free hormones were separated on Ultrogel AcA44 columns (0.9 × 18 cm). GHBP activity in unknown sera was expressed as the percent specific binding relative to that of an adult reference serum (%RSB). The interference of binding by endogenous GH was corrected by comparison with an inhibition curve established by adding increasing concentrations of rhGH to the reference serum.

For Scatchard analysis, the concentration of unlabeled hGH was corrected by the addition of endogenous GH values in the sera. Interassay variability for sera with %RSB 31, 51.5, and 110 was 11.0%, 6.1%, and 6.0%, respectively. Plasma GH was determined by radioimmunoassay (RIA) using hGH-RP-1 (NPA, MD) as standard. The sensitivity of the method was 0.5 ng/ml; the intra- and interassay variability were 12.5% and 16.5%, respectively. Serum IGF-I was determined by RIA after acid-ethanol extraction followed by cryoprecipitation. The RIA incorporated anti-IGF-I antiserum BO1066S and recombinant IGF-I as the standard (Fujisawa Pharm. Co.). The sensitivity was 1.5 nmol/liter; intra- and interassay variability were 4.7% and 8.5%, respectively.

Statistical Analysis. Results are given as the mean ± SEM. Comparisons were made using paired Student's *t* test.

Results

Prior to treatment, serum IGF-I levels ranged from 1.5 to 2.8 nmol/liter (normal values, age and sex matched: 10–25 nmol/liter). Four hours after injection, IGF-I levels reached a peak of 25–30 nmol/liter. Levels of GHBP activity and fasting GH before and during IGF-I treatment are shown in Table I. Basal GHBP values were within normal range for age. GHBP activity was reduced to 62% of basal values ($P < 0.001$) after 1 week of therapy in Sib 1 and 3, and after 3 months in Sib 2. Fasting GH levels decreased during therapy (Sib 1 and 2) or remained constant (Sib 3), but there was no consistent relationship between GHBP activity and GH levels.

Scatchard analysis of the binding of GH to GHBP in sera from a healthy male adult and from Patient 3, before and after 1 week, are represented in Fig. 1. The plots are linear and give association constants (K_a) of 1.55–1.80 × 10^9 M^{-1} for normal and patients' sera.

Table I. Serum GHBP (%RSB) and GH (ng/ml) in Three Laron Syndrome Siblings Before and During IGF-I Therapy

IGF-I treatment	Sibling 1		Sibling 2		Sibling 3	
	GHBP	GH	GHBP	GH	GHBP	GH
None	43 ± 2	9	57 ± 2	10	66 ± 2	3
1 week	27 ± 2[a]	16	49 ± 3	6	40 ± 2[a]	4
3 months	23 ± 1[a]	5	37 ± 2[a]	6	54 ± 1[a]	3

[a] $P < 0.001$ treated vs untreated. Mean GHBP %RSB values were obtained from 4–9 determinations on each serum. Normal GHBP levels: 3–5 years, 60 ± 6; 6–10 years, 76 ± 5 (9).

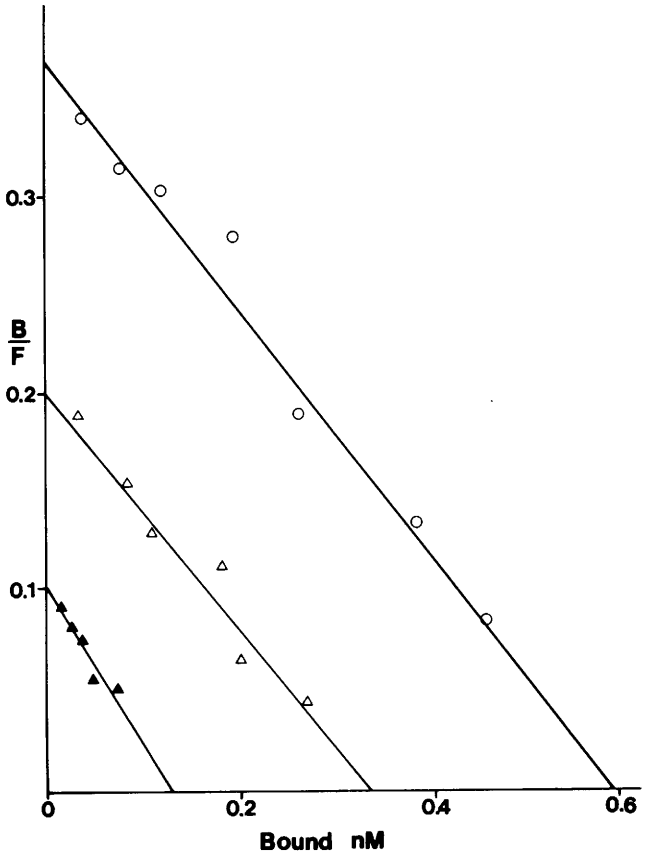

Figure 1. Scatchard analysis of the specific binding of [^{125}I]hGH to the GHBP in sera from Sib 3 before (open triangles) and after (closed triangles) 1 week IGF-I and a male adult (ref. serum) (open circles). The concentration of unlabeled hGH was corrected for by the addition of endogenous GH. K_a values are listed in Table II.

Table II presents the K_a and binding capacities (BC) of sera from the LS siblings (including data from Fig. 1). It is evident that the K_a of the LS patients' sera are similar to those of healthy controls and that the variations in GHBP binding are due to changes in the binding capacity.

Discussion

We have demonstrated that serum GHBP activity is reduced by the administration of recombinant IGF-I

Table II. Serum GHBP Activity, Binding Affinity (K_a), and Binding Capacity (BC) in LS Siblings and in Healthy Controls

Serum	IGF-I	%SB[a]	K_a ($\times 10^9\ M^{-1}$)	BC (n*M*)
Sib 1	None	10.2	1.53	0.23
Sib 2	None	13.5	1.75	0.25
Sib 3	None	16.9	1.55	0.39
Sib 3	1 week	9.7	1.80	0.13
Sib 3	3 months	14.9	1.68	0.27
Male (10 years)	None	17.1	1.80	0.27
Male (adult)	None	25.1 ± 0.9[b]	1.52 ± 0.15[b]	0.58 ± 0.02

[a] GHBP activity is expressed as the % specific binding of [^{125}I]hGH.
[b] Three determinations.

in three GHBP-positive LS patients. These changes resulted from a reduction in the binding capacity of the GHBP, whereas the binding affinity remained constant. These patients have typical clinical features of LS and extremely low IGF-I levels which are refractory to exogenous hGH. Nevertheless, normal serum GHBP activity indicates that the extracellular portion of the GH receptor is intact, since GHBP is thought to be derived from the receptor (4). Moreover, the IGF-BP3 carrier protein, which is GH dependent, increased after hGH administration (7), suggesting normal functioning of the GH receptor signal for the synthesis of this protein. We have suggested that there exists a post-GH receptor defect mechanism for GH-stimulated IGF-I synthesis in these patients.

The evidence that GH and/or IGF-I modulates GHBP levels in man is controversial (10). In normal boys, GHBP is inversely related to the 24-hr GH secretion rate (11). However, in children with idiopathic short stature (12), the low GHBP levels rose after GH therapy and were correlated to serum IGF-I (12). The response to GH treatment in GH deficient children has been variously reported as having no effect (13) or to elevate GHBP (14).

On the other hand, active acromegalics have low GHBP levels which are partially reversed in those patients responding to octreotide therapy by a decrease in IGF-I levels (15).

Exogenous IGF-I may act directly on GHBP levels by inhibiting the "shedding" of GHBP from the receptor. Since GHBP is thought to be a measure of tissue GH receptors (5, 10), it can be inferred that IGF-I causes down-regulation of the receptor itself which, in turn, limits GH action. Alternately, the effect of IGF-I may be indirect, possibly via a decrease in GH secretion which occurs after acute (16) and chronic (17) administration of IGF-I in LS patients. While the mechanism of IGF-I action on GHBP is unclear, the data presented in this communication suggest that IGF-I may participate in the modulation of serum GHBP in man.

This study was supported in part by a grant from Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan. The authors thank Ms. C. Børnbach of Novo/Nordisk, Gentofte, Denmark for the generous gift of [^{125}I]hGH and rhGH.

Zvi Laron is Incumbent of the Irene and Nicholas Marsh Chair in Endocrinology and Juvenile Diabetes, Tel Aviv University, Israel.

1. Laron Z. Laron Syndrome—From description to therapy. Endocrinologist **13**:21–28, 1993.
2. Godowski PJ, Leung SW, Meacham LR, Galgani JP, Hellmiss R, Keret R, Rotwein PS, Parks JS, Laron Z, Wood WI. Characterization of the human growth hormone receptor gene and demonstration of partial gene deletion in two patients with Laron-type dwarfism. Proc Natl Acad Sci USA **86**:8083–8087, 1987.
3. Berg MA, Guevara-Aguirre J, Rosenbloom A, Rosenfeld RG, Francke U. Laron Syndrome in Ecuador: Point mutation in the growth hormone receptor gene causes deletion of 8 amino acids from the extracellular domain. In: Laron Z, Parks JS, Eds. Lessons from Laron Syndrome (LS) 1966–1992—A Model of GH and IFG-I Action and Interaction. Pediatric and Adolescent Endocrinology. Basel: Karger, Vol **24**:pp140–152, 1993.
4. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature **330**:537–543, 1987.
5. Daughaday WH, Trivedi B. Clinical aspects of GH binding proteins. Acta Endocrinol **124**:27–32, 1991.
6. Silbergeld A, Keret R, Selman-Almonte A, Klinger B, Laron Z. Serum growth hormone binding protein in Laron Syndrome patients and their relatives. In: Laron Z, Parks JS, Eds. Lessons from Laron Syndrome (LS) 1966–1992—A model of GH and IGF-I action and interaction. Pediatric and Adolescent Endocrinology. Basel: Karger, Vol **24**:pp153–159, 1993.
7. Laron Z, Klinger B, Eshet R, Kaneti H, Karasik A, Silbergeld A. Laron Syndrome (LS) due to a post-receptor defect response to IFG-I treatment. Isr J Med Sci **29**:757–761, 1993.
8. Maheshwari HG, Clayton PE, Mughal Z, Price DA, Norman M. Laron-type dwarfism: The GH-BP positive phenotype. In: Laron Z, Parks JS, Eds. Lessons from Laron Syndrome (LS) 1966–1992. Pediatr Adolesc Endocrinol. Basel: Karger, Vol 24: pp160–166, 1993.
9. Silbergeld A, Lazar L, Erster B, Keret R, Tepper R, Laron Z. Serum growth hormone binding protein activity in neonates, children and young adults: Correlation with age, height and weight. Clin Endocrinol **31**:295–303, 1989.
10. Baumann G. Growth hormone-binding proteins (43550B). Proc Soc Exp Biol Med **202**:392–400, 1993.
11. Martha PM, Rogol AD, Blizzard RM, Shaw MA, Baumann G. Growth hormone-binding protein activity is inversely related to

24-hour growth hormone release in normal boys. J Clin Endocrinol Metab **73**:175–181, 1991.
12. Fonturra M, Mugnier E, Brauner R, Rappaport R, Postel-Vinay MC. Effect of growth hormone on the low level of growth hormone binding protein in idiopathic short stature. Clin Endocrinol **37**:249–253, 1992.
13. Martha PM, Reiter EO, Davila N, Shaw MA, Holcombe JH, Baumann G. Serum growth hormone (GH)-binding protein receptor: An important determinant of GH responsiveness. J Clin Endocrinol Metab **75**:9.1464–9.1469, 1992.
14. Hochberg Z, Barkey RJ, Even L, Peleg J, Youdim MBH, Amit T. The effect of human growth hormone therapy on GH binding protein in GH-deficient children. Acta Endocrinol **125**:23–27, 1991.
15. Roelen CAM, Donker GH, Thijssen JHR, Koppeschaar HPF, Blankenstein MA. High affinity growth hormone binding protein in plasma of patients with acromegaly and the effect of octreotide treatment. Clin Endocrinol **37**:373–378, 1992.
16. Laron Z, Klinger B, Anin S. Effect of acute administration of insulin-like growth factor-I in patients with Laron type dwarfism. Lancet **2**:1170–1172, 1988.
17. Laron Z, Klinger L, Jensen T, Erster B. Biochemical changes induced by one week of administration of rIGF-I to patients with Laron type dwarfism. Clin Endocrinol **35**:145–150, 1991.