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Research article

Return to fertility after extended chemical castration with a GnRH antagonist

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Abstract

Background: Antagonistic analogues of GnRH for the treatment of prostate cancer may be used clinically in persons for whom return to fertility after such treatment is important or desirable. The purpose of this study was, therefore, to evaluate the effects of a long term treatment with orntide, a GnRH antagonist, on testosterone levels and fertility in male rats.

Methods: Two groups of male rats received either 120-day orntide microspheres (8.8 mg orntide/kg/120 days) or vehicle alone (control group). Serum orntide and testosterone levels in both groups were monitored at certain intervals for 9 months from the initiation of treatment. After recovery of normal serum testosterone levels in the treated animals, each rat was housed with two proven breeder, but drug-naive, females.

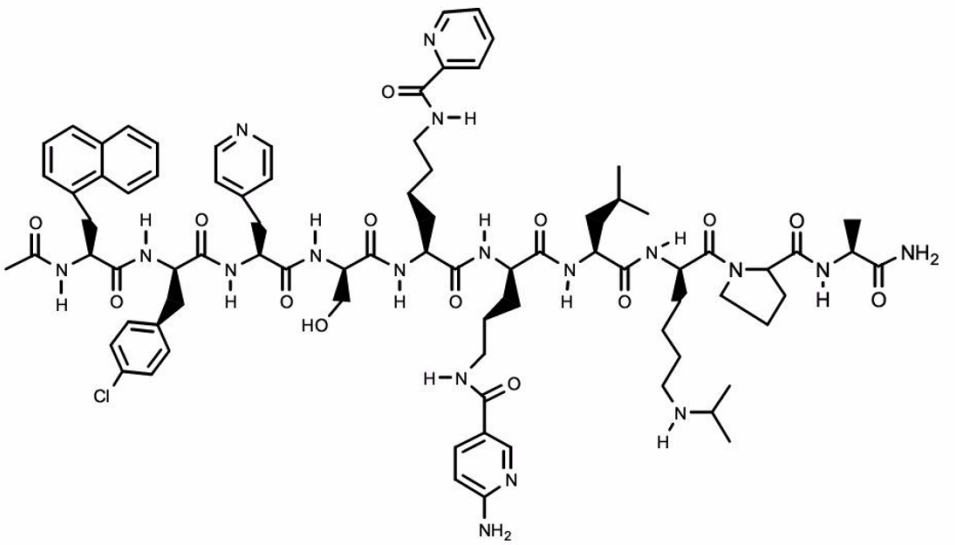
Results: All mates of treated rats achieved pregnancy as rapidly as the mates of control rats although two of the control rats did not sire a litter with either female and one sired only one litter. The mean size of the litters of treated (12.3 offspring per litter) and control (10.6 offspring per litter) were similar. All offspring were grossly normal morphologically and behaviorally during the time to weaning.

Conclusions: These results suggest that lack of fertility due to testosterone suppression is reversible after cessation of treatment with this GnRH antagonist.

Background

Analogues of gonadotrophin releasing hormone (GnRH) are being used for a variety of hormone-dependent human disease states. Prominent among these are prostate cancers and endometriosis [1–5]. One class of these analogues, GnRH superagonists, such as leuprorelin, triptorelin, goserelin, and buserelin, have been in use for some years. They act by hyper-stimulation of GnRH receptors on the gonadotroph cells of the pituitary, resulting in an initial phase of elevated levels of testosterone and androgens, followed by down-regulation of the receptors. This eventually results in blocking release of LH and FSH and secondarily preventing synthesis/release of testosterone or estrogen from the gonads. The superag-

A. Orntide acetate ($C_{81}H_{106}N_{18}O_{14}Cl$, MW_{avg} 1591.31)



AcDNal[1]-DpClPhe[2]-D3Pal[3]-Ser[4]-PicLys[5]-D(6Anic)Orn[6]-Leu[7]-IprLys[8]-Pro[9]-DAla[10]-NH_2

AcDNal	N-acetyl-3-(12-naphtyl) alanine
DpClPhe	4-(4-chlorophenyl)-2-amino-butyric acid
DPal	3-(3-pyridyl) alanine
PicLys	Nε-picolinoyllysine
D6AnicOrn	6-aminonicotinoyl ornithine
IprLys	Nε-isopropyllysine

B. GnRH

Pyro-Glu[1]-His[2]-Trp[3]-Ser[4]-Tyr[5]-Gly[6]-Leu[7]-Arg[8]-Pro[9]-Gly[10]-NH_2

C. Leuprolide

5-oxo-Pro[1]-His[2]-Trp[3]-Ser[4]-Tyr[5]-D-Leu[6]-Leu[7]-Arg[8]-Pro[9]-NHEt

Figure 1
Structure of orntide acetate (A) and amino acid sequences for GnRH (B) and leuprolide (C)

onists have a long safety record and are successfully used clinically in persons, who later have children. In this class of GnRH analogues which are commercially available, return to fertility does not appear to be a problem.

A new class of GnRH analogues has reached the clinical testing phase. These are GnRH antagonists, which immediately block the GnRH receptors to access by native GnRH peptides. Clinically the antagonists differ from the superagonists in that the initial hyper-stimulation and release of LH and FSH do not occur [6]. While the net result of the treatments by agonists and antagonists appears to be the same, two characteristic of the antagonists make it essential to determine whether return to fertility may be a problem and of regulatory interest. The first is the necessity for a loading dose at the beginning (the first day or two) of a treatment regimen and the considerably lower dose requirement thereafter to sustain suppression. The second characteristic is the propensity of at least some of the antagonists to be ligands for extra-pituitary GnRH receptors such as those in prostate tissue and elsewhere [7–11]. There is concern that, whatever the mechanism might be, protracted administration of effective antagonist doses might make a return to fertility more difficult. The concern is heightened by the likelihood that antagonists will be used for treatment of benign prostatic hypertrophy, a condition in men who might wish at some future time to sire offspring.

A potent GnRH antagonist, orntide, that shares with other GnRH analogues the need for a loading dose, is being developed in depot formulations (microspheres) for long term administration (months-years) [12–14]. One such formulation, a potential four month microsphere preparation in PLGA, was evaluated in male rats for its release kinetics and effectiveness of castration [14]. This report is an evaluation of the effect of a long term treatment with a GnRH antagonist, orntide acetate, on the fertility of male rats. Treated male rats which recovered from chemical castration and untreated controls were mated with breeder females.

Materials and Methods

Materials

Orntide acetate (Fig. 1A, with comparison to native GnRH (B) and super-agonist leuprolide (C)) was supplied by California Peptide Research, Inc. (Napa, CA) and was 99% pure. The polymer for microsphere formulation, 85:15 poly(D,L-lactide-co-glycolide) (PLGA) was obtained from Birmingham Polymers, Inc. (Birmingham, AL). Orntide microspheres were prepared as previously described [13]. Proven breeder female Sprague Dawley rats weighing approximately 230 g were purchased from Harlan (Indianapolis, IN) and used immediately after the quarantine period required by a University of Kentucky Animal Research Facility approved protocol. Sprague Dawley male rats were those that had been treated with a 4 month dose of orntide [14] and allowed a sufficient period of time for wash out.

Methods

In vivo evaluation of orntide microspheres

Two groups of male Sprague Dawley rats (n = 6) were used in this study. Group I (rats 1–6) received 120-day orntide microspheres (8.8 mg orntide/kg) prepared with the polymer (16% drug load) in vehicle, and animals in Group II (rats 7–12) were used as controls and received vehicle (1% carboxymethylcellulose and 2% mannitol (w/v)) alone. The injections were administered subcutaneously with an 18G needle between the scapulae. Blood samples were collected from the tail vein at predetermined time points. The serum was separated and frozen until analysis. The animals were challenged with 10 μg/kg LHRH after routine sampling at days 77 and 91 and additional samples were taken at 2 and 24 hours after the challenges. Following the 120 day treatment period, the orntide microsphere treated rats were subjected to a washout period until they resumed and maintained similar testosterone levels as the control before the fertility assessment. A challenge dose of LHRH was administered during the washout period at 173 days.

Serum orntide levels in rats were measured using a radioimmunoassay[1]. Tyrl-orntide was radioiodinated by the lactoperoxidase method and the labeled ligand was purified by HPLC. Orntide was conjugated by the carbodiimide method and the antibody to orntide was produced in rabbits. The lower detection limit of the assay was 0.008 ng/ml. The intra- and interassay coefficients of variation were 6% and 9%, respectively.

Serum testosterone levels were assayed using Active™ Testosterone RIA DSL-4000 kits (Diagnostic Systems, Inc., Webster, TX). The lower limit of detection for this assay was 0.08 ng/ml and the intra- and interassay coefficients of variation were 10 and 9%, respectively.

Fertility assessment

After orntide levels were no longer detectable as shown in Figure 2 and the testosterone levels of the treated animals had returned to and been maintained at normal levels (Figure 3), each male rat from Groups I and II was housed in a cage with two proven breeder females. A proven breeder female is defined as having given birth to at least two offsprings. The study was continued until most of the females gave birth. After completion of the study, the baby rats were counted and separated by sex with observation for abnormality at the time of weaning.

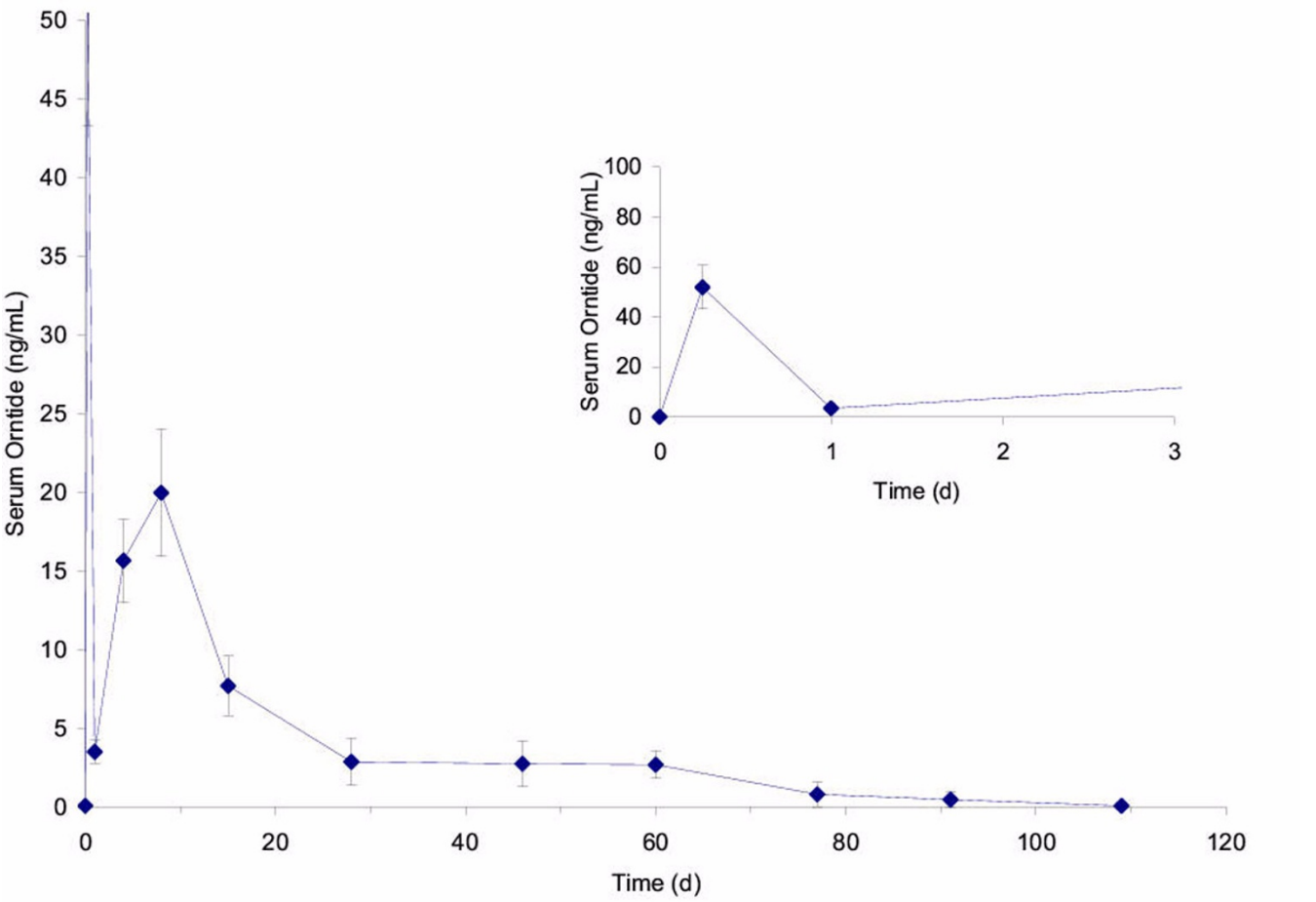


Figure 2
Mean serum orntide levels in rats after administration of 120-day orntide ms (Group I). The insert shows initial serum orntide levels

Statistical evaluation of data

Drug and testosterone data are presented as means ± standard deviation. For values below the assay detection limit the limit was used for calculations.

Results and Discussion

Release kinetics and pharmacodynamics

Figures 2 and 3 show the release kinetics of these microspheres and the resultant rapid and profound testosterone suppression that resulted in the treated animals. This information confirms that the treated animals were, indeed, rendered incapable of producing offspring during the treatment. As shown in Figure 2 administration of orntide microspheress initially resulted in high serum drug levels in rats (Cmax 52 ng/mL after 6 h). Subsequent release resulted in a peak level of 20 ng/mL on day 8 after which orntide levels decreased to approximately 3 ng/mL and remained there for at least 60 days before decreasing gradually to below 1 ng/mL at day 109. The initial orntide levels may be due to the release of the fraction of the peptide residing at or near the accessible surfaces of the polymeric matrix in this formulation. The second elevation in the orntide release profile may be attributed to a diffusion-controlled release. The remainder of the release profile is likely a result of erosion-controlled peptide release from the microspheres [12–14].

Figures 3 shows serum testosterone levels in groups I and II, respectively. The immediate testosterone suppression seen in group I was associated with the significant release of GnRH antagonist from the microspheres. Complete suppression of testosterone was observed through 109 days and chemical castration (defined as testosterone < 0.5 ng/ml) persisted to 145 days for four of the orntide treated animals. Challenges at 77 and 91 days with LHRH (10 μg/kg) did not elevate the testoster-

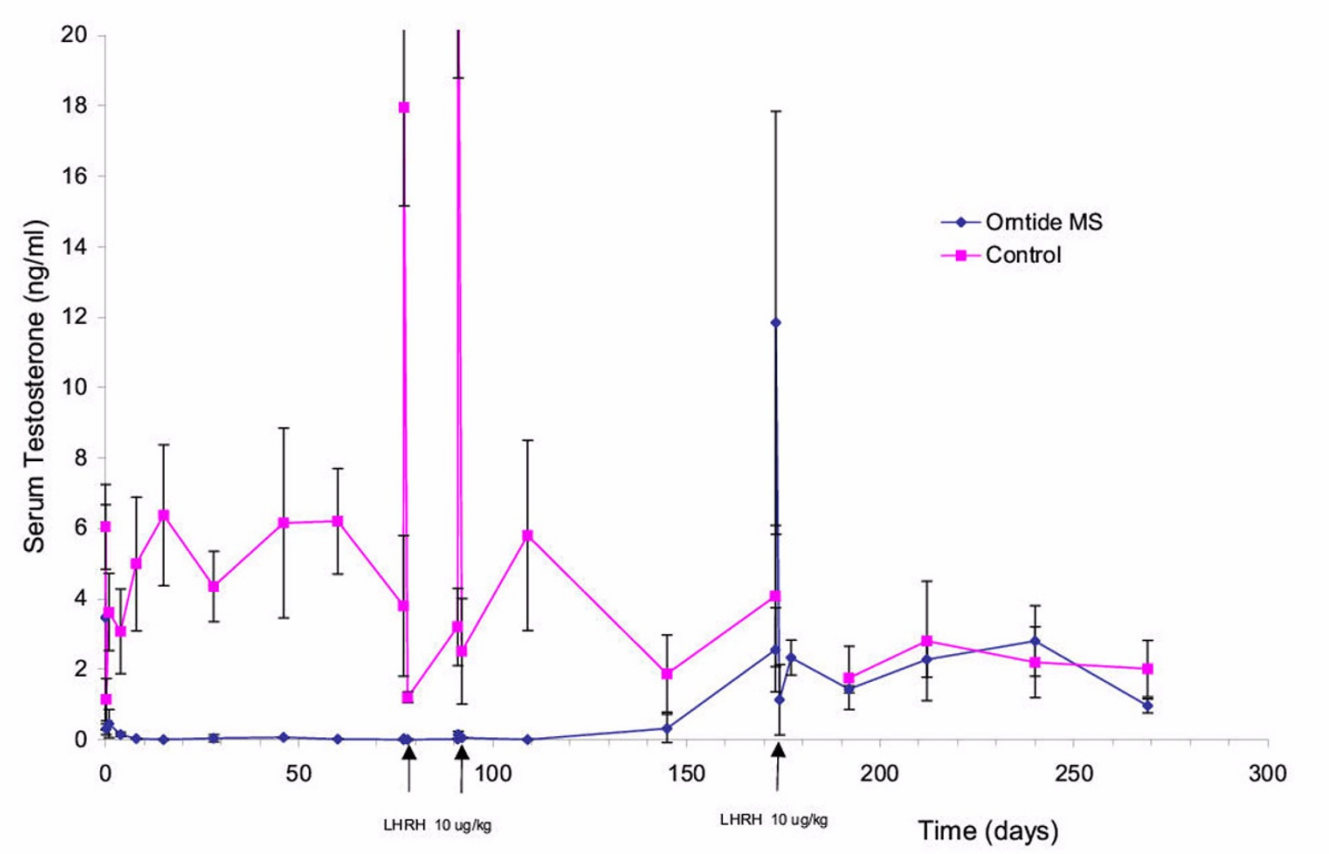


Figure 3
Mean serum testosterone levels in rats treated with 120-day orntide ms (Group I) and in control animals (Group II)

one levels indicating that good suppression was maintained at those time points. The levels elevated above 1 ng/ml sometime between 145 days and 173 days. The return to normal was confirmed by a challenge at 173 days which showed the same testosterone elevation as that seen in the untreated animals at 77 and 91 days. Figure 3 showed that during the period from 173 days to 269 days, the testosterone levels of treated and untreated were similar, fluctuating in a manner that is typical of normal rats.

Fertility assessment

At 269 days after the initial injections, the animals were placed with the females to assess fertility. By this time the animals were more than a year old, and problems with fertility would not have been a surprise.

Table 1I shows the number of offsprings born to each of the female rats that were housed with the designated male rat. All of the females housed with the treated males gave birth. A total of 123 offspring (62 male, 61 female) average 12.3 offspring per litter (range 3–17) and 24.6 offspring per male (range 17–33). The control group was seemingly less productive in that the females housed with males #7 and #12, and one of those housed with #10 had no offspring. Eliminating those with no offspring, the average number of offspring per male was 21.1 (range 10–28), similar to that from treated males, and the offspring per litter was 10.6 (range 3–15), also close to the treated.

All of the offspring survived to the time of weaning and were morphologically and behaviorally normal. Figure 4 is a photograph showing the offspring of female 2 mated with male rat #11 (vehicle treated) and of female 2 mated with male rat #3 (orntide microspheres treated) along with their mothers. The dates of birth were two days apart.

Conclusions

Adult male rats that had been rendered chemically castrate for 4 months or more with orntide released from a

Table I: Number of offspring produced by orntide treated and drug-free rats

	Male Rat #	Female Rat #	Male offspring	Female offspring
Group I	1	1	5	6
Treated		2	6	4
with	2	1	3	10
Orntide MS		2	12	5
	3	1	2	1
		2	3	11
	4	1	10	7
		2	9	7
	5	1	8	5
		2	4	5
	6*	--	--	--
		--	--	--
Group II	7	1	0	0
Control		2	0	0
	8	1	7	6
		2	7	8
	9	1	3	4
		2	6	7
	10	1	5	5
		2	0	0
	11	1	2	1
		2	9	4
	12	1	0	0
		2	0	0

* Male rat #6 died on day 192 during anesthesia

Figure 4
Left: Offspring produced by male rat #11 (control) and female #2 Right: Offspring produced by male rat #3 (orntide treated) and female #2.

depot formulation were able to return to fertility. This suggests that this GnRH antagonist may be safely used in patients who may wish to regain their capacity for reproduction after hormonal treatment.

Competing Interests

The microsphere technology described in this paper is patented by the University of Kentucky Research Foundation and has been licensed to a Pharmaceutical Company. Should the product in this technology be commercialized by the company the University will receive royalties on any income. Additionally, the company has provided funding to the University for preclinical formulation research and development of peptides using the microsphere technology.

[1]The RIA method was developed at Tulane University School of Medicine by Dr. Brower and the assay were carried out by his research lab.

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