Lack of effects of recombinant human GH on spermatogenesis in the adult cynomolgus monkey (Macaca fascicularis)

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Abstract

Objective: The effects on male reproductive parameters after 1 year of treatment with recombinant human GH to the cynomolgus monkey were investigated.
Design: Twenty-four male cynomolgus monkeys were given daily subcutaneous doses of 0 (vehicle) (n = 7), 0.4 (n = 5), 2.0 (n = 5) and 10.0 (n = 7) IU/kg bodyweight for 52 weeks. At completion of the treatment period two control and two high-dose animals were left for a 12-week treatment-free period.
Methods: Before and during the treatment period and during the recovery period, sperm analyses, testicular volume measurements and hormone analyses of prolactin (PRL), LH, FSH, testosterone and IGF-I in serum, and analysis of serum antibodies against human GH were performed. Testicular morphology was monitored by biopsies, predose and on day 15 of the study, and with light microscopy on organ samples collected at time of death, at the end of the treatment, and during recovery periods respectively.
Results: Of all studied parameters, alterations were observed only in serum levels of IGF-I and PRL. IGF-I showed a dose-dependent increase throughout the treatment, with a normalisation during the treatment-free period. PRL decreased significantly in animals given 10.0 IU/kg per day from week 14 of treatment and throughout the study but with a normalisation upon cessation of treatment. Spermatogenesis, as judged from semen analysis, testicular volume measurements and testicular morphology was not affected.
Conclusion: This controlled preclinical study demonstrates that high doses of human GH do not alter male reproductive parameters in a non-human primate model.

European Journal of Endocrinology 140 350–357

Introduction

The role of growth hormone (GH) in male reproduction has been elucidated in recent years. It is obviously of importance for normal pubertal development and subsequent fertility (1). The development of the genital organs is poor in GH-deficient boys and administration of GH increases the growth of the genitalia (2, 3). In children with dwarfism due to resistance to the action of GH (Laron-type dwarfism) puberty is delayed (4). Data from experimental animal studies are in line with those clinical data. The GH-deficient dwarf mouse has reduced testicular weight, seminiferous tubular diameter and germ cell number: however, with GH administration during the postnatal period these parameters can be normalised (5). In the prepubertal boar exogenous GH increased Sertoli cell size, tubular lumen formation and initiated spermatogenesis earlier than in controls (6). Reduced seminal fluid volume and total sperm number/ejaculate compared with reference groups were found in patients with isolated GH deficiency (7). Treatment with GH for 4 months did not improve semen quality, probably due to too short a treatment period. However, in some infertile patients with hypogonadotrophic hypogonadism Shoham et al. (8) observed that co-treatment with gonadotrophins and GH resulted in increased serum testosterone concentrations and a normalisation of sperm parameters. If gonadotrophins were given alone, these effects were not seen.

In man, an effect of GH on Leydig cell function has been demonstrated in pubertal boys (9). The postulated mechanism for the effect of GH on testicular function is an increase of local insulin-like growth factor-I (IGF-I) production which leads to an augmentation of the follicle-stimulating hormone (FSH) acting directly on Sertoli and indirectly on Leydig cells. Studies in animals and man have elucidated the interaction between GH and sex steroids (10, 11). In studies performed as a part of the safety documentation, high doses of human (h) GH have been given to
normal intact animals. In the monkey and the rat, hGH has been shown to have few adverse effects (12, 13) after up to 3 months of treatment. However, in the male dog severe testicular alterations and reduced plasma levels of testosterone, luteinizing hormone (LH) and prolactin (PRL) have been observed in preclinical safety studies after 3–4 weeks of treatment (14).

To evaluate possible effects on spermatogenesis of hGH in the cynomolgus monkey, male reproductive parameters were carefully monitored before and during the treatment period and at the end of a 12-week recovery period in this 1 year preclinical safety study. The reproductive system of humans and Old World non-human primates share many similarities. Hence, several macaque species were tested in studies of reproductive physiology and reproductive toxicology and were proved to be appropriate preclinical animal models (15, 16). The doses used were 10–250 times the recommended therapeutic dose in adult GH-deficient patients and 4–100 times the doses used in GH-deficient children.

Materials and methods

Animals and treatment

Twenty-four adult male cynomolgus monkeys (Macaca fascicularis) weighing between 3.7 and 5.4 kg at the start of dosing were used for the study. Although the precise age of the animals was unknown, they were considered to be sexually mature at the start of the treatment, due to historical data regarding the relationship between body weight and age. The monkeys were divided into four groups and treated with recombinant human (rh) GH (Genotropin; 12 IU: Pharmacia AB), or vehicle (glycine/phosphate-buffered solution). The doses were 0 (vehicle) (n = 7), 0.4 (n = 5), 2.0 (n = 5) and 10.0 (n = 7) IU/kg per day corresponding to 0.013, 0.65 and 3.23 mg/kg per day respectively.

The animals were treated once daily in the morning for 52 weeks by subcutaneous bolus injections on the back. The administered dose volume (2 ml/kg) was divided into two equal doses which were injected on the same occasion at two different sites. Six sites were used and these were alternated. Five animals/group were killed after the treatment period, whereas two animals in the control and high-dose groups, respectively, were given a 12-week treatment-free period before being killed. The monkeys were housed individually in stainless steel cages in rooms maintained at a temperature of 20–25 °C and a relative humidity of 40–70%. They were exposed to artificial light for 12 h (0600–1800 h) during a 24-h light/darkness cycle and fed twice daily with about 70 g/animal of a pelleted diet. Additional fruit supplement was also given. Food was withdrawn overnight prior to blood sampling. Maintenance and experimentation with animals was performed in accordance with the German Law for the Care and Use of Laboratory Animals.

Analyses, measurements and morphological examinations

Individual body weight was recorded before initiation of treatment and then weekly throughout the treatment and recovery periods and on the day of necropsy. Sperm analysis was performed three times before the treatment period at intervals of at least 4 days, in weeks 13, 26, 39 and 52 and at the end of the treatment-free period. Ejaculates were obtained by rectal probe stimulation under light anaesthesia with ketamine hydrochloride (Ketavet; Parke-Davis, Germany). The weight and volume of each ejaculate was recorded and the coagulum — if present — was digested using a 2% trypsin solution. The sperm number was determined in both exudate and coagulum using a haemacytometer and expressed as spermatozoa x 10^6/ejaculate. Sperm motility was evaluated microscopically in the exudate. Sperm morphology was assessed from Papanicolaou-stained exudate smears according to the WHO guidelines (17). Testicular size measurements using an adjustable stage were carried out in a blinded fashion twice before the start of treatment, and in weeks 13, 28, 40 and 52, and at the end of the treatment-free period. Unilateral surgical testicular biopsy was performed under sedation once before the treatment period and on day 15 of the study on three animals from the control and high-dose groups and on two animals from the low- and intermediate-dose groups. Biopsies were performed as described earlier (18). The biopsy material was fixed in 5.25% glutaraldehyde in 0.067 mol/l sodium cacodylate buffer (pH 7.4) and embedded in Epon. Semithin sections (about 0.5 μm) were stained with toluidine blue and evaluated by light microscopy.

Blood samples for hormone analyses were collected from the brachial vein in the morning during the pretest period and before dosing in weeks 14, 27, 39/40 and 52 and after the recovery period for serum analysis of IGF-I, PRL, testosterone, FSH and LH. However, the week-52 samples for LH and testosterone for the animals in the groups given 0.4 and 2.0 IU/kg per day respectively were not analysed. Blood for analysis of IGF-I was also sampled on days 5 and 10. For gonadotrophins and testosterone, blood was sampled at the same time in the morning for 3 successive days on each occasion. In the Figures median values are given. The following assays were used: IGF-I, a competitive RIA following acid–ethanol treatment, detection limit 20 μg/L, coefficient of variation 3.1% (intra-assay) and 10% (interassay) (19); PRL, an RIA, detection limit 2 ng/ml, coefficient of variation 3.2% (intra-assay) and 7.5% (interassay) (Testpack DPC; Hermann Biermann GmbH Diagnostica); testosterone, an RIA following ether extraction, detection limit 0.2 nmol/L, coefficient of variation 8.5% (intra-assay) and 13.0% (interassay).
(20); FSH, an RIA, detection limit 0.1 mg/l, coefficient of variation 8.4% (intra-assay) and 10.8% (interassay) (21); LH, an in vitro mouse Leydig cell bioassay, detection limit 2.7 U/l, coefficient of variation 13.1% (intra-assay) and 16.9% (interassay) (22).

Blood samples were collected from all animals during the pre-test period, and in weeks 13 and 26, and at necropsy for determination of serum antibodies against hGH which was determined by a competitive RIA (23).

After 52 weeks of treatment or after an additional 12-week treatment-free period, the animals were killed by an intravenous injection of pentobarbital sodium and immediate exsanguination.

A full macroscopic examination of all tissues and organs was performed. During necropsy, epididymides, prostate, seminal vesicles and testes were weighed before fixation. Samples of epididymides, prostate and seminal vesicles were fixed in 4% buffered formaldehyde solution and testes were fixed in Bouin’s fluid. In addition, samples of the above organs were fixed in 5% glutaraldehyde in 0.067 mol/l sodium cacodylate buffer (pH 7.4). The formaldehyde-fixed tissue was embedded in paraffin wax, sectioned and stained with haematoxylin and eosin (HE). The Bouin-fixed testes were embedded in methacrylate (Historesin) and the glutaraldehyde-fixed samples were embedded in Epon. Sections were stained with HE, periodic acid-Schiff or toluidine blue (Epon sections) and examined under the light microscope.

Statistical evaluation of body weight, body weight change and organ weights was performed with Bartlett’s test for homogeneity of variances, followed by a rank transformation and Bartlett’s test in case of heterogeneity only (P < 0.05, equivalent to 95% probability). For hormonal data and sperm analysis a rank transformation was performed, followed by Bartlett’s test for homogeneity of variances. For homogeneous data, the one-way analysis of variance (ANOVA) was performed. In the event of significant results for the ANOVA (P < 0.05, equivalent to 95% probability), Dunnett’s two-tailed t-test was used to compare each treated group against the control group. In the case of heterogeneity of the rank-transformed data, Kruskal–Wallis test was performed together with Wilcoxon rank-sum test to compare each treated group against the control group.

The statistical evaluation was performed with the standard software package SAS (Statistical Analysis System) release 6.04. Data are expressed as means ± S.E.M.

Results

One low-dose male was killed in extremis on day 210 of the study due to chronic enteritis. This was considered to be a spontaneous disease and not related to the treatment with rhGH. In the further analysis this animal was not accounted for.

Body weight was within historical control data for the animals in all groups, although the group 3 animals (2 IU/kg per day) showed the lowest mean body weight throughout the experimental period and including the pre-test period. There was no effect on ejaculate weight, sperm motility and morphology (Figs 1 and 2), weight of genital organs (Table 1), sperm count or testicular volume throughout the treatment period, or during the pre-test period.
recovery period (Fig. 3). Already on day 5 and throughout the treatment period, serum IGF-I levels showed a dose-related increase in all dose groups (Fig. 4). At the end of the recovery period the IGF-I levels were comparable to predose values (not shown).

Although there was a great individual variation in PRL serum concentrations, statistically significant reductions ($P < 0.05$) were found in the high-dose group from week 14 and throughout the treatment period (Fig. 5). PRL levels were normalised at the end of the treatment-free period. There were large variations in serum testosterone and LH levels in all groups (Fig. 6). However, the values were considered to be within the normal limits of the background data for this species (24, 25, 26) and/or in the range of the pre-test data. FSH levels in serum collected at different time-points were under the detection limit in many cases. However, for those samples that had measurable values (two to four/group) there were no apparent treatment-related effects on serum FSH throughout the experimental period (data not shown). No significant amounts of rhGH antibodies appeared in treated animals. Microscopic examination of the testicular biopsies, collected

Table 1 Group mean organ weight (g) at necropsy.

<table>
<thead>
<tr>
<th>Organ</th>
<th>At completion of treatment</th>
<th>After recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (0 IU/kg per day)</td>
<td>Group 2 (0.4 IU/kg per day)</td>
</tr>
<tr>
<td>Testes Mean</td>
<td>35.53</td>
<td>32.74</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>3.39</td>
<td>6.93</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Epididymes Mean</td>
<td>7.34</td>
<td>8.69</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>1.24</td>
<td>0.57</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Seminal vesicles Mean</td>
<td>9.94</td>
<td>10.44</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>1.53</td>
<td>2.16</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Prostate Mean</td>
<td>1.79</td>
<td>2.17</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.12</td>
<td>0.27</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>45.68</td>
<td>46.38</td>
</tr>
</tbody>
</table>

Figure 3 Testicular volume (ml) (upper panel) and sperm number (million (mill)/ejaculate) (lower panel) during the treatment period in controls (●), animals treated with 0.4 IU/kg per day (○), 2 IU/kg per day (△), or 10 IU/kg per day (★).

Figure 4 Serum IGF-I levels (μg/l) during the treatment period in controls (●), animals treated with 0.4 IU/kg per day (○), 2 IU/kg per day (△), or 10 IU/kg per day (★).
before the treatment started, revealed normal spermatogenesis in the examined animals. Treatment with rhGH did not induce any morphological testicular changes as judged from biopsies taken after 15 days or organ samples collected after 52 weeks of treatment or after the 12-week recovery period (Fig. 7).

Discussion

In earlier preclinical safety studies in dogs where high doses of hGH have been administered for 20–28 days, severe testicular changes have been observed in primary and secondary spermatocytes and spermatids (14). The changes were accompanied by reduced plasma levels of LH, testosterone and PRL and resembled those seen after hypophysectomy or treatment with gonadotrophin-releasing hormone (GnRH) agonists. In the present study, serum testosterone levels were highly variable. Although a trend towards lower values might be apparent in GH-exposed animals, this is likely a matter of chance rather than a true biological effect for the following considerations: lack of corresponding changes in the secretion of bioactive LH and lack of effect on the testosterone-dependent accessory organs (prostate, epididymis, seminal vesicles). Cynomolgus monkeys lack a clear-cut reproductive seasonality in captivity (27, 28) and under outdoor conditions (29) so it is improbable that the testosterone variations reflect such an effect. Reduced PRL serum levels were seen in the monkeys. The low serum PRL concentrations did not cause any alterations in testicular function as judged from testicular weight, size, morphology and sperm analyses. The reason for the reduction of PRL levels is not known, but since hGH has a lactogenic activity (30), a negative feedback on PRL production is possible at the high doses given to the monkeys. We are, however, not aware of hGH binding to monkey or dog PRL receptors. PRL has a role in spermatogenesis in seasonal breeders, rodents and men (31, 32). In several species which breed throughout the year, PRL enhances gonadal responsiveness to gonadotrophin stimulation by increasing the number of LH receptors (33). In men, a 4-week-long bromocriptine-induced hypoprolactinaemia significantly reduced the maximal response of plasma testosterone to human chorionic gonadotrophin stimulation (34). To our knowledge, the effects of hypoprolactinaemia on testicular function in the cynomolgus monkey have not been described.

The reason for the difference between the cynomolgus monkey and the beagle dog in effect on spermatogenesis after hGH administration is not known. The most probable explanation is that hGH is structurally different from canine GH and thereby does not act biologically as GH in the dog. A comparison of the canine and human GH renders 68.3% identity in amino acid sequence. By contrast, macaque GH has 96.3% identity (35). In toxicity studies with GH in the dog, porcine GH (structurally similar to canine GH) has been administered in doses of 0.025, 0.1 and 1.0 IU/kg per day to dogs for 14 weeks, without any adverse effects on testes or other male reproductive organs being reported (36). However, increased pituitary weights were recorded and quantitative analysis of immunostained cells in pars distalis showed an increase in the absolute volume of GH-, PRL- and adrenocorticotropic-containing cells.
However, effects on serum/plasma levels of PRL were not measured or reported (37). Plasma levels of PRL were found to be unaffected in beagles after administration of a non-peptidyl secretagogue which induced increased levels of GH (38).

Another explanation for the species difference is that spermatogenesis in the dog seems to be far more vulnerable than in the cynomolgus monkey. This is evident from studies using GnRH agonists which induced complete testicular involution within about 3–4 weeks (39), whereas the achievement of such an effect requires a few months in macaque monkeys (40).

Serum IGF-I levels showed a dose-dependent increase seen already on day 5 of treatment and throughout the treatment period. At the end of the recovery period, IGF-I serum concentrations were comparable to those seen predose. The IGF-I response is considered to be due to the exogenous hGH administered (41), and shows that the hGH was biologically active during the study period.

Neither decreased nor increased spermatogenesis was observed. It should be noted, however, that the monkeys used were adult normal, non-GH-deficient animals. The results are in accordance with those presented in an earlier study where Crawford & Handelsman (42) showed that in prepubertal baboons without GH deficiency, treatment with neither GH nor IGF-I was able to enhance the inhibin response to FSH.

To conclude, administration of high doses of hGH to adult male cynomolgus monkeys for 1 year caused a dose-dependent increase of IGF-I serum levels throughout the study and a reduction of PRL serum levels from week 14 in monkeys given 10 IU/kg per day. Both changes were reversible upon cessation of treatment. Testicular parameters were unaffected at all doses.

Figure 7 Photomicrograph from testis from a monkey treated with rhGH (10 IU/kg per day) for 52 weeks. Spermatogenesis appears normal. HE stain; × 830.
Acknowledgements

Appreciation is expressed to Mrs Ingrid Sandström for preparing the slides and to Professor Leif Ploien, Professor Martin Ritzen and Dr Hans-Erik Johansson for scientific discussions while planning and performing this study.

References

2. Sheikholslam BM & Stempfel RS Jr. Hereditary isolated somatotropin deficiency: effects of human growth hormone administra-
13. Hult C, Mendel-Hartvig L, Vangbo B & Ohlander C. A radioimmunoassay (RIA) for quantification of IGF-1 in acid-


Received 31 August 1998
Accepted 14 January 1999