Aldosterone Synthase Inhibitor

Preclinical and Early Clinical Profile of a Highly Selective and Potent Oral Inhibitor of Aldosterone Synthase (CYP11B2)

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See Editorial Commentary, pp 11–12

Abstract—Primary hyperaldosteronism is a common cause of resistant hypertension. Aldosterone is produced in the adrenal by aldosterone synthase (AS, encoded by the gene CYP11B2). AS shares 93% homology to 11β-hydroxylase (encoded by the gene CYP11B1), responsible for cortisol production. This homology has hitherto impeded the development of a drug, which selectively suppresses aldosterone but not cortisol production. This homology has hitherto impeded the development of a drug, which selectively suppresses aldosterone but not cortisol production, as a new treatment for primary hyperaldosteronism. We now report the development of RO6836191 as a potent (Ki 13 nmol/L) competitive inhibitor of AS, with in vitro selectivity >100-fold over 11β-hydroxylase. In cynomolgus monkeys challenged with synthetic adrenocorticotropic hormone, single doses of RO6836191 inhibited aldosterone synthesis without affecting the adrenocorticotropic hormone–induced rise in cortisol. In repeat-dose toxicity studies in monkeys, RO6836191 reproduced the adrenal changes of the AS−/− mouse: expansion of the zona glomerulosa; increased expression of AS (or disrupted green fluorescent protein gene in the AS−/− mouse); hypertrophy, proliferation, and apoptosis of zona glomerulosa cells. These changes in the monkey were partially reversible and partially preventable by electrolyte supplementation and treatment with an angiotensin-converting enzyme inhibitor. In healthy subjects, single doses of RO6836191, across a 360-fold dose range, reduced plasma and urine aldosterone levels with maximum suppression at a dose of 10 mg, but unchanged cortisol, on adrenocorticotropic hormone challenge, up to 360 mg, and increase in the precursors 11-deoxycorticosterone and 11-deoxycortisol only at or >90 mg. In conclusion, RO6836191 demonstrates that it is possible to suppress aldosterone production completely in humans without affecting cortisol production.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01995383.

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Primary hyperaldosteronism is recognized as a common cause of resistant hypertension and contributes to heart and kidney diseases.1-3 Mineralocorticoid receptor (MR) antagonists are currently included among the treatments of choice. However, they are not always well tolerated and induce a counterregulatory increase in aldosterone production, which may limit the efficacy of the MR blockade.4,5 Furthermore, experimental data suggest that some of the deleterious effects of aldosterone may occur through nongenomic pathways.5,6 These limitations have led to the exploration of alternative approaches to antagonize aldosterone effects by inhibition of aldosterone synthase (AS, encoded by the gene CYP11B2).7 AS, expressed within the zona glomerulosa (ZG) of the adrenal cortex, catalyzes the final 3 steps in aldosterone synthesis from 11-deoxycorticosterone (11-DOC; sequentially to corticosterone and 18-OH-cortico-}
stimulates the adrenal glands to release cortisol, and negative feedback of cortisol on ACTH secretion occurs at the hypothalamic and pituitary levels.\textsuperscript{11}

The only AS inhibitor to enter phase 2 clinical development was LCI699. This compound reduced plasma aldosterone in healthy subjects and in patients with primary hyperaldosteronism.\textsuperscript{12,13} It decreased blood pressure in patients with primary hyperaldosteronism, essential hypertension, and resistant hypertension.\textsuperscript{13–15} However, lack of sufficient selectivity led to cortisol suppression within the clinical efficacious dose range and consequently apparent termination of the development program in hypertension.\textsuperscript{15} Indeed, LCI699 is currently under development for the treatment of Cushing syndrome.\textsuperscript{16,19}

RO6836191 was designed as a highly selective and potent AS inhibitor, belonging to the series with a novel tetrahydroisoquinoline structure.\textsuperscript{20} We have investigated the selectivity, efficacy, and safety of RO6836191 in various preclinical test systems, including in vitro cells expressing recombinant CYP11B1 and CYP11B2 enzymes. Because there is only 68% homology between rat and human CYP11B2, preclinical pharmacological characterization and chronic toxicity studies were performed in cynomolgus monkeys.\textsuperscript{21} Then we conducted a first-in-man single ascending dose study during which we measured the extent of translation from preclinical into clinical.

**Methods**

**In Vitro Assay of CYP11B2 and CYP11B1 Inhibition**

The in vitro effect of RO6836191 on CYP11B2 and CYP11B1 proteins was assessed in human renal leiomyoblastoma cells (ATCC CRL1440) expressing recombinant human or cynomolgus CYP11B1 and CYP11B2 enzymes as described for an earlier compound in the series.\textsuperscript{20} Further details are provided in the online-only Data Supplement.

**In Vivo Monkey Pharmacology Study**

The effect of RO6836191 in cynomolgus monkeys was assessed as described for an earlier compound in the series.\textsuperscript{20} Animals received an oral gavage of vehicle and RO6836191 (0.035, 3, or 30 mg/kg, 2 monkeys per dose). At 1 hour postdose, 0.0145 mg/kg Synacthen (ACTH challenge) was given intramuscularly. Serial blood samples were collected pre- and postdose for analysis of RO6836191, cortisol, aldosterone, and precursors.

**In Vivo Monkey Preclinical Safety Studies**

A 4-week high-dose study and 4-week mechanistic study were performed. The high-dose study explored doses from 0 to 40 mg/kg and included a treatment-free period to assess the reversibility, persistence, or delayed occurrence of any changes noted at 40 mg/kg. At 40 mg/kg, clinical signs of dehydration (skin tenting, urine drinking, and increase water consumption) after 16 days of dosing necessitated a suspension of dosing for 3 days. Animals received free access to electrolyte solution bottles from day 17 onward, which resulted in rapid reversal of dehydration status and allowed redosing of the animals until end of the 4-week treatment period.

A 4-week mechanistic study explored doses from 0 to 1 mg/kg, testing whether adrenal changes in the high-dose study were entirely because of salt depletion and consequent activation of the renin–angiotensin system, and investigating the reversibility of any remaining changes in the adrenal ZG. Animals received either vehicle alone or RO6836191 0.15 or 1 mg/kg, with electrolyte supplementation and with or without angiotensin-converting enzyme (ACE) inhibitor (lisinopril 0.5 mg/kg). An additional group received 1 mg/kg RO6836191 alone without electrolyte supplementation or ACE inhibitor. In both studies, recovery animals were dosed for 4 weeks and then maintained undosed for a further 4 weeks to assess recovery.

The studies were performed at contract research organizations accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) study procedures such as necropsy and are in compliance with AAALAC accreditation. Further details are provided in the online-only Data Supplement.

**Histology and Immunohistochemistry**

Serial sections (nominal thickness of ≈5 µm) were prepared from formalin-fixed, paraffin-embedded adrenal gland (left) of all animals. The adrenal gland was stained routinely with hematoxylin-eosin, as well as for Ki-67 (marker for proliferative activity), CYP11B2 (marker for AS), and TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling; marker for apoptosis). Further details are provided in the online-only Data Supplement.

**First-In-Man Study**

This was an adaptive, single-center, randomized, blinded, single-ascending dose, placebo-controlled study to investigate safety, tolerability, pharmacokinetic effects, and pharmacodynamic effects of RO6836191 in healthy male subjects (unique identifier: NCT01995383) conducted in the Netherlands. RO6836191 was administered as oral solution to fasted subjects on day 1. Subjects were advised to consume a minimum of 1.5 L fluid daily while in clinic. The study was conducted in 2 parts. In part 1, a total of 64 subjects received single ascending oral doses of 1, 3, 10, 30, 90, 180, or 360 mg RO6836191 or matching placebo (the active treatment/placebo ratio was 3/1) under normal-salt diet conditions with ACTH challenge (n=56) or without ACTH challenge (n=8). In part 2, a total of 24 subjects received single oral doses of 1, 3, or 10 mg RO6836191 or matching placebo (the active treatment/placebo ratio was 3/1). Each subject was studied under low-salt and normal-salt diet conditions, in a 2-way crossover design with a 2- to 3-week wash out, with no change in treatment assignment. Dosing under low-salt diet conditions required a 3-day in-clinic run-in period in which subjects adhered to a low-salt diet of 50 mmol sodium/d and 70 to 100 mmol potassium/d, which was confirmed by urinary sodium and creatinine assessments. The low-salt diet was continued until 48 hours postdose. Adverse events, vital signs, orthostatic vital signs, ECG, standard laboratory safety data (hematology, coagulation, blood chemistry, and urinalysis), and body weight were monitored at baseline and postdose. The AS inhibition was assessed with different procedures to stimulate aldosterone release: ACTH challenge (intravenous administration of 0.25 mg Cortrosyn, 1 hour postdose in part 1 only), postural change (assessed at 6 hours postdose in part 1 and 2, subjects were required to be standing or moving for at least 30 minutes before sampling, whereas at other timepoints pharmacodynamic samples were taken in supine position), and low-salt diet.

Serial blood samples (predose and up to maximum 96 hours postdose) were taken for the assessment of plasma aldosterone, cortisol, and their precursors, plasma renin activity (PRA), plasma ACTH, and plasma electrolytes. Serial blood samples were taken before and up to 4 hours post-ACTH challenge for the measurement of cortisol and precursors. Urine (collected from day −1 up to 48 hours postdose) was analyzed for electrolytes, aldosterone, tetrahydroidaldosterone, and cortisol. All pharmacodynamic assessments and procedures were performed at baseline (day −1, time-matched with day 1 assessments) and at multiple time points postdose. Serial blood samples for RO6836191 pharmacokinetic analysis were collected up to 96 hours postdose, and a sample was taken on day 9 and at the follow-up visit. The study was approved by the local independent ethics committees and health authorities and conducted according to the provisions of the Declaration of Helsinki. Written informed consent was obtained from each study participant before conducting any protocol-related procedures. Further details are provided in the online-only Data Supplement.

**Pharmacokinetic and Statistical Analysis**

Results are reported as mean and standard deviation. Estimates of ANOVA models are reported together with 90% confidence interval (CI).
Percentage change from baseline was calculated as follows: (PD treated – PD baseline) × 100/PD baseline, where PD refers to a pharmacodynamic marker concentration at a specific time point or over a specific time interval.

Pharmacokinetic parameters for RO6836191 and area under the plasma concentration versus time curve (AUC) for the pharmacodynamic markers were estimated by noncompartmental methods, and exploratory pharmacokinetic and pharmacodynamic (PKPD) analysis was performed using WinNonlin software (Phoenix version 6.2; Pharsight Corporation, Mountain View, CA).

Plasma concentrations for aldosterone and precursors that were below limit of quantification were set to the lower limit of quantification value to allow further calculations.

GraphPad Prism 6, version 6.04 (GraphPad Software, Inc, La Jolla, CA) was used for creation of figures and for calculation of in vitro Ki values. To calculate the inhibition constant (Ki) value, the amount produced was plotted versus the substrate concentration for every inhibitor concentration tested; the resulting data set was then analyzed using the mixed model equation that is part of the statistic program GraphPad Prism.

For the monkey study, individual percentage change from vehicle in analytes in plasma at 2 hours postdose (or 1 hour after Synachten application) was calculated.

To test the effect of dose on plasma aldosterone, cortisol, and their precursors in the clinical study, an ANOVA model was applied on results from part 1 on the change from baseline for the log-transformed AUC. Baseline AUC (as a continuous variable) and dose (as a categorical variable) were included as fixed effects in the model. The 90% CIs were derived for the percentage change from baseline on AUC for each dose and for the placebo-adjusted percentage change from baseline on AUC for each active dose of RO6836191.

An exploratory pharmacokinetic and pharmacodynamic analysis using data from the clinical study was applied on percentage change from baseline in plasma aldosterone, 11-DOC, and 11-deoxycortisol concentrations versus the corresponding RO6836191 plasma concentration (details of the analysis are in Table SIII in the online-only Data Supplement).

Analytical Methods
Details are provided in the online-only Data Supplement.

Results
Estimation of In Vitro Selectivity of RO6836191
Cortisol or aldosterone production by human renal leiomyoblastoma cells expressing recombinant human or monkey CYP11B1 or CYP11B2 was measured in the presence of the respective substrate for these enzymes. The in vitro Ki values of RO6836191 in these cell lines showed that RO6836191 is a potent, selective, and competitive inhibitor of AS (Table 1 and Figure SII). Its inhibition of AS was 800- and 100-fold more potent than that of 11β-hydroxylase (CYP11B1) in the monkey and human cell lines, respectively.

Histopathologic Effect of RO6836191 on Adrenal ZG in Monkey
The ZG of the adrenal gland was the main affected tissue, and changes occurred at the lowest dose tested, that is, 1 mg/kg, which was then used as the high dose in the mechanistic study. Results of the semiquantitative analysis from both studies are in Table SI. There was drug-related expansion of the ZG, hypertrophy, and increased AS (CYP11B2) expression of ZG cells at 1 or 7 mg/kg. At 40 mg/kg RO6836191, the width of the ZG and CYP11B2 expression was reduced compared with the lower doses. The typically unstrained transitional zone at the junction of the ZG and zona fasciculata present in vehicle-treated monkeys was absent or indistinct in RO6836191-treated animals. Furthermore, both apoptosis and proliferation of ZG cells were increased in dosed animals compared with vehicle controls. After a 4-week treatment-free period, there was a trend toward reversibility of increased CYP11B2 expression, while apoptosis and proliferation of ZG cells persisted (see Figure 1A).

Changes in clinical chemistry and urinalysis were consistent with the predicted natriuretic action of RO6836191, notably PRA was increased in both sexes at 1 and 7 mg/kg. At these dose levels, aldosterone levels were clearly decreased without changing cortisol. At the highest dose of 40 mg/kg, there was no change in renin, most probably because high-dose animals received rescue electrolyte supplementation. At this dose, a decrease in cortisol was observed in addition to the expected decrease in aldosterone, indicating a loss of selectivity against 11β-hydroxylase (CYP11B1). All values returned to control group values by the end of the 4-week treatment-free period (see Figure SIIIA).

In the mechanistic study, supplementation with electrolytes ameliorated the histopathologic findings in the adrenal gland (see Figure 1B), which had occurred in the previous study at doses of 1 mg/kg and higher, namely, resulting in only minimally reduced ZG expansion, CYP11B2 expression, and cell proliferation. In addition, coadministration of an ACE inhibitor in monkeys treated with 1 mg/kg RO6836191 with electrolytes conferred further protection, notably with the retention of the transitional zone.22 At 0.15 mg/kg, no expansion of the ZG was observed. PRA was increased only for doses >0.15 mg/kg. PRA was still high when electrolyte supplementation was present, but almost comparable with control values in the group receiving electrolyte supplementation and ACE inhibitor treatment.

Measurements of aldosterone and cortisol showed the expected decrease and no change, respectively, at all doses. The expected decrease of aldosterone was also observed in the group treated with ACE inhibitor alone (see Figure SIIIB).

Table SII shows that the efficacity and selectivity of the 0.15 and 1 mg/kg doses are consistent with the RO6836191 exposures being equal or above the Ki for CYP11B2 and below Ki for CYP11B1.
Pharmacological Characterization in Cynomolgus Monkeys

The effect of a single oral dose RO6836191 (0–30 mg/kg) on aldosterone, cortisol, and precursors after an ACTH challenge is shown in Figure 2. RO6836191 blunted aldosterone production at all doses tested compared with vehicle, with a 70% to 90% decrease in aldosterone from the 0.035 mg/kg to the 30 mg/kg dose, respectively. The precursors 11-DOC and 11-deoxycortisol were increased only by high doses (up to 600% at 30 mg/kg), but cortisol and corticosterone levels were not different from vehicle. The reduction in aldosterone, but absence of change in cortisol levels post-ACTH challenge across the 1000-fold dose range of RO6836191, was consistent with the selectivity ratio predicted from in vitro experiments. The observed aldosterone reduction at the lowest dose and the related free drug exposure are in line with the in vitro Ki value for AS. By contrast, the rise in 11-DOC and 11-deoxycortisol was consistent with the free drug exposure at the highest dose being comparable with the in vitro Ki value for 11β-hydroxylase (see Table 2). RO6836191 plasma exposure data are provided in Table SII.

Proof of Mechanism in Healthy Subjects

Safety, Tolerability, and Pharmacokinetics

RO6836191 was well tolerated at all doses tested. The most frequently reported adverse events were headache, nasopharyngitis, asthenia, and diarrhea, which were also reported in the placebo group and which were all of mild intensity. There was no dose-related increase in incidence or in the severity of reported adverse events. There were no other apparent treatment-related trends or any clinically significant findings in any other safety assessments. Mean time-matched blood pressure and pulse rate changes from baseline up to day 3 did not show a trend with time, dose, or diet, and no difference from placebo was observed.

RO6836191 was rapidly absorbed reaching maximum plasma concentrations between 0.5 and 2 hours postdose. Exposure of RO6836191 increased dose-proportionally. Mean half-life was 29 hours, and approximately 10% of the dose was recovered unchanged in the urine (RO6836191 plasma exposure data are provided in Table SII).

Pharmacological Characterization in Healthy Subjects

With ACTH Challenge and Comparison With Monkey Data

The time course of plasma aldosterone and cortisol in healthy subjects after single ascending doses of RO6836191 or placebo with ACTH challenge is shown in Figure 3. RO6836191 increased plasma aldosterone and cortisol levels, with a postural change induced an immediate surge in plasma aldosterone and cortisol levels, which peaked =0.5 and 2 hours after the challenge, respectively. The postural change induced an immediate surge in plasma aldosterone and cortisol levels, which peaked =0.5 and 2 hours after the challenge, respectively.
aldosterone to a similar level as induced by the ACTH challenge. After RO6836191 administration, plasma aldosterone was decreased dose-dependently with the maximum effect reached by 10 mg onward (mean change in AUC from baseline −84.9% [90% CI, −86.6 to −82.8%]; different from placebo \( P < 0.0001 \)). Corticosterone and cortisol were unchanged at all doses compared with placebo, and there were no reductions in plasma cortisol on ACTH challenge. At doses >90 mg, there were dose-dependent (and different from placebo \( P < 0.01 \)) increases in 11-DOC (maximum mean change in AUC from baseline +194% [90% CI, 160 to 232]) and 11-deoxycortisol (maximum mean change in AUC from baseline +193% [90% CI, 157 to 234]). 18-Hydroxycorticosterone decreased at 1 mg with a maximum effect at 10 mg onward (\( P < 0.01 \); maximum mean change in AUC from baseline −58.6% [90% CI, −64.3 to −52.0]). These results were consistent with the in vitro and monkey findings, with the absence of change in cortisol levels post-ACTH challenge across the 360-fold dose range predicted by the drug’s selectivity for CYP11B2. The increase in plasma concentration of precursors 11-DOC (substrate for CYP11B1 and CYP11B2) and 11-deoxycortisol (substrate for CYP11B1) at doses >90 mg were proportionately less than the reduction in aldosterone and as in monkey was absent or insubstantial except at concentrations of drug inhibiting 11β-hydroxylase.

### Table 2. Comparison of Human and Monkey In Vivo and In Vitro Potency and Selectivity

<table>
<thead>
<tr>
<th>Species</th>
<th>Aldosterone Synthase</th>
<th>11β-Hydroxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ki in Vitro, ng/mL</td>
<td>RO6836191-Free C&lt;sub&gt;avg&lt;/sub&gt; at Minimum Effective Dose, ng/mL</td>
</tr>
<tr>
<td>Monkey</td>
<td>1.45</td>
<td>0.74; 0.94*</td>
</tr>
<tr>
<td>Human</td>
<td>4.7</td>
<td>5.2 (0.57)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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Ki (see Table 1) units were converted to ng/mL for comparison with RO6836191 plasma concentrations. RO6836191 free C<sub>avg</sub> was calculated from total C<sub>avg</sub> taking into account the free fraction of 0.26 for human and of 0.33 for monkey; C<sub>avg</sub> is expressed as individual data per monkey or mean and SD for human. Change in plasma exposure for aldosterone and 11-deoxycortisol is expressed as % change from vehicle for monkey (individual values per monkey, \( n=2 \)) and as % change from day 1 baseline for human (represented as mean and 90% confidence interval). The minimum effective dose was defined as the dose at which ≥75% of the maximum observed effect was reached. \( a, b, c, d, e, \) and \( f \) are parameters for dose groups 0.035 mg/kg, 3 mg/kg, 30 mg/kg, 3 mg, 10 mg, and 360 mg, respectively.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Dose-dependent effect of RO6836191 on plasma aldosterone, cortisol, and precursors in healthy subjects (study part 1). Aldosterone (A) and cortisol (B) plasma concentration-time profiles after single ascending doses of RO6836191 (0–360 mg) administered orally to healthy subjects 1 hour before the adrenocorticotropic hormone (ACTH) challenge. Downward arrow indicates time of ACTH challenge (1 hour postdose) and upward arrow time of postural change (6 hours postdose). Data represent mean plasma concentrations (+SD) by dose group (\( n=6/dose \) group and \( n=12 \) for placebo). Below limit of quantification (BLQ) values were set to lower limit of quantification (LLOQ; 5 pg/mL), for doses >10 mg, aldosterone concentrations were BLQ from 2.5 hours postdose onward. Effect of RO6836191 on plasma aldosterone (C), cortisol (D), and precursors after a single oral dose (0–360 mg) in healthy subjects administered 1 hour before the ACTH challenge. Data represent mean (and 90% confidence interval) % change from baseline in plasma exposure (AUC<sub>0-24</sub>) for aldosterone and precursors (left graph) and cortisol and precursors (right graph) by dose group (\( n=6/dose \) group and \( n=12 \) for placebo). Filled symbols indicate \( P < 0.01 \) compared with placebo. 11-DOC indicates 11-deoxycorticosterone.
Consistent with the decrease in plasma aldosterone, urinary excretion of both aldosterone and its metabolite, tetrahydroaldosterone, was decreased dose-dependently (Figure SV). No dose-dependent effect on urinary cortisol was apparent.

The pattern of RO6836191-induced changes in aldosterone, cortisol, and precursors was qualitatively and quantitatively comparable between monkey and human, relative to the respective Ki values. A comparison of monkey and human RO6836191 plasma exposures leading to a similar effect on aldosterone and 11-deoxycortisol is provided in Table 2 and demonstrate that the effective free plasma concentrations are consistent with the in vitro potency and selectivity of RO6836191 in the respective species.

**PKPD Characterization in Healthy Subjects and Comparison With In Vitro Cell-Based Potency**

Assessment of the effect of RO6836191 on aldosterone and precursors in subjects who received study drug under normal-salt or low-salt conditions without an ACTH challenge (part 2) resulted in a similar dose response as observed with ACTH challenge (part 1). The exploratory PKPD analysis indicated that aldosterone decreases, and both 11-DOC and 11-deoxycortisol levels increase RO6836191 concentration dependently as a direct effect. The PKPD model parameters provide a good estimate of the concentration range where RO6836191 is both efficacious and selectively inhibits aldosterone but not cortisol production. The half-maximal inhibitory concentration (IC50) values were similar to the Ki values from human in vitro assays (Table SIII).

**Effect on Electrolytes, Renin, and ACTH in Healthy Subjects**

Low-salt diet conditions caused the expected increases in plasma aldosterone and PRA levels (Figures SIVB and SVIID). Urinary sodium excretion was dose-dependently (0–10 mg) increased by RO6836191 (up to 100% from baseline (BL)) under low-salt conditions (Figure SVB). An increase in urinary sodium (0–360 mg) was also observed under uncontrolled, normal-salt conditions, but without any difference from placebo (see Figure SVIA). Only minor changes in potassium urinary excretion (≤20%) were observed (see Figure SVIC and SVID). However, an increase in the urinary sodium/potassium ratio was apparent, under controlled low-salt diet (Figure 4). No change in urine creatinine or in plasma electrolytes (potassium, sodium) was apparent. The single-dose administration of RO6836191 resulted in minor (-2-fold increase) changes in PRA (Figure SVIC and SVIID), and there were no dose- or diet-dependent changes in ACTH (Figure SVIIA and SVIIB).

**Discussion**

Our studies demonstrate that, despite the close homology of the 2 enzymes catalyzing the final steps in aldosterone and cortisol synthesis, it is possible for 1 molecule to completely inhibit the former without effect on the latter and that preclinical experiments conducted on cell lines and then in monkeys predict to a remarkable degree the doses in humans, which selectively inhibit aldosterone production. The therapeutic range of RO6836191 for optimal plasma aldosterone suppression has to be defined in subsequent clinical studies.

However, a high suppression is achieved by single doses of 3 mg and virtually complete aldosterone suppression at 10 mg. By contrast, the precursors only start to increase at a dose of 90 mg and cortisol remained unchanged up to the highest dose tested (360 mg).

We think that of critical importance in the evaluation of selectivity, and its impact on the potential clinical utility of an AS inhibitor, is the demonstration of a wide dose range at which aldosterone itself is suppressed without any increase in its precursor, 11-DOC. Aldosterone and 11-DOC have approximately equal and high affinities for the MR and circulate at roughly similar total concentrations under normal conditions; however, the free fraction of aldosterone (fu 0.30–0.50) is greater than that of 11-DOC (fu <0.05). In the case of imbalance between free aldosterone and free 11-DOC, the mineralocorticoid effect of 11-DOC may become relevant, with high levels of circulating 11-DOC negating the value of aldosterone inhibition. Because 11-DOC is a substrate for both CYP11B1 and CYP11B2 (Figure SI), intact activity of the former now seems essential in preventing accumulation of the precursor after inhibition of the latter. This observation may explain the relatively low clinical effect on blood pressure, when compared with MR blockade, of an AS inhibitor with less selectivity than RO6836191. Thus, after treatment with LCI699, the ACTH-stimulated adrenal gland produced a 10-fold increase in 11-DOC. RO6836191 did not affect the ability of CYP11B1 to transform 11-DOC into corticos-terone; however, to date, we do not have data to confirm that the 11β-hydroxylase activity of CYP11B2 is inhibited by RO683619.

The large selectivity ratio of RO6836191 in vitro led us to study a wide range of doses in vivo, allowing the quantification of the dose range over which it can be expected to suppress aldosterone but not cortisol. Although the single-dose first-in-man study cannot establish the clinical range, the use of conventional PKPD analyses enabled us to correlate doses at which biochemical responses were observed...
with the previously measured Ki of RO6836191 for the 2 enzymes, CYP11B1 and CYP11B2. Some other selective AS inhibitors have recently been reported, but the only one to date tested in humans did not reduce plasma aldosterone by >70%, even at high doses. Previous AS inhibitors to enter clinical development were LCI699 and fadrozole. Of these, LCI699 has been repurposed for the treatment of Cushing disease, where excess of cortisol is the cardinal clinical feature, and fadrozole is an aromatase inhibitor used for the treatment of breast cancer.

We undertook extensive preclinical studies in the cynomolgus monkey to understand changes observed in adrenal ZG. Interestingly, RO6836191 reproduced the adrenal changes of the AS−/− mouse, namely, expansion of the ZG; increased expression of AS (or of enhanced green fluorescent protein, which was used to disrupt the CYP11B2 gene in the AS−/− mouse); hypertrophy, proliferation, and apoptosis of ZG cells. The partial amelioration of apoptotic and other adaptive changes in the ZG layer, by electrolyte supplementation and ACE inhibition, is consistent with a counteraction of the RO6836191-induced salt loss at doses ≥1 mg/kg. However, not all the effects on ZG of RO6836191 were reversed by ACE inhibition and electrolyte supplementation. We cannot exclude the possibility that the dose of the ACE inhibitor, or the electrolyte replacement, was insufficient to fully prevent sodium depletion. However, the residual increase in CYP11B2 expression and apoptosis in the ZG layer are consistent with adrenal changes reported in the AS−/− mouse, which suggested that ZG cells migrate to the medullary junction and undergo apoptosis when unable to synthesize aldosterone. Increased renin production is observed in the AS−/− mouse, in response to sodium depletion, as in our monkeys treated with RO6836191 but not receiving saline supplementation. But the increased PRA, reported in Figure SIII, is unlikely to be the sole or principal cause of changes in the adrenal, especially when the renin elevation is partly because of the inhibition by lisinopril of angiotensin production. Only minor increases in PRA were observed after a single dose in the clinical study. We would anticipate an increase in PRA in future, multiple dosing, studies, given the expected natriuresis and the trend we observed both in the preclinical data and the low-salt diet group in the clinical study. Although there was no significant evidence of natriuresis after a single dose of RO6836191, we did observe a similar increase in the Na/K ratio as reported after a single dose of MR antagonist.

A degree of apoptosis may be beneficial in patients with hyperaldosteronism, particularly if this occurred in the so-called aldosterone-producing cell clusters that are often the only residual sites of aldosterone production or in actual aldosterone-producing adenomas. By contrast, drugs that block the MR (ie, spironolactone, eplerenone, and newer aldosterone receptor antagonists) cause an increase in aldosterone production. The MR knockout mouse has shown increased circulating aldosterone levels, and no reports were found of involution of aldosterone-producing cells.

A limitation of our study for predicting clinical utility of AS inhibition is the single-dose nature of the clinical study. It was not expected that in healthy subjects a transient natriuresis would lower blood pressure, and several doses may be required for a compensatory activation of PRA to be detectable. We have yet to show any effect on blood pressure, which was not measured in the cynomolgus studies addressing biochemical and pathological effects of RO6836191 and did not change after the single dose in healthy volunteers. Clearly, therefore, predictions regarding clinical potential are untested until multiple-dose studies have been undertaken, and the effect of selective AS inhibition on blood pressure is demonstrated in patients with primary aldosteronism. However, an important first step was to show whether the hurdle of 93% homology between AS and 11β-hydroxylase could be overcome, and if so, what the consequences are likely to be for circulating levels of substrate and product of the 2 enzymes. Our findings open the door to suppression of aldosterone production being investigated as a treatment option and provide some confidence that further selective inhibitors of AS can be developed using the monkey as the relevant preclinical species for mechanistic exploration of aldosterone inhibition.

In conclusion, selective AS inhibition can be achieved in humans, with selectivity preventing not only the inhibition of cortisol production but also the compensatory increase in 11-DOC. Preclinical data underline the probable importance of aldosterone in regulating ZG cell turnover, with the possibility that accelerated turnover may contribute to chronic efficacy of AS inhibitors in comparison with the receptor blockers, which elevate aldosterone secretion.

**Perspectives**

The homology between the 2 enzymes catalyzing the final steps in aldosterone and cortisol synthesis has been a block in the development of drugs, which inhibit aldosterone secretion rather than block its response. We show that in vitro selectivity can translate into clinical practice, providing that, as in our cynomolgus studies, the single-dose findings are predictive of those on multiple dosing. An important discovery is that the plasma concentration of deoxycorticosterone, the biologically active substrate for AS, does not increase at selective doses of inhibitor. This predicts greater natriuretic and antihypertensive efficacy than achieved by less selective inhibitors.

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**Disclosures**


**References**


Novelty and Significance

What Is New?

• We report that it is possible to completely inhibit the enzyme-producing aldosterone without causing any inhibition of the 93% homologous enzyme-producing cortisol.

• This inhibition reduced plasma aldosterone to below detectable levels, without accumulation of deoxycorticosterone, the substrate of aldosterone synthase.

What Is Relevant?

• No drug is currently available for the treatment of hyperaldosteronism, which works through inhibition of aldosterone production rather than blocking aldosterone response. This study now permits the consequenc- es of selective aldosterone inhibition to be investigated in patients.

Summary

An inhibitor of aldosterone synthase was tested over multiple doses in cynomolgus monkeys and after a single dose in humans. Its 100-fold selectivity in vitro (for aldosterone versus cortisol inhibition) translated into 100-fold difference in doses required to inhibit aldosterone and cortisol in vivo.
Preclinical and Early Clinical Profile of a Highly Selective and Potent Oral Inhibitor of Aldosterone Synthase (CYP11B2)

Katrijn Bogman, Dietmar Schwab, Marie-Laure Delporte, Giuseppe Palermo, Kurt Amrein, Susanne Mohr, Maria Cristina De Vera Mudry, Morris J. Brown and Philippe Ferber

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http://hyper.ahajournals.org//subscriptions/
SUPPLEMENTARY DATA

Pre-clinical and early clinical profile of a highly selective and potent oral inhibitor of aldosterone synthase (CYP11B2)

Katrijn Bogman1*, Dietmar Schwab1, Marie-Laure Delporte1, Giuseppe Palermo1, Kurt Amrein1, Susanne Mohr1, Maria Cristina De Vera Mudry1, Morris J. Brown2#, Philippe Ferber1,3#

Running Title: An oral selective inhibitor of aldosterone synthase

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Methods

**In vitro assay of CYP11B2 and CYP11B1 inhibition**

Cells were incubated for 16 hrs in the presence of different substrate concentrations (0.25-4 µM of 11-DOC for CYP11B2 and 0.5-12 µM of 11-deoxycortisol for CYP11B1) and different inhibitor concentrations (0-100 nM for CYP11B2 and 0-10000 nM for CY11B1). The steroid products (aldosterone for CYP11B2; cortisol for CYP11B1) were measured using homogenous time-resolved fluorescence (HTRF) assays from CisBio. The assay was performed in 5 and 8-13 replicates for CYP11B2 and CYP11B1 respectively.

**In vivo monkey pre-clinical safety studies**

Animals were housed in pens, socialized in groups of the same dose group whenever possible in compliance with “Code of practice for the housing and care of animals used in scientific procedures’ (Home Office, London, 1989) and European Regulations (Directive 2010/63/EU). In the 4-week high dose study 4 groups of cynomolgus monkeys (3 males and 3 females per group) were dosed with RO6836191 (vehicle, 1, 7 or 40 mg/kg/day) once daily by oral gavage for four weeks. Additionally, RO6836191 was tested in a 4-week mechanistic study to investigate the mechanism of RO6836191-related changes in the adrenal zona glomerulosa. Five groups, each consisting of 3 males, received either vehicle alone, or RO6836191 0.15 or 1 mg/kg/day, with or without lisinopril 0.5 mg/kg/day. These five groups received electrolyte supplementation. The sixth group was treated with 1 mg/kg/day RO6836191 alone without electrolyte supplementation or ACE inhibition. In both studies, additional groups each comprising 2 animals per sex were similarly dosed for four weeks, and then maintained un-dosed for a further four weeks to assess recovery. In addition to standard safety assessments, the focus was on understanding findings in the adrenal gland zona glomerulosa, i.e. endocrinology, histology and immunohistochemistry in both studies.

**Histology and Immunohistochemistry**

For immunohistochemistry, the sections were incubated in a rabbit monoclonal anti-Ki-67 (Ventana 790-4286; prediluted) or a rabbit monoclonal anti-CYP11B2 (Sigma HPA049171; 1:200) primary antibodies, followed by incubation in a biotinylated donkey anti-rabbit IgG secondary antibody (Jackson Immuno Research, Ref: 711-065-152; 1:100). The primary antibody was omitted for the negative control. All immunostains were revealed with Ventana DAB Map Detection Kit (Ventana, Reference No. 760-124), and counterstained with hematoxylin. Immunohistochemistry was performed using Ventana Discovery XT platform (Ventana Medical Systems, Tucson, AZ). TUNEL staining was performed manually.
Immunohistochemistry staining was graded on a scale of 1-4 based on the number of positively stained cells (1, minimal < 2, slight < 3, moderate < 4, marked).

First-in-man study

Subjects were screened within 4 weeks prior to the first dose administration. Screening assessments included medical history, physical examination, height and weight, 12 lead electrocardiograms (ECG), vital signs including blood pressure and body temperature, drugs of abuse and alcohol test, blood and urine clinical laboratory safety tests (hematology, coagulation, chemistry, urinalysis, serology).

Pulse rate and blood pressure were obtained in a quiet room at a comfortable temperature, with the subject’s arm unconstrained by clothing or other material. All measurements were obtained from the same arm and, with the same cuff size, using an automatic instrument with a digital readout, throughout the study. The measurements were taken after the subject had been resting in a supine position for at least 10 minutes. All blood pressure measurements were taken in triplicate (with approximately 2 minutes interval).

Analytical methods

For the pre-clinical studies, plasma cortisol, aldosterone and precursors from the monkey studies were analyzed with a qualified liquid chromatography coupled to mass spectrometry (LC-MS) method, with estimated detection limit of 0.3 nM and LLOQ of 1nM (the method has been reported previously by Martin RE et al.¹). Plasma renin activity was measured by radio-immunoassay (RIA), according to the manual from the manufactures (Renin activity RIA Kit RENCTK P2721 (DiaSorin)).

For the clinical study, the following PD markers were quantified using validated LC-MS-MS methods with LLOQ of 5 pg/mL for plasma 11-DOC, 250 pg/mL for plasma corticosterone, 50 pg/mL for plasma 18-hydroxycorticosterone, and 5 pg/mL for plasma aldosterone, 40 pg/mL for plasma 11-deoxycortisol, 0.5 ng/mL for plasma total cortisol, 1.00 ng/mL for urine cortisol, 0.20 ng/mL for urine aldosterone, and 1.00 ng/mL for urine tetrahydroaldosterone. Plasma ACTH was measured with a solid-phase, two-site sequential chemiluminescent immunometric assay on the Immulite 2000. Plasma renin activity (PRA) was measured by a RIA kit (REN-CT2 (Cis Bio International)). Electrolytes (sodium and potassium) in plasma and urine were measured with an Ion Selective (ISE) method on the ADVIA 1800 Clinical Chemistry analyzer.

Plasma and urine concentrations of RO6836191 were quantified using LC-MS-MS methods. The LLOQ was 1 ng/mL for the assay used for toxicokinetic plasma
samples and was 0.5 and 0.05 ng/mL, for the assay used for analysis of monkey and human plasma and urine samples respectively.

References

Table S1. Semi-quantitative analysis results of immunohistochemistry of CYP11B2 expression, apoptosis (TUNEL), and cell proliferation (Ki-67) from the 4-week cynomolgus monkey toxicology studies

A

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
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<th>7</th>
<th>40</th>
<th>Recovery</th>
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<td>A</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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B

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P=Present; A=Absent
0 none, 1 minimal, 2 mild

(A) Semi-quantitative assessment from all monkeys (averaged for males and females in each group) included in the 4-week “high dose study.” P=Present; A=Absent, 0 none, 1 minimal, 2 mild, 3 moderate

(B) Semi-quantitative assessment from all monkeys included in the 4-week “mechanistic study” administered RO6836191 at 0, 0.15, or 1 mg/kg/day with electrolytes (E) or electrolytes and ACE inhibitor (EA), followed by a 4-week recovery period. P=Present; A=Absent, 0 none, 1 minimal, 2 mild
Table S2. Plasma exposure to RO6836191 after oral administration in cynomolgus monkey and human

Exposure to RO6836191 in cynomolgus monkeys in the pre-clinical studies

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0.15</th>
<th>1</th>
<th>7</th>
<th>40</th>
<th>0.035</th>
<th>3</th>
<th>30</th>
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<tr>
<td>n</td>
<td>5</td>
<td>17</td>
<td>6</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>40.6 (11.9)</td>
<td>296 (65.6)</td>
<td>4170 (1320)</td>
<td>12800 (7280)</td>
<td>15.3; 18.3</td>
<td>1420; 2700</td>
<td>13900; 15400</td>
</tr>
<tr>
<td>C&lt;sub&gt;avg&lt;/sub&gt; (ng/mL)</td>
<td>12 (3.0)</td>
<td>75.1 (19.3)</td>
<td>967 (458)</td>
<td>3771 (2142)</td>
<td>2.2; 2.9</td>
<td>357; 567</td>
<td>5731; 5975</td>
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</table>

Exposure to RO6836191 in the clinical study in healthy subjects (single dose PK)

<table>
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<tr>
<th>Dose (mg)</th>
<th>1</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>90</th>
<th>180</th>
<th>360</th>
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<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>10.1 (1.21)</td>
<td>31.9 (4.42)</td>
<td>125 (14.8)</td>
<td>386 (78.5)</td>
<td>1380 (259)</td>
<td>2490 (399)</td>
<td>5750 (412)</td>
</tr>
<tr>
<td>C&lt;sub&gt;avg&lt;/sub&gt; (ng/mL)</td>
<td>6 (0.9)</td>
<td>20 (2.2)</td>
<td>66 (5.9)</td>
<td>219 (39.4)</td>
<td>762 (83.8)</td>
<td>1383 (221)</td>
<td>3050 (305)</td>
</tr>
</tbody>
</table>

Pharmacokinetic (PK) parameters are calculated for total RO6836191 in plasma; free exposure can be calculated taking into account fu of 0.26 for human and 0.33 for monkey; C<sub>max</sub> = maximum observed plasma concentration; C<sub>avg</sub> = average plasma concentration within a 24 hr time interval calculated as AUC<sub>0-24</sub>/24; values are reported as mean (± SD) except if n=2 in which case individual values are reported.
Table S3. Pharmacokinetic-pharmacodynamic model parameter estimates for aldosterone, 11-DOC and 11-deoxycorticisol

**Aldosterone**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low salt diet</th>
<th>Normal salt diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC(_{50}) free (CV) [ng/ml]</td>
<td>4.1 (32%)</td>
<td>1.1 (34.4%)</td>
</tr>
<tr>
<td>(I_{max}) (CV) [%]</td>
<td>103.2 (10.3%)</td>
<td>70.7 (6.9%)</td>
</tr>
<tr>
<td>(E_0) (CV) [%]</td>
<td>7.8 (31.3%)</td>
<td>-1.7 (99.8%)</td>
</tr>
</tbody>
</table>

**11-DOC**

<table>
<thead>
<tr>
<th>Parameter</th>
<th></th>
<th>11-deoxycorticisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC(_{50}) free (CV) [ng/mL]</td>
<td>136 (48.8%)</td>
<td>343 (58.2%)</td>
</tr>
<tr>
<td>(E_{max}) (CV) [%]</td>
<td>210 (15.3%)</td>
<td>273 (25.7%)</td>
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</table>

A maximum inhibition (\(I_{max}\)) model with the following equation was fitted to the individual time-matched change in plasma aldosterone (all doses pooled from Part 2 of the study grouped by diet, n=24): \(E=E_0-(I_{max}*C)/(C+IC_{50})\); where C is the RO6836191 plasma concentration, \(E_0\) is the % change from baseline in plasma aldosterone concentration when C=0, \(I_{max}\) is the maximum inhibitory effect and \(IC_{50}\) is the RO6836191 concentration causing half of the maximum inhibitory effect. Model parameters for aldosterone (IC\(_{50}\) free calculated from total IC\(_{50}\) using \(f_u\) of 0.26, \(I_{max}\) and \(E_0\) and CV% are listed by diet group and provide a measure of the RO6836191 potency to inhibit CYP11B2 (for comparison in vitro Ki= 4.7 ng/mL).
An $E_{\text{max}}$ model with the following equation was fitted to the individual % change from day-1 baseline in plasma exposure (based on $\text{AUC}_{0\text{-}24}$) for the precursors 11-DOC and 11-deoxycortisol (all doses pooled from Part 1 of the study, n=56):

$$E = \frac{E_{\text{max}} C}{C + EC_{50}};$$

where $C$ is the plasma exposure (based on $C_{\text{avg}}$) for RO6836191, where $E_{\text{max}}$ is the maximum effect and $EC_{50}$ is the RO6836191 exposure causing half of the maximum effect. Model parameters for 11-DOC and 11-deoxycortisol ($EC_{50}\text{ free}$ calculated from total $EC_{50}$ using $fu$ of 0.26, $E_{\text{max}}$) and CV% are listed by analyte and provide an estimate of potential unselectivity for CYP11B1 (for comparison in vitro $Ki = 476 \text{ ng/mL}$)
**Figure S1.** Steps in the biosynthetic pathways for aldosterone and cortisol
**Figure S2.** Ki determination of RO6836191 in human renal leiomyoblastoma cells expressing recombinant human CYP11B2 or CYP11B1 enzyme

**hCYP11B2**

- Ki = 15.2 nM; alpha ~ 1.8e+13;
- Vmax = 62.2 nM; Km = 1228 nM;

**hCYP11B1**

- Ki = 1372 nM; alpha = 2.7;
- Vmax = 269.8 nM; Km = 1301 nM

Ki determination from one representative experiment using the fitting algorithm provided by the program GraphPad Prism™. Ki is the inhibition constant, Vmax is the maximum enzyme velocity without inhibitor, Km is the Michaelis-Menten constant. Alpha determines mechanism, the reported alpha value is above 1 which suggests competitive inhibition.
**Figure S3.** Plasma renin activity (PRA), aldosterone and cortisol data in cynomolgus monkey “high dose” and “mechanistic” safety studies

A

<table>
<thead>
<tr>
<th>PRA</th>
<th>Aldosterone</th>
<th>Cortisol</th>
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<td><img src="image2.png" alt="Graph B" /></td>
<td><img src="image3.png" alt="Graph C" /></td>
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<td><img src="image5.png" alt="Graph B" /></td>
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</table>

(A) PRA, aldosterone and cortisol from “high dose” study. High-dose animals (40 mg/kg) received rescue electrolyte supplementation. Data are represented as median and individual values (n=9, 8, 3 and 3, for control, 1, 7 and 40 mg/kg, respectively, at baseline and week 4, n=3 and 1 for control and 40 mg/kg at week 8, respectively); horizontal line at ratio equals to 1.

(B) PRA, aldosterone and cortisol from the “mechanistic study”. RO6836191 was administered at 0.15 - 1 mg/kg/day for 4 weeks, with or without electrolytes or ACE inhibitor Lisinopril 0.5 mg/kg/day. Data are represented as individual values at 7 h post-dose for Days 1 and 28 vs baseline time 0, horizontal dash represents the median by dose group.
Figure S4. Mean (+SD) aldosterone plasma concentration time profiles after single doses of RO6836191 (0-10 mg) in healthy subjects (Part 2) administered without ACTH challenge under a normal salt (A) or low salt diet (B)

The upward arrow indicates time of postural change (6 hr post-dose), data represent mean + SD by dose group (n=6/dose group), BLQ values were set to LLOQ (5 pg/mL)
Figure S5 Dose dependent effect of RO6836191 on urine aldosterone, tetrahydro-aldosterone and cortisol in healthy subjects

Aldosterone tetrahydro-aldosterone and cortisol excreted in the 24hr-urine from healthy subjects (Part 1) who received a single oral dose of RO6836191 (0-360 mg) 1 hr prior to the ACTH challenge under a normal salt diet, data represent mean % change from day -1 baseline by dose group and 90% CI (n=6/dose group and n= 12 for placebo)
**Figure S6.** Urinary excretion of sodium and potassium following single dose administration of RO6836191 to healthy subjects (Parts 1 and 2). Box whiskers show interquartile range (box) and min-max (whiskers).

A  **Sodium (Part 1)**

B  **Sodium (Part 2)**
(A) Urinary excretion of sodium under normal salt diet following single dose administration of 1 - 360 mg RO6836191 or placebo (Part 1) to healthy subjects. Data are presented as ratio to baseline for days 1 and 2 (Clinical study Part 1, n=16, 9, 9, 6, 6, 6, 6 for placebo and 1, 3, 10, 30, 90, 180 and 360 mg, respectively). Mean (SD) baseline sodium was 144 (34) mmol across all subjects (n=64).

(B) Urinary excretion of sodium under low salt diet or normal salt diet following single dose administration of 1, 3, 10 mg RO6836191 or placebo to healthy subjects (Part 2) in a cross-over design (Clinical study Part 2, n=6/dose group). Data are presented as baseline values and ratio to baseline for days 1 and 2.

(C) Urinary excretion of potassium under normal salt diet following single dose administration of 1 - 360 mg RO6836191 or placebo to healthy subjects (Part 1). Data are presented as ratio to baseline for days 1 and 2 (Clinical study Part 1; n=16, 9, 9, 6, 6, 6, 6 for placebo and 1, 3, 10, 30, 90, 180 and 360 mg, respectively). Mean (SD) baseline potassium was 87.4 (21.0) mmol across all subjects (n=64)
(D) Urinary excretion of potassium under low sodium diet or normal diet following single dose administration of 1, 3, 10 mg RO6836191 or placebo to healthy subjects (Part 2) in a cross-over design (Clinical study Part 2, n=6/dose group). Data are presented as baseline values and ratio to baseline for days 1 and 2.

(E) Urinary sodium-to-potassium ratio under normal salt diet following single dose administration of 1 - 360 mg RO6836191 or placebo to healthy subjects (Part 1) and under low sodium diet or normal diet following single dose administration of 1, 3, 10 mg RO6836191 or placebo to healthy subjects (Part 2) in a cross-over design.
Figure S7. Plasma ACTH and PRA following single dose administration of RO6836191 in healthy subjects. Box whiskers show interquartile range (box) and min-max (whiskers).

A  ACTH (Part 1)

B  ACTH (Part 2)
(A) Plasma ACTH following single dose administration of 1 - 360 mg RO6836191 or placebo (Clinical study Part 1; n=16, 9, 9, 4, 6, 6, 6, 6 for placebo and 1, 3, 10, 30, 90, 180 and 360 mg, respectively). Baseline values and ratio of 24 h post-dose and pre-dose (baseline).

(B) Plasma ACTH under low salt diet or normal diet following single dose administration of 1, 3, 10 mg RO6836191 or placebo in a cross-over design (Clinical study Part 2; n=6/dose group): Baseline values and ratio of 24 h post-dose value to baseline.

(C) PRA following single dose administration of 1 - 360 mg RO6836191 or placebo (Clinical study Part 1; n=15, 9, 8, 6, 5, 3, 6, 5 for placebo and 1, 3, 10, 30, 90, 180 and 360 mg, respectively). Baseline values and ratio of 24 h post-dose and pre-dose (baseline). For the 90 mg dose group, data from only 3 subjects were available.

(D) PRA under low sodium diet or normal diet following single dose administration of 1, 3, 10 mg RO6836191 or placebo in a cross-over design (Clinical study Part 2). Baseline values and ratio of 24 h post-dose value to baseline, box plots shown only if at least 5 data points available, otherwise individual data shown; for the 1 mg normal salt diet group, no data were available to calculate the ratio.