A Selective Androgen Receptor Modulator for Hormonal Male Contraception

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Received July 31, 2004; accepted September 3, 2004

ABSTRACT

The recent discovery of nonsteroidal selective androgen receptor modulators (SARMs) provides a promising alternative for testosterone replacement therapies, including hormonal male contraception. The identification of an orally bioavailable SARM with the ability to mimic the central and peripheral androgenic and anabolic effects of testosterone would represent an important step toward the “male pill.” We characterized the in vitro and in vivo pharmacologic activity of (S)-3-(4-chloro-3-fluorophenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethylphenyl)propionamide (C-6), a novel SARM developed in our laboratories. C-6 was identified as an androgen receptor (AR) agonist with high AR binding affinity (Ki = 4.9 nM). C-6 showed tissue-selective pharmacologic activity with higher anabolic activity than androgenic activity in male rats. The doses required to maintain the weight of the prostate, seminal vesicles, and levator ani muscle to half the size of the maximum effects (i.e., ED50) were 0.78 ± 0.06, 0.88 ± 0.1, and 0.17 ± 0.04 mg/day, respectively. As opposed to other SARMs, gonadotropin levels in C-6-treated groups were significantly lower than control values. C-6 also significantly decreased serum testosterone concentration in intact rats after 2 weeks of treatment. Marked suppression of spermatogenesis was observed after 10 weeks of treatment with C-6 in intact male rats. Pharmacokinetic studies of C-6 in male rats revealed that C-6 was well absorbed after oral administration (bioavailability 76%), with a long (6.3 h) half-life at a dose of 10 mg/kg. These studies show that C-6 mimicked the in vivo pharmacologic and endocrine effects of testosterone while maintaining the oral bioavailability and tissue-selective actions of nonsteroidal SARMs.

Worldwide population growth and social awareness of reproductive health have stimulated a great deal of research in contraception. Although contraceptive pills have been available for women for decades, male contraception remains restricted to physical methods, such as condoms and vasectomy. However, many polls show that men would be more willing to be involved in family planning if appropriate contraceptive methods (i.e., a male contraceptive pill) were available (Anderson and Baird, 1997; Martin et al., 2000). Although the mechanism of spermatogenesis is not completely understood, inhibition of LH and FSH production is antithetical to GTx, Inc. J. Dalton is a consultant to GTx, Inc. D. Miller is an employee of GTx, Inc. These studies were supported in part by grants from the National Institutes of Health (R01 DK58800) and GTx, Inc. (Memphis, TN). J. Dalton is a consultant to GTx, Inc. D. Miller is an employee of GTx, Inc. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.104.075424.

ABBREVIATIONS: LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin hormone-releasing hormone; SARM, selective androgen receptor modulator; C-6, (S)-3-(4-chloro-3-fluorophenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethylphenyl)propionamide; MIB, mibolerone; PBS, phosphate-buffered saline; SD, Sprague-Dawley; AR, androgen receptor; DHT, dihydrotestosterone; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; PEG 300, polyethylene glycol 300; TP, testosterone propionate; EIA, enzyme immunosassay; HPLC, high-performance liquid chromatography; CL, clearance; AUC, area under the concentration-time curve; MRT, mean residence time; S-1, (S)-3-(4-fluorophenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethylphenyl)propionamide; ANOVA, analysis of variance.
transdermal patch. In addition to their inconvenient means of administration, steroidal androgens are associated with a variety of undesirable side effects. In fact, the major dose-limiting side effects observed in recent clinical trials of male contraception were androgen-related (Kamischke and Ni-eschlag, 2004).

Several approaches to overcome the limitations of using testosterone preparations have been explored. One major approach focused on structural modifications of steroidal androgens to develop long-acting androgens. Another approach is nonsteroidal selective androgen receptor modulators (SARMs). In 1998, during our search for androgen receptor (AR) affinity labels, we discovered and reported a group of nonsteroidal androgens that are derivatives of two known antiandrogens, bicalutamide (Fig. 1) and flutamide (Dalton et al., 1998). In recent years, we determined important structure-activity relationships for AR binding affinity and transcriptional activation (He et al., 2002; Yin et al., 2003b) and pharmacokinetics and key metabolism pathways of SARMs (Yin et al., 2003c). We also identified dozens of SARMs that demonstrate tissue-selective anabolic and androgenic in vivo pharmacologic effects but are devoid of the side effects commonly associated with testosterone therapy (Yin et al., 2003a; Marhefka et al., 2004). The discovery of SARMs therefore provides a unique alternative for androgen replacement therapy with advantages including oral bioavailability, flexibility of structural modification, AR specificity, lower activity in the prostate, and the lack of steroid-related side effects.

Earlier studies demonstrated that minor structural modification of SARMs could result in dramatic changes in their in vitro and in vivo pharmacologic activity. To date, most of the SARMs reported in our laboratories are able to mimic various pharmacologic activities of testosterone in peripheral tissues but are unable to do so in the central nervous system (Yin et al., 2003a). We hypothesized that novel SARMs can also be designed and synthesized to mimic the effects of testosterone on the hypothalamus-pituitary-testis axis. Such compounds would represent an important step toward the discovery and development of SARMs for hormonal male contraception. The present studies were designed to characterize the preclinical pharmacology of one such novel SARM.

Materials and Methods

Chemicals and Animals. The S-isomer of C-6 (Fig. 1) was synthesized in our laboratory with a purity greater than 99% using previously described methods (He et al., 2002; Marhefka et al., 2004).

\[\text{[17\alpha-Methyl-\textsuperscript{3}H]mibolerone (\textsuperscript{3}H\textsuperscript{[MIB]; 84 Ci/mol]) and unlabeled MIB were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Phosphate-buffered saline (PBS; catalog number P5368, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Male Sprague-Dawley (SD) rats were purchased from Harlan Bio-products for Science Inc. (Indianapolis, IN). All animals were maintained on a 12-h light/dark cycle with food and water available ad libitum. The animal protocol was reviewed and approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.}

In Vitro Pharmacologic Activity. Rat prostate cytosolic AR was prepared from the ventral prostates of castrated male SD rats using established methods (Mukherjee et al., 1999). The AR binding affinity of C-6 was determined using an in vitro radioligand competitive binding assay as previously described (Yin et al., 2003b). Briefly, an aliquot of AR cytosol was incubated with 1 nM \([\textsuperscript{3}H\textsuperscript{MIB}]\) and 1 mM triamcinolone acetonide at 4°C for 18 h in the absence or presence of increasing concentrations of dihydrotestosterone (DHT) or C-6 \((10^{-1}\text{ to }10^{-4} \text{ M})\). Nonspecific binding of \([\textsuperscript{3}H\textsuperscript{MIB}]\) was determined by adding excess unlabeled MIB \((1000 \text{ nM})\) to the incubate. The separation of bound and free radioactivity at the end of incubation was achieved by the hydroxyapatite method as previously described (Yin et al., 2003b); then the radioactivity was counted in a liquid scintillation counter (Model LS6500; Beckman Coulter, Inc., Fullerton, CA). The specific binding of \([\textsuperscript{3}H\textsuperscript{MIB}]\) at each concentration of DHT or C-6 \((B)\) was further calculated by subtracting the nonspecific binding of \([\textsuperscript{3}H\textsuperscript{MIB}]\), and expressed as the percentage of the specific binding in the absence of DHT or C-6 \((B_0)\). Competitive displacement curves of DHT and C-6 were constructed with \(B\) on the vertical axis and ligand concentration \(C\) on the horizontal axis. The concentration of compound that reduced the \(B_0\) by 50% \((IC_{50})\) was determined by computer-fitting the data to the following equation using WinNonlin (Pharsight Corporation, Mountain View, CA): 

\[B = B_0 \times \left[1 - C/(IC_{50} + C)\right]\]

The equilibrium binding constant \((K_d)\) of DHT or C-6 was calculated by 

\[K_d = K_{d0} \times IC_{50}/(K_{d0} + L)\]

where \(K_{d0}\) was the dissociation constant of \([\textsuperscript{3}H\textsuperscript{MIB}]\) \((0.19 \pm 0.01 \text{ nM}; \text{ previously determined by Mukherjee et al., 1999})\), and \(L\) was the concentration of \([\textsuperscript{3}H\textsuperscript{MIB}]\) used in the experiment \((1 \text{ nM})\).

The in vitro AR-mediated transcriptional activity of C-6 was measured using a modification of the method of (Yin et al., 2003b). Monkey kidney cells, CV-1, were maintained at 37°C in a humidified atmosphere of 5% CO\textsubscript{2}/95% air in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). At ~90% confluence, cells were transfected in serum-free medium using LipofectAMINE (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Cells in each T-150 flask were transfected with 0.8 g of an androgen-dependent luciferase reporter construct (pMMTV-Luc; gener...
ously provided by Dr. Ron Evans, Salk Institute, San Diego, CA, and 8 μg of a β-galactosidase expression construct (pSV-β-galactosidase; Promega, Madison, WI). After 3 to 5 h of transfection, cells were washed with serum-free medium and allowed to recover for 10 h in Dulbecco’s modified Eagle’s medium containing 0.2% FBS. Transfected cells were trypsinized, centrifuged, and counted. Cells were then plated into 24-well plates at a density of 8 × 10^4 cells/well. Before drug treatment, cells were allowed to attach to plates for 12 h. The agonist activity and antagonist activity of C-6 were determined by incubating cells with C-6 (1, 10, 100, and 1000 nM) in the absence and presence of DHT (1 nM), respectively. In each experiment, vehicle control and positive control (activity induced by 1 nM DHT) were included. For each treatment, compounds were dissolved in ethanol and then diluted with medium to the desired concentrations. The final concentration of ethanol in each well was <0.05%.

After 24-h drug treatment, medium was removed by aspiration. Cells were washed twice with ice-cold PBS and lysed with 110 μl/well of Reporter Lysis Buffer (Promega) at room temperature for 30 min. An aliquot (50 μl) of cell lysate from each well was used for β-galactosidase assays and the other 50 μl of cell lysate was used for luciferase assays using the method previously described (Yin et al., 2003b). All results were expressed as the ratio of luciferase activity/β-galactosidase activity to avoid variations caused by cell number and transfection efficiency.

In Vivo Pharmacologic Activity. In vivo androgenic and anabolic activities of C-6 in castrated and intact male rats. Forty-five male SD rats weighing approximately 200 g were purchased and randomly divided into nine groups with five rats per group. One day before the start of treatment, groups 1 through 8 were castrated via a scrotal incision under anesthesia. Group 9 served as an intact vehicle control. C-6 was dissolved in vehicle containing DMSO (5% v/v) in PEG 300. Groups 1 through 7 received daily subcutaneous injections of C-6 at a dose rate of 0.1, 0.3, 0.5, 0.75, 1, and 3 mg/kg/day, respectively. Groups 8 and 9 received vehicle. In another study, groups of intact male rats were treated with vehicle, testosterone propionate (TP), or C-6 at a dose rate of 0.5 mg/day. All rats were killed 14 days after the i.v. dose. Blood samples (−200 μl) were then withdrawn through the jugular vein catheter at 5, 10, 30, 60, 90, 120, 180, 360, 720, 1440, and 1800 min after the i.v. dose and at 20, 30, 60, 90, 120, 240, 360, 480, 720, 1080, 1440, and 1800 min after the p.o. dose. Blood samples were immediately centrifuged at 1000g for 10 min at 4°C. Plasma samples were prepared and stored at −20°C until HPLC analysis.

HPLC Methods. An aliquot (100 μl) of each plasma sample from the pharmacokinetic studies was spiked with 10 μl of an internal standard (a structural analog of C-6) and mixed well with 1 ml acetonitrile. After centrifugation at 16,000g for 10 min at 4°C, the supernatant was collected and evaporated. The residues were reconstituted in 120 μl of mobile phase. An aliquot of each sample was injected into a Nova-pak C18 column (3.9 × 150 mm, 4-μm particle size) purchased from Waters Corporation (Milford, MA). The HPLC system consisted of a model 515 HPLC pump (Waters), a model 717 plus autosampler (Waters), and a model 486 absorbance detector (Waters). HPLC separation was performed using an isocratic mobile phase (H2O:acetonitrile, 46:54 v/v) at a flow rate of 1 ml/min. The UV absorbance of eluents was monitored at 298 nm. Calibration standards were prepared in blank rat plasma with C-6 concentrations ranging from 0.1 to 50 μg/ml. The recoveries of this compound over the calibration range were from 93.4 to 100.4%. The intra- and interday coefficients of variation of the assay were lower than 2.9 and 8.9%, respectively. The limit of quantitation of the HPLC assay was 0.1 μg/ml.

Pharmacokinetic Data Analysis. The plasma concentration-time data were analyzed using noncompartmental methods and WinNonlin software (Pharsight Corporation). The terminal half-life (t1/2) was calculated as t1/2 = 0.693/λ, where λ is the terminal elimination constant. The area under the plasma concentration-time curve (AUCt-∞) was calculated using the trapezoidal method with extrapolation to time infinity. The plasma clearance (CL) was calculated as CL = dosei.v./AUCt-∞, where dosei.v. and AUCt-∞ are the intravenous dose and corresponding area under the plasma concentration-time curve from time 0 to infinity, respectively. The apparent volume of distribution at equilibrium (Vdss) was calculated as Vdss = CL · MRT, where MRT is the mean residence time following the intravenous bolus dose. The peak plasma concentration (Cmax) and time to reach the peak concentration (tmax) after a p.o. dose were obtained directly from the plasma concentration-time curves. Oral bioavailability (Fp.o.) was defined as Fp.o. = (AUC0-∞ · p.o. · dosei.v.)/(AUC0-∞ · i.v. · dosei.v.), where dosei.v. and AUC0-∞ · p.o. are the oral dose and corresponding area under the plasma concentration-time curve from time 0 to infinity after p.o. administration, respectively.

Statistical Analyses. All statistical analyses were performed using single-factor ANOVA, with the α value set a priori at p < 0.05.

Results

In Vitro Pharmacologic Activity. Previous studies demonstrated that structural modification(s) of the A-ring, link-
age, and/or the B-ring of nonsteroidal SARMs affected their in vitro and in vivo pharmacologic activity. Compared with bicalutamide, C-6 has a NO$_2$ at the para-position of the A-ring, an oxygen linkage, and a chloro and a fluoro substituent at the para- and meta-position of the B-ring, respectively (Fig. 1). The AR binding affinity of C-6 was determined using a competitive binding assay. DHT and C-6 displaced $[^3]$H MI B from AR binding sites (Fig. 2). The $K_i$ values of DHT and C-6 were 0.45 $\pm$ 0.2 and 4.9 $\pm$ 0.3 nM, respectively. Although the binding affinity of C-6 was less than one-tenth that of DHT, it bound the AR with 2-fold higher affinity than R-bicalutamide (i.e., $K_i$ bicalutamide $= 11.0$ nM) (Mukherjee et al., 1996) and 4- to 8-fold higher affinity than hydroxyflutamide (Furr et al., 1987; Teutsch et al., 1994), indicating that C-6 interacted to the AR with high binding affinity.

Upon binding to the AR, C-6 induced a concentration-dependent increase in AR-mediated transcriptional activation (Fig. 3A). The transcriptional activity induced by C-6 plateaued at concentrations equal to or greater than 10 nM ($\sim$100% of activity that was induced by 1 nM DHT). The activity in the vehicle-treated wells was less than 3% of the transcriptional activation induced by 1 nM DHT. C-6 did not significantly inhibit transcriptional activity induced by DHT (Fig. 3B) even at a concentration as high as 1000 nM. These results clearly demonstrated that C-6 binds to the AR with high affinity and potently stimulates AR-mediated transcriptional activity in vitro.

**In Vivo Pharmacologic Activity.** *In vivo androgenic and anabolic activities.* We then examined the in vivo androgenic and anabolic activities of C-6. In castrated male rats, the androgenic activity was evaluated by the ability of C-6 to maintain the weights of the ventral prostate and seminal vesicle, whereas the levator ani muscle weight was used to assess anabolic activity. Castration resulted in a significant reduction in the weights of the ventral prostate, seminal vesicle, and levator ani muscle to 8.5, 6.5, and 34% of that observed in intact animals, respectively. The administration of C-6 increased the weights of these tissues in a dose-dependent manner (Fig. 4A). At the highest dose rate (3 mg/day), the weights of all three organs were increased to about 130% of those observed in intact controls. Nonlinear regression analysis of the dose-response relationships showed that the ED$_{50}$ values of C-6 were 0.77 $\pm$ 0.06, 0.88 $\pm$ 0.1, and 0.17 $\pm$ 0.04 mg/day in the ventral prostate, seminal vesicle, and levator ani muscle, respectively (Fig. 4B). These results clearly revealed the potent and efficacious androgenic and anabolic activity of C-6 in male rats. The ED$_{50}$ value of C-6 in the levator ani muscle (0.17 $\pm$ 0.04 mg/day) was about 4- to 5-fold less than its ED$_{50}$ values in the prostate and seminal vesicle (0.77 $\pm$ 0.06 and 0.88 $\pm$ 0.14 mg/day, respectively). At a dose rate of 0.3 mg/day, C-6 maintained the weight of the levator ani muscle at a weight different from that observed in intact controls. The effect of C-6 in the ventral prostate and seminal vesicle was about 30% of that observed in intact controls at this dose rate. Results suggested that C-6 acts as a tissue-selective androgen with more potent anabolic activity than androgenic activity. The efficacy (maximum effect) of C-6 in anabolic and androgenic tissues was about the same.

We also determined the serum levels of LH and FSH in animals treated with C-6 and compared them to the levels of those hormones observed in intact and castrated control animals. As shown in Table 1, castration led to a significant elevation in LH (8.4 $\pm$ 2.5 ng/ml) and FSH (63.4 $\pm$ 5.0 ng/ml) levels due to the lack of negative feedback inhibition by testosterone. C-6 caused a dose-dependent decrease in LH and FSH levels and restored the levels of these hormones back to physiological levels at dose rates as low as 0.3 and 0.5 mg/day, respectively.

These data led us to immediately examine the pharmacologic activity of C-6 in intact male rats to answer an important question: would C-6 mimic the ability of exogenous testosterone to inhibit the synthesis of endogenous testosterone? We chose a dose rate of 0.5 mg/day for this study. This dose was the minimum dose required to fully suppress LH and FSH and yet maintain normal muscle mass in castrated rats. In a 14-day study (Fig. 5), TP stimulated growth in all three organs, whereas C-6 demonstrated tissue-selective pharmacologic effects in intact rats. The weights of the ventral prostate and seminal vesicles were significantly decreased to about 50% of those observed in intact controls, whereas the weights of the levator ani muscle remained the same as those observed in intact controls. Surprisingly, the pharmacologic effects of C-6 in intact rats were nearly identical to those found in castrated rats at this dose rate (0.5 mg/day) (Fig. 5). Based on this finding, we predicted that C-6 reduced serum testosterone concentration to levels similar to those observed in castrated rats. Results showed that the average testosterone concentration in the intact control group was 4.47 $\pm$ 1.66 ng/ml. Testosterone concentrations in three animals of the C-6-treated group were below the detection limit (0.04 ng/ml) of the assay, whereas testosterone concentrations in the two remaining animals were 0.11 and 0.46 ng/ml. Clearly, C-6 potently and rapidly inhibited endogenous testosterone synthesis in male rats.

**In vivo antireproductive activity.** In this pilot study, we evaluated the effects of C-6 on the reproductive system after long-term (1 mg/day, 10 weeks) treatment in adult male rats. Body and organ weights in control and C-6-treated rats are...
Fig. 3. AR-mediated transcriptional activity induced by DHT and C-6. CV-1 cells were transfected with a human AR plasmid, an androgen-responsive luciferase reporter plasmid, and a constitutively expressed β-galactosidase plasmid in a T-150 flask using LipofectAMINE. After transfection, cells were plated onto 24-well plates and allowed to recover for 12 h before drug treatment. Cells were then treated with vehicle or increasing concentrations of C-6 alone or together with 1 nM DHT for 24 h. Luciferase activity in each well was normalized with the β-galactosidase activity and then expressed as the percentage of that induced by 1 nM DHT. Each bar represents mean ± S.D. (n = 3).

Fig. 4. A, androgenic and anabolic activities of C-6 in castrated male rats. Castrated male rats received C-6 at a dose rate ranging from 0.05 to 3 mg/day by daily subcutaneous injection for 14 days. Organ weights were normalized with body weight and expressed as the percentage of the weights in the intact control group. Each bar represents the mean ± S.D. (n = 5/group). The letters I and C above each error bar represent a significant difference between the group and intact control group or castrated control group, respectively, as analyzed by single-factor ANOVA with p < 0.05. B, dose-response relationship of C-6 in castrated male rats. Dose-response curves, E_max, and ED_{50} in this figure were obtained by nonlinear least-square regression analysis.
TABLE 1
Effects of C-6 on LH and FSH in castrated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>&lt;0.2</td>
<td>11.2 ± 2.0</td>
</tr>
<tr>
<td>Castrated control</td>
<td>8.4 ± 2.5</td>
<td>63.4 ± 5.0</td>
</tr>
<tr>
<td>C-6, 0.1 mg/day</td>
<td>&lt;0.2</td>
<td>53.0 ± 5.1</td>
</tr>
<tr>
<td>C-6, 0.3 mg/day</td>
<td>&lt;0.2</td>
<td>26.6 ± 8.9</td>
</tr>
<tr>
<td>C-6, 0.5 mg/day</td>
<td>&lt;0.2</td>
<td>12.4 ± 1.1</td>
</tr>
<tr>
<td>C-6, 0.75 mg/day</td>
<td>&lt;0.2</td>
<td>12.8 ± 1.6</td>
</tr>
</tbody>
</table>

The average testosterone concentration in intact control animals was 1.06 ± 0.78 ng/ml. These concentrations are within the normal range of testosterone concentration reported from male rats with a similar age (Sun et al., 1989; Lue et al., 2000). Animals in C-6-treated group showed significantly suppressed testosterone levels that were lower than the quantitation limit (0.04 ng/ml) of the EIA kits. Clearly, the reduction of endogenous testosterone production was the main reason for the suppressed reproductive organ weights and sperm number in intact animals. Although a high dose rate (1 mg/day) of C-6 was used in this study, low activity was observed in the prostate and seminal vesicles due to the tissue selectivity of C-6. In conclusion, C-6 was able to mimic the effects of exogenous testosterone on spermatogenesis in male rat while maintaining its tissue selectivity after chronic treatment. Additional studies over a wider dose range are warranted to more fully describe the effects of C-6 on spermatogenesis and male reproductive activity.

Pharmacokinetics of C-6 in Male Rats. To develop a possible male contraceptive pill, high oral bioavailability and a suitable half-life of the compound are required. Therefore, the pharmacokinetics of C-6 were examined after i.v. and p.o. administration to male rats with a single dose of 10 mg/kg.

The time-courses of the changes in plasma concentrations of C-6 following i.v. and p.o. administration are shown in Fig. 7, and the pharmacokinetic parameters are listed in Table 3. Following intravenous administration, C-6 concentrations declined and remained detectable until 30 h after the dose. The terminal t1/2 of C-6 in male rats was 6.3 h. The systemic clearance (CL) and steady-state volume distribution (Vdss) were 0.72 ml/min · kg and 473 ml/kg, respectively. C-6 appeared rapidly (within 5 min) in the systemic circulation after p.o. administration, suggesting gastric absorption of the drug. Oral absorption was prolonged with maximum plasma concentrations forming a plateau over 6 to 12 h postdose. Plasma concentrations of C-6 diminished with a mean terminal t1/2 of 5.6 h. This terminal t1/2 was smaller than that observed after i.v. administration, an observation which was likely due to individual variation of plasma drug concentrations after p.o. administration; however, statistical analysis revealed no significant difference between these two values. The bioavailability of C-6 following oral administration was 76%.

Discussion

Earlier studies revealed that known nonsteroidal AR ligands (mainly bicalutamide derivatives) could be structurally modified to obtain a series of compounds with diverse in vitro (Yin et al., 2003b) and in vivo pharmacologic activities (Yin et al., 2003a). Subsequent research in our laboratories has focused on the discovery of novel SARMS as alternatives for testosterone replacement therapy and the treatment of muscle wasting diseases, osteoporosis, prostate hyperplasia, and hormonal male contraception. However, unlike testosterone, the SARMS that we have reported to date showed little to no effects on the hypothalamic-pituitary-testis axis, precluding their ability to regulate gonadotropins and serum testosterone levels or to serve as components of a male contraceptive regimen. Our previous studies showed that the aromatic B-ring is much more amenable to structural modification than other positions in the structure. We used this and other structure-activity relationships uncovered in our research to design and synthesize additional series of SARMS in the hopes of identifying compounds with enhanced central activity. Typically, these compounds were S-isomers with a structure that bears an NO2 and CF3 at the para- and meta-position of the A-ring, respectively, a CH3 linked to the chiral carbon, an oxygen linkage, and an electronegative substituent(s) in the aromatic B-ring. C-6 was one of these AR ligands.
notably, at a concentration of 10 nM, C-6 showed 2- to 3-fold higher transcriptional activation than S-1 (i.e., 118 versus 43% of the activity induced by 1 nM DHT). These findings were consistent with our prediction regarding the flexibility of structural modification in the B-ring. When C-6 was tested in the castrated male rat model, it produced more potent and efficacious in vivo pharmacologic activity than S-1. Although C-6 exhibited profound effects on all three androgen-dependent organs, it remained tissue-selective. The potency (i.e., \( ED_{50} \)) and efficacy (i.e., \( E_{\text{max}} \)) of C-6 and TP (Yin et al., 2003a) in the levator ani muscle were comparable, whereas the potency of C-6 in the prostate and seminal vesicles was 6- to 7-fold less than that of TP. Furthermore, the \( ED_{50} \) value for C-6 in the levator ani muscle was 0.17 mg/day, or about 5-fold lower than that in the prostate and seminal vesicles. Thus, in castrated rats, a dose of C-6 (i.e., 0.3 mg/day) that will fully maintain the weight of the levator ani muscle will only partially maintain the weight of the prostate and seminal vesicles. The enhanced pharmacologic activity of C-6 compared with our previously reported SARM (i.e., S-1) might have resulted from several factors, including binding affinity, transcriptional activity, drug disposition, metabolism rate, and/or nuclear retention time. During in vitro studies, C-6 showed higher transcriptional activity but similar binding affinity to S-1. The in vivo total body clearance of C-6 was 0.72 ml/min/kg, which was significantly smaller than that of S-1 (J. Chen, D. J. Hwang, C. E. Bohl, D. D. Miller, J. T. Dalton, and D. Wu, unpublished data). Therefore, the increased efficacy and potency of C-6 in rats may have been due to its higher intrinsic activity and greater in vivo exposure.

Due to the detection limit of the LH radioimmunoassay and limited sample volumes, LH concentrations in the high-dose drug-treated and intact groups were undetectable. It was clear, however, that the antigonadotropic activity of C-6 was dose-dependent and more potent than TP (Yin et al., 2003a). Others showed that testosterone and DHT inhibit LH secretion in response to luteinizing hormone-releasing hormone stimulation in rats (Debeljuk et al., 1974) in cultures of rat anterior pituitary fragments (Schally et al., 1973) and
anterior pituitary cells (Liang et al., 1984). Studies using 5α-reductase inhibitors revealed that the conversion of testosterone to DHT is not critical to the antigonadotropic activity of testosterone. This conclusion is further supported by our current studies since C-6 does not undergo 5α reduction.

It is well known that exogenous testosterone interferes with spermatogenesis indirectly by decreasing pituitary gonadotropin secretion via negative feedback on the hypothalamic-pituitary-testis axis in different species (Berndtson et al., 1974; Moger, 1976; Bansal and Davies, 1986; Matsumoto, 1990); however, the effects of testosterone on spermatogenesis were biphasic depending on the dosage of androgen administered (Walsh and Swerdloff, 1973; Ewing and Robaire, 1978). At low doses, androgen suppresses gonadotropin secretion, decreases testicular steroidogenesis, and then subsequently inhibits spermatogenesis. At higher androgen doses, endogenous testosterone levels remain suppressed, but the exogenous androgen is sufficient to directly support spermatogenesis. In our pilot antireproductive study, we demonstrated for the first time that the administration of a nonsteroidal androgen receptor modulator (C-6) to adult male rats resulted in marked suppression of spermatogenesis. C-6 treatment reduced peripheral testosterone concentrations to undetectable levels, significantly suppressed spermatogenesis, reduced the size of the testis and epididymis, and increased the weight of the levator ani muscle. The magnitude of antireproductive effects of C-6 could, like testosterone, be dose-dependent. Only one dose rate was examined in this pilot study. Although the androgenic activity of C-6 was much lower than testosterone and its esters, this dose of C-6 may still be sufficient to partially maintain spermatogenesis in rats. To date, our knowledge about the role of SARMs on the reproductive system is limited. Dose-response relationships of C-6 on spermatogenesis, fertility, and libido are presently being investigated in our laboratory to study the potential use of C-6 for hormonal male contraception.

In conclusion, C-6 is a novel SARM that was identified during studies to define structure-activity relationships for AR binding. C-6 bound the AR with high affinity and stimulated AR-mediated transcriptional activation to a similar extent as DHT. In vivo studies showed that C-6 demonstrated tissue-selective pharmacologic activity with more potent anabolic than androgenic activity. The high oral bioavailability and potent ability of C-6 to suppress gonadotropins, testosterone, and spermatogenesis suggest that C-6 is a promising candidate for hormonal male contraception either alone or in combination with a progestin.

References


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