Archives of Disease in Childhood, 1979, **54**, 609–613

Use of growth hormone-gel

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SUMMARY We evaluated the efficacy of a depot preparation of growth hormone (GH) in a 15% gelatin solution (GH-gel) in the treatment of 15 growth hormone-deficient children. The studies were designed to see if prolonging absorption of GH to achieve lower more physiological concentrations of GH in plasma would decrease the frequency of injection, reduce the amount of GH needed for effective therapeutic response, and improve the response to long-term treatment. We found that after a single dose of GH-gel the plasma concentrations of GH were lower than those achieved after the standard aqueous preparation. The preparation was efficacious in promoting growth and our 1st study of 6 patients suggested that GH-gel given twice a week had a growth response equal to that of the three-times a week aqueous schedule. However both schedules resulted in the frequently observed decreased growth rate during the second treatment year. Our 2nd study, attempting to ameliorate this waning effect by using the GH-gel preparation twice a week in a weight-adjusted dose during the entire second year did not resolve the problem. Thus, GH in depot gel results in more physiological plasma concentrations of GH and may be beneficial in reducing the quantity of hormone needed and the injection frequency but it does not offer a solution to the waning response to the long-term administration of GH.

The administration of growth hormone (GH) extracted from pituitaries has provided successful treatment for children with growth hormone deficiency. However, the optimal dose and administration schedule has yet to be established (Preece *et al.*, 1976; Frasier *et al.*, 1977). The quantity of GH given to each child is limited by its availability and cost, and the need to give the preparation repeatedly by the intramuscular (IM) route.

The usual method of administration results in an unphysiological pattern of the concentration of GH in plasma. After a single IM injection of GH in aqueous solution, peak concentrations are reached in 2–4 hours. The peak levels of GH are in the range usually found in patients with acromegaly. After this very high peak there is a rapid decline in the concentration of GH and the hormone disappears from plasma within 12–24 hours (Frasier *et al.*, 1969).

We thought that a depot preparation of GH might prolong absorption from the site of injection and maintain lower but more physiological plasma concentrations for longer periods of time, thus decreasing the amount of GH needed to maintain an effective therapeutic response, and improving the therapeutic response to long-term GH administration. Our experience with GH in a 15% gelatin solution (GH-gel) in the treatment of 15 patients with growth hormone deficiency is reported.

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Materials and methods

Patient population. Patients with growth hormone deficiency diagnosed by testing as previously described (Lippe *et al.*, 1971) and followed up at the UCLA Pediatric Endocrine Clinic were studied under an approved National Pituitary Agency (NPA) protocol. The patients ranged in age from 7–15 years, were all prepubertal at the time of starting GH treatment, and were all growing at a rate of 3 cm or less in the year before treatment.

Preparation of GH for injection. Aqueous growth hormone was prepared by adding sterile, distilled water to GH as provided by the NPA. Gelatin (gel) USP was prepared as a 15% weight/volume solution

in freshly distilled water and packed in 10 ml rubber-stoppered vials without preservative. The vials of gelatin were sterilised by autoclaving. To prepare GH for injection, the gelatin vial was warmed to liquidity under warm, running water by the patients' parents and used as diluent for the NPA GH in the same way that sterile water was used. Once mixed, each GH-gel vial provided material for 4–6 injections. The material was refrigerated between injections and rewarmed to liquidity before administration of each dose.

Laboratory methods. The concentration of GH in plasma was measured by a double antibody radioimmunoassay (Boden and Soldner, 1967). Binding antibodies to GH were measured as previously described (Frasier *et al.*, 1974).

GH blood levels. The concentrations of GH achieved in plasma, after a single IM injection of either 2 international units (IU) GH in aqueous solution (GH-AQ) or 2 IU GH-gel, were measured in 6 patients. All patients had an indwelling scalp vein needle with a heparin lock placed in a hand vein and blood was sampled at 0, 1, 2, 4, 6, 8, 12, 18, 24, and 30 hours after the injection. In those patients receiving GH-gel, sampling continued at 36, 42, 54, 60, and 72 hours. Plasma was separated and stored at −20°C until assay.

Treatment protocols.

Study 1

Six patients entered an initial 30-month crossover study to test the efficacy of the gel preparation and to examine the therapeutic response to a lower dose of hormone in the depot form. Cases 1–3 were started on 2 IU of GH three-times a week (6 IU/week) in aqueous solution (GH-AQ) for 6 months followed by 2 IU twice a week (4 IU/week) in gel for 6 months. After a 6-month period during which no GH was administered, these patients resumed treatment with 6 months of GH-gel (2 IU twice a week) followed by 6 months of GH-AQ (2 IU three-times a week). Cases 4–6 followed a reverse schedule. They received GH-gel for 6 months followed by GH-AQ for 6 months. After a 6-month period of the treatment they received GH-AQ followed by GH-gel.

Study 2

After the crossover study had been completed, a second group of 9 patients (Cases 7–15) began a 30-month regimen of GH therapy with doses adjusted for weight. Therapy was begun with GH-AQ in a dose of 0·1 IU/kg per injection, administered three-times a week (0·3 IU/kg per week). After one year of this schedule and a 6-month period of no treatment, GH-gel was administered at a dose of 0·15 IU/kg twice a week. Thus, patients received the same weekly dose as during the first year (0·3 IU/kg per week) in two injections. The dose of GH-gel was again adjusted to body weight at the beginning of the second year.

Results

GH blood levels. The mean plasma concentrations of GH reached after single injections of GH-AQ and GH-gel are shown in Table 1. The GH-AQ preparation resulted in a rapid increase in GH by one hour, a wide peak of 128·4 ± 77·5 mU/l at 2 hours, and concentrations in excess of those obtained after the gel preparation until 8 hours after the injection. By contrast, there was no clear peak after the gel preparation, and the concentrations achieved in the first 8 hours were much lower than with the aqueous, with maximum concentrations of only 37·9 ± 8·8 mU/l. By between 12 and 18 hours the gel preparation had maintained levels in excess of the aqueous preparation and by 24 hours both resulted in virtually undetectable concentrations. When we looked at the peak concentrations of GH achieved by each patient, irrespective of time, the patients receiving GH-AQ had a peak of 158·6 ± 62·2 mU/l (SEM) with a range of 95·7–283·1 mU/l, while the mean peak of those receiving GH-gel was 46·6 ± 11·9 mU/l and the range 26·7–68·0 mU/l.

While the number of patients in each group is small and the variability great (precluding meaningful statistical analysis), there are clear differences in the pattern and concentrations of GH achieved in blood after the two modes of administration. The levels and

Table 1 *Plasma growth hormone (GH) concentrations after single injections of GH-AQ and GH-gel*

Time (hours)	*Plasma GH (mU/l)**	
	GH-AQ (n=3)	*GH-gel (n=3)*
0	<4·0	<4·0
1	94·9 ± 66·9	8·5 ± 4·6
2	128·4 ± 77·5	22·1 ± 9·5
4	79·9 ± 12·1	37·1 ± 15·8
6	68·0 ± 13·9	37·4 ± 8·8
8	35·5 ± 3·1	32·5 ± 7·7
12	19·6 ± 5·6	29·6 ± 5·1
18	<4·0	17·5 ± 8·0
24	<4·0	<4·0
30	<4·0	<4·0
36	—	6·9 ± 3·7
42	—	<4·0
48	—	<4·0
54	—	<4·0
60	—	<4·0
72	—	<4·0

*Mean ± SEM.

Conversion: SI units to traditional units—1 mU/l = 0·6 ng/ml.

pattern after GH-AQ are similar to those which we previously reported (Frasier *et al.*, 1969). In contrast, the administration of GH-gel leads to a slower increase in blood levels, a lower and more prolonged peak in growth hormone concentration, and persistence of detectable growth hormone for a longer time.

Study 1

Table 2 shows the growth rates for Cases 1–6 while they received alternate courses of GH-AQ and GH-gel. The results of the first year of this study were confounded by the development of hypothyroidism in 3 patients (Lippe *et al.*, 1975). However, when the 6-month periods during which patients with untreated hypothyroidism are eliminated from consideration, it is clear that in the first year of treatment there was no overall difference in the rate of growth during the administration of GH-AQ ($3 \cdot 9 \pm 0 \cdot 2$ cm/6 months) and during the administration of GH-gel ($3 \cdot 9 \pm 0 \cdot 3$ cm/6 months). The small numbers preclude meaningful statistical comparison of the effects of the sequence in which each GH preparation was given.

In the second year of treatment there was also no difference in the rate of growth during the administration of GH-AQ ($3 \cdot 2 \pm 0 \cdot 2$ cm/6 months) and the administration of GH-gel ($3 \cdot 1 \pm 0 \cdot 2$ cm/6 months). The sequence in which the two GH preparations were given did not significantly alter the overall response. Patients who received GH-AQ first grew $6 \cdot 2 \pm 0 \cdot 1$ cm/year and patients who received GH-gel first grew $6 \cdot 4 \pm 0 \cdot 2$ cm/year.

However, there was a decrease in the rate of growth during the second year of GH administration. The growth rate for all treatment periods in which hypothyroidism was not a variable ($n = 9$) during the first year was $3 \cdot 9 \pm 0 \cdot 2$ cm/6 months. During the second year of treatment the growth rate for all treatment periods ($n = 12$) decreased to $3 \cdot 1 \pm 0 \cdot 2$ cm/6 months ($P < 0 \cdot 01$).

Table 2 *Growth rate (cm/6 months) while receiving alternate courses of GH-AQ and GH-gel*

Case	*First year*		*Second year*	
	GH-AQ *1st 6 months*	*GH-gel* *2nd 6 months*	*GH-AQ* *2nd 6 months*	*GH-gel* *1st 6 months*
1	4·5	4·5	2·6	3·2
2	4·3	4·2	3·7	3·5
3	3·5	*	2·9	2·7
	2nd 6 months	*1st 6 months*	*1st 6 months*	*2nd 6 months*
4	3·7	*	2·9	3·6
5	*	3·7	4·0	2·1
6	3·3	3·3	3·2	3·2
Mean±SEM	3·9±0·2	3·9±0·3	3·2±0·2	3·1±0·2

*Hypothyroidism developed during these 6-month intervals.

Study 2

Table 3 shows the growth rates of Cases 7–15 during the year they received GH-AQ (0·1 IU/kg three-times a week) and during the year they received GH-gel (0·15 IU/kg twice a week). Growth rates for the 6-month 'off' period between the two treatment years have been extrapolated to a 12-month period and are shown for comparison. As hypothyroidism had been a problem in the 1st study, these patients were frequently monitored and in the one patient in whom the T4 decreased precipitously (Case 7) supplementary thyroxine was instituted within weeks.

The growth rate of $8 \cdot 5 \pm 0 \cdot 4$ cm/year during the first year of GH-AQ compares favourably with the therapeutic responses seen in other studies. In the second year with the weight-adjusted GH-gel preparation, there was a significant decrease ($P < 0 \cdot 001$) in rate to $5 \cdot 6 \pm 0 \cdot 4$ cm/year.

Sera obtained from 7 patients after 6–12 months of GH-gel were tested for binding antibody to GH. Significant antibody was present in two samples.

Discussion

The most commonly used treatment regimens for the administration of GH to hypopituitary children are based on empiric observations of successful therapy (Raben, 1958; Escamilla *et al.*, 1961). Actual dose schedules, however, vary between countries and investigative protocols. Some schedules use doses which vary with body size or weight (Ferrandez *et al.*, 1970; Hall and Olin, 1972; Kirkland *et al.*, 1973; Frasier *et al.*, 1977), while others do not (Tanner *et al.*, 1971; Aceto *et al.*, 1974; Preece *et al.*, 1976). The variables of patient heterogeneity, additional hormonal requirements, and age at start of treatment have made the analysis of results difficult and have all contributed to less than ideal recommendations for the initial dose of GH. Administration of aqueous preparations results in radioimmunoassayable concentrations of GH which quickly reach very high

Table 3 *Growth rate (cm/year) while receiving GH-AQ followed by GH-gel*

	Cases									*Mean± SEM*
	7	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	
GH-AQ growth	7·8	9·4	7·6	7·7	7·0	8·4	10·7	8·5	9·0	8·5±0·4
No treatment	0·4	1·3	1·0	1·1	3·3	2·6	2·0	1·6	3·0	1·8±0·3
GH-gel growth	4·0	7·3	5·0	5·0	5·8	6·5	5·9	5·1	8·0*	5·6±0·4

*Entered puberty, rate not included in mean.
†$P < 0 \cdot 001$, ‡$P < 0 \cdot 001$ v. either treatment year.

levels and then fall rapidly. Long periods ensue before the next injection during which GH is undetectable (Frasier *et al.*, 1969). Thus, the present methods of administration result in abnormal patterns of plasma hormone levels characterised by peak concentrations, similar to those seen in acromegaly.

The current study was undertaken to test the efficacy of a depot GH-gelatin preparation designed to prolong time of absorption. We hoped to achieve adequate growth with less frequent injections and to reduce the total amount of hormone administered. We also studied the effect of GH-gel in ameliorating the deceleration in growth rate experienced by most patients after the initial rapid growth of the first year of treatment.

Our results demonstrate that the IM administration of a single injection of GH in gelatin reduces the peak concentration of GH and prolongs its absorption when compared with an injection of the same amount of GH in aqueous solution. The peak concentrations achieved after the injection of GH-gel resemble those seen in association with sleep in normal children (Underwood *et al.*, 1971; Eastman and Lazarus, 1973).

Although the number of observations was limited by the development of hypothyroidism in 3 patients, our initial study appears to indicate that GH-gel (2 IU twice a week) is as effective as an aqueous solution of GH (2 IU three-times a week) during both the first and second year of treatment. Thus, the administration of GH in gelatin appears to have stimulated the same rate of growth as aqueous GH with a significant saving in the quantity of hormone. Theoretically these savings would provide treatment for one more patient for every 2 currently being treated. At the same time, the number of injections and cost of treatment would be reduced. Because the number of patients treated in our study is small, these conclusions must be considered tentative. A larger group of patients needs to be treated with GH in gelatin to test the validity of our preliminary results.

As in previous studies in which GH was given for more than one year, the response waned during the second 12 months of GH therapy (Soyka *et al.*, 1970; Kirkland *et al.*, 1973; Aceto *et al.*, 1974; Frasier *et al.*, 1977). In our 2nd study we attempted to ameliorate this waning effect by administering GH-gel in the same total weekly dose as in the earlier GH-AQ therapy, using twice-weekly rather than three-weekly injections. We were not successful. The growth rate in patients receiving GH-gel was significantly less than that observed during the previous year of therapy with GH-AQ. In view of the comparable effects of the two GH preparations during the second year of our initial study, this diminished response is most likely due to time and not to the preparation. As previous analysis (Frasier *et al.*, 1977) indicates that a three-times a week dose schedule may be preferable to twice a week, we might have been more successful if GH-gel had been given three-times a week in a dose of 0·1 IU/kg. This hypothesis remains to be tested. A solution to the problem of waning response as observed in this and other studies remains to be found.

We thank Ms Jean Hilburn for technical assistance, Ms Sandra Dills for help with this manuscript, and Dr S. Raiti and the National Pituitary Agency for supplying the human growth hormone for this study. The study was supported in part by a grant from the USPHS, RR865.

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Received 14 November 1978