

Pharmacokinetics and Pharmacodynamics of LGD-3303 [9-Chloro-2-ethyl-1-methyl-3-(2,2,2-trifluoroethyl)-3H-pyrrolo[3,2-f]quinolin-7(6H)-one], an Orally Available Nonsteroidal-Selective Androgen Receptor Modulator

Eric G. Vajda, Francisco J. López, Peter Rix, Robert Hill, Yanling Chen, Kyoung-Jin Lee, Z. O'Brien, William Y. Chang, Martin D. Meglasson, and Yong-Hee Lee

Discovery Research, Ligand Pharmaceuticals, Inc., San Diego, California

Received September 29, 2008; accepted November 17, 2008

ABSTRACT

Selective androgen receptor modulators (SARMs) are a new class of molecules in development to treat a variety of diseases. SARMs maintain the beneficial effects of androgens, including increased muscle mass and bone density, while having reduced activity on unwanted side effects. The mechanisms responsible for the tissue-selective activity of SARMs are not fully understood, and the pharmacokinetic (PK)/pharmacodynamic (PD) relationships are poorly described. Tissue-specific compound distribution potentially could be a mechanism responsible for apparent tissue selectivity. We examined the PK/PD relationship of a novel SARM, LGD-3303 [9-chloro-2-ethyl-1-methyl-3-(2,2,2-trifluoroethyl)-3H-pyrrolo[3,2-f]quinolin-7(6H)-one], in a castrated rat model of androgen deficiency. LGD-

3303 has potent activity on levator ani muscle but is a partial agonist on the preputial gland and ventral prostate. LGD-3303 never stimulated ventral prostate above intact levels despite increasing plasma concentrations of compound. Tissue-selective activity was maintained when LGD-3303 was dosed orally or by continuous infusion, two routes of administration with markedly different time versus exposure profiles. Despite the greater muscle activity relative to prostate activity, local tissue concentrations of LGD-3303 were higher in the prostate than in the levator ani muscle. LGD-3303 has SARM properties that are independent of its pharmacokinetic profile, suggesting that the principle mechanism for tissue-selective activity is the result of altered molecular interactions at the level of the androgen receptor.

Androgens play a vital role in sexual dimorphism, reproduction, and maintenance of the male phenotype. Androgen supplementation increases bone density, lean body mass, and libido in men and women (Behre et al., 1997; Flicker et al., 1997; Snyder et al., 2000; Wang et al., 2000; Davis et al., 2006). However, widespread clinical use of androgens has been limited because of potential safety concerns (Rhoden and Morgentaler, 2004). In men, stimulation of the prostate is a significant concern, whereas virilization is a potential side effect in women. Liver toxicity is also a concern, although this is primarily an issue with 17 α -alkylation of steroidal androgens. Steroidal androgens without this chemical modification have reduced impact on liver enzymes or serum

lipid levels but must be administered parenterally or topically (Snyder et al., 2000; Wang et al., 2000).

Selective androgen receptor modulators (SARMs) are androgen receptor (AR) ligands that have tissue-specific effects (Negro-Vilar, 1999). The ideal SARM would be orally available without stimulatory effects in prostate and skin while maintaining the beneficial effects of androgen treatment. Multiple SARMs have been described, but the precise mechanism responsible for their tissue-selective activity is unknown (Yin et al., 2003; Martinborough et al., 2007; Ostrowski et al., 2007; Page et al., 2008; Piu et al., 2008). It has been postulated that the selectivity is the result of altered cofactor recruitment. Like other nuclear receptors, AR dimerizes after ligand binding, binds to DNA, and recruits cofactor proteins to initiate transcription. In theory, SARMs place AR in a conformation that is sufficiently different from the native ligands that assembly of the transcriptional complex is

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.
doi:10.1124/jpet.108.146811.

ABBREVIATIONS: SARM, selective androgen receptor modulator; AR, androgen receptor; DHT, dihydrotestosterone; LGD-3303, 9-chloro-2-ethyl-1-methyl-3-(2,2,2-trifluoroethyl)-3H-pyrrolo[3,2-f]quinolin-7(6H)-one; ORDX, orchidectomized; LH, luteinizing hormone; FITC, fluorescein isothiocyanate; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; LC, liquid chromatography; MS, mass spectrometry; AUC, area under the plasma concentration curve; ANOVA, analysis of variance; LGD2226, 6-[bis-(2,2,2-trifluoroethyl)amino]-4-trifluoromethyl-1H-quinolin-2-one.

altered in a tissue-specific manner. Some evidence exists to support the cofactor hypothesis. Testosterone and dihydrotestosterone (DHT) induce very subtle differences in AR conformation, yet this alters biological activity. Testosterone induces a weaker interaction than DHT between AR and LXXLL coactivator motifs, accounting for a faster dissociation constant (Askew et al., 2007). Although X-ray crystal structures of SARMs bound to AR reveal conformations quite similar to DHT, subtle differences may affect recruitment of LXXLL motifs and other cofactors that can vary among tissues (Bohl et al., 2005; Ostrowski et al., 2007). One SARM has been reported to alter AR interaction with GRIP1 in comparison with DHT (Miner et al., 2007). The precise cofactors responsible for tissue-selective activity have not been identified.

Plausible, alternative explanations for the tissue selectivity of SARMs exist. Tissue selectivity might be because of differences in tissue distribution of the compounds. For example, relatively greater accumulation of compound within muscle compared with prostate would appear as enhanced muscle activity. The prostate gland is known to contain enzymes involved in drug metabolism and energy-dependent drug transporters. Phase I cytochrome P450 drug-metabolizing enzymes and phase II drug-metabolizing enzymes such as UDP-glucuronosyltransferases are present in the prostate (Obligacion et al., 2006; Barbier and Bélanger, 2008). The multidrug resistance-associated proteins (MRP) 1–4 and the multiple drug resistance protein (MDR-1) are also expressed in the prostate. It is possible that through metabolic inactivation or active transport, the concentration of a SARM in the prostate might differ appreciably from its systemic exposure. It has been postulated that the lack of tissue-selective activity observed with testosterone is the result of high prostate levels of 5 α -reductase, the enzyme that converts testosterone to the more potent metabolite DHT (Gao and Dalton, 2007).

Some steroid hormones, including testosterone, are secreted in a pulsatile manner, which suggests that the duration or timing of receptor stimulation may play an important role in determining their biological responses (Veldhuis et al., 2000; Koch et al., 2006). Cyclic patterns of gene transcription have been described for nuclear receptors in response to cognate agonists (Munck and Holbrook, 1984; Aiyar et al., 2004; Lightman et al., 2008). These patterns of cyclical transcription are postulated to occur because of depletion of coactivators, recruitment of corepressors, and ligand-induced receptor turnover or receptor stabilization. AR demonstrates stabilization of the AR-DNA binding complex in response to agonists, and it is conceivable that there may be a time component involved in the biological response (Black and Paschal, 2004; Farla et al., 2004). Subtle variations in the temporal component might lead to altered tissue sensitivity in comparison with the pulsatile secretion of testosterone under normal physiological conditions. These variations might occur in a tissue-specific manner.

There are relatively few publications with correlated pharmacokinetics and pharmacodynamics of SARMs across a range of doses (for review, see Gao et al., 2006). The pharmacological activity has been described for several chemical series, but pharmacokinetic data have not been published (Hanada et al., 2003; Martinborough et al., 2007; Miner et al., 2007; Ostrowski et al., 2007; Diel et al., 2008; Piu et al.,

2008). More extensive pharmacokinetic data have been published for the aryl propionamide analogs over a limited number of oral and intravenous doses (Kearbey et al., 2004; Chen et al., 2005; Kim et al., 2005). However, pharmacodynamic data were collected in separate studies using a subcutaneously administered compound (Yin et al., 2003; Chen et al., 2005; Kim et al., 2005). Thus, it remains possible that pharmacokinetics and tissue distribution of compound could vary with dose administered and route of administration and play a role in the observed tissue-selective pharmacodynamics. We have discovered a nonsteroidal SARM, LGD-3303, with potent activity on muscle but greatly reduced partial agonist activity on the prostate (Vajda et al., 2008). We evaluated the role of systemic and tissue pharmacokinetics in the selectivity of this SARM in castrated male rats. We report the effects of different exposure profiles on pharmacological activity and the tissue distribution of the compound in relation to its tissue-selective activity.

Materials and Methods

Compounds. LGD-3303 is a nonsteroidal androgen receptor agonist that binds the androgen receptor with high potency and activates gene transcription (Fig. 1). LGD-3303 has minimal binding or transcriptional activity on related nuclear receptors (Vajda et al., 2008). LGD-3303 was synthesized at Ligand Pharmaceuticals (San Diego, CA).

Animal Model. Male Sprague-Dawley rats (7–8 weeks old, 200 g) were purchased from Harlan (Indianapolis, IN) and housed two or three rats per cage. A 12-h light/dark cycle was maintained throughout the course of the experiment with lights on at 6:00 AM. Animals were acclimated for a minimum of 3 days before performing any experimental procedures. Animals were fed standard laboratory rodent chow and water ad libitum. The diet contained 1.36% calcium, 1.01% phosphorus, and 2.40 IU/g vitamin D. Rats were anesthetized by isoflurane anesthesia and sham-operated or orchidectomized (ORDX) through a scrotal incision. In all experiments, rats were treated for 14 days. All procedures involving animals were approved by Ligand's Institutional Animal Care and Utilization Committee, and the animals were maintained in accordance with the *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

Oral High-Dose Experiment. Rats were sorted by weight, assigned to experimental groups ($n = 5/\text{group}$), and surgery was performed as described above. Experimental groups consisted of LGD-3303 (doses ranged from 0.1–300 mg/kg/day) or vehicle. LGD-3303 was administered in a suspension of Tween 80, polyethylene glycol-400, and 0.1% carboxy-methyl cellulose in water (0.005:10:89.995%) by once daily oral gavage in a volume of 4 ml/kg. On the 14th day, blood was collected into lithium heparin tubes (Becton Dickinson, Franklin Lakes, NJ) by jugular puncture at 0, 0.5, 1, 2, 4, and 6 h postdosing from the animals in the high-dose groups. Blood was centrifuged, and plasma was stored at -20°C for pharmacokinetic analysis. On the 15th day, rats were killed by decapitation, and trunk blood was collected, allowed to clot in serum separator tubes (BD Biosciences, San Jose, CA), and centrifuged, and serum was

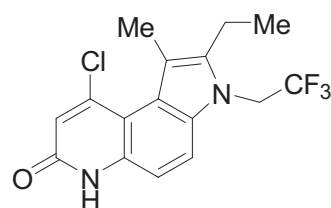


Fig. 1. Chemical structure of LGD-3303.

stored at -80°C for future analysis of serum luteinizing hormone (LH) levels. The wet weights of the ventral prostate, levator ani muscle, and preputial gland were measured at necropsy.

Oral and Infusion Dosing. Rats were sorted by weight, assigned to experimental groups ($n = 5/\text{group}$), and surgery was performed as described above. Experimental groups consisted of vehicle, orally dosed LGD-3303 (0.3–100 mg/kg/day), or constant infusion of LGD-3303 via an osmotic minipump (Alzet model 2ML1; Alzet, Cupertino, CA; doses ranged from 0.01–10 mg/kg/day). Minipumps were surgically implanted in the intrascapular subcutaneous tissue while the rats were under anesthesia for ORDX. After 7 days, rats were anesthetized, and the minipumps were surgically removed and replaced with new minipumps. LGD-3303 was formulated for oral administration as described above and formulated for minipump administration in 50% polyethylene glycol-400:50% dimethyl sulfoxide. On the 14th day of the experiment, blood was collected into lithium heparin tubes (Becton Dickinson) by jugular puncture at 0, 0.5, 1, 2, 4, 8, 12, and 24 h postdosing. Blood was centrifuged, and plasma concentration of LGD-3303 was measured. Rats were killed on the morning of the 15th day, and the wet weights of the ventral prostate and levator ani muscle were measured.

Tissue Distribution of LGD-3303. Rats were sorted by weight, assigned to experimental groups ($n = 3/\text{group}$), and surgery was performed as described above. Rats received either LGD-3303 (30 mg/kg/day) or vehicle by once daily oral gavage. On the 14th day of dosing, rats were killed at 2, 4, or 8 h postdosing by cardiac exsanguination under isoflurane anesthesia. Thirty minutes before necropsy, rats received an intravenous injection of 10 mg/ml high-molecular mass dextran labeled with fluorescein isothiocyanate (FITC-dextran, 150 kDa; Sigma-Aldrich, St. Louis, MO) at a volume of 2 ml/kg body weight. Extravasation of large-molecular mass FITC-dextran is slow, and it serves as a marker of blood volume for acute studies.

Luteinizing Hormone Immunoassay. Serum samples collected at necropsy were assayed for LH with a double antiserum procedure using reagents from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; National Hormone and Peptide Pro-

gram, Dr. Parlow, Torrance, CA). In brief, samples and standards (NIDDK-rLH-RP-3) in a total volume of 200 μl were incubated at room temperature for 2 to 3 days with 100 μl of primary antiserum (rabbit NIDDK-anti-rLH-S-11) diluted 1:100,000. Thereafter, 100 μl of ^{125}I -labeled LH (MP Biomedicals, Irvine, CA) diluted to 200,000 to 300,000 cpm/ml was added to the tubes and incubation continued for an additional 24-h period. Bound hormone was separated from free hormone by precipitation with a specific goat anti-rabbit serum (Antibodies Inc., Davis, CA). For this purpose, 50 μl of 4% normal rabbit serum was added to each incubation tube, after which an additional 50 μl of a 1:10 goat anti-rabbit serum solution was added. The tubes were vortexed and incubated overnight at 4°C . The assay was terminated by centrifugation, the supernatants were decanted and discarded, and the pellets were counted in a 10-channel gamma counter. The assay has a minimal detectable amount of 0.001 ng/tube, and the intra- and interassay variability is less than 10%. To eliminate interassay variability, all samples from a single study were run in the same assay.

Quantitation of LGD-3303 in Plasma and Tissue Samples.

For the determination of plasma concentration, the standard solution of LGD-3303 was spiked in blank rat plasma; calibration standards were constructed from 0.001 to 10 $\mu\text{g}/\text{ml}$; and 50 μl of calibration standard and 50 μl of plasma samples were extracted by the protein precipitation method using 250 μl of acetonitrile containing LGD-2226 as an internal standard (Miner et al., 2007). After centrifugation, the supernatant was analyzed for LGD-3303 via liquid chromatography (LC) followed by tandem mass spectrometry. For the determination of tissue concentration, the standard solution of LGD-3303 was spiked in blank rat tissue with 2:1 ratio (v/w) of standard solution and tissue. The calibration standards (from 0.01–5 $\mu\text{g}/\text{ml}$) were constructed in an extraction solution [acetonitrile/water = 70:30 (v/v)], containing LGD-2226 as an internal standard. Tissue samples were extracted with 2 volumes of extraction solution containing internal standard. After overnight extraction, the supernatant was analyzed for LGD-3303 via LC-tandem mass spectrometry. Analysis was done via electrospray ionization-mass spectrometry (MS) utilizing Applied Biosystems API 4000 or API 4000/Q-trap

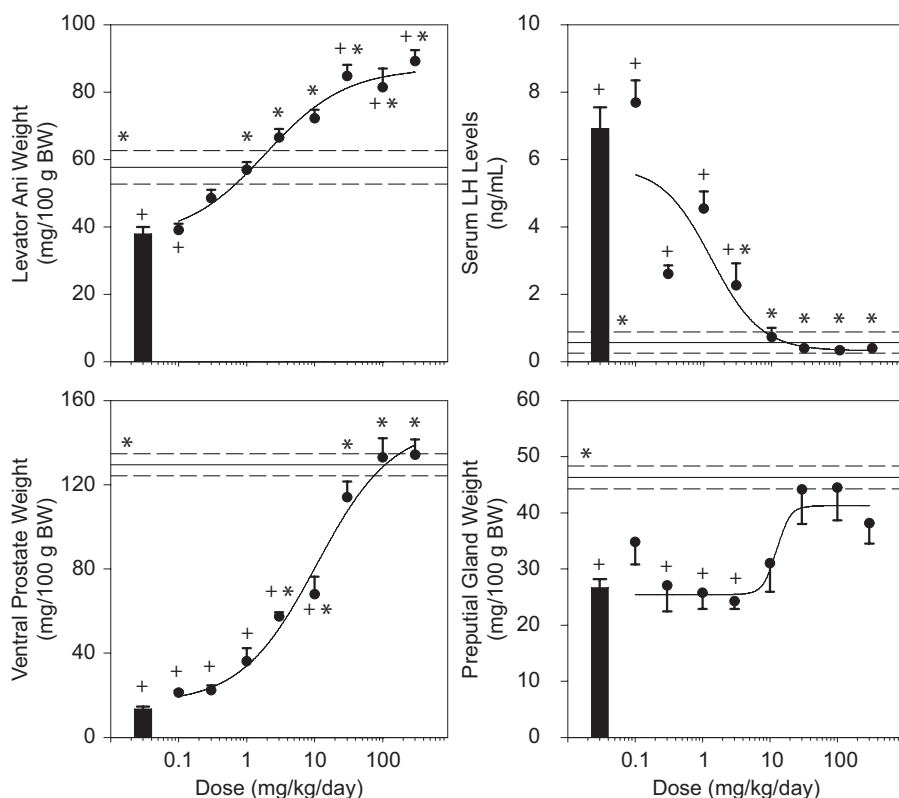


Fig. 2. Pharmacological effects of LGD-3303 in ORDX male rats treated for 14 days by oral gavage. Castration significantly reduces levator ani muscle weight, ventral prostate weight, and preputial gland weight but elevates serum LH. LGD-3303 inhibits the effects of ORDX on levator ani weight at a dose of 1 mg/kg/day and significantly increases muscle weight above the eugonadal level at higher doses. LGD-3303 suppresses serum LH to eugonadal levels. LGD-3303 is less potent on the ventral prostate, maintaining the ventral prostate at eugonadal levels with a dose of 100 mg/kg/day or greater. Black bar, ORDX controls; solid horizontal line, sham-operated controls \pm S.E.M. (dashed horizontal lines). *, $p < 0.05$ versus ORDX controls; +, $p < 0.05$ versus sham-operated intact controls by one-way ANOVA.

TABLE 1

Mean (\pm S.E.M.) exposure of LGD-3303 from the oral high-dose experiment

Dose	AUC ₀₋₆
	$\mu\text{g} \cdot \text{h}/\text{ml}$
10 mg/kg/day	0.15 ± 0.05
30 mg/kg/day	0.24 ± 0.10
100 mg/kg/day	0.56 ± 0.08
300 mg/kg/day	0.85 ± 0.07

(Applied Biosystems, Foster City, CA). Samples were analyzed by injecting 10 to 20 μl of the supernatant into the LC/MS instrument. The LC instrument was fitted with a C8 Octyl, MOS guard column (4 \times 2 mm; Phenomenex, Torrance, CA) and a Gemini C6-Phenyl chromatographic column (50 \times 2.0 mm, 5 μm ; Phenomenex). The mobile phases A and B were 0.1% formic acid in H₂O and 0.1% formic acid in acetonitrile, respectively. A multiple-reaction monitoring scan of 343.1/245.3 atomic mass units was used to monitor LGD-3303 in a positive mode.

Quantitation of FITC-Dextran in Serum and Tissue Samples. An aliquot of serum was diluted 1:10 in water. One hundred microliters of the diluted serum was pipetted into a microtiter plate and analyzed in a fluorescence plate reader (Wallac Victor 1420; PerkinElmer Life and Analytical Sciences, Waltham, MA) to determine the FITC-dextran concentration. Blank serum was spiked with FITC-dextran and serially diluted (with blank serum/water, 10:90) to yield a calibration curve. Calibration standards were analyzed on the same microtiter plate as the samples to reduce variability. Serum was protected from light exposure throughout the experimental procedure.

FITC-dextran concentration in the tissue was measured after quantification of LGD-3303 concentration as described above. The remaining tissue and extraction solution were homogenized and centrifuged. One hundred microliters of supernatant solution (acetonitrile/water = 70:30, v/v) was pipetted into a 96-well microtiter plate. Blank prostate and levator ani extracts were spiked with FITC-dextran and serially diluted to yield calibration standards. Individual calibration curves were established for each tissue. Calibration standards were analyzed within the same microtiter plate as tissue samples to reduce variability. Tissue was protected from light exposure throughout the experimental procedure. Serum FITC-dextran concentration and local tissue FITC-dextran concentration were

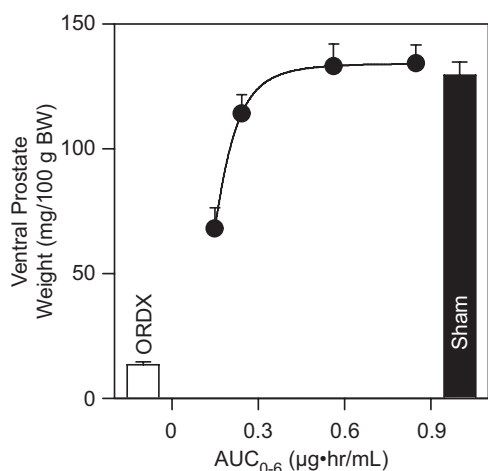


Fig. 3. Pharmacokinetic analysis of plasma samples from ORDX male rats treated for 14 days with LGD-3303 by oral gavage. Exposure to LGD-3303 (AUC₀₋₆) increased with dose from 10 to 300 mg/kg/day in ORDX male rats after 14 days of dosing via oral gavage. Despite the increase in exposure, ventral prostate weight never exceeded the levels of sham-operated controls and reached an apparent plateau in pharmacological response.

used to estimate residual blood volume within the tissues after cardiac exsanguination. Tissue concentrations of LGD-3303 measured as described above were corrected for residual blood volume.

Pharmacokinetic Analysis. Plasma concentration-time data for each animal were analyzed using WinNonlin (version 5.0; Pharsight, Mountain View, CA) by noncompartmental pharmacokinetic methods (Gibaldi and Perrier, 1982). The highest observed concentration and the corresponding sampling time were defined as C_{max} and t_{max} , respectively. The area under the plasma concentration time curve (AUC) was calculated by the trapezoidal method, and either AUC₀₋₆ or AUC₀₋₂₄ was used in this study. The elimination half-life ($t_{1/2}$) was estimated from $t_{1/2} = \ln 2/\lambda$, where λ is the slope of the regression line

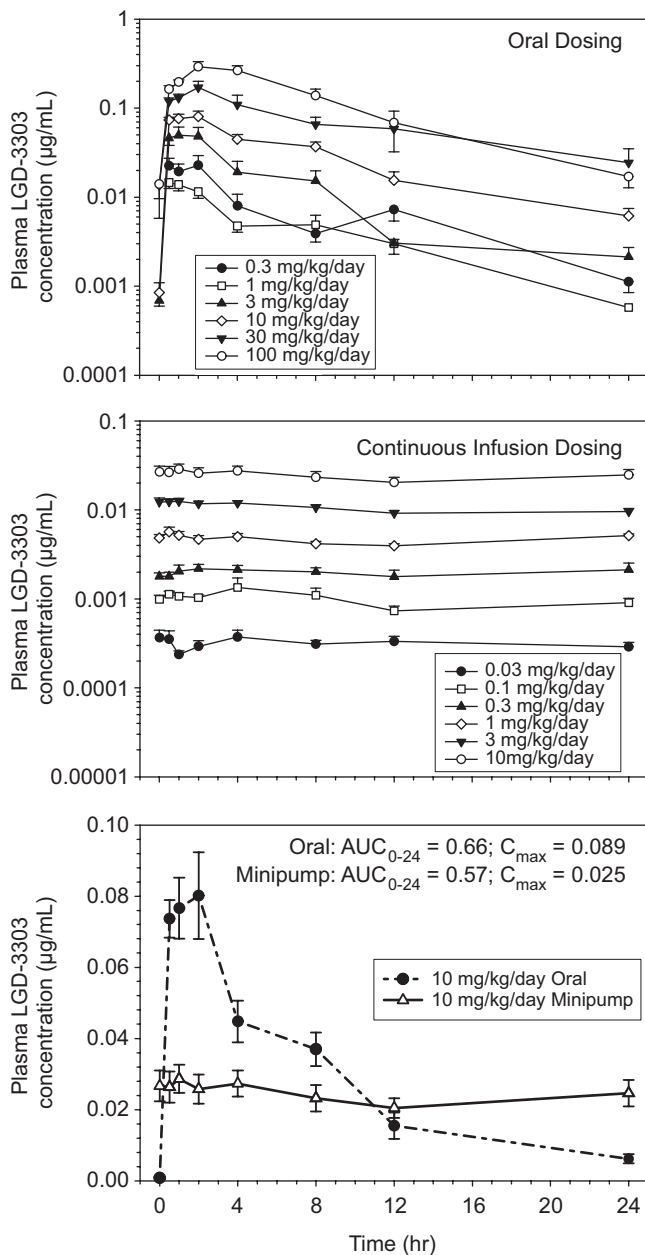


Fig. 4. Plasma exposure profile of LGD-3303 in a comparative study of oral gavage versus subcutaneous continuous infusion dosing in ORDX male rats. The exposure profile is markedly different after dosing by oral gavage (circle) or continuous infusion via osmotic minipump (triangle). Plasma was collected by jugular puncture at 0, 0.5, 1, 2, 4, 8, 12, and 24 h postdosing on the 14th day of dosing. LGD-3303 was extracted from plasma with acetonitrile and analyzed by liquid chromatography with tandem mass spectrometry.

that best fits the terminal portion of the log-linear concentration time curve.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test. When necessary, transformations were performed to ensure that variances were homogeneous among groups and that the residuals of the one-way analysis of variance model followed a Gaussian (normal) distribution (Box and Cox, 1964; Box and Hill, 1974). For the tissue distribution study, concentrations of LGD-3303 in the ventral prostate and levator ani were compared by two-tailed paired Student's *t* test. Statistical analysis was performed using commercially available software (JMP, SAS Institute, Cary, NC; Microsoft Excel, Microsoft, Redmond, WA). A *p* value less than 0.05 was considered statistically significant. When possible, data were fitted to a modified four-parameter logistic equation (Ghosh et al., 1998). All data are expressed as mean \pm S.E.M.

Results

Androgen deprivation (ORDX) significantly increased serum LH levels and reduced ventral prostate weight, preputial gland weight, and levator ani muscle weight in male rats. LGD-3303 completely inhibited the loss of muscle weight with an oral dose of 1 mg/kg/day (Fig. 2). At higher doses, LGD-3303 significantly increased levator ani muscle weight above eugonadal levels. In contrast, LGD-3303 had greatly reduced potency and efficacy on the other measured endpoints. LGD-3303 did not maintain eugonadal levels of serum LH at doses less than 10 mg/kg/day. LGD-3303 maintained eugonadal prostate weight only at doses of 100 mg/kg/day or greater and never fully returned the mean preputial gland weight to eugonadal levels at any tested dose (Fig. 2). In no case did LGD-3303 restore LH, prostate, or preputial gland weights to supraphysiological levels significantly exceeding sham-operated controls. The ventral prostate, in particular, demonstrated a greatly reduced response to LGD-3303. At the muscle normalizing dose (1 mg/kg/day), ventral prostate weight was not significantly increased above the level of ORDX control rats (20% efficacy relative to intact rats). At the highest doses tested, ventral prostate never significantly exceeded eugonadal levels and reached an apparent plateau with minimal increase in prostate weight as dosing escalated from 30 to 300 mg/kg/day. To investigate this apparent plateau in pharmacological activity, we analyzed plasma concentrations of LGD-3303 from the highest dose groups. Exposure to LGD-3303 (AUC_{0-6}) monotonically increased with

dose from 10 to 300 mg/kg/day (Table 1), although the increases were less than dose proportional. Despite the increase in plasma exposure, ventral prostate weight did not continue to increase above eugonadal levels (Fig. 3).

Many previous studies with androgens have been performed with subcutaneously injected compounds or compounds administered in a sustained release formulation. To determine whether the tissue-selective activity we observed was related to a different exposure profile and variations in tissue uptake after oral dosing, we examined the role of pharmacokinetic exposure profile on pharmacological activity. We repeated the oral dosing experiment over a slightly narrower dose range and included a subcutaneously dosed arm within the same experiment. Oral dosing led to a rapid increase in plasma exposure levels, with a t_{max} near 2 h and a C_{max} increasing with dose administered (Fig. 4; Table 2). AUC_{0-24} similarly increased with dose administered. The elimination phase ($t_{1/2}$) was relatively consistent across dosed groups (Table 2). Subcutaneous dosing via osmotic minipump yielded a much different profile. After minipump dosing, plasma concentrations were constant throughout the 24-h period, and plasma concentration increased with dose administered (Fig. 4; Table 2). When equivalent doses were compared between the two dosing routes, a clear and distinct difference could be observed. Oral dosing led to a higher C_{max} (approximately 3-fold greater) than minipump dosing, yet AUC_{0-24} was comparable by both routes at doses from 1 to 10 mg/kg/day (Fig. 4).

Despite the difference in exposure profile, the tissue-selective activity of LGD-3303 was unaffected by route of administration. By both routes, LGD-3303 significantly increased levator ani muscle weight above eugonadal levels at high doses while not stimulating ventral prostate weight above eugonadal levels (Fig. 5). At the dose and AUC_{0-24} that restored levator ani to eugonadal levels, ventral prostate was maintained at less than 50% of intact controls by both routes of administration. The one major effect of subcutaneous constant infusion dosing was to increase the potency of the compound. Continuous infusion maintained levator ani and ventral prostate at eugonadal levels with exposures (AUC_{0-24}) of approximately 0.065 and 0.56 $\mu\text{g} \cdot \text{h}/\text{ml}$, respectively. In contrast, exposures of approximately 0.20 and 2.4 $\mu\text{g} \cdot \text{h}/\text{ml}$ were required to maintain eugonadal levels after oral dosing.

We also investigated the local tissue concentration of LGD-

TABLE 2

Mean (\pm S.E.M.) pharmacokinetic parameters of LGD-3303 from animals on day 14 ($n = 5/\text{group}$)

Dose	AUC_{0-24} $\mu\text{g} \cdot \text{h}/\text{ml}$	C_{max} $\mu\text{g}/\text{ml}$	t_{max} <i>h</i>	$t_{1/2}$
Oral dosing				
0.3 mg/kg/day	0.162 \pm 0.023	0.025 \pm 0.006	0.9 \pm 0.3	5.2 \pm 0.8
1 mg/kg/day	0.093 \pm 0.002	0.016 \pm 0.002	0.7 \pm 0.1	5.8 \pm 1.0
3 mg/kg/day	0.283 \pm 0.030	0.055 \pm 0.010	2.5 \pm 1.4	4.3 \pm 1.1
10 mg/kg/day	0.658 \pm 0.014	0.089 \pm 0.010	1.0 \pm 0.3	6.3 \pm 0.7
30 mg/kg/day	1.62 \pm 0.44	0.173 \pm 0.028	1.6 \pm 0.2	8.7 \pm 2.0
100 mg/kg/day	2.67 \pm 0.30	0.317 \pm 0.038	2.8 \pm 0.5	5.0 \pm 0.6
Continuous infusion dosing				
0.03 mg/kg/day	0.008 \pm 0.001	0.0003 \pm 0.0000	N.A.	N.A.
0.1 mg/kg/day	0.023 \pm 0.003	0.0010 \pm 0.0001	N.A.	N.A.
0.3 mg/kg/day	0.047 \pm 0.06	0.0020 \pm 0.0002	N.A.	N.A.
1 mg/kg/day	0.109 \pm 0.06	0.0048 \pm 0.0004	N.A.	N.A.
3 mg/kg/day	0.246 \pm 0.016	0.011 \pm 0.001	N.A.	N.A.
10 mg/kg/day	0.566 \pm 0.081	0.025 \pm 0.004	N.A.	N.A.

N.A., not applicable.

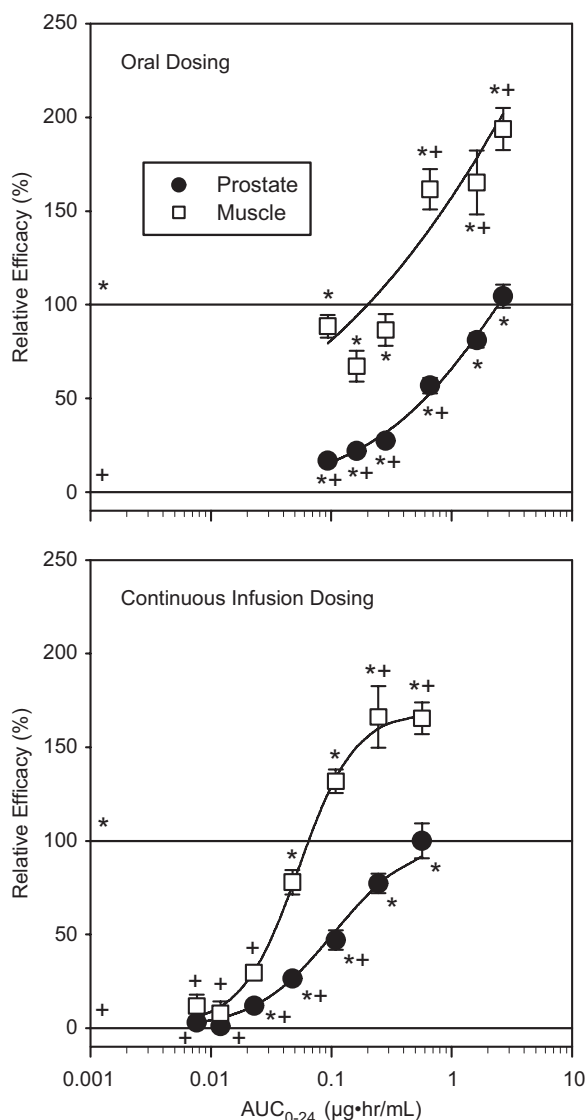


Fig. 5. Tissue-selective activity of LGD-3303 in ORDX male rats in a comparative study of oral gavage versus subcutaneous continuous infusion dosing. LGD-3303 was administered for 14 days by once daily oral gavage or by continuous infusion via a subcutaneously implanted osmotic minipump. Ventral prostate and levator ani muscle weight were measured at the end of the experiment. The route of administration did not alter tissue-selective activity of LGD-3303. Data are normalized relative to sham-operated intact control rats and ORDX control rats receiving vehicle. The organ weights of ORDX rats were defined to be 0% efficacy, and the organ weights of sham-operated rats were defined to be 100% efficacy. Organ weight data for LGD-3303-treated animals were scaled relative to the sham and ORDX controls allowing for direct comparisons of different organs and dosing regimens. *, $p < 0.05$ versus ORDX controls; +, $p < 0.05$ versus sham-operated intact controls by one-way ANOVA.

3303 to determine whether differential drug distribution played a role in the tissue-selective pharmacology. Consistent with our earlier experiments, an oral dose of 30 mg/kg/day significantly increase levator ani above eugonadal levels but maintained the ventral prostate at only 53% of eugonadal levels (Fig. 6). For ventral prostate and levator ani, local tissue concentrations of LGD-3303 increased slightly from 2 to 4 h and substantially declined by 8 h, a pattern similar to the serum concentration. At all time points, local concentration of LGD-3303 was greater in the ventral prostate than

the levator ani (Fig. 6). The difference was statistically significant at the 2-h time point.

Discussion

LGD-3303 is a tissue-selective androgen receptor agonist that stimulates muscle anabolism to supraphysiological levels. LGD-3303 has greatly reduced activity on the prostate and reaches a pharmacological plateau, never increasing prostate weight above eugonadal levels. In rats with established hypogonadism, the tissue-selective effects are even more pronounced as LGD-3303 failed to restore ventral prostate to more than 50% of eugonadal levels at doses that had robust effects on muscle and bone (Vajda et al., 2008). This is in strong contrast to testosterone, which hyperstimulates both prostate and muscle in castrate rats with similar potency whether administered at the time of ORDX (Martinborough et al., 2007; Miner et al., 2007) or after androgen deprivation and hypogonadism has been established (Ostrowski et al., 2007; Vajda et al., 2008). The plateau in prostate activity is unique among androgens and demonstrates a true partial agonist activity. Other SARMs have varying degrees of tissue-selective activity, but some increase prostate weight above eugonadal levels at high doses (Chen et al., 2005; Ostrowski et al., 2007), whereas others have been evaluated over a very narrow dose range, making it difficult to assess the partial agonist activity (Diel et al., 2008; Page et al., 2008; Piu et al., 2008). Pharmacokinetic data from these studies have not been published, making it difficult to confirm that partial agonist activity is not an artifact of saturated compound exposure. Pharmacokinetic data that are available were obtained in separate experiments that used different dosing routes (Kearbey et al., 2004; Chen et al., 2005; Kim et al., 2005). One of the strengths of this study is the broad dose range, characterizing the effects of LGD-3303 over a 3-log interval. Throughout the entire dose range, LGD-3303 maintains greater efficacy on muscle rather than prostate. We have characterized the pharmacokinetics of LGD-3303 at high doses and observed that the plateau in prostate activity cannot be the result of saturated exposure because concentrations of LGD-3303 in the systemic circulation continued to increase through 300 mg/kg/day. In addition, these partial agonist effects occur at doses that significantly increase muscle weight above intact levels. This is an important observation because previous studies have claimed partial agonist activity of SARMs without displaying a clear plateau in pharmacological activity and have not examined the compound exposure within the experimental animals.

Studies demonstrating a lack of tissue selectivity by testosterone have been performed by subcutaneous administration using either daily injection (Miner et al., 2007) or constant infusion (Yin et al., 2003), both of which can lead to different pharmacokinetic profiles and alterations in the endogenous circadian rhythm of testosterone. To investigate the potential role of pharmacokinetics and diurnal variations on tissue selectivity of LGD-3303, we administered LGD-3303 by two different routes, once daily oral gavage or sustained release in a minipump. The oral route produced a much higher C_{max} but a similar AUC, indicating that the majority of the compound exposure occurred at the beginning of the day with compound exposure steadily decreasing

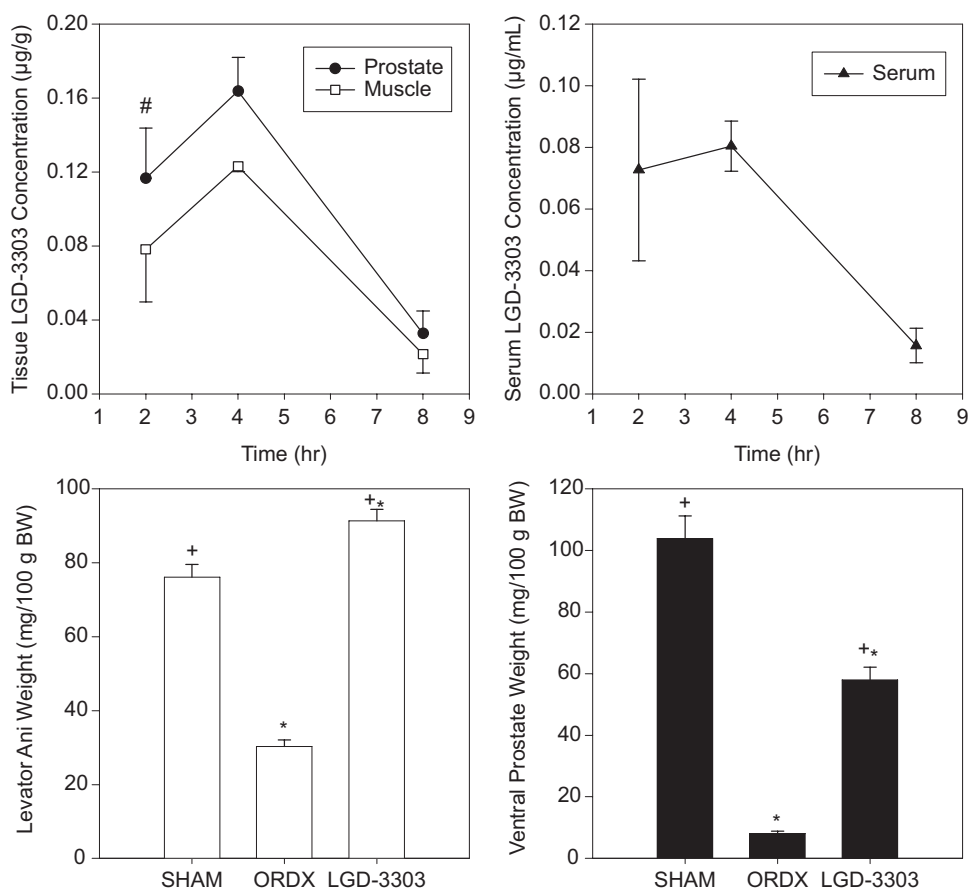


Fig. 6. Concentration of LGD-3303 in the ventral prostate and levator ani muscle after 14 days of dosing via oral gavage in ORDX male rats. Separate groups of rats ($n = 3$) were killed 2, 4, or 8 h postdosing, and levator ani and ventral prostate were collected. Concentration of LGD-3303 was determined by LC/tandem mass spectrometry after acetonitrile extraction. LGD-3303 concentration was higher in the ventral prostate than the levator ani muscle despite a greater effect of LGD-3303 on muscle weight than ventral prostate weight. *, $p < 0.05$ versus ORDX controls; +, $p < 0.05$ versus sham-operated intact controls by one-way ANOVA. #, $p < 0.05$ prostate versus levator ani tissue concentration by paired Student's t test.

through the remainder of the dosing interval. In contrast, minipump dosing maintained lower peak concentrations but steady exposure throughout the day. The altered dosing regimen did not have any effect on tissue-selective activity. This is particularly important given that pharmacokinetics often vary among species and could be different in humans. A compound with tissue-selective activity based upon pharmacokinetics would be less desirable. Constant infusion did increase potency, suggesting that sustained compound at the receptor will enhance the activation of this pathway. This suggests that sustained release formulations or continuous drug delivery could potentially improve the potency of SARM compounds but would not affect tissue-selective activity.

As a final experiment, we examined local tissue concentrations of LGD-3303 in muscle and prostate. Concentrations of LGD-3303 were higher in the prostate despite reduced activity in this tissue compared with skeletal muscle. This provides compelling evidence that the tissue-selective effects are not the result of tissue-specific exposure but are likely to represent altered interaction at the level of the AR. This is encouraging because it suggests that the tissue specificity will be maintained in humans or other species where the pharmacokinetics of LGD-3303 may differ from that in rats. Although drug-metabolizing enzymes and transporters are present in the prostate, this does not seem to play a role in LGD-3303 pharmacology. This may not be true for steroidal androgens or other nonsteroidal SARMS. The lack of tissue-selective activity observed with other androgens such as testosterone could potentially be the result of tissue-selective metabolism or distribution. Testosterone is metabolized to

the more potent androgen DHT in the prostate by 5α -reductase enzymes (Pelletier, 2008). As a result, DHT levels in the prostate may be higher than in muscle or the systemic circulation. It is conceivable that other SARMS and steroidal androgens have pharmacological profiles unlike LGD-3303 as a result of uneven tissue distribution, and similar evaluation of those compounds would be required to make a full comparison with LGD-3303.

In conclusion, we have demonstrated that LGD-3303 is a full agonist on muscle with partial agonist activity on the prostate. The partial agonist activity is not the result of saturated exposure, tissue-specific compound distribution, or alterations to the normal circadian rhythms of androgen exposure. The tissue selectivity of LGD-3303 is probably the result of altered interactions at the level of the AR.

Acknowledgments

We thank Dr. Arjan van Oeveren (Medicinal Chemistry Department, Ligand Pharmaceuticals) for preparing LGD-3303 for the study and Kelven Burnett, Li Wang, and Robert Srijemac (Pharmacology Department, Ligand Pharmaceuticals) for technical assistance.

References

- Aiyar SE, Sun JL, Blair AL, Moskaluk CA, Lu YZ, Ye QN, Yamaguchi Y, Mukherjee A, Ren DM, Handa H, and Li R (2004) Attenuation of estrogen receptor alpha-mediated transcription through estrogen-stimulated recruitment of a negative elongation factor. *Genes Dev* **18**:2134–2146.
- Askew EB, Gampe RT Jr, Stanley TB, Faggart JL, and Wilson EM (2007) Modulation of androgen receptor activation function 2 by testosterone and dihydrotestosterone. *J Biol Chem* **282**:25801–25816.
- Barbier O and Bélanger A (2008) Inactivation of androgens by UDP-glucuronosyl-

- transferases in the human prostate. *Best Pract Res Clin Endocrinol Metab* **22**: 259–270.
- Behre HM, Kliesch S, Leifke E, Link TM, and Nieschlag E (1997) Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. *J Clin Endocrinol Metab* **82**:2386–2390.
- Black BE and Paschal BM (2004) Intracellular organization and function of the androgen receptor. *Trends Endocrinol Metab* **15**:411–417.
- Bohl CE, Miller DD, Chen J, Bell CE, and Dalton JT (2005) Structural basis for accommodation of nonsteroidal ligands in the androgen receptor. *J Biol Chem* **280**:37747–37754.
- Box GE and Cox DR (1964) An analysis of transformations. *J R Stat Soc Ser B* **26**:211–252.
- Box GH and Hill WJ (1974) Correcting in homogeneity of variance with power transformation weighting. *Technometrics* **16**:385–389.
- Chen J, Hwang DJ, Bohl CE, Miller DD, and Dalton JT (2005) A selective androgen receptor modulator for hormonal male contraception. *J Pharmacol Exp Ther* **312**:546–553.
- Davis SR, van der Mooren MJ, van Lunsen RH, Lopes P, Ribot C, Ribot J, Rees M, Moufargue A, Rodenberg C, Buch A, et al. (2006) Efficacy and safety of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. *Menopause* **13**:387–396.
- Diel P, Friedel A, Geyer H, Kamber M, Laudenschlager U, Schänzer W, Schleipen B, Thevis M, Vollmer G, and Zierau O (2008) The prohormone 19-norandrostenedione displays selective androgen receptor modulator (SARM) like properties after subcutaneous administration. *Toxicol Lett* **177**:198–204.
- Farla P, Hersmus R, Geverts B, Mari PO, Nigg AL, Dubbink HJ, Trapman J, and Houtsmuller AB (2004) The androgen receptor ligand-binding domain stabilizes DNA binding in living cells. *J Struct Biol* **147**:50–61.
- Flicker L, Hopper JL, Larkins RG, Lichtenstein M, Buirski G, and Wark JD (1997) Nandrolone decanoate and intranasal calcitonin as therapy in established osteoporosis. *Osteoporos Int* **7**:29–35.
- Gao W and Dalton JT (2007) Ockham's razor and selective androgen receptor modulators (SARMs): are we overlooking the role of 5 α -reductase? *Mol Interv* **7**:10–13.
- Gao W, Kim J, and Dalton JT (2006) Pharmacokinetics and pharmacodynamics of nonsteroidal androgen receptor ligands. *Pharm Res* **23**:1641–1658.
- Ghosh K, Shen ES, Arey BJ, and López FJ (1998) A global model to define the behavior of partial agonists (bell-shaped dose-response inducers) in pharmacological evaluation of activity in the presence of the full agonist. *J Biopharm Stat* **8**:645–665.
- Gibaldi M and Perrier D (1982) *Pharmacokinetics*, pp 271–318, 409–417, Marcel Dekker, New York.
- Hanada K, Furuya K, Yamamoto N, Neishima H, Ichikawa K, Nakamura T, Miyakawa M, Amano S, Sumita Y, and Oguro N (2003) Bone anabolic effects of S-40503, a novel nonsteroidal selective androgen receptor modulator (SARM), in rat models of osteoporosis. *Biol Pharm Bull* **26**:1563–1569.
- Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals*, 7th ed, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC.
- Kearbey JD, Wu D, Gao W, Miller DD, and Dalton JT (2004) Pharmacokinetics of S-3-(4-acetyl-amino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide in rats, a non-steroidal selective androgen receptor modulator. *Xenobiotica* **34**:273–280.
- Kim J, Wu D, Hwang DJ, Miller DD, and Dalton JT (2005) The para substituent of S-3-(phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide is a major structural determinant of in vivo disposition and activity of selective androgen receptor modulators. *J Pharmacol Exp Ther* **315**:230–239.
- Koch A, Hoppen HO, Dieleman SJ, Kooistra HS, and Günzel-Apel AR (2006) Effects of the dopamine agonist cabergoline on the pulsatile and TRH-induced secretion of prolactin, LH, and testosterone in male beagle dogs. *Theriogenology* **65**:1666–1677.
- Lightman SL, Wiles CC, Atkinson HC, Henley DE, Russell GM, Leendertz JA, McKenna MA, Spiga F, Wood SA, and Conway-Campbell BL (2008) The significance of glucocorticoid pulsatility. *Eur J Pharmacol* **583**:255–262.
- Martinborough E, Shen Y, Oeveren A, Long YO, Lau TL, Marschke KB, Chang WY, López FJ, Vajda EG, Rix PJ, et al. (2007) Substituted 6-(1-pyrrolidino)quinolin-2(1H)-ones as novel selective androgen receptor modulators. *J Med Chem* **50**: 5049–5052.
- Miner JN, Chang W, Chapman MS, Finn PD, Hong MH, López FJ, Marschke KB, Rosen J, Schrader W, Turner R, et al. (2007) An orally active selective androgen receptor modulator is efficacious on bone, muscle, and sex function with reduced impact on prostate. *Endocrinology* **148**:363–373.
- Munck A and Holbrook NJ (1984) Glucocorticoid-receptor complexes in rat thymus cells: rapid kinetic behavior and a cyclic model. *J Biol Chem* **259**:820–831.
- Negro-Vilar A (1999) Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *J Clin Endocrinol Metab* **84**:3459–3462.
- Obligacion R, Murray M, and Ramzan I (2006) Drug-metabolizing enzymes and transporters: expression in the human prostate and roles in prostate drug disposition. *J Androl* **27**:138–150.
- Ostrowski J, Kuhns JE, Lupisella JA, Manfredi MC, Beehler BC, Krystek SR Jr, Bi Y, Sun C, Seethala R, Golla R, et al. (2007) Pharmacological and x-ray structural characterization of a novel selective androgen receptor modulator: potent hyper-anabolic stimulation of skeletal muscle with hypostimulation of prostate in rats. *Endocrinology* **148**:4–12.
- Page ST, Marck BT, Tolliver JM, and Matsumoto AM (2008) Tissue selectivity of the anabolic steroid, 19-nor-4-androstenediol-3 β ,17 β -diol in male Sprague Dawley rats: selective stimulation of muscle mass and bone mineral density relative to prostate mass. *Endocrinology* **149**:1987–1993.
- Pelletier G (2008) Expression of steroidogenic enzymes and sex-steroid receptors in human prostate. *Best Pract Res Clin Endocrinol Metab* **22**:223–228.
- Piu F, Gardell LR, Son T, Schlienger N, Lund BW, Schiffer HH, Vanover KE, Davis RE, Olsson R, and Bradley SR (2008) Pharmacological characterization of AC-262536, a novel selective androgen receptor modulator. *J Steroid Biochem Mol Biol* **109**:129–137.
- Rhoden EL and Morgentaler A (2004) Risks of testosterone-replacement therapy and recommendations for monitoring. *N Engl J Med* **350**:482–492.
- Snyder PJ, Peachey H, Berlin JA, Hannoush P, Haddad G, Dlewati A, Santanna J, Loh L, Lenrow DA, Holmes JH, et al. (2000) Effects of testosterone replacement in hypogonadal men. *J Clin Endocrinol Metab* **85**:2670–2677.
- Vajda EG, Hogue A, Griffiths KN, Chang WY, Burnett K, Chen Y, Marschke K, Mais DE, Pedram B, Shen Y, et al. (2008) Combination treatment with a selective androgen receptor modulator (SARM) and a bisphosphonate has additive effects in osteopenic female rats. *J Bone Miner Res* doi:10.1359/JBMR.081007.
- Veldhuis JD, Iranmanesh A, Godschalk M, and Mulligan T (2000) Older men manifest multifold synchrony disruption of reproductive neurohormone outflow. *J Clin Endocrinol Metab* **85**:1477–1486.
- Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, and Berman N (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. *J Clin Endocrinol Metab* **85**:2839–2853.
- Yin D, Gao W, Kearbey JD, Xu H, Chung K, He Y, Marhefka CA, Veverka KA, Miller DD, and Dalton JT (2003) Pharmacodynamics of selective androgen receptor modulators. *J Pharmacol Exp Ther* **304**:1334–1340.

Address correspondence to: Dr. Eric G. Vajda, 10275 Science Center Drive, San Diego, CA 92121. E-mail: evajda@ligand.com