Changes in Levels of Serum and Urinary Collagen-Related Substances in Growth Hormone Deficient Children Following Discontinuation of Growth Hormone Therapy

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THE effect of GH on bone formation and resorption *in vivo* has been reported recently [1, 2]. We reported that urinary pyridinoline excretion rapidly decreased in patients with GH deficiency when the administration of GH was stopped, and then concluded that exogenous GH appears to stimulate bone resorption in those patients [3]. In order to further clarify the effect of GH on collagen metabolism, we herein examined changes in the levels of another four types of collagen-related substances.

Subjects and Methods

We studied 26 patients, 21 males and 5 females, aged 5 to 16 years, with complete or partial GH deficiency. They had been treated with 0.5 IU/kg/week of GH in 6–7 divided doses, for periods of one to five years. 16 male patients and all the female patients were prepubertal and the rest were at the pubertal stage. In particular, all who showed an increase of 6 cm a year or more in growth velocity during the first year of GH administration compared to before it, had been prepubertal during their first year of treatment. All patients had open metaphyses. After informed consent was obtained, early morning urine and serum specimens were collected while the patients received GH and then

for 14 days after discontinuing it.

Total urinary hydroxyproline was assayed by high-performance liquid chromatography with a cation-exchange column. Serum specimens were assayed for both prolylhydroxylase (EC 1.14.11.2) and type IV collagen with solid-phase enzyme immunoassay kits provided by Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan, and for type III procollagen propeptide with a solid-phase immunoradiometric assay kit provided by CIS Bio International, Gif-sur-Yvette Cedex, France.

Results

Total urinary hydroxyproline excretion levels corrected for creatinine were 169 ± 8.3 mmol/mol creatinine (mean $\pm$ SEM) during GH administration and 145 ± 6.4 mmol/mol creatinine (mean $\pm$ SEM) after discontinuation of GH. Wilcoxon signed-rank test showed a significant decrease in total urinary hydroxyproline excretion levels after discontinuation of GH ($P < 0.005$). Fig. 1A shows the relationship between the increase in growth velocity during the first year of GH administration compared to before it, and the ratio of the levels of urinary hydroxyproline while receiving GH and after discontinuation. Linear regression analysis revealed no significant relationship ($P = 0.19$). Levels of serum prolylhydroxylase also significantly decreased from 76.9 ± 2.6 ng/ml to 63.7 ± 3.2 ng/ml in 14 days after discontinuing therapy ($P < 0.005$). Fig. 1B shows the relationship between the increase in growth velocity and the ratio of the levels of serum prolylhydroxylase while receiving GH and

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Fig. 1. A: Relationship between the increase in growth velocity during the first year of GH administration compared to before it, and the ratio of urinary hydroxyproline excretion levels corrected for creatinine while receiving GH and for 14 days after discontinuation. B: Relationship between the increase in growth velocity and the ratio of serum prolylhydroxylase levels while receiving GH and for 14 days after discontinuation. C: Relationship between the increase in growth velocity and the ratio of serum type III procollagen propeptide levels while receiving GH and for 14 days after discontinuation. D: Relationship between the increase in growth velocity and the ratio of serum type IV collagen levels while receiving GH and for 14 days after discontinuation.

after discontinuation. No significant regression was found between them ($P=0.96$). Levels of serum type III procollagen propeptide also significantly decreased from 1.36 ± 0.075 ng/ml to 1.24 ± 0.071

ng/ml in 14 days after discontinuing therapy ($P<0.025$). Fig. 1C shows the relationship between the increase in growth velocity and the levels of serum type III procollagen propeptide

while receiving GH and after discontinuation. A significant positive correlation was seen in the linear regression analysis ($r^2=0.278$, $P=0.0033$). Levels of serum type IV collagen also significantly decreased from 5.20 ± 0.22 ng/ml to 4.65 ± 0.21 ng/ml in 14 days after discontinuing therapy ($P<0.01$). Fig. 1D shows the relationship between the increase in growth velocity and the ratio of the levels of serum type IV collagen while receiving GH and after discontinuation. No significant regression was found between them ($P=0.16$). In addition, linear regression analyses were calculated between the peak value of GH in arginine-loading test or insulin hypoglycemia test, and each of the ratio of the levels of the above four substances while receiving GH and after discontinuation. No significant regression was found between them.

Discussion

As shown above, the levels of the four types of collagen-related substances were increased while receiving GH. Only the effects of GH on type III procollagen propeptide have been reported [4]. The ratios of levels of the four types of substances while receiving GH compared to levels after discontinuation shown above are thought to represent the degree of activation of collagen

metabolism by the exogenous GH. A significant positive correlation was observed between the increase in growth velocity and the ratio of levels of serum type III procollagen propeptide (Fig. 1C). Thus, exogenous GH seems to stimulate type III procollagen propeptide in patients with GH deficiency. In prepubertal subjects, the increase in growth velocity during the first year of GH administration compared to before it is thought to be inversely correlated with the patients' endogenous GH secretion [5]. Therefore, it is possible that the degree of activation of type III procollagen propeptide, as well as urinary pyridinoline excretion [3], by exogenous GH is inversely correlated to patients' endogenous GH secretion. Another three types of substances also seemed to be weakly stimulated by exogenous GH. Exogenous GH is considered to activate collagen metabolism in osteoblasts and osteoclasts and in the whole-body extracellular matrix. The action on collagen metabolism may be mostly mediated by insulin-like growth factor I, but also may be in part due to the action of GH itself on the liver or fibroblasts [6]. The ratio of levels of serum type III procollagen propeptide provides informations about patients' endogenous GH secretion, which may not be completely clarified by pharmacological provocation tests performed before receiving GH.

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