Aldosterone Breakthrough During Angiotensin II Receptor Antagonist Therapy in Stroke-Prone Spontaneously Hypertensive Rats

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Abstract—Aldosterone breakthrough during ACE inhibitor therapy has been reported. This study investigates changes in plasma aldosterone concentration (PAC) and its mechanism and effects on target organ damage during long-term angiotensin II type 1 (AT1) receptor antagonist (AT1A) therapy in hypertensive rats. An AT1A (candesartan, 1 mg/kg per day PO) was administered in stroke-prone spontaneously hypertensive rats from 4 weeks of age for 34 weeks. PAC was significantly decreased during the first 4 weeks but showed aldosterone breakthrough after 8 weeks of AT1A administration. Plasma angiotensin II concentration was significantly elevated, whereas no change was seen in plasma ACTH or serum potassium. The mechanism(s) of aldosterone breakthrough were investigated by giving high doses of candesartan (3 mg/kg per day PO), dexamethasone (200 μg/kg per day IP), or the AT2 antagonist (PD123319, 10 mg/kg per day SC) during the last week of the 24-week AT1A treatment period. Dexamethasone and AT2 antagonist but not high-dose AT1A produced a significant decrease in PAC, with a larger decrease produced by the AT2 antagonist. To clarify the effects of the residual aldosterone, effects of coadministration of low-dose spironolactone (10 mg/kg per day SC), an aldosterone antagonist, on left ventricular hypertrophy and expression of brain natriuretic peptide mRNA were determined. Low-dose spironolactone further improved left ventricular hypertrophy and brain natriuretic peptide mRNA expression despite no additional depressor effect. These results suggest that aldosterone breakthrough occurs during long-term AT1A therapy, mainly by an AT2-dependent mechanism. Residual aldosterone may attenuate the cardioprotective effects of AT1A. (Hypertension. 2002;40:28-33.)

Key Words: aldosterone ■ angiotensin II ■ rats, inbred SHR ■ receptors, angiotensin II

Since the renin/angiotensin (RA) system plays an important role in the maintenance of blood pressure and development of cardiovascular diseases in hypertension, blockade of the RA system has been the mainstay of treatment. Pitt et al,1 however, demonstrated in the Randomized Aldactone Evaluation Study (RALES) that administration of ACE inhibitors reduced morbidity and mortality rates more in patients with severe heart failure if combined with the aldosterone antagonist spironolactone (SPRL). These findings indicate that physiologically active levels of aldosterone persist even during long-term ACE inhibition.

More recently, an angiotensin II type 1 (AT1) receptor-specific antagonist (AT1A) has been clinically in use as a blocker of the RA system.2 There are two major subtypes of angiotensin (Ang) II receptor: AT1 and angiotensin II type 2 (AT2). Although Ang II stimulates aldosterone synthesis and secretion through the AT1 receptor in the adrenal cortex,3 expression of the AT2 receptor has also been demonstrated in human adrenal tissue.4 In addition, we have demonstrated that the AT2 receptor may be involved in the stimulation of aldosterone secretion.5 Selective antagonism of the AT1 receptor is known to be associated with a substantial increase in plasma Ang II levels.6 It is therefore suggested that the increased plasma Ang II may stimulate aldosterone secretion through the AT2 receptor with the AT1 receptor blocked. Since evidence has accumulated to support a pathological role of aldosterone in the development of cardiovascular lesions,7–9 plasma aldosterone could be of potential importance in hypertensive target organ damage during AT1A administration.

The aim of this study was to elucidate changes in plasma aldosterone, its mechanism(s), and pathophysiological significance during long-term administration of AT1A. We administered AT1A with and without SPRL in stroke-prone spontaneously hypertensive rats (SHR-SP) and determined changes in plasma aldosterone concentration (PAC), cardiac...
weight, and cardiac mRNA expression of brain natriuretic peptide (BNP) as a marker of cardiac overload. Our data show that aldosterone breakthrough occurs during AT1A administration through an AT2-dependent mechanism and that combination with SPRL facilitates the cardioprotective effects of AT1A.

Methods

Animals

Male, 4-week-old SHR-SP (SHR-SP/Izm) and Wistar-Kyoto (WKY/Izm) rats obtained from the Disease Model Cooperative Research Association (Kyoto, Japan) were used. All experiments were conducted in accordance with the guidelines for the Care and Use of Animals approved by the Tokyo Women’s Medical University. Blood and cardiac tissues were collected under pentobarbital anesthesia (40 mg/kg IP).

Experimental Protocols

Effects of Long-Term AT1A Administration on PAC

Candesartan cilexetil (Takeda Chemical Industry) (1.0 mg/kg per day PO) (AT1A group) or vehicle (vehicle-treated SHR-SP group) was given to SHR-SP/Izm from 4 weeks of age for 34 weeks. Systolic blood pressure was measured by the tail-cuff method. At 4, 6, 8, 12, 16, 20, and 38 weeks of age (n=10 in each group), blood was collected for assay of plasma Ang II, PAC, ACTH, corticosterone, and potassium. Ang II, PAC, ACTH, and corticosterone levels were measured by radioimmunoassay and serum potassium by autoanalyzer.

Mechanism(s) of Changes in PAC During AT1A Administration

Involvement of the AT1 receptor, AT2 receptor, and the pituitary adrenal axis was studied. Saline as a vehicle was given to one group (vehicle-treated SHR-SP group, n=5) and candesartan (1.0 mg/kg per day PO) was given to 5 groups of 5 rats each from 4 weeks of age for 24 weeks. In the last week, a higher dose of candesartan (3.0 mg/kg per day PO) (high-dose AT1A group) or dexamethasone (200 μg/kg per day IP) (AT1A+DEX group) was given to 5 rats each for 4 weeks of age for 24 weeks. In the last week, a higher dose of candesartan (3.0 mg/kg per day PO) (high-dose AT1A group) or dexamethasone (200 μg/kg per day IP) plus PD123319 (10 mg/kg per day SC) (AT1A+DEX+PD group), or vehicle (AT1A group) was given in each of the original candesartan groups, respectively. PD123319 was given by osmotic minipump (2 ML1; Alza Corp). At 28 weeks of age, blood for assay of PAC was collected.

Effects of Combination of AT1A With SPRL on Cardiac Hypertrophy and Expression of BNP mRNA

To determine whether residual aldosterone plays an important role in target organ damage, the effect of blocking aldosterone action was investigated. Low-dose SPRL (10 mg/kg per day SC) was given with candesartan (1.0 mg/kg per day PO) (AT1A+SPRL group, n=5) to SHR-SP/Izm from 4 weeks of age for 24 weeks. Results were compared with those in the age-matched WKY/Izm (n=5) and SHR-SP/Izm groups in which vehicle (vehicle-treated SHR-SP group; n=5) or candesartan (1.0 mg/kg per day PO) (AT1A group; n=5) was given for the same period.

Systolic blood pressure, PAC, cardiac morphology, and left ventricular (LV) expression of BNP mRNA were determined. LV weight with septum was measured after removal of the heart. Transverse sections at the level of the maximum diameter of the heart were stained with hematoxylin and eosin, and the area of transverse section of each heart was determined on a personal computer with NIH image software. Expression of BNP mRNA was determined by reverse transcriptase–polymerase chain reaction according to a protocol described previously. Expression levels of BNP mRNA were compared semiquantitatively by correcting the expression level with that of GAPDH mRNA.

Results

Effects of Long-Term Administration of AT1A on PAC in SHR-SP

Systolic blood pressure was significantly decreased in the AT1A group compared with that in the untreated SHR-SP group during the entire period of AT1A administration. There was no significant difference in body weight between the groups (Figures 1a and 1b). Plasma Ang II concentration was significantly increased in the AT1A group during the first 4 weeks of administration. After 8 weeks of administration, it showed a rebound to levels similar to those observed before AT1A administration and to those of the vehicle-treated SHR-SP group (Figure 2a). No significant changes were seen in plasma ACTH, plasma corticosterone, or serum potassium concentrations between the AT1A and vehicle-treated SHR-SP groups over the entire period of AT1A administration (Figures 3a, 3b, and 3c).

Effects of Higher Dose of AT1A, Dexamethasone, and PD123319 on PAC in AT1A-Treated SHR-SP

As shown in Figure 4, there was no significant difference in PAC between the vehicle-treated SHR-SP group and the AT1A group after 24 weeks of administration. PAC in the high-dose AT1A group did not show any significant difference from that in the AT1A group. In contrast, PAC showed...
a significant decrease in both the AT1A+DEX group and AT1A+PD group compared with that in the AT1A group. The extent of the decrease was significantly larger for PD123319 than for DEX, and there was an additive decrease in PAC in the AT1A+DEX+PD group.

**Effects of SPRL on LV Hypertrophy and Expression of BNP mRNA in AT1A-Treated SHR-SP**

Systolic blood pressure was significantly higher in the vehicle-treated SHR-SP group than in the age-matched WKY/Izm group and was significantly decreased in the AT1A group. Systolic blood pressure in the AT1A+SPRL group did not show any significant difference from that in the AT1A group (Figure 5a). LV weight (Figure 5b) and cardiac transverse sectional area (Figure 5c) were significantly higher in the vehicle-treated SHR-SP group than in the age-matched WKY/Izm group and were significantly decreased in the AT1A group. In addition, both cardiac morphologic parameters in the AT1A+SPRL group showed a slight but significant reduction compared with that in the AT1A group.

LV expression of BNP mRNA in the vehicle-treated SHR-SP group was significantly higher than that in the age-matched WKY/Izm group and showed a significant decrease in the AT1A group. In addition, there was a further decrease of LV expression of BNP mRNA in the AT1A+SPRL group compared with that in the AT1A group (Figure 6).

**Discussion**

Since AT1A specifically blocks the AT1 receptor, the major receptor subtype involved in the secretion of aldosterone, it is expected that PAC would be decreased during administration of AT1A. In agreement with this, PAC showed a significant decrease after 2 weeks of AT1A administration in SHR-SP. During longer-term AT1A administration, however, PAC increased, reaching levels similar to those measured before AT1A administration and in the vehicle-treated SHR-SP group. Grossman et al. reported that PAC decreased after 1 year of losartan administration but returned to pretreatment levels when hydrochlorothiazide was combined to further lower blood pressure. Long-term effects of AT1A alone on PAC, however, have not been reported to date. The present results provide the first evidence for a possible aldosterone...
breakthrough with AT1A, a phenomenon similar to that described during long-term administration of the ACE inhibitor captopril.\textsuperscript{16,17}

Although the mechanism(s) responsible for aldosterone breakthrough during long-term AT1 antagonism remains to be elucidated, a possible contribution of physiological factors regulating aldosterone secretion should be taken into account. Renin secretion is under negative feedback regulation by the AT1 receptor on juxtaglomerular cells.\textsuperscript{18} It is therefore expected that plasma renin would be increased during long-term administration of AT1A, resulting in an increased generation of Ang II. In agreement with this, plasma Ang II levels were significantly elevated in the AT1A group. The first potential mechanism of aldosterone breakthrough is that the increased plasma Ang II stimulates aldosterone secretion through the AT1 receptor by overriding the receptor blockade by AT1A. This was investigated by increasing the dosage of AT1A to 3-fold higher than that in the AT1A group. The higher dose of AT1A, however, did not affect PAC, suggesting that increased plasma Ang II is not overriding the AT1 receptor blockade and acting through the AT1 receptor to stimulate aldosterone secretion.

It has been demonstrated that AT1 and AT2 receptors counteract each other in their biological actions on the cardiovascular system.\textsuperscript{19,20} However, it has also been shown that the AT2 receptor acts in concordance with the AT1 receptor in collagen synthesis in cultured vascular smooth muscle cells,\textsuperscript{21} in proliferative effects in mesenteric artery,\textsuperscript{22} and in smooth muscle cell growth and extracellular matrix expression in the aorta.\textsuperscript{23} In adrenal tissues, both the AT2 receptor and the AT1 receptor are thought to stimulate aldosterone secretion.\textsuperscript{5} Although AT1 is the predominant receptor subtype, accounting for 80% of the Ang II receptors in rat and bovine adrenal glands,\textsuperscript{24} a contribution of the AT2 receptor could be potentiated if the AT1 receptor is chronically blocked. Therefore, the second potential mechanism of aldosterone breakthrough in our study was that the increased Ang II stimulates aldosterone secretion through the AT2 receptor. This was investigated by concomitant administration of the AT2 receptor antagonist PD123319 with AT1A in SHR-SP rats. The data clearly show that the AT2 antagonist significantly decreases PAC in the AT1A group. Since the dose of PD123319 has been shown to be effective in blocking the AT2 receptor without affecting the AT1 receptor,\textsuperscript{11} the current results indicate that the AT2-mediated mechanism is involved in aldosterone breakthrough during long-term administration of AT1A.

Other major factors regulating aldosterone secretion are plasma ACTH and serum potassium. Plasma ACTH level did not show a significant change during the entire period of AT1A administration. Although PAC was partially suppressed by dexamethasone in the AT1A group, it was
suppressed to greater extent by dexamethasone in the untreated SHR-SP group (data not shown). These results suggest that an ACTH-dependent secretion is not a major mechanism of aldosterone breakthrough in the AT1A group. In addition, the ACTH-mediated mechanism and AT2-mediated mechanism are independent from each other since the effects on PAC were additive when both PD123319 and dexamethasone were concomitantly administered.

Serum potassium concentration did not show any significant change during the entire period of AT1A administration. These results agree with the previous findings by Bakris et al., in which serum potassium level did not show any significant change during administration of AT1A. Serum potassium is therefore not likely to be involved in aldosterone breakthrough.

Recent studies have disclosed effects of aldosterone on nonepithelial tissues such as the heart. Aldosterone facilitates cardiac fibrosis and cardiac hypertrophy without affecting blood pressure. We have reported that prevalence and severity of LV hypertrophy is larger in patients with primary aldosteronism than in Cushing’s syndrome and pheochromocytoma with similar blood pressure levels. These studies suggest that aldosterone may play an important role in the development of target organ damage in hypertension. To elucidate the pathophysiological significance of aldosterone breakthrough during long-term administration of AT1A, effects of coadministration of low-dose SPRL with AT1A were investigated. Blood pressure, LV weight, area of cardiac transverse section, and expression of BNP mRNA all showed a remarkable decrease after long-term administration of AT1A, supporting the cardioprotective effects of AT1A. In addition, coadministration of low-dose SPRL with AT1A elicited a further decrease in LV weight, area of cardiac transverse section, and expression of BNP mRNA. Since SPRL at the dose used in this study did not affect blood pressure, its effects are likely to be direct on the heart. The present results suggest that aldosterone breakthrough during long-term administration of AT1A may have harmful effects on target organs by attenuating the cardioprotective effects of AT1A.

In conclusion, this study demonstrates for the first time that aldosterone breakthrough occurs during long-term administration of AT1A mainly by an AT2-dependent mechanism. Residual aldosterone modifies the hypertensive target organ damage and the cardioprotective effects of AT1A.

Perspectives
It was clearly demonstrated that aldosterone breakthrough occurs during long-term AT1A therapy mainly by an AT2-dependent mechanism. In addition, the residual aldosterone was shown to attenuate the cardioprotective effects of AT1A. The results may provide experimental rationale for a combination therapy of AT1A and the aldosterone antagonist SPRL in the treatment of hypertension and related cardiovascular diseases. Whether aldosterone breakthrough occurs with AT1A administration in human hypertension, however, awaits further investigation.

Acknowledgments
We wish to thank Takeda Chemical Industries for providing candesartan cilexetil. This work was supported in part by research grants from the Japanese Ministry of Education, Science, and Culture, the Toshiko Yamakawa Research Award of Tokyo Women’s Medical University for Akiyo Tanabe, and the Young Investigator Award from the Tokyo Hypertension Conference (Tokyo, Japan) for Akiyo Tanabe.

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Hypertension. 2002;40:28-33; originally published online June 3, 2002;
doi: 10.1161/01.HYP.0000022606.52221.2F
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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