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ANTIGENICITY AND EFFICACY OF AUTHENTIC SEQUENCE RECOMBINANT HUMAN GROWTH HORMONE (SOMATROPIN): FIRST-YEAR EXPERIENCE IN THE UNITED KINGDOM

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SUMMARY

Twenty-one children were treated for GH deficiency with authentic sequence biosynthetic human GH (somatropin), 60 μg/kg body weight, subcutaneously, three times weekly for 1 year or longer. The magnitude of growth response and rise in serum insulin-like growth factor I levels were similar to those expected from experience with pituitary GH and somatrem. Three patients developed serum antibodies to GH with a binding capacity >0·02 mg/l, but in only one patient was the GH binding capacity >1·0 mg/l and he showed no attenuation of growth response. *Escherichia coli* polypeptide antibodies did not rise significantly and no clinically important side-effects occurred. Somatropin is safe, effective and of low immunogenicity in the treatment of GH deficiency.

Human pituitary GH has been the preferred treatment for GH deficiency since its efficacy was first reported 30 years ago (Raben, 1958). This treatment was withdrawn in most countries in 1985 following the deaths from Creutzfeldt-Jakob disease of four patients who had received pituitary GH between 1965 and 1975 (Preece, 1986a). Recombinant DNA technology led initially to the synthesis of methionyl-GH (somatrem) (Goeddel *et al.*, 1979), which was introduced for clinical use in 1981 (Kaplan *et al.*, 1986), and latterly to somatropin which has an amino-acid sequence identical to human GH. Experience with somatrem has shown it to be equipotent to pituitary GH and without significant side-effects (Kaplan *et al.*, 1986; Flodh, 1987; Milner *et al.*, 1987) but the associated incidence of GH antibodies, which may rarely be associated with attenuation of growth response, has been relatively high (Preece, 1986b; Tyllström *et al.*, 1986; Milner *et al.*, 1987). It was hoped that somatropin would prove, in comparison, to have reduced or absent antigenicity.

We report our experience during the first clinical trial in the United Kingdom of somatropin, with particular regard to the immunogenic aspects.

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PATIENTS AND METHODS

Children selected for this study were GH deficient as defined by conventional clinical and biochemical criteria (Milner & Burns, 1982) and had not previously received GH treatment. Children with active malignancy, significant psychosocial disturbance or chronic illness other than the primary study condition were excluded. All patients attended the Growth Clinic at the Hospital for Sick Children, Great Ormond Street. Twenty-one children entered the study, commencing treatment between March and October 1986. Their clinical features are shown in Table 1. Three patients (nos 18–20) were pubertal on entry into the study and three patients (nos 15, 17 & 21) entered puberty within 1 year of starting somatropin treatment. Patients with multiple pituitary hormone deficiency were established on appropriate therapy with hydrocortisone, thyroxine or desmopressin before commencing GH treatment.

Informed written parental consent was obtained together with that of the patient when of appropriate age; approval of the study protocol was obtained from the Standing Committee on Ethical Practice of the Hospitals for Sick Children, Great Ormond Street.

Patients were reviewed 1 month after commencing treatment, to check compliance, then at 3-monthly intervals from the start of the study for clinical examination and venepuncture for assessment of biochemical and haematological parameters, thyroid function, insulin-like growth factor I (IGF I), *Escherichia coli* polypeptide (ECP) antibodies, and GH antibodies. A urine sample was obtained for analysis. Growth assessment was by trained observers using standard techniques (Cameron, 1984). Height and height velocity for prepubertal children were expressed as standard deviation scores (SDS) for chronological age (Milner *et al.*, 1979). Radiographs of the left hand and wrist were taken at entry and 6-monthly thereafter for determination of bone age by the TWII-RUS method (Tanner *et al.*, 1983).

Somatropin (Eli Lilly, Basingstoke, England) was provided as lyophilized powder in vials of 1·48 mg (4 IU) and reconstituted in 1·0 ml of solvent (0·3% metacresol, 0·2% phenol in water). At each clinic visit treatment was adjusted to maintain a dose of 60 μg/kg body weight, subcutaneously, three times per week. Injections were given by the family doctor or nurse until a parent or the patient was competent.

Assays

Serum IGF I was measured by radioimmunoassay using a polyclonal rabbit antiserum (R557A) (Morrell *et al.*, 1989).

Antibodies to somatropin were determined at the Lilly Research Laboratories, Indianapolis, USA (Method 1; J. R. Sportsman, C. L. Winely & W. C. Smith, personal communcation, 1986; Sportsman *et al.*, 1987) and independently, blind at the Middlesex Hospital, London (Method 2; Di Silvio *et al.*, 1987) using polyethyleneglycol precipitation techniques. GH binding was considered specific if it was 50% inhibitable by unlabelled somatropin. Samples with specific binding greater than twice that of normal serum were subjected to a full displacement assay (Sportsman & Winely, personal communication, 1986) to determine serum GH-binding capacity (Munson & Rodbard, 1980).

Antibodies to ECP were measured by solid-phase radioimmunoassay (Sportsman & Smith, personal communication, 1986). Monkey normal and immune sera were used as

antibody controls and a normal human serum pool was used as an internal control. Antibody levels were compared with the untreated normal controls and a patient's own pretreatment value.

Statistical comparisons were made by Student's paired *t*-test or Wilcoxon tests according to distributional constraints.

RESULTS

Growth response

Twenty-one patients commenced somatropin treatment and 20 have been treated with somatropin for 1 year or longer. Patient 12 had no increase in height velocity after 9 months (2·3 cm/year pre-study; 2·9 cm/year over 9 months), so treatment was stopped. He had three 'partial' GH responses to insulin-induced hypoglycaemia and the cause of his poor growth remains under review; his compliance was considered satisfactory and data on his serum IGF I and GH antibody status, but not growth response, are included in the analyses which follow. Patient 10 had poor compliance, missing 11 weeks of treatment in 1 year; he was excluded from statistical analyses.

Table 1. Clinical data for 21 patients with GH deficiency at entry to trial of somatropin treatment

Patient	Sex	Age (years)	Bone age ('years')	Diagnosis		Height SDS for CA	Height velocity SDS
1	M	5·4	3·7	IGHD		−3·9	−2·5
2	F	5·8	3·7	MPHD		−2·9	−1·8
3	M	5·9	3·1	MPHD		−3·7	−3·4
4	F	6·0	4·3	MPHD	Histiocytosis X	−3·9	−5·7
5	M	7·1	3·7	IGHD		−4·0	−2·2
6	M	7·3	5·8	IGHD	TB meningitis	−3·1	−2·4
7	M	7·5	4·2	MPHD	Craniopharyngioma; surgery	−3·4	−5·0
8	M	8·1	5·1	IGHD		−2·3	−1·3
9	F	8·5	6·0	IGHDP	Histiocytosis X	−3·6	−1·0
10	M	9·3	5·7	IGHD		−4·2	−2·3
11	M	9·6	10·2	IGHD	Craniopharyngioma; XRT	−1·1	−5·3
12	M	9·7	7·2	IGHDP		−3·4	−3·7
13	M	10·1	10·6	MPHD	Craniopharyngioma; surgery	−2·3	−0·4
14	M	10·2	9·9	IGHD	ALL; craniospinal XRT	−2·5	−2·4
15	F	11·0	8·2	MPHD	Septo-optic dysplasia	−3·0	*
16	M	11·5	9·8	IGHD		−2·7	−1·5
17	M	12·7	11·4	MPHD	Craniopharyngioma; surgery	−1·2	*
18	F	12·8	9·1	MPHD	Optic glioma; XRT	−1·5	*
19	M	14·4	11·9	IGHDP		−3·6	*
20	F	15·5	13·9	MPHD	Cushing's disease; surgery	−3·1	*
21	M	16·6	13·9	MPHD	Craniopharyngioma; surgery	−3·3	*

M male; F female; IGHD isolated growth hormone deficiency; MPHD multiple pituitary hormone deficiency; TB tuberculous; XRT irradiation therapy; ALL acute lymphoblastic leukaemia; IGHDP 'partial' IGHD; SDS standard deviation score; CA chronological age.

* Height velocity SD scores not applicable because of age and/or pubertal status.

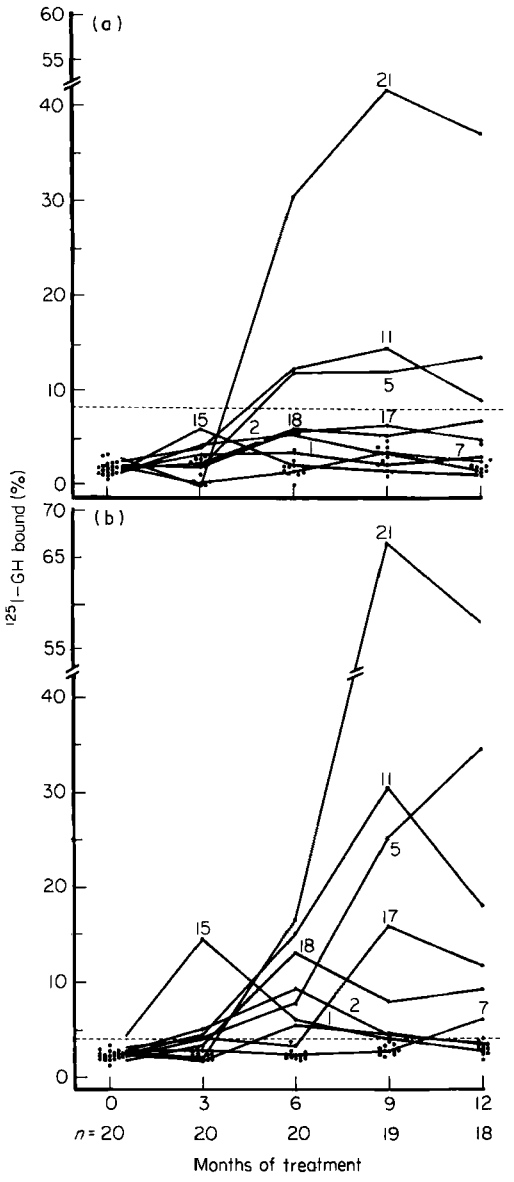

Fig. 1. Percentage serum binding of ^{125}I-GH by two methods of polyethyleneglycol immunoprecipitation assay ((a) Method 1, (b) Method 2) in 20 GH-deficient children during the first 12 months of somatropin treatment. Individual patients are identified by number. Normal range upper limit for non-immune control sera (- - - - -) is shown for each method.

Thirteen prepubertal children have completed 1 year of treatment. Their height velocity SDS increased from a pretreatment mean of −2·7 (SD 1·7) to +4·1 (2·0) ($P < 0{\cdot}001$). The six pubertal children increased their height velocities from a mean of 2·8 (SD 1·9) cm/year before treatment to 8·6 (2·3) cm/year after 1 year. Patient 21 entered puberty after 6

months of treatment and his subsequent growth acceleration cannot be interpreted meaningfully with respect to the concurrent rise in his serum level of GH antibodies.

The mean ratio of bone age advancement to chronological age advancement during the first year of treatment was $1{\cdot}0 \pm 0{\cdot}5$ (SD) 'years'/year ($n = 19$).

Insulin-like growth factor I

Serum IGF I levels before treatment were low in both the prepubertal (median 0·16, range 0·05–0·44 U/ml) and pubertal (0·23, 0·18–0·36 U/ml) children, with no significant difference between these groups. IGF I levels in both groups were higher after 3 months (prepubertal 0·28, 0·10–0·69 U/ml, $P < 0{\cdot}005$; pubertal 0·69, 0·49–0·79, $P < 0{\cdot}05$), the increase being greater in the pubertal group ($P < 0{\cdot}01$). At 12 months a further rise in IGF I was evident in the prepubertal group, reaching a median of 0·40(0·18–1·17) U/ml. Patient 12, who showed no growth response to 9 months of treatment, had the highest pretreatment IGF I level (0·44 U/ml) and this showed only a small increase during treatment (0·52–0·71 U/ml over 9 months).

Immunogenicity

The GH antibody binding levels for the first year of somatropin treatment are shown in Fig. 1. Patient 10, excluded because of poor compliance, had normal GH binding. There was a strong correlation between the results of the two GH-binding assays; the regression of log (per cent GH binding + 1) by Method 2 on Method 1 was: $y = 0{\cdot}51 + 0{\cdot}82x$ ($n = 97$, $r = 0{\cdot}83$, SE 0·39).

Method 1 showed only three of the 20 patients to have raised GH binding, none before 6 months of treatment. The pattern of GH binding for these three patients was similar, although reaching higher levels, using Method 2, which showed nine of the 20 patients to have increased GH binding. Five of these patients first had elevated GH binding at 3 months, three after 6 months, and one after 12 months. Of these nine patients, only four (patients 5, 17, 18 and 21) did not return to normal levels of GH binding during continued somatropin treatment for periods of between 15 and 21 months from entry to the study (data not shown).

Sera from the three patients with elevated GH binding by both methods had specific GH binding. Patient 11 had a serum GH-binding capacity of 0·04 mg/l at 6 months and 0·05 mg/l at 9 months, but this had fallen to $< 0{\cdot}02$ mg/l by 15 and 18 months. Patient 5 had a serum GH binding capacity $< 0{\cdot}02$ mg/l, reaching 0·07 mg/l at 12 months and rising to 0·12 mg/l after 15 months. Patient 21 showed the highest GH-binding capacity with an abrupt rise after 6 months of treatment to 1·00 mg/l. This reached a peak of 3·81 mg/l after 12 months but fell to 0·15 mg/l 3 months later. GH-binding levels were not associated with changes in height velocity between 6 and 12 months of treatment.

No patient had a significant rise in ECP antibodies.

Side-effects of somatropin therapy

No clinically important side-effects due to somatropin treatment were observed during the study. There were no significant abnormalities in haematological or biochemical parameters or urine tests. Five patients experienced periodic injection site pain and four

developed generalized rashes (two maculopapular, one pustular, one urticarial) which lasted for a few days and resolved spontaneously. One child (patient 2) developed a desquamative erythematous rash of the hands and feet 2 months after starting treatment. She had concurrent symptoms of upper respiratory tract infection but extensive serological studies failed to identify a cause. The rash resolved without withdrawal of somatropin.

DISCUSSION

This study has shown somatropin to be as effective in promoting growth in GH-deficient children as both pituitary GH (Milner *et al.*, 1979) and somatrem (Kaplan *et al.*, 1986; Milner *et al.*, 1987). Serum IGF I levels rose during treatment, in keeping with previous studies with pituitary GH and somatrem (Kaplan *et al.*, 1986). Somatropin was well tolerated and no clinically significant side-effects were observed. Antibodies to ECP did not rise significantly.

The development of antibodies to GH has been a feature common to treatment with both pituitary and biosynthetic GH preparations. Their importance is related to their binding capacity with growth attenuation becoming likely with serum GH-binding capacity above 1 mg/l (Kaplan *et al.*, 1974; Milner *et al.*, 1979). Serum GH-binding capacity $>0{\cdot}02$ mg/l was shown in only the three patients whose sera had the highest binding by both assay methods. The peak GH-binding capacities were nonetheless low in two of these patients (at 0·05 and 0·12 mg/l) and although the third patient reached a peak binding capacity of 3·81 mg/l, this had fallen considerably 3 months later. None of our patients showed attenuation of their response to treatment. It remains to be seen how the antibody levels change with continued somatropin treatment but most patients who have developed clinically significant GH antibodies have done so within 1 year of starting GH, although antibodies may first develop as late as 2 years after beginning treatment (Kaplan *et al.*, 1974; Milner *et al.*, 1979).

The incidence of GH antibodies to pituitary GH preparations has ranged from less than 10 up to 66% (Preece, 1986b) and seemed to relate to the extraction procedures and purity of the preparation. Thus the incidence of antibodies to somatropin is comparable to that associated with the less immunogenic pituitary GH preparations. Growth attenuation occurred in the presence of such antibodies in about 5% of patients receiving the Raben-type preparation (Preece, 1986b). Preparations of somatrem have been associated with antibodies to GH in 30 to 75% of patients within 6 to 9 months of starting treatment (Kaplan *et al.*, 1986; Tyllström *et al.*, 1986; Thompson & Draper, 1987) yet growth attenuation has been reported in only two children, both of whom received early preparations with a relatively high ECP content (Kaplan *et al.*, 1986; Takano & Shizume, 1986). Thompson and Draper (1987), using a dose of GH (somatrem) and methods of antibody assessment similar to those in this study, reported antibody-binding capacities greater than 0·02 mg/l in 50% of patients and 1 mg/l or more in 20% of patients. Although somatrem can be distinguished immunologically from natural human GH (Aston *et al.*, 1985) there has been only one reported instance of a somatrem-treated child developing antibodies specific to methionyl-GH (Kaplan *et al.*, 1986). The mechanisms responsible for the induction of GH antibodies in response to GH treatment remain undefined, although an association with impurities, such as ECP, in the GH preparation is apparent (Kaplan *et al.*, 1986). Even recent preparations of somatrem provoked significant

increases in ECP antibodies (Milner *et al.*, 1987) whereas we observed no such effect with somatropin.

Except when there is evidence of growth attenuation, the significance of these antibodies in the long term remains unknown but should nonetheless be considered when selecting the biosynthetic GH preparation for clinical use.

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