Journal of Pharmacological and Toxicological Methods 71 (2015) 137–146

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Rhesus monkey model for concurrent analyses of in vivo selectivity, pharmacokinetics and pharmacodynamics of aldosterone synthase inhibitors

Tian-Quan Cai [a,*], Sloan Stribling [a], Xinchun Tong [b], Ling Xu [b], Thomas Wisniewski [c], Jane A. Fontenot [d], Mary Struthers [c], Karen O. Akinsanya [c]

[a] In Vivo Pharmacology, Kenilworth, NJ, USA
[b] Pharmacokinetics and Drug Metabolism, Kenilworth, NJ, USA
[c] Cardiovascular Diseases of Merck Research Laboratories, Kenilworth, NJ, USA
[d] New Iberia Primate Research Center, New Iberia, LA, USA

ARTICLE INFO

Article history:
Received 18 July 2014
Accepted 30 September 2014
Available online 7 October 2014

Keywords:
Aldosterone
Animal model
Glucocorticoids
Pharmacodynamics
Pharmacokinetics

ABSTRACT

Introduction: In vivo profiles of aldosterone synthase inhibitors (ASIs) have been investigated utilizing various rodent models. Due to lack of CYP17 activity, rodents produce corticosterone rather than cortisol as that of humans, which raised concern to their effectiveness in translational pharmacological characterization of ASI. **Methods**: A rhesus monkey model that combines a low sodium diet with adrenocorticotropin (ACTH) treatment was developed. Plasma concentrations of steroid metabolites associated with reactions catalyzed by CYP11B2 and CYP11B1 were measured concurrently by a UPLC/MS method. **Results**: Plasma concentration of aldosterone in regular diet fed rhesus monkeys was low at 109 pg/mL. Aldosterone concentrations were increased to 252 pg/mL when animals were maintained on a low sodium diet for 3 weeks, and to 300 pg/mL with ACTH treatment at 0.3 mg/kg. The combination of low sodium diet with ACTH treatment further increased plasma concentration of aldosterone to 730 pg/mL and other steroid metabolites at various levels. Intravenous administration of ASI, fadrozole (0.001–1 mg/kg) or LCI699 (0.003–3 mg/kg), led to dose-dependent reductions in aldosterone and 18-hydroxycorticosterone, increases in 11-deoxycorticosterone and 11-deoxycortisol, and bell-shaped changes in cortisol and corticosterone. In vivo selectivity of CYP11B2/CYP11B1 for fadrazole was 26-fold and LCI-699 was 27-fold, which was consistent with relative selectivity using in vitro values from recombinant cells transfected with rhesus monkey CYP11B2 and CYP11B1. **Discussion**: This model enables concurrent characterization of pharmacokinetics, pharmacodynamics and selectivity of CYP11B2 over CYP11B1 inhibition in the same animal. It may be used as a translational model for pharmacological characterization of ASI.

1. Introduction

Aldosterone is a key mediator of salt and water homeostases. Excess levels of aldosterone are linked to the pathogenesis of hypertension and congestive heart failure (Rocha & Funder, 2002; Struthers, 2004; Weber, 2001). Aldosterone may mediate its effects via genomic and non-genomic mechanisms (Funder & Mihailidou, 2009). The genomic effects of aldosterone are mediated via the activation of an intracellular mineralocorticoid receptor (MR), whereas the nongenomic effects may be mediated via the action of MR or other receptors (Funder & Mihailidou, 2009). Benefits of targeting the action of aldosterone were demonstrated in the clinic via antagonists of MR, including spironolactone and eplerenone (Fiebeler, Muller, Shagdarsuren, & Luft, 2007; Funder, 2006; Funder & Mihailidou, 2009). A widespread use of these MR antagonists, however, has been limited due to efficacy and tolerability issues (Brown, Quirk, & Kirkpatrick, 2003; Jeunemaitre et al., 1987).

Aldosterone synthase (CYP11B2) is the enzyme responsible for the final critical steps of aldosterone biosynthesis in the adrenal gland (Bureik, Lisurek, & Bernhardt, 2002; Jansen, van den Meiracker, & Jan Danser, 2009). Inhibiting CYP11B2 will decrease concentrations of

Abbreviations: ASI, aldosterone synthase inhibitor; ACTH, adrenocorticotropin; MR, mineralocorticoid receptor; LSD, low sodium diet; DOC, 11-deoxycorticosterone; RSS, 11-deoxycortisol; 18-OHB, 18-hydroxycorticosterone; PD, pharmacodynamics; PK, pharmacokinetics; Fadrozole (FAD286), (+)-(5R)-4-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-5-yl]benzonitrile hydrochloride; LCI699, ((R)-4-(6,7-dihydro-5H-pyrrolo[1,2-c]imidazol-5-yl)-3-fluorobenzonitrile.

* Corresponding author at: In Vivo Pharmacology, Merck Research Laboratories, K15-MW312, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA. Tel.: +1 908 740 5544; fax: +1 908 740 4151.
E-mail address: tianquan_cai@merck.com (T.-Q. Cai).

http://dx.doi.org/10.1016/j.vascn.2014.09.011
1056-8719/

aldosterone, which could mitigate both aldosterone receptor-dependent and -nondependent actions. Human CYP11B2 shares 93% sequence homology with CYP11B1 (11β-hydroxylase), an enzyme critical for the biosynthesis of cortisol (Bureik et al., 2002; Kawamoto et al., 1992). Cortisol is the main glucocorticoid in human and it regulates stress responses and is involved in immune responsiveness of the human body (Bureik et al., 2002). Selectivity for CYP11B2 inhibition over CYP11B1 is essential for a successful development of aldosterone synthase inhibitors (ASIs).

The in vivo profile of ASI activity has largely been investigated utilizing various rodent models (Ménard, Gonzalez, Guyene, & Bissery, 2006; Rigel et al., 2010). While these rodent models may be effective in profiling inhibitory activity of ASI on synthesis of aldosterone (Ménard et al., 2006; Rigel et al., 2010) or studying effect of ASI on aldosterone-mediated organ functions (Fiebeler et al., 2005; Mulder et al., 2008), the ability to fully characterize the cross reactivity against CYP11B1 in rodents is a concern. There is a major difference in adrenal steroid metabolism between humans and rodents. Unlike humans, rats lack CYP17 activity in the adrenal (Bush, 1953; van Weerden, Bierings, van Steenbrugge, de Jong, & Schröder, 1992). As a result, rats produce only negligible amounts, if any, of cortisol, and instead produce corticosterone, which is a precursor to aldosterone biosynthesis in humans and acts as the major glucocorticoid in rats.

The purpose of this study was to develop a non-human primate model that will enable concurrent characterization of pharmacokinetics, pharmacodynamics and selectivity of ASI in vivo. Four model conditions were investigated, including rhesus monkeys on: 1) regular diet, 2) low sodium diet (LSD), 3) regular diet plus adrenocorticotropic hormone (ACTH) treatment, and 4) LSD plus ACTH treatment. Plasma concentrations of adrenal steroid metabolites associated with reactions catalyzed by CYP11B2 and CYP11B1 (see Fig. 1), including aldosterone, 11-deoxycorticosterone (DOC), 11-deoxycortisol (RSS), cortisol, corticosterone and 18-hydroxycorticosterone (18-OHB), were measured concurrently from the same sample via a newly developed UPLC/MS method. Effects of two ASIs, fadrozole (FAD286, (+)-(5R)-4-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-5-yl]benzonitrile hydrochloride, Furet et al., 1993) and LCI699 ((R)-4-(6,7-dihydro-5H-pyrrolo[1,2-c]imidazol-5-yl)-3-fluorobenzonitrile, Menard, Watson, Rebello, Zhang, & Dole, 2010; Hu, Yin, & Hartmann, 2014), were investigated in LSD plus ACTH treated animals. PK/PD relationship and in vivo potency/selectivity profiles of these two ASIs were generated.

2. Materials and methods

2.1. In vitro analyses of rhesus monkey CYP11B1 and CYP11B2 activities

V79 cell lines stably expressing either the rhesus CYP11B2 or CYP11B1 enzyme were generated by transfecting the cells with plasmids of pTriEx3-Hygro-rhesus CYP11B2 or pTriEx3-Hygro-rhesus CYP11B1 using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). Single cell clones were generated by infinite dilution. Clones that exhibit high activities toward aldosterone (CYP11B2) or cortisol (CYP11B1) production were selected. On the day of assay, cells were harvested at 80% confluency with 0.5% Trypsin-EDTA, washed once in PBS, and reconstituted in cell suspension media (DMEM with 0.1% BSA) at 4×10^5 cells/mL.

Activities of CYP11B2 were assayed by measuring conversion of DOC into aldosterone in 384-well plates. 25 μL per well of cells at 4×10^5 cells/mL stock concentration and 0.3 μL per well of increasing concentrations of test articles dissolved 100% DMSO stock were transferred to 384-well plates. Following 1 h incubation in a 37 °C humidified incubator at 5% CO_2, 5 μL per well of DOC with stock concentration of 750 nM prepared in the same cell suspension media (DMEM media with 0.1% BSA) were added to each well. After 3 h of incubation, culture supernatants were harvested and stored at −80 °C for measurement of aldosterone. Concentrations of aldosterone were measured using an aldosterone HTRF assay kit as instructed by the manufacturing company (Cisbio, France). Compounds were tested and assayed in triplicates.

Measurement of CYP11B1 enzyme activity was carried out in a manner similar to that of CYP11B2 assay, except that 5 μL per well of substrate DOC was replaced with 5 μL per well of substrate RSS with stock concentration of 1500 nM, and V79 cells expressing rhesus CYP11B1 were used. Concentrations of cortisol were measured using a cortisol HTRF assay kit as instructed by the manufacturing company (Cisbio, France).

2.2. In vivo experiments

2.2.1. Animal use

All animal procedures were conducted in accordance with approved Institutional Animal Care and Use Committee protocols. 45 healthy male rhesus macaques (age: ~3–13 years, body weight between 5 and 14 kg) were selected for study use from established in-house colonies at New Iberia Primate Research Center (New Iberia, LA). Animals were pair housed in temperature and humidity controlled rooms with fixed light/dark cycles of 12 h duration. Animals were fed with a commercial fiber-plus monkey diet that has sodium content of 0.22% (#5049, LabDiet, St. Louis, MO) twice daily, and provided fresh water ad libitum. Animals were food fasted overnight (approximately 16 h) prior to study participation. In studies involving low sodium conditions, the diet was replaced with a similar commercial fiber-plus monkey diet but without added sodium leading to a final sodium content of 0.04% (#1810052, TestDiet, St. Louis, MO). Animals were maintained on the same diet for at least 3 weeks before enrolling in studies.

2.2.2. Experimental procedures

Animals were sedated by the injection of telazol (5 mg/kg, IM), starting at 7:00 AM sharp to minimize potential circadian rhythm related influences to the study animals. Intravenous catheters were then placed in saphenous or cephalic veins. Animal sedation was maintained for the duration of the procedure by constant infusion of ketamine HCL at 0.2 mg/kg/min delivered via an infusion pump and initiated upon IV catheter placement. Routine vital signs, including heart rate, respiratory rate, body temperature and pulse-oximetry, were monitored via a Surgivet monitor (Smiths Medical PM, Norwell, MA). ACTH at 0.3 mg/kg was administered via IM at 0.3 mL/kg, divided over two sites. Test compounds were administered via intravenous injection at 0.3 mL/kg. Blood samples were collected periodically during the studies for ex vivo measurements of plasma concentrations of adrenal steroid metabolites and test compounds. Approximately 1 mL of blood sample was collected from each animal into EDTA tubes at each time point. Plasma samples were prepared immediately by centrifugation of blood samples at 3000 rpm for 10 min, and stored at −80 °C for analytical use. At the end of the study, animals were recovered and returned to their home cages.

2.2.3. Experimental protocols

2.2.3.1. Assessment of ACTH effects in regular- and low sodium-diet fed animals. Animals on regular or low sodium diet were sedated by injection of telazol and maintained anesthetized by constant infusion of ketamine throughout the study. ACTH at 0.3 mg/kg was administered. Blood samples were collected at 0 (right before ACTH administration) and 30, 60, 90, 120, 150 and 180 min post-ACTH administration. Plasma samples were prepared and stored at −80 °C for the measurement of plasma concentrations of adrenal steroid metabolites.

2.2.3.2. Assessment of fadrozole or LCI699 effects in low sodium-diet fed animals treated with ACTH. Animals on low sodium diet were anesthetized. Vehicle (saline, 0.3 mL/kg, IV) or increasing doses of fadrozole