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Persistence of Infertility in GnRH Immunized Male Rats Treated with Subdermal Implants of Dihydrotestosterone (DHT)

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Male hormonal contraception has been limited to date because two fundamental requirements have not been concurrently satisfied, these are, consistent and dependable azoospermia and infertility coupled with maintenance of libido. The objective of this study was to determine the extent to which implants of potent androgen (DHT) will restore androgenization and spermatogenesis in hypogonadotropic infertile male rats. Twenty-five sexually mature male rats of proven fertility were actively immunized against gonadotropin releasing hormone (GnRH) to induce azoospermia. After azoospermia was achieved, GnRH immunized rats received subdermal DHT-filled Silastic implants of 2, 4, 6, or 8 cm, or empty implants (n = 5/group). Five untreated control rats received empty capsules. Eight weeks later, fertility was evaluated, sperm number was obtained from the testis, and weights of androgen-dependent organs were measured. The results indicate that immunoneutralization of GnRH induced complete azoospermia, and subsequent treatment with DHT implants of 2 or 4 cm for 8 wk restored accessory organ weights, but did not restore spermatogenesis or fertility. In addition, DHT implants of 6 to 8 cm partially restored spermatogenesis, but not fertility. We conclude that low-dose DHT supplementation of GnRH-immunized rats may be a suitable alternate therapy able to maintain androgenization in the face of persistent azoospermia in the rat. This may be an effective model for development of a male contraceptive.

Key Words: DHT; GnRH immunization; spermatogenesis; azoospermia; fertility.

Introduction

Theoretically, effective hormonal contraception in the male depends on complete azoospermia. Whereas severe oligospermia may be clinically equivalent, efficacy will always be questioned if spermatozoa are present in the ejaculate. Previous studies have reported that GnRH analog treatment with testosterone (T) replacement can produce azoospermia in both laboratory animals, as well as in humans (Linde et al., 1981; Doelle et al., 1983; Rea et al., 1986; Bhasin et al., 1988). However, reversible oligospermia rather than azoospermia is far more common, and the degree of suppression is paralleled by the degree of sexual function (Linde et al., 1981; Doelle et al., 1983; Rea et al., 1986). Active immunization against GnRH is a promising male contraceptive approach because of its ability to predictably induce hypogonadism and azoospermia, and because it is reversible (Awoniyi et al., 1989, 1990a, 1990b, 1992). Once antibodies against GnRH are actively generated, animals usually remain suppressed for a long period of time, and require only periodic booster injections to maintain the antibody titer and spermatogenic suppression. Compared to GnRH analog treatment, GnRH immunization is far cheaper and requires much less frequent dosing to consistently suppress spermatogenesis.

We have previously reported that T administration by Silastic implants of 8 to 24 cm can restore quantitatively and qualitatively normal spermatogenesis in GnRH immunized hypogonadotropic rats. However, only 40% of the GnRH-immunized animals receiving 4 cm T implants and 0% receiving 2 cm T implants were fertile (Awoniyi et al., 1992). Thus, the administration of low-dose T to GnRH-immunized animals seems to represent a theoretically viable contraceptive approach. In fact, in 4 cm, and 2 cm T replaced rats, azoospermia was seen in 3 of 5, and 5 of 5, respectively, whereas copulatory activity was normal in all of them (Awoniyi et al., 1993). Although these results are promising, we have not yet met the goal of developing a male contraceptive combination that produces complete

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Table 1
Effects of Treatment of GnRH Immunized Rats with DHT Implants (2–8 cm) on the Testis, Epididymis, Prostate, and Seminal Vesicle Weights (g)[a]

Group	Testis	Epididymis	Prostate	Seminal vesicle
Control	2.10 ± 0.05	0.71 ± 0.03	1.4 ± 0.04	1.8 ± 0.07
GnRH-I[b]	0.33 ± 0.01[d]	0.16 ± 0.03[d]	0.3 ± 0.02[d]	0.1 ± 0[d]
GnRH-I + 2[c]	0.37 ± 0.01[d]	0.34 ± 0.04[d]	0.9 ± 0.07[d]	0.9 ± 0.09[d]
GnRH-I + 4	0.42 ± 0.04[d]	0.42 ± 0.01[d]	1.6 ± 0.06	1.3 ± 0.05[d]
GnRH-I + 6	1.16 ± 0.02[d]	0.53 ± 0.03[d]	1.7 ± 0.04[d]	1.9 ± 0.10
GnRH-I + 8	1.27 ± 0.07[d]	0.59 ± 0.01[d]	1.7 ± 0.06[d]	2.5 ± 0.09[d]

[a]Values are mean ± SEM.
[b]GnRH-I = GnRH immunization.
[c]DHT implant length in cm.
[d]Indicate significant differences of $p < 0.05$ from controls.

azoospermia with minimal or no effect on sexual activity. In this study, we examined the extent to which another androgen supplement, DHT, can inhibit spermatogenesis and maintaining androgenization. DHT was chosen with the hope that there would be a greater difference between the dose required to allow androgenization and the dose that restored spermatogenesis than we observed with T supplementation in GnRH-immunized rats. We report herein that DHT implants of 2, 4, 6, or 8 cm were all unable to restore fertility, or normal spermatogenesis in GnRH-immunized male rats.

Results

Organ Weights

Testis, prostate, and seminal vesicle weights were used to assess the degree to which androgenization was restored. Testis, prostate, and seminal vesicle weights were significantly reduced in GnRH-immunized rats when compared to controls (Table 1). Whereas administration of DHT implants of 2 and 4 cm did not induce any significant increase in the testis and epididymis weights when compared to GnRH-immunized rats with empty implant, 6 and 8 cm DHT implants restored the testis weights to 58% and 60%, and epididymis weights to 75% and 82% of control weights, respectively. DHT implants of all sizes (2–8 cm) increased the prostate and seminal vesicle weights in proportion to the implant size (Table 1). DHT implants of 4 cm restored the prostate to control values, and DHT implants of 6 cm restored the seminal vesicle weights back to control values.

Sperm Counts

Figure 1 shows the effect of GnRH immunization and subsequent DHT administration on the testicular sperm content. In control rats, the testicular sperm content was $232 \pm 21 \times 10^6$. In contrast, there were no detectable spermatozoa in the testis of GnRH immunized rats that received empty implants, 2 or 4 cm DHT implants. Testicular sperm numbers were $4.2 \pm 1 \times 10^6$, and $26 \pm 3 \times 10^6$ in rats that received 6 and 8 cm DHT implants, respectively.

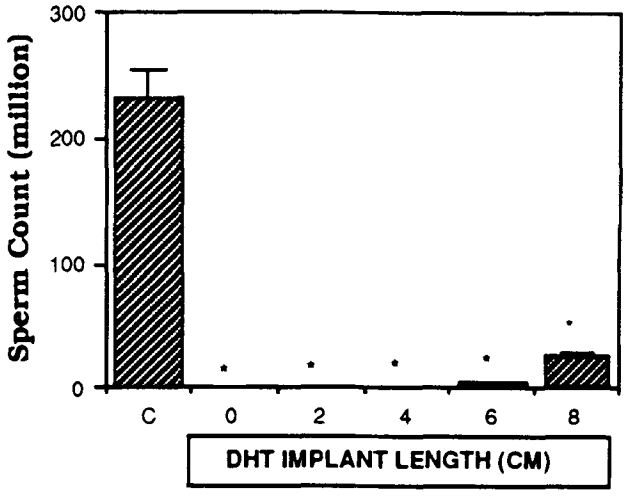

Fig. 1. Advanced spermatid numbers (mean ± SEM) per testis in controls (C), and in GnRH-immunized rats treated with empty implants (0), or with DHT implants of 2–8 cm. Asterisks (*) indicate significant differences of $p < 0.05$ from controls.

Serum Testosterone and DHT Levels

Table 2 shows the effects of GnRH immunization and subsequent DHT treatment on serum levels of testosterone and DHT. Serum testosterone levels was 1.2 ± 0.05 ng/mL in control rats, but was nondetectable in GnRH-immunized rats regardless of whether they received DHT implants or not. Serum DHT levels was 0.14 ± 0.02 ng/mL in control, but was nondetectable in GnRH-immunized rats that received empty implants. In GnRH-immunized rats that received DHT implants, serum DHT concentrations increased in an escalating pattern relative to the increase in DHT implant length. With DHT implants of 8 cm, their serum levels of DHT was 0.38 ± 0.05 ng/mL, approximately threefold greater than control value.

Table 2
Effects of Treatment of GnRH Immunized Rats with DHT Implants (2–8 cm) on Serum Levels of Testosterone and DHT (ng/mL)[a]

Group	Testosterone	DHT
Control	1.2 + 0.05	0.14 ± 0.06
GnRH-I[b]	ND	ND
GnRH-I +2[c]	ND	0.13 ± 0.03
GnRH-I +4	ND	0.18 ± 0.04[d]
GnRH-I +6	ND	0.27 ± 0.05[d]
GnRH-I +8	ND	0.38 ± 0.05[d]

[a]Values are mean±SEM. ND = nondetectable (below detectable limit; two standard deviations at zero dose).
[b]GnRH-I = GnRH immunization.
[c]DHT implant length in cm.
[d]Indicate significant differences of $p < 0.05$ from controls.

Table 3
Effects of Treatment of GnRH Immunized Rats with DHT Implants (2–8 cm) on Restoration of Fertility when Exposed to Females

Group	No. of Fertile males	Embryos[a]	Corpora Lutea[a]
Control	5 of 5	13.8 ± 0.6	14.5 ± 0.7
GnRH-I[b]	0 of 5	0	14.9 ± 1.2
GnRH-I +2[c]	0 of 5	0	13.8 ± 0.5
GnRH-I +4	0 of 5	0	12.9 ± 1.7
GnRH-I +6	0 of 5	0	14.1 ± 0.5
GnRH-I +8	0 of 5	0	14.6 ± 0.9

[a]Values are mean ± SEM.
[b]GnRH-I = GnRH immunized.
[c]DHT implant length in cm.

Fertility Trial

The results of the fertility trial are presented in Table 3. All control rats impregnated female rats when cohabitated for 5 d, whereas, none of the GnRH-immunized rats treated with DHT implants were able to impregnate female rats, regardless of the length of the implants (2–8 cm). The mean corpora lutea number in females was identical (Table 3), confirming that an equal number of oocytes was available for fertilization.

Discussion

Although considerable progress has been made in the development of highly effective, acceptable, and reversible methods of birth control for women, progress in the development of a male contraceptive has been slow. One strategy for regulating male fertility is to suppress production of testicular androgens, primarily testosterone, which is required for spermatogenesis. The greatest obstacle to this approach, however, is that testosterone supports important physiological androgenic functions, including libido and potency, external male characteristics, and physical strength. Thus, any method that blocks sperm production by interfering with testosterone production or action will potentially produce unacceptable side effects of hypogonadism and, therefore, would require suitable androgen replacement to be clinically useful.

We have consistently shown that active immunization against GnRH is capable of inducing azoospermia in rats and that restoration of quantitatively normal spermatogenesis and sexual activity can be effected by exogenous administration of testosterone (Awoniyi et al., 1989, 1992). However, in an earlier study, we found that lower doses of testosterone administered via Silastic implants failed to restore fertility in all animals. In fact, up to 40% of GnRH immunized azoospermic rats remained infertile at this level of androgen supplementation (Awoniyi et al., 1992). Thus, low-dose testosterone replacement in GnRH-immunized animals was felt to be a viable approach to contraception. For GnRH immunization to be an acceptable form of male contraceptive, the androgen replacement regimen should sustain androgenization without restoring sperm production. In this study, we examined the extent to which DHT supplementation of GnRH-immunized rats restored spermatogenesis and fertility as a possible alternative to testosterone replacement. GnRH immunization in all experimental rats led to azoospermia, and none of the DHT implants used restored spermatogenesis to a level that induced fertility. At the same time, DHT restored androgenization as measured by weight of sex accessory organs to control values. It is, therefore, possible that these doses of DHT may be sufficient to restore other manifestations of androgenicity such as libido while being insufficient to restore spermatogenesis.

One might argue that the doses of DHT used in the present study did not induce male interest in females despite the effect of androgenization of sex accessory organs. In fact, some studies have shown that DHT alone failed to restore sexual behavior, or support ejaculation in castrated male rats (McDonald et al., 1970; Feder 1971; Johnston and Davidson, 1972). However, there are also evidence to the contrary. For example, peripheral injections of DHT have been shown to stimulate mating in castrated pigs (Alsum and Goy, 1975), rhesus monkey (Phoenix, 1974), and rabbits (Beyer and Rivaud, 1981). Others have also reported significant elevations of sexual activity in castrated rats given large doses of DHT (Parrott 1975; Paup et al., 1975; Sodersten, 1975; Olsen, 1979; Olsen and Whalen, 1984). This issue requires further investigation.

Dihydrotestosterone is known to be the active intracellular androgen responsible for prostatic morphogenesis and growth (Aumuller 1983; Berry and Isaac, 1984). In humans and in dogs, studies have shown that benign prostatic hyperplasia is associated with high tissue concentration of DHT and increased in 5 alpha-reductase activity (Isaac and Coffey, 1981; Isaac et al., 1983). Therefore, the clinical applicability of the data presented herein may be obscured by the fear of increase in prostate size that may occur with DHT therapy. However, there are no data that suggest that modest elevation of serum DHT levels would necessarily produce enlargement of the prostate. In fact, the data presented in this study suggest that a suitable low dose of DHT supplement in these animals produces persistent azoospermia without a significant increase in prostate weight (2 and 4 cm implants). Therefore, the advantages of evaluating low-dose DHT for male contraceptive purposes following GnRH immunization, as used in this animal model, seem to out-weigh the disadvantages.

Several reports in the literature (Linde et al., 1981; Doelle et al., 1983; Rea et al., 1986; Bhasin et al., 1988) have used GnRH analogs together with subsequent T replacement to accomplish both suppression of sperm production and support sexual function in laboratory animals as well as in humans. In some of these studies, reversible oligospermia, but not azoospermia was achieved (Linde et al., 1981; Rea et al., 1986). Active immunization against GnRH, like GnRH analog administration, is a promising male contraceptive because of its ability to induce azoospermia and because it is reversibile. In a broader sense, however, DHT as the androgenic supplement to GnRH-immunized rats represents only one of a number of alternative choices. There are theoretical advantages of DHT. For example, the concentration of DHT in seminiferous tubule fluid (the milieu to which germ cells are exposed) in intact rats is less than 5% that of testosterone (Turner et al., 1984). Thus, there may be a better chance of DHT not supporting spermatogenesis compared to testosterone. This fact is supported by our observation that none of the DHT implants we used restored quantitative spermatogenesis or fertility, whereas in our previous studies (Awoniyi et al., 1993), testosterone implants as small as 4 cm partially restored spermatogenesis and fertility. Perhaps new androgens, as they become available, will be even more efficient at producing a broad gap between the low dose required for androgenization, and the higher dose required for spermatogenesis. The results of this study are particularly significant because it will now be possible to evaluate GnRH immunization and DHT supplementation on maintenance of sexual activity, perhaps the most relevant androgenic function. By knowing the appropriate DHT doses that will support and maintain androgenization, but still be inadequate to produce spermatogenesis, we can further develop the threshold concept of male contraception using GnRH vaccine.

Materials and Methods

Fertile adult male Sprague Dawley rats (300–350 g) were housed under standard controlled temperature (22°C) and lighting (14 h of light, 10 h of darkness) and had free access to rat chow. To confirm fertility, each rat was placed with two normal cycling sexually mature female rats for 5 d. Twenty days after mating, the female rats were euthanized, and the number of embryos was determined. A male rat was considered fertile if one or both of the females became pregnant, and infertile if neither of the two females became pregnant.

Twenty-five male rats that successfully passed this fertility test were actively immunized against GnRH to render them azoospermic as previously described (Awoniyi et al., 1992). Briefly, 100 μg of GnRH conjugated to human serum globulin was dissolved in 0.2 mL sterile saline and emulsified with an equal volume (0.2 mL) of Freund's adjuvant (GIBCO BRL, Grand Island, NY). After emulsification, the total dose of immunogen (0.4 mL/rat) was injected intradermally at 12–15 sites. Primary immunization was given in Freund's complete adjuvant (Gibco-BRL), and boosters were given in Freund's incomplete adjuvant at weeks 4, and 6, thereafter. Five control rats received injections of saline and adjuvant throughout the trial. Two weeks after the second booster injection (i.e., week 8), GnRH-immunized rats received subdermal implantation of DHT-filled Silastic implants of 2, 4, 6, or 8 cm, or empty implants (n = 5/group). The five control rats received empty implants. DHT implants were prepared according to a previously described method (Stratton et al., 1973). Two months after implantation, each male rat was placed with three female rats for 5 d. After 20 d, female rats were euthanized. The ovaries and uterine horns were removed and the number of corpora lutea, embryos, and implantation sites were determined. All GnRH-immunized and control rats were euthanized by rapid decapitation after the fertility trial. Testes, ventral prostate, and seminal vesicle were removed, and weights were determined. One testis from each rat was used to determine the number of advanced spermatids by the hemacytometric counting of testicular homogenate under phase contrast microscopy (Robb et al., 1978). Serum levels of testosterone and DHT were determined by standard RIA procedure using commercial kits obtained from Amersham Life Science (Arlington Heights, IL) according to the protocol provided with the kit. The detection limit (two standard deviations at zero dose) was approx 0.003 ng and 0.005 ng per tube for testosterone and DHT, respectively. All animal were treated according to animal care protocols approved by the Animal Care Committee at The University of Colorado Health Sciences Center.

All data were analyzed by one-way analysis of variance. Duncan's multiple range test was used to identify differences between the treatment groups (Snedecor and Cochran, 1967). Values were considered significant at $p < 0.05$.

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