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Clinical and Pharmacotherapeutic Relevance of the Double-Chain Domain of the Angiotensin II Type 1 Receptor Blocker Olmesartan

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Abstract

We previously reported that the angiotensin II type 1 (AT₁) receptor blocker (ARB) olmesartan has two important interactions to evoke inverse agonism (IA). We refer to these interactions as the “double-chain domain (DCD).” Since the clinical pharmacotherapeutic relevance of olmesartan is still unclear, we examined these effects in rats and humans. We analyzed the effects at an advanced stage of renal insufficiency in Dahl salt-sensitive hypertensive rats (Study 1). Rats were fed a high-salt diet from age 9 weeks and arbitrarily assigned to three treatment regimens at age 16 to 21 weeks: olmesartan (2 mg/kg/day) with DCD, a compound related to olmesartan without DCD (6 mg/kg/day, R-239470) or placebo. We also compared the depressor effects of olmesartan to those of other ARBs in patients with essential hypertension (Study 2). Thirty essential hypertensive outpatients who had been receiving ARBs other than olmesartan were recruited for this study. Our protocol was approved by the hospital ethics committee and informed consent was obtained from all patients 12 weeks prior to switching from ARBs other than olmesartan to olmesartan. In Study 1, olmesartan induced a more prominent suppression of the ratio of urinary protein excretion to creatinine at age 21 weeks without lowering blood pressure among the three groups. In Study 2, the depressor effect of olmesartan was significantly stronger than those of other ARBs, which do not contain the DCD. These additive effects by olmesartan may be due to DCD.

Keywords

double-chain domain; angiotensin II type 1 receptor blocker; olmesartan; renal dysfunction; Dahl salt-sensitive rat

INTRODUCTION

Currently used angiotensin II (Ang II) receptor antagonists (ARBs) are highly selective for the Ang II type 1 (AT₁) receptor, which is a member of the G protein-coupled receptor (GPCR) superfamily, and block the diverse effects of Ang II. In addition to their blood pressure (BP)-lowering effects in hypertensive patients, ARBs have been shown to promote

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the regression of left ventricular hypertrophy and decrease cardiovascular morbidity and mortality in patients with heart failure or hypertensive diabetic nephropathy with proteinuria (1). Many ARBs are available for clinical use. Since not all ARBs have the same effects, some benefits conferred by ARBs may not be class effects (2).

Inverse agonists, such as the opioid receptor ligand ICI174864 (3), block agonist-independent signal transduction by GPCRs. The AT₁ receptor shows slight constitutive activity (2). A recent study demonstrated that the wild-type (WT) AT₁ receptor is activated by mechanical stretching of cultured rat myocytes without the involvement of Ang II, and this was suppressed by an inverse agonist (4). We previously reported that EXP3174, an active metabolite of losartan, but not losartan itself, can act as an inverse agonist in the inositol phosphate (IP) production assay using a constitutively active AT₁-N111G mutant (5). Although most ARBs have biphenyltetrazole and imidazole groups as common chemical moieties, EXP3174 has a carboxyl group in the imidazole ring. This difference in the chemical structure may be important for tightly binding to the receptor and for producing inverse agonistic activity. Olmesartan, a potent ARB, has a hydroxyl group in the imidazole ring in addition to the carboxyl group and exhibits strong inverse agonism. We previously reported that the interactions between the AT₁ receptor and the hydroxyl and carboxyl groups of olmesartan play an important role in inverse agonism (6). We refer to these important interactions as the “double-chain domain (DCD) (Figure 1).” We suggest that the BP-depressor effect of olmesartan is stronger than those of other ARBs since the hydroxyl and carboxyl groups may strongly bind to an AT₁ receptor and induce strong inverse agonism.

Chronic kidney disease (CKD) has received considerable attention in the management of hypertension (7) and is an independent risk factor for cardiovascular disease in patients with hypertension (8). Proteinuria is a clinical parameter that is used to diagnose renal damage, especially glomerular hypertension, and is a risk factor for and predictor of cardiovascular events (9). Ohishi et al. (10) reported that the maximum permitted dose of valsartan is more effective than the maximum allowable dose of candesartan for reducing BP and proteinuria. These notions represent an exciting new area in ARB-based therapy, which holds the promise of reducing the incidence of cardiovascular and renal disease.

Therefore, we hypothesized that the DCD of olmesartan contributed to the prevention of renal dysfunction and the reduction in BP. In the present study, we analyzed the renoprotective effects of olmesartan at an advanced stage of renal insufficiency in Dahl salt-sensitive (Dahl-S) rats (Study 1). We also compared the depressor effects of olmesartan to those of other ARBs without DCD in patients with essential hypertension (Study 2).

METHODS

Study Design-Study 1

Materials—Olmesartan and a related compound, R-239470, were synthesized in the Research Laboratories of Sankyo Co., Ltd. The chemical structure of olmesartan is shown in Figure 1.

Animal Preparation and Protocol—After weaning, male Dahl-S rats (Kyudo Co., LTD., Saga, Japan) were fed a 0.3% NaCl diet until the age of 8 weeks. Thereafter, they were fed a diet containing 4% NaCl and arbitrarily assigned to three treatment regimens at age 16 to 21 weeks (Figure 2A): olmesartan (2 mg/kg/day, n = 9) with DCD, a compound related to olmesartan without DCD (6 mg/kg/day, R-239470, n = 8) or placebo (n = 6). In the present study, since we investigated the renoprotective effect using ARB with or without DCD independent of a BP-lowering effect and we determined a nondepressor dose of each

ARB. The ARBs were administered orally using a gastric tube (Muromachi Kikai Co., Ltd., Tokyo, Japan) everyday during age 16 to 21 weeks. Blood samples were obtained by exsanguination, and tissues were harvested at the end of the study. Tissues were immediately fixed overnight in 4% paraformaldehyde in 1 mol/L phosphate buffer. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996), and the use of animal tissues was approved by the Fukuoka University Internal Review Committee.

BP and Cardiac Function Measurements—We performed systolic BP (SBP) and heart rate (HR) measurements by the tail-cuff method with a BP monitor (MK1100, Muromachi Kikai Co., Ltd., Tokyo, Japan) in conscious rats at 8, 15, and 21 weeks. We also performed transthoracic echocardiography (NEMIO SSA-550A, Toshiba, Tokyo, Japan) after BP measurements at 8, 15, and 21 weeks. Dahl-S rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg) before we performed echocardiography. Short- and long-axis 2D and M-mode views at the level of papillary muscle were analyzed in real-time and recorded on a magneto-optical disk for off-line analysis by a sonographer who was blinded to the study treatments. Left ventricular (LV) internal dimensions were measured and ejection fraction (EF) was calculated from the composite LV internal diastolic (LVDd) and LV internal systolic (LVDs) dimensions as $EF = [(LVDd - LVDs) / LVDd] \times 100$ from M-mode long-axis views as previously described (11).

Urine Collection—Twenty-four-hour urine samples were collected from rats in metabolic cages at 8, 15, and 21 weeks for the measurement of urinary volume (UV). U-electrolyte (U_{Na} and U_K), U-urea nitrogen (U_{UN}), U-protein ($U_{protein}$), and U-creatinine (U_{Cr}) were analyzed by standard methods.

Study 2

Study Population and Protocol—Thirty essential hypertensive outpatients at Fukuoka University Hospital, who had been receiving ARBs other than olmesartan, were recruited for this study. We applied a changeover of ARBs, switching from valsartan 40 and 80 mg/day, telmisartan 20 and 40 mg/day, candesartan 4 and 8 mg/day, and losartan 25 and 50 mg/day to olmesartan 10 and 20 mg/day, respectively. At the onset of the study, the patients had not achieved the optimal BP [i.e., SBP < 140 mmHg or diastolic BP (DBP) < 90 mmHg]. Use of other anti-hypertensive drugs was permitted, and the doses of those drugs were not changed during the study. Patients who had suffered a stroke or cardiovascular event within 6 months, had severe renal dysfunction, severe liver dysfunction, were pregnant or had olmesartan hypersensitivity were excluded from the study. Our protocol was approved by the hospital ethics committee and informed consent was obtained from all patients 12 weeks prior to switching from ARBs other than olmesartan to olmesartan.

BP and Biochemical Parameter Measurements—Blood pressure was measured at the doctor's office in the right arm after a 5-minute resting period, and an average of three consecutive BP measurements was used for analysis. A total of seven BP and pulse rate (PR) measurements were obtained for each patient during hospital visits at 12 weeks, 8 weeks, and 4 weeks before changeover, at changeover, and at 4 weeks, 8 weeks, and 12 weeks after changeover. We also examined biochemical parameters, including complete blood cell count, liver function, renal function, lipid profile, glucose, and electrolytes at changeover (0 week) and at 12 weeks after changeover. Blood samples were collected following an overnight fast for the measurement of lipid and glucose profiles by standard techniques.

Data Analysis—All data are given as the mean $\pm$ standard error. In Study 1, the significance of differences between mean values was evaluated by an unpaired *t*-test or one-way analysis of variance (ANOVA) followed by Fisher's protected-least-significant-difference test, as appropriate. In Study 2, differences in results between treatment with olmesartan and other ARBs were assessed using unpaired *t*- or paired *t*-tests. A value of $p < 0.05$ was considered significant.

RESULTS

Study 1

Changes in Body Weight and Heart Weight—There were no significant differences in body weight (BW), heart weight (HW), or the ratio of HW to BW (olmesartan, 0.51 ± 0.03 ; R-239470, 0.47 ± 0.02 ; placebo, 0.51 ± 0.05) between at baseline and at end of the study among the three groups.

Changes in BP and Cardiac Function—There was no significant difference in BP at 8 weeks, 15 weeks, and 21 weeks (Figure 2B). There was no significant difference in cardiac function among the three groups before the experiments (data not shown). The data on changes in cardiac function at pre- and post-treatment are shown in Table 1. Interventricular septum thickness (IVST) in the olmesartan and R-239470 groups at post-treatment were significantly lower than that in the placebo group. There was a significant suppression of IVST in the olmesartan and R-239470 groups (2.2 ± 0.1 mm and 2.2 ± 0.1 mm, respectively) compared to the placebo group (2.6 ± 0.1 mm). There were no significant differences in LVDd, LVDs, posterior wall thickness (PWT), or EF. These results indicate that ARB therapy may prevent left ventricular hypertrophy without a reduction in BP at post-treatment.

Changes in Serum and Urine Biochemical Parameters—There were no significant differences in serum biochemical parameters among the three groups (Table 2). Although there were no differences in UV, $U_{Na}V$, U_KV , and $U_{UN}V$ between the placebo and olmesartan groups, the R-239470 group was significantly increased in these parameters compared to the olmesartan group. The ratios of $U_{protein}$ to U_{Cr} at 8 weeks, 15 weeks, and 21 weeks are shown in Figure 2C. There was a significant suppression of the ratio of $U_{protein}$ to U_{Cr} in the olmesartan group compared to the placebo and R-239470 groups at post-treatment. This result indicates that olmesartan suppresses the excretion of $U_{protein}$ compared to R239470.

Study 2

Patient Characteristics—A total of 30 patients completed the changeover study, and 18 were female. The subjects had a mean age of 64 ± 2 years and a body mass index (BMI) of 26 ± 1 kg/m². The patients had taken valsartan [average dose 69 ± 5 mg/day, 40 mg/day ($n = 4$) and 80 mg/day ($n = 11$)], telmisartan [34 ± 3 mg/day, 20 mg/day ($n = 3$) and 40 mg/day ($n = 6$)], candesartan [6 ± 1 mg/day, 4 mg/day ($n = 2$) and 8 mg/day ($n = 3$)], and losartan (25 mg/day, $n = 1$) for 15 ± 3 months before the study. A total of 15 subjects had complications, i.e., hyperlipidemia ($n = 10$), diabetes mellitus ($n = 2$), or both ($n = 3$). One patient developed hyperkalemia as a side effect of olmesartan and one patient complained of palpitations. Hyperkalemia and palpitations were improved immediately after olmesartan cancellation. In addition, a patient changed from olmesartan at 10 mg/day to 20 mg/day during the study. These three patients were excluded from further analysis.

BP and PR Before and After Changeover—The subjects received 18 ± 1 mg of olmesartan after changeover. Pulse rate did not differ between before and after changeover,

while SBP and DBP were significantly reduced after changeover. In fact, BP was $150 \pm 3/86 \pm 2$ mmHg at changeover and $142 \pm 2/81 \pm 2$ mmHg at 4 weeks and $139 \pm 2/80 \pm 1$ mmHg at 12 weeks after changeover (Figure 3). The BP-depressor effect of olmesartan was stronger than those of valsartan, telmisartan, and candesartan ($-12^*/-5^*$ mmHg, $-7/-6^*$ mmHg, and $-11/-8$ mmHg, respectively; $*p < 0.05$).

Biochemical Parameters—There were no significant differences in biochemical parameters including complete blood cell count, liver function (aspartate aminotransferase, 18 ± 3 IU/l and 17 ± 1 IU/l; alanine aminotransferase, 15 ± 3 IU/l and 18 ± 4 IU/l before and at 3 months after changeover, respectively), renal function (Cr, 0.8 ± 0.04 mg/dl and 0.8 ± 0.05 mg/dl, respectively), lipid profile, glucose, and electrolytes (potassium, 4.2 ± 0.1 mEq/l and 4.4 ± 0.1 mEq/l, respectively).

DISCUSSION

In Study 1, there was no difference in the reduction in BP among the three groups throughout the study period due to the administration of low doses of ARBs in rats. Compared to R-239470, olmesartan induced a more prominent suppression of UV and the ratio of U_{protein} to U_{Cr} at age 21 weeks. Finally, in Study 2, the depressor effect of olmesartan was significantly stronger than those of other ARBs, which do not contain DCD in hypertensive patients. This beneficial effect induced by DCD may be very important because a major therapeutic goal is to design strategies for preventing cardiovascular and renal diseases.

We demonstrated that olmesartan induced a more prominent suppression of the ratio of U_{protein} to U_{Cr} compared to R-239470. Several large clinical trials have evaluated the effects of ARBs on renoprotective effects. The Microalbuminuria Reduction with Valsartan (MARVAL) study suggested that valsartan lowered urine albumin excretion more effectively than amlodipine in patients with type 2 diabetes (12). Brenner et al. (13) reported that losartan conferred significant renal protective benefits in patients with type 2 diabetes and nephropathy. In a double-blind, randomized, controlled, and prospective trial, an ultra-high dose of the ARB candesartan (64 mg/day) produced a greater anti-proteinuric effect than doses of 16 or 32 mg/day. This effect did not seem to be related to BP (14). In our results, olmesartan induced more suppression of the ratio of U_{protein} to U_{Cr} than R-239470 did. Since olmesartan contains DCD, olmesartan may have stronger renoprotective effects than other ARBs without BP-reduction. Although we did not perform a histological analysis in the kidney, Ohtake et al. (15) indicated that the mesangial matrix expansion ratio and the interstitial fibrosis ratio were significantly reduced by treatment with ARBs. Varagic et al. (16) reported that ARBs given concomitantly with an excess of dietary salt ameliorated salt-related structural and functional cardiac and renal abnormalities in spontaneously hypertensive rats without reducing arterial pressure. In addition, Katayama et al. (17) demonstrated that ARBs significantly decreased urinary albumin excretion without a reduction in BP, indicating that ARB has a renoprotective action independent of any change in BP. These data suggest that an AT_1 receptor plays an important role as a pressure-independent factor in hypertensive organ damage, and such damage might be prevented more by ARB with DCD.

In the present study, olmesartan had renoprotective effects without BP-reduction compared to other ARB without DCD in rats. There were no significant differences in serum biochemical parameters among the three groups. Since serum levels of Cr and UN were increased in Dahl-S rats compared to those in Wistar rats (UN: 21.41 ± 2.75 mg/dl, Cr: 0.21 ± 0.05 mg/dl, data from Kyudo Co., LTD., Saga, Japan), we thought that Dahl-S rats in this study were renal insufficient models. Although we did not analyze the renoprotective

mechanism, Fliser et al. (18) reported that a biochemical marker of oxidative stress and (intra)renal vascular resistance decreased significantly upon treatment with olmesartan and renal perfusion increased despite significant BP reduction. In addition, Abadir et al. (19) indicated that the production of nitric oxide (NO) is mediated mainly via the AT₂-B2 receptor cascade, and blockade of the AT₁ receptor enhances the production of NO via the AT₂ receptor. Nitric oxide can be produced by two alternative pathways: directly through the AT₁ receptor or indirectly from AT₁ receptor stimulation of bradykinin via the B2 receptor. These effects of olmesartan may contribute to its beneficial long-term renal effects.

The olmesartan and R-239470 groups showed significantly suppressed hypertrophy in IVST compared to the placebo group. In acute and chronic hypertrophic conditions, there is evidence of impaired angiotensinergic stimulation (20,21). Ang II can promote the growth of human cardiac muscle cells *in vivo* and is a mediator of stretch-induced myocyte hypertrophy. Ang II also promotes fibroblast proliferation, collagen deposition, and a decrease in heart compliance. In fact, blockade of the renin-angiotensin system is a highly effective therapy for hypertension and heart failure (22). Since both the olmesartan and R-239470 groups showed significantly suppressed hypertrophy in IVST, but not PWT in our study, DCD may not be related to the suppression of myocardial hypertrophy. Although we do not know the mechanism by which olmesartan and R239470 differentially affect cardiac hypertrophy and renal function, stronger inverse agonism and more insurmountability induced by DCD of olmesartan (6) might induce the differential effects.

In Study 2, we found that the depressor effect of olmesartan is significantly stronger than those of other ARBs, which do not contain DCD. Smith et al. (23) reported that ambulatory BP (ABP) was significantly greater for patients receiving olmesartan than for those receiving losartan or valsartan. Compared to recipients of losartan or valsartan, a significantly higher percentage of patients treated with olmesartan achieved the 24-hour ABP goal of < 130/85 mm Hg. Brunner and Arakawa (24) reported that greater proportions of olmesartan-treated patients (20 mg/day) achieved goals of < 135/80 mm Hg over 24 h compared to those treated with candesartan 8 mg/day. The superior BP control of olmesartan was also reflected in the larger proportions of olmesartan-treated patients who achieved these goals during the early morning surge period. Our present study clearly showed that the BP-depressor effect of olmesartan, which contained DCD, was significantly stronger than those of other ARBs in the same patients. Olmesartan with DCD strongly binds to AT₁ receptors compared to ARBs without DCD (6). Since the AT₁ receptor has constitutive activity (2), DCD also suppresses its activity and contributes to a strong anti-hypertensive effect. Therefore, it may be important for ARBs to contain DCD, since our study indicated that olmesartan significantly suppressed BP compared to other ARBs.

There are several limitations in the present study. In Study 1, one possible limitation is that the advanced stage of renal insufficiency in Dahl-S rat does not accurately reflect the conditions in humans. A second limitation is that echocardiography was performed using anesthetized rats. Although this measurement may not provide the same quantitative data as in a conscious rat, the important issue is that we found significant changes in wall thickness in each rat. In addition, we do not know the reason why only IVST was reduced by ARBs treatment. A third limitation is that we showed the ratio of U_{protein} to U_{Cr} , but not 24-h U_{protein} . However, the ratio of U_{protein} to U_{Cr} is commonly used to evaluate U_{protein} excretion. We believe that our study should contribute to understanding the beneficial effects of olmesartan in an advanced stage of renal insufficiency in rats. The most important possible limitation of Study 2 was the protocol, since only one changeover was performed to compare anti-hypertensive effects of ARBs. A crossover or randomized study is necessary to properly compare olmesartan and other ARBs. In addition, olmesartan was not compared with R-239470 because it was not clinically used in Japan. Therefore, we used alternative

ARBs that were permitted in Japan. Although the permitted maximum doses of ARBs are low compared to the doses used in major clinical trials in the world and the higher doses of ARBs may have clinical benefits in hypertensive patients, we would like to compare the BP-lowering effects in the range of permitted doses of ARBs in Japan. Finally, we should include another ARB without DCD in Study 1 and analyze U_{protein} in Study 2 for understanding the beneficial effect by ARB with DCD in rat and humans.

In conclusion, the additive effects with DCD to ARB likely occur through the more prominent suppression of renal dysfunction in rats and a reduction in BP in hypertensive patients. A better understanding of the molecular mechanisms and pharmacotherapeutic relevance of this DCD could be useful for the development of new G protein-coupled receptor antagonists with DCD.

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FIGURE 1.

Molecular modeling of interaction between olmesartan and the AT₁ receptor. The chemical structure of olmesartan contains a “double-chain domain (DCD).” The hydrogen bonds and electrostatic interactions proposed by the molecular modeling study are indicated by dotted lines.

FIGURE 2.

(A) Experimental protocol of Study 1. (B) Changes in BP in the Olmesartan, R-239470 and placebo groups. Abbreviation: NS not significant. (C) Changes in the ratio of urinary protein and urinary creatinine ($U_{\text{protein}}/U_{\text{Cr}}$) in the Olmesartan, R-239470 and placebo groups. $p < 0.05$ vs. R-239470 and placebo groups.

FIGURE 3. Changes in systolic and diastolic blood pressure (SBP, DBP) and pulse rate (PR). *p < 0.05 vs. 0 week. Abbreviation: NS not significant.

TABLE 1

Changes in cardiac function

	Pre			Post		
	Olmesartan	R-239470	Placebo	Olmesartan	R-239470	Placebo
LVDd, mm	6.4 ± 0.2	6.3 ± 0.3	7.1 ± 0.3	7.0 ± 0.3	6.5 ± 0.3	7.5 ± 0.7
LVDs, mm	3.6 ± 0.2	3.5 ± 0.2	3.9 ± 0.4	3.7 ± 0.3	3.4 ± 0.2	4.4 ± 0.6
IVST, mm	2.6 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	2.2 ± 0.1*	2.2 ± 0.1*	2.6 ± 0.1
PWT, mm	2.6 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
EF, %	80 ± 2	81 ± 1	81 ± 4	84 ± 2	83 ± 2	78 ± 3

Abbreviations: LVDd-left ventricular internal diastolic diameter; LVDs-left ventricular internal systolic diameter; IVST-interventricular septum thickness; PWT-posterior wall thickness; EF-ejection fraction.

* p < 0.05 vs. placebo.

TABLE 2

Biochemical parameters in serum and urine after treatment

	Olmesartan	R-239470	Placebo
Serum			
Cr, mg/dl	0.50 ± 0.05	0.47 ± 0.06	0.48 ± 0.06
UN, mg/dl	28 ± 4.1	24 ± 1.4	36 ± 8.1
Na, mEq/l	150 ± 2.5	146 ± 2.4	152 ± 2.8
K, mEq/l	9.1 ± 0.5	9.9 ± 1.0	7.7 ± 0.4
Cl, mEq/l	93 ± 0.9	90 ± 1.9	95 ± 1.7
Urine			
UV, ml/day	58 ± 8.1 **	89 ± 12 *	56 ± 11
U _{Na} V, mEq/d	11 ± 2.1 **	20 ± 0.8 *	11 ± 2.4
U _K V, mEq/d	4.0 ± 0.5 **	5.9 ± 0.2 *	3.8 ± 0.6
U _{UN} V, mg/d	374 ± 35 **	486 ± 19 *	282 ± 52

* p < 0.05 vs. placebo

** p < 0.05 vs. R-239470.