Poster #SAT-547

Pharmacology of COR-003 (Levoketoconazole), an Investigational Treatment for Endogenous Cushing's Syndrome

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ABSTRACT

Ketoconazole is a 50/50 racemic mixture of 2 enantiomers (2S,4R and 2R,4S) used off-label in the United States for the treatment of endogenous Cushing's syndrome by virtue of adrenal cortisol synthesis inhibition. Ketoconazole has a black box warning for liver toxicity. COR-003 (levoketoconazole) is the single (2S,4R) enantiomer of ketoconazole, with data indicating a better therapeutic index, and is currently being investigated in a multinational phase 3 study for the treatment of endogenous Cushing's syndrome.

Compared to the 2R,4S enantiomer, COR-003 more potently inhibits key enzymes of adrenal cortisol synthesis: CYP11B1 (IC_{50}, 116 nM; 46×), CYP17 (IC_{50}, 48 nM; 37.×), and CYP21 (IC_{50} 1,000 nM; 10×) in cell lines expressing recombinant human enzymes. As published previously (Rotstein, 1992), COR-003 also inhibits CYP11A1 (IC_{50}, 1,240 nM; 4.4×, bovine adrenal mitochondria) and CYP51 (IC_{50}, 48 nM; 2.5×, rat liver microsomes) with slightly higher potency than the 2R,4S enantiomer. In rats, after a single oral application of increasing doses of COR-003 or 2R,4S-ketoconazole, COR-003 more potently (50% inhibition at about 100 mg/kg) and more effectively reduces serum cortisone, the main glucocorticoid in this species, measured 4 hours post-dose.

In a 3-period crossover study in 24 healthy subjects dosed with either placebo, COR-003, or ketoconazole at a dose of 400 mg QD, a significant decrease of serum cortisol (AUC_{0-6h} after dosing) was evidenced following COR-003 administration by the morning of the 4th day, $P \leq 0.0019$ versus placebo and $P \leq 0.0429$ versus ketoconazole. In the same study, plasma concentrations of the 2 enantiomers were measured on Day 5 after 4-day QD oral dosing with 400 mg ketoconazole and a single 80-mg dose of atorvastatin on Day 5. Maximal plasma concentration of COR-003 (6.1 µg/mL; coefficient of variation, 40.7%) was about 3-fold higher than that of the 2R,4S enantiomer, potentially indicative of reduced hepatic metabolism of the COR-003 enantiomer. Study treatments were safe and well tolerated. Headache, nausea, and back pain were the most frequently reported adverse events.

Ketoconazole is metabolized in the liver and eliminated predominantly by biliary excretion. Ketoconazole inhibits hepatic CYP7A, a key enzyme for bile acid synthesis, which may interfere with biliary elimination of ketoconazole and its metabolites. 2R,4S-ketoconazole is 12 times more potent for inhibition of CYP7A (IC_{50} = 195 nM) than COR-003 in a microsome preparation from rat liver.

Taken together, nonclinical and clinical data suggest that, compared with ketoconazole, COR-003 more potently inhibits cortisol synthesis, reaches higher plasma concentrations potentially indicating reduced hepatic metabolism, and interferes less with bile acid synthesis and thus certain drug eliminations. Mechanistic nonclinical studies are being conducted to further investigate the differentiated profile of COR-003.

INTRODUCTION

- Endogenous Cushing's syndrome is a rare and serious endocrine disease caused by chronic excessive exposure to cortisol[1]
- Treatment options for Cushing's syndrome include neurosurgery, radiotherapy, and medical treatment[2]
- Ketoconazole is a 50/50 racemic mixture of 2 enantiomers (2S,4R and 2R,4S), used off-label in the United States for the treatment of endogenous Cushing's syndrome due to its inhibitory effect on adrenal cortisol synthesis[3-5]
  - Ketoconazole has a black box warning for liver toxicity[6]
- COR-003 (levoketoconazole) is the single (2S,4R) enantiomer of ketoconazole, with data suggesting an improved therapeutic index compared with ketoconazole
  - COR-003 is currently being investigated in a multinational phase 3 study for the treatment of endogenous Cushing's syndrome

OBJECTIVE

- To describe preclinical and clinical pharmacology findings for COR-003

METHODS

Preclinical Pharmacology of COR-003

Primary Pharmacology of COR-003 in In Vitro Assays

- Half-maximal inhibitory concentration (IC_{50}) values for various enzyme systems were evaluated for racemic ketoconazole, COR-003, and 2R,4S-ketoconazole using recombinant AD293 cell lines expressing human CYP17, CYP21, and CYP11B1
  - CYP17 activity was measured using a radiometric acetic acid release assay (AARA) with [21-^{3}H] 17α-hydroxypregnenolone as a substrate
  - CYP21 and CYP11B1 activity were measured based on the formation of 11-deoxycortisol from 17α-hydroxyprogesterone and the formation of cortisol from 11-deoxycortisol, respectively, using liquid chromatography-mass spectrometry
- CYP11A1 and CYP51 activity were evaluated as described in a prior study using microsomes prepared from the livers of male rats[5]

Corticosterone Effects in Rat Studies

- Effects of COR-003 on corticosterone (the major glucocorticoid in rats) levels were evaluated in a dose-ranging study where male Sprague Dawley rats (10/group) received a single oral dose of COR-003, 2R,4S-ketoconazole, or racemic ketoconazole via a gastric tube at 1 of 5 doses (50, 100, 200, 400, or 600 mg/kg) and were sacrificed 4 hours later
- In a separate time-course study, male Sprague Dawley rats (10/group) received a single oral dose of COR-003, 2R,4S-ketoconazole, or racemic ketoconazole via a gastric tube at a dose of 200 mg/kg and were sacrificed at 4, 8, 12, 16, 20, and 24 hours after dosing

Clinical Pharmacology of COR-003

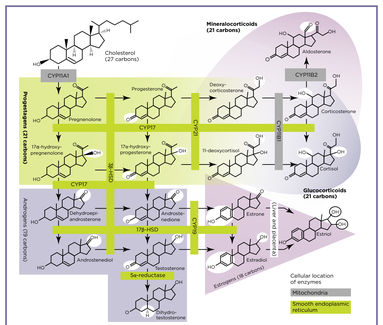
- In a 3-period, crossover, phase 1, drug-interaction study, healthy subjects (N = 24) received 400 mg of COR-003, 400 mg of racemic ketoconazole, or placebo once daily for 7 days
  - On Day 5, all subjects received a single 80-mg dose of atorvastatin
  - Blood samples were taken for determining cortisol levels during each of the 3 study periods (placebo, COR-003 400 mg, and ketoconazole 400 mg) on Days 1, 4, and 5 after dosing with study drug in the morning, and after co-administration with atorvastatin at 1, 2, 4, 6, and 24 hours
  - Serum cortisol levels were evaluated before and during administration of atorvastatin using a chemiluminescence assay
  - Pharmacokinetics of the 2 enantiomers were evaluated
    - Plasma samples were assayed using a validated chiral assay
    - Area under the curve (AUC), maximum concentration (C_{max}), and time to maximum concentration (T_{max}) were calculated

RESULTS

Preclinical Pharmacology of COR-003

Inhibition of Steroidogenic Enzymes In Vitro

- Cytochrome P450 (CYP) enzymes play important roles in the synthesis of steroid hormones (**Figure 1**)



CYP, cytochrome P450; HSD, hydroxysteroid dehydrogenase.

Figure 1. Role of cytochrome P450 enzymes in the corticosteroid synthesis pathway.[7]

- COR-003 more potently inhibits CYP11B1 (46× greater potency), CYP17 (37× greater potency), and CYP21 (10× greater potency), key enzymes of adrenal cortisol synthesis, compared with the 2R,4S enantiomer (**Table 1**)
- COR-003 has also been shown to inhibit CYP11A1 (cholesterol side-chain cleavage enzyme; IC_{50}, 1,240 nM) and CYP51 (lanosterol-14α-demethylase; IC_{50}, 48 nM) with 4.4 and 2.5 times greater potency, respectively, than the 2R,4S enantiomer in microsomal assays[5]

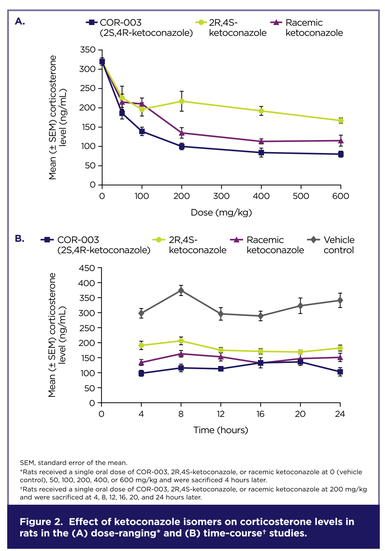
Table 1. IC_{50} Values for Racemic Ketoconazole, COR-003, and 2R,4S-ketoconazole Towards Key Enzymes of Adrenal Cortisol Synthesis*

	IC_{50} (nM)		
Enzyme	Racemic ketoconazole	COR-003 (2S,4R-ketoconazole)	2R,4S-ketoconazole
CYP17	93	48	1,800
CYP11B1	840	116	5,300
CYP21	2,450	1,000	10,000

IC_{50}, half maximal inhibitory concentration; CYP, cytochrome P450.
*Assays were performed using recombinant AD293 cell lines expressing human CYP17, CYP11B1, and CYP21.

Suppression of Corticosterone in Rats

- After a single oral application of increasing doses of COR-003 or 2R,4S-ketoconazole, COR-003 more potently and more effectively reduced serum corticosterone, measured 4 hours post-dose (**Figure 2A**)
  - COR-003 showed 50% inhibition of corticosterone at the 100-mg/kg dose
  - The mean (± standard error of the mean [SEM]) concentration of corticosterone at 4 hours at the highest administered dose (600 ng/mL) was 80 ± 7.8 ng/mL with COR-003, 167 ± 6.8 ng/mL with the 2R,4S enantiomer, and 115 ± 14.3 ng/mL with racemic ketoconazole
- The time-course study in which test compounds were administered at a fixed dose of 200 mg/kg showed that maximal suppression of serum corticosterone across groups was reached within 4 hours and maintained over 24 hours; COR-003 was the most effective in reducing corticosterone with ~70% reduction (**Figure 2B**)
  - Serum concentrations of corticosterone (mean ± SEM) ranged from 98 ± 10 ng/mL to 136 ± 12 ng/mL with COR-003, and from 169 ± 7 ng/mL to 206 ± 13 ng/mL with the 2R,4S enantiomer

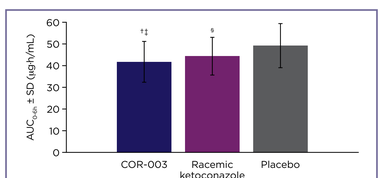


SEM, standard error of the mean.
*Rats received a single oral dose of COR-003, 2R,4S-ketoconazole, or racemic ketoconazole at 0 (vehicle control), 50, 100, 200, 400, or 600 mg/kg and were sacrificed 4 hours later.
†Rats received a single oral dose of COR-003, 2R,4S-ketoconazole, or racemic ketoconazole at 200 mg/kg and were sacrificed at 4, 8, 12, 16, 20, and 24 hours later.

Figure 2. Effect of ketoconazole isomers on corticosterone levels in rats in the (A) dose-ranging* and (B) time-course† studies.

Clinical Pharmacology of COR-003

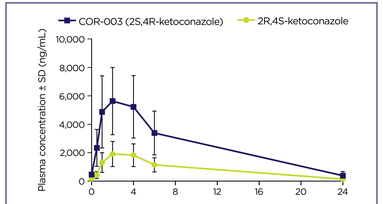
- In healthy subjects after 4 days of treatment, prior to administration of atorvastatin, a significant decrease in serum cortisol (AUC_{0-6h}) was seen with COR-003 versus placebo (P = 0.0019) and versus racemic ketoconazole (P = 0.0429; **Figure 3**)



AUC_{0-6h}, area under the curve from 0 to 6 hours after dosing; SD, standard deviation.
*Cortisol levels were determined from blood samples collected on Day 4 after dosing with study drug in the morning.
†P = 0.0429 vs racemic ketoconazole.
‡P = 0.0019 vs placebo.
§P = 0.0100 vs placebo.

Figure 3. AUC_{0-6h} at Day 4 after COR-003, racemic ketoconazole, and placebo dosing.*

- After 5 days of dosing with racemic ketoconazole, maximal plasma concentration of COR-003 (6.1 µg/mL; coefficient of variation, 40.7%) was ~3-fold higher compared with the 2R,4S enantiomer (**Figure 4**)



SD, standard deviation.
*Enantiomer concentrations were evaluated from blood samples after 5 days of dosing with racemic ketoconazole.

Figure 4. Plasma concentration of COR-003 and 2R,4S-ketoconazole over time in healthy subjects dosed with racemic ketoconazole.*

- COR-003 was generally well tolerated in healthy subjects
  - Headache, back pain, and nausea were the most frequently reported adverse events (**Table 2**)
  - No clinically significant variations in liver function values were noted in the active treatment groups

Table 2. Summary of AEs Reported by ≥10% Subjects*

	Subjects, n (%)			
AE	COR-003 + atorvastatin (N = 24)	Racemic ketoconazole + atorvastatin (N = 23)	Placebo + atorvastatin (N = 23)	Total (N = 24)
Headache	6 (25.0%)	5 (21.7%)	4 (17.4%)	11 (45.8%)
Back pain	3 (12.5%)	2 (8.7%)	0 (0%)	4 (16.7%)
Nausea	3 (12.5%)	3 (13.0%)	0 (0%)	4 (16.7%)

AE, adverse event.
*COR-003, racemic ketoconazole, or placebo was administered daily on Days 1 to 4 and concomitantly with atorvastatin on Day 5.

SUMMARY

- Compared to the 2R,4S enantiomer, COR-003 more potently inhibits key enzymes of adrenal cortisol synthesis, CYP11B1, CYP17, and CYP21, in cell lines
  - Inhibition of CYP17 has been shown to inhibit testosterone production,[8] and COR-003 showed more potent inhibition of testosterone than 2R,4S-ketoconazole in rats (data on file)
- COR-003 more potently and more effectively reduces serum corticosterone than 2R,4S-ketoconazole in rats
- COR-003 administration was followed by a significant decrease of serum cortisol by Day 4 in healthy volunteers
- In healthy subjects, the serum concentration of COR-003 was substantially higher than the 2R,4S enantiomer after oral administration of racemic ketoconazole, which could potentially indicate lower hepatic metabolism of COR-003
  - Similarly increased plasma levels for COR-003 were also found in a phase 2a study in patients with type 2 diabetes[9]
  - In nonclinical studies, the 2R,4S enantiomer has been shown to be 12 times more potent than COR-003 for inhibition of CYP7A, a key enzyme for bile acid synthesis[5,9]; this inhibition may interfere with biliary elimination of ketoconazole and its metabolites
- These findings suggest that COR-003 is a more potent inhibitor of cortisol synthesis compared with the 2R,4S enantiomer and may potentially have less liver exposure as well as less interference with metabolite elimination, resulting in a potentially improved therapeutic index compared with the racemic mixture
- Ongoing mechanistic studies will provide further insight into the pharmacodynamic and pharmacokinetic profile of COR-003

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