Contrasting effects of aliskiren versus losartan on hypertensive vascular remodeling

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ABSTRACT

Background: Hyperactivation of the renin–angiotensin system contributes to hypertension-induced upregulation of vascular matrix metalloproteinases (MMPs) and remodeling, especially in the two kidney, one clip (2K1C) hypertension model. We hypothesized that the AT1R antagonist losartan or the renin inhibitor aliskiren, given at doses allowing similar antihypertensive effects, could prevent in vivo vascular MMP upregulation and remodeling, and collagen/elastin deposition found in 2K1C hypertension by preventing the activation of extracellular matrix metalloproteinases (MMPs), especially MMP-2, gelatinolytic activity, and expression of phospho-ERK 1/2 and transforming growth factor-β1 (TGF-β1).

Methods: 2K1C rats were treated with aliskiren (50 mg.kg⁻¹.day⁻¹), or losartan (10 mg.kg⁻¹.day⁻¹), or both by gavage during 4 weeks.

Results: Aliskiren, losartan, or both drugs exerted similar antihypertensive effects when compared with 2K1C rats treated with water. Aliskiren reduced plasma renin activity in both sham and 2K1C rats. Losartan alone or combined with aliskiren, but not aliskiren alone, abolished 2K1C-induced aortic hypertrophy and hyperplasia, and prevented the increase in aortic collagen/elastin content, MMP-2 levels, gelatinolytic activity, and expression of phospho-ERK 1/2 and TGF-β1. No significant differences were found in the aortic expression of the (pro)renin receptor.

Conclusions: These findings show that although losartan and aliskiren exerted similar antihypertensive effects, only losartan prevented the activation of vascular profibrotic mechanisms and MMP upregulation associated with vascular remodeling in 2K1C hypertension. Our findings also suggest that aliskiren does not enhance the protective effects exerted by losartan.

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1. Introduction

Hyperactivation of the renin–angiotensin system (RAS) contributes to hypertension and promotes cardiovascular remodeling [1]. Mounting evidence indicates that a proteolytic imbalance involving upregulated matrix metalloproteinases (MMPs), especially MMP-2 [2], plays a major role in these alterations, especially in the two kidney, one clip (2K1C) hypertension model [3,4]. Indeed, angiotensin II increases MMP-2 expression and activity [5,6] via activation of angiotensin II type 1 receptors (AT-R) [7,8] and therefore promotes cardiovascular remodeling [2]. Moreover, increased renin and prorenin formation activates the (pro)renin receptor (PRR) [9], which is widely expressed in renal mesangial and in vascular smooth muscle cells (VSMCs) [10,11]. Importantly, PRR activation exerts dual molecular functions by stimulating signaling pathways that are both dependent and independent of angiotensin II (ANG II) generation [12]. The last one elicits intracellular signaling that upregulates profibrotic pathways including extracellular regulated kinase 1/2 (ERK 1/2) and transforming growth factor-β1 (TGF-β1) [13,14], which contribute to hypertensive cardiovascular remodeling.

While antihypertensive drugs interfering with the RAS exert beneficial effects against the cardiovascular remodeling associated with hypertension [1], it is not known whether AT1R antagonists affect profibrotic pathways and vascular MMP upregulation in hypertensive animals or humans. Moreover, although aliskiren (a direct renin inhibitor) decreases angiotensin II levels and improves cardiac remodeling [15], its effects on hypertension-induced vascular MMP upregulation and remodeling are not known. While compelling results suggest that aliskiren enhances the protective effects of AT1R antagonists [16,17], the effects of this drug combination on the increases in vascular MMPs and remodeling associated with hypertension are not known.

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In this study, we hypothesized that the AT₁R antagonist losartan or the renin inhibitor aliskiren prevents in vivo vascular MMPs upregulation and remodeling, and elastin/collagen deposition found in 2K1C hypertension [4,18] by preventing the activation of profibrotic pathways involving ERK 1/2 and TGF-β1. Because increased transmural pressure is sensed by integrins [19], which transform signals into adaptive vascular remodeling, thus affecting vascular MMP activities [20], we compared the effects of losartan and aliskiren given at doses that allowed very similar antihypertensive effects in pilot studies. Moreover, we hypothesized that aliskiren could enhance the effects of losartan, as previously suggested [16].

2. Methods

2.1. Animals and treatments

This study was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, and the animals were handled according to the guiding principles published by the National Institutes of Health. Male Wistar rats (180–200 g) were maintained on 12-h light/dark cycle at 25 °C with free access to rat chow and water.

2K1C hypertension was induced by clipping the left renal artery with a silver clip (0.2 mm). Sham-operated rats underwent the same surgical procedure (under general anesthesia with ketamine 100 mg/kg and xylazine 10 mg/kg i.p.), except for the placement of the renal artery clip. The systolic blood pressure (SBP) was measured by tail-cuff plethysmography weekly, and the rats were considered hypertensive when SBP > 160 mm Hg two weeks after the surgery.

Animals were randomly assigned to one of eight groups: 2K1C and Sham groups that received tap water; 2K1C and Sham groups that received losartan (Cozaar®; Merck Sharp & Dohme) at 10 mg/kg per day [8]; 2K1C and Sham groups that received aliskiren at 50 mg/kg [21] (Rasilez®; Novartis) per day; and 2K1C and Sham groups that received the combination of aliskiren 50 mg/kg + losartan 10 mg/kg per day. The treatments were started two weeks after 2K1C hypertension was induced and maintained for additional four weeks. All treatments were given daily by oral gavage and distilled water was used as vehicle.

2.2. Plasma renin activity assay

Blood samples were collected in tubes containing 5.0 mmol/L of EDTA. Blood samples were centrifuged at 2000 × g for 30 min at 4 °C. Plasma renin activity was determined by assessing the generation of angiotensin I by reversed phase HPLC after blood samples were centrifuged at 2000 × g for 10 minutes. The proteins were then transferred onto nitrocellulose membranes and blocked with TBST (NaCl 100 mmol/L; Tris–Cl 100 mmol/L; Tween 0.1%) containing 5% bovine serum albumin. The membranes were incubated overnight at 4 °C with anti-MMP-2 (1:1,000, Chemicon), anti-TGF-β1 (1:1,000, Chemicon), anti-phosphorylated-ERK 1/2 (anti-p-ERK 1/2; 1:2,000, Santa Cruz Biotechnology) or anti-prorenin receptor (anti-PRR; 1:250, Abcam) antibodies. Anti-β-actin (1:10,000, Millipore) was used as a loading control. The antibodies were washed in TBST and incubated with horseradish peroxidase (HRP)-secondary goat anti-mouse antibody (1:2,000, Millipore) or HRP-secondary goat anti-rabbit antibody (1:1,000, Millipore) for 1 h. Immunoabeled proteins were visualized using chemiluminescence ECL (Millipore) and registered by ImageQuant 350 detection system (GE Healthcare). The signal intensities were quantified using ImageJ Program (NIH – National Institute of Health).

2.7. Statistical analysis

Results are expressed as means ± S.E.M. Comparisons between groups were assessed by two-way or one way ANOVA followed by the Bonferroni test. A probability value < 0.05 was considered significant.

3. Results

3.1. Effects of aliskiren and losartan on SBP levels and plasma renin activity

We found similar baseline SBP in all experimental groups and no effects on SBP in the Sham groups (Fig. 1). While SBP increased progressively in 2K1C rats treated with water (208 ± 2 mm Hg), treatment of 2K1C rats with aliskiren, losartan, or both drugs exerted similar antihypertensive effects, especially after 4 weeks of treatment (SBP = 157 ± 2 mm Hg, 158 ± 2 mm Hg, 146 ± 2 mm Hg, respectively; Fig. 1, P < 0.05). Similar body weight gain was found in the eight

Fig. 1. Systolic blood pressure (mm Hg) measured by tail-cuff method (Panel A) and plasma renin activity (Panel B) in all experimental groups at the end of six weeks of antihypertensive treatment. Data are shown as mean ± S.E.M (n = 11–15 per group). * P < 0.05 for the 2K1C groups versus the Sham groups. ** P < 0.05 versus the Sham + vehicle or versus 2K-1C + vehicle. * P < 0.05 for the 2K1C group versus the other 2K1C groups.
Experimental groups (P > 0.05; data not shown). The plasma renin activity was significantly reduced in sham rats treated with aliskiren when compared with Sham + vehicle group (Fig. 1B, P < 0.05). Treatment with aliskiren significantly attenuated plasma renin activity in 2K1C rats when compared with 2K1C rats treated with vehicle (Fig. 1B, P < 0.05).

3.2. Aliskiren treatment did not prevent the vascular remodeling

2K1C hypertension induced vascular remodeling as compared to sham-operated rats, and the antihypertensive drugs had no effects in the sham groups (Fig. 2A-D). Whereas losartan alone or combined with aliskiren abolished the increases in VSMCs number, CSA, and

![Image](image-url)

**Fig. 2.** Effects of aliskiren and/or losartan on the structural modifications induced by 2K1C hypertension in the aortas from rats. Panel A shows representative photomicrographs of aortic samples (400×) stained by hematoxylin and eosin (H&E). Panel B shows the values for vascular smooth muscle cell number per length of aorta. Panel C shows the values for thoracic aorta medial cross-sectional area (CSA). Panel D shows the values for media to lumen ratio (M/L). Data are shown as mean ± S.E.M. (n = 5–6 per group). *P < 0.05 versus the Sham groups and versus the 2K1C + LOS and the 2K1C + ALK/LOS groups.

![Image](image-url)

**Fig. 3.** Effects of aliskiren and/or losartan on collagen and elastin content in the aortic media. Panels A and C show representative photomicrographs of aortic samples (400×) stained by Sirus red and Orceine, respectively. Panel B and Panel D show the values for surface area of collagen and elastin in the aortic media. Data are shown as mean ± S.E.M. (n = 4–6 per group). *P < 0.05 versus the Sham groups and versus the 2K1C + LOS and the 2K1C + ALK/LOS groups.
M/L ratio found in hypertensive rats (P<0.05; Fig. 2A–D), aliskiren alone exerted no significant effects, even though all treatments had similar antihypertensive effects. In parallel with these findings, we found increased total collagen fiber and elastin content per cross-sectional area in 2K1C rats compared with the sham groups (P<0.05; Fig. 3A–D). Treatment with losartan or aliskiren + losartan, but not with aliskiren alone, attenuated the increases in these parameters (P<0.05; Fig. 3).

3.3. Assessment of aortic MMP-2 levels by zymography

Gelatin zymography was used to assess MMP-2 levels in aortic extracts because this protease has been implicated in hypertensive vascular remodeling [4,18]. 2K1C hypertension increased the aortic levels of the 75 kDa, 72 kDa, and 64 kDa MMP-2 forms (P<0.05; Fig. 4). While all treatments reduced 75-kDa MMP-2 levels, only losartan or aliskiren + losartan treatments abolished hypertension-induced increases in the 64-kDa MMP-2 form (P<0.05; Fig. 4).

3.4. Assessment of aortic gelatinolytic activity and MMP-2 levels

Hypertension significantly increased aortic gelatinolytic activity, as revealed by increased green fluorescence in Fig. 5A. While treatment with losartan or with aliskiren + losartan abolished the increases in aortic gelatinolytic activity found in 2K1C rats (P<0.05; Fig. 5A–B), treatment with aliskiren alone exerted no such effect (P>0.05; Fig. 5A–B). Interestingly, aortic MMP-2 levels assessed by immunofluorescence (red fluorescence in Fig. 5 A) paralleled and co-localized with aortic gelatinolytic activity (Fig. 5A–C).

3.5. Aortic expression of MMP-2, PRR, p-ERK 1/2 and TGF-β1, protein levels

Hypertension significantly increased the vascular expression of MMP-2, p-ERK 1/2, and TGF-β1, as shown in Fig. 6 (all P<0.05). While treatment of 2K1C rats with losartan or with aliskiren + losartan abolished the increases in aortic expression of MMP-2, ERK 1/2, and TGF-β1 (all P<0.05; Fig. 6A, C, and D, respectively), treatment with aliskiren alone exerted no such effect (P>0.05; Fig. 6A, C, and D).

We found no significant changes in PRR expression in hypertensive rats compared with the Sham group, and no significant effects for drug treatments (P>0.05; Fig. 6B).

4. Discussion

This is the first study to show that the antihypertensive effects of losartan are associated with prevention against activation of vascular profibrotic mechanisms and MMP-2 upregulation found in 2K1C hypertension. Another important finding is that aliskiren, given at a dose exerting similar antihypertensive effects to those found with losartan, was not effective in preventing profibrotic mechanisms and vascular remodeling. Moreover, aliskiren did not improve the protective effects exerted by losartan.

Clinical studies suggested that both AT1R antagonists or aliskiren exert comparable antihypertensive effects [17,25,26]. While losartan and aliskiren exerted similar effects on blood pressure in the present study, the combination of both drugs did not lower blood pressure significantly more than either monotherapy, and this finding apparently contrasts with clinical findings [17]. However, it is reasonable to believe that the differences between treatments with respect to vascular remodeling, MMP-2, or other profibrotic factors are not due to differences in antihypertensive effects and therefore differences in transmural pressure, which affects vascular MMPs [20].

As expected from previous studies [23,24,27], 2K1C hypertension induced vascular hypertrophy, increased vascular collagen and elastin contents, and enhanced MMP-2 levels, probably as a result of increased angiotensin II levels [6,28]. These findings were associated with increased expression of profibrotic factors including p-ERK 1/2 and TGF-β1, which clearly contribute to VSMC growth and rearrangement of extracellular matrix. The increased vascular MMP-2 levels in 2K1C rats may activate TGF-β1 and enhance TGF-β1 intracellular signaling, thus leading to fibrosis with increased deposition of collagen and elastin [29,30]. The antigrowth effects exerted by losartan in the present study are supported by previous results indicating similar effects on small resistance arteries from rats treated with angiotensin II [8]. Consistent with the notion that the AT1R enhances collagen gene expression via ERK 1/2 signaling [31], we found increased levels of p-ERK 1/2 and collagen deposition in the aorta from 2K1C rats, and these changes were prevented with losartan, thus strongly suggesting a major role for the AT1R in the vascular alterations of 2K1C hypertension.
In contrast with losartan, aliskiren monotherapy was not effective in preventing the structural and biochemical alterations discussed above, even though similar antihypertensive effects were found with both drugs. In fact, the lack of significant effects for aliskiren on p-ERK 1/2 and on TGF-β1 levels aligns with the lack of significant effects on MMP-2 levels and on the other biochemical and morphological parameters studied here. Moreover, it is intriguing that aliskiren did not improve the protective effects exerted by losartan, thus contrasting with previous experimental studies[16]. These findings suggest that renin inhibition may not have significant effects on fundamental mechanisms promoting hypertensive vascular remodeling, even though antihypertensive effects are found with aliskiren.

In line with this suggestion above, previous studies have shown that the concentrations of aliskiren necessary to block rat renin are approximately 100 times higher than those required to block human renin [32]. However, in the present study, aliskiren clearly suppressed plasma renin activity in 2K-1C rats, thus confirming previous findings from a study using aliskiren at the same dose used here [21]. In addition, the decreased plasma renin activity that we found in 2K-1C, untreated rats is in agreement with previous studies showing that plasma renin activity is reduced after four weeks, during the more chronic phase of 2 K-1 C hypertension [33,34].

The lack of significant antigrowth effects for aliskiren may be attributed to a compensatory elevation in plasma renin concentration in 2K1C hypertension [35] and augmented PRR activation. While overexposure of the PRR to renin/prorenin could downregulate PRR [13,36,37], we found that 2K1C hypertension or the antihypertensive treatments exerted no effects on the expression of PRR in the aorta. Therefore, it is possible that treatment with aliskiren enhanced PRR signaling and activated mitogen-activated protein kinases (MAPK)-dependent pathways. This results in VSMCs proliferation [14] and overexpression of matrix proteins and profibrotic proteins including ERK 1/2 and TGF-β1 [38–40], especially in a context of unblocked AT1R, which contributes to MAPK activation [41]. In contrast, while the treatment with losartan may also increase renin concentrations, this AT1R blocker directly prevents the activation of AT1R and MAPK signaling pathways [41], probably exerting more effective antigrowth effects as consistently suggested by our results.

In conclusion, our findings support the idea that the antihypertensive effects exerted by losartan and aliskiren are associated with contrasting antigrowth effects. Only losartan prevented in vivo vascular MMP upregulation and remodeling, and the profibrotic alterations found in 2K1C hypertension. Thus, our results suggest that blockade of AT1R promotes better protection against the vascular remodeling of 2K1C hypertension than renin inhibition, and that aliskiren does not improve the effects exerted by losartan.

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Fig. 6. Expression of MMP-2 (panel A), PRR (panel B), p-ERK1/2 (panel C), and TGF-β1 (panel D) in the thoracic aorta from Sham and 2K1C rats after treatment with vehicle, aliskiren (ALK), losartan (LOS) or aliskiren + losartan (ALK/LOS). Densitometric intensity corresponding to each band was normalized using β-actin expression. Data are expressed as a ratio between the expression interest protein and β-actin expression. Data are shown as mean ± S.E.M. (n = 3–5 per group). * P < 0.05 versus the Sham groups and versus the 2K1C + LOS and the 2K1C + ALK/LOS groups.

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