Suppression of Ang II-mediated Up-regulation of Renal αENaC mRNA in Renal ATRAP Tg Mice

Effects of Ang II infusion on the mRNA expression of major sodium transporters (NHE3, NKCC2, NCC and ENaC subunits) in the kidney of Wt and ATRAP Tg mice. Values are expressed as the mean±SE (N=6 in each group). *P<0.05, versus vehicle.

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The Physiology and Pathophysiology of a Novel Angiotensin Receptor-binding Protein ATRAP/*Agtrap*

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Abstract: The Ang II type 1 receptor (AT1R)-associated protein (ATRAP/*Agtrap*) is a molecule specifically interacting with the carboxyl-terminal domain of AT1R. The results of *in vitro* studies showed that ATRAP suppresses Ang II-mediated pathological responses in cardiovascular cells by promoting AT1R internalization. With respect to the tissue distribution and regulation of ATRAP expression *in vivo*, ATRAP is broadly expressed in many tissues as is AT1R. Accumulating evidence indicates that a tissue-specific regulatory balancing of ATRAP and AT1R expression may be involved in the modulation of AT1R signaling at local tissue sites and also in the pathophysiology of hypertension and its associated end-organ injury. Furthermore, the activation of ATRAP in transgenic-models inhibited inflammatory vascular remodeling and cardiac hypertrophy in response to Ang II stimulation. These results suggest the clinical potential benefit of an ATRAP activation strategy in the treatment of hypertension and related organ injury.

Keywords: Gene expression/regulation, hypertension, receptor internalization, receptor signaling, renin-angiotensin system, target organ injury.

1. INTRODUCTION

Recent progress in molecular research in the fields of cardiovascular and renal medicine has identified several interesting molecules which interact with Ang II type 1 receptor (AT1R) or Ang II type 2 receptor (AT2R) to modulate respective receptor functions [1-3]. Particularly, a lot of preceding investigation aimed to identify molecules which directly bind to AT1R or AT2R and regulate activity of downstream signaling pathways, and a novel molecule which interacts with the carboxyl-terminal domain of AT1R was identified for the first time and named AT1R-associated protein (ATRAP/*Agtrap*) [4].

2. IDENTIFICATION OF ATRAP AS A NOVEL INTERACTING MOLECULE WITH AT1R

The G protein-coupled receptors (GPCRs) interact with different classes of intracellular proteins, including heterotrimeric G proteins, kinases, and arrestins [5-7]. Although the intracellular third loop of a number of GPCRs plays an important role as a structural determinant of coupling of the receptor to heterotrimeric G proteins, accumulated experimental results also highlighted the functional importance of the carboxyl-terminal cytoplasmic domain in receptor signaling and internalization [8-10]. Employing yeast two-hybrid screening of a mouse kidney cDNA library, with the carboxyl-terminal cytoplasmic domain of the mouse AT1R as a bait, a novel protein, with an open reading frame of 483 base pairs in its cDNA and with a predicted molecular mass of 18 kDa, was isolated and named ATRAP/*Agtrap* (for AT1R–associated protein) [4] (Fig. 1). The ATRAP did not interact with the carboxyl-terminal cytoplasmic domains of the AT2R and those of several Gq-coupled receptors such as m_3 muscarinic, bradykinin B_2, and endothelin B receptors, nor did it associate with the Gs-coupled β_2-adrenergic receptor. Thus, ATRAP is likely to be an AT1R-specific binding molecule to date. The human ATRAP/*Agtrap* cDNA was also cloned and the deduced polypeptide product of the cDNA was 22 kDa in size [11]. The human ATRAP/*Agtrap* cDNA and amino acid sequences were 85 and 77% identical to those of the mouse ATRAP/*Agtrap* gene, respectively.

3. PREDICTED DOMAIN STRUCTURE OF ATRAP

Characterization using cultured cells revealed ATRAP as a transmembrane protein localized in intracellular trafficking vesicles and plasma membrane [4, 12]. With respect to the domain structure, ATRAP is predicted *in silico* to contain three hydrophobic domains at the amino-terminal end of the protein, encompassing the amino acid residues 14-36, 55-77, and 88-108 and a hydrophilic cytoplasmic carboxyl-terminal tail from residues 109-161. The first transmembrane domain consists of a mixture of apolar and polar amino acid residues; the second and third transmembrane domains are composed mainly of hydrophobic residues with some polar amino acid residues.

4. WIDE TISSUE DISTRIBUTION OF ATRAP

To date several polyclonal anti-ATRAP antibodies, which are able to recognize the ATRAP protein specifically, were produced to examine the tissue distribution of ATRAP *in vivo* [13-17]. The results of Western blot analysis showed that the ATRAP antibody detected a major protein band at 18 kDa in the mouse tissues and that ATRAP was widely distributed in the mouse tissues as was AT1R [15]. The mouse ATRAP protein was expressed at relatively high levels in the kidney, lung, and testis, followed by the spleen, but at lower levels in the heart, brain, liver, skeletal muscles, and aorta. Similarly, the human ATRAP mRNA was most abundantly expressed in kidney, heart, pancreas and thyroid [11].

The results of immunohistochemistry using the polyclonal anti-ATRAP antibody within kidney sections from normal adult mouse and humans showed a relatively high level of ATRAP immunoreac-

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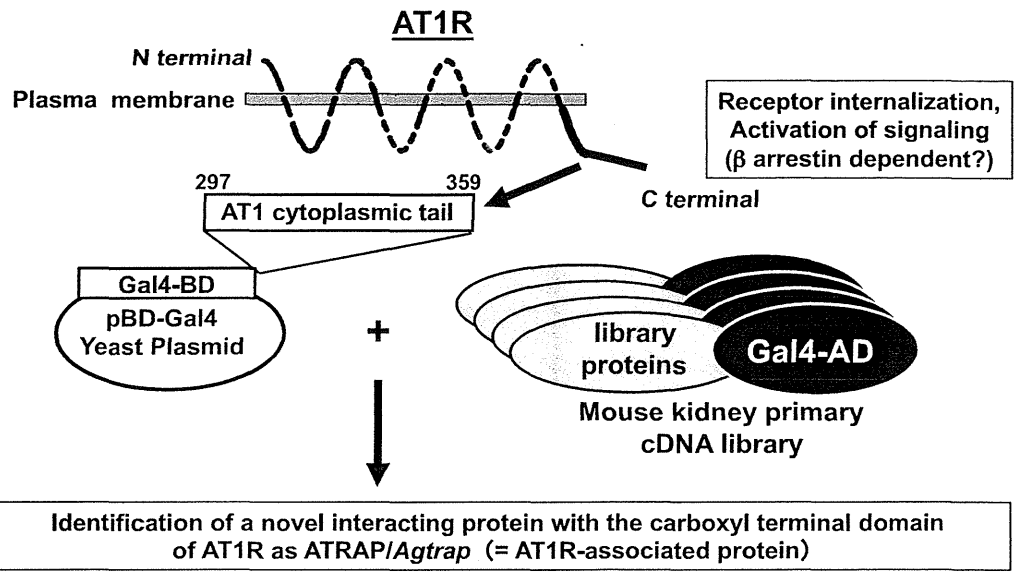

Fig. (1). Employing a yeast two-hybrid screening system, we previously cloned a novel AT1R-associated protein (ATRAP/*Agtrap*) that is predicted to have three transmembrane domains and specifically interacts with the carboxyl-terminal cytoplasmic domain of the AT1R.

tivity in Bowman's capsules, the proximal convoluted tubules (PCT), the proximal straight tubules (PST), and the distal convoluted tubules (DCT). A lower level of ATRAP immunostaining was also detected in other nephron segments. However, significant staining was not found in the glomeruli, in the vasculature, (including arcuate artery, interlobular arteries, and arterioles), or in the interstitial cells [15, 18].

5. PROMOTING EFFECT OF ATRAP ON AT1R INTERNALIZATION

The results of analysis of intracellular distribution of ATRAP showed a particulate distribution; electron microscopy reveals the presence of ATRAP in prominent perinuclear vesicular membranes; and co-localization analysis by immunofluorescence shows that ATRAP co-localizes in an intracellular vesicular compartment corresponding to endoplasmic reticulum, Golgi, and endocytic vesicles [12].

With respect to the interaction of ATRAP with AT1R and effects of ATRAP on AT1R internalization in cells, the results of immunoprecipitation assay, BRET analysis, and immunofluorescence staining in cultured cells including cardiovascular cells indicate that ATRAP is able to interact with AT1R even without Ang II stimulation and that Ang II stimulation significantly facilitated the interaction of these proteins [19]. The results of real-time trafficking analysis of ATRAP vesicles also showed a constitutive translocation of ATRAP from intracellular vesicle compartments to the periphery of the cell, which was not affected by the treatment with Ang II [12].

Taken together, these results suggest that ATRAP is actually able to bind to the AT1R under baseline conditions but that ATRAP interacts mainly with the AT1R that is internalized from the cell surface into the endocytic vesicles on Ang II stimulation to keep the receptor internalized even after the removal of Ang II. Furthermore, the quantitative analysis of immunofluorescence staining indicated that almost all of the internalized AT1R were associated with ATRAP, indicating that a major function of ATRAP in cultured cells including cardiovascular cells is to promote the constitutive internalization of AT1R [13, 19, 20]. Furthermore, a transgenic model increase in renal ATRAP expression beyond baseline *in vivo* was accompanied by a constitutive reduction of renal plasma membrane AT1R expression and by the promotion of renal AT1R internalization in response to Ang II [21]. Furthermore, another study also showed that a genetic deficiency of ATRAP in mice caused an enhanced surface expression of AT1R in the kidney, which is consistent with these results [17].

6. PUTATIVE FUNCTIONAL ROLE OF ATRAP IN CULTURED CELLS

Initially, this protein has been found to modulate AT1R function in transformed African green monkey kidney fibroblast (COS-7) cells and human embryonic kidney (HEK) 293 cells [4, 12]. Overexpression of ATRAP in COS-7 cells caused a marked inhibition of AT1R-mediated activation of phospholipase C, and functional analysis of the effects of ATRAP on Ang II-induced AT1-receptor signaling in HEK293 cells reveals a moderate decrease in the generation of inositol lipids, a marked decrease in Ang II–stimulated transcriptional activity of the c-fos promoter luciferase reporter gene, and a decrease in cell proliferation.

In cardiomyocytes, overexpression of ATRAP by adenoviral gene transfer significantly decreases the number of AT1R on the surface of cardiomyocytes, and it also decreases the degree of p38 mitogen–activated protein kinase phosphorylation, the activity of the c-fos promoter, and protein synthesis upon Ang II stimulation in cardiomyocytes. In addition, in vascular smooth muscle cells (VSMC) and in distal convoluted tubule cells (mDCT), overexpression of ATRAP inhibited Ang II–mediated increases in TGF-β mRNA expression and TGF-β production into the medium [18-20]. On the other hand, ATRAP knockdown by small-interference RNA in VSMC activated Ang II-induced c-fos gene expression, which was effectively inhibited by valsartan, an AT1R-specific antagonist [19].

The nuclear factor of activated T cells (NFAT) transcription factor, which is dephosphorylated by the phosphatase calcineurin activated by the calcium signaling regulator and cyclophilin-binding protein, calcium-modulating cyclophilin ligand (CAML), has received broader interest in relation to various signaling events, in addition to regulating T cell receptor signaling [22]. It is expressed in cardiomyocytes, endothelial, and VSMC and is implicated in Ang II signaling through the AT1R [23]. Several findings have shown that the calcineurin/NFATsignaling pathway induced

by Ang II regulates cell growth and cardiovascular hypertrophy, contributing to pathological cardiovascular remodeling [24]. The CAML has been shown as an ATRAP partner, and the N-terminal hydrophilic domain of CAML (the amino acid residues 1-189) mediates a specific interaction between ATRAP and CAML. The amino acid residues 40-82 of ATRAP contribute to this interaction. Functionally, overexpression of ATRAP decreased Ang II–mediated and CAML–induced activation of calcineurin-NFAT pathway and inhibited cardiomyocyte hypertrophic response and VSMC senescence process [25, 26]. These results indicate that ATRAP significantly promotes the constitutive internalization of the AT1R and further attenuates certain Ang II–mediated pathological responses in cardiovascular and renal cells (Fig. 1).

7. REGULATION OF ATRAP EXPRESSION IN PATHOPHYSIOLOGICAL CONDITIONS

To understand the pathophysiological roles of ATRAP in hypertension and its related cardiovascular and renal disease, it should be important to investigate the regulation of endogenous expression of ATRAP gene in response to pathological stimuli and in the diseased conditions. Although the ATRAP mRNA and protein are abundantly and widely distributed along the renal tubules, including the distal and proximal tubules, a subpressor or pressor infusion of Ang II in mice causes a significant suppression of intrarenal ATRAP expression and that this response is dependent on the activation of AT1R [21]. Unilateral ureteral obstruction (UUO) is a well-established experimental model of progressive tubulointerstitial fibrosis. UUO leads to changes in renal hemodynamics, inflammatory responses in the kidney, and tubular hypertrophy and interstitial fibrosis of the affected kidney. The renin-angiotensin system is also known to be activated in UUO, and a recent study showed a significant down-regulation of ATRAP expression, with a concomitant decrease in Runx3 which is an activator of ATRAP gene transcription, in the affected kidney [27].

In the normal human kidney, both ATRAP mRNA and protein were widely and abundantly distributed along the renal tubules from Bowman's capsule to the medullary collecting ducts. In all renal tubular epithelial cells, the

ATRAP protein co-localized with the AT1R. In renal biopsy specimens with IgA nephropathy, a significant positive correlation between ATRAP and AT1R gene expression was observed. Furthermore, there was also a positive relationship between tubulointerstitial ATRAP expression and the estimated glomerular filtration rate in patients with IgA nephropathy, indicating that renal ATRAP expression appears to be influenced by renal functional status without significant compensatory up-regulation of endogenous ATRAP expression under the renal pathological condition [18].

In addition to the kidney, ATRAP is expressed in the cardiovascular tissues. As in the kidney, a subpressor or pressor infusion of Ang II in mice caused a significant suppression of cardiac ATRAP expression with a concomitant development of cardiac hypertrophy [21, 28], and in the vasculature, the cuff-mediated vascular injury in mice was shown to down-regulate the vascular ATRAP expression with atherosclerotic lesion development [14].

8. POSSIBLE INVOLVEMENT OF AT1R IN THE MODULATION OF TISSUE ATRAP EXPRESSION

From the accumulated preceding results *in vitro* and *in vivo*, we hypothesized that the tissue-specific balancing of ATRAP and AT1R expression may be an important regulator in the pathogenesis of hypertension and its related cardiovascular and renal disease (Fig. 2). In spontaneously hypertensive rats (SHR), concomitant with blood pressure increase and cardiac hypertrophy, there was a constitutive decrease in the ratio of cardiac expression of ATRAP to AT1R [16, 29]. However, treatment with AT1R–specific blocker

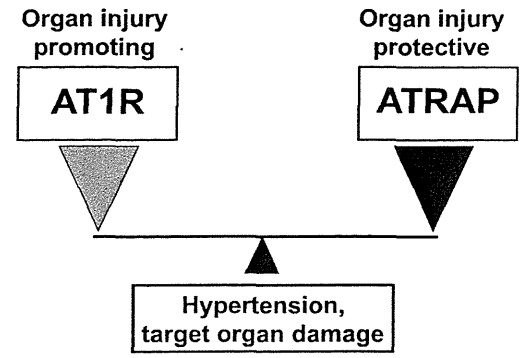

Fig. (2). ATRAP is widely expressed in many tissues as is AT1R, and tissue expression balance between ATRAP and AT1R may play a role in the determination of progression or suppression of hypertension and/or its related target organ damage.

(ARB), either at a depressor or subdepressor dose, recovered the suppressed cardiac ATRAP to AT1R ratio, which was accompanied by a decrease in AT1R density, an inhibition of p38 mitogen-activated protein kinase activity, and a regression of cardiac hypertrophy in SHR.

We also examined the regulation of endogenous ATRAP expression and effects of ARB in a genetic model of salt-sensitive hypertension. In Dahl Iwai salt-sensitive rats, renal ATRAP expression was suppressed concomitant with up-regulation of renal oxidative stress, inflammation and fibrosis-related markers such as p22phox, TGF-β, fibronectin, MCP-1 and type 1 collagen. However, prepubertal as well as continuous ARB treatment recovered the suppressed renal ATRAP expression and inhibited the renal activation of p22phox, TGF-β, fibronectin, MCP-1 and type 1 collagen [30]. These results showed that activation of AT1R signaling is one of major depressant of tissue ATRAP expression but indicated that prepubertal transient blockade of AT1R signaling exerts a long-term therapeutic effect on salt-induced hypertension and renal injury in Dahl Iwai salt-sensitive rats, partly through a ARB-mediated sustained enhancement of renal ATRAP expression (Fig. 3). Furthermore, these results demonstrated that there is a tissue-specific regulatory balancing of the expression of ATRAP and AT1R during the development of hypertension and related cardiovascular and renal disease.

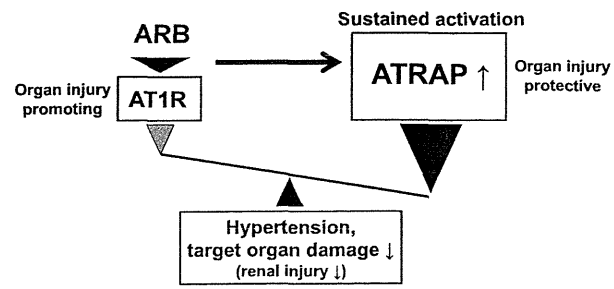

Fig. (3). Prepubertal transient blockade of renal AT1R signaling by ARB may exert a sustained activation of renal ATRAP expression to exhibit a long-term therapeutic effect even after withdrawal of ARB.

9. PUTATIVE FUNCTIONAL ROLE OF ATRAP *IN VIVO*

To examine the ATRAP-mediated effect on tissue AT1R internalization and AT1R signaling by a different strategy *in vivo*, several kinds of ATRAP transgenic mice were produced and analyzed to date. A transgenic model increase in renal ATRAP expression

beyond baseline was accompanied by a constitutive reduction of renal plasma membrane AT1R expression and by the promotion of renal AT1R internalization as well as the decreased induction of angiotensinogen gene expression in response to Ang II [21]. In another transgenic model increase in whole body ATRAP expression, there was a substantial attenuation of inflammatory vascular remodeling in a cuff injury-mediated atherosclerotic lesion [14].

Cardiac-specific ATRAP transgenic mice were also produced to examine a possible cardiac protective effect of ATRAP [28]. These ATRAP transgenic mice at baseline displayed no evident anatomical abnormality or alteration in physiological parameters, such as blood pressure and renal function. However, in cardiac-specific ATRAP transgenic mice, the development of cardiac hypertrophy, activation of p38 mitogen-activated protein kinase, and expression of hypertrophy-related genes in response to chronic Ang II infusion were completely suppressed, in spite of there being no significant difference in blood pressure between the transgenic mice and wild-type mice. These results demonstrate that cardiomyocyte-specific overexpression of ATRAP *in vivo* protected from the cardiac hypertrophy provoked by chronic Ang II infusion [28].

On the other hand, a previous study examined phenotypic changes in ATRAP deficient mice generated by gene-trap strategy and showed that these ATRAP deficient mice had increased arterial pressure and plasma volume, although the strain background of these ATRAP deficient mice and mice strain of wild-type mice used as controls were not identified in the study [17]. This study suggests that ATRAP acts as a negative regulator of AT1R in the tubular system of the kidney.

10. FUTURE DIRECTIONS OF ATRAP RESEARCH

Accumulated results showed that the ATRAP gene is abundantly and widely expressed in many tissues under normal condition and that there is a tissue-specific regulatory balancing of the expression of ATRAP and AT1R during the development of hypertension and related cardiovascular and renal disease. The activation of tissue ATRAP in transgenic models in which

ATRAP expression was increased beyond baseline promoted Ang II-mediated internalization of the AT1R, inhibited the pathological activation of certain but not all AT1R signaling pathways in the local tissues, and attenuated hypertension and tissue injury. Therefore, ATRAP may be a novel molecular target in hypertension and cardiovascular and renal injury (Fig. **4**), and it is important to elucidate the molecular mechanism of the tissue-specific regulation of ATRAP gene expression to determine the regulatory machinery for the tissue ATRAP level and/or ATRAP activity under both physiological and pathological conditions [27].

In addition, recent evidence has revealed that GPCRs may form dimers as part of their normal trafficking and function and GPCRs dimerization may have a functional impact on the diversity of receptor signaling [31]. The Ang II receptors are also reported to form homodimers and heterodimers, and undergo complex formation with other GPCRs [1, 32, 33]. Recent studies have reported that AT1R is able to form homodimers, as well as heterodimeric complexes with AT2R, MAS receptor, B2 bradykinin receptor, and β2 adrenergic receptor [34]. For example, a previous study reported that intracellular factor XIIIA transglutaminase crosslinks AT1R homodimers on monocytes via glutamine 315 in the carboxylterminal tail of the AT1R [35]. Such elevated levels of crosslinked AT1R dimers could be related to the onset of atherosclerosis. Although it should be determined whether the ATRAP activation promotes the formation of AT1R homodimer, the results of our preliminary study revealed that ATRAP is able to form homodimers and further studies are warranted to investigate the possible effects of ATRAP dimerization as a modulator of AT1R signaling. Furthermore, inves-

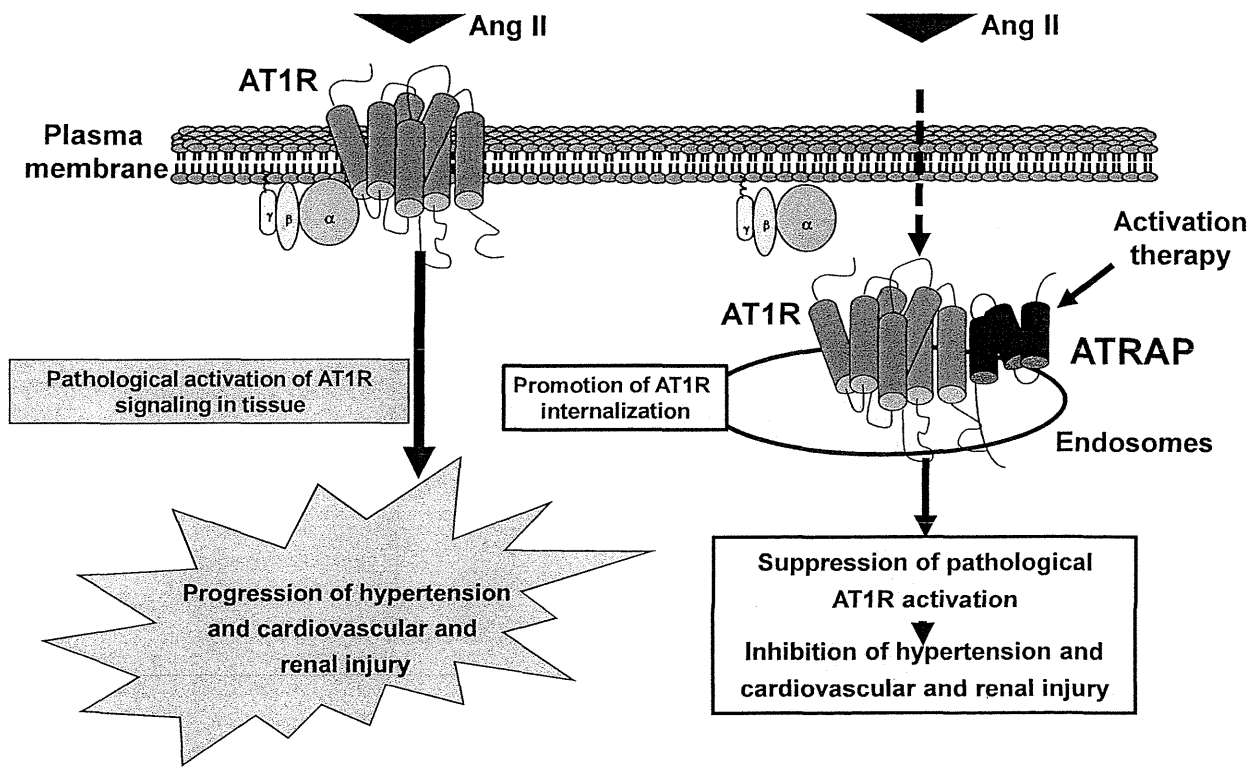

Fig. (4). ATRAP promotes constitutive internalization of tissue AT1R to intracellular endosomes and suppresses pathological activation of AT1R signaling in local tissue sites. Thus, ATRAP may be an interesting target in hypertension and related cardiovascular and renal injury.

tigation for possible functional interplay among ATRAP and other Ang II receptor-binding molecules such as ARAP1 (another AT1R-associated protein which promotes AT1R recycling to the plasma membrane) and ATIP (AT2R-interacting protein which enhances neural differentiation and inhibits vascular atherosclerosis) is warranted [3, 7, 36-38].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ORIGINAL ARTICLE

The angiotensin II type 1 receptor blocker olmesartan preferentially improves nocturnal hypertension and proteinuria in chronic kidney disease

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Accumulated evidence suggests that an altered ambulatory blood pressure (BP) profile, particularly elevated nighttime BP, reflects target organ injury and is a better predictor of further cardiorenal risk than the clinic BP or daytime BP in hypertensive patients complicated by chronic kidney disease (CKD). In this study, we examined the beneficial effects of olmesartan, an angiotensin II type 1 receptor blocker (ARB), on ambulatory BP profiles and renal function in hypertensive CKD patients. Forty-six patients were randomly assigned to the olmesartan add-on group ($n=23$) or the non-ARB group ($n=23$). At baseline and after the 16-week treatment period, ambulatory BP monitoring was performed and renal function parameter measurements were collected. Although the baseline clinic BP levels and the after-treatment/baseline (A/B) ratios of clinic BP levels were similar in the olmesartan add-on and non-ARB groups, the A/B ratios of ambulatory 24-h and nighttime BP levels in the olmesartan add-on group were significantly lower. Furthermore, the A/B ratios of urinary protein, albumin and type IV collagen excretion in the olmesartan add-on group were significantly lower than those in the non-ARB group (urinary protein excretion, 0.72 ± 0.41 *vs.* 1.45 ± 1.48, $P=0.030$; urinary albumin excretion, 0.73 ± 0.37 *vs.* 1.50 ± 1.37, $P=0.005$; urinary type IV collagen excretion, 0.87 ± 0.42 *vs.* 1.48 ± 0.87, $P=0.014$) despite comparable A/B ratios for the estimated glomerular filtration rate in the two groups. These results indicate that in hypertensive patients with CKD, olmesartan add-on therapy improves the ambulatory BP profile via a preferential reduction in nighttime BP with concomitant renal injury inhibition.
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Keywords: ambulatory blood pressure; angiotensin receptor blocker; estimated glomerular filtration rate; proteinuria

INTRODUCTION

Clinical trials have shown that strict blood pressure (BP) control is critical for preventing target organ damage and cardiovascular mortality in hypertensive patients.[1,2] Hypertensive patients with chronic kidney disease (CKD) are increasing in number, and cardiovascular complications are the most common cause of death in these patients. Thus, it is of considerable importance to identify therapeutic targets in cases of hypertension accompanied by the complication of CKD. Recent studies have indicated that the ambulatory and the clinic BP profiles are important for proper estimation of BP control. In particular, ambulatory BP monitoring has allowed accurate diagnosis of hypertension[3,4] and determination of the circadian BP rhythm under different pathophysiological conditions, including CKD, and thus may have prognosis predictability superior to clinic BP measurement.[5,6] The circadian BP pattern in hypertensive patients with CKD has been found to exhibit a blunted nocturnal BP decrease, which is associated with autonomic neuropathy and nephropathy.[7,8] Conversely, the loss of nocturnal BP dipping is considered a risk factor for nephropathy progression and is of prognostic value with respect to target organ damage and cardiovascular morbidity in these CKD patients.[6,9–12]

Activation of the renin–angiotensin system has been demonstrated to be involved in CKD pathogenesis and its cardiovascular complications through the generation of angiotensin II (Ang II), a key regulator of cardiovascular homeostasis. The Ang II type 1 receptor (AT1R) is responsible for most Ang II-mediated pathophysiological effects, and inhibition of the renin–angiotensin system by angiotensin-converting enzyme inhibitors (ACEIs) and AT1R-specific blockers (ARBs) has been shown to exert various protective effects against CKD progression and cardiovascular complications, at least partially through a reduction in urinary protein/albumin excretion.[13–16]

Furthermore, recent clinical study results and a meta-analysis of several large-scale cohort studies indicated that preservation of the estimated glomerular filtration rate (eGFR), as well as reduction

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in proteinuria/albuminuria are important for the suppression of CKD progression and cardiovascular complications in hypertensive CKD patients.[17–19] Of the clinically available ARBs, olmesartan has been reported to exert a long-lasting BP-lowering effect via its characteristic 'double-chain domain' structure[20] and is expected to efficiently improve the altered ambulatory BP profile of hypertensive CKD patients.[21] Previous studies have shown that nocturnal hypertension is closely related to an increase in urinary protein excretion and renal function deterioration in CKD patients. We hypothesized that olmesartan may effectively lower nocturnal BP levels with concomitant renal protective effects such as improvements in proteinuria and markers of renal injury. Therefore, in this study, we examined the therapeutic effects of olmesartan add-on therapy on the ambulatory BP profile and renal function of hypertensive patients with CKD.

METHODS

Study design

This was a randomized open-label parallel-group controlled study; it was conducted at the outpatient clinic of the Department of Internal Medicine, Yokohama City University Hospital (Yokohama, Japan). The study consisted of a 2-week run-in period and a 16-week active treatment period. This study was approved by the Ethics Committees of Yokohama City University Hospital, and written informed consent was obtained from every participant.

Study participants

Inclusion criteria were an age of 20 years or older, a history of mild-to-moderate hypertension (clinic systolic BP $\geqslant$130 mm Hg and/or diastolic BP $\geqslant$80 mm Hg or receiving antihypertensives) and CKD. When patients were already being treated for hypertension, anti-hypertensive drugs other than ARBs were continued during the run-in period, and then, if the hypertension was still uncontrolled (BP $\geqslant$130/80 mm Hg), the patients were considered for recruitment into the study. CKD was diagnosed by the presence of albuminuria (urinary albumin excretion rate (UACR) $\geqslant$30 mg per g creatinine), proteinuria (urinary protein excretion rate (UPCR) $\geqslant$0.15 g per g creatinine), or eGFR $<$60 ml min per 1.73 m^2 for a period of more than 3 months. We calculated the eGFR using a revised equation for the Japanese population: eGFR (ml min^{-1} per 1.73 m^2) = 194 $\times$ serum creatinine$^{-1.094}$ $\times$ age$^{-0.287}$ $\times$ 0.739 (*if female*).[22] Exclusion criteria included patients who were on dialysis, women who were nursing or pregnant, and patients with clinically significant heart disease, stroke, renal artery stenosis, hepatic dysfunction or known hypersensitivity to any ingredient in the study medications.

Study treatment

After the run-in period and the discontinuation of any previous ARB treatment, eligible patients were randomized to the olmesartan add-on group or the non-ARB group. Patients in the olmesartan add-on group were initially given 10 mg of olmesartan once daily in the morning; the dose of olmesartan was titrated up to 40 mg daily, as needed, during the 16-week active treatment period. Patients in the non-ARB group were given either an increased dose of their existing treatment or an additional conventional treatment other than ARB to achieve the BP goal (BP $<$130/80 mm Hg).

Clinic BP measurement and 24-h ambulatory BP monitoring

Clinic BP was measured at the trough of the medication cycle (24 $\pm$ 2 h post dose) using a calibrated standard mercury sphygmomanometer and the recommended cuff sizes in a sitting position.[23] Two measurements were taken at 1- to 2-min intervals, and their average was used to calculate the clinic BP.

The ambulatory BP and heart rate were monitored every 30 min with a fully automated device (TM-2425, A&D, Tokyo, Japan), as described previously.[24–30] Ambulatory BP monitoring was repeated in patients who had $>$20% of values missing, $>$30% error rate for the total readings, or values missing for more than 2 consecutive hours. The following readings were considered technical artifacts and were omitted: systolic BP $>$250 mm Hg or $<$70 mm Hg, diastolic BP $>$130 mm Hg or $<$30 mm Hg, pulse pressure $>$160 mm Hg or $<$20 mm Hg, systolic differences $>$60 mm Hg or diastolic differences $>$30 mm Hg compared with the immediately preceding or subsequent values. The patients were instructed to fill out a diary to record the time of sleeping, rising and other daytime activities. Therefore, the terms 'daytime' and 'nighttime' in the present study reflect the average period during which the subjects were awake/upright and asleep/supine, respectively. We defined the morning BP as the average BP during the initial 2 h after awakening, as described previously.[31–33]

Table 1 Patient baseline characteristics

Variable	*Olmesartan*, n = *22*	*Non-ARB*, n = *23*	P-*value*
Age (years); mean (s.d.)	64.7 $\pm$ 10.4	67.0 $\pm$ 7.9	0.413
Male (%)	86	87	1.000
Waist circumference (cm); mean (s.d.)	88.3 $\pm$ 7.7	90.1 $\pm$ 10.1	0.892
Diabetes (%)	41	51	0.554
CKD stage (n)			*0.167*
Stage 1	0	0	
Stage 2	6	1	
Stage 3	10	16	
Stage 4	3	4	
Stage 5	3	2	
Serum creatinine (mg dl^{-1}); mean (s.d.)	1.73 $\pm$ 1.04	1.73 $\pm$ 1.10	0.742
eGFR (ml min^{-1} per 1.73 m^2); mean (s.d.)	43.0 $\pm$ 22.2	40.5 $\pm$ 17.9	0.751
UPCR (g per g Cr); mean (s.d.)	2.12 $\pm$ 2.58	1.58 $\pm$ 2.23	0.517
UACR (mg per g Cr); mean (s.d.)	1605.3 $\pm$ 1889.7	1182.0 $\pm$ 1614.9	0.388
hs-CRP (mg dl^{-1}); mean (s.d.)	0.14 $\pm$ 0.17	0.07 $\pm$ 0.05	0.175
Urinary AGT (μg per g Cr); mean (s.d.)	514.8 $\pm$ 843.3	454.3 $\pm$ 811.3	0.835
Urinary 8-OHdG (ng per mg Cr); mean (s.d.)	3.60 $\pm$ 3.53	2.65 $\pm$ 2.74	0.455
Urinary type IV collagen (μg per g Cr); mean (s.d.)	12.70 $\pm$ 12.72	8.78 $\pm$ 8.85	0.301

Abbreviations: ARB, angiotensin II type 1 receptor blocker; AGT, angiotensinogen; CDK, chronic kidney disease; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity c-reactive protein; 8-OHdG, 8-hydroxydeoxyguanosine; UACR, urinary albumin-to-creatinine ratio; UPCR, urinary protein-to-creatinine ratio.

Laboratory measurements

Blood and urine sampling was performed between 0800 and 1000 hours after an overnight fast. After the patients had spent 30 min at quiet rest in a recumbent position, blood samples were collected to determine laboratory parameters using routine methods in the Department of Clinical Chemistry, Yokohama City University Hospital. The urinary concentrations of type IV collagen and 8-hydroxydeoxyguanosine (8-OHdG) were determined using an enzyme immunoassay kit (SRL laboratory, Tokyo, Japan) and an ELISA kit (Japan Institute for Control of Aging, Shizuoka, Japan), respectively.[34] Urinary angiotensinogen (AGT) levels were measured using a sandwich ELISA, as described previously.[35,36] Urinary type IV collagen, 8-OHdG and AGT concentrations were normalized to urinary creatinine concentration (Table 1).

Statistical analysis

The quantitative data are expressed as the mean ± s.d. To examine the effects of anti-hypertensive treatment, the values of the variables after the 16-week active treatment period were normalized to those of their respective variables at baseline and were expressed as after-treatment/baseline (A/B) ratios. For the statistical analysis of the difference between the olmesartan add-on group and non-ARB group, the Mann–Whitney *U*-test was performed for continuous variables, and the χ^2 test was performed for qualitative variables using SPSS (Statistical Package for the Social Sciences) software (Version 16.0, Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

Forty-six hypertensive patients with CKD were enrolled between October 2007 and March 2010. The causes of CKD were hypertensive nephrosclerosis ($n=17$), diabetic nephropathy ($n=14$), chronic glomerulonephritis ($n=6$), polycystic kidney disease ($n=1$), other renal diseases ($n=4$) or of unknown cause ($n=4$). Patients were randomly assigned to the olmesartan add-on group ($n=23$) or the non-ARB group ($n=23$). One patient in the olmesartan add-on group was discontinued from the study; therefore, a total of 45 patients completed the study. This patient was unavailable for follow-up due to a transfer to another hospital. Table 1 shows the demographics of the study participants, including the number of participants in each CKD stage and the baseline characteristics of the participants.

The olmesartan add-on therapy was well tolerated without any significant adverse events, and the average additional dose of olmesartan was 15.9 ± 8.9 mg per day after a 16-week treatment period. The additional treatments in both groups were primarily calcium channel blockers, diuretics, ACEIs and statins; more patients in the non-ARB group were treated with ACEIs than the olmesartan add-on group during the study period (Table 2). Although ~50% of patients in both groups had diabetes, there were no significant differences in the use of oral hypoglycemic agents and insulin.

Effects of olmesartan add-on therapy on the BP profile

At baseline, the mean clinical BP values were similar in the olmesartan add-on and non-ARB groups. After the 16-week active treatment period, the A/B ratios of the two groups were comparable (Table 3). With respect to the ambulatory BP profile, the baseline daytime systolic/diastolic BP levels were comparable in the olmesartan add-on and non-ARB groups, while the baseline 24-h and nighttime systolic/diastolic BP levels were significantly higher in the olmesartan add-on group (Table 4). However, compared with the non-ARB group, the 24 h diastolic BP and nighttime systolic/diastolic BP level A/B ratios in the olmesartan add-on group were significantly lower after the 16-week active treatment period (Table 4; 24-h diastolic BP, 0.92 ± 0.07 *vs.* 0.98 ± 0.07, $P=0.017$; nighttime systolic BP, 0.91 ± 0.10 *vs.* 1.00 ± 0.08, $P=0.001$; nighttime diastolic BP 0.90 ± 0.11 *vs.* 0.98 ± 0.10, $P=0.024$), and the 24-h systolic BP A/B ratio tended to be decreased in the olmesartan add-on group (Table 4; 24-h systolic BP, 0.93 ± 0.07 *vs.* 0.99 ± 0.08, $P=0.053$). However, the daytime systolic/diastolic BP A/B ratios were comparable in the olmesartan add-on group and the non-ARB group (Table 4; daytime systolic BP, 0.94 ± 0.07 *vs.* 0.98 ± 0.09, $P=0.261$; daytime diastolic BP, 0.93 ± 0.07 *vs.* 0.97 ± 0.07, $P=0.105$). In addition, although the differences did not reach statistical significance, there was a trend toward a decrease in morning systolic/diastolic BP A/B ratios in the olmesartan add-on group compared with the non-ARB group (Table 4).

Effects of olmesartan add-on therapy on markers of renal function

At baseline, serum creatinine, eGFR, UPCR and UACR were similar in the olmesartan add-on and non-ARB groups (Table 1). After the 16-week active treatment period, serum creatinine and eGFR A/B ratios were comparable in the two groups (Figure 1). In contrast, the UPCR and UACR A/B ratios were significantly lower in the olmesartan add-on group than in the non-ARB group (Figure 1; UPCR, 0.72 ± 0.41 *vs.* 1.45 ± 1.48, $P=0.030$; UACR, 0.73 ± 0.37 *vs.* 1.50 ± 1.37, $P=0.005$) after the 16-week active treatment period. Overall, the results of correlation analysis showed that there were significant positive associations between the A/B ratios of nighttime systolic BP and UPCR ($R=0.440$, $P=0.004$), and between the A/B ratios of nighttime systolic BP and UACR ($R=0.472$, $P=0.002$) in all patient groups.

Table 2 Medications used during the study

Medication	*Olmesartan*, n = *22*	*Non-ARB*, n = *23*	P-*value*
Calcium channel blockers (%)	77	96	0.096
Diuretics (%)	36	57	0.175
ACEI (%)	14	57	0.003
Statins (%)	23	35	0.372
Oral hypoglycemic agents (%)	32	17	0.187
Insulin (%)	18	4	0.260

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II type 1 receptor blocker.

Table 3 Effects of anti-hypertensive therapy on clinic BP

Variable	*Olmesartan*, n = *22*	*Non-ARB*, n = *23*	P-*value*
Clinic BP			
Systolic BP			
Baseline (mm Hg); mean (s.d.)	144.1 ± 13.7	144.1 ± 17.8	0.955
A/B ratio; mean (s.d.)	0.99 ± 0.12	0.96 ± 0.11	0.714
Diastolic BP			
Baseline (mm Hg); mean (s.d.)	79.7 ± 19.0	84.6 ± 11.5	0.474
A/B ratio; mean (s.d.)	1.36 ± 1.61	0.95 ± 0.12	0.182

Abbreviations: A/B ratio, after-treatment/baseline ratio; ARB, angiotensin II type 1 receptor blocker; BP, blood pressure.

Table 4 Effects of anti-hypertensive therapy on the ambulatory BP profile

Variable	*Olmesartan, n = 22*	*Non-ARB, n = 23*	*P-value*
24 h			
Systolic BP			
Baseline (mm Hg); mean (s.d.)	142.6 ± 14.4	135.0 ± 11.3	0.036
A/B ratio; mean (s.d.)	0.93 ± 0.07	0.99 ± 0.08	0.053
Diastolic BP			
Baseline (mm Hg); mean (s.d.)	83.6 ± 10.4	78.2 ± 7.5	0.045
A/B ratio; mean (s.d.)	0.92 ± 0.07	0.98 ± 0.07	0.017
Daytime			
Systolic BP			
Baseline (mm Hg); mean (s.d.)	146.6 ± 14.8	140.5 ± 11.5	0.180
A/B ratio; mean (s.d.)	0.94 ± 0.07	0.98 ± 0.09	0.261
Diastolic BP			
Baseline (mm Hg); mean (s.d.)	86.3 ± 10.8	81.5 ± 8.4	0.104
A/B ratio; mean (s.d.)	0.93 ± 0.07	0.97 ± 0.07	0.105
Nighttime			
Systolic BP			
Baseline (mm Hg); mean (s.d.)	133.4 ± 15.9	123.9 ± 13.9	0.031
A/B ratio; mean (s.d.)	0.91 ± 0.10	1.00 ± 0.08	0.001
Diastolic BP			
Baseline (mm Hg); mean (s.d.)	77.8 ± 10.7	71.8 ± 7.8	0.013
A/B ratio; mean (s.d.)	0.90 ± 0.11	0.98 ± 0.10	0.024
Morning			
Systolic BP			
Baseline (mm Hg); mean (s.d.)	144.6 ± 16.8	138.9 ± 16.9	0.107
A/B ratio; mean (s.d.)	0.95 ± 0.11	1.02 ± 0.15	0.095
Diastolic BP			
Baseline (mm Hg); mean (s.d.)	84.1 ± 12.2	80.9 ± 10.5	0.340
A/B ratio; mean (s.d.)	0.95 ± 0.12	1.03 ± 0.13	0.068

Abbreviations: BP, blood pressure; A/B ratio, after-treatment/baseline ratio.

Effects of olmesartan add-on therapy on markers of inflammation, the renal renin–angiotensin system, oxidative stress and fibrosis

At baseline, high-sensitivity C-reactive protein (hs-CRP), urinary AGT, 8-OHdG and type IV collagen levels were similar in the olmesartan add-on and non-ARB groups (Table 1). The hs-CRP and urinary 8-OHdG A/B ratios after the 16-week active treatment period were comparable in both groups (Figure 2). The urinary AGT A/B ratio after the 16-week active treatment period was lower in the olmesartan add-on group than in the non-ARB group, but the difference did not reach statistical significance (Figure 2; urinary AGT, 1.35 ± 1.59 *vs.* 4.43 ± 7.39, $P = 0.172$). In contrast, the urinary type IV collagen A/B ratio following the 16-week treatment was significantly suppressed in the olmesartan add-on group compared with the non-ARB group (Figure 2; urinary type IV collagen, 0.87 ± 0.42 *vs.* 1.48 ± 0.87, $P = 0.014$). The correlation analysis demonstrated that there was a significant positive relationship between the A/B ratios of nighttime systolic BP and urinary type IV collagen ($R = 0.451$, $P = 0.003$), and further revealed a trend toward a positive association between the A/B ratios of nighttime systolic BP and urinary AGT ($R = 0.332$, $P = 0.051$).

Comparison of renal function parameters between patients with and without ACEI treatment in the non-ARB group

As shown in Table 2, there was a significant difference in the percentage of patients in the olmesartan add-on group and non-ARB group that were prescribed ACEIs (14% *vs.* 57%, $P = 0.003$). Previous studies have shown that ACEIs exert renoprotective effects in CKD patients, and thus, ACEIs could also affect the renal function parameters measured in the present study.[37] Therefore, we compared the renal function parameters between patients in the non-ARB group that were either treated with ACEIs (ACEI + patients) or not treated with ACEIs (ACEI − patients). In the non-ARB group, the UPCR and UACR in the ACEI − patients both showed an increasing trend after the 16-week active treatment period, and the UPCR and UACR A/B ratios after treatment in the ACEI − patients were higher than those in the ACEI + patients (Table 5; UPCR, 2.27 ± 2.08 *vs.* 0.89 ± 0.37, $P = 0.060$; UACR, 2.27 ± 1.80 *vs.* 0.92 ± 0.43, $P = 0.015$). These differences were not observed between ACEI + patients and ACEI − patients when the olmesartan add-on group and all of the subjects were analyzed (data not shown). These results support the renoprotective effects of ACEIs in hypertensive CKD patients.

DISCUSSION

Accumulated evidence suggests that elevated nighttime BP levels reflect target organ injury and are a better predictor of further cardiovascular and renal risk than daytime or 24-h BP levels in hypertensive patients with diabetes and CKD.[6,38] In addition, efficient reduction in nighttime BP by methods such as bedtime dosing, as determined by ambulatory BP monitoring, has been shown to reduce cardiovascular risk in cases of resistant hypertension and hypertension complicated by diabetes or CKD.[39–41] The results of the present study showed that olmesartan add-on therapy exerted a significantly better BP-lowering effect than non-ARB therapy, particularly during the nighttime period; similar daytime BP lowering was observed in the olmesartan add-on group and non-ARB group.

The results of this study show that both the olmesartan add-on therapy and non-ARB anti-hypertensive therapy were well tolerated in hypertensive patients with CKD. Although the baseline clinic BP levels were similar in the olmesartan add-on and non-ARB groups, the baseline 24-h and nighttime BP levels from the ambulatory BP recording were significantly higher in the olmesartan add-on group than the non-ARB group (Table 4). Thus, to strictly compare the effects of the two treatment regimens after the 16-week active treatment period, the values of the variables, including the ambulatory BP parameters, were normalized to those of the respective variables at baseline and were expressed as A/B ratios.

In this study, although more patients were treated with ACEIs in the non-ARB group than in the olmesartan add-on group, the olmesartan add-on therapy significantly decreased urinary protein, albumin and type IV collagen excretion. Thus, these data indicate that the benefit of olmesartan add-on therapy for patients with hypertension and CKD results from inhibition of proteinuria/albuminuria and pathological renal injury, and these beneficial effects of olmesartan are exerted, at least in part, through a preferential reduction in nocturnal BP, which is an important potential therapeutic target for cardiorenal protection in hypertension with CKD.

The role of the renin–angiotensin system in promoting hypertension- and CKD-related organ damage is well established. Reducing proteinuria/albuminuria is critically important for the regression of CKD,[42] and the evidence indicates that ARBs and ACEIs reduce cardiovascular and renal risks primarily by reducing proteinuria/albuminuria.[13,16,43] In a recent, large-scale ORIENT clinical trial,

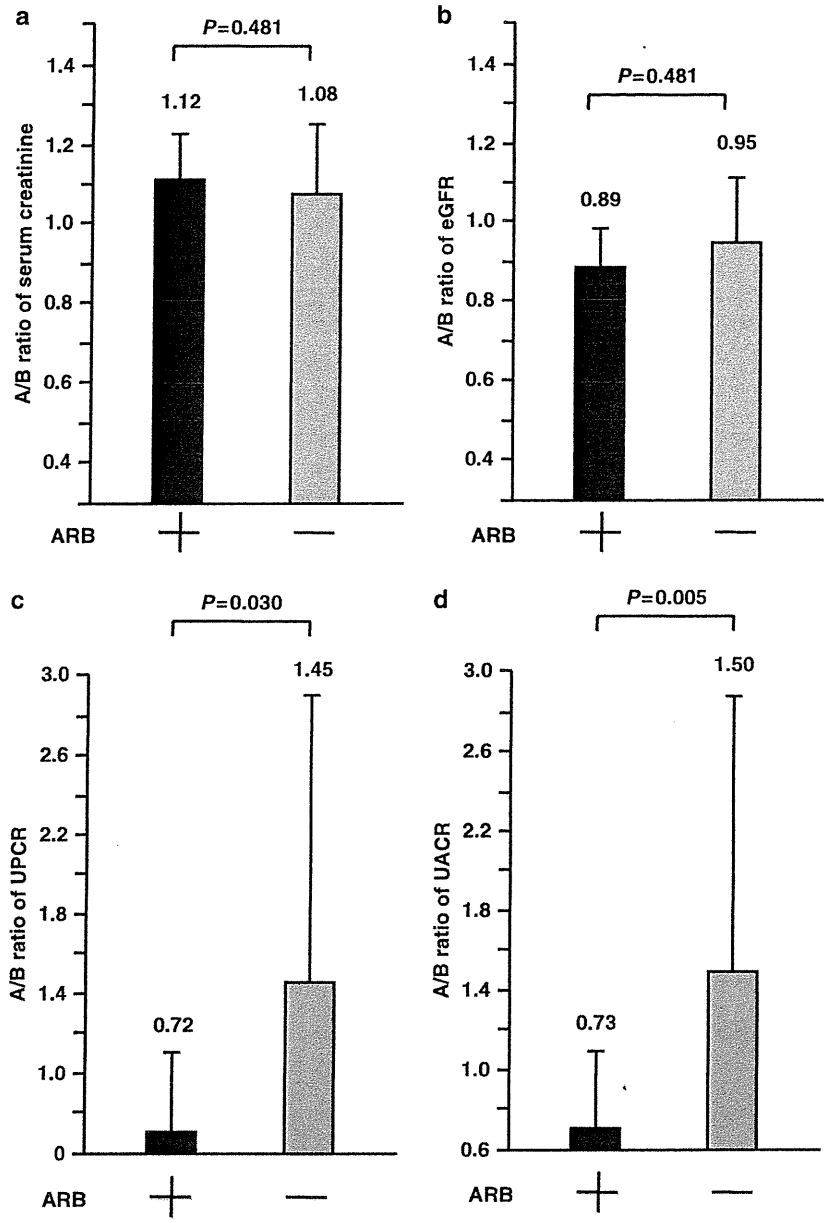

Figure 1 Effects of olmesartan add-on therapy on the after-treatment/baseline (A/B) ratios of (**a**) serum creatinine, (**b**) eGFR, (**c**) UPCR and (**d**) UACR. Forty-six hypertensive patients with CKD were randomly assigned to the olmesartan add-on group (ARB+) or the non-ARB group (ARB−). At baseline and after the 16-week treatment period, measurements were collected. To compare the effects of anti-hypertensive treatment in each group, the values of the variables after the 16-week active treatment period were normalized to the respective variables at baseline and were expressed as the A/B ratio. The values of the A/B ratios are expressed as the mean ± s.d.

olmesartan treatment significantly decreased clinic BP and proteinuria compared with the control anti-hypertensive treatment that included ACEIs, and there was no renal dysfunction acceleration in type 2 diabetic patients with overt nephropathy.[44] The present study included non-diabetic glomerulopathy patients as well as diabetic nephropathy patients. The results of the present study demonstrate that a preferential decrease in nighttime BP levels was accompanied by decreases in urinary protein and albumin excretion and urinary type IV collagen excretion, an important marker of renal injury in diabetic nephropathy and non-diabetic renal disease,[45,46] without any decrease in eGFR resulting from the olmesartan add-on therapy. The results of the ACCOMPLISH study indicated that not only a reduction of albuminuria but also long-term preservation of eGFR are important for the suppression of CKD progression and cardiovascular complications.[17,19] Therefore, it is likely that olmesartan effectively inhibits renal deterioration through improvement in the circadian BP rhythm and maintenance of eGFR, in addition to its general BP-lowering effect in hypertensive patients with CKD.

In contrast, proteinuria/albuminuria showed an increasing trend after the treatment period in the non-ARB group. We previously showed that urinary protein excretion correlated positively with nighttime BP in hypertensive CKD patients with diabetic

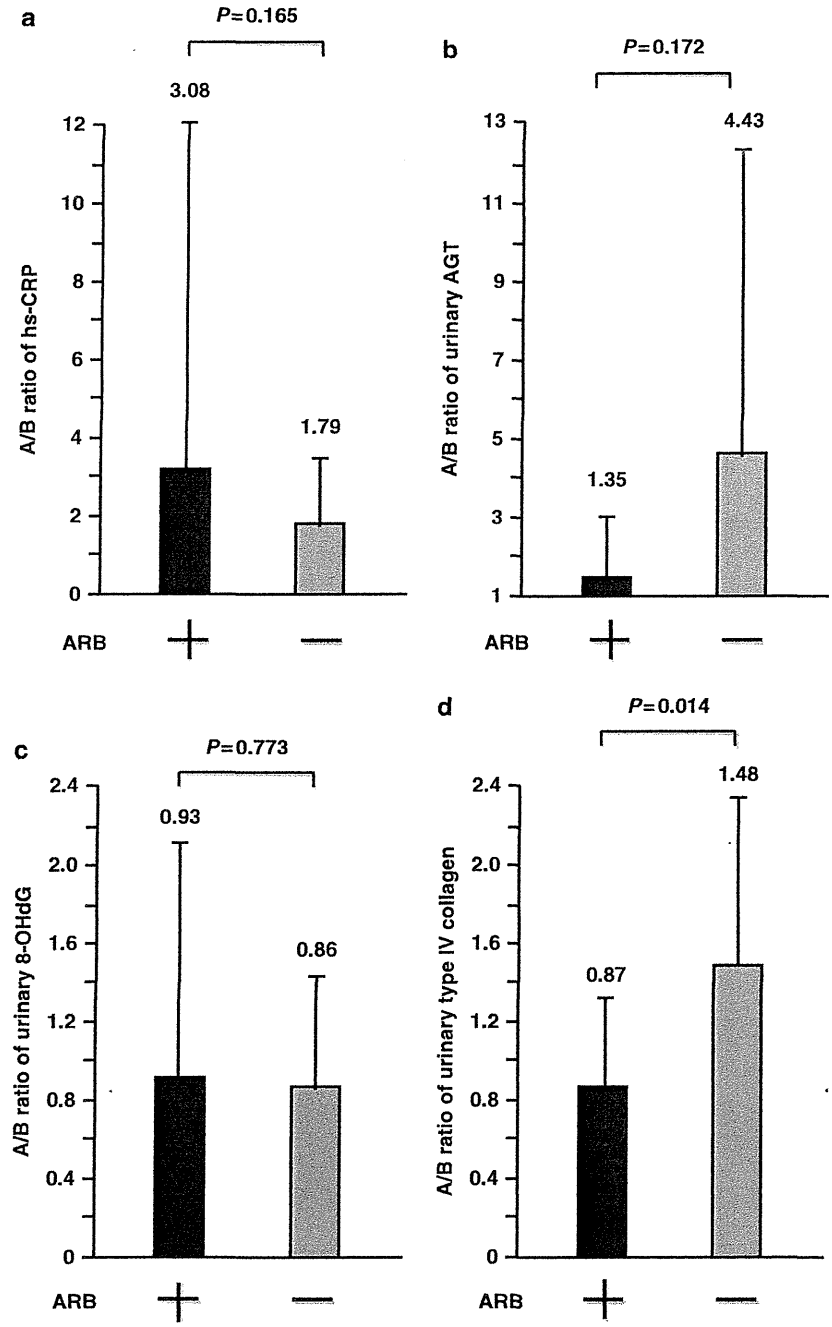

Figure 2 Effects of olmesartan add-on therapy on the after-treatment/baseline (A/B) ratios of (**a**) hs-CRP, (**b**) urinary AGT, (**c**) urinary 8-OHdG and (**d**) urinary type IV collagen. Forty-six hypertensive patients with CKD were randomly assigned to the olmesartan add-on group (ARB+) or the non-ARB group (ARB−). Measurements were collected at baseline and after the 16-week treatment period. To compare the effects of anti-hypertensive treatment in each group, the values of the variables after the 16-week active treatment period were normalized to those of the respective variables at baseline and are expressed as A/B ratios. The values of the A/B ratios are expressed as the mean ± s.d.

nephropathy or non-diabetic glomerulopathy,[25] and a recent study reported that nocturnal BP reduction is critically important for the inhibition of CKD progression.[41] Therefore, the lack of a decrease in nighttime BP may have caused the increasing trend in proteinuria/albuminuria in the non-ARB group after treatment. In addition, proteinuria/albuminuria in the ACEI− patients in the non-ARB group showed an increasing trend after the 16-week active treatment period, and urinary protein/albumin excretion after treatment was higher in the ACEI− patients than in the ACEI+ patients (Table 5), thereby also supporting the renoprotective effects of ACEIs in hypertensive CKD patients.

What might the underlying mechanism be that is responsible for the efficient nocturnal BP-lowering effects of olmesartan? In comparison with other ARBs, olmesartan is reported to exert a long-lasting BP-lowering effect via its characteristic 'double-chain domain' structure[20] and to efficiently improve the altered ambulatory BP profile in

Table 5 Comparison of UPCR, UACR and urinary type IV collagen between ACEI + patients and ACEI − patients in the non-ARB group

Variable	*ACEI+, n=13*	*ACEI−, n=10*	P-*value*
UPCR			
Baseline (g per g Cr); mean (s.d.)	1.97±2.18	1.09±2.30	0.343
A/B ratio; mean (s.d.)	0.89±0.37	2.27±2.08	0.060
UACR			
Baseline (mg per g Cr); mean (s.d.)	1511±1507	788±1730	0.123
A/B ratio; mean (s.d.)	0.92±0.43	2.27±1.80	0.015
Urinary type IV collagen			
Baseline (μg per g Cr); mean (s.d.)	11.05±10.33	5.83±5.66	0.148
A/B ratio; mean (s.d.)	1.28±0.84	1.76±0.87	0.186

Abbreviations: A/B ratio, after-treatment/baseline ratio; ACEI, angiotensin-converting enzyme inhibitor; UACR, urinary albumin-to-creatinine ratio; UPCR, urinary protein-to-creatinine ratio.

hypertensive patients, even if olmesartan is only administered once in the morning.[21] In addition, sodium retention due to a reduced GFR and impaired renal sodium excretion capacity are characteristic pathophysiological states in hypertensive patients with CKD. Recent animal studies reported that activation of AT1R in the kidney mediates chronic hypertensive effects; by stimulation of the intrarenal renin–angiotensin system, sodium reabsorption from the renal tubules is promoted.[47,48] Furthermore, previous clinical studies showed that the urinary AGT level closely reflects renal renin–angiotensin system activity in hypertension and CKD.[36,49–51] In this regard, a recent study suggested that inhibition of renal sodium reabsorption may be an important mechanism involved in the olmesartan-mediated improvement in the circadian BP rhythm via a preferential lowering of nocturnal BP levels in CKD patients.[52] Although the decrease in urinary AGT excretion by the olmesartan add-on therapy did not reach statistical significance in the current study, there was also a trend toward a positive relationship between nocturnal BP and urinary AGT. Therefore, olmesartan-mediated preferential reduction in nocturnal BP may be effected via the prevention of renal tubular sodium reabsorption through the inhibition of renal tubule AT1R.

The limitations of the present study include the background difference in ambulatory BP levels between the olmesartan add-on and non-ARB groups, although the clinic BP levels in these groups at baseline were comparable. To examine the effects of olmesartan add-on therapy on ambulatory BP levels, the values of the variables after the 16-week active treatment period were normalized to the respective variables at baseline and were expressed as A/B ratios. Furthermore, the sample size is relatively small, which limits our ability to determine significance.

In summary, the results of this study indicate that olmesartan add-on therapy improves the ambulatory BP profile by preferential reduction in nighttime BP and may afford protective renal effects in patients with hypertension and CKD. Further studies are needed to examine the mechanistic basis of the olmesartan-mediated therapeutic effects on ambulatory BP profiles and renal function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Effects of Aliskiren-Based Therapy on Ambulatory Blood Pressure Profile, Central Hemodynamics, and Arterial Stiffness in Nondiabetic Mild to Moderate Hypertensive Patients

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Aliskiren is a direct renin inhibitor that exerts its effect at the rate-limiting step of the renin-angiotensin system. This study was performed to examine the beneficial effects of aliskiren-based antihypertensive therapy on the ambulatory blood pressure (BP) profile, central hemodybamics, and arterial stiffness in untreated Japanese patients with mild to moderate hypertension. Twenty-one Japanese nondiabetic patients with untreated mild to moderate essential hypertension were initially given aliskiren once daily at 150 mg, and the dose was titrated up to 300 mg as needed. After 12 weeks of aliskiren-based therapy, the clinic, ambulatory, and central BP values as well as brachial-ankle pulse wave velocity (baPWV) were all significantly decreased compared with baseline (clinic systolic BP, 151±11 mm Hg vs 132±11 mm Hg; clinic diastolic BP, 91±13 mm Hg vs 82±9 mm Hg; 24-hour systolic BP, 144±12 mm Hg vs 133±11 mm Hg; 24-hour diastolic BP, 88±8 mm Hg vs 81±9 mm Hg; central BP, 162±16 mm Hg vs 148±14 mm Hg; baPWV, 1625±245 cm/s vs 1495±199 cm/s; $P<.05$). These results show that aliskiren, as a first-line regimen, improves the ambulatory BP profile and may have protective vascular effects in Japanese nondiabetic patients with untreated mild to moderate essential hypertension. *J Clin Hypertens (Greenwich).* 2012; 14:522–529. ©2012 Wiley Periodicals, Inc.

Hypertension is highly prevalent worldwide and is one of the major risk factors for cardiovascular and renal diseases. In Japan, the first-line antihypertensive drugs are calcium channel blockers (CCBs), angiotensin II (Ang II) type 1 receptor (AT1R) blockers (ARBs), angiotensin-converting enzyme (ACE) inhibitors, diuretics, and β-blockers (including α-/β-blockers), according to the 2009 Japanese Society of Hypertension Guidelines for the Management of Hypertension.[1]

Experimental and clinical evidence has indicated that activation of the renin-angiotensin system (RAS) is involved in the pathogenesis of hypertension and the related target organ damage, and multiple studies have proven the usefulness of RAS blockade induced by ACE inhibitor and ARB for the management of hypertension. Both ACE inhibitor and ARB function downstream of the rate-limiting step in the RAS cascade, which involves the renin-catalyzed conversion of angiotensinogen to angiotensin I (Ang I). This leads to the promotion of renin release and to an increase in plasma renin activity (PRA) via the intrarenal short feedback loop due to lack of AT1R-mediated suppression of renin production in the juxtaglomerular cells of kidney. Renin inhibition is a means for blocking the RAS. Aliskiren is a direct renin inhibitor that acts at the rate-limiting step of the RAS cascade, inhibiting the formation of Ang I from angiotensinogen. Therefore, unlike either ACE inhibitors or ARBs, aliskiren does not induce a compensatory increase in PRA, but rather, reduces it.[2,3]

Recent evidence has shown that ambulatory as well as clinic BP profiles are important for a proper estimation of BP control. In particular, ambulatory BP monitoring has allowed a more accurate diagnosis of hypertension and a determination of the circadian rhythm of BP under different pathophysiologic conditions.[4,5] The central hemodynamics (ie, the central systolic BP [cSBP], augmentation index [AI]) and pulse wave velocity (PWV), a marker of large artery stiffness, do not always correlate with the peripheral brachial BP value, but do reflect the pressure load in the major organs. Several previous studies demonstrated that these variables (cSBP, AI, and PWV) are more closely related to organ damage than brachial BP.[6–9] Previous meta-analyses also showed that the cSBP, AI, and PWV are independent risk factors for cardiovascular disease, and that these variables may reflect the different characteristics of the pathophysiologic abnormalities related to arterial stiffness.[10] Thus, this study aimed to examine the beneficial effects of aliskiren-based antihypertensive therapy on ambulatory BP profile, central hemodybamics, and PWV in

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nondiabetic Japanese patients with untreated mild to moderate essential hypertension.

METHODS

Study Participants and Design

Patients with untreated hypertension from the outpatient clinic of the Department of Internal Medicine, Yokohama City University Hospital (Yokohama, Japan) were enrolled from January 2010 to April 2011. Hypertension was defined as an average clinic systolic BP (SBP) of at least 140 mm Hg or diastolic BP (DBP) of at least 90 mm Hg or both on two different occasions (with at least a 2-week interval) during the run-in period (4 weeks) in advance of entry. Inclusion criteria were age 20 years or older and older than 80 years, mild to moderate essential hypertension (<140 mm Hg clinic SBP <180 mm Hg or <90 mm Hg clinic DBP <110 mm Hg), and no treatment for at least 4 weeks before the first hospital visit. Exclusion criteria included patients who exhibited severe hypertension (clinic SBP ≥180 mm Hg and/or DBP ≥110 mm Hg), secondary hypertension, type 1 or type 2 diabetes mellitus, arrhythmia, clinically significant cardiovascular disease, cerebrovascular disease or neuropathy, renal dysfunction (estimated glomerular filtration <60 mL/min/1.73 m^2), overt proteinuria (urinary protein to creatinine ratio ≥300 mg/g-creatinine), taking cyclosporine, or women who were nursing or pregnant.

Determination of the clinic BP, ambulatory BP, baPWV, and cSBP were performed at baseline and 12 weeks after the start of aliskiren-based antihypertensive treatment. Venous blood and urine samples for the hematological, biochemical, and renal parameters were drawn and collected the morning after an overnight fast at baseline and 12 weeks after treatment. This study was approved by the ethics committees of Yokohama City University Hospital, and written informed consent was obtained from every participant prior to the start of the study.

The patients were initially given 150 mg of aliskiren once daily in the morning and the dose was titrated up to 300 mg daily 4 weeks after the treatment to reach the BP target if necessary. In addition, an optional addition of concomitant medication (either a thiazide diuretic at low dose or a CCB) was used to achieve the target BP control 8 weeks after the treatment as needed.

Clinic BP and 24-Hour Ambulatory BP Monitoring

The clinic sitting BP was measured at trough levels (24±2 hours postdose) using a calibrated standard mercury sphygmomanometer and the recommended cuff sizes.[11] Two measurements were taken at 1- to 2-minute intervals, and their average was used for the subsequent calculation.

Ambulatory BP and heart rate (HR) were monitored every 30 minutes with a fully automated device (TM-2425; A&D, Tokyo, Japan), essentially as described previously.[12–18] Ambulatory BP monitoring was repeated in patients who had >20% (missing) of the values missing out of the expected number of readings, a >30% error rate for the total readings, or values that were missing for >2 consecutive hours. The following readings were omitted because of technical artifacts: SBP >250 mm Hg or <70 mm Hg, DBP >130 mm Hg or <30 mm Hg, pulse pressure >160 mm Hg or <20 mm Hg, a systolic difference >60 mm Hg, or a diastolic differences >30 mm Hg compared with the immediately preceding or subsequent values. The patients were instructed to fill out a diary to record the time of sleeping, rising, and other daytime activities. Therefore, the term "day" and "night" hours in the present study reflect the average period during which the patients were awake/upright and asleep/supine, respectively. Short-term BP variability, which is comprised of the coefficients of variation of the BP values obtained from ambulatory BP monitoring, is defined as the within-patient standard deviation (SD) of all of the systolic and diastolic readings at 30-minute intervals divided by the mean BP during the course of the measurement periods.[14–16,19–21] HR variability, which is comprised of the HR coefficients of variation, is defined as the within-patient SD of all the HR values at 30-minute intervals divided by the mean HR.[14–16,19–21]

Central Hemodynamics

The cSBP and AI were measured using an HEM-9000AI (Omron Healthcare, Kyoto, Japan) with an automatic tonometry probe wrapped onto the wrist to record radial waveforms, which were calibrated against the contralateral arm cuff brachial BP taken immediately after tonometry. An algorithm based on a linear regression model was then applied to estimate the cSBP from the "late systolic shoulder" (pSBP2) of the radial pulse waveform, which has been shown to closely agree with the cSBP.[22–25] This device uses the maxima of the "multidimensional derivatives" on the recorded pressure waveforms to detect the first and second inflection points corresponding to the early and late systolic (pSBP2) pressure readings.

BaPWV

The baPWV values were determined with a PP analyzer (model BP-203RPEII; Nihon Colin, Tokyo, Japan) as described previously.[14,15] The baPWV values obtained by this method are reported to significantly correlate with the aortic PWV determined by the catheter method.[26]

Laboratory Measurements

Blood sampling was performed after the patients had spent 30 minutes at quiet rest in a semirecumbent position. PRA, plasma aldosterone, and plasma atrial natriuretic peptide were measured by radioimmunoassay. Other parameters were determined by routine methods in the Department of Clinical Chemistry, Yokohama

City University School Hospital. We calculated the estimated glomerular filtration rate (eGFR) with an application of the revised equation for the Japanese population: eGFR (mL/min/1.73 m^2)=194×serum creatinine$^{-1.094}$×Age$^{-0.287}$×0.739 (if female).[27]

Statistical Analysis

The quantitative data are expressed as mean±SD. For the statistical analysis of the difference between baseline and 12 weeks of therapy, analysis of variance was compared by a paired comparison *t* test. A *P* value <.05 was considered statistically significant. Clinic BP and pulse rate were analyzed by one-way repeated measures analysis of variance. Analysis was performed with STATISTICA 6.0 (StatSoft, Inc, Tulsa, OK).

RESULTS

Patient Characteristics

Twenty-one patients with untreated mild to moderate essential hypertension were enrolled. Four patients were discontinued from the study, so 17 patients completed the study. The reasons patients were lost to follow-up included referral to other hospitals (two patients) and protocol deviations (two patients). Table I shows the demographic and baseline characteristics of the participants. Mean age was 57±9 years, and there were 10 men and 7 women. Body mass index (BMI) was 24±2 kg/m^2 and clinic SBP/DBP was 151±11/91±13 mm Hg, suggesting that the participants corresponded to marginally obese untreated hypertensive patients. No patients had any impairment of renal function, including reduced eGFR and albuminuria, and glucose and lipid metabolism. There were no detectable abnormalities on electrocardiography or chest x-ray. Thus, the participants were ultimately characterized as middle-aged, marginally obese, mild to moderate hypertensive patients without complications.

TABLE I. Patient Baseline Characteristics

Variables	Mean (SD) or %
Age, y	57 (9)
Men/women, No.	*10/7*
Body mass index, kg/m^2	24 (2)
Current smoking	71
Diabetes	0
Dyslipidemia	47
Antihypertensive therapy within 4 weeks	0
Clinic SBP, mm Hg	151 (11)
Clinic DBP, mm Hg	91 (13)
Clinic PR, beats per min	69 (11)
LDL cholesterol, mg/dL	127.1 (4.4)
HDL cholesterol, mg/dL	68.4 (18.5)
Triglyceride, mg/dL	136.9 (77.7)
Glycated hemoglobin, %	5.3 (0.4)
Fasting plasma glucose, mg/dL	107.4 (25.7)
HOMA	2.6 (3.2)
Serum creatinine, mg/dL	0.7 (0.2)
eGFR, mL/min/1.73 m^2	77.8 (12.2)
Uric acid, mg/dL	5.8 (1.3)
Serum sodium, Eq/L	142.7 (1.5)
Serum potassium, Eq/L	4.1 (0.3)
Serum chloride, Eq/L	106.8 (1.9)
hs-CRP, mg/dL	0.2 (0.3)
UACR, mg/g-Cr	15.6 (14.6)

Abbreviations: CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA, homeostasis model assessment, LDL, low-density lipoprotein; PR, pulse rate; SBP, systolic blood pressure; SD, standard deviation; UACR, urinary albumin-to-creatinine ratio.

Aliskiren-based therapy was well tolerated in all of the patients without any significant adverse events and the average aliskiren dose was 212±74 mg daily after a period of 12 weeks of treatment. At the end of the study, 12 patients (71%) were taking aliskiren monotherapy (aliskiren 150 mg daily, n=10; 300 mg daily, n=2) and the remaining patients were taking combination therapy (aliskiren 300 mg daily/CCB, n=5; aliskiren/diuretic, n=0). The CCBs used were amlodipine (n=4, average dose 3.75 mg daily) and benidipine (n=1, 2 mg daily).

Effects of Aliskiren-Based Therapy on BP Profile

From baseline to week 4, aliskiren-based therapy significantly decreased the clinic SBP (151±11 vs 132±11, *P*<.001) and DBP (91±13 vs 82±9, *P*=.001) without any change in the clinic PRA (69±11 vs 70±8, not significant) (Figure 1). These decreases in the SBP and DBP by aliskiren were maintained at week 8 and week 12. At week 12, the ambulatory SBP/DBP during the 24-hour, daytime, and nighttime periods were significantly lowered without changes in the ambulatory HR during any of these periods (Table II). With respect to ambulatory short-term BP variability, nighttime diastolic short-term BP variability was slightly but significantly increased at week 12. The hourly ambulatory BP values at week 12 are shown in Figure 2 and indicate that the aliskiren-based therapy exerts a sustained reduction in the ambulatory SBP and DBP during 24 hours.

Effects of Aliskiren-Based Therapy on Endocrine and Vascular Function

Concerning the endocrine function parameters, aliskiren significantly decreased PRA, with a change from baseline to week 12 of −0.4±0.3 ng/mL/h (*P*<.001) (Figure 3), which is consistent with previous studies.[28] In addition, there was a trend toward a decrease in the plasma aldosterone concentration (PAC, a change from baseline to week 12 of −9.3±25.9 pg/mL, *P*=.1217) and plasma brain natriuretic peptide (BNP, a change from baseline to week 12 of −5.9±11.7 pg/mL, *P*=.1004). With respect to vascular function, aliskiren-based therapy for 12 weeks dramatically improved both cSBP (a change from baseline to week 12 of −14.3±9.8 mm Hg, *P*<.001) and baPWV

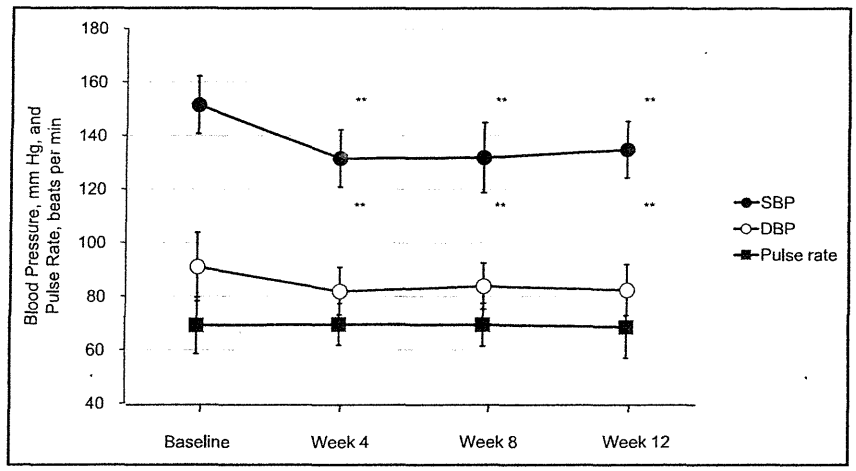

FIGURE 1. Mean clinic systolic blood pressure (SBP), clinic diastolic blood pressure (DBP), and pulse rate induced by aliskiren-based treatment during the study period (12 weeks) (n=17). $^{**}P<.001$ from baseline.

TABLE II. Effects of Aliskiren-Based Therapy on Ambulatory BP Profile

	Baseline	Week 12	*P* Value
24-Hour			
SBP (SD), mm Hg	144 (12)	133 (11)	<.001
DBP (SD), mm Hg	88 (8)	81 (9)	.002
HR (SD), beats per min	66 (9)	66 (8)	NS
SBP variability (SD), %	14.4 (4.4)	14.4 (4.3)	NS
DBP variability (SD), %	13.8 (4.2)	14.7 (4.2)	NS
HR variability (SD), %	15.6 (4.1)	16.7 (3.1)	NS
Daytime			
SBP (SD), mm Hg	150 (14)	140 (14)	<.001
DBP (SD), mm Hg	91 (8)	85 (10)	.003
HR (SD), beats per min	70 (11)	69 (8)	NS
SBP variability (SD), %	12.7 (3.7)	12.1 (4.2)	NS
DBP variability (SD), %	12.4 (3.7)	12.0 (4.1)	NS
HR variability (SD), %	15.6 (5.6)	15.8 (3.5)	NS
Nighttime			
SBP (SD), mm Hg	127 (11)	119 (11)	.011
DBP (SD), mm Hg	80 (7)	74 (8)	.015
HR (SD), beats per min	62 (11)	61 (10)	NS
SBP variability (SD), %	9.8 (2.7)	10.9 (4.8)	NS
DBP variability (SD), %	10.3 (2.6)	12.9 (4.1)	.019
HR variability (SD), %	9.5 (3.6)	9.4 (4.2)	NS

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; HR, heart rate; NS, not significant; SBP, systolic blood pressure; SD, standard deviation.

(a change from baseline to week 12 of −130.0±119.7 cm/s, $P<.001$), although AI was not affected (a change from baseline to week 12 of −1.9%±6.8%, $P=.286$) (Figure 4).

At the end of the study, 12 patients (71%) were taking aliskiren monotherapy (aliskiren 150 mg daily, n=10; 300 mg daily, n=2), and aliskiren monotherapy significantly improved both cSBP (a change from baseline to week 12 of −12.6±10.7 mm Hg, $P=.002$) and baPWV (a change from baseline to week 12 of −121.0±136.0 cm/s, $P=.010$), although the AI was not affected (a change from baseline to week 12 of −2.6±7.2%, $P=.250$) (Figure 5).

DISCUSSION

The main finding of this study is that aliskiren-based antihypertensive therapy effectively lowers ambulatory BP throughout the 24-hour period in untreated nondiabetic Japanese patients with mild to moderate essential hypertension. In addition, the aliskiren-based therapy resulted in a significant improvement in vascular function. These therapeutic effects achieved with aliskiren merit further consideration.

With respect to the BP-lowering effects of aliskiren, while several previous studies showed that the reduced clinic BP-lowering responses to aliskiren tended to be more common in hypertensive patients with a low PRA at baseline, as is the case with other RAS inhibitors such as ARBs and ACE inhibitors,[29,30] other studies reported that the antihypertensive effect of aliskiren was independent of the baseline PRA.[28] Japanese hypertensive patients are reported to have a lower overall PRA than western hypertensive patients due to the high dietary salt intake in Japanese patients.[31] In this study, although PRA was relatively low at baseline (0.7 ng/mL/h), the aliskiren-based therapy successfully caused substantial lowering of the clinic and ambulatory BP, not only for the 24-hour and daytime periods, but also for the nighttime period.

What might be the mechanism underlying the efficient BP-lowering effects of aliskiren in patients with relatively low renin profile? Previous animal studies demonstrated the critical role of the activation of the kidney AT1R in the pathogenesis of hypertension and its cardiovascular complications and suggested that the major mechanism of action of RAS inhibitors in hypertension was the attenuation of Ang II effects in

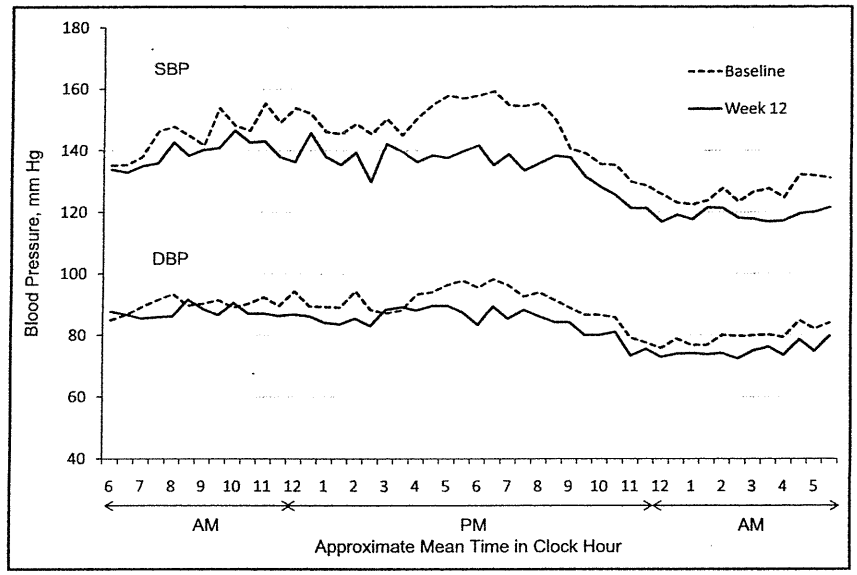

FIGURE 2. Hourly ambulatory systolic blood pressure (SBP) and diastolic blood pressure (DBP) at baseline and after 12 weeks of aliskiren-based therapy (week 12) (n=17).

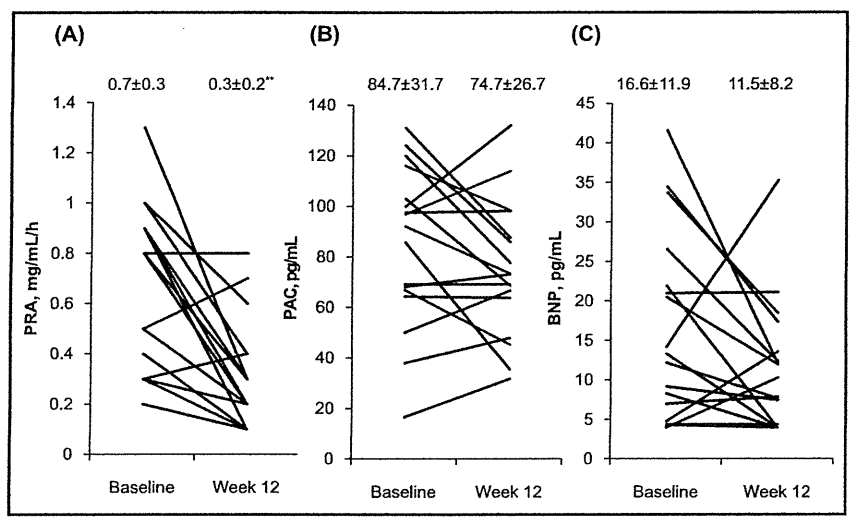

FIGURE 3. (A) Plasma renin activity (PRA), (B) plasma aldosterone concentration (PAC), and (C) brain natriuretic peptide (BNP) at baseline and after 12 weeks of aliskiren-based therapy (week 12) (n=17). $^{**}P<.001$ from baseline.

the kidney.[32,33] On the other hand, the (pro)renin receptor (PRR) is an emerging RAS component and is abundantly expressed in the kidney and cardiovascular system. PRR-bound renin and prorenin display enzymatic cleavage of angiotensinogen to Ang I, and the subsequently produced Ang II activates AT1R signaling at local tissue sites, including the kidney.[34,35] Aliskiren was shown to be extensively distributed in kidney tissues and to efficiently inhibit the production of Ang II derived from PRR-bound renin and prorenin at the local tissue sites.[36,37] Thus, it is possible that aliskiren exerts substantial BP-lowering effects through an effective blockade of kidney AT1R signaling, irrespective of the activity levels of circulating RAS, as reported recently.[38] A recent study showed that, in the presence of a high sodium and low potassium diet, which suppresses renin release, circulating angiotensinogen concentrations are more closely related to aldosterone and BP than in patients receiving a low-sodium and high-potassium diet.[39] Further studies are needed to examine the mechanistic basis of the aliskiren-mediated therapeutic effects on low renin hypertension.

This study also shows that aliskiren exerted a beneficial effect on central hemodynamics (cSBP) and arterial stiffness (baPWV). Dihydropyridine CCBs, either