The Response of Insulin-like Growth Factor Binding Protein-3 Complex to Growth Hormone

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Abstract. Biochemical markers of response to growth hormone (GH) have been determined by many groups for use as long term predictors of growth response. We have examined the changes in insulin-like growth factor binding protein (IGFBP)-3 by RIA in three groups of children (biochemical GH Deficiency (GHD, n=36), Chronic Renal Failure (CRF, n=22), Idiopathic Short Stature (ISS, n=101)) during six months treatment. Additionally, the alpha subunit of IGFBP-3 (ALS) was measured in the last group. Ten GHD children showed during the first 24 hours a mean IGFBP-3 increase of 37% and this change strongly related to the change in the sum of $IGF_1 + IGF_{11}$ ($r=0.882$). The change at three and six months for the GHD group was variable and did not relate to an individual's growth response. IGFBP-3 was high in CRF group at baseline and did not rise during therapy. Both mean IGFBP-3 and ALS showed a dose dependent rise when two doses of GH were given for six months in ISS group but this change was less than seen with mean IGF_1 and could not be used to confidently predict subsequent growth response. These studies confirm the GH dependence of IGFBP-3 but its routine measurement for prediction of future growth is not indicated.

Key words: growth hormone deficiency, insulin-like growth factor 1, IGF binding protein 3, chronic renal failure, idiopathic short stature

Introduction

The major circulating binding protein for the insulin-like growth factors (IGF- I and IGF-II) in children and adults is IGFBP-3. It is recognised as existing in a ternary complex [1,2] consisting of alpha, beta and gamma subunits. Beta subunit is the core protein which has a molecular weight of nearly 29 kDa

[3], but after glycosylation, has an approximate molecular weight of 50 kDa [4]. This protein was first purified by Baxter and will be referred to in this review as IGFBP-3. The alpha subunit is an acid-labile 85 kDa glycoprotein (ALS, 5). The gamma subunit refers to IGF- I or IGF-II and binding alpha subunit to the beta subunit can only occur in the presence of the IGF- I or IGF-II. Baxter has shown that the total molarity of IGF- I and IGF-II in the plasma is equal to the molarity of BP-3 and that plasma ALS is in excess [5].

Several studies have shown that IGFBP-3

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is age and growth hormone dependent, with high values in acromegaly and low values in growth hormone deficiency [6,7]. This pattern is similar to the observations on plasma IGF- I , but unlike IGF- I , there have been few studies of the changes of IGFBP-3 or ALS during growth hormone therapy. We have examined the changes in IGF- I , IGFBP-3 and ALS during the first 24 hours and during the first six months of growth hormone therapy in three groups of children. The relation between the changes in the IGFBP-3 complex and growth velocity has been examined to determine its potential as a predictor of the growth response to GH.

Methods

Assays

IGF- I , IGFBP-3 and ALS were all measured by radioimmunoassay as previously described [6,8,9].

Study Population

Study A

Ten children with GH deficiency (GHD) defined as peak growth hormone response to two provocative tests being < 20 mU/l, as well as auxological features of GHD, participated. The age range was 8–14 years. Methionyl hGH (Somatonorm, Kabi Pharmacia) 4 IU was given subcutaneously at 4.00pm. Hourly samples were taken for IGF- I , IGF-II, IGFBP-1 and IGFBP-3 for 24 hours in six children. In the remaining four children, samples were taken for the first four hours and the last eight hours of the 24 hour sample period.

Study B - GHD and Chronic Renal Failure (CRF) group

Thirty-six children, 23 males and 13 females with proven GHD (peak provocative GH to 2 stimulation tests < 20 mU/l) had samples drawn for measurement of IGF- I at commencement of GH therapy and after three and six months of therapy. The mean age for

the GHD group was 11.8 years with range 1.3–16.6 years. The mean growth hormone doses were 0.4 U/kg/week with a range of 0.2–0.8 U/kg/week.

Twenty three children with CRF, 15 males and eight females had measurement of IGF- I and IGFBP-3 at the time of commencement of GH and after six and 12 months of GH therapy. The age range of the CRF group was 2–16.5 years. At the time of entering the study, six of the group had undergone a transplant, 10 were on peritoneal dialysis and seven had CRF but were not requiring dialysis. rhGH (Genotropin, Kabi Pharmacia) was given in a dose of 1 U/kg/week divided into six daily doses.

Study C - idiopathic short stature (ISS)

One hundred four children who did not have evidence of biochemical growth hormone deficiency, but were short (height < 3 rd percentile) and slowly growing (growth velocity < 25 th centile for bone age), but who did not have a recognisable disorder were enrolled in a study comparing the effect of two doses of rhGH (Genotropin, Kabi Pharmacia) with placebo during the first six months. The injections were given six times/week and two doses of growth hormone used were 0.6 U/kg/week and 1.2 U/kg/week. The mean age of the study group was 9.4 years with a range of 3–15 years. IGF- I , IGFBP-3 and ALS were measured at the commencement of the study and at 1, 3, 6 months after commencement.

Results

Study A

The mean increase ($\pm$ SEM) after 24 hours in IGF- I was $93 \pm 16\%$ and for IGFBP-3 was $37 \pm 13\%$. Most children showed a pattern of IGF- I commencing to rise four hours after the GH injection with a plateau after 16 hours. The change in IGFBP-3 was less obvious, but occurred later and may not have peaked at the end of the sampling times. Mean profiles are shown in figure 1a and 1b.

IGF- I and IGFBP-3 Response to hGH

Fig 1a.

Fig 1b.

The means + SD for IGF- I (fig 1a) and IGFBP-3 (fig 1b) for 6 individuals with GHD are shown. hGH 4 IU, was given subcutaneously at 4pm (time 0) and samples were collected hourly for 24 hours.

Study B

IGF- I and IGFBP-3 were generally low compared to the reference range prior to commencing therapy. The mean increase in IGF- I was 166% and BP-3 was 67% six months after commencing growth hormone. Although the mean percentage increase was large, not all individuals showed an impressive rise in IGF- I or IGFBP-3 ; 3 showed a decrease in IGFBP-3 (Fig 2, 3). The mean growth velocity increased from 3.4 cm/year pretherapy to 9.9 cm/year during the first 6 months in the GHD group.

In the CRF group at time of commencement, the IGF- I values were normal for their age. The IGFBP-3 generally were elevated. After six months, the mean change was a 46% increase in IGF- I, but a 8% decrease for IGFBP-3 (Fig 2, 3). Four out of the six who had a transplant had normal values for IGFBP-3 prior to commencing GH. The mean growth velocity for the group increased from 2.7 cm/year pre-therapy to 7.1 cm/year during the first six months.

Study C

Following randomisation, 27 children received placebo injections during the first six months, 37 children received 0.6 U/kg/week and 40 children received 1.2 U/kg/week. All of the placebo group, 34 of the 0.6 U/kg/week group and 39 of the 1.2 U/kg/week group had

IGF- I and their binding proteins measured. The mean ($\pm$ SEM) change for IGF- I was 4 ± 5 , 85 ± 11 , $139 \pm 14\%$ for IGF- I in the respective groups, 14 ± 3 , 27 ± 4 and $43 \pm 5\%$ for IGFBP-3 in the respective groups and 7 ± 3 , 35 ± 4 and $50 \pm 6\%$ for ALS. In the same time period, the mean growth velocity ($\pm$ SEM) for the respective groups was 5.25 ± 0.19 , 8.72 ± 0.30 and 10.80 ± 0.29 cm/year.

Use of changes in the IGFBP-3 complex as predictor of growth

The changes in IGF- I, IGFBP-3 and ALS during the first six months in the ISS group who received growth hormone were examined in relation to the growth velocity achieved in these children during the first 12 months of therapy. There were 68 children who had growth velocity and IGFBP-3 complex data available for analysis.

At baseline there was a strong relation between IGF- I and IGFBP-3 ($R=0.74$, $P=0.0001$, $N=100$) and with ALS ($R=0.67$, $P=0.0001$, $N=99$). All subunits of the IGFBP-3 complex were age dependant at baseline with the relation between age and IGF- I, IGFBP-3 and ALS being $R=0.44$, $R=0.41$, $R=0.42$ ($P=0.0001$ for all relations) respectively.

The change in IGF- I either in nmol/l or as a percentage provided the strongest rela-

Fig 2. IGF- I values (nmol/l) are shown at baseline and after six months of hGH therapy for 36 children with GHD (left panel) and 23 children with CRF (right panel).

Fig 3. IGFBP-3 values (ug/ml) are shown at baseline and after six months of hGH therapy for 36 children with GHD (left panel) and 23 children with CRF (right panel).

IGF- I and IGFBP-3 Response to hGH

tion with either growth velocity during the first year or change in growth velocity during the first year (Table 1). Relation were also seen between growth velocity during the first year and a change in IGFBP-3 and ALS in the first six months as shown in Table 1.

Discussion

Previous studies have indicated that the IGFBP-3 complex is growth hormone dependent [6,10]. This study confirms these observations with low values observed in the children with GHD, whereas more normal values were seen in children who are ISS, IGFBP-3 was elevated in children with CRF as has been noted previously [6,11].

Acute changes in both IGF- I and IGFBP-3 were observed 24 hours following an injection of growth hormone in children with GHD. IGF- I appeared to rise after about four hours and almost doubled during the 24 hours with plateau achieved after 16 hours. The rise in IGFBP-3 was more modest and occurred many hours after the rise in IGF- I . In this study it was not clear that a plateau had been reached, and in another study [12] IGFBP-3 continued to rise after the first injection of GH. In molar terms the rise in IGFBP-3 was sufficient to account for all of the increase in IGF- I , which is consistent with the previous observation that nearly all of a circulating IGF- I is accounted for by binding to IGFBP-3.

Changes in IGF- I , IGFBP-3 and ALS after six months of GH therapy in the ISS group indicate that their increase is dose dependent. Percentage increases in both IGFBP-3 and ALS were similar for both treatment groups, indicating that they may be under synchronous regulation. There was no significant increase seen in the placebo group. The percentage increase seen in the subunits of the IGFBP-3 complex in the ISS group was less than that seen in children with GHD. This is possibly because the children with GHD had very low baseline values of both IGF- I and IGFBP-3.

Table 1. Relation between IGFBP-3 complex changes at 6 months and growth velocity during first 12 months.

	GROWTH VELOCITY	
	Actual	Change
IGF- I Change	0.41 (0.0005)	0.49 (0.0001)
%IGF- I Change	0.49 (0.0001)	0.45 (0.0001)
BP-3 Change	0.21 (0.1)	0.18 (0.15)
%BP-3 Change	0.28 (0.02)	0.27 (0.03)
ALS Change	0.29 (0.02)0	0.36 (0.004)
%ALS Change	0.31 (0.01)	0.27 (0.03)

Data are r values (p values in bracket)
Change = treatment period - baseline.

The children with CRF present a different picture. There was a rise in IGF- I , but it was more modest than in the children with GHD or ISS despite the use of a high dose of growth hormone. This could in part be related to the fact that their baseline IGF was in the normal range, or it may indicate partial resistance to the growth hormone action in this disorder. The changes in IGFBP-3 in the CRF group are difficult to interpret. Most had high values at baseline, many decreased during treatment. One possible explanation for this observation would be that the degree of renal impairment improved, but this was not observed during the study. Further studies are required to examine the changes in the IGFBP-3 complex in children with CRF and determine their relevance to the growth of these children while undergoing GH therapy.

Clinicians have long been interested in determining the biochemical markers that may reflect the biological response to GH. Significant relations were found between the growth velocity during the first 12 months of the ISS group and the subunits of the IGFBP-3 complex. Despite the significant relations, it would be difficult in an individual to use change in either IGF- I , IGFBP-3 or ALS when either expressed as a whole number or as a percentage to indicate their likely subsequent growth. For example, a change in

IGF-I of 100% could represent a growth velocity during the first 12 months between 6.5 and 12.5 cm/year, a variation which would not be clinically useful.

This study extends the previous observation that IGF-I and IGFBP-3 are growth hormone dependent. Additionally, the third subunit of the IGFBP-3 complex, ALS, is also GH dependent. The percentage increase of the binding proteins of IGF-I is significantly less than the percentage rise in IGF-I, as would be expected, as both binding proteins are present in the circulation in a higher concentration than IGF-I. Measurement of these binding proteins in children receiving GH provides valuable information regarding the regulation of the IGFBP-3 complex, but cannot be used to determine an individual child's growth during therapy.

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