Chronic Direct Renin Inhibition With Aliskiren Prevents the Development of Hypertension in Cyp1a1-Ren2 Transgenic Rats With Inducible ANG II-Dependent Hypertension

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Abstract: *Introduction:* This study was performed to determine whether chronic direct renin inhibition can prevent the development of slowly progressive angiotensin (ANG) II-dependent hypertension and the associated derangements in renal function in Cyplal-Ren2 transgenic rats with inducible expression of the *Ren2* gene. *Methods:* Male Cyplal-Ren2 rats (n = 6) were fed a normal diet containing 0.15% indole-3-carbinol (I3C) for 16 days to induce slowly progressive ANG II-dependent hypertension. Conscious systolic blood pressure was measured daily using tail-cuff plethysmography. The rats were then anesthetized with pentobarbital sodium and surgically prepared for the measurement of mean arterial pressure (MAP) and renal hemodynamics and excretory function. *Results:* In rats induced with I3C, systolic blood pressure increased by day 3 (130 ± 7–160 ± 5 mm Hg, $P < 0.01$) and continued to increase to 191 ± 6 mm Hg ($P < 0.001$) by day 16. In a separate group of rats (n = 6), chronic administration of the direct renin inhibitor, aliskiren (30 mg/kg/d, sc), prevented the development of hypertension (113 ± 5 versus 114 ± 5 mm Hg, not significant). Rats treated with aliskiren exhibited significantly lower mean arterial pressure (138 ± 4 versus 201 ± 6 mm Hg, $P < 0.001$), renal vascular resistance (23 ± 4 versus 38 ± 3 mm Hg/mL/min · g, $P < 0.01$), urine flow (17.6 ± 1.4 versus 25.1 ± 2.9 μL/min, $P < 0.05$) and urinary sodium excretion (1.11 ± 0.32 versus 2.35 ± 0.28 μEq/min, $P < 0.05$) and higher renal plasma flow (4.22 ± 0.23 versus 2.56 ± 0.21 mL/min · g, $P < 0.01$) and glomerular filtration rate (1.19 ± 0.07 versus 0.78 ± 0.08 mL/min · g, $P < 0.01$), compared with induced rats not treated chronically with aliskiren. *Conclusions:* The present findings demonstrate that chronic direct renin inhibition with aliskiren prevents the development of ANG II-dependent hypertension and the associated derangements in renal hemodynamics and excretory function in Cyplal-Ren2 transgenic rats.

Key Indexing Terms: Kidney; Renin-angiotensin system; Renin inhibitor; Renal hemodynamics; Sodium excretion. **[Am J Med Sci 2012;344(4):301–306.]**

The Cyplal-Ren2 transgenic rat line was created by inserting the mouse *Ren2* renin gene, fused to an 11.5-kb fragment of the cytochrome P450 l a l promoter, into a neutral genomic site on the Y chromosome of the Fischer 344 rat.[1] Cyplal, which catalyzes the oxidation of a wide range of endogenous lipophilic compounds and xenobiotics,[2–4] is not constitutively expressed but is highly inducible on exposure to various aryl hydrocarbons such as indole-3-carbinol (I3C).[2–8] Induction of Cyplal is mediated by the aryl hydrocarbon receptor, which is a basic helix-loop-helix-transcription factor that binds to specific DNA elements in the Cyplal promoter. [2,4,9] Rats transgenic for the Cyplal-Ren2 construct do not constitutively express the *Ren2* renin gene. Rather, the *Ren2* gene is expressed, primarily in the liver, only on induction of the Cyplal promoter by dietary administration of aryl hydrocarbons such as I3C.[1] Previous studies have demonstrated that chronic dietary administration of I3C increases blood pressure in a dose-dependent fashion.[1,10] At a dose of 0.15% (w/w), dietary I3C administration leads to hypertension that develops gradually over the course of 14 to 16 days.[10,11] In contrast, at a higher dose of 0.3% (w/w), dietary I3C induces malignant hypertension characterized by rapid development of hypertension, severe loss of body weight, lethargy and piloerection.[10,12,13] In addition, dietary I3C increases plasma renin activity (PRA) and plasma angiotensin (ANG) II levels in a dose-dependent manner.[10]

Recent investigations showed that AT_1 receptor blockade both prevents and treats the increase in blood pressure in Cyplal-Ren2 rats induced with 0.3% I3C.[14–16] These findings have strongly suggested that the hypertension Cyplal-Ren2 animals induced with I3C has been mediated via an ANG II-dependent mechanism. However, little information is available regarding the blood pressure and renal effects of the new pharmacologic targets to the renin-angiotensin system such as aliskiren, the first direct renin inhibitor in the U.S. market, in slowly progressive forms of ANG II-dependent hypertension. Aliskiren binds to the $S3^{bp}$ binding pocket of renin, its active site, thereby preventing the conversion of angiotensinogen to ANG I.[17,18] Although originally designed to bind to human renin, animal studies have shown that aliskiren binds mouse renin effectively in models that upregulate mouse renin.[18] Human clinical trials have shown that aliskiren suppresses both PRA and hypertension. Chronic administration of aliskiren in TGR(mRen2)27 rats that constitutively express the mouse *Ren2* renin gene has shown that aliskiren suppresses hypertension, reduces renal and cardiac pathologies associated with increased blood pressure and prevents the increases in both kidney and plasma ANG II levels.[19,20] Acute administration of aliskiren in the Cyplal-Ren2 transgenic rat model with inducible malignant hypertension demonstrated that aliskiren normalizes mean arterial pressure (MAP) and renal plasma flow (RPF) without altering glomerular filtration rate (GFR).[21] However, little is known about the effects of chronic administration of a direct renin inhibitor on the prevention of blood pressure and the associated derangements in renal hemodynamics and sodium excretory function in slowly progressive form of ANG II-dependent hypertension. This study was performed to address the hypothesis that chronic direct renin inhibition with aliskiren prevents increases in arterial blood pressure and associated derangements

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in renal hemodynamics and sodium excretory function in Cyplal-Ren2 transgenic rats with slowly progressive ANG II-dependent hypertension.

METHODS

The experimental procedures in this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Tulane University Health Sciences Center. Experiments were performed on adult male transgenic rats [TGR(Cyplal-Ren2)] with inducible expression of the mouse *Ren2* renin gene.[1] All transgenic rats used in this study were adult male Cyplal-Ren2 transgenic rats (249–280 g) bred at the Tulane University School of Medicine from stock animals supplied by Harlan UK Limited, Bicester, UK. The experimental animals were divided into 3 groups. Group 1 (noninduced; n = 6) were maintained on a normal, non-I3C rat diet (diet TD 99414; Harlan-Teklad, Madison, WI) for 16 days. Group 2 (0.15% I3C; n = 6) Cyplal-Ren2 rats were fed a normal diet containing 0.15% I3C for 16 days to induce slowly progressive ANG II-dependent hypertension. Group 3 (I3C+Aliskiren; n = 6) Cyplal-Ren2 rats were implanted with an Alzet 2ML4 osmotic minipump administering aliskiren (30 mg/kg/d, subcutaneously) 3 days before initiating dietary administration of 0.15% I3C. Sham surgeries were performed on groups 1 and 2 to control for the minor surgical trauma experienced by group 3. The dose of aliskiren used in this study was chosen on the basis of the previous observation that chronic administration of this dose of aliskiren elicited a pronounced and prolonged decrease in blood pressure in hypertensive TGR(mRen2)27 transgenic rats, which constitutively express high levels of the mouse *Ren2* renin gene.[22] They were fed a normal diet containing 0.15% I3C for 16 days. The rats were allowed free access to food and tap water until day 16.

Measurement of conscious systolic blood pressure (SBP) was obtained in conscious rats using tail-cuff plethysmography (Model 6R22931; IITC Life Science, Woodland Hills, CA). All rats were trained for 1 week before the beginning of the experiment in order to habituate them to this procedure. Blood pressures were measured daily throughout the duration of the study. Body weight was also measured daily throughout the course of the study.

At the conclusion of the treatment period, all animals were surgically prepared for assessment of renal hemodynamics and continuous measurement of arterial blood pressure as described previously.[10] Briefly, the rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal) and placed on a surgical table thermostatically controlled to maintain body temperature at 37°C. A tracheostomy was performed, and the animals were allowed to breathe humidified air enriched with oxygen. The left jugular vein was cannulated to allow infusion of solutions and additional anesthetic. The rats were infused at a constant rate of 1.2 mL/hr with isotonic saline containing 6% albumin (bovine; Sigma Chemical, St. Louis, MO) during surgery and thereafter with isotonic saline containing 1% albumin, 7.5% polyfructosan (Inutest; Fresenius Kabi Austria GmbH, Linz, Austria) and 1.5% p-aminohippurate sodium (PAH; Merck Sharp & Dohme, West Point, PA). The carotid artery was cannulated to allow measurement of arterial blood pressure. Blood pressure was monitored with a Statham pressure transducer (model P23DC) and recorded using a computerized data acquisition system (MP100 System; BIOPAC Systems, Santa Barbara, CA) with the AcqKnowledge Software Package (version 3.7.3; BIOPAC Systems). A suprapubic incision was made and the bladder was exposed by blunt dissection through the abdominal wall. The bladder was catheterized to allow timed urine collections to be made.

After a 45-minute recovery period, urine was collected during 2, 30-minute periods followed by a blood sample (~300 μL) to allow assessment of whole kidney hemodynamics and excretory function. At the end of each experiment, both kidneys were removed, decapsulated, blotted dry and weighed. Urine volume was determined gravimetrically. Sodium concentration in urine and plasma were measured using flame photometry. Inulin and PAH concentrations in both urine and plasma were measured by standard spectrophotometry. GFR and RPF were estimated from the clearance of inulin and PAH, respectively. Renal blood flow was calculated as RPF/(1 – hematocrit). Renal vascular resistance (RVR) was determined from the quotient of MAP and calculated renal blood flow.

Statistical analyses were performed within groups using 1-way repeated measures analysis of variance followed by Student-Newman-Keuls test and between groups using 1-way analysis of variance followed by Student-Newman-Keuls test. All statistical analyses were performed using SigmaPlot for Windows (version 11; Systat Software, San Jose, CA). Statistical significance was defined as $P < 0.05$. All data are expressed as mean ± standard error of mean.

RESULTS

Conscious SBPs and changes in body weights of Cyplal-Ren2 transgenic rats are summarized in Figures 1 and 2. Chronic administration of 0.15% I3C resulted in an increase in SBP by day 3 (130 ± 7–160 ± 5 mm Hg, $P < 0.01$) and continued to increase incrementally to a peak of 191 ± 6 mm Hg ($P < 0.001$) by day 16 (Figure 1). The hypertensive animals did not exhibit signs of malignant hypertension, such as piloerection or severe lethargy.

Hypertensive animals did not exhibit weight loss, and they did not experience a weight gain as seen in animals concurrently treated with aliskiren or maintained on a non-I3C diet (Figure 2). In this regard, noninduced rats increased their body weights by 14 ± 1% ($P < 0.01$), and induced rats treated chronically with aliskiren increased their body weights by 14 ± 1% ($P < 0.01$) throughout the course of the study. In contrast,

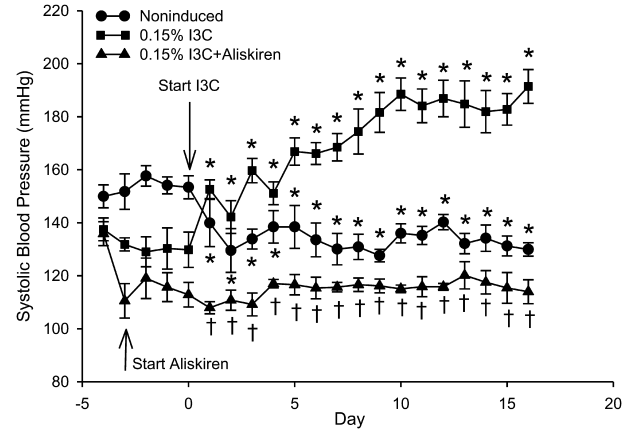

FIGURE 1. Conscious systolic blood pressures in noninduced (circle), 0.15% I3C-induced (square) and 0.15% I3C + aliskiren-treated (triangle) Cyp1a1-Ren2 rats. *$P < 0.05$ versus day 0 within group. †$P < 0.05$ versus 0.15% I3C-induced rats on corresponding day.