ONLINE DATA SUPPLEMENT

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/mUI 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. 1 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. 3 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) 3 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. 3
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-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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<th>Parameters</th>
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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.