Suppression of spermatogenesis to azoospermia by combined administration of GnRH antagonist and 19-nortestosterone cannot be maintained by this non-aromatizable androgen alone

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BACKGROUND: For male hormonal contraception, combined administration of gonadotrophin-releasing hormone (GnRH) antagonists and androgens effectively suppresses spermatogenesis to azoospermia. In non-human primates this suppression can be maintained more easily by androgens alone. METHODS: A clinical trial with six healthy volunteers was performed to test this approach in man. Loading doses of 10 mg/day of the GnRH antagonist cetrorelix were given subcutaneously for 5 days, followed by maintenance doses of 2 mg/day up to week 12. At 2 weeks after the first GnRH antagonist injection, androgen substitution was initiated with a loading dose of 400 mg 19-nortestosterone hexyloxyphenylpropionate (19NT-HPP) intramuscularly, followed by injections of 200 mg 19NT-HPP every 3 weeks up to week 26. RESULTS: Serum concentrations of LH, FSH and testosterone were effectively suppressed by cetrorelix administration. Within 12 weeks, azoospermia was achieved in all six volunteers. After cessation of cetrorelix injections in week 12, gonadotrophins and testosterone increased significantly despite continued 19NT-HPP injections. In parallel, spermatogenesis was restimulated in five of six volunteers. CONCLUSIONS: Combined administration of cetrorelix and 19NT-HPP leads to azoospermia within 3 months. However, complete azoospermia cannot be maintained by continued injections of the non-aromatizable 19NT-HPP alone.

Key words: GnRH antagonist/gonadotrophins/male contraception/19-nortestosterone/spermatogenesis

Introduction
Of all the different experimental approaches to male contraception, hormonal methods are the most advanced in terms of clinical testing of safety and efficacy. The principle of hormonal male contraception is based on the suppression of LH and FSH and substitution of peripheral testosterone to maintain androgenicity (Nieschlag et al., 2000). Two WHO multicentre efficacy trials of male contraception involving 670 men have convincingly demonstrated that suppression of spermatogenesis to azoospermia results in highly effective contraception with a low Pearl index of 0.8 (WHO, 1990) or 0.0 (WHO, 1996).

With testosterone injections alone [even with high doses of 200 mg testosterone enanthate (TE) per week], spermatogenesis was suppressed to azoospermia only in two-thirds of Caucasian men within 6 months (WHO, 1995). The attempt to augment suppression of gonadotrophins by simply increasing the dose of TE has failed (Matsumoto, 1988). In order to accelerate the onset of testosterone effectiveness and to increase azoospermia rates, testosterone is combined with other gonadotrophin-suppressing substances such as gestagens and GnRH antagonists.

GnRH antagonists cause an immediate and highly effective suppression of both gonadotrophins in normal men. Five clinical trials of male contraception using combined administration of TE and the experimental gonadotrophin-releasing hormone (GnRH) antagonist Nal-Glu demonstrated a rapid and highly effective suppression of gonadotrophins and spermatogenesis (Nieschlag et al., 2000).

However, the need for daily subcutaneous administration of GnRH antagonists of even the newest generation over extended periods of time are not feasible and too expensive for contraception. It has been demonstrated previously in non-human primates that the suppression of spermatogenesis by the combination of a GnRH antagonist and testosterone can be maintained by
Materials and methods

Study design and volunteers

The study protocol was approved by the Ethics Committee of the University and the local State Medical Board. Male volunteers aged between 18 to 45 years were recruited for the study. Detailed information about the experiment was provided, and written informed consent was obtained before commencement of the study. Criteria for participation included an uneventful medical history, and normal results of physical examination, semen analysis, hormone concentrations, blood chemistry and haematology at the two baseline examinations. Taking of additional drugs was not allowed during the study.

Initially, eight male volunteers were enrolled in the study, but two left the study during the injection phase for personal reasons. Ultimately, six normal healthy men (mean ± SD, age 26.0 ± 2.9 years; body weight 71.0 ± 7.6 kg; height 1.76 ± 0.08 m; body mass index 23.0 ± 1.2 kg/m²) completed the injection phase and were included in the final analysis.

After the baseline control examinations, the GnRH antagonist cetrorelix was injected subcutaneously in all volunteers into adipose tissue at the abdominal wall laterally to the rectus abdominis muscle. For the first 5 days the volunteers received 10 mg cetrorelix per day, given as two injections of 5 mg at two sites (Figure 1). These loading-dose injections were followed by maintenance injections of 2 mg cetrorelix per day given at one site up to the end of study week 12 (Behre et al., 1997). At 14 days after the first cetrorelix injection, all volunteers received 400 mg 19NT-HPP intramuscularly. After this loading dose, maintenance dose injections of 200 mg 19NT-HPP were given every 3 weeks up to the end of the treatment period in study week 26. Follow-up examinations were performed every 3 weeks up to week 47, and thereafter in weeks 52 and 60 (Figure 1).

Medication

GnRH antagonist

The GnRH antagonist cetrorelix ([Ac-D-Nal(2)1, D-Phe(4Cl)2, D-Pal(3)3, D-Cit8, D-Ala10]GnRH; SB-75) was synthesized and provided by Asta Medica AG (Frankfurt am Main, Germany). Before injection, cetrorelix was dissolved in bacteriostatic water containing 5.2% (w/v) mannitol to a final concentration of 1.0 g/l.

Androgen

19NT-HPP (Anadur; Pharmacia Arzneimittel GmbH, Ratingen, Germany) is a long-acting androgen derivative. Injection of 200 mg 19NT-HPP every 3 weeks has been shown to support sexual function in male volunteers with hormonally suppressed endogenous testosterone secretion (Knuth et al., 1985; Behre et al., 1992).

Local side effects

Local side effects after subcutaneous cetrorelix administration were documented daily on transparency paper. The erythema area was determined in a blinded manner applying a digital planimeter (Haff GmbH, Pfronten, Germany).

Evaluation of sexual function

For evaluation of possible effects on sexuality, a questionnaire on sexual thoughts and fantasies, sexual interest and desire, satisfaction with sexuality, frequency of erections and number of morning erections and ejaculations was used every week up to study week 14, then every 3 weeks up to study week 47, and finally in study weeks 52 and 60 (Behre et al., 1992).

Blood samples

Blood samples for hormone determinations were withdrawn between 08:00 and 10:00 at two pretreatment control examinations, shortly before the first cetrorelix injection (baseline level), on study days 2, 4, 7, 9, 12, 14, 16, 18, 21, 23, 25, 28, 30 and 35, then weekly up to study week 17, then every 3 weeks up to study week 47, and finally at week 52 and 60 (Figure 1). Blood samples for hormone determinations were separated by centrifugation at 800 g and the serum stored at −20°C until assayed. Blood samples for haematology and clinical chemistry (including lipids) were withdrawn after 12 h of fasting at the two control examinations, weekly up to study week 12, every 3 weeks from week 14 to 47, and finally at weeks 52 and 60.

Immunooassays

Serum LH, FSH, prolactin, sex hormone-binding globulin (SHBG), prostate-specific antigen (PSA), testosterone, oestradiol and inhibin B concentrations were determined as described previously (Behre et al., 1997; Eckardstein et al., 1999). In the authors’ laboratory, the normal ranges for LH are 2–10 IU/l, for FSH 1–7 IU/l, for SHBG 11–71 nmol/l and for inhibin B 94–327 pmol/l. The lower normal limit for testosterone is 12 nmol/l. The upper normal limit for prolactin is 500 mIU/l, for PSA 4 µg/l, and for oestradiol 250 pmol/l.

Semen analysis

Semen analyses were performed according to the WHO guidelines at the two pretreatment control examinations, weekly up to study week 12, every 2 weeks up to week 20, every 3 weeks up to week 47, and finally in weeks 52 and 60. The volunteers were requested to abstain from sexual activity for 48 h to 7 days before investigation.

Testicular and prostate volumes

Changes in testicular volume were determined by scrotal sonography as described previously (Behre et al., 1989) at the second pretreatment control examination, then weekly up to week 5, every 3 weeks from study week 8 to 47, and finally in week 52. Prostate volume was measured by transrectal ultrasonography (Behre et al., 1994) before the first cetrorelix injection, then weekly up to study week five, every 3 weeks from week 8 to 47, and finally in study week 52. Volume was calculated applying the ellipsoid method.

Statistical analysis

Significant variations over time of any parameter were evaluated by analysis of variance (ANOVA) for repeated measures. In case of a general effect over time, values at single time points were analysed in more detail by comparison with the pretreatment baseline value using the Duncan multiple comparison test for repeated measures. For LH, FSH, testosterone, oestradiol, SHBG, PSA, ejaculate and psychosexual variables the values shortly before the first cetrorelix injection were defined as baseline levels. For inhibin B, testicular
volume, prostate volume, clinical chemistry including lipids, haematology and physical examinations the values at the second pretreatment control examination were defined as baseline levels. When necessary, analysis was performed on logarithmically transformed data. A P value < 0.05 was considered significant. Unless otherwise stated, results are given as mean ± SEM.

Results

During the study period, no significant changes were observed upon physical examination or in body weight and vital signs. Injection volumes of up to 2×5 ml in the loading-dose period were well tolerated by all men. On most occasions during the first 5 days, injections of cetrorelix caused local erythema at the injection site that generally resolved within 60 min. The erythema size was very variable between volunteers and, in one individual volunteer, between study days. The mean size of the combined erythema areas (two sites of injection during the 10 mg/day application) at 20 min after subcutaneous injection varied between 0.2 ± 0.1 cm² and 2.2 ± 1.0 cm². During the maintenance-dose phase with daily injections of 2 mg cetrorelix, erythema occurred only sporadically with a maximal size of 1.7 cm² in one volunteer. Neither pruritus nor induration occurred in any volunteer; none of the volunteers expressed any discomfort or required any specific treatment. Local side effects caused by intramuscular injections of 19NT-HPP were not observed.

Ejaculate parameters

Abstinence times remained constant throughout the study. Ejaculate volume decreased significantly from a baseline of 3.0 ± 0.2 ml to 1.7 ± 0.3 ml in study week 2, and returned to the normal baseline range following week 3.

A significant suppression of sperm concentration was first seen in week 3 (Figure 2, upper panel). The first volunteer achieved azoospermia in week 3, the second in week 6, two more in week 8, one in week 9 and the last in week 12 (Figure 2, lower panel). During the injection phase with 19NT-HPP alone only one volunteer remained continuously azoospermic up to study week 38. In four of the six volunteers spermatozoa reappeared in the ejaculate in week 14, an additional one in week 16 (Figure 2, lower panel). One of these five volunteers was again suppressed to azoospermia from week 26 to 35. At study week 52 all volunteers showed sperm concentrations back in the normal range.

During the cetrorelix injection period a significant decrease of progressive sperm motility (WHO grade ‘a’ and ‘b’) in the non-azoospermic volunteers was seen following week 2 compared with baseline control. The percentage of normally formed spermatozoa was significantly decreased following week 3. Whereas progressive sperm motility had consistently returned to the normal range following week 35, the mean percentage of normally formed spermatozoa first returned to the normal range in week 60.

Gonadotrophins

Serum LH concentrations were significantly suppressed by cetrorelix injections to the assay detection limit up to the end of study week 12 (Figure 3, upper panel). After cessation of the GnRH antagonist injections, LH concentrations increased within 1 week and were in the normal range in week 14. During continued 19NT-HPP injections, LH concentrations declined again to a nadir in week 29, without achieving the same degree of suppression which had been seen constantly during the GnRH antagonist injection period. Serum concentrations of LH in the prestudy range were seen following week 44.

Similar to LH, FSH concentrations were significantly suppressed up to the end of study week 12 (Figure 3, lower panel). After cessation of the GnRH antagonist injections, FSH concentrations increased within 1 week back to the normal range, and were in the prestudy range at week 14. Thereafter, FSH concentrations declined again but did not reach the degree of suppression which was seen during the cetrorelix injection period. Serum concentrations of FSH in the prestudy range were seen following week 35.

Testosterone, oestradiol, SHBG and prolactin

Serum concentrations of testosterone mirrored LH concentrations. Cetrorelix injections suppressed serum concentrations of testosterone to a nadir of 2.1 ± 0.2 nmol/l on day 9 (Figure 4, upper panel). Mean serum concentrations of testosterone remained constantly low during the first 12 study weeks. At 2 weeks after cessation of cetrorelix injections testosterone concentrations were restimulated to a maximum of 11.4 ± 3.1 nmol/l. During continued 19NT-HPP injections of study week 12 (Figure 3, upper panel). After cessation of the GnRH antagonist injections, LH concentrations increased within 1 week and were in the normal range in week 14. During continued 19NT-HPP injections, LH concentrations declined again to a nadir in week 29, without achieving the same degree of suppression which had been seen constantly during the GnRH antagonist injection period. Serum concentrations of LH in the prestudy range were seen following week 44.

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Testosterone, oestradiol, SHBG and prolactin

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mean serum concentrations declined again and then rose to the normal range following study week 41.

Oestradiol concentrations were significantly suppressed during cetrorelix injections to levels near the detection limit of the assay (Figure 4, lower panel). Serum concentrations remained low during cetrorelix injections, but rose again 2 weeks after cessation of the GnRH antagonist, in parallel with the LH-driven restimulated endogenous testosterone (Figure 4, upper panel). Thereafter, serum concentrations declined again to the assay detection limit and returned to the prestudy range in week 52.

Serum concentrations of SHBG remained statistically unchanged throughout the study course (Figure 5, upper panel). No significant change was seen in prolactin concentrations throughout the study course, with all individual values remaining within the normal range.

**Inhibin B**

Although serum concentrations of inhibin B showed some fluctuations during the study course, no significant change was detected during the treatment phase compared with baseline (Figure 5, lower panel).

**Sexual function**

Standardized questionnaires revealed a decrease in the frequency of morning erections during the first two injection weeks without androgen supplementation, and in study week 3 (Figure 6). This effect was paralleled by a decrease of sexual thoughts and fantasies. In view of the high variability, these changes did not reach statistical significance. Thereafter, mean levels of sexual function variables were restored to the prestudy range and remained unchanged throughout the rest of the study.

**Testicular volume**

Total testicular volume as measured by ultrasonography decreased significantly to a nadir value in study week 11 (Figure 7, upper panel). During the recovery phase, testicular volume gradually increased to pretreatment values.

**Prostate evaluation**

Prostate volume decreased significantly to 12.5 ± 1.3 ml at the end of study week 2 (Figure 7, lower panel). Prostate volume re-increased during 19NT-HPP injections, without achieving baseline control values. During the recovery phase, prostate volume gradually increased and returned to baseline.

No significant change was seen in serum concentrations of PSA throughout the study course. Individual values never exceeded the upper normal limit for PSA, with mean concentrations remaining constantly below 1 µg/l.

**Haematology, lipids and clinical chemistry**

Haemoglobin decreased significantly during the first 2 weeks of cetrorelix injections (Figure 8, upper panel). During 19NT-HPP administration, haemoglobin concentration increased...
Figure 5. Mean (± SEM) serum concentrations of sex hormone-binding globulin (SHBG) (upper panel) and inhibin B (lower panel). The cetrorelix injection period is indicated by the shaded bar, and the 19NT-HPP injection period by the cross-hatched bar. Horizontal dashed lines indicate normal ranges.

Figure 6. Mean (± SEM) number of morning erections per week. The cetrorelix injection period is indicated by the shaded bar, and the 19NT-HPP injection period by the cross-hatched bar.

significantly to maximal values at the end of the injection period. Subsequently, mean concentrations decreased and returned to baseline at the end of the recovery period. A similar pattern was noted for erythrocyte concentrations and for the haematocrit. No significant changes were seen in either leukocyte or platelet concentrations during the study course.

No significant changes were noted for cholesterol and triglyceride concentrations throughout the study. During 19NT-HPP injections, high-density lipoprotein (HDL)-cholesterol concentrations were significantly suppressed to nadir values in study week 29 (Figure 8, lower panel). Low-density lipoprotein (LDL)-cholesterol increased significantly during 19NT-HPP injections (Figure 8, lower panel). All other parameters of clinical chemistry showed no systematic change throughout the study course.

Discussion

Although the first studies with GnRH agonists and androgens for male contraception were performed only 8 years after their identification and synthesis, the results were disappointing, with few subjects achieving azoospermia (Behre and Nieschlag, 1999).

In contrast to GnRH agonists, GnRH antagonists administered to men produce a precipitous and prolonged fall in serum concentrations of both LH and FSH. To date, the results of six clinical trials using GnRH antagonists for male contraception have become available (Pavlou et al., 1991, 1994; Tom et al., 1992; Bagatell et al., 1993; Swerdloff et al., 1998; and the present study). Within these studies, 47 of 55 volunteers (85%) became azoospermic, demonstrating the high efficacy of the GnRH antagonist–testosterone combination for complete suppression of spermatogenesis. In addition, the mean time to achieve azoospermia (8 weeks in the present study) was considerably shorter than when testosterone was used alone (mean 17 weeks in Caucasian men; WHO, 1995).

Although GnRH antagonists are very effective for suppression of spermatogenesis, the high costs and need for daily
subcutaneous administration renders them impracticable for widespread and long-term contraceptive use in males. Therefore, an approach to reduce the dose and duration of GnRH antagonist administration seems mandatory. One possible dose schedule, which was first tested in cynomolgus monkeys, showed that spermatogenesis could be suppressed by daily subcutaneous administration of 450 µg/kg body weight of cetrorelix, and maintained after withdrawal of the GnRH antagonist by using long-acting testosterone buciclate (Weinbauer et al., 1994).

In a clinical trial, 10 mg/day of the second-generation GnRH antagonist Nal-Glu in combination with weekly intramuscular injections of 100 mg TE were given to 15 healthy male volunteers (Swerdloff et al., 1998). At study week 12, 10 of the 15 volunteers had achieved azoospermia, and four were suppressed to a detectable sperm concentration lower than 3 × 10⁶/ml. The 14 volunteers with severely suppressed spermatogenesis during the induction phase of the Nal-Glu plus TE thereafter received weekly injections of 100 mg TE for another 20 weeks (Swerdloff et al., 1998). During this maintenance period, eight volunteers showed persistent azoospermia, whereas five remained severely oligozoospermic and one escaped suppression, showing sperm concentration in the normal range while receiving TE alone.

In the present study, all six volunteers were suppressed to azoospermia within 12 weeks, and with a much lower dose of the modern GnRH antagonist cetrorelix. While receiving 19NT-HPP alone, only one of the six men remained consistently azoospermic. This restimulation of spermatogenesis can be explained by the restimulated serum concentrations of LH and FSH after study week 12. Injections of 19NT-HPP at the given dose and injection interval were unable to maintain the significant suppression of gonadotrophins achieved by the GnRH antagonist. Similar restimulated LH and FSH concentrations were seen in the volunteer who escaped suppression of spermatogenesis in the maintenance phase of the Nal-Glu plus TE study (Swerdloff et al., 1998). The finding of unchanged inhibin B concentrations in the current study corresponds to similar results in recent trials with gestagens and androgens for male contraception (Büchter et al., 1999; Martin et al., 2000).

One possible explanation for the difference in the maintenance rate of gonadotrophin suppression and azoospermia in the two clinical studies might be the nature of the different androgens, testosterone versus 19-nortestosterone. Because of the different conversion rate to the 5α-reduced form and the minimal metabolism to oestrogens, 19-nortestosterone has a different spectrum of biological actions compared with testosterone, and can be regarded as a selective androgen (Toth and Zakar, 1982). The selectivity of 19-nortesterone effects on various organs was demonstrated in the current study. Administration of the GnRH antagonist to study volunteers reduced prostate volume significantly, by one-third. The rapid and pronounced suppression of prostate size has also been seen in cetrorelix-treated cynomolgus monkeys (Kamischke et al., 1997) and, to a lesser degree, in patients with benign prostatic hyperplasia (Comaru-Schally et al., 1998). Delayed

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**Figure 8.** Mean (± SEM) serum concentrations of haemoglobin (upper panel) and HDL-cholesterol (○) and LDL-cholesterol (□) (lower panel). The cetrorelix injection period is indicated by the shaded bar, and the 19NT-HPP injection period by the cross-hatched bar. Horizontal dashed lines indicate the normal range for haemoglobin and the lower normal limit for HDL-cholesterol.
administration of 19-nortestosterone to the cetrorelix-treated volunteers induced only minimal stimulation of prostate growth, in contrast to the significant stimulation of haemoglobin concentration and the pronounced suppression of HDL-cholesterol. Similar prostate-sparing effects have been described for other selective androgens which cannot be converted to 5-dihydrotestosterone (Cummings et al., 1998) or oestrogens (Swerdlow and Wang, 1998).

The lack of oestrogenic activity of 19-nortestosterone could explain the less suppressive effect on gonadotrophins in the current study. It has been shown that, in addition to a hypothalamic effect (Hayes et al., 2000; Vanderschueren and Bouillon, 2000), oestrogens are potent inhibitors of GnRH responsiveness in the pituitary gland (Finkelstein et al., 1991). Recently, it has also been demonstrated that the negative feedback regulation of testosteronon FSH appears to be mediated largely by aromatization to oestradiol (Hayes et al., 2001). The additional suppressive effect of oestradiol on gonadotrophin secretion has recently been demonstrated in an experimental trial of male contraception (Handelsman et al., 2000). Similarly, long-acting testosterone preparations plus gestagens with significant aromatization to oestrogens (such as norethisterone enanthate; Kuhnz et al., 1997) are highly effective combinations for male contraception (Kamischke et al., 2001).

It should be noted that the same dose of 19NT-HPP given without GnRH analogues consistently suppressed gonadotrophins (Behre et al., 1992). However, it has been shown that after cessation of GnRH antagonist administration, a rebound increase of gonadotrophins to concentrations exceeding the baseline control occurs in normal men (Behre et al., 1997). It might be speculated that the selective androgen 19-nortestosterone is not capable of suppressing this temporarily increased activity of the pituitary gland after cessation of the GnRH antagonist (Hayes et al., 2001), in contrast to natural testosterone which is significantly metabolized to oestradiol.

In conclusion, this first study using a modern and now (at least in some countries) clinically available GnRH antagonist for male contraception demonstrated the high efficacy of suppressing both gonadotrophins as well as spermatogenesis to azoospermia. This effective suppression could not be maintained by the selective androgen 19NT-HPP. Different testosterone preparations given at varying application intervals and doses should be tested in combination with initial administration of modern GnRH antagonists to further exploit this promising approach to hormonal male contraception.

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