Aldosterone Synthase Inhibition With LCI699
A Proof-of-Concept Study in Patients With Primary Aldosteronism

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Catherine Watson, Pierre-François Plouin

Abstract—We report the first administration of an orally active aldosterone synthase inhibitor, LCI699, to 14 patients with primary aldosteronism. After a 2-week placebo run-in, patients received oral LCI699 (0.5 mg BID) for 2 weeks, LCI699 (1.0 mg BID) for 2 weeks, and placebo for 1 week. We assessed changes in hormone concentrations, plasma potassium levels, and 24-hour ambulatory systolic blood pressure and safety. The supine plasma aldosterone concentration decreased from 540 pmol/L (95% CI: 394 to 739 pmol/L) to 171 pmol/L (95% CI: 128 to 230 pmol/L) after 0.5 mg of LCI699 (−68%; P<0.0001) and to 133 pmol/L (95% CI: 100 to 177 pmol/L) after 1.0 mg of LCI699 (−75%; P<0.0001). Plasma 11-deoxycorticosterone concentrations increased by 710% after 0.5 mg of LCI699 (P<0.0001) and by 1427% after 1.0 mg of LCI699 (P<0.0001). The plasma potassium concentration increased from 3.27±0.31 to 4.03±0.33 mmol/L (P<0.0001) after only 1 week on 0.5 mg of LCI699. Twenty-four–hour ambulatory systolic blood pressure decreased by −4.1 mm Hg (95% CI: −8.1 to −0.1 mm Hg) after 4 weeks of treatment (P=0.046). Basal plasma cortisol concentrations remained unchanged, whereas plasma adrenocorticotropin hormone concentrations increased by 35% after 0.5 mg of LCI699 (P=0.08) and by 113% after 1.0 mg of LCI699 (P<0.0001), and the plasma cortisol response to an adrenocorticotropin hormone test was blunted. All of the variables except plasma 11-deoxycorticosterone concentration returned to initial levels after the placebo. LCI699 was well tolerated. In conclusion, the administration of LCI699, up to 1.0 mg BID, effectively and safely inhibits aldosterone synthase in patients with primary aldosteronism. This 4-week treatment corrected the hypokalemia and mildly decreased blood pressure. The effects on the glucocorticoid axis were consistent with a latent inhibition of cortisol synthesis. (Hypertension. 2010;56:831-838.)

Key Words: hypertension • inhibitors • hormones • potassium • enzymes • aldosterone
organs. Fadrozole, an aromatase inhibitor with inhibitory properties against aldosterone synthase,\textsuperscript{18,19} and its dextro-enantiomer FAD286 have been experimentally tested preclinically,\textsuperscript{20–24} but this potential therapeutic approach has never been investigated in patients.

LCI699 is the first orally active aldosterone-synthase inhibitor similar in structure to FAD286 that has been developed for human use. It was shown to cause a dose-dependent decrease in plasma and urinary aldosterone concentrations without affecting basal cortisol levels compared with placebo and to be well tolerated after oral administration over 2 weeks, for doses of <3 mg/d in healthy normotensive men on a controlled salt diet.\textsuperscript{25}

This proof-of-concept study investigated the BP, hormonal effects, tolerability, and safety of inhibiting aldosterone synthesis with LCI699 for 4 weeks, in hypertensive patients with primary aldosteronism, the most common cause of secondary hypertension.\textsuperscript{26} Excess aldosterone in this clinical condition is considered to be responsible for hypokalemia and, at least partly, for BP increase.\textsuperscript{27}

Methods

The full Patients and Methods section is described in the online Data Supplement (please see http://hyper.ahajournals.org). The protocol was approved by the “Comité de Protection des Personnes” (Paris-Ile de France III, France). All of the investigations were performed according to the Declaration of Helsinki principles and Title 45, US Code of Federal Regulations, Part 46. Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. The procedures followed were in accordance with institutional guidelines.

Patients

Eligible patients were male and/or postmenopausal female hypertensive patients aged 18 to 70 years diagnosed with primary aldosteronism within the last 3 years at the Georges Pompidou Hypertension Clinic, based on the combination of spontaneous hypokalemia (plasma potassium ≤3.5 mmol/L), hypertension, and a high aldosterone and low renin status, as reported previously (see Table 1).\textsuperscript{28}

Study Design

After signing an informed consent form and the initial screening assessment (days −56 to −16), patients entered a 2- to 6-week washout period to stop all antihypertensive medication interfering with the renin-angiotensin-aldosterone system and to stabilize their BP measured at home ≤170/105 mm Hg using calcium channel blockers and/or slow release prazosin (see below). After the baseline inclusion visit on day −15, patients entered a single-blind, placebo-controlled, sequential, and forced-titration study\textsuperscript{29} that lasted 7 weeks and included 4 consecutive phases: a 2-week placebo run-in phase (day −14 to −1); a 2-week treatment phase with 0.5 mg of LCI699 bid (day 1 to 14); a 2-week treatment phase with 1.0 mg of LCI699 bid (day 15 to 29); and a 1-week placebo phase (day 30 to 36). Patients were instructed to follow a low-sodium (~50 to 100 mmol/d) and high-potassium (~70 to 100 mmol/d) isocaloric diet throughout the study.

Rationale for LCI699 Dose Selection

The doses of 0.5 and 1.0 mg were selected from results of the phase 1 studies.\textsuperscript{25} Both doses induced potent aldosterone inhibition, as assessed by the decrease in plasma and urinary aldosterone concentrations, without clinical or biological abnormalities. The twice-daily dosing scheme was selected based on the pharmacokinetic parameters of LCI699 observed in this phase 1 study (time to peak concentration of 1 hour and an elimination half-life of ~4 hours)\textsuperscript{24} and the high aldosterone levels expected in the patient with primary aldosteronism.

Table 1. Clinical and Biological Characteristics of the 14 Patients With PA at the Time of Diagnosis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients With PA (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>50.3±6.7</td>
</tr>
<tr>
<td>Male sex, n/N</td>
<td>13/14</td>
</tr>
<tr>
<td>Office SBP, mm Hg</td>
<td>151±17</td>
</tr>
<tr>
<td>Office DBP, mm Hg</td>
<td>91±12</td>
</tr>
<tr>
<td>Antihypertensive treatment score, n</td>
<td>2 (1; 3)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6±3.0</td>
</tr>
<tr>
<td>Unilateral adrenal tumor on CT scan and/or lateralized secretion of aldosterone during adrenal venous sampling, n/N</td>
<td>8/14</td>
</tr>
<tr>
<td>Time interval between diagnosis and inclusion, mo</td>
<td>6 (0.3; 25)</td>
</tr>
<tr>
<td><strong>Biological characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Lowest plasma potassium, mmol/L</td>
<td>3.0 (2.5; 3.5)</td>
</tr>
<tr>
<td>Plasma potassium at time of diagnosis, mmol/L</td>
<td>3.1 (2.6; 4.1)</td>
</tr>
<tr>
<td>Supine plasma aldosterone, pmol/L</td>
<td>630 (359; 997)</td>
</tr>
<tr>
<td>Supine plasma renin, μmol/L</td>
<td>4.5 (1.0; 9.5)</td>
</tr>
<tr>
<td>Supine plasma aldosterone/renin ratio, pmol/μmol</td>
<td>123 (84; 553)</td>
</tr>
<tr>
<td>Upright plasma aldosterone, pmol/L</td>
<td>816 (552; 1459)</td>
</tr>
<tr>
<td>Upright plasma renin, μmol/L</td>
<td>4.7 (1.0; 12.1)</td>
</tr>
<tr>
<td>Upright plasma aldosterone/renin ratio, pmol/μmol</td>
<td>142 (93; 816)</td>
</tr>
<tr>
<td>Urine aldosterone excretion, mmol/24 h</td>
<td>79 (60; 135)</td>
</tr>
</tbody>
</table>

Data are mean±SD or median (minimum; maximum). PA was defined by a supine or upright plasma aldosterone/renin ratio ≥64 pmol/μmol on 2 separate occasions and an elevated supine plasma aldosterone concentration ≥500 pmol/L or 24-hour urinary free extractable aldosterone at pH1 excretion ≥63 mmol/d after a 2- to 6-week washout of drugs interfering with the renin-angiotensin-aldosterone system.\textsuperscript{28} SBP/DBP indicates systolic/diastolic BP.

Concomitant Antihypertensive Treatment and Oral Potassium Supplements

From the screening visit onward, patients received antihypertensive treatment not interfering with the renin-angiotensin-aldosterone system (5 to 10 mg/d of amiodipine or 240 to 300 mg/d of diltiazem and/or 2.5 to 10.0 mg/d of slow-release prazosin), to ensure that BP measured at home using an automated system remained ≤170/105 mm Hg, and oral KCl supplements (3 to 6 g/d), to ensure that plasma potassium levels measured weekly at the clinic remained ≥3.0 mmol/L. The antihypertensive drugs and doses were adjusted if needed until day −15 but were kept constant thereafter until day 36. If needed, the oral KCl dose was adjusted at each visit according to the plasma potassium concentration. From the start to the end of the study, 2 patients received 5 to 10 mg of amiodipine alone, 8 received a combination of 10 mg of prazosin with 5 to 10 mg of amiodipine (n=6) or 300 mg of diltiazem (n=2), and 4 received no additional antihypertensive treatment.

Follow-Up

On days 8, 15, 22, 29, and 36, patients reported to the center at approximately 8:30 AM, without having taken their morning dose, to undergo safety, BP, and biological assessments.

BP Measurements

BP was measured using an adapted cuff placed on the nontodominant arm. Measurements were taken with a calibrated and validated semiautomatic oscillometric electronic device equipped with an electronic memory enabling storage of the BP measurements (UA-
The clinical and biological characteristics of the 14 patients with primary aldosteronism, n*

| Variables                                      | Placebo, Day – 15 | Day 1 (Baseline) | Day 8 (0.5 mg BID) | Day 15 (0.5 mg BID) | Day 22 (1.0 mg BID) | Day 29 (1.0 mg BID) | Placebo, Day 36 | P

| Plasma aldosterone, pmol/L                     | 395 (300 to 520) | 540 (394 to 739) | 173 (131 to 230)*  | 171 (128 to 230)*   | 132 (100 to 173)*   | 133 (100 to 177)*   | 400 (315 to 508) | <0.0001
| 24-h urine aldosterone excretion, nmol/24 h   | 82 (61 to 109)   | 93 (70 to 123)   | 21 (15 to 29)*     | 17 (12 to 24)*      | 10 (7 to 15)*       | 11 (8 to 15)*†     | 71 (64 to 94)    | <0.0001
| Plasma 11-deoxycorticosterone, pmol/L         | 156 (99 to 246)  | 170 (107 to 268) | 1373 (1059 to 1779)*| 1723 (1329 to 2234)*| 2968 (2414 to 3648)*| 2590 (2101 to 3193)*| 585 (425 to 804)*| <0.0001
| Plasma renin concentration, mU/L               | 10 (7 to 13)     | 11 (8 to 15)     | 13 (10 to 18)      | 14 (11 to 19)       | 15 (11 to 19)       | 15 (12 to 18)      | 12 (9 to 16)     | 0.0007
| Plasma cortisol, nmol/L                        | 298 (258 to 345) | 285 (251 to 323) | 307 (273 to 346)   | 306 (277 to 339)    | 298 (263 to 337)    | 272 (232 to 319)   | 283 (253 to 316) | 0.73
| Plasma 11-deoxycortisol, nmol/L                | 1.44 (1.44 to 1.44) | 1.65 (1.38 to 1.96) | 1.94 (1.56 to 2.43) | 3.31 (2.40 to 4.57)* | 6.82 (4.94 to 9.43)* | 6.42 (4.93 to 8.34)*† | 1.62 (1.39 to 1.89) | <0.0001
| Plasma ACTH, pmol/L                            | 5 (3 to 7)       | 4 (3 to 6)       | 5 (3 to 6)         | 6 (4 to 8)          | 8 (6 to 12)*        | 9 (6 to 12)*‡      | 5 (4 to 6)       | <0.0001

Data are geometric means (95% CI). *Data show P<0.0001 vs day 1. †Data show P<0.001 vs day 15. ‡Data show P<0.01 vs day 15. §Data show P value for global time effect by ANOVA.

767PC, A&D Co) either in the office during outpatient visits36 or at home. Twenty-four-hour ambulatory BP monitoring was performed with a validated Spacelabs 90207 monitor (SpaceLabs Medical) placed on the nondominant arm before and after 4 weeks of LCI699 intake, as described previously.31

Laboratory Methods

Plasma electrolytes and blood hormone levels were measured after patients had rested in the supine position for 1 hour. Great care was taken in drawing blood to avoiding any artificial increase in plasma potassium. Twenty-four-hour urine samples were collected at home throughout the study for hormone determination. In addition, plasma aldosterone and cortisol response to an adrenocorticotropic hormone (ACTH) stimulation test (IV injection of 250 μg of Synacthen) were investigated on days 15 (baseline), 30 (12 hours after 1.0 mg of LCI699 intake on day 29), and 36 (1 week washout, cortisol only) at 9:00 AM. The methods for measuring plasma and urinary hormone levels are indicated in the online Data Supplement (please see http://hyper.ahajournals.org). Statistical Methods

The main objectives of the study were to determine whether the inhibition of aldosterone synthase by LCI699 in patients with primary aldosteronism would decrease aldosterone production, lower the mean 24-hour ambulatory systolic BP (SBP) and increase the plasma potassium concentration.

Statistical analyses were performed on the intent-to-treat population. A paired t test was used to compare ambulatory BP values after 4-week LCI699 treatment with baseline values. All of the other variables were analyzed using a linear mixed-effects model with time as the fixed effect and a modeling covariance structure within subjects. Pairwise comparisons between different days were tested using the Holm procedure.

SAS Statistical Software 8.2 was used for statistical analysis. Data are expressed as geometric means with 95% CIs or medians (minimum; maximum) for nonnormal data and as means±1 SD for normally distributed data or otherwise specified. A P value of <0.05 was considered to be significant.

Results

We screened 181 consecutive patients who had been diagnosed previously with primary aldosteronism between 2005 and 2008 at our clinic. Among the 181 patients screened for the study, 133 patients did not meet the inclusion criteria for various reasons: nonmenopausal women (n=44), comorbidities (n=43), age >70 years old (n=9), body mass index >34 kg/m² (n=8), unconfirmed diagnosis of primary aldosteronism (n=12), identified as noncompliant (n=12), and inability to undergo follow-up (n=5). Among the 48 eligible patients, 28 declined to participate because of the constraint of the weekly visits to the hospital. Six of the 20 patients who signed the informed consent form were excluded before receiving LCI699 on day 1 (abnormal test result, n=3; acute atrial fibrillation, n=1; BP increase >170/105 mm Hg, n=1; unconfirmed diagnosis of primary aldosteronism, n=1). The 14 remaining patients completed the study. The main reasons for noninclusion and study discontinuation are described in Figure S1 in the online Data Supplement (please see http://hyper.ahajournals.org). The clinical and biological characteristics of the 14 patients with primary aldosteronism who completed the study are described in Table 1. All had hormonal and biological signs of primary aldosteronism recurrence before LCI699 administration on day 1 (Tables 2 and 3).

Plasma and Urinary Aldosterone, Plasma 11-Deoxycorticosterone, and Plasma Renin Concentration

As early as day 8, patients given 0.5 mg of LCI699 BID showed a marked and significant decrease in both plasma aldosterone concentrations and 24-hour urinary aldosterone excretion (Table 2 and Figure 1). Doubling the LCI699 dose further decreased 24-hour urinary aldosterone excretion (relative change from day 1: −88% [95% CI: −92% to −84%]; day 29 versus day 15, P=0.0003; Table 2 and Figure 1) but not the plasma aldosterone concentration (relative change from day 1: −75% [95% CI: −84 to −63]; day 29 versus day 15, P=0.09). Both variables returned to values not significantly different from baseline levels on day 36 (Table 2 and Figure 1). The time course change in plasma 11-deoxycorticosterone concentrations mirrored that of plasma aldosterone concentrations. Approximately 50% decrease was seen by day 22 of 0.5 mg BID and by day 29 of 1.0 mg BID (Figure 1).
Table 3. Ambulatory and Office BP and Plasma Potassium Changes Before and After LCI699 Administration in 13 Patients With PA

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo, Day –15</th>
<th>Day 1 (Baseline)</th>
<th>Day 8 (0.5 mg BID)</th>
<th>Day 15 (0.5 mg BID)</th>
<th>Day 22 (1.0 mg BID)</th>
<th>Day 29 (1.0 mg BID)</th>
<th>Placebo, Day 36</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulatory BP</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24-h SBP, mm Hg</td>
<td>144.9±9.3</td>
<td>140.8±9.3</td>
<td>0.046†</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>24-h DBP, mm Hg</td>
<td>89.3±7.0</td>
<td>87.2±6.9</td>
<td>0.080†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime SBP, mm Hg</td>
<td>149.2±10.0</td>
<td>145±9.5</td>
<td>0.036†</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Daytime DBP, mm Hg</td>
<td>92.4±6.9</td>
<td>90.9±7.2</td>
<td>0.187†</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nighttime SBP, mm Hg</td>
<td>135.3±9.3</td>
<td>131.4±10.5</td>
<td>0.178†</td>
<td></td>
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<tr>
<td>Nighttime DBP, mm Hg</td>
<td>82.2±7.5</td>
<td>79±7.5</td>
<td>0.092†</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Office BP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>145.8±16.2</td>
<td>145±10.7</td>
<td></td>
<td>138.4±11.5</td>
<td>138.4±12.0</td>
<td>138.4±13.0</td>
<td>135.5±11.3</td>
<td>0.044‡</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>90.1±7.9</td>
<td>91.2±8.6</td>
<td></td>
<td>88.7±11.5</td>
<td>89.3±10.8</td>
<td>90.3±10.5</td>
<td>86.3±8.5</td>
<td>0.284‡</td>
</tr>
<tr>
<td>Plasma potassium, mmol/L</td>
<td>3.44±0.53</td>
<td>3.27±0.31</td>
<td>0.046†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.03±0.33*</td>
<td></td>
<td>4.10±0.34*</td>
<td>3.92±0.33*</td>
<td>3.89±0.35*</td>
<td>3.38±0.35</td>
<td>&lt;0.0001†</td>
</tr>
</tbody>
</table>

DBP indicates diastolic BP. Data are mean ± SD. One patient who entered the active phase with no antihypertensive treatment had an asymptomatic increase in home BP of >170/105 mm Hg on day 15 while receiving LCI699 0.5 mg BID. The decision was made to up-titrate LCI699 to 1.0 mg BID and to add 5.0 mg/day amlodipine, which decreased his BP levels. His office and ambulatory BP measurements were excluded for the statistical analysis.

*P<0.001 vs day 1.
†Data show P value by paired t test.
‡Data show P value for global time effect by ANOVA.

Plasma aldosterone concentrations. On day 8, patients had significantly higher plasma 11-deoxycorticosterone concentrations (relative change from day 1: +710% [95% CI: +411% to +1183%]; P<0.0001; Table 2 and Figure 1), which further increased on day 29 (relative change from day 1: 1427% [95% CI: +863% to +2323%]; day 29 versus day 15, P=0.0039). In contrast to plasma aldosterone concentrations, plasma 11-deoxycorticosterone concentrations remained significantly higher than baseline concentrations on day 36 (+345% [95% CI: +218% to +546%]; day 36 versus day 1, P<0.0001; Table 2 and Figure 1).

Plasma aldosterone response to ACTH was strongly blunted in each individual patient on day 30 in comparison with day −15 (P<0.0001; Figure 2). The mild increase in plasma renin concentration from day 1 (26% to 39%) onward was significant in the ANOVA (P=0.0007; Table 2 and Figure 1).

Ambulatory and Office BP

Office BP was similar at the beginning and end of the 2-week placebo run-in period for all of the patients (Table 3). Treatment with LCI699 over 4 weeks reduced the 24-hour ambulatory SBP by −4.1 mm Hg (95% CI: −8.1 to −0.1 mm Hg; P=0.046; Table 3) but not the 24-hour ambulatory diastolic SBP (−2.1 mm Hg [95% CI: −4.5 to +0.3 mm Hg; P=0.08]). Similar observations were made for daytime SBP/diastolic BP (Table 3). Systolic/diastolic office BP decreased by −7.0 mm Hg (95% CI: −12.4 to +0.2 mm Hg)/−1.9 mm Hg (95% CI: −6.7 to −3.0 mm Hg) on day 15 and by −9.5 mm Hg (95% CI: −16.7 to −2.4 mm Hg)/−4.9 mm Hg (95% CI: −11.0 to +1.3 mm Hg) on day 29 (Table 3). The decrease in office BP was significant for SBP (P=0.044) but not for diastolic BP (P=0.284; Table 3). Office BP returned to levels not significantly different from baseline 1 week after LCI699 discontinuation.

Plasma Potassium Concentrations

Before the administration of LCI699 on day 1, the plasma potassium concentration was low in all of the patients (3.27±0.31 mmol/L), despite a median oral intake of 5 g of KCl per day (range: 4 to 6 g). The oral KCl dose was kept constant until day 15.

Plasma potassium concentration increased by 0.76 mmol/L (95% CI: 0.58 to 0.94 mmol/L; P<0.0001) within 1 week of administering 0.5 mg of LCI699 BID. It exceeded 3.5 mmol/L as early as day 8 in all of the patients (4.03±0.33 mmol/L on day 8; Table 3), allowing oral KCl treatment to be stopped in 13 of 14 patients on day 15. The plasma potassium concentration remained stable until day 29 (3.89±0.35 mmol/L; P<0.0001 versus day 1; Table 3). It decreased by 0.51 mmol/L (95% CI: 0.33 to 0.69 mmol/L) on day 36 with reoccurrence of hypokalemia (3.38±0.35 mmol/L; P<0.0001 versus day 29 and P=0.47 versus day 1; Table 3).

Plasma Cortisol, 11-Deoxycorticisol, and ACTH

Morning plasma cortisol concentrations remained stable in the normal range throughout the study. In contrast, plasma 11-deoxycorticisol concentrations showed a significant and dose-dependent increase from baseline, reaching up to 4-fold greater than baseline values on day 29 (Table 2 and Figure 1). Moreover, morning plasma ACTH concentrations increased significantly from baseline in all of the patients; indeed, ACTH concentrations were 2-fold greater than baseline values on day 29 (Table 2 and Figure 1). In contrast to the plasma 11-deoxycorticosterone concentrations, which remained elevated, the plasma 11-deoxycorticisol and ACTH concentrations returned to baseline levels 1 week after LCI699 discontinuation (Table 2 and Figure 1). On day 30, plasma cortisol response to ACTH was significantly blunted in all 14 patients (P<0.0001 versus day −15; Figure 2) but showed recovery 7 days after stopping LCI699 (data not shown).

Safety and Tolerability

LCI699 administration was clinically and biologically well tolerated; no subjects discontinued after LCI699 treatment was started (please see Table S1).
Discussion

A 4-week treatment with the aldosterone-synthase inhibitor LCI699 induced a potent, rapid, sustained, tolerable, and reversible decrease in plasma and urinary aldosterone concentrations, of up to $\approx 70\%$ to $80\%$, in patients with primary aldosteronism characterized by a high aldosterone and low renin status associated with sustained hypertension and spontaneous hypokalemia. Because aldosterone synthase is responsible for $11\beta$-hydroxylation of 11-deoxycorticosterone to corticosterone and its subsequent 18-hydroxylation and 18-oxidation, we observed a dose-dependent accumulation of 11-deoxycorticosterone in plasma after treatment with LCI699. The plasma 11-deoxycorticosterone concentration remained elevated 1 week after LCI699 withdrawal, suggesting a long-lasting biological inhibition of aldosterone synthase within the adrenal glands. We could not ascertain whether the increase in plasma 11-deoxycorticosterone was attributable only to aldosterone synthase inhibition in the zona glomerulosa after 4 weeks of LCI699 administration, because we did not measure the plasma corticosterone and dehydroepiandrosterone/dehydroepiandrosterone sulfate concentrations because of blood volume constraints. There are, however, some arguments in favor of CYP11B2 inhibition with LCI699 in our patients. First, LCI699 inhibits human recombinant CYP11B2 product, aldosterone synthase, in vitro with a half maximal inhibitory concentration (IC$_{50}$) in the nanomolar range, and it significantly decreased plasma and urine aldosterone concentrations in our patients. Second, although 11-deoxycorticosterone is produced by the zona fasciculata of the adrenal glands under ACTH control and is converted to corticosterone by the CYP11B1 gene product $11\beta$-hydroxylase, the very rapid and marked increase in plasma 11-deoxycorticosterone occurred on day 8, that is, before the significant increase in plasma 11-deoxycortisol (a marker of $11\beta$-hydroxylase inhibition, see below) and ACTH concentrations, which occurred only on days 15 and 22, respectively. Conversely, the plasma 11-deoxycorticosterone concentration remained significantly higher than the baseline value 1 week after LCI699 withdrawal, at a time where plasma 11-deoxycortisol and ACTH concentrations had returned to baseline levels. Therefore, the dissociation between the kinetics of the increase in plasma 11-deoxycorticosterone, 11-deoxycortisol, and ACTH suggests that, at least during the first 15 days of exposure to the lowest LCI699 dose (0.5 mg BID) and 1 week after LCI699 withdrawal, the increased plasma 11-deoxycorticosterone concentrations were mainly

Figure 1. Hormonal changes after LCI699 administration in 14 patients with primary aldosteronism. Percentage changes from day 1 are the ratio of geometric means (95% CI). *P<0.0001 vs day 1; †P<0.001 and ‡P<0.01 vs day 15.
attributed to inhibition of aldosterone synthase. However, from day 22 to day 29, we could not exclude the additive effect of both 11β-hydroxylase inhibition and increased ACTH concentrations in stimulating 11-deoxycorticosterone secretion from the zona fasciculata of the adrenal glands. An extensive study of all precursors of aldosterone, cortisol, and androgens synthesized from cholesterol in the adrenal glands is now necessary, both in animals and humans, to better characterize the effects of LCI699 and other aldosterone-synthase inhibitors.

In patients with primary aldosteronism, 70% to 80% aldosterone synthase inhibition was associated with the hypokalemia correction that occurred within a week of patients being given 0.5 mg of LCI699 BID, allowing the discontinuation of KCl supplementation in 13 of 14 patients on day 15. This effect was observed while patients were on low-sodium (≈50 to 100 mmol/d) and high-potassium (≈70 to 100 mmol/d) diets, and, thus, its magnitude may be less marked in other conditions of sodium and potassium intake. By contrast, the BP-lowering effects of LCI699 were modest (≈4 mm Hg decrease in 24-hour SBP on day 29), and the resultant increase in the plasma renin concentration, an indirect index of sodium depletion, was mild. LCI699 was administered at the highest tolerable dose that was defined based on phase I studies. A >80% decrease in aldosterone synthesis, if not limited by cortisol synthesis impairment (see below), or treatment of a longer duration, may induce greater effects on the sodium balance (ie, increase in plasma renin concentration and decrease in BP).

In summary, the 70% to 80% decrease in the aldosterone concentration achieved over 4 weeks was, thus, sufficient to normalize plasma potassium levels in our sodium and potassium diet conditions but not BP in patients with primary aldosteronism. An increase in the plasma potassium concentration consistently occurs after surgery in patients with an aldosterone-producing adenoma or after MR blockade in essential hypertensive patients, but it does not correlate with the decrease in BP, which also depends on several nonhormonal factors, such as age, body mass index, and cardiovascular remodeling.

Figure 2. Top, Individual plasma aldosterone and cortisol response to an ACTH test (250 µg IV) at screening (day −15) and after 4 weeks of LCI699 administration (day 30) in 14 patients with primary aldosteronism. Open circles are geometric means and 95% CI. Bottom, Individual changes in plasma aldosterone and cortisol concentration after an ACTH test (250 µg IV) at screening (day −15) and after 4 weeks of LCI699 administration (day 30) in 14 patients with primary aldosteronism. Horizontal bars are medians with interquartile range.
LCI699 administered to 14 primary aldosteronism patients over 4 weeks was both clinically and biologically well tolerated. However, although no change in basal/morning cortisol levels was observed, dose-dependent accumulation of basal plasma 11-deoxycortisol, the cortisol precursor, and impairment of ACTH-stimulated cortisol synthesis were apparent after treatment with LCI699, suggesting a latent inhibition of cortisol synthesis. This was confirmed by the 2-fold increase in plasma ACTH concentrations (although still within the normal physiological range) on day 29. This mild compensatory stimulation of ACTH secretion, consistent with activation of the cortisol negative feedback loop at the level of the hypothalamo-hypophysial axis, allowed the maintenance of normal plasma cortisol levels throughout the study. These results demonstrated that LCI699 is not fully selective for aldosterone synthase in vivo, in accordance with the in vitro data. Indeed, in cell systems stably expressing CYP11B2 or CYP11B1, as well as human adrenodoxin and adrenodoxin reductase, LCI699 inhibits the human recombinant CYP11B2 product, aldosterone synthase, in vitro with an IC_50_ in the nanomolar range and inhibits to a lesser extent the CYP11B1 product, 11β-hydroxylase (which converts 11-deoxycortisol to cortisol) with a 3-fold higher IC_50_. Our results similarly indicated that LCI699 was a more potent and longer acting compound in inhibiting aldosterone synthase in vivo than 11β-hydroxylase. Indeed, plasma 11-deoxycorticosterone concentrations remained significantly higher (+345% [95% CI: +218 to +546%]) than baseline values, whereas plasma 11-deoxycorticosterone concentrations returned to baseline levels 1 week after LCI699 withdrawal. These data are in agreement with a recent study, which shows that FAD286 is ≈50-fold more selective at reducing plasma aldosterone than plasma cortisol concentrations, whereas the 11β-hydroxylase inhibitor metyrapone is only ≈3-fold more selective in rodent models of secondary hyperaldosteronism and corticosteronism.

The presence of a mild reversible impairment in cortisol synthesis during LCI699 treatment had no clinical or biological adverse consequences in the short-term study, and all of the patients recovered normal glucocorticoid adrenal function after LCI699 was stopped for a week. However, the long-term effects of suppressing the adrenal response, particularly in response to stressful situations and those of a 2-fold increase in plasma ACTH on adrenal steroid synthesis and tissue changes, are unknown.

Study Limitations
The sample size and the short treatment period are both limitations of the study. Because LCI699 was in an early stage of clinical development at the start of this study, study beyond 4-week duration was not allowed. Consequently, we selected a single-blind sequential placebo-controlled forced-titration design and administered LCI699 to a small sample of a carefully selected population of patients with primary aldosteronism in which the probability to detect the expected effect was the greatest while ensuring the maximal safety. This clinical condition offers a unique opportunity to investigate the effects of this drug in a situation in which aldosterone production at the level of the adrenal glands is considered to be, at least partly, responsible for an increase in BP associated with increased exchangeable sodium, plasma volume and total body water, reduced exchangeable potassium, and renin suppression. The design and sample size used in our study allowed us to demonstrate the effects of LCI699 on lowering the concentration of aldosterone, on potassium sparing, and on lowering BP.

Perspectives
A 4-week administration of LCI699 (up to 1 mg BID), in 14 hypertensive patients with primary aldosteronism, safely induced a sustained, low aldosterone status, which had a greater impact on the correction of low plasma potassium concentrations than on low plasma renin concentration and high BP levels. The effects on the glucocorticoid axis were consistent with a latent inhibition of cortisol synthesis. Our findings suggest that aldosterone synthase inhibition with this first in-class aldosterone synthase inhibitor, LCI699, may offer a new medical treatment alternative to MR blockade or adrenal surgery in patients with primary aldosteronism. White et al. have reported recently that the antihypertensive effect of LCI699 1 mg QD was not statistically different from that of eplerenone 50 mg BID in patients with stage 1 and 2 hypertension. The observation that the selected doses of LCI699 permanently decreased aldosterone concentrations by >80% with the expected biological effects, including a good short-term tolerability and safety, indicates that this new therapeutic approach provides a feasible method for neutralizing the effects of aldosterone. It should be further investigated in patients with cardiovascular and renal diseases, particularly those with heart failure, postmyocardial infarction, and proteinuric nephropathies. Indeed, in contrast to the MR antagonist, exposure of organs sensitive to aldosterone, such as the heart, the blood vessels, and the kidneys, in the presence of high dietary sodium intake is decreased by an aldosterone-synthase inhibitor. This decrease, achieved in the absence of a change in basal cortisol levels, may, in addition to the BP and electrolytic effects, contribute to the neutralization of harmful genomic and nongenomic effects of aldosterone and the aldosterone-dependent effects of the small GTP binding protein Rac1, a potent activator of MR signal transduction, which could lead to long-term improvement in tissue injury effects of aldosterone.

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Disclosures
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References


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Aldosterone synthase inhibition with LCI699: a proof of concept study in patients with primary aldosteronism.

Short title: Aldosterone synthase inhibition
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Methods

The protocol (ClinicalTrials.gov Identifier: NCT00732771) was approved by the “Comité de Protection des Personnes” (Paris-Ile de France III, France) and the “Agence Française de Sécurité Sanitaire des Produits de Santé”. All investigations were performed according to the Declaration of Helsinki principles and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. The procedures followed were in accordance with institutional guidelines.

Patients

Eligible patients were male and/or post-menopausal female hypertensive patients aged 18 to 70 years diagnosed with primary aldosteronism within the last three years at the Georges Pompidou Hypertension Clinic, based on the combination of spontaneous hypokalemia (plasma potassium ≤3.5 mmol/L), hypertension and a high aldosterone and low renin status. Primary aldosteronism was defined by two occurrences of a supine or upright plasma aldosterone/immunoreactive renin ratio ≥ 64 pmol/mU and an elevated supine plasma aldosterone concentration ≥ 500 pmol/L or 24h urinary free extractable aldosterone at pH1 excretion ≥ 63 nmol/24h, as reported previously. Main exclusion criteria included severe grade III hypertension, persistent hypokalemia < 3.0 mmol/L despite oral administration of KCl (6 g/day), estimated creatinine clearance < 60 ml/min, type 1 or uncontrolled (HbA1c ≥ 8%) type 2 diabetes mellitus, and a history of any severe cardiac, cerebrovascular or life-threatening disease.

Study design

After signing an informed consent form and the initial screening assessment (Days -56 to -16), patients entered a two- to six-week washout period, to stop all antihypertensive medication interfering with the renin-angiotensin-aldosterone system and to stabilize their BP measured at home ≤170/105 mmHg using calcium channel blockers and/or slow release prazosin (see below). After the baseline inclusion visit on Day -15, patients entered a single-blind, placebo-controlled, sequential, and forced-titration study 2 that lasted seven weeks and included four consecutive phases: a two-week placebo run-in phase (Day -14 to -1); a two-week treatment phase with 0.5 mg LCI699 bid (Day 1 to 14); a two-week treatment phase with 1 mg LCI699 bid (Day 15 to 29); and a one-week placebo phase (Day 30 to 36). Throughout the study, patients were kept blinded to the placebo and LCI699 capsules, which were similar in appearance. Patients were instructed to follow a low sodium (≈ 50 to 100 mmol/day) and high potassium (≈ 70 to 100 mmol/day) isocaloric diet throughout the study.

Rationale for LCI699 dose selection.

The doses of 0.5 and 1 mg were selected from the two-week, randomized, double-blind, placebo-controlled, multiple-ascending-dose (0.5, 1, 3 and 10 mg daily) study in healthy men on a controlled sodium diet with eplerenone 100 mg qd, as an active comparator. Both doses induced potent aldosterone inhibition, as assessed by the decrease in plasma and urinary aldosterone concentrations, without clinical or biological abnormalities. Additionally, aldosterone inhibition by LCI699 (0.5-3 mg) was accompanied by a dose-dependent increase in trough plasma renin activity with the 0.5 mg dose stimulating renin-angiotensin system counter-regulation to a similar degree to eplerenone 100 mg, suggesting comparable inhibition of the aldosterone pathway. The twice-daily dosing scheme was selected based on the pharmacokinetic parameters of LCI699 observed in this phase I study (Tmax of 1 h and an elimination half-life of ~4 h) and the high aldosterone levels expected in the patient with primary aldosteronism. We did not select higher doses since during the multiple oral dose phase I study, signs of hypoaldosteronism (postural tachycardia, decreased body weight and mild hyponatremia) were detected in some subjects at the 3 mg qd dose.
Concomitant antihypertensive treatment and oral potassium supplements

From the screening visit onwards, patients received 1) antihypertensive treatment not interfering with the renin-angiotensin-aldosterone system (5-10 mg/day of amlodipine or 240-300 mg/day of diltiazem and/or 2.5-10 mg/day of slow-release prazosin), to ensure that BP measured at home using an automated system remained ≤170/105 mmHg, and 2) oral KCl supplements (3 to 6 g/day), to ensure that plasma potassium levels measured weekly at the clinic, remained ≥3.0 mmol/L. The antihypertensive drugs and doses were adjusted if needed until Day -15, but were kept constant thereafter until Day 36. If needed, the oral KCl dose was adjusted at each visit according to the plasma potassium concentration.

From the start to the end of the study, two patients received 5 to 10 mg amlodipine alone, eight received a combination of 10 mg prazosin with 5 to 10 mg amlodipine (n=6) or 300 mg diltiazem (n=2), and four received no additional antihypertensive treatment.

Follow-Up

On Days 8, 15, 22, 29, and 36, patients reported to the centre around 08:30 am, without having taken their morning dose, to undergo safety, BP and biological assessments. During each visit, adverse events, concomitant medication, and treatment compliance assessed by pill counting were recorded.

BP measurements

BP was measured using an adapted cuff placed on the non-dominant arm. Measurements were taken with a calibrated and validated semi-automatic oscillometric electronic device equipped with an electronic memory enabling storage of the BP measurements (UA-767PC, A&D Co, Tokyo, Japan) either in the office during out-patient visits or at home.

Twenty four-hour ambulatory BP monitoring was performed with a validated Spacelabs 90207 monitor (Spacelabs Medical, Redmond, Wash.), placed on the non-dominant arm before and after 4 weeks of LCI699 intake, as described previously. Measurements included mean 24-hour systolic and diastolic blood pressures, as well as mean daytime values (measured every 15 minutes from 7 a.m. to 10 p.m.) and night-time values (measured every 20 minutes from 10 p.m. to 7 a.m.), all of which served as primary outcome measures.

Laboratory methods

Plasma electrolytes and blood hormone levels were measured after patients had rested in the supine position for one hour. Great care was taken in drawing blood by avoiding a tourniquet, fist clenching, and any condition that might artificially increase plasma potassium. Twenty-four-hour urine samples were collected at home throughout the study for hormone determination. Additionally, plasma aldosterone and cortisol response to an ACTH stimulation test (IV injection of 250 µg of Synacthen®) were investigated on Days -15 (baseline), 30 (12 h after last 1mg LCI699 intake on Day 29) and 36 (1 week washout, cortisol only) at 09:00 am.

Plasma aldosterone and urinary free extractable aldosterone at pH1 concentrations were measured using a commercially available radioimmunoassay kit (DPC, France). Plasma cortisol, 11-deoxycortisol and 11-deoxycorticosterone were quantified simultaneously using a specific liquid chromatography followed by tandem mass spectroscopy method. Plasma ACTH was measured using a commercially available immunoradiometric kit (Imunotech, France). The plasma immunoreactive active renin concentration was measured using a commercially available immunoradiometric kit (CisBio, France).

Statistical Methods

The main objectives of the study were to determine whether the inhibition of aldosterone synthase by LCI699 in patients with primary aldosteronism would decrease aldosterone
production, lower the mean 24-hour ambulatory systolic BP (SBP) and increase the plasma potassium concentration.

Statistical analyses were performed on the intent-to-treat population. A paired t-test was used to compare ambulatory BP values after four-week LCI699 treatment with baseline values. All other variables were analyzed using a linear mixed effects model with time as the fixed effect, and a modelling covariance structure within subjects. Pairwise comparisons between different days were tested using the Holm procedure.

SAS Statistical Software 8.2 (Cary, NC 27513, USA) was used for statistical analysis. Data are expressed as geometric means with 95 % confidence intervals (CI) or medians (min; max) for non-normal data and as means ± one standard deviation (SD) for normally distributed data or otherwise specified. A P value of less than 0.05 was considered to be significant.
References


Table S1: Safety and tolerability of 4 week-administration of LCI699 in 14 patients with primary aldosteronism.

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181 patients assessed for eligibility

133 patients not meeting inclusion criteria
- 44 non-menopausal women
- 20 patients with other disease
- 16 patients with cardiovascular disease
- 9 patients aged > 70 years old
- 7 patients with renal insufficiency
- 8 patients with a body mass index >34 kg/m²
- 5 patients living abroad
- 12 patients with unconfirmed primary aldosteronism
- 12 patients known as non compliant

48 eligible patients

28 patients refused to participate

20 patients entered washout and run in period

6 patients excluded
- 3 for abnormal test procedure result
- 2 for adverse events (1 episode of acute atrial fibrillation, 1 BP >170/105 mmHg)
- 1 for protocol violation (unconfirmed diagnosis of primary aldosteronism)

14 patients entered the active phase

14 patients completed the study

Figure S1: Flow chart of the study.