Aliskiren, a Human Renin Inhibitor, Ameliorates Cardiac and Renal Damage in Double-Transgenic Rats


Abstract—We tested the hypothesis that the renin inhibitor aliskiren ameliorates organ damage in rats transgenic for human renin and angiotensinogen genes (double transgenic rat [dTGR]). Six-week-old dTGR were matched by albuminuria (2 mg per day) and divided into 5 groups. Untreated dTGR were compared with aliskiren (3 and 0.3 mg/kg per day)-treated and valsartan (Val; 10 and 1 mg/kg per day)-treated rats. Treatment was from week 6 through week 9. At week 6, all groups had elevated systolic blood pressure (BP). Untreated dTGR showed increased BP (202±4 mm Hg), serum creatinine, and albuminuria (34±5.7 mg per day) at week 7. At week 9, both doses of aliskiren lowered BP (115±6 and 139±5 mm Hg) and albuminuria (0.4±0.1 and 1.6±0.6 mg per day) and normalized serum creatinine. Although high-dose Val lowered BP (148±4 mm Hg) and albuminuria (2.1±0.7 mg per day), low-dose Val reduced BP (182±3 mm Hg) and albuminuria (24±3.8 mg per day) to a lesser extent. Mortality was 100% in untreated dTGR and 26% in Val (1 mg/kg per day) treated rats, whereas in all other groups, survival was 100%. dTGR treated with low-dose Val had cardiac hypertrophy (4.4±0.1 mg/g), increased left ventricular (LV) wall thickness, and diastolic dysfunction. LV atrial natriuretic peptide and β-myosin heavy chain mRNA, albuminuria, fibrosis, and cell infiltration were also increased. In contrast, both aliskiren doses and the high-dose Val lowered BP to a similar extent and more effectively than low-dose Val. We conclude that in dTGR, equieffective antihypertensive doses of Val or aliskiren attenuated end-organ damage. Thus, renin inhibition compares favorably to angiotensin receptor blockade in reversing organ damage in dTGR. (Hypertension. 2005;46:569-576.)

Key Words: renin ■ rats, transgenic ■ hypertrophy

Renin is the rate-limiting step in the generation of angiotensin II (Ang II).1 Thus, inhibiting this step reduces Ang II levels. Historically, renin inhibitors have not been clinically successful because of lack of potency or bioavailability. The new nonpeptidic renin inhibitor aliskiren is a potent human renin inhibitor (IC50=0.6 nmol/L).2 Because renin displays species specificity for its substrate, human renin inhibitors cannot be tested efficiently in conventional hypertensive rat models. To circumvent this problem, transgenic rats and mice were developed harboring the human renin and the human angiotensinogen genes.3,4 Human renin does not effectively cleave rat angiotensinogen, and similarly, rat renin cleaves human angiotensinogen poorly.5 Consequently, the single transgenic rats and mice (ie, transgenic for either human angiotensinogen or renin) are normotensive. However, when cross-bred, the double transgenic rat (dTGR) offspring develop hypertension with severe organ damage and do not live beyond the seventh or eighth week of age. We extensively studied these animals; the injury features nuclear factor κB (NF-κB) and activator protein-1 transcription factor activation, upregulation of surface adhesion molecules, cytokines, and the influx of inflammatory cells.6–9 This investigation is the only animal study using the human renin inhibitor aliskiren to test the efficacy of the compound in preventing progression of pre-existing albuminuria and target organ damage. The only other conceivable model might be the marmoset, a small primate. However, those animals must first be made hypertensive, which has not been done to our knowledge. For various reasons, the marmoset is unsuitable. Our protocol examined the possibility of ameliorating target organ injury, a phenomenon not yet shown for aliskiren.

Methods

All procedures complied with guidelines from the American Physiology Society, and a local review board approved the study (permission No. G237/02). Male dTGR were allowed to develop hypertension and were placed in metabolic cages at 5.5 weeks of age. Systolic
blood pressure (BP; tail-cuff) and 24-hour albumin excretion (ELISA; CellTrend) were measured as described previously. The dTGR were matched at week 6 in terms of 24-hour albumin excretion and distributed in 5 groups of 19 rats each. Treatments began when the rats were 6 weeks of age. The rats received vehicle treatment, aliskiren at 0.3 mg/kg per day and 3 mg/kg per day (by subcutaneous minipump), and valsartan (Val) at 1 mg/kg per day and at 10 mg/kg per day (given in the food). Val doses were selected on the basis of a previous study showing that 10 mg/kg per day of Val provided complete end-organ protection (D.N. Müller, unpublished data, 2004). The 1 mg/kg per day dose of Val was selected as a subthreshold treatment to reduce mortality yet only minimally effect BP and organ damage. We knew from previous studies that vehicle-treated animals would not survive beyond 8 weeks of age, and thus, this low-dose Val group would then serve as a control group at 9 weeks. This study was not designed to compare the effects of aliskiren to either dose of Val. Echocardiography (M-mode tracings in the parasternal long-axis view and both septal and lateral regions), range, gain, and filter settings were optimized to detect low velocities, and the pulsed-wave Doppler spectrum was displayed at 200 mm/s. The measurements represent velocities of peak early (Ea) and late (Aa) diastolic expansion velocities. The Ea/Aa ratio is reported as an index of diastolic function.

Rats were killed at 9 weeks of age by decapitation. Serum was collected for further analysis. Kidneys and hearts were removed and washed with ice-cold saline, blotted dry, and weighed. Serum samples were used to measure Ang I formation toward assessing the efficacy of renin inhibition by aliskiren. Human renin estimated as de novo Ang I formation was measured by the in vitro enzyme-kinetic assays described by Bohlender et al. Samples were incubated for 1 hour in the presence and absence of the renin inhibitor remikiren (1 μmol/L) with an excess of human angiotensinogen. The reaction was stopped by the addition of (1 μmol/L) remikiren. De novo Ang I production was calculated as the difference between untreated sample and remikiren-treated sample (ng/mL per hour). The 24-hour urine albumin excretion (Figure 1B) averaged 0.2±0.2 mg per day in all dTGR groups before randomization. The 24-hour urine albumin excretion of Sprague Dawley rats at this point time was 0.2±0.05 mg per day (data not shown; P<0.05). At 7 weeks, albuminuria in untreated dTGR was increased to 36.4±4.6 mg per day. By the end of the study, albuminuria had increased in the 1 mg/kg per day Val dTGR group. Albuminuria remained constant (10 mg/kg per day Val) or actually decreased to 1.6±0.6 mg per 24 hours or 0.4±0.2 mg per 24 hours in the 0.3 mg/kg per day and 3 mg/kg per day aliskiren (P<0.05) groups. Serum creatinine was maintained at normal levels by aliskiren doses and high-dose Val (Figure 1C). Systolic BP increased (Figure 1D) in the vehicle-treated dTGR and 1 mg/kg per day Val dTGR groups but decreased in the other groups. The 3 mg/kg per day aliskiren-treated group completely normalized BP at all times to the level of the nontransgenic Sprague Dawley rats. We assessed renal fibrosis by immunostaining against collagen IV. Increased collagen IV expression of the Bowman's capsule and tubular basement membranes was observed in 1 mg/kg per day Val (Figure 1E). Collagen IV semiquantification demonstrated that high doses of aliskiren and Val were slightly more effective compared with 0.3 mg/kg per day aliskiren. The 1 mg/kg per day Val group showed the highest score (a score of 5+ for dTGR+1 mg/kg per day Val, 1+ for dTGR+3 mg/kg per day aliskiren, 1+ for dTGR+10 mg/kg per day Val, and 3+ for dTGR+0.3 mg/kg per day aliskiren).

Using an in vitro enzyme-kinetic assay, we next determined whether the capacity of serum samples to generate Ang I was altered by treatment of dTGR with the different aliskiren doses. Therefore, we incubated serum samples with an excess of human angiotensinogen. De novo Ang I formation was dose-dependently reduced in both aliskiren groups compared with both Val groups (Figure 2A). We also measured renal Ang I and II. The 10 mg/kg per day Val dose and both aliskiren doses reduced renal Ang I and II content (Figure 2B and 2C).

At death, the cardiac hypertrophy index score (Figure 3A) decreased in the 10 mg/kg per day Val, 0.3 mg/kg per day aliskiren, and 3 mg/kg per day aliskiren groups (P<0.05). However, the cardiac hypertrophy index was significantly lower in 3 mg/kg per day aliskiren treated compared with 10 mg/kg per day Val-treated dTGR. Echocardiography shows a 1 mg/kg per day Val animal with concentric hypertrophy and...
wall thickness of 3.4 mm with a normal LV end-diastolic diameter (Figure 3B). Treatment with aliskiren (3 mg/kg per day) or 10 mg/kg per day Val reduced wall thickness to 2.2 mm and 2.7 mm, respectively (Figure 3C). Tissue Doppler measurements (Figure 4A and 4B) showed an Ea/Aa ratio of 0.68 ± 0.1 in the 1 mg/kg per day Val group, whereas 10 mg/kg per day Val improved Ea/Aa quotient (1.0 ± 0.1). High and low aliskiren doses increased Ea/Aa values (1.4 ± 0.1 and 1.5 ± 0.1, respectively), demonstrating improved diastolic filling. We also investigated untreated
dTGR at week 7 just before death and found increases in LV thickness (3.5 mm) and Ea/Aa ratio of 0.48/0.1, indicating diastolic dysfunction (data not shown). With RT-PCR, we examined α-MHC mRNA and β-MHC expression in the left ventricles (Figure 5A through 5C). The 10 mg/kg per day Val treatment and both aliskiren treatments prevented the shift from α-MHC expression to the fetal β-MHC isoform. LV ANP mRNA expression was reduced by both aliskiren treatments compared with 1 mg/kg per day Val-treated dTGR. The 10 mg/kg per day Val dose reduced the expression of this gene, but not to a significant degree.

To measure markers of tissue inflammation, we quantified macrophage (Figure 6A), CD4 T cell (Figure 6B), CD8 T cell (Figure 6C), dendritic cell (Figure 6D), CD86+ cell (Figure 6E), and MHC II+ cell (Figure 6F) infiltration in the kidneys. The 10 mg/kg per day Val, 0.3 mg/kg per day aliskiren, and 3 mg/kg per day aliskiren doses prevented cell infiltration completely.
Discussion

This study demonstrates that the human renin inhibitor aliskiren can inhibit BP and organ damage even after organ damage has occurred and hypertension has been established. Thus, aliskiren and high-dose Val are capable of not only ameliorating but also reversing albuminuria and reducing mortality in dTGR. Our findings also underscore the utility of the dTGR model for studying human renin inhibitors in rats. Our positive control in this study was the Ang II type 1 (AT1) receptor blocker Val. We showed previously that treatment with Val or angiotensin-converting enzyme (ACE) inhibitor effectively protects dTGR from organ damage. In the current study, we selected 2 doses for Val: a low 1 mg/kg per day subthreshold dose chosen solely to prolong survival until the ninth week and a 10 mg/kg per day dose. Aliskiren was administered by constant infusion, maintaining plasma concentrations of the agent at constant levels. Val was administered in the food. Thus, we did not address the differences in pharmacokinetic properties or renin-angiotensin system-blocking potencies of the 2 agents. Therefore, we cannot conclude that aliskiren was more effective than Val. Both treatments were highly effective.

We used echocardiography to assess the benefit of renin inhibition on LV wall thickness and function. The data showed that the 1 mg/kg per day Val animals had severe LV hypertrophy with marked diastolic dysfunction (diastolic heart failure). The LV hypertrophy was markedly ameliorated with 10 mg/kg per day Val dose still showed similarly deep Ea and Aa waves indicating diastolic dysfunction (Ea/Aa 1.0). The 0.3 mg/kg per day and 3 mg/kg per day Alisk doses show deeper Ea than Aa waves, indicating appropriate diastolic filling (Ea/Aa=1.5).

Figure 4. Tissue Doppler assessment of diastolic filling in dTGR at 9 weeks of age is given. Ea wave (early diastolic filling) and the Aa wave (atrial contraction) of the same animals as in Figure 3 were measured at the same time point. The 1 mg/kg per day Val dose shows a deeper Aa than Ea wave, indicating a severe diastolic dysfunction (Ea/Aa=0.66). The 10 mg/kg per day Val dose still showed similarly deep Ea and Aa waves indicating diastolic dysfunction (Ea/Aa 1.0). The 0.3 mg/kg per day and 3 mg/kg per day Alisk doses show deeper Ea than Aa waves, indicating appropriate diastolic filling (Ea/Aa=1.5).
Renin inhibitors prevent the formation of Ang I and Ang II and so may act differently from AT1 receptor blockers and ACE inhibitors. We found that aliskiren reduced renal Ang I and II levels. Val, at high doses, reduced renal Ang II levels in agreement with studies by Nussberger et al15 and Campbell et al compared aliskiren with enalapril in human volunteers, demonstrating decreased plasma and urinary aldosterone levels, induced natriuresis, and unchanged potassium excretion with aliskiren therapy.24 Stanton et al compared aliskiren (37.5, 75, 150, and 300 mg per day) to losartan (100 mg per day) in a 4-week blinded study of 226 hypertensive patients. Aliskiren was well tolerated and lowered BP as effectively as ACE inhibitors and AT1 receptor blockers. In this regard, Hollenberg et al performed pioneering studies on early renin inhibitors (enalikiren and zankiren) and renal blood flow on human subjects. They found that renin inhibitors and Ang II antagonists induced renal vasodilation to a greater extent than did ACE inhibition,26 despite their expectation that ACE inhibition might be superior in inducing vasodilation through kinin generation. Thus, renin inhibition may confer renoprotection (through vasodilation) beyond that of ACE inhibition.

Our investigations into the mechanisms related to Ang II–induced organ damage have implicated reactive oxygen species generation, adhesion molecule upregulation, cytokine and chemokine release, and the initiation of NF-κB dependent gene expression.6,7,9 Exploring the effect of aliskiren on these parameters was beyond the scope of the current study and will be the subject of future investigations. BP reduction is definitively an important issue for target organ protection. Nevertheless, we believe that high BP is not the sole cause for damage. We published previously that blockade of the renin-angiotensin system compared to BP lowering with triple therapy of hydralazine, hydrochlorothiazide, and reserpine, was more protective than merely lowering BP.27 We also demonstrated in this model that anti-inflammatory treatment prevented renal damage despite BP levels of 200 mm Hg.7 In that study, dexamethasone raised BP but nonetheless reduced mortality to 0.

Perspectives

Our animal data suggest that aliskiren is able to ameliorate established organ damage. Particularly impressive were the
effects on ventricular filling. In humans, aliskiren blocks plasma renin activity, reduces BP, and reduces plasma aldosterone levels and aldosterone excretion.24,25 The current animal data suggest that renin inhibition in humans will provide a valuable addition to antihypertensive treatments that interrupt the renin-angiotensin system.

Acknowledgments

The NGFN (Nationales Genom Netzwerk) and Novartis Institutes for BioMedical Research USA supported the study. D.N.M. holds a Helmholtz fellowship. The Deutsche Forschungsgemeinschaft (DFG) supported D.N.M. and F.C.L. The authors thank Mathilde Schmidt, Reika Langanki, Astrid Schiche, Jutta Meisel, Jana Czychi, Ute Gerhard, and Andrea Weller for excellent technical assistance.

References

10. Mazak I, Fiebeler A, Muller DN, Park JK, Shagdarsuren E, Lindschau C, 
        Dechend R, Viedt C, Pilz B, Haller H, Luft FC. Aldosterone potentiates 
        angiotensin II-induced signaling in vascular smooth muscle cells. 
        Murakami K, Luft FC, Ganten D. High human renin hypertension in 
12. van Kats JP, Danser AH, van Meegen JR, Sassen LM, Verdouw PD, 
        Schalekamp MA. Angiotensin production by the heart: a quantitative 
        study in pigs with the use of radiolabeled angiotensin infusions. 
13. Mervaala E, Dehmel B, Gross V, Lippoldt A, Bohlender J, Milia AF, 
        Ganten D, Luft FC. Angiotensin-converting enzyme inhibition and AT1 
        receptor blockade modify the pressure-natriuresis relationship by additive 
        mechanisms in rats with human renin and angiotensinogen genes. J Am Soc 
14. Wellner M, Dechend R, Park JK, Shagdarsuren E, Al-Saadi N, Kirsch T, 
        Gratze P, Schneider W, Meiners S, Fiebeler A, Haller H, Luft FC, Muller 
        DN. Cardiac gene expression profile in rats with terminal heart failure and 
15. Mazzolai L, Pedrazzini T, Nicoud F, Gabbiani G, Brunner HR, 
        Nussberger J. Increased cardiac angiotensin II levels induce right and left 
        ventricular hypertrophy in normotensive mice. Hypertension. 2000;35: 
        985–991.
16. Campbell DJ, Kladis A, Valentinj AJ. Effects of losartan on angiotensin 
        and bradykinin peptides and angiotensin-converting enzyme. J Cardiovasc 
17. van Kats JP, de Lannoy LM, Jan Danser AH, van Meegen JR, Verdouw 
        PD, Schalekamp MA. Angiotensin II type 1 (AT1) receptor-mediated 
        accumulation of angiotensin II in tissues and its intracellular half-life in 
18. Zhuo JL, Imig JD, Hammond TG, Oreno G, Benes E, Navar LG. Ang II 
        accumulation in rat renal endosomes during Ang II-induced hypertension: 
19. van Kats JP, Schalekamp MA, Verdouw PD, Duncker DJ, Danser AH. 
        Intra renal angiotensin II: interstitial and cellular levels and site of produc- 
20. Brasier AR, Ron D, Tate JE, Habener JF. A family of constitutive 
        C/EBP-like DNA binding proteins attenuate the IL-1 alpha induced, NF 
        kappa B mediated trans-activation of the angiotensinogen gene 
        specific and long-acting renin inhibitor in the marmoset. Hypertension. 
22. Clozel JP, Fischli W. Comparative effects of three different potent renin 
23. Wood JM, Schnell CR, Cumin F, Menard J, Webb RL. Aliskiren, a novel, 
        orally effective renin inhibitor, lowers blood pressure in marmosets and 
24. Nussberger J, Wuerzner G, Jensen C, Brunner HR. Angiotensin II sup- 
        pression in humans by the orally active renin inhibitor aliskiren (SPP100): 
        in essential hypertension with an oral renin inhibitor, aliskiren. Hypertension. 
26. Hollenberg NK, Fisher ND, Price DA. Pathways for angiotensin II 
        generation in intact human tissue: evidence from comparative pharmaco- 
27. Mervaala EMA, Muller DN, Schmidt F, Park J-K, Bader M, Breu V, 
        Ganten D, Haller H, Luft FC. Blood pressure-independent effects in rats 
        with human renin and angiotensinogen genes. Hypertension. 2000;35: 
        587–594.
Aliskiren, a Human Renin Inhibitor, Ameliorates Cardiac and Renal Damage in Double-Transgenic Rats


_Hypertension._ 2005;46:569-576; originally published online August 15, 2005; doi: 10.1161/01.HYP.0000179573.91016.3f

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/3/569

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/