Abstract #184

Galeterone, Abiraterone, Orteronel and Ketoconazole Exhibit Differential Inhibitory Effects on CYP17 and Steroidogenesis

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Abstract

Background: Because CYP17 has a central role in converting progestogens to androgens, inhibitors have been developed to treat CRPC. CYP17 has both hydroxylase and lyase catalytic functions. However, selective hydroxylase inhibition causes build-up of progestogens and mineralocorticoids, resulting in secondary mineralocorticoid excess (ME), edema, hypokalemia and hypertension (treated clinically with prednisone). We analyzed four inhibitors for their effects on CYP17 hydroxylase and lyase activities, and more globally on the steroidogenic pathway.

Methods: Human CYP17 expressed in yeast microsomes was incubated with pregnenolone or 17α-hydroxypregnenolone and products quantitated using LC/MS/MS. Galeterone, abiraterone, orteronel or ketoconazole were added, and hydroxylase or lyase IC_{50} values calculated. In a separate experiment, H295R adenocarcinoma cells were incubated with drugs at 1 μM for 24 hours and media was analyzed for steroid production by LC/MS/MS.

Results: All drugs inhibited CYP17. However, potency and selectivity for hydroxylase and lyase varied significantly. Galeterone was the most potent and selective CYP17 lyase inhibitor. Abiraterone most selectively inhibited hydroxylase, while orteronel and ketoconazole were less potent lyase inhibitors than galeterone. In the cell-based assay, all drugs inhibited testosterone synthesis ≥94% at 1 μM. However, at this concentration, evidence of hydroxylase inhibition was supported by significant elevation of progesterone (abiraterone increased 293-fold), mineralocorticoids (orteronel increased DOC 74-fold), or reductions in cortisol (abiraterone, orteronel, ketoconazole reduced by 91%, 70% and 94%, respectively). In contrast, galeterone produced minimal changes in cortisol (decreased 14%) and other intermediate precursors.

Conclusions: Galeterone is a selective and potent CYP17 lyase inhibitor that also antagonizes and degrades the AR, and exhibits minimal evidence of deleterious steroid changes associated with ME. These experimental data recapitulate Phase 1 clinical experience where no ME was observed or prednisone required in CRPC patients.

Introduction

It has long been known that androgens (testosterone and dihydrotestosterone) fuel tumor growth in prostate cancer, and therapies that reduce circulating levels are the standard of care in early-stage disease (e.g., leuprolide). Ketoconazole, an antifungal agent, has been used off-label to treat prostate cancer because of its inhibition of CYP17 lyase. Recently, drug developers have targeted CYP17 inhibition to reduce the biosynthesis of androgens and one, abiraterone acetate, is approved in combination with prednisone for treating Castration Resistant Prostate Cancer (CRPC).

CYP17A1 is a single enzyme that has both 17α-hydroxylase and 17,20-lyase catalytic functions (Figure 1). CYP17 lyase inhibition reduces circulating testosterone and dihydrotestosterone (DHT) levels. However, CYP17 hydroxylase inhibition causes a reduction in cortisol, leading to a compensatory increase in ACTH that stimulates the steroidogenic pathway. This in turn elevates progestogens and mineralocorticoids that lie upstream of the CYP17 hydroxylase blockade, resulting in secondary mineralocorticoid excess (ME), a clinical syndrome which includes edema, hypokalemia and hypertension. As a result of ME, abiraterone must be co-administered with prednisone and orteronel is also being dosed with prednisone in Ph3 clinical studies. Abiraterone has been shown to cause symptoms of ME in approximately 90% of patients when prednisone was not used concomitantly (Attard, 2009), and in 55% of patients with the use of prednisone (de Bono, 2011).

Galeterone is a proprietary oral small molecule compound that disrupts AR signaling via a novel triple mechanism of action: (1) selective inhibition of CYP17 lyase; (2) competitive antagonism of androgen (testosterone, DHT) binding to AR; and (3) degradation of the AR protein itself. Patients treated with galeterone at doses that produced radiographic responses and reductions in PSA have to date not exhibited dose-limiting toxicities or signs of ME and have not required concomitant prednisone (Montgomery, 2012). We therefore have performed experiments to better understand the biological basis for these clinical observations. Our data show that galeterone is a selective and potent CYP17 lyase inhibitor that exhibits minimal evidence of deleterious steroid changes associated with ME.

Methods

Effects of CYP17 Inhibitors on human CYP17 (OpAns, Durham, NC)
Microsomes were prepared from a yeast strain that expresses human CYP17 and human P450 oxidoreductase, according to methods described in Sherbet, 2003. On ice, microsomes (~5 μg, 1 pmol P450) were added to 50 mM potassium-phosphate buffer, pH 7.4 containing the steroid substrate at 1 μM (either pregnenolone for measuring CYP17 hydroxylase or 17α-hydroxypregnenolone for measuring CYP17 lyase). The test compounds were also added at concentrations ranging from 2.1 nM to 20 μM for hydroxylase measurements or 0.42 nM to 4 μM for lyase measurements. For measuring lyase activity, 2 pmol human cytochrome b5 was also added. The reaction was pre-incubated for 2 minutes at 37ºC and then NADPH was added as part of the BD Biosciences GENTEST NADPH Regeneration System. The reaction was carried out for 10 minutes at 37ºC and then the sample was extracted with acetonitrile. The products in the organic phase were analyzed using an Agilent 6410 LC/MS/MS system consisting of a Halo RP-Amide column with mobile phases of methanol and water, each with 0.1% formic acid, and a MultiMode ion source in APCI mode. Under these conditions, the untreated control yielded approximately 20% conversion to product.

Effects of CYP17 Inhibitors on Steroidogenesis in NCI-H295R Adrenocortical Carcinoma Cells (Charles River Labs, Morrisville, NC)
H295R cells (ATCC) were cultured in DMEM:F12 medium containing 0.00625 mg/ml insulin, 0.00625 mg/ml transferrin, 6.25 ng/ml selenium, 1.25 mg/ml bovine serum albumin, 0.00535 mg/ml linoleic acid, and 2.5% Nu-Serum. Cells were plated into 6 well dishes and cultured for 48 hours. The media was then removed and the cells washed twice in 1x PBS prior to addition of the test compounds in serum-free media at 1 μM. In addition, two wells contained serum-free media alone and served as untreated controls. One micromolar drug concentration was chosen because this concentration approximates the peak blood concentration in patients treated with abiraterone and galeterone (Ryan, 2010). In addition, it is the lowest concentration of all drugs that inhibits testosterone biosynthesis by greater than 90% (Fig. 3). Galeterone, abiraterone, orteronel and ketoconazole were supplied by: Tokai Pharmaceuticals, Univ. Maryland (Dr. Vincent Njar), Organix, Inc, and Sigma Chemicals, respectively. After 24 hours of incubation at 37ºC, the media was removed, centrifuged to remove any debris, and stored at -80ºC. The cells remaining on the plate were assessed for viable cell number using CellTiter-Glo® (Promega #G7571).

Media Steroid Concentration Determination using LC/MS/MS (OpAns, Durham, NC)
Steroid levels in the media were quantitated using LC/MS/MS by first extracting the media with acetonitrile. The steroid products in the organic phase were analyzed using an Agilent 6410 LC/MS/MS system consisting of a Halo RP-Amide column with mobile phases of methanol and water, each with 0.1% formic acid, and a MultiMode ion source in APCI mode. The steroid concentrations were corrected for variation of viable cell number relative to untreated control wells.

Figure 1. Steroidogenic Pathway

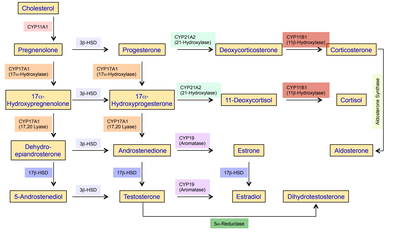


Table 1. CYP17A1 Enzyme Inhibition

Compound	Hydroxylase IC_{50} (nM)	Lyase IC_{50} (nM)	Lyase:Hydroxylase Selectivity
Galeterone	73	23	3.2
Abiraterone	7	12	0.6
Orteronel	348	64	5.4
Ketoconazole	190	31	6.1

Figure 2. CYP17A1 Enzyme Inhibition

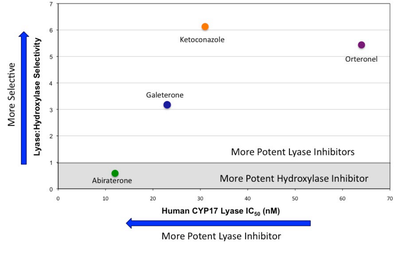
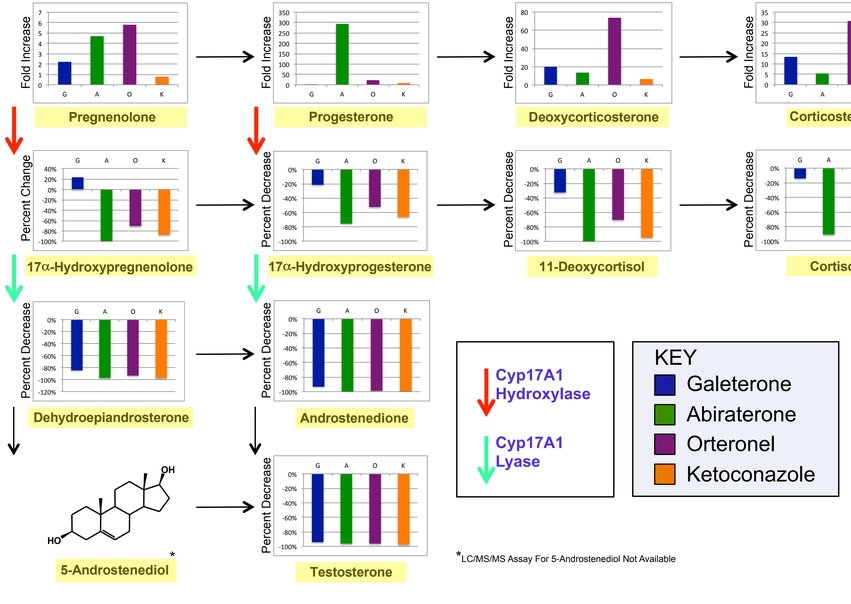


Figure 3. Drug Effects on Steroidogenesis at 1 μM



*LC/MS/MS Assay For 5-Androstenediol Not Available

Summary (Table 1, Figures 2 and 3)

1. Galeterone:
- was the most potent CYP17 inhibitor that was also selective for CYP17 lyase
- caused only moderate elevations of pregnenolone, deoxycorticosterone and corticosterone
- had the smallest effect on the levels of CYP17A1 hydroxylase products, especially cortisol

These experimental data may explain the Phase 1 clinical experience with galeterone where no Mineralocorticoid Excess (ME) was observed or prednisone required.

2. Abiraterone:
- was the most potent CYP17 inhibitor, but was more selective for CYP17 hydroxylase than lyase
- reduced cortisol and elevated pregnenolone, progesterone, and to a lesser extent deoxycorticosterone and corticosterone

These changes are consistent with the ME observed clinically.

3. Orteronel:
- was a less potent, selective CYP17 lyase inhibitor
- elevated CYP17A1 hydroxylase precursors (especially deoxycorticosterone and corticosterone) and reduced CYP17A1 hydroxylase products, including cortisol

The effects are consistent with inhibition of CYP17A1 hydroxylase which could cause ME.

4. Ketoconazole:
- was a less potent, selective CYP17 lyase inhibitor
- exhibited dose-dependent inhibition of CYP17A1 hydroxylase and lyase, but also caused off-target effects in the pathway (data not shown)

Non-specific effects of ketoconazole on other CYP450 enzymes limit its clinical use due to toxicity.

Conclusions

Clinical experience with galeterone has shown no signs of Mineralocorticoid Excess. Galeterone has not required concomitant prednisone or eplerenone use to date. We have demonstrated that the differential clinical profile of galeterone is ascribed to its highly selective and potent CYP17 lyase inhibition, while minimally impacting CYP17 hydroxylase function.

References:

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