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SHORT COMMUNICATION

Oral administration of the GnRH antagonist acyline, in a GIPET®-enhanced tablet form, acutely suppresses serum testosterone in normal men: single-dose pharmacokinetics and pharmacodynamics

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Abstract
Purpose GnRH analogs are useful for the treatment of prostate cancer, but require parenteral administration. The peptide GnRH antagonist acyline potently suppresses luteinizing hormone (LH) and testosterone in man; however, its clinical utility is limited by the requirement for frequent injections. The use of a proprietary enhancer system called GIPET®, which is based on medium-chain fatty acids, facilitates the oral bioavailability of peptides. We hypothesized that GIPET® enhancement would allow for the safe oral dosing of acyline for the treatment of prostate cancer.
Methods We enrolled eight healthy young men in a pharmacokinetic and pharmacodynamic study of 10, 20 and 40 mg doses of GIPET®-enhanced oral acyline. Blood for measurement of serum LH, FSH, testosterone and acyline was obtained prior to each dose of GIPET®-enhanced oral acyline and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h after dosing.
Results Serum LH, FSH and serum testosterone were significantly suppressed by all doses of GIPET®-enhanced oral acyline after 6 h, with suppression reaching a nadir 12 h after dosing. In addition, the 20 and 40 mg doses demonstrated sustained suppression of testosterone for 12–24 h. All hormone concentrations returned to normal 48 h after administration. There were no treatment-related serious adverse events, and laboratory assessments, including liver function tests and creatinine, were unaffected by treatment.
Conclusions Oral administration of GIPET®-enhanced acyline significantly suppresses testosterone and gonadotropins in normal men without untoward side effects and might have utility in the management of prostate cancer.

Keywords Prostate cancer · Testosterone · LH · FSH · Pharmacokinetics · Pharmacodynamics

Introduction

Gonadotropin releasing hormone (GnRH) stimulates the pituitary release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in both males and females [1]. GnRH antagonists are analogs of GnRH that inhibit the action of the endogenous hormone via competitive inhibition of GnRH binding to receptors on pituitary gonadotropes [2]. GnRH antagonists produce immediate and sustained declines in gonadotropin and sex steroid levels, and therefore, might have a role in treatment of hormone-dependent conditions such as prostate and breast cancer, or in the treatment of benign conditions such as endometriosis. However, most antagonists are peptides that require subcutaneous injection for administration. Such injections frequently require medical personnel and can precipitate histamine release and worrisome allergic reactions. As a result, there is a need for orally active GnRH antagonists.

Acyline is a potent GnRH antagonist that has been studied extensively in humans [3–6]. After subcutaneous injection, acyline produces a rapid and sustained suppression of gonadotropin and testosterone levels that can persist for up to 2 weeks at the highest doses tested in man [4]. No

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toxicological effects of subcutaneous acyline have been observed in preliminary studies when used at the highest tested dose (300 μg/kg SQ every 14 days) for up to 12 weeks [6].

A proprietary formulation of medium-chain fatty acids called GIPET® (GI enhancing permeability technology) can allow for the oral administration of otherwise poorly bioavailable compounds such as peptides in a solid dosage form [7]. As GIPET® technology might allow for oral dosing of acyline, we sought to ascertain the single-dose pharmacokinetics and pharmacodynamics of oral GIPET®-enhanced acyline in man.

Methods

Subjects

Eight men, 18–55 years of age, in good health, were recruited through local newspapers and campus flyers. After informed consent was obtained, subjects underwent screening procedures consisting of a medical history, a physical examination, and measurements of serum hormone levels, blood counts and serum chemistries. Specific exclusion criteria included body-mass index greater than 35, history or current use of testosterone, infertility, poor general health, significantly abnormal laboratory results, history of testicular disease or severe testicular trauma, major psychiatric disorders, use of illicit drugs or the use of more than three alcoholic beverages daily. The Western Investigational Review Board (WIRB) approved all aspects of this study. The trial was registered in advance at clinicaltrials.gov as study # NCT00471185.

Study procedures

Oral acyline administration occurred on three occasions, each separated by 1 week. The subjects received progressively increasing doses of 10, 20 and 40 mg of GIPET®-enhanced oral acyline after an overnight fast, and continued to fast for 4 h after dosing. On each dosing day, a baseline blood sample was drawn immediately prior to dosing between 0800 and 1000 hours. Blood samples for measurement of serum hormones were then obtained 30, 60, 90 min, 2, 3, 4, 6, 8, 12, 24, 48 h and 7 days after each dose. Complete blood counts and comprehensive metabolic panels were obtained 24 h after each dose for assessment of safety.

Measurements

Serum FSH and LH concentrations were measured by immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland) as reported previously [4]. The normal ranges were: LH (1.2–7.3 IU/L) and FSH (1.1–6.7 IU/L). Serum total testosterone was measured by a radioimmunoassay (Diagnostic Products Corporation, Webster, TX) [4]. The normal range was 8.7–33 nmol/L. Serum acyline concentrations were measured using liquid chromatography/tandem mass spectroscopy (LC/MS). Serum was extracted with 4 ml of dichloromethane/IPA 80/20, mixed for 15 min, and centrifuged at 3,500 rpm for 10 min. The solvent layer was dried under nitrogen, reconstituted and 100 μl was injected into the LC/MS/MS (API 4000; Applied Biosystems, Foster City, CA, USA). The limit of quantitation (LOQ) for acyline was 0.2 ng/ml. Blood for hematology, routine chemistries and hepatic function tests were measured in the University of Washington Clinical Laboratory.

Statistical analysis

Due to non-normality, changes in serum hormone concentrations from baseline and between doses were analyzed in a non-parametric fashion using a Kruskal–Wallis analysis of variance (ANOVA) with a Bonferroni correction for multiple comparisons. For the analysis of the serum acyline concentrations, maximum concentration after dosing (Cmax), time to maximum concentration (Tmax), area-under-the curve (AUC) and elimination phase half-life ($T_{1/2}$) were calculated using a computer program (WinNonlin, Pharsight, Mountain View, CA). Pharmacokinetic parameters between doses were compared using ANOVA. All statistical analyses were performed using STATA (College Park, TX, USA) or the General Linear Models procedure of SAS (SAS Institute, Cary, NC). For all comparisons, an alpha of 0.05 was considered significant.

Results

Subjects

Nine men were screened and met all inclusion criteria, and the first eight were enrolled in the study. Subjects had a mean age of 31 ± 8.2 and had a mean body-mass index of 23 ± 1.8 kg/m^2. All subjects completed the study, and none was lost to follow-up. Five of the subjects reported seven adverse events during the study. Two subjects reported headaches, one subject reported an upper respiratory infection, one subject reported an episode of nausea with vomiting, and one subject had a bicycle accident resulting in a fractured collarbone. One subject died unexpectedly 6 days after receiving the 40-mg dose of oral acyline. An autopsy by the medical examiner revealed the cause of death to be an accidental narcotic drug overdose. The death was not

considered study-related, as serum acyline levels were undetectable, and serum hormone concentrations were normal 4 days prior to his death.

Acyline pharmacokinetics

Mean serum acyline concentrations rose immediately after oral dosing with all three doses. The maximum concentration, time of maximum concentration, AUC and half-life of serum acyline are listed in the table. There were no significant differences in the pharmacokinetic parameters between doses, due to the large degree of variability between subjects. Serum acyline was undetectable in all subjects 48 h after dosing, except for one subject in the 40-mg group. Acyline was not detectable in the serum of any subject in any dose group 7 days after dosing (Table 1).

Serum hormones

Baseline serum concentrations of LH, FSH and testosterone did not differ prior to dosing of 10, 20 or 40 mg of oral acyline. All doses of oral acyline significantly suppressed serum LH 6 h after dosing (Fig. 1a). Significant suppression of serum LH was maintained through 12 h after dosing and returned to baseline 2 days after dosing. Serum LH was significantly more suppressed with the 40 mg dose compared to the 10 mg dose, 8–12 h after dosing. Serum FSH was significantly suppressed 6–12 h after dosing with all three doses of oral acyline; however, the average suppression of serum FSH 12 h after dosing was 28 ± 5% compared with 70 ± 10% suppression of serum LH ($P < 0.001$; Fig. 1a, b).

Suppression of serum testosterone closely mimicked suppression of serum LH, being significantly suppressed with all doses between 6 and 12 h after dosing, and with greater suppression with the 40 than the 10 mg dose, 8 and 12 h after dosing (Fig. 1c). All eight subjects had a serum testosterone concentration below the lower limit of the normal range (<8.4 nmol/L) 12 h after the 40-mg dose.

Table. 1 Acyline pharmacokinetics after administration of a single dose to normal men ($N = 8$)

Dose (mg)	10	20	40
*C*max (ng/ml)	15.3 ± 10.3	20.7 ± 18.6	17.1 ± 12.7
*T*max (h)	1.6 ± 0.7	7.7 ± 16	2.3 ± 1.1
AUC_{0-24} (ng h/ml)	61 ± 48	175 ± 188	100 ± 87
$T_{1/2}$ (h)	7.4 ± 3.1	9.2 ± 6.3	10.7 ± 3.2

All data are means ± SD

Cmax maximum concentration after dosing, *Tmax* time of maximum concentration, AUC_{0-24} area-under-the-curve 0–24 h, $T_{1/2}$ terminal half-life

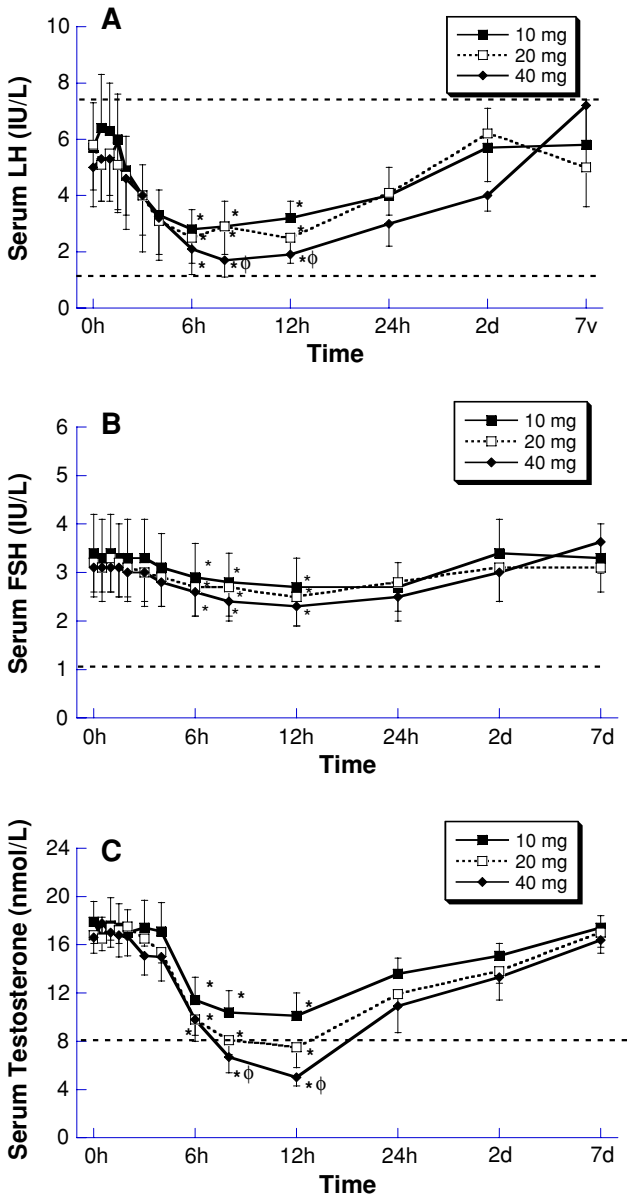

Fig. 1 Serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone in eight normal men administered increasing doses of 10, 20 and 40 mg of GIPET®-enhanced oral acyline. All values are means ± SEM. *Asterisk* $P < 0.05$ compared with baseline; *phi* $P < 0.05$ compared with 10-mg dose. *Dotted lines* represent the upper and lower limit of the normal range in 100 normal men. Please note that the *X*-axis is non-linear

Discussion

Currently, GnRH *agonists*, not antagonists are the primary form of hormone suppression therapy due to superior dosage forms, which include long-acting injections and implants. The major drawbacks of agonists are the initial gonadotropin-driven hormone release, the need for parenteral administration and the lag period of 2–3 weeks before

hormonal suppression is achieved. GnRH antagonists might be clinically superior to agonists as they can provide immediate hormone suppression. Clinical use of existing antagonists, however, is limited by very short half-lives and the need for frequent injections.

In this report, the single-dose pharmacokinetics and pharmacodynamics of oral dosing of the potent GnRH antagonist acyline are described. Like most antagonists, acyline is a decapeptide, which if administered orally would be subject to degradation within the gastrointestinal tract. Therefore, in this study, oral absorption was made possible by formulating the acyline with a proprietary mixture of medium-chain fatty acids, called GIPET® (GI enhancing permeability technology), which markedly improves the oral bioavailability of compounds such as peptides in a solid dosage form [7]. To our knowledge, this is the first report of an orally active peptide GnRH antagonist in humans, and only the second report in the literature [8] of an orally active GnRH antagonist tested in humans.

In this study, all three doses of GIPET®-enhanced oral acyline significantly suppressed serum gonadotropins and testosterone for up to 12 h after oral dosing. Intriguingly, suppression of serum FSH was less pronounced than suppression of LH and testosterone. This discrepancy between LH and FSH suppression has been observed previously with the GnRH antagonists Nal-Glu [9], cetrorelix [10] and abarelix [11] as well as acyline [3, 4] when all are administered by subcutaneous injection. This difference is likely due to the fourfold greater (320 vs. 80 min) serum half-life of FSH compared to LH [12].

Importantly, the pharmacokinetics and pharmacodynamics of oral acyline suggest it may have clinical utility for hormone suppression in patients with hormonally responsive tumors, such as breast and prostate cancers or in the treatment of benign conditions such as endometriosis. Future multiple-dose pharmacokinetic and pharmacodynamic studies of oral acyline will be required to determine the optimal dose and dosing frequency of this approach to hormone suppression. Given the testosterone suppression observed in this study, twice-daily dosing and/or higher doses may be required to achieve the castrate levels of serum testosterone required for prostate cancer therapy.

In addition, we have demonstrated that GIPET® is a novel, effective method of enhancing the absorption of peptides from the gastrointestinal tract. GIPET® technology might be useful for enhancing the absorption of other peptides and compounds with poor bioavailability.

Currently, hormonal suppression is widely used clinically in the treatment of both breast [13] and prostate cancer [14]. Intriguingly, GnRH antagonists may also exert anti-tumor effects independent of their reduction of hormone levels, as anti-proliferative effects on the division of cancer cells in culture have been observed [15]. This may be due to inhibition of epidermal growth factor-induced signal transduction [16] or inhibition of the plasminogen-activator system [17]. Regardless of the mechanism, it is increasingly apparent that early hormonal suppression is associated with significantly lower mortality in the setting of locally advanced prostate cancer [18].

In conclusion, we have demonstrated that oral administration of the peptide GnRH antagonist acyline, in a GIPET®-enhanced tablet form, can acutely suppress serum gonadotropin and testosterone biosynthesis in normal men without adverse side effects. Further study of this novel form of hormone suppression in hormone responsive cancers, benign disease states, such as endometriosis, and for indications such as hormonal contraception is warranted.

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