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Radioimmunoassay for Type I Procollagen in Growth Hormone-deficient Children before and during Treatment with Growth Hormone

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ABSTRACT. Type I procollagen concentrations were measured by radioimmunoassay in sera from 14 growth hormone-deficient children before and during 12 months of treatment with human growth hormone. Basal procollagen levels were lower than those of control children and comparable to those of normal adults. With treatment, the mean procollagen level increased into the range of the control children and was significantly greater than the baseline level at 1, 2, 3*, and 12 months ($p < 0.01$; * $p < 0.05$). Although there was no significant statistical corre-

lation between the growth velocity during treatment and the serum procollagen level, there was a suggestion that a high basal procollagen may be predictive of a less than optimal response to human growth hormone. (*Pediatr Res* 19: 8-11, 1984)

Abbreviations

RIA, radioimmunoassay
hGH, human growth hormone
ANOVA, analysis of variance

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Somatic growth results from the generation of new supporting and connective tissues. Since collagen is the major protein con-

stituent of connective tissue, its synthesis must be a prerequisite for normal growth. A RIA for human type I procollagen showed that sera from newborn cord blood had about 12-fold higher concentrations of the antigen than those in adult sera (1). This finding is presumed to reflect the greater degree of collagen synthesis in rapidly growing newborns as compared to fully grown adults. The serum procollagen level, by virtue of its intermediate position between growth promoting hormones and growth itself, might, therefore, have clinical usefulness as a nonspecific biochemical marker of the response to growth stimuli. In order to evaluate the relationship between the serum procollagen and growth, we studied procollagen levels in 14 growth hormone-deficient children prior to and during replacement therapy with hGH.

MATERIALS AND METHODS

Subjects. Written, informed consent was obtained from the parents of the subjects. Clinical data on the 14 children with growth hormone deficiency who participated in the study are summarized in Table 1. The diagnosis of growth hormone deficiency was made by demonstrating failure of the serum growth hormone concentration to rise to at least 7 ng/ml after administration of two of the following: insulin-induced (0.1 unit/kg) hypoglycemia; arginine infusion (0.5 g/kg); or L-dopa (500 mg) (2). Eleven children had idiopathic growth hormone deficiency. The remaining three had organic hypopituitarism and had undergone both neurosurgery and cranial irradiation. Children with multiple hormone deficiencies were given appropriate replacement therapy prior to the initiation of the study. The patients were prepubertal except for one girl with Tanner II pubic hair. All of the girls had 46XX karyotypes.

Experimental protocol. Basal control samples were obtained from 52 normally growing children being evaluated for short stature in the Growth Disorders Clinic at the Newington Children's Hospital. All control children had a skeletal age between 2 and 13 years, were well nourished, and had a diagnosis of either familial short stature, constitutional delay of maturation, or a combination. Native hGH was supplied by the National Hor-

mone and Pituitary Program. Treatment consisted of intramuscular injections of hGH three times per wk in a dose of 0.1 IU/kg. Serum for determination of immunoreactive procollagen was obtained prior to the initiation of therapy and at 1 wk and 1, 2, 3, 6, and 12 months during treatment. All seven determinations were completed in 8 of 14 subjects.

Procollagen assay. The RIA for human type I procollagen was performed essentially as reported (1, 3, 4). The source of the antigen was type I procollagen precipitated from the medium of cultured human diploid fibroblasts. The triple-helical portion of the procollagen molecule was digested with bacterial collagenase and the collagenase-resistant carboxy terminal propeptide fragment was then recovered by DEAE-cellulose ion exchange chromatography (5, 6). The isolated fragment, a disulfide-linked three-chain molecule of 100,000 daltons was used as the antigen. Rabbits were immunized with the antigen by conventional procedures. The serum IgG was then isolated and purified by affinity chromatography.

The antigen (25 μ g) was labeled with 125 I by the chloramine-T method (7). Antisera and IgG were titered against the radiolabeled antigen, and an IgG concentration (~3 ng) that bound 50% of the input radioactivity (100,000 cpm) was used in each tube of the RIA. Assay tubes containing dilutions of standard unlabeled antigen or dilutions of unknown sera were prepared in triplicate. All tubes contained carrier normal rabbit serum; control tubes omitted unlabeled antigen, and unlabeled antigen and antibody, respectively. After the antibody additions, the tubes were incubated for 2 h at 37°C. The radiolabeled antigen (100,000 cpm/tube) was then added and the tubes were incubated for 1 h at 37°C, and then for 18 h at 4°C. Bound antigen was precipitated with sheep anti-rabbit IgG antiserum and the centrifuged pellets were counted in a gamma spectrometer.

Figure 1 shows typical curves generated by additions of unlabeled procollagen antigen and dilutions of subject serum. The serum dilutions produced parallel displacement of label relative to standard antigen. Sera were diluted so that displacements were measured in the 20–70% range and antigen concentrations were read from the standard curve. The coefficients of variation (standard deviation $\times$ 100/mean) were 3.6 and 7.8 for within assay, and between assay measurements, respectively.

Statistical analysis (8). ANOVA for repeated measures was used to assess the effect of time as a variable. Cases D.W. and T.B. were excluded from the ANOVA (missing baseline values) and data for eight empty cells were estimated using the Yates method. Comparison of each time with baseline was done using Tukey's honestly significant difference method for multiple com-

Table 1. Clinical data on children with growth hormone deficiency*

Patient	Sex	Age (years)	Bone age (years)	Comments
A. A.	F	3-0/12	6/12	TSH/ACTH deficient on T ₄ and cortisol; IGR and dysmorphic
M. B.	F	11-1/12	7-10/12	
M. V.	M	6-0/12	3-9/12	
S. C ₁	M	7-5/12	3-5/12	
C. R.	F	13-0/12	10-0/12	Tanner II pubic hair
N. R.	M	7-1/12	7-0/12	Ependymoma of 4th ventricle
J. H ₁	M	11-8/12	7-6/12	Craniopharyngioma; pan-hypopituitarism with DI on replacement
C. G.	F	5-7/12	3-6/12	TSH deficient on T ₄
A. G.	M	10-5/12		Craniopharyngioma; pan-hypopituitarism with DI on replacement
S. C ₂	M	9-8/12	6-0/12	
R. P.	M	9-4/12	6-6/12	
J. H ₂	M	7-5/12	5-0/12	
D. W.	F	12-5/12	8-5/12	TSH deficient T ₄
T. B.	M	15-8/12	13-3/12	Gonadotropin deficiency-not replaced

* TSH, thyroid stimulating hormone; ACTH, adrenocorticotrophic hormone; IGR, intrauterine growth retardation; DI, diabetes insipidus; T₄, thyroxine.

Fig. 1. Procollagen radioimmunoassay: competitive displacement by reference antigen and a subject serum. Increasing amounts of unlabeled propeptide antigen (solid line) or serum (12.5 $\times$ dilution, dashed line) were incubated with antibody followed by incubation with radioiodinated propeptide antigen. Immune complexes were precipitated with sheep anti-rabbit IgG and the radioactivity in the pellets was measured. The curves were fitted visually through average data points. Illustrated subject serum contains 57 μ g/dl, by calculation from the standard curves.

parisons. The relationship of treated growth velocity to basal, peak, and incremental serum procollagen levels was analyzed using Pearson's R coefficient.

RESULTS

The mean ($\pm$ SE) serum procollagen concentrations in the basal state and during hGH therapy are shown in Figure 2. The mean procollagen concentration in untreated growth hormone-deficient children of 11.9 ± 2.0 $\mu\text{g/dl}$ was significantly less than that of the child controls of 31.9 ± 1.3 $\mu\text{g/dl}$ ($p < 0.001$) and within the adult range (2–14 $\mu\text{g/dl}$). The mean value increased to 16.5 $\mu\text{g/dl}$ at the end of the 1st wk of therapy and to 22.9 $\mu\text{g/dl}$ at the end of 1 month. Thereafter, the mean procollagen level remained greater than the baseline with values of 22.9 $\mu\text{g/dl}$ at 2 months, 21.6 $\mu\text{g/dl}$ at 3 months, 19.0 $\mu\text{g/dl}$ at 6 months, and 23.6 $\mu\text{g/dl}$ at 1 year. ANOVA appropriate for repeated measures designs showed a significant effect of the time variable ($F = 5.07$, $p < 0.01$). Differences from baseline became significant at 1 month and remained significant at 2, 3, and 12 months. The mean values at 1, 2, 3, and 12 months were within the lower portion of the range for child controls (19.5 to 59 $\mu\text{g/dl}$). The mean value at 6 months was 60% above the baseline. However, it was just below the range for child controls and not significantly different from the baseline. The procollagen level more than doubled in 10 of the 12 growth hormone-deficient children for whom both basal and peak values were available (Fig. 3). The mean peak procollagen level was 29.2 ± 2.9 $\mu\text{g/dl}$. This value was not significantly different than the mean level in control children. One child had no increment in procollagen. Procollagen levels in the two children in whom basal values were not available (open circles, Fig. 3) were similar to the mean value for the 12 children after treatment.

There was no significant correlation between treated growth velocity and either basal ($r = -0.060$), peak ($r = -0.148$), or incremental ($r = -0.125$) serum procollagen levels. Table 2 lists the growth velocity during therapy as well as the basal procollagen levels.

DISCUSSION

Collagen is synthesized in a precursor form, procollagen, distinguished by the presence of extension propeptides at the ends of the helical collagen α chains. The propeptides are subject to excision in the course of secretion and formation of extracellular collagen fibrils. Our RIA is specific for the carboxy terminal

Fig. 2. Serum procollagen levels in control children, adults, and growth hormone-deficient children before and during 1 year of hGH treatment. Control bars show the mean $\pm$ 2SD range of values. Results in hGH-deficient children are shown as mean $\pm$ SE.

Fig. 3. Basal and peak serum procollagen concentrations in the hGH-treated children. Basal and peak values were available for 10 of the 12 children. The open circles show the peak levels in the two treated children whose basal values were not available.

Table 2. Comparison of individual basal procollagen levels to growth velocity during hGH therapy

Subject	Post-hGH growth rate (cm/year)	Basal procollagen ($\mu\text{g/dl}$)
A. A.	3.0	12.6
M. B.	4.9	14.5
M. V.	5.8	13.5
S. C ₁	6.4	21.2
C. R.	7.6	5.6
N. R.	8.0	4.2
J. H ₁	8.9	7.2
C. G.	8.9	13.6
A. G.	9.1	7.0
S. C ₂	10.3	6.3
R. P.	10.8	10.2
J. H ₂	10.8	27.0

propeptides of human type I procollagen, as they exist in the intact precursor molecule, or after they are physiologically excised as a disulfide-linked, three-chain fragment of 100,000 daltons. Current evidence favors the view that the carboxy terminal propeptide fragment is excised before the procollagen is incorporated into fibrils in the extracellular space (9, 10). The concentration of the propeptide in serum should reflect recently synthesized collagen rather than resorption of previously formed collagen fibrils. Measurements of serum procollagen levels, therefore, do not evaluate the same aspects of collagen metabolism as the measurement of urinary hydroxyproline. The latter reflects both synthesis and turnover of collagen molecules and fibrils of all genetic types, as well as the dietary intake of collagenous proteins (11).

Because type I collagen is the most abundant collagen type and the major matrix protein of bone, we sought a correlation between serum procollagen values and linear growth. For this purpose, we chose to evaluate children treated for growth hormone deficiency. We found the mean pretreatment serum procollagen level of growth hormone-deficient children to be within

the normal range for adults and significantly lower than levels in short children without growth failure. All but two individual basal procollagen levels were below the range of the child controls and within the range of adult controls. Replacement therapy with exogenous hGH produced a sustained increase in the mean serum procollagen level and 12 of 14 peak levels were within the range of child controls. Overall, the data demonstrate that poor growth is accompanied by low serum procollagen levels similar to those in nongrowing adults and that stimulation of growth with hGH in growth hormone-deficient children results in an increase in serum procollagen to levels more comparable to that of children with normal growth velocity. The results suggest that type I collagen synthesis is diminished in growth hormone-deficient children and is restored toward normal during therapy which promotes linear growth. Complete restoration may not have been obtained since the mean procollagen levels during therapy remained in the lower portion of the control range.

Decreased collagen synthesis in association with growth failure appears not to be limited to growth hormone deficiency since we have found low basal procollagen levels in poorly growing children with primary hypothyroidism (12) and inflammatory bowel disease (13). The serum procollagen level may, therefore, be a nonspecific biochemical marker of biologic response to growth stimuli.

A biochemical marker for growth would be most useful if it predicted growth within a time frame shorter than that needed for the measurement of linear growth. Because enhanced collagen synthesis is a prerequisite for growth, we speculated that the serum procollagen response to hGH therapy would be predictive of the linear growth response. Statistical analysis revealed, however, that there was no significant correlation between the growth velocity and the basal, incremental, and peak serum procollagen values during treatment with hGH. Other investigators have reported a lack of correlation between linear growth response to hGH and levels of serum somatomedin-C (14, 15) or urinary epidermal growth factor (16).

We observed, with the exception of subject J. H₂, a clustering of higher basal procollagen values in the poorer growth responders (post-hGH growth velocity < 6.5 cm/year) and the lower values in better responders. Also noteworthy is the observation that three subjects with organic hypopituitarism (N. R., J. H₁, and A. G.), had very low basal procollagen levels and each had a good growth response to hGH. These data suggest that a "high" basal procollagen level may be predictive of a poor or less than optimal response to hGH.

The lack of statistical correlation between serum procollagen levels and linear growth responses in the present study possibly could be explained by variation in the timing of blood sampling in relation to hGH administration or an unrecognized lack of homogeneity in the growth hormone-deficient population. Strict

temporal coupling of sampling to hormone administration and expansion of the study population might clarify this relationship.

Despite the lack of significant correlation between growth velocity and serum procollagen response, the positive change in serum procollagen following hGH treatment suggests that serum procollagen may be a marker for an anabolic response *viz.* collagen synthesis and secretion. Further investigations are necessary to establish age-related norms for procollagen and to assess procollagen levels in other disorders associated with growth failure.

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