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Plasma growth hormone-binding activity is low in uraemic children

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Abstract. Plasma growth hormone-binding protein (GH-BP) activity was evaluated in two groups of prepubertal children with chronic renal failure (CRF) who had been treated with recombinant human GH (rhGH). Group 1 consisted of eight children (mean chronological age 10.8 years) with advanced renal failure; group 2 consisted of nine children (mean chronological age 6 years) presenting with end-stage renal disease, who were on dialysis. Before treatment the specific binding of (^{125}I)hGH to high-affinity GH-BP was low in the two groups (group 1, 17.3 ± 1.6% of radioactivity; group 2, 14.2 ± 1.4%) compared with the mean value obtained in normal prepubertal children (24.8 ± 1.7%). No significant changes in GH-BP activity were found during the 1st year of GH therapy, although growth velocity and plasma levels of insulin-like growth factor-I increased significantly in both groups. The low GH-binding activity found in children with CRF supports the state of GH resistance. The reason for the absence of a GH-BP response to GH therapy has to be clarified.

Key words: Growth hormone – Growth hormone-binding protein – Chronic renal failure

Introduction

Hormonal, nutritional and other factors contribute to the growth failure in uraemia. The role of growth hormone (GH) and insulin-like growth factor (IGF) has to be clarified. Elevated levels have been reported in uraemia [1, 2]; low somatomedin bioactivity and normal immunoreactive serum IGF-I concentrations have also been found [3, 4]. On the basis of possible resistance to GH and IGF, high doses of GH were given to uraemic rats and improved growth significantly [5]. Consequently, trials with recombinant human GH (rhGH) have been undertaken in children with chronic renal failure (CRF) [6, 7].

Measurement of GH receptors was previously not possible in man due to their inaccessibility. However, the GH-binding protein (GH-BP) which has recently been identified in human serum is the extracellular domain of the membrane GH receptor [8, 9]; identity of the amino-acid sequence of the two proteins has been shown in the rabbit, mouse and rat. Therefore the GH-BP can be considered a soluble fragment of the GH receptor and its measurement is a possible approach for the evaluation of the GH receptor in humans.

GH-BP varies as a function of age [10]; it is probably regulated through multihormonal control. Very recently. GH was shown to increase the concentration of the GH-BP in children with isolated GH deficiency [11]. In the present study, plasma GH-BP was evaluated in two groups of children with CRF; the children were treated with rhGH, and GH-BP was measured before and after several months of GH therapy.

Materials and methods

Patients. Two groups of children with CRF were studied. Group 1 (Paris group) consisted of six boys and two girls aged 8.2–12.1 years (mean chronological age ± SEM, 10.8 ± 0.6 years). All were prepubertal at the beginning of treatment. Their mean height, expressed as standard deviation score (SDS) was –4.3 ± 0.4 SDS. All children presented with advanced renal failure with a mean creatinine clearance of 20.4 ± 1.3 ml/min per 1.73 m^2 body surface area (range 15.2–24.2 ml/min per 1.73 m^2).

Group 2 (Heidelberg group) consisted of seven boys and two girls, aged 2.6–10.4 years (mean chronological age 6 ± 0.9 years. Their height ranged from –2 to –4.3 SDS, with a mean value of –3.2 ± 0.2 SDS. These children were treated by continuous ambulatory peritoneal dialysis, or by haemodialysis. More clinical data from group 2 patients have been published previously [7].

rhGH therapy. All children received rhGH subcutaneously every day, at a dose of 1–1.3 IU/kg per week (Genotropin, Kabi Vitrum, Stockholm Sweden).

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Table 1. Specific binding of (^{125}I) human growth hormone (hGH) to high-affinity GH-binding protein, before and during GH therapy in uraemic children[a]

Patients		Specific binding of (^{125}I)hGH (% total radioactivity)			
		Before treatment	GH treatment		
			3 months	6 months	12 months
Group 1	1	19.6	–	25.9	20.6
	2	19.4	18.9	21.0	18.7
	3	6.1	12.6	14.7	14.0
	4	18.9	–	–	24.1
	5	20.3	8.8	18.3	20.9
	6	18.6	–	–	–
	7	17.6	26.0	15.8	15.4
	8	17.6	14.6	15.3	13.8
Mean ± SEM		17.3 ± 1.6	16.2 ± 2.9	18.5 ± 1.7	18.2 ± 1.5
Group 2	1	10.3	10.0	10.6	11.5
	2	12.4	10.6	14.1	8.3
	3	6.7	10.4	10.3	13.6
	4	15.5	16.5	15.4	–
	5	16.8	11.8	13.9	16.0
	6	13.1	13.3	12.8	10.3
	7	18.9	16.4	–	–
	8	19.2	–	–	–
	9	14.9	10.0	–	–
Mean ± SEM		14.2 ± 1.4	12.4 ± 1.0	12.9 ± 0.8	11.9 ± 1.3

[a] The mean value for 15 normal prepubertal children is 24.8 ± 1.7%

Measurement of GH-BP. In GH-treated children, blood was withdrawn 12–15 h after the hormone injection. Serum or plasma was stored at –20°C prior to assay. As previously described [10], 200 µl plasma or serum was incubated for 20 h at 4°C with 200 µl 0.1 M potassium phosphate, pH 7.0, containing bovine serum albumin 0.1% and (^{125}I)hGH (2×10^5 cpm). After filtration through a 0.45-mm Millipore minifiltre, the entire incubation mixture was injected onto a high-performance liquid chromatography Protein Pak 300 sw column. (Waters, Milford MA) Elution was performed isocratically using a degassed buffer (sodium sulphate 0.1 M, potassium phosphate 0.1 M, pH 7.0) pumped at a rate of 0.5 ml/min. The binding of hGH was expressed as the radioactivity in the individual peak divided by the total radioactivity in peaks I, II and III. To evaluate non-specific binding to peak II-BP, 5 µg GH was added to the plasma incubation.

In plasma samples containing high levels of GH (> 6 µg/l), a correction was made for the estimation of peak II-BP: the control plasma was supplemented with GH to yield a GH concentration identical to that in the patient's plasma; the decrease in the binding activity was then evaluated.

Statistical analysis. The unpaired Student's *t*-test was used for statistical evaluations. Differences were considered statistically significant when *P* was less than 0.05.

Results

The specific binding of (^{125}I)hGH to the high-affinity GH-BP (peak II-BP) was low in children with CRF. As shown in Table 1, the mean value before treatment in group 1 was 17.3 ± 1.6% of radioactivity; this value is significantly lower than that of our control group of prepubertal children 24.8 ± 1.7% ($P < 0.01$).

Uraemic children from group 2 were younger than group 1 patients and they were treated by dialysis their mean serum GH-binding activity before GH therapy was lower than in group 1, 14.2 ± 1.4% of radioactivity ($P < 0.001$ versus controls).

GH-BP was evaluated 3, 6 and 12 months from the beginning of GH treatment. No significant changes in mean values were found in either group: the mean value remained low at 3, 6 and 12 months of treatment (Table 1). However, two patients (no. 3 of group 1 and no. 3 of group 2) showed a twofold and a 1.6-fold increase, respectively. This was observed at 3 months of treatment and did not change thereafter. These two patients had a very low level of plasma GH-binding activity before beginning treatment (Table 1).

In children from group 1, the growth velocity which was 3.8 ± 0.4 cm/year before treatment, increased to 8.9 ± 0.4 cm/year after 12 months of GH therapy. In this group the mean IGF-I plasma level was 0.4 ± 0.07 units/ml before treatment; under GH therapy it increased in all patients with a mean value of 2.3 ± 0.3 units/ml at 12 months. Patient no. 3 of group 1 responded to GH with an increase in growth velocity from 3 to 8.8 cm/year. Her IGF-I plasma level was 0.15 units/ml before treatment; it reached 1.5 units/ml after 12 months of GH-therapy.

Discussion

Our study demonstrates that plasma GH-binding activity is low in uraemic children. This finding could explain the state of GH resistance presented by children with CRF. Elevated circulating GH levels are associated with low or normal immunoreactive IGF-I concentrations and reduced somatomedin bioactivity in patients with CRF [1–4]. The

IGF-BP, which has been shown to be increased in uraemia, could act as an IGF-I inhibitor by reducing the concentration of unbound IGF-I [7].

The regulation of cell membrane GH receptors cannot be studied in man since no assay for GH receptors in accessible cells is available. However, in rats with CRF the number of liver membrane GH receptors has been shown to be extremely low [12]. Our results in uraemic children support a low number of liver GH receptors. The plasma level of GH-BP could mirror the concentration of liver membrane GH receptors. The mechanism of GH-BP generation is inknown in man; it has been proposed that the BP, which is the extracellular domain of the membrane receptor, could be generated from the receptor by proteolytic cleavage [9].

The factors which are responsible for the decreased number of GH receptors in rats with CRF, or for the low level of GH-BP in children with CRF, remain to be clarified. In rats, high levels of circulating GH are associated with an increased number of liver GH receptors [13]. To our knowledge, there is no in vivo situation in which GH down-regulates its receptors.

Plasma GH-binding activity was lower in patients from group 2 who were on dialysis, than in group 1 patients who were not. Both the younger age and the different treatment for the severe renal disease could explain the different results in the two groups.

Children from the two groups were treated with high doses of rhGH and responded to the hormone, as judged by the increase in growth velocity and IGF-I plasma levels. The results are comparable in the two groups of patients [7]. Although growth velocity is accelerated and plasma IGF-I increases under GH, plasma GH-binding activity remains low; it is not affected by GH therapy in most patients.

We have recently shown that in prepubertal children with isolated GH deficiency, the plasma GH-BP is low; when these children are treated with GH, GH-BP increases [11]. Thus, GH has a role in the regulation of GH-BP, as well as in that of the receptor. This regulation is also under a complex control, through multiple factors and hormones. The discrepancy between the response to GH in terms of IGF-I levels and growth, and the absence of changes in GH-BP activity in children with CRF remains to be elucidated. It should be stressed that the uraemic state can alter the binding equilibrium of circulating complexes; the presence of binding inhibitors cannot be excluded. Elevated levels of GH have been found in the urine of patients with renal insufficiency [14]. Very recently GH-BP has been found in human urine [15]. In order to better understand the effect of GH on growth parameters in CRF, the renal handling of GH has to be further investigated.

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