Suppression of spermatogenesis in a nonhuman primate (Macaca fascicularis) by concomitant gonadotropin-releasing hormone antagonist and testosterone treatment

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Abstract. The effects of concomitant testosterone (T)-supplementation on gonadotropin-releasing hormone (GnRH) antagonist-induced testicular regression in cynomolgus monkeys (M. fascicularis) were investigated. Four adult monkeys were infused via osmotic minipumps with daily amounts of 2 mg of a potent GnRH antagonist (N-Ac-D-Nal(2), D-pC1-Phe2, D-Trp3, D-hArg(12), D-Ala10)-GnRH (RS-68439) for a period of 104 days. Androgen substitution was provided via T-filled Silastic capsules implanted at initiation of GnRH antagonist treatment. Within 1–4 days of GnRH antagonist administration, serum concentrations of bioactive LH became undetectable. The implants maintained serum T at 50–80% of pre-treatment levels. Sperm production decreased in three out of four monkeys. One animal became azoospermic by the 13th week of treatment and the ejaculates of two other monkeys contained less than $5 \times 10^6$ sperm. In the fourth monkey, spermatogenesis was less affected. Testicular histology, judging from biopsies at termination of GnRH antagonist treatment, was typical of the hypogonadotropic status in 3 of the 4 monkeys. The most affected tubules contained only spermatogonia and Sertoli cells. Although comparison with GnRH antagonist treatment alone in a previous study indicated a delay of spermatogenic inhibition with testosterone, the present study confirms the potential of GnRH antagonist for male fertility regulation.

Gonadotropin-releasing hormone (GnRH) analogues are capable of inhibiting pituitary and testicular function. Thus, they can be used to treat androgen-dependent diseases. Moreover, they have a potential for regulating male fertility (Sandow 1982; Nieschlag et al. 1986). GnRH agonists provoke an elevation, although transitory, of serum gonadotropins and testosterone (T) followed by a decrease after 3 to 4 weeks of treatment (Bint Akhtar et al. 1983b; Mann et al. 1984; Schürmeyer et al. 1984; Bashir et al. 1985). In contrast, GnRH antagonists induce an immediate and precipitous decline of reproductive hormone concentrations (Weinbauer et al. 1984; Bint Akhtar et al. 1985; Adams et al. 1986).

Treatment with both kinds of GnRH analogues is associated with a fall of serum T concentrations into the castrate range (Bint Akhtar et al. 1983b; Weinbauer et al. 1984; Bint Akhtar et al. 1985). Their use in fertility regulation will require T-substitution in order to prevent the untoward effects of T-deficiency. A combined treatment regimen of GnRH agonist and T has revealed that the concomitant T supplementation attenuated the antifertility effects of the GnRH analogue in monkeys (Bint Akhtar et al. 1983a) and in men (Doelle et al. 1983). In these studies, azoospermia could not be attained, whereas the GnRH agonist alone led to complete inhibition of spermatogenesis (Bint Akhtar et al. 1983b; Linde et al. 1981).

In monkeys, complete spermatogenic arrest has been induced with GnRH antagonists alone (Weinbauer et al. 1984; Bint Akhtar et al. 1985). The object of the present investigation was to see...
whether spermatogenic inhibition can also be induced by a GnRH antagonist when serum T levels are maintained in the physiological range.

Materials and Methods

Animals

Six male cynomolgus monkeys (M. fascicularis), weighing 4.5–7.3 kg, were caged individually under controlled laboratory conditions (20 ± 1°C room temperature; 50–60% relative humidity, lights on from 7.00–19.00 h). The animals were provided with defined amounts of pelleted food and tap water in order to monitor food and water intake.

Study design

Four animals (4.5–6.2 kg), which had been subjected to GnRH antagonist treatment one year ago, received daily amounts of 2 mg of the GnRH antagonist (N-Ac-D-Nal(2), D-pCl-Phe², D-Trp³, D-hArg(Et)⁶, D-Ala¹⁹)-GnRH (RS-68439, Nestor et al. 1983) for a period of 104 days. The compound was administered via sc implanted mini-osmotic pumps (Weinbauer et al. 1984) which were changed every 16 days (model 2002, 4 pumps per animal, Alza Corp, Palo Alto, CA) except for the last week of treatment, when a 1-week pump (model 2ML1) was used. Two animals (5.3 and 7.3 kg) were infused with the vehicle, 50:50 propylene glycol/distilled water (vol/vol). Pump performance was assessed by comparing the fluid volume left in the pump upon removal with the volume dispensed at a constant release rate of approximately 0.5 µl/h. When GnRH antagonist treatment was begun, 3 animals (C, 3919, 3911) were implanted with a 2 cm-long T-filled Silastic implant (DOW Corning Corp, Medland, MI, inner diameter 0.132 in; outer diameter 0.188 in) whereas the fourth animal (4) received a 1 cm-long T-filled capsule. The capsule length was chosen by previous experience with this mode of T-supplementation (Bint Akhtar et al. 1983a, 1985) in order to achieve serum levels in the lower range of 31 pre-treatment values obtained from each monkey. The control animals were implanted with 2 cm-long empty capsules.

Blood samples were obtained from the femoral vein once or twice a week under ketamine hydrochloride anaesthesia (8–12 mg/kg Ketavet® Parke-Davis, Munich, FRG) for determination of T and bioactive LH, stored overnight at 4°C. The serum was kept at −20°C until assayed. GnRH tests were performed at 2- to 3-week intervals. Testicular volumes were measured biweekly and a testicular biopsy was performed before and at the end of GnRH antagonist treatment. Ejaculates were collected twice before treatment and thereafter at 2- to 4-week intervals. The ejaculations, biopsies and GnRH-stimulation tests were not performed in the two control animals.

Hormone assays

T was determined in unchromatographed serum by an established radioimmunoassay (Schürmeyer et al. 1983). The sensitivity of the assay was 25 pg/tube. The intra- and inter-assay coefficients of variation were 5.2 and 8%, respectively.

Serum LH was measured using a mouse Leydig cell bioassay (Wickings et al. 1979). The minimal LH activity detected was 2.7 IU/l MRC 64/109. The intra- and inter-assay coefficients of variation were 7.7 and 22.2%, respectively.

GnRH stimulation tests

Blood was collected at zero time and 15, 30 and 60 min after iv injection of 50 µg of GnRH (HOE-471, Hoechst AG, Frankfurt, FRG) for determination of bioactive LH and T.

Semen collection

Semen from sedated monkeys was collected by electro-ejaculation and evaluated for weight and volume, motility, and number of sperm as described previously (Wickings & Nieschlag 1980).

Testicular morphology

Testicular volumes (ml) were estimated from caliper measurements of the testis length and width using the

Body weight of two monkeys during infusion with the GnRH antagonist vehicle (propylene glycol). Arrows indicate implantation of mini-osmotic pumps. The first symbol indicates the 99% confidence interval around the mean of 9 pre-treatment values collected over a period of 12 weeks.

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Fig. 1.
formula for a regular ellipsoid, i.e. \((w^2 \times l \times \pi)/6\) (\(w =\) width and \(l =\) length).

Testicular biopsies were performed on the right testis before and on the left testis at termination of treatment as previously reported in detail (Weinbauer et al. 1984). The tissue was fixed in 2.5% glutaraldehyde, postfixed in 2% osmium tetroxide, and after dehydration embedded in epoxy resin. Semithin sections were cut at 1 \(\mu m\) and stained with 1% toluidine blue.

**Statistical evaluation**
For analysis of the body weight trend along with treatment period the Spearman correlation coefficient (\(r_s\)) was tested for significance using two-sided probability levels.

**Results**

**Body weight**
Infusion of the vehicle had no effect on body weight (Fig. 1) whereas all GnRH antagonist-treated animals lost body weight along with the treatment (6.6–14.2%, 280–660 g) compared with the pre-treatment average (Fig. 2). This was also reflected by a significant trend in body weight loss with duration of GnRH antagonist treatment in 3 of the 4 monkeys (\(r_s = -0.83\) to -0.91; \(P < 0.025\)). Body weight recovered within 4–6 weeks after cessation of treatment. Average daily food consumption (mean ± SEM) before, during and after termination of GnRH antagonist treatment was 192 ± 20, 200 ± 19 and 210 ± 22 g, respectively. Average daily water intake was 375 ± 14, 397 ± 14 and 434 ± 12 ml. Thus the body weight decrease appeared unrelated to food and water consumption.

**Serum hormones**
Infusion of the GnRH antagonist vehicle (propylene glycol) had no effects on serum levels of bioactive LH and T (Fig. 3), whereas under GnRH antagonist treatment, serum concentrations of bioactive LH (Fig. 4) became undetectable within 1–4 days and remained undetectable (<2.7 IU/l) throughout most of the study period in 3 animals. Monkey C had repeated episodes of measurable bioactive LH beginning with the 6th week of treatment. During week 7 there was evidence of incomplete delivery of the GnRH antagonist-solution in this particular animal as judged from the residual volume collected at removal of the mini-osmotic pump. The implants maintained T levels at 50–80% of pre-treatment range (Fig. 4, Table 1). Serum LH values returned to pre-treatment within 1–2 weeks after withdrawal of GnRH antagonist treatment.

**GnRH stimulation tests**
Following an iv bolus injection of 50 \(\mu\)g of GnRH, bioactive LH concentrations peaked after 15 and 30 min and T levels after 30 and 60 min (Fig. 5). During GnRH antagonist treatment, the pituitary and testicular responses to GnRH declined progressively and became severely blunted or abolished in two monkeys. In these two animals, the inhibition persisted even during the first post-treatment week. In the third animal, the responses were less affected, and they remained

![GnRH-antagonist](image)

**Fig. 2.**
Body weight of four monkeys during GnRH antagonist infusion. Arrows indicate the implantation of mini-osmotic pumps. The first symbol indicates the 99% confidence interval around the mean of 9 pre-treatment values collected over a period of 12 weeks.
Serum concentrations of bioactive LH (closed circles) and testosterone (open circles) of two monkeys during infusion of the GnRH antagonist vehicle (propylene glycol). Arrows indicate the implantation of mini-osmotic pumps.

Table 1.
Serum testosterone levels of monkeys before and during treatment with GnRH antagonist-vehicle (propylene glycol) or GnRH antagonist plus testosterone. Testosterone was administered via silastic capsules. The capsule length is given in brackets. The figures represents means (± SEM) of 29 to 31 measurements in each animal and period, except for * where only 12 values were obtained.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>27.6 ± 5.1*</td>
<td>25.7 ± 3.7</td>
<td>18.9 ± 2.2</td>
</tr>
<tr>
<td>#3918</td>
<td>16.4 ± 4.4*</td>
<td>12.5 ± 1.7</td>
<td>12.9 ± 2.02</td>
</tr>
<tr>
<td>#3909</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH antagonist plus testosterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#4 (1 cm)</td>
<td>15.8 ± 1.5</td>
<td>8.8 ± 0.6</td>
<td>24.9 ± 1.9</td>
</tr>
<tr>
<td>#3911 (2 cm)</td>
<td>33.7 ± 2.8</td>
<td>14.5 ± 0.5</td>
<td>32.4 ± 1.7</td>
</tr>
<tr>
<td>#C (2 cm)</td>
<td>24.8 ± 2.1</td>
<td>16.9 ± 1.2</td>
<td>20.5 ± 1.8</td>
</tr>
<tr>
<td>#3919 (2 cm)</td>
<td>29.5 ± 3.3</td>
<td>24.3 ± 1.1</td>
<td>25.8 ± 2.1</td>
</tr>
</tbody>
</table>

almost unaltered in the fourth. By the 7th post-treatment week, pre-treatment responses had reappeared in all animals.

Testicular volumes
No effects on testicular volumes could be seen during infusion of the vehicle. Testicular volumes in two antagonist-treated monkeys steadily declined to 20 and 30% of pre-treatment values. In the other two animals, volumes started to flatten out from the 7th treatment week and onwards, and could not be suppressed below 40% of pre-treatment values (Fig. 6). Within a period of 7–9 weeks after termination of GnRH antagonist treatment, testicular volumes had fully recovered.

Seminal parameters
Sperm production in monkey 4 was markedly reduced (4.46 x 10^6 sperm/ejaculate) and 2 weeks after cessation of treatment an azoospermic ejaculate could be obtained (Fig. 7). Monkey 3911 produced azoospermic ejaculates from week 13.
and onwards. Lowest sperm counts from monkey 3919 ranged from $4 - 7 \times 10^6$ sperm/ejaculate during weeks 9–13, but azoospermia never occurred. The seminal parameters of monkey C were less affected. Pre-treatment sperm concentrations were obtained within 9–14 weeks after termination of treatment.

On several occasions, sperm motility (Fig. 7) was comparable to the pre-treatment value in spite of highly reduced sperm numbers. The ejaculate volumes and weights were maintained at 70–100% of pre-treatment values by the T-implants.

Testicular histology

Testicular histology correlated with the status of ejaculates. In the most of the regressed tubules, spermatogenesis was arrested at the level of spermatogonia and spermatocytes. The Leydig cells were consistently shrunken and atrophic. Accumulation of lipid and lipofuscin granula was found in tubules with disrupted spermatogenesis. The testicular wall in these tubules was markedly thickened, mainly owing to increased folding of the basal lamina and enlargement of the connective tissue layer. Testicular histology of monkey C remained unaffected.

Discussion

The results of the present study, conducted in order to assess the potential of a GnRH antagonist for suppression of testicular function when T levels are maintained, demonstrate that sperm production can be reversibly inhibited or severely

Serum concentrations of bioactive LH (closed circles) and testosterone (open circles) of four monkeys during GnRH antagonist infusion. Arrows indicate the implantation of mini-osmotic pumps. The animals received T-filled silastic implant. The first symbol indicates the 95% confidence interval of 31 pre-treatment values for testosterone.
Serum concentrations of bioactive LH (broken line) and testosterone (solid line) of four monkeys following a 50 µg iv bolus injection of GnRH. ○ indicate bioactive values below assay detection limit (<2.7 IU/l).

Fig. 5.

Impaired by a combined GnRH antagonist/T treatment. Azoospermia or severe oligospermia could be induced in 3 of 4 animals.

The data confirm the potential of the GnRH antagonist for male fertility regulation. The observation, however, that sperm motility was largely maintained despite highly reduced sperm counts suggests that the epididymal function has been maintained in monkeys treated with the GnRH antagonist and physiological T. The crucial role of T in the functional integrity of the epididymis is well documented (Orgebin-Christ et al. 1975). For purposes of male fertility regulation with GnRH analogues, T-substitution would presumably necessitate the achievement of azoospermia for complete inhibition of fertility.

Since the same monkeys were subjected to the same GnRH antagonist alone a year earlier (Weinbauer et al. 1984), a comparison of the effects of T-supplementation on GnRH antagonist-induced testicular regression is possible. Treatment with GnRH antagonist alone induced azoospermia in 3 of the 4 monkeys within 9 weeks, whereas during T-supplementation only one animal became consistently azoospermic, but not before week 13 of treatment. Thus it appears that T-substitution delayed the achievement of testicular suppression.

The delay in testicular regression could not be attributed to discrepancies in body weight or sperm production at the initiation of the two studies. The attenuating effect of testosterone could be explained by the following possibilities: 1) T alone, when injected as T-ester in amounts raising serum T levels 8–25 times over pre-injection values, is known to maintain (Marshall et al. 1986) and to reinitiate spermatogenesis in mon-
keys (Marshall et al. 1983). In the present investigation, serum T was kept at relatively low levels. It remains to be shown whether such low, but constant T concentrations have similar effects on spermatogenesis as injection of high amounts of T. 2) We have recently shown that in GnRH antagonist-treated rats, T maintains pituitary and serum FSH as well as spermatogenesis (Rea et al. 1986). Thus, in rats, it appears that in this condition T or its metabolites have a direct effect on pituitary FSH synthesis and release. If such a mechanism could also be demonstrated in primates, the 'attenuating effect' of T could be easily explained. Unfortunately, the available assays for monkey FSH are too insensitive to investigate this possibility.

In the present study a reduction of body weight (7—14%) occurred during GnRH antagonist plus T treatment. With GnRH antagonist treatment alone, the body weight decrease in the same monkeys ranged from 9—20% (Weinbauer et al. 1984). Thus T-supplementation did not completely prevent body weight loss. Since a similar weight loss was also observed with another GnRH antagonist in monkeys (Bint Akhtar et al. 1985), the effect appears to be related to this class of compounds and deserves further attention in addition to the acute side effects caused by histamin release at the beginning of treatment (Karten & Rivier 1986).

In summary, the present investigation shows that under chronic GnRH antagonist treatment, extreme oligospermia or azoospermia can be attained despite concomitant T-substitution. However, the suppressive effects of GnRH antagonist on the testis during T-substitution seem delayed when compared with treatment with GnRH antagonist alone.

![Graph showing testicular volumes](image)

Fig. 6.

Testicular volumes (both testes combined) in four monkeys during GnRH antagonist infusion. Arrows indicate the implantation of mini-osmotic pumps. The starting volume is the mean of four pre-treatment values.
Sperm count ($\times 10^6$/ejaculate) (bars) and sperm motility (solid line) in four monkeys during GnRH antagonist infusion (■). • Sperm number too low for valid quantification. * No sperm found in ejaculate.

Fig. 7.

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