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Orteronel (TAK-700), a novel non-steroidal 17,20-lyase inhibitor: Effects on steroid synthesis in human and monkey adrenal cells and serum steroid levels in cynomolgus monkeys

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ABSTRACT

Surgical or pharmacologic methods to control gonadal androgen biosynthesis are effective approaches in the treatment of a variety of non-neoplastic and neoplastic diseases. For example, androgen ablation and its consequent reduction in circulating levels of testosterone is an effective therapy for advanced prostate cancers. Unfortunately, the therapeutic effectiveness of this approach is often temporary because of disease progression to the 'castration resistant' (CRPC) state, a situation for which there are limited treatment options. One mechanism thought to be responsible for the development of CRPC is extra-gonadal androgen synthesis and the resulting impact of these residual extra-gonadal androgens on prostate tumor cell proliferation. An important enzyme responsible for the synthesis of extra-gonadal androgens is CYP17A1 which possesses both 17,20-lyase and 17-hydroxylase catalytic activities with the 17,20-lyase activity being key in the androgen biosynthetic process. Orteronel (TAK-700), a novel, selective, and potent inhibitor of 17,20-lyase is under development as a drug to inhibit androgen synthesis. In this study, we quantified the inhibitory activity and specificity of orteronel for testicular and adrenal androgen production by evaluating its effects on CYP17A1 enzymatic activity, steroid production in monkey adrenal cells and human adrenal tumor cells, and serum levels of dehydroepiandrosterone (DHEA), cortisol, and testosterone after oral dosing in castrated and intact male cynomolgus monkeys. We report that orteronel potently suppresses androgen production in monkey adrenal cells but only weakly suppresses corticosterone and aldosterone production; the IC_{50} value of orteronel for cortisol was ~3-fold higher than that for DHEA. After single oral dosing, serum levels of DHEA, cortisol, and testosterone were rapidly suppressed in intact cynomolgus monkeys. In castrated monkeys treated twice daily with orteronel, suppression of DHEA and testosterone persisted throughout the treatment period. In both in vivo models and in agreement with our in vitro data, suppression of serum cortisol levels following oral dosing was less than that seen for DHEA. In terms of human CYP17A1 and human adrenal tumor cells, orteronel inhibited 17,20-lyase activity 5.4 times more potently than 17-hydroxylase activity in cell-free enzyme assays and DHEA production 27 times more potently than cortisol production in human adrenal tumor cells, suggesting greater specificity of inhibition between 17,20-lyase and 17-hydroxylase activities in humans vs monkeys. In summary, orteronel potently inhibited the 17,20-lyase activity of monkey and human CYP17A1 and reduced serum androgen levels in vivo in monkeys. These findings suggest that orteronel may be an effective therapeutic option for diseases where androgen suppression is critical, such as androgen sensitive and CRPC.

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Abbreviations: 17-OHP, 17α-hydroxyprogesterone; ACTH, adrenocorticotropic hormone; AR, androgen receptor; AUC, area under the curve; β-NADP+, β-nicotinamide adenine dinucleotide phosphate, oxidized form; BA, bioavailability; CI, confidence interval; C_{max}, maximum plasma concentration; CRPC, castration-resistant prostate cancer; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; DMEM, Dulbecco's modified Eagle's medium; G-6-P, glucose 6-phosphate; GnRH, gonadotropin-releasing hormone; h, hour; KRBGA, Krebs–Ringer buffer containing glucose and bovine serum albumin solution; LH, luteinizing hormone; LSC, liquid scintillation; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; PSA, prostate-specific antigen; RIA, radioimmunoassay; SCC, side chain cleavage enzyme; SD, standard deviation; SEM, standard error of the mean; $t_{1/2}$, half life; TLC, thin layer chromatography; T_{max}, time to reach maximum plasma concentration.

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1. Introduction

Multiple human diseases, such as prostate and breast cancers, benign prostate hyperplasia, endometriosis, and polycystic ovarian syndrome, are driven by the biological effects of steroid hormones. For example, progression of prostate cancer, one of the leading causes of cancer death in men worldwide [1], was shown more than 50 years ago to be dependent on circulating levels of testosterone [2]. Since then, surgical castration (hormone ablation therapy) has constituted a major medical treatment for recurrent and locally advanced or metastatic prostate cancers, for which radical prostatectomy is no longer an option. As an alternative to surgical castration, medical castration (i.e., treatment with gonadotropin-releasing hormone [GnRH] agonists such as leuprorelin) has been shown to suppress gonadal androgen production with similar efficacy and is now first-line therapy for this disease. Unfortunately, while most prostate cancers initially respond to androgen ablation therapy, they almost uniformly acquire resistance to this approach within 2–3 years, associated with a rise in prostate specific androgen (PSA). Of interest, disease progression following hormone ablation therapy is still driven by circulating androgens despite castration levels of testosterone (<50 ng/dL). Prostate cancer under these conditions is called castration-resistant (CRPC) and the prognosis is poor [3,4]. Until recently, docetaxel was the only chemotherapeutic compound shown to have survival benefit in patients with CRPC [5] despite its moderate efficacy and frequent adverse effects. Clearly new treatment options for CRPC are urgently needed.

Studies have established the importance of androgen receptor (AR) signaling in all stages of prostate cancer, including CRPC. In CRPC, several mechanisms such as AR activation by non-gonadal (adrenal and/or intratumoral) androgens, AR hypersensitivity through over-expression of AR, and associated proteins [6–9], or promiscuous/constitutive activation of mutant AR [10–19] have been proposed to explain ongoing AR activation in the face of castrate androgen levels. Regarding the role for extragonadal androgen synthesis, it is interesting to note that expression levels of the enzymes responsible for converting adrenal androgens to testosterone and dihydrotestosterone (DHT) are up-regulated in CRPC tissues [20].

Biochemically, both testicular and adrenal androgens are synthesized from cholesterol through a multi-step process in which the 17,20-lyase activity of CYP17A1 is essential [21]. Thus, inhibition of the 17,20-lyase activity of CYP17A1 might be a rational approach to inhibit extragonadal androgen production and prostate cancer progression. In this regard, the antifungal agent ketoconazole possesses CYP17A1 inhibitory activity [22] and has been tested clinically in patients with CRPC. However, ketoconazole treatment is often discontinued due to toxicity which stems from its ability to potently inhibit the activity of other CYP enzymes in addition to CYP17A1 [22] and then usefulness of CYP17A1 inhibitor for CRPC was not fully established. More recently the steroid analogue abiraterone acetate, a more specific CYP17A1 inhibitor, has been reported to show clinically significant antitumor activity in up to 70% of prostate cancer patients [23,24]. We have generated a series of non-steroidal, naphthylmethylimidazole derivatives expressly designed to specifically inhibit the 17,20-lyase activity of human CYP17A1 to reduce impact on steroid levels other than androgen, which originally targeted hormone-dependent prostate cancer. Preliminary studies to assess potency and specificity of this series have identified the lead analogue, orteronel (TAK-700) (6-[(7*S*)-7-hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl]-*N*-methyl-2-naphthamide [25] (Fig. 1), as a potential clinical candidate.

The synthesis and secretion of adrenal androgens is apparently unique to humans and nonhuman primates, and requires the expression of key mediators such as CYP17 [26]. Endocrine control of CYP17 expression in rhesus monkeys is comparable to that in humans [27]. An analysis of baboon cDNA for CYP17 showed 96% homology with human CYP17, with only 28 predominantly conservative amino acid differences between the two [28]. Similarly, chimpanzee and rhesus cDNA sequences have been shown to be near identical to human CYP17 [29]. Thus a nonhuman primate model would appear suitable for analysis of 17,20-lyase inhibition in the development of orteronel. As such, here we report the effects of orteronel on steroid hormone production in monkey adrenal cells and human adrenal tumor cell lines, and serum dehydroepiandrosterone (DHEA), cortisol, and testosterone levels after oral dosing in both castrated and intact male cynomolgus monkeys. Our findings reveal that orteronel effectively inhibits the 17,20-lyase activity of CYP17A1 in vitro and in vivo and suggest that orteronel may be an effective therapeutic option for both androgen-dependent and CRPC.

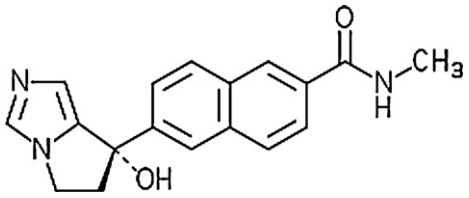

Fig. 1. Chemical structure of orteronel (6-[(7*S*)-7-hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl]-N-methyl-2-naphthamide).

2. Materials and methods

2.1. Chemicals

Orteronel was synthesized by Takeda Pharmaceutical Company Limited (Osaka, Japan). Ketoconazole was purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA, USA) or LKT Laboratories, Inc. (St Paul, MN, USA). Abiraterone (CB7598) was prepared by Medicinal Chemistry Research Laboratories, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited based upon its published structure and synthetic methods [30]; its chemical structure was confirmed by element analysis and melting point. Glucose 6-phosphate (G-6-P), β-nicotinamide adenine dinucleotide phosphate, oxidized form (β-NADP+) and G-6-P dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Osaka, Japan).

17-α-Hydroxy-[1,2(n)-^{3}H]-pregnenolone (1 mCi/mL, 41.9 Ci/mmol) was obtained from Amersham Pharmacia Biotech UK Ltd. (Buckinghamshire, UK). [7-^{3}H (N)]-Pregnenolone (1 mCi/mL, 17.5 Ci/mmol) was obtained from NEN (Waltham, MA, USA). 6-[(7*S*)-7-Hydroxy-6,7-dihydro-5*H*-[^{14}C]pyrrolo[1,2-*c*]imidazol-7-yl]-*N*-methyl-2-naphthamide ([^{14}C]orteronel) with specific radioactivity of 183 μCi/mg was synthesized by Amersham Pharmacia Biotech UK Ltd. (Buckinghamshire, UK).

2.2. Animals

Intact male cynomolgus monkeys were obtained from Siconbrec, Inc. (Tanay, Philippines), Nafovanny Resources Ltd. (Tam-Phuoc, VietNam), Keari Inc. (Wakayama, Japan), Shin Nippon Biomedical Laboratories, Ltd. (Kagoshima, Japan) and China National Scientific Instrument & Materials Import/Export Corporation (Beijing, China), aged 5, 5, 7, 4–7 or 5–7 years, respectively. These monkeys were housed in a temperature-controlled room (26 ± 2 °C) with a 12 h:12 h light/dark cycle (illumination from 6:00 a.m. to 6:00 p.m.) at Shin Nippon Biomedical Laboratories, Ltd. Six castrated male cynomolgus monkeys were also used in specific

studies. These monkeys (aged 6–12 years) were obtained from Keari Inc. and housed in a temperature-controlled room (23 ± 2 °C) with a 12 h:12 h light/dark cycle (illumination from 7:00 a.m. to 7:00 p.m.). All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of the Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited.

2.3. Assay of inhibitory activity on monkey 17,20-lyase/17-hydroxylase

17,20-Lyase inhibitory activity was determined as described [25,31] with some modifications. Adrenals excised from two intact 5-year-old male cynomolgus monkeys were homogenized in 10 mmol/L HEPES buffer (pH 7.4) containing 250 mmol/L sucrose, 25 mmol/L KCl, 0.5 mM EDTA 2 K, 1 mmol/L dithiothreitol, 0.02 mg/mL phenylmethylsulfonyl fluoride and 20% (v/v) glycerol. Adrenal microsomes were then separated by centrifugation and suspended in 50 mmol/L Tris·Cl buffer (pH 7.4) containing 5 mmol/L $MgCl_2$ and 25% (v/v) glycerol. The protein concentration of the microsome fraction was determined using the Bio-Rad protein assay kit (Hercules, CA, USA). To assess steroid biosynthesis, a reaction mixture containing 50 mmol/L Tris–HCl buffer (pH 7.4), 5 μmol/L 17-α-hydroxypregnenolone (Sigma–Aldrich Co. LLC) including equivalent of 0.05 μL/tube 17-α-hydroxy-[1,2(n)-^{3}H]-pregnenolone (1 mCi/mL, 41.9 Ci/mmol), NADPH generating system (0.6 mmol/L β-NADP+, 10 mmol/L glucose-6-phosphate, 5 mmol/L magnesium chloride, 1.5 unit/mL G-6-P dehydrogenase), 2.5% (v/v) propylene glycol, microsomal protein (50 μg/mL, final concentration) and test compounds in a total volume of 200 μL was employed. The reaction mixture was incubated for 120 min at 37 °C. Reaction substrate and products (DHEA, androstenedione) were separated by thin layer chromatography (TLC, Whatman LHPK) in hexane:tetrahydrofuran (4:1) solvent system. Identification of appropriate regions on the TLC plate and measurement of the radioactivity were performed with a BAS 2000 II Bio-image analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan) and enzyme activity was expressed as nmol/h/mg protein.

The assay for 17-hydroxylase activity was similar to that described above. Five μmol/L pregnenolone (ICN Biomedicals) including equivalent of 0.05 μL/tube [7-^{3}H (N)]-pregnenolone (1 mCi/mL, 17.5 Ci/mmol) replaced 17-α-hydroxypregenolone as substrate and the reaction mixture was incubated for 5 min. The substrate and products (17-α-hydroxypregnenolone, 17α-hydroxyprogesterone (17-OHP), DHEA, androstenedione, deoxycortisol) were separated by TLC in cyclohexane:ethyl acetate (3:2) solvent system and activity determined and expressed as noted above.

2.4. Assay of 11-hydroxylase activity

Inhibitory activities on monkey 11-hydroxylase were determined according to methods described elsewhere [32] with some modifications. Adrenals were excised as described above. Adrenal mitochondria were prepared by centrifugation and suspended in 50 mmol/L Tris·Cl buffer (pH 7.4) containing 5 mmol/L $MgCl_2$. The protein concentration of the mitochondrial fraction was determined using the Bio-Rad protein assay kit (Hercules, CA, USA). The reaction mixture contained 50 mmol/L Tris–HCl buffer (pH 7.4), 2 μmol/L 11-deoxycortisol (Pfaltz & Bauer, CT, USA) including equivalent of 0.05 μL/tube hydroxy-11-deoxycorticosterone, [1,2-^{3}H(N)] (NEN, 56.8 Ci/mmol), NADPH generating system (0.6 mmol/L β-NADP+, 10 mmol/L glucose-6-phosphate, 5 mmol/L magnesium chloride, 1.5 unit/mL G-6-P dehydrogenase), 10 mmol/L calcium chloride, 5% (v/v) propylene glycol, mitochondrial protein (50 μg/mL, final concentration) and the test compounds in a total volume of 200 μL. The reaction mixture was incubated for 120 min at 37 °C. The substrate and product (^{3}H-cortisol) were separated in the toluene-acetone (3.5:1) solvent system. All other procedures were performed as above and activity was expressed as nmol/h/mg protein.

2.5. Preparation of monkey adrenal cells for in vitro studies

Adrenals from one 7-year-old male cynomolgus monkey were minced in ice-cold Krebs–Ringer buffer containing glucose and bovine serum albumin solution (KRBGA) and adrenal cells were disaggregated by treatment with KRBGA solution supplemented with DNase I 0.15 mg/mL and collagenase 2 mg/mL at 37 °C with shaking (100 cycles/min). After 50 min, undigested debris was removed by filtration with a cell strainer (Nylon, 70 μm). The resulting cell suspension was kept on ice for 10 min and then centrifuged at 112 RCF for 5 min. The cells were washed three times with KRBGA solution at room temperature, resuspended in Dulbecco's modified Eagle's medium (DMEM) at a density of 100,000 cells/mL, and seeded in 48-well plates (1 mL/well) at room temperature. Varying concentrations of orteronel (0.1–100,000 nmol/L) or ketoconazole (0.1–100,000 nmol/L) were then added. After 10 min of preincubation at 37 °C, the cells were stimulated with adrenocorticotropic hormone (ACTH, 10 nmol/L) for 3 h at 37 °C to induce steroid hormone production. Steroid production was terminated by heating the cells at 60 °C for 30 min, and the supernatant was collected by centrifugation (112 RCF at 4 °C for 5 min) and stored at −30 °C until assayed by RIA.

2.6. Pharmacokinetic study

[^{14}C]orteronel was suspended in 0.5% methylcellulose solution for oral administration to fed monkeys at a dose of 1 mg (183 μCi)/kg. For intravenous dosing, [^{14}C]orteronel was dissolved in a mixture of dimethyl acetamide and physiological saline (1:4 by vol.) for injection at a dose of 0.5 mg (91 μCi)/kg (weight, 2.38–3.08 kg). At 5, 10 (intravenous dosing only), 15, 30 min, and 1, 2, 3, 4, 6, 8, and 24 h after drug administration, blood was taken from the femoral vein and centrifuged to obtain plasma that was then frozen at −20 °C until analysis. To quantify plasma [^{14}C]orteronel, plasma was extracted with methanol (5 vol.) and then evaporated to dryness under a nitrogen gas stream at room temperature. The residue was dissolved in 80% (by vol.) aqueous methanol. The solution components were separated using TLC plates pre-coated with silica gel 60F254 (0.25-mm thick; E. Merck, Darmstadt, Germany), which were developed one-dimensionally in chloroform–methanol–28% ammonia water (10:4:0.2, by vol.). After development, the radioactive regions on the plate were located by radioluminography using a Bio-image Analyzer (BAS-2000 or BAS-2000II; Fuji Photo Film Co., Ltd., Tokyo, Japan) and imaging plate (BAS III; Fuji Photo Film Co., Ltd.), or by the UV absorption of orteronel added to the test samples as internal standards, or both. The silica gel sections corresponding to orteronel were scraped off the plate, the radioactivity of each section determined by liquid scintillation (LSC-5100 and LSC-6100, Aloka Co., Ltd., Tokyo, Japan; LS-6000, Beckman Coulter, Inc., CA, USA; and 3100TR, Packard Instrument Co., Inc., IL, USA), and the concentration of orteronel was calculated from the specific radioactivity. Values for maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were calculated directly from the data. Half life ($t_{1/2}$) in the plasma and area under the plasma concentration time curve (AUC) were calculated by the linear regression analysis and trapezoidal rule, respectively, using Microsoft Excel' 95. The bioavailability (BA) was determined after dose normalization. The pharmacokinetic parameters for [^{14}C]orteronel in the plasma were expressed as the

mean values or the mean values with standard deviations (SD) for the results of three animals, unless otherwise indicated.

2.7. Dosing and blood sampling in pharmacodynamic studies

In all oral dosing experiments, serum DHEA and testosterone were measured by RIA (as described above) before dosing to establish baseline values [25].

In the single dose experiment, 20 monkeys were randomly distributed into 5 groups ($n = 4$). Each group received a different dose of orteronel or vehicle (0.5% methylcellulose, 3 mL/kg). For oral administration, orteronel was suspended in 0.5% methylcellulose and administered at doses of 0.3, 1, 3, or 10 mg/kg at 10 a.m. Blood samples were collected 48, 24 h, and immediately before dosing, and 2, 5, 10, 24, and 48 h after dosing and the serum was stored at −30 °C.

For the multiple dose experiment, 20 monkeys were randomly distributed into 4 groups ($n = 5$). Each group received a different orteronel dose. Orteronel was suspended in 0.5% methylcellulose and administered orally for 7 days at doses of 3, 7.5, and 15 mg/kg twice daily at about 10 a.m. and 10 p.m. for the first 6 days, and once at about 10 a.m. on the last day; one group received vehicle (0.5% methylcellulose, 3 mL/kg) only. Blood samples were collected twice daily (at approximately 2 p.m. and 7 p.m., to compensate for circadian variations) from 3 days before the onset of dosing until 9 h after the final dose. After collection, the serum was stored at −30 °C.

A multiple dosing study was also conducted in castrated male monkeys. For this study, six castrated monkeys were divided into 2 groups ($n = 3$), with each group receiving a different orteronel dose. Orteronel was suspended in 0.5% methylcellulose and administered orally (3 mL/kg) for 7 days at either 7.5 or 15 mg/kg twice daily (about 8 a.m. and 8 p.m.) for the first 6 days and once at about 8 a.m. on the final day. Blood samples were collected twice daily (at approximately 12 p.m. and 5 p.m.) from 3 days before the onset of dosing until 9 h after the final administration of orteronel. Approximately 1 month following the final orteronel dose, five of the six castrated monkeys received vehicle alone (0.5% (w/v) methylcellulose solution, 3 mL/kg) by the same administration regimen employed to administer orteronel, and whole blood was collected and serum processed as described during orteronel dosing. As such, baseline data were collected 1 month following orteronel dosing to avoid the possibility that oral administration alone affects the hormonal circadian patterns. Steroid concentrations in all serum samples were measured by RIA as described previously.

2.8. Culture of human tumor cells

Human adrenocortical tumor cell line NCI-H295R was purchased from American Type Culture Collection (Manassas, VA), and cultured in DMEM/F-12 medium (Life Technologies, Grand Island, NY) supplemented with 2.5% Nu-Serum I (Collaborative Biomedicals, Bedford, MA) and 1% ITS-plus (BD Biosciences, Bedford, MA).

2.9. Expression of human CYP17A1

Human CYP17A1 (17-hydroxylase/17,20-lyase) was expressed in *Escherichia coli* with N-terminal sequence modification (MALLLAVF) as described previously [33]. The vector pCWori was obtained as a generous gift from Dr. FW Dahlquist (University of Oregon, Eugene, OR). The membrane fraction prepared from *E. coli* expressing human 17,20-lyase was used for the following assay.

2.10. Assay of inhibitory activity on human 17,20-lyase/17-hydroxylase

Inhibitory activities on the human enzyme were determined as described in Section 2.3. The reaction mixture contained 50 mmol/L potassium phosphate buffer (pH 7.4), 50 pmol/mL of recombinant human CYP17A1, 50 pmol/mL of recombinant cytochrome b5 (Pan Vera, Madison, WI), 50 μunit/mL of recombinant NADPH-cytochrome P450 reductase (Pan Vera), 5 μmol/L 17-α-hydroxypregnenolone (Sigma–Aldrich Co. LLC) including equivalent of 0.05 μL/tube 17-α-hydroxy-[1,2(n)-^{3}H]-pregnenolone (1 mCi/mL, 41.9 Ci/mmol), NADPH generating system (0.5 mmol/L β-NADP+, 5 mmol/L G-P-6, 5 mmol/L magnesium chloride, 1.5 unit/mL G-6-P dehydrogenase), and the test compound in a total volume of 200 μL. The reaction mixture was incubated for 60 min at 37 °C. The substrate and product (DHEA) were separated in cyclohexane:ethyl acetate (3:2) solvent system. Identification of appropriate regions on the TLC plate and measurement of the radioactivity were performed with a BAS 2000 II Bio-image analyzer and enzyme activity was expressed as nmol/h/mg protein.

The assay for 17-hydroxylase activity was similar to that described above. Five μmol/L pregnenolone (ICN Biomedicals, Solon, OH) including equivalent of 0.05 μL/tube [7-^{3}H (N)]-pregnenolone (1 mCi/mL, 17.5 Ci/mmol) was used as the substrate. The reaction mixture was incubated for 120 min. The substrate and products (17-α-hydroxypregnenolone, DHEA) were separated by TLC in the same solvent system and activity determined and expressed as noted above.

2.11. Steroid production by human cells

NCI-H295R cells were seeded in 24-well plates at a density of 50,000 cells/0.5 mL of DMEM/F-12 medium supplemented with 2.5% Nu-Serum I and 1% ITS-plus/well at day 0, and cultured at 37 °C in 5% CO_2/atmosphere. At day 3, the medium was changed to a new DMEM/F-12 medium supplemented with 2.5% Nu-Serum I, 1% ITS-plus and 20 μmol/L forskolin (Wako Pure Chemical Industries, Osaka, Japan). At day 5, after the cells were washed with 500 μL of DMEM/F-12 medium supplemented with 1% ITS-plus twice, 500 μL of DMEM/F-12 medium containing 1% ITS-plus, 20 μmol/L forskolin and varying concentrations of the compounds (orteronel and ketoconazole: 3–3000 nmol/L, abiraterone: 0.3–300 nmol/L) were added to the cells. The medium was collected 24 h later and stored at −30 °C until assayed by RIA.

2.12. Determination of steroid concentrations by RIA

Concentrations of testosterone, androstenedione, 17-hydroxyprogesterone, progesterone, DHEA, cortisol, corticosterone, and aldosterone were assayed with a Testosterone I-125 kit (Dia Sorin s.r.l., Saluggia, Italy), an Androstenedione RIA kit (Diagnostics System Laboratories, Webster, TX), a DPC 17α-OH progesterone kit (Diagnostic Products Corporation, Los Angeles, CA), a progesterone RIA kit (bioMérieux, Craponne, France, or cisbio international, Bagnols-sur-Cèze, France), a DHEA RIA kit (Diagnostics System Laboratories), an Amerlex cortisol RIA kit (Ortho Clinical Diagnostics, Amersham, UK), a [^{125}I] Rat corticosterone assay system (Amersham), and an aldosterone RIA kit II (Dainabott, Tokyo, Japan), respectively, according to the manufacturer's directions.

2.13. Statistics

For in vitro studies, the concentrations of orteronel estimated to induce 50% suppression of the production of testosterone,

androstenedione, DHEA, cortisol, corticosterone, or aldosterone (IC_{50} ± 95% confidence interval [CI]) were calculated by linear regression analysis over the descending linear portion of the log dose–response curves. As serum steroid concentrations are highly variable among individuals, show circadian variations, and are affected by stress, serum steroid concentrations were expressed as percentages of the mean pretreatment values (mean of values 48, 24 h, and immediately before dosing) in single oral dosing experiments. In multiple oral dosing experiments using intact male monkeys, statistical significance of the difference in steroid levels between vehicle- and orteronel-treated groups at 2 pm on the last treatment day was analyzed by Dunnett's test (where the variance is homogenous) or Steel's test (where the variance is heterogenous). *P*-Values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Enzyme inhibition

Orteronel inhibited monkey 17,20-lyase and 17-hydroxylase activities with IC_{50} values of 27 (95% CI: 24–32) and 38 (95% CI: 30–48) nmol/L, respectively (Table 1). For comparison, the IC_{50} values of abiraterone in the same assays were 16 (95% CI: 14–18) and 25 (95% CI: 23–28) nmol/L, respectively. IC_{50} values of ketoconazole, which has been used 'off-label' to inhibit adrenal androgen production clinically [4], were 750 (95% CI: 560–1100) and >10,000 nmol/L, respectively. These findings reveal that in this model system, both orteronel and abiaterone possess comparable inhibitory activities against 17,20-lyase and 17-hydroxylase and both agents are more potent inhibitors than ketoconazole. For monkey 11-hydroxylase, a key downstream enzyme that is proximal in corticosterone as well as cortisol biosynthesis, 50% inhibition was not achieved with orteronel concentrations of up to 10,000 nmol/L (Table 1) while IC_{50} values of abiraterone and ketoconazole were 600 (95% CI: 480–760) and 270 (95% CI: 190–380) nmol/L, respectively.

Orteronel inhibited human 17,20-lyase and 17-hydroxylase activities with IC_{50} values of 140 (95% CI: 120–170) and 760 nmol/L, respectively (Table 1). The IC_{50} values of abiraterone in the same assays were 27 (95% CI: 24–30) and 30 (95% CI: 27–34) nmol/L, respectively. IC_{50} values of ketoconazole were 110 (95% CI: 88–130) and 580 (95% CI: 530–640) nmol/L, respectively. Although abiraterone showed the most potent inhibitory activity for human 17,20-lyase and 17-hydroxylase, orteronel showed higher specificity of inhibition for human 17,20-lyase vs 17-hydroxylase in comparison with abiraterone. Ketoconazole demonstrated more potent inhibitory activity towards human 17,20-lyase compared with monkey 17,20-lyase and showed some specificity between human 17,20-lyase and 17-hydroxylase. However, it potently inhibited monkey 11-hydroxylase as well (Table 1).

In summary, orteronel inhibited the key target enzyme 17,20-lyase with similar potency to abiraterone, while both abiraterone and ketoconazole more potently inhibited the off-target steroidogenic enzyme 11-hydroxylase than did orteronel.

3.2. Effects of orteronel and ketoconazole on steroid production in monkey adrenal cells

In monkey adrenal cells, pretreatment with orteronel suppressed the ACTH stimulated production of DHEA and androstenedione with IC_{50} values of 110 nmol/L (95% CI: 90–140 nmol/L) and 130 nmol/L (95% CI: 96–170 nmol/L), respectively (Fig. 2A). Cortisol and aldosterone production were also suppressed by orteronel, with IC_{50} values of 310 nmol/L (95% CI: 190–450 nmol/L) and 4400 nmol/L (95% CI: 3600–5500 nmol/L), respectively (Fig. 2A). Orteronel increased the production of corticosterone and progesterone in a concentration-dependent manner (Fig. 2B and C) while 17-OHP production was suppressed by orteronel at concentrations >100 nmol/L (Fig. 2D). These findings support the contention that orteronel has weaker inhibitory activity for 11-hydroxylase and aldosterone synthase than for 17,20-lyase in this monkey model.

We also evaluated the effects of ketoconazole in this model system. Ketoconazole suppressed production of DHEA, androstenedione, cortisol, and aldosterone with IC_{50} values of 340 nmol/L (95% CI: 290–400 nmol/L), 580 nmol/L (95% CI: 450–750 nmol/L), 340 nmol/L (95% CI: 240–480 nmol/L), and 350 nmol/L (95% CI: 290–410 nmol/L), respectively (Fig. 3A). Corticosterone production also was inhibited by ketoconazole with an IC_{50} value of 1500 nmol/L (95% CI: 1000–2600 nmol/L; Fig. 3B). The production of 17-OHP was markedly increased by ketoconazole at a concentration of 1000 nmol/L, but decreased in a concentration-dependent manner between 3000 and 10,000 nmol/L (Fig. 3C). Progesterone production peaked with ketoconazole 1000 nmol/L, but decreased at higher concentrations (Fig. 3D). These findings suggest that in addition to inhibiting monkey 17,20-lyase, ketoconazole also inhibits other enzymes such as 11- or 21-hydroxylase and side chain cleavage enzyme (SCC) at higher concentrations. Taken together, these data indicate that, in this primate model, orteronel is more potent than ketoconazole and also possesses greater specificity at inhibiting CYP17.

3.3. Pharmacokinetics

[^{14}C]orteronel was administered orally to intact monkeys for pharmacokinetic analysis. When a 1 mg/kg dose was administered, the T_{max}, C_{max}, $t_{1/2}$ and $AUC_{0-24\,h}$ were observed to be 1.7 h, 0.147 μg/mL, 3.8 h and 0.727 μg h/mL, respectively (Table 2). Collectively, orteronel showed high BA and reasonable $t_{1/2}$ which are important parameters for oral dosing. Considering that concentrations >300 nmol/L (>0.1 μg/mL) were observed to be required to inhibit DHEA production in vitro in monkey adrenal cells (Fig. 2A), twice-daily oral dosing at 5–15 mg/kg was predicted to give minimum trough orteronel levels to maintain 17,20-lyase inhibition and produce a substantial reduction of serum DHEA levels (assuming linear pharmacokinetic characteristics).

3.4. Impact of single dose orteronel on serum DHEA, cortisol, and testosterone levels in intact male monkeys

In vehicle-treated animals, serum DHEA was found to decrease continuously after the 10 a.m. (0 h after treatment) blood sampling until it reached its nadir at approximately 8 p.m. (10 h after treatment) and thereafter increased in a pattern thought to reflect an intrinsic circadian variation (Fig. 4A [34]). After single oral dosing of orteronel (0.3–10.0 mg/kg), serum DHEA levels decreased rapidly and in a dose-dependent manner in the 10 h post-dosing period and remained suppressed (vs control) at 24 h post-dosing (Fig. 4A). Serum DHEA levels in all orteronel groups returned towards those seen in the vehicle-treated animals by 48 h after drug dosing (Fig. 4A).

Serum cortisol levels in the vehicle-treated group showed a similar circadian pattern to that observed for DHEA (Fig. 4A and B [35]). After oral dosing of orteronel (0.3–10.0 mg/kg), serum cortisol levels also decreased rapidly and in a general dose-dependent manner in the 10 h period after drug administration (Fig. 4B). The magnitude of decrease in cortisol was, however, smaller than that observed for DHEA and serum cortisol levels in all drug treated groups returned towards that seen in the vehicle-control group 24 h after dosing (Fig. 4B).

The circadian variation in serum testosterone was out of phase from that seen for serum DHEA or cortisol (Fig. 4A–C),

Table 1
Inhibition of 17,20-lyase, 17-hydroxylase, and 11-hydroxylase in different species by the 17,20-lyase inhibitors orteronel, abiraterone, and ketoconazole.

Species	Enzyme	IC_{50} (95% CI) (nmol/L)		
		Orterenol	Abiraterone	Ketoconazole
Human	17,20-Lyase	140 (120–170)	27 (24–30)	110 (88–130)
	17-Hydroxylase	760 (640–910)	30 (27–34)	580 (530–640)
Monkey	17,20-Lyase	27 (24–32)	16 (14–18)	750 (560–1100)
	17-Hydroxylase	38 (30–48)	25 (23–28)	>10,000
	11-Hydroxylase	>10,000	600 (480–760)	270 (190–380)

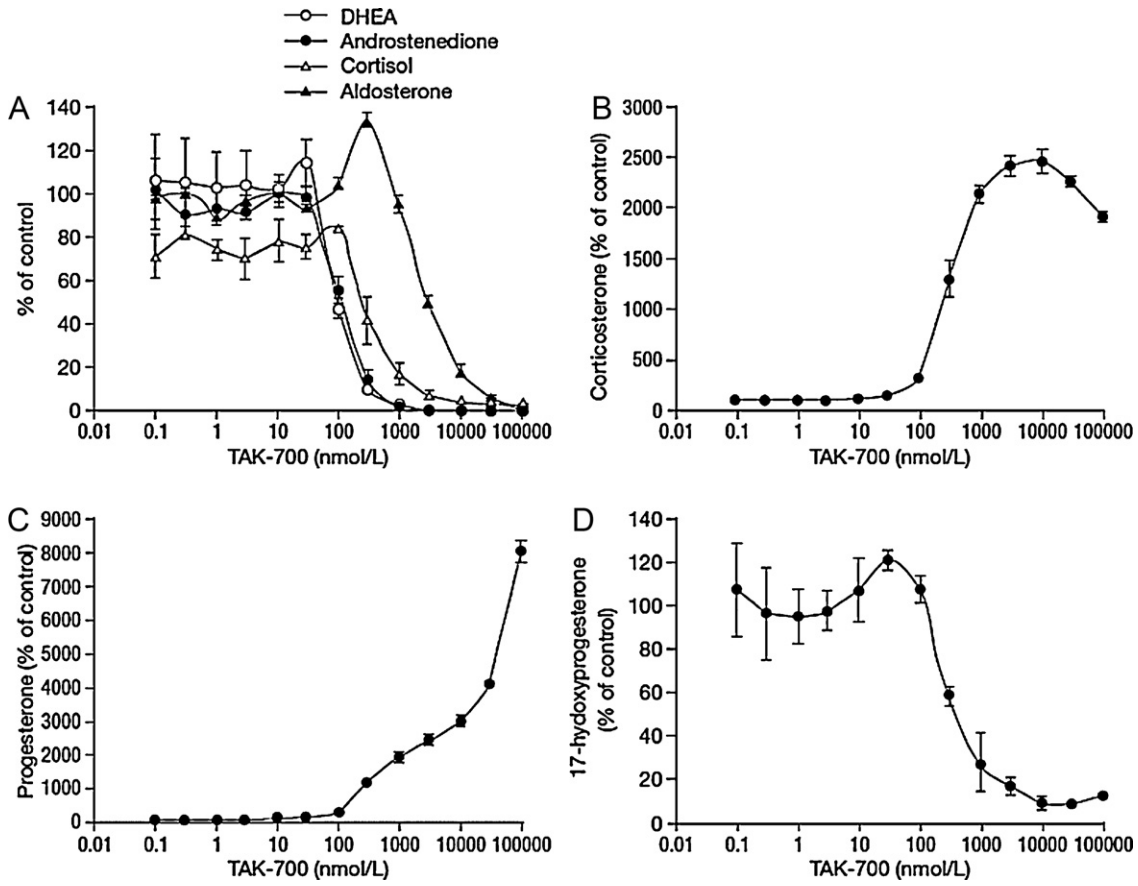

Fig. 2. Effects of orteronel on ACTH-stimulated production of steroid hormones in monkey adrenal cells. Orteronel was added 10 min before adrenocorticotropic hormone (ACTH) stimulation. The concentrations of dehydroepiandrosterone (DHEA), androstenedione, cortisol, and aldosterone (A), corticosterone (B), progesterone (C), and 17α-hydroxyprogesterone (17-OHP) (D) in conditioned media 3 h after ACTH stimulation were determined by radioimmunoassay (RIA). Mean ± standard error of the mean (SEM [$n = 3$]).

presumably reflecting the fact that different pituitary hormones, namely ACTH or luteinizing hormone (LH), govern secretion patterns of DHEA/cortisol or testosterone, respectively [34,36]. In the vehicle-treated group, the highest testosterone level was observed at approximately 8 p.m., 10 h after dosing (Fig. 4C). After oral dosing with orteronel (0.3–10.0 mg/kg), serum testosterone levels decreased rapidly and reached their nadir (for the 3 and 10 mg/kg doses) or were maintained at low levels (for the 1 mg/kg dose) 10 h after dosing, corresponding to the period when the highest testosterone level was observed in the vehicle-treated group (Fig. 4C).

3.5. Impact of multiple dosing of orteronel on steroid hormone levels in intact male monkeys

As observed in the single dose studies, serum DHEA levels in the vehicle-treated group showed a clear circadian pattern before and during the treatment period in all groups (Fig. 5B). However, under

Table 2
Selected pharmacokinetic data of orteronel in monkeys.

Species	Dosage route	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	$t_{1/2}$ (h)	$AUC_{0-24\,h}$ (μg h/mL)	Bioavailability (%)
Monkey	Oral	1	1.7 ± 0.6	0.147 ± 0.049	3.8 ± 0.4	0.727 ± 0.162	70.5 ± 9.6
	IV	0.5	–	–	ND	0.516 ± 0.096	–

Mean ± SD ($n = 3$).
AUC: area under the curve; C_{max}: maximum plasma concentration; IV: intravenous; $t_{1/2}$: half life; SD: standard deviation; T_{max}: time to reach maximum plasma concentration; ND: not determined.

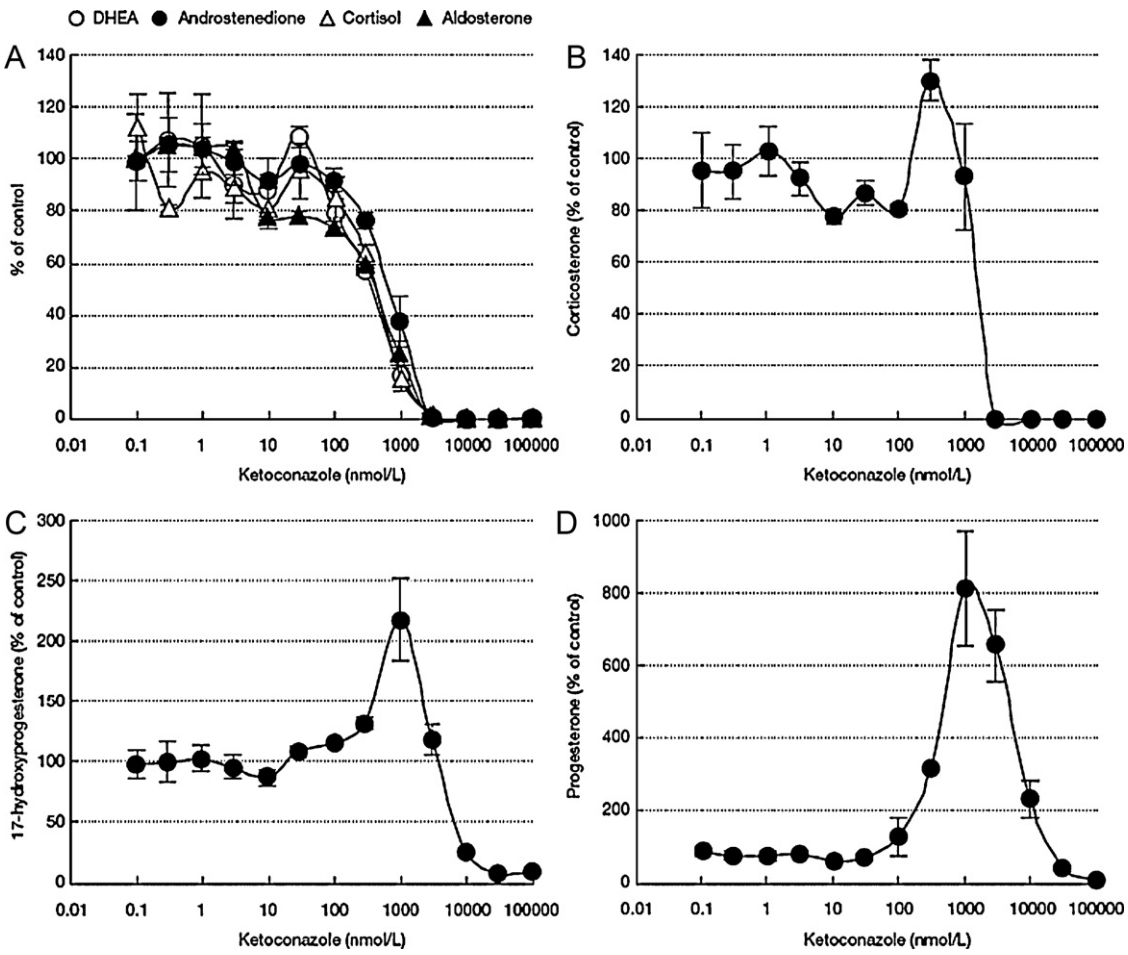

Fig. 3. Effects of ketoconazole on ACTH-stimulated production of steroid hormones in monkey adrenal cells. Ketoconazole was added 10 min before adrenocorticotropic hormone (ACTH) stimulation. The concentrations of dehydroepiandrosterone (DHEA), androstenedione, cortisol, and aldosterone (A), corticosterone (B), 17α-hydroxyprogesterone (17-OHP) (C), and progesterone (D) in conditioned media 3 h after ACTH stimulation were determined by radioimmunoassay (RIA). Mean ± standard error of the mean (SEM [$n = 3$]).

this twice-daily × 7 day administration regimen the magnitude of the circadian variation was noticeably reduced and stayed at low levels throughout the treatment period (Fig. 5A and B). Mean serum DHEA levels during the treatment period in the groups administered 3, 7.5 and 15 mg/kg twice daily vs the mean serum DHEA levels before treatment were 17.0%, 8.1% and 6.5%, respectively (Fig. 5B), and this effect appeared to be maintained through the treatment period (Fig. 5A and B). The difference in DHEA levels between the vehicle group and each of the orteronel treatment groups at 2 p.m. on the last treatment day was statistically significant (Fig. 5A). Of interest, DHEA levels in the vehicle control group were also reduced slightly as a consequence of this treatment regimen.

As expected, serum cortisol in the vehicle-treated group also showed a circadian pattern before and during the treatment period (Fig. 5D). Cortisol levels in the groups treated with orteronel (3, 7.5, or 15 mg/kg twice daily) showed similar circadian rhythms before and after the onset of treatment, although absolute serum levels and magnitude of circadian variation were reduced at treatment onset and remained reduced throughout the treatment period (Fig. 5C and D). Mean serum cortisol in the groups treated with 3, 7.5, and 15 mg/kg orteronel twice daily vs the mean serum cortisol levels before the onset of the treatment were 46.5%, 28.1%, and 23.6%, respectively, and were reduced by a smaller degree than that observed for serum DHEA under these conditions (Fig. 5A and C). The difference in cortisol levels between the vehicle group and each of the orteronel treatment groups at 2 p.m. of the last treatment day was statistically significant (Fig. 5C). Again there was a slight reduction in serum cortisol as a function of vehicle administration by this regimen. Serum testosterone in the vehicle-treated group also revealed a circadian variation (Fig. 5F [37]), although it was not as striking as that seen in serum DHEA (Fig. 5B). Consistent with the results of the single oral dosing experiment, orteronel treatment at all doses decreased serum testosterone levels potently on the first day (day 0) of treatment (Fig. 5E). However, unlike the effects observed in serum DHEA, the effectiveness of testosterone reduction appeared to be reduced as a function of time during the drug dosing regimen (Fig. 5E), an effect that may reflect activation of the hypothalamus–pituitary–gonadal axis under these conditions (Fig. 5E and F [38,39]). Only the difference in testosterone levels between vehicle group and orteronel 7.5 mg/kg treatment group at 2 p.m. on the last treatment day was statistically significant (Fig. 5E).

3.6. *Impact of multiple dosing of orteronel on serum DHEA, cortisol and testosterone levels in castrated male monkeys*

Castration eliminates testicular testosterone but has no effect on DHEA and cortisol levels and it is this DHEA, generated by the 17,20-lyase activity of CYP17A1, that can be converted into testosterone outside the adrenals. Consequently, castration has no effect on this extra-gonadal source of testosterone. By inhibition of 17,20-lyase activity, orteronel is predicted to be effective at reducing DHEA levels and disrupting this extra-gonadal source of testosterone. To

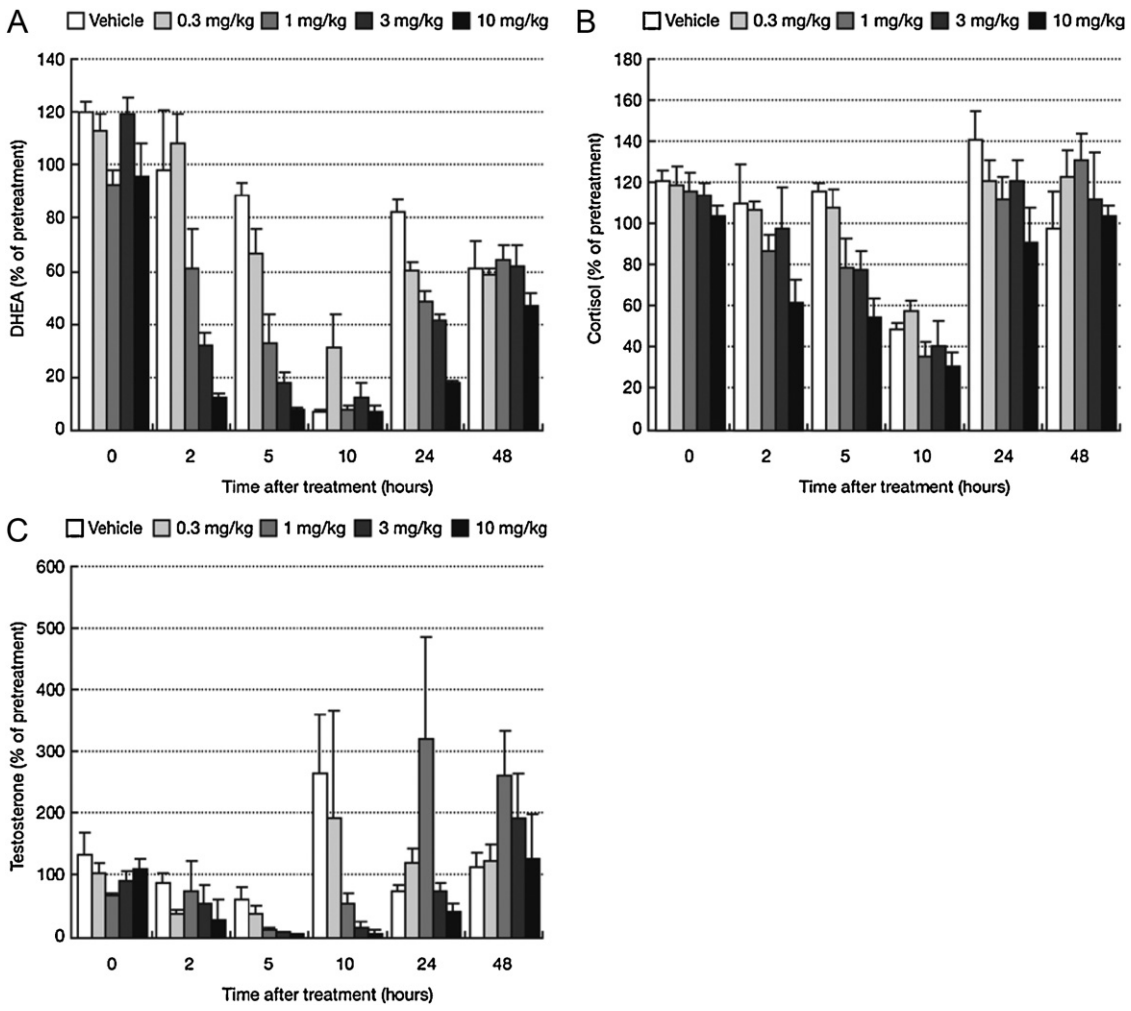

Fig. 4. Impact of single oral dosing of orteronel in male monkeys. Impact of orteronel on serum dehydroepiandrosterone (DHEA) (A), cortisol (B), and testosterone (C) levels. Serum steroid levels were determined by radioimmunoassay (RIA) and were expressed as percentages of the mean pretreatment values. Each value represents mean ± standard error of the mean (SEM [$n = 4$]).

assess this possibility we chose to evaluate the impact of orteronel on hormone levels in castrated male monkeys since this model is thought to closely mimic the hormonal milieu found in surgically or medically castrated CRPC patients.

In multiple dosing experiments (twice-daily × 7 days) in castrated monkeys, serum DHEA levels were markedly reduced with the onset of treatment with orteronel (7.5 and 15 mg/kg, twice daily) by 91.0% and 93.1%, respectively, vs DHEA levels before treatment. Further, in castrated monkeys, these reduced DHEA levels were maintained throughout the treatment period (Fig. 6A). When the same monkeys subsequently received vehicle treatment, serum DHEA levels showed a clear circadian pattern similar to that observed in the multiple dosing study in intact animals, before and during the vehicle treatment period (Figs. 5B and 6A).

In this model, serum cortisol levels were also reduced with the onset of treatment with orteronel 7.5 and 15 mg/kg (twice daily). Mean serum cortisol levels of the groups treated with orteronel 7.5 and 15 mg/kg twice daily were reduced by 74.0% and 82.7%, respectively, vs mean serum cortisol levels before the onset of treatment and these orteronel-induced low levels were maintained throughout the treatment period (Fig. 6C). In vehicle control animals, as expected, circadian patterns of cortisol during vehicle treatment were similar to that observed in the multiple dosing experiment using intact male monkeys (Figs. 5D and 6C).

Importantly, castrate levels of serum testosterone were further reduced with the onset of treatment with orteronel (7.5 or 15 mg/kg twice daily). Furthermore, these very low testosterone levels (0.2–0.3 ng/mL) were maintained throughout the treatment period unlike that observed in intact monkeys (Fig. 6E). This maintained effectiveness under these conditions may reflect the fact that, in contrast to intact monkeys, serum testosterone in castrated monkeys is not of testis origin and not under the control of hypothalamus-pituitary-gonadal axis. As expected, when the same monkeys subsequently received vehicle treatment, serum testosterone levels ranged between 0.2 and 0.8 ng/mL (Fig. 6F) and showed a circadian pattern similar to that seen in serum DHEA levels before and during the treatment period (Fig. 6B).

3.7. Impact on steroid production by human adrenocortical tumor line H295R cells

The human adrenocortical tumor cell line NCI-H295R produces mineralocorticoid, glucocorticoid and adrenal androgen [40]. Orteronel potently inhibited DHEA production in NCI-H295R cells with an IC_{50} of 37 nmol/L (95% CI: 31–42) (Fig. 7A). This was comparable with the IC_{50} for 17,20-lyase enzyme inhibition in our assay system (Table 1). Orteronel also inhibited cortisol production with an IC_{50} of 990 nmol/L (95% CI: 720–1400). Thus, the potency for inhibition of DHEA production is 27-fold higher than for cortisol,

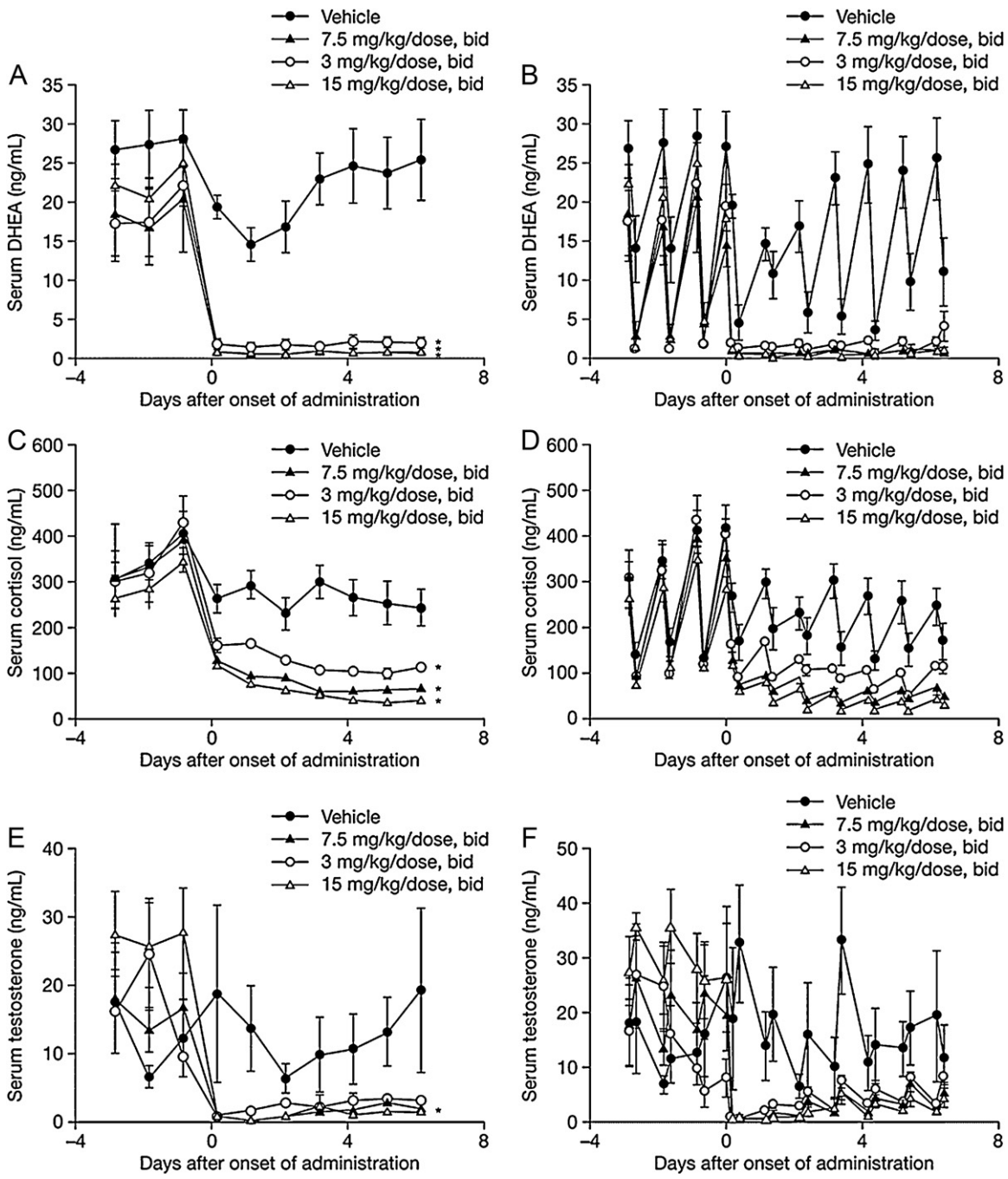

Fig. 5. Impact of multiple oral dosing of orteronel in male cynomolgus monkeys. Impact of orteronel on serum levels of dehydroepiandrosterone (DHEA) (A, B), cortisol (C, D), and testosterone (E, F). Dosing and sampling is described in Materials and Methods. Serum levels of DHEA, cortisol, and testosterone were determined by specific radioimmunoassay (RIA). Only the steroid levels at 2 pm are shown in A, C and E. Each value represents mean ± standard error of the mean (SEM [$n = 5$]). bid: twice daily, $^{*}P \leq 0.05$ vs the vehicle control group by Steel's test.

suggesting some specificity between DHEA and cortisol production. This might be partly explained by the fact that orteronel inhibited 17,20-lyase activity 5.4 times more potently than 17-hydroxylase activity in cell-free enzyme assays, even though the single enzyme has both 17,20-lyase and 17-hydroxyase activity [41]. This selectivity might be due to the difference between 17-hydroxylase and 17,20-lyase activities, as suggested by studies showing the existence of a partial congenital deficiency that lacks only 17,20-lyase activity [42], and the requirement of cytochrome b5 for 17,20-lyase activity through a non-electron transfer mechanism [43].

Orteronel demonstrated dose-dependent inhibition of androstenedione production, another adrenal androgen, with an IC_{50} of 54 nmol/L (95% CI: 48–61) (Fig. 8A). The dose-inhibition curve was similar to that for DHEA. Orteronel inhibited 17-hydroxyprogesterone production in a dose-dependent manner with an IC_{50} of 1700 nmol/L (95% CI: 1100–4000) (Fig. 8A). There was a tendency for orteronel to increase 17-hydroxyprogesterone production at concentrations <100 nmol/L. This again suggests some specificity of inhibition between 17,20-lyase and 17-hydroxylase activities.

Progesterone production increased in a dose-dependent manner with addition of orteronel, possibly through inhibition of 17,20-lyase and 17-hydroxylase activities (Fig. 9A). Aldosterone production also increased in a dose-dependent manner with

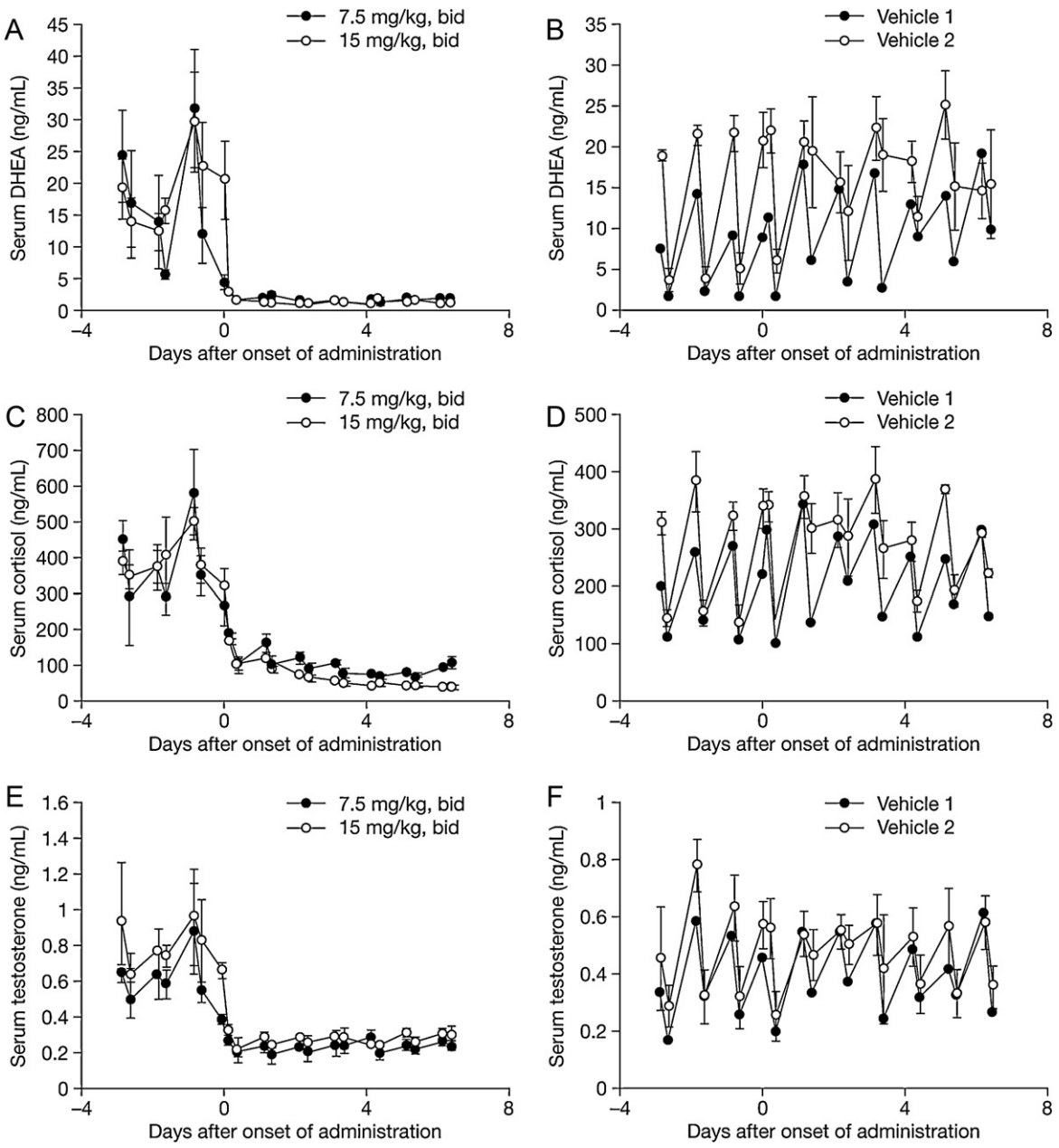

Fig. 6. Impact of multiple oral dosing of orteronel in castrated male cynomolgus monkeys. Impact of orteronel on serum dehydroepiandrosterone (DHEA) (A), cortisol (C), and testosterone (E) levels. Serum DHEA (B), cortisol (D), and testosterone (F) levels in castrated male cynomolgus monkeys treated with vehicle following multiple oral dosing of orteronel. Serum levels of DHEA, cortisol, and testosterone were determined by specific radioimmunoassay (RIA). The monkeys in the vehicle 1 group had been previously treated with orteronel 7.5 mg/kg twice daily (bid). The monkeys in the vehicle 2 group had previously received 15 mg/kg bid. Each value represents mean ± standard error of the mean (SEM [$n = 3$]), except vehicle 1 group (mean, $n = 2$); one monkey was omitted from the vehicle treatment experiment due to poor health.

addition of orteronel, and then tended to decrease slightly above 3000 nmol/L (Fig. 9A). The accumulation of progesterone is likely responsible for this observed increase. The increase in aldosterone production is unlikely to occur in vivo since different layers in adrenal glands are responsible for aldosterone, cortisol, and DHEA production, and zona glomerulosa, which primarily produce aldosterone, contain lower levels of 17,20-lyase and 17-hydroxylase than zona fasciculata and zona reticularis [44]. In NCI-H295R cells, each cell is able to produce all three steroids. All the changes in steroid production seen with orteronel could be explained by its inhibitory activity on 17,20-lyase and 17-hydroxylase.

Abiraterone inhibited DHEA and cortisol production with IC_{50} values of 2.7 (95% CI: 2.5–2.9) and 23 (95% CI: 17–37) nmol/L (Fig. 7B). Although the potency of abiraterone to inhibit DHEA production is higher than that of orteronel, the ratio of IC_{50} values for inhibition of DHEA:cortisol production was slightly lower than that with orteronel (8.5 vs 27). Abiraterone inhibited androstenedione production in a dose-dependent manner, with an IC_{50} of 2.9 nmol/L (95% CI: 2.7–3.1) (Fig. 8B), which was similar to that for DHEA production, and also inhibited 17-hydroxyprogesterone production in a dose-dependent manner, with an IC_{50} of 68 nmol/L (95% CI: 46–110), which was similar to that for cortisol production (Fig. 8B). Abiraterone increased progesterone production in a dose-dependent manner, possibly through inhibition of 17,20-lyase and 17-hydroxylase activities (Fig. 9B). Aldosterone production also increased in a dose-dependent manner with addition of abiraterone

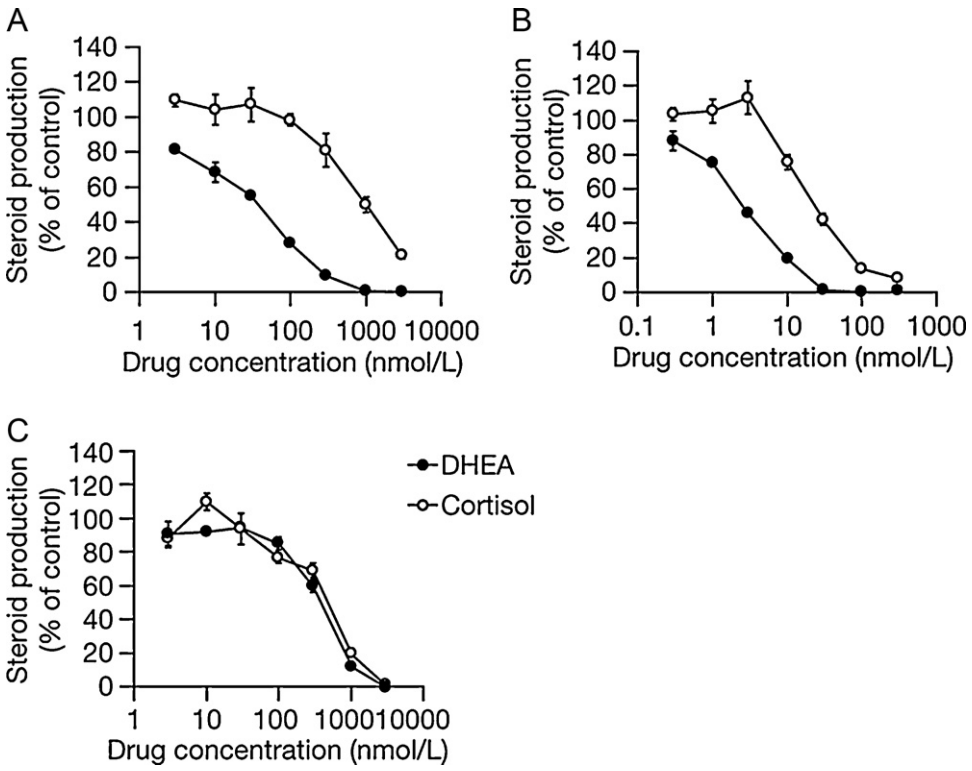

Fig. 7. Impact of orteronel on DHEA and cortisol production in human tumor cells. Inhibition of DHEA and cortisol production in NCI-H295R cells by the 17,20-lyase inhibitors (A) orteronel, (B) abiraterone, and (C) ketoconazole. Steroid production expressed as percentage of mean control (no drug added) value. Each point represents mean ± SEM, $n = 3$.

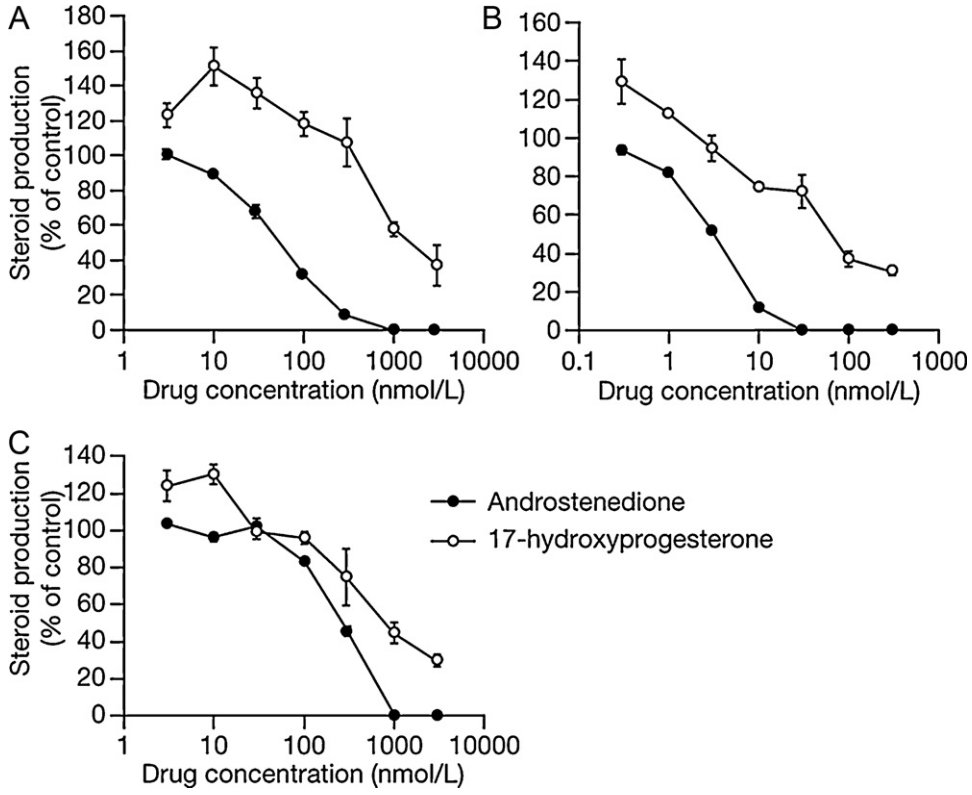

Fig. 8. Impact of orteronel on androstenedione and 17-hydroxyprogesterone production in human tumor cells. Inhibition of androstenedione and 17-hydroxyprogesterone production in NCI-H295R cells by the 17,20-lyase inhibitors (A) orteronel, (B) abiraterone, and (C) ketoconazole. Steroid production expressed as percentage of mean control (no drug added) value. Each point represents mean ± SEM, $n = 3$.

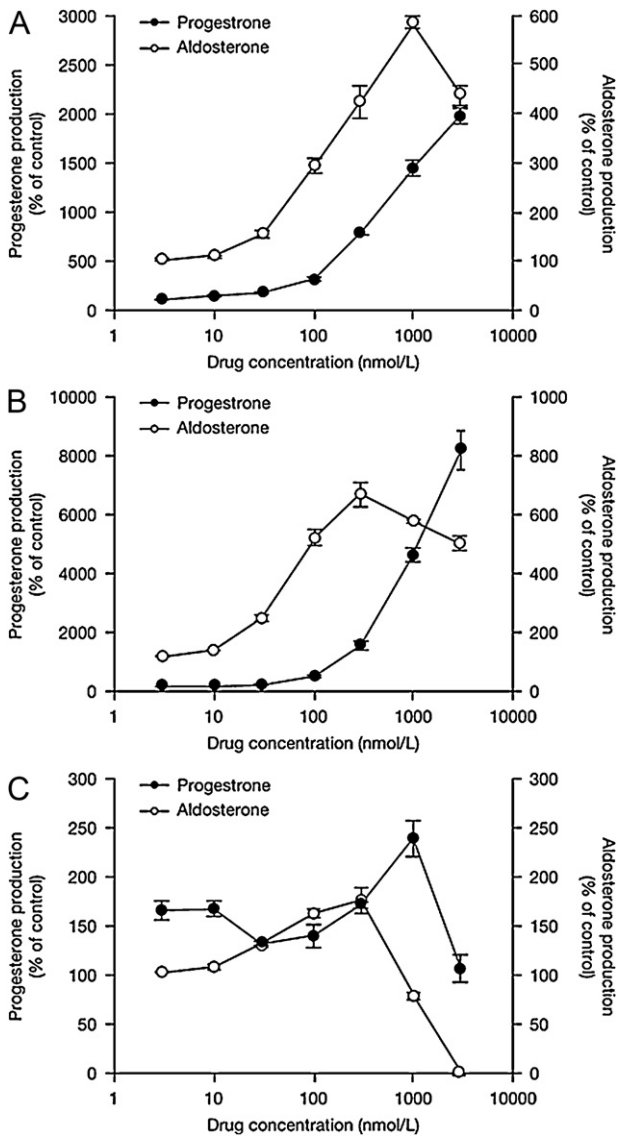

Fig. 9. Impact of orteronel on progesterone and aldosterone production in human tumor cells. Inhibition of progesterone and aldosterone production in NCI-H295R cells by the 17,20-lyase inhibitors (A) orteronel, (B) abiraterone, and (C) ketoconazole. Steroid production expressed as percentage of mean control (no drug added) value. Each point represents mean ± SEM, $n = 3$.

and then tended to decrease slightly at 100 nmol/L; however, aldosterone production at 300 nmol/L was still higher than the control value (Fig. 9B).

Ketoconazole inhibited DHEA and cortisol production with IC_{50} values of 330 (95% CI: 280–400) and 490 (95% CI: 350–630) nmol/L, respectively (Fig. 7C). Among the 17,20-lyase inhibitors tested, ketoconazole showed the lowest potency for inhibition of DHEA production and the lowest specificity of inhibition between DHEA and cortisol (IC_{50} ratio: 1.5). Ketoconazole inhibited androstenedione production in a dose-dependent manner, with an IC_{50} of 250 nmol/L (95% CI: 240–270) (Fig. 8C), which was similar to that for DHEA production, and also inhibited 17-hydroxyprogesterone production in a dose-dependent manner, with an IC_{50} of 820 nmol/L (95% CI: 460–3800), which was similar to that for cortisol production (Fig. 8C). However, the dose–response curve of ketoconazole for progesterone production differed from those of the other compounds (Fig. 9C). This is probably related to the low specificity of ketoconazole [22]. In addition, ketoconazole completely inhibited aldosterone production at 3000 nmol/L (Fig. 9C), which is approximately 9 times higher than the IC_{50} for DHEA production.

4. Discussion

Orteronel is a novel, non-steroidal, naphthylmethylimidazole derivative expressly designed to specifically inhibit the 17,20-lyase activity of CYP17A1 [25]. Continuing the evaluation and development of this compound, here we investigated its biochemical effects as well as its in vitro and in vivo pharmacological activity in the cynomolgus monkey model system and in human adrenal tumor cells. In enzyme assays, orteronel showed potent inhibition for both 17,20-lyase and 17-hydroxylase activities of monkey adrenal CYP17, an effect that was comparable to abiraterone. This similarity in enzyme inhibitory activity vs 17,20-lyase and 17-hydroxylase most likely reflects low selectivity of inhibition for DHEA production vs cortisol production specifically in this non-human primate system [45], thus it is difficult to say whether the clinical effects of cortisol inhibition would be significant. It is important to note that the IC_{50} ratio between 17,20-lyase and 17-hydroxylase for orteronel was 27:38 nmol/L (approximately 1:1.4) in monkeys but 140:760 nmol/L (approximately 1:5.4) in humans, thus it can be expected that orteronel will have a lower effect on 17-hydroxylase in humans relative to monkeys and relative to 17,20-lyase inhibition. Abiraterone on the other hand, had an IC_{50} ratio of 27:30 nmol/L (approximately 1:1.1), suggesting less specificity and thus potentially a greater effect on 17-hydroxylase relative to 17,20-lyase inhibition. Furthermore, abiraterone potently inhibited cortisol synthesis with an IC_{50} of 23 nmol/L. Based on current phase 1/2 clinical data, single-agent orteronel did not appear to result in clinically meaningful changes in cortisol [46] at a dose level effective for testosterone lowering (300 mg twice daily), while single-agent abiraterone has been reported to be associated with a 2-fold decrease in serum cortisol levels [47,48]. Further investigation will be required to determine if differences in the inhibitory effects of orteronel for 17,20-lyase vs 17-hydroxylase activity are clinically significant. The antifungal agent ketoconazole, which also inhibits CYP17A1 and has been occasionally used for CRPC [4], showed much lower inhibition of these key target enzymatic activities. For 11-hydroxylase activity, which is necessary for cortisol and corticosterone production but not testosterone production, orteronel showed significantly lower inhibitory activity when compared to either abiraterone or ketoconazole, suggesting that utilizing orteronel to reduce serum androgens should have reduced effects on glucocorticoids.

In in vitro studies conducted in monkey adrenal cells, production of DHEA and androstenedione was suppressed by orteronel with IC_{50} values similar to those determined in the cell-free enzyme assays conducted in this model and reported above. Aldosterone production was also suppressed by orteronel, while at higher concentrations, corticosterone and progesterone production was increased. The increase in progesterone production is thought to be accumulation of substrate by inhibition of 17-hydroxylation leading to the increase in corticosterone levels. These findings support the contention that orteronel has weaker inhibitory activity for 11-hydroxylase and aldosterone synthase than for 17,20-lyase in monkeys as 11-hydroxylase activity is necessary for corticosterone production. In contrast, ketoconazole suppressed production of DHEA, androstenedione, cortisol, and aldosterone with similar IC_{50} values suggesting that ketoconazole also effectively inhibits enzymes such as 11- or 21-hydroxylase and SCC enzyme. Taken together, these data support the notion that orteronel has more potent and more specific CYP17 inhibitory activity than ketoconazole.

From our in vivo studies and previously reported findings [25], we have found that orteronel possesses excellent oral BA. Indeed, single oral dosing of orteronel at doses of 0.3–10.0 mg/kg decreased serum DHEA levels in a dose-dependent manner. Of interest, DHEA levels did not return to levels comparable to that observed in the vehicle-treated group until approximately 48 h after orteronel dosing. Conversely, at the 1 mg/kg dose, testosterone levels did reach and briefly exceed pre-treatment levels at 24 h. However, this might have resulted from an increased LH level, and potentially suggest that more regular dosing or higher doses than 1 mg/kg may be required to maintain testosterone suppression. Studies to more clearly define tissue distribution and clearance are under consideration to better define the pharmacological basis of this extended activity and help inform our ongoing clinical program.

For recurrent prostate cancer after radical prostatectomy, hormone ablation therapy remains the mainstay of medical treatment. However, prostate cancer inevitably acquires resistance to this approach. While several molecular and/or cellular mechanisms have been proposed to be responsible for this resistance [6–8,10–15,49,50], it is clear that circulating androgens drive prostate cancer progression, even following surgical or medical castration. Thus reductions in residual testosterone after castration may be an effective treatment for some CRPCs. Currently, antiandrogens such as bicalutamide, in combination with castration, are used to block the action of the residual androgens. However, treatment with bicalutamide often leads to the emergence of resistance possibly associated with the over-expression of the AR or AR co-factors [8,9,51], or the emergence of mutated AR [13,14]. Based on the encouraging data seen in phase 1/2 clinical trials of abiraterone acetate, a steroidal compound that inhibits both 17,20-lyase and 17-hydroxylase activities of CYP17A1 [48,52,53], the progression of a significant proportion of CRPC may depend on extra-gonadal androgen production [23,24,54–56]. In this study, we show that orteronel, by virtue of its enzyme specificity and potency might be an alternate therapeutic option to suppress circulating levels of residual androgens and consequently inhibit the progression of CRPC while inducing minimal off-target effects on other CYP metabolizing enzymes [25]. To this end, the safety and efficacy of orteronel is currently being investigated in phase 1/2 clinical trials in patients with metastatic CRPC.

Disclosure statement/funding

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