Original Article

Effects of Benidipine and Candesartan on Kidney and Vascular Function in Hypertensive Dahl Rats

Kozo YAO, Hitoshi SATO *, Rie SONODA, Yasuhiro INA, Kazuo SUZUKI *, and Tetsuji OHNO

We examined the effect of the dihydropyridine calcium channel blocker (CCB) benidipine, the angiotensin II type 1 receptor blocker (ARB) candesartan, and the combination of these drugs on blood pressure and kidney and vascular function in rats with salt-induced hypertension. Dahl salt-sensitive (DS) rats were fed with a high-salt (8% NaCl) diet from 7 weeks of age. Benidipine (1, 3 mg/kg), candesartan (1, 3 mg/kg), benidipine (3 mg/kg) combined with candesartan (3 mg/kg), or vehicle was administered orally after the start of the feeding. Relaxant responses to acetylcholine (an endothelium-dependent vasodilator) and sodium nitroprusside (an endothelium-independent vasodilator) were measured to examine the vascular function. DS rats fed the high-salt diet showed an increase in systolic blood pressure (SBP), which was accompanied by glomerular sclerosis and an increase in urinary albumin excretion. Relaxant responses to acetylcholine and sodium nitroprusside were impaired in superior mesenteric arterial rings from the hypertensive DS rats. SBP was significantly lower in all of the drug-treated groups than in the vehicle-treated group. The antihypertensive effect of benidipine at 3 mg/kg was more potent than that of candesartan at 3 mg/kg. The albuminuria was significantly decreased in the benidipine and benidipine plus candesartan groups, but not in the candesartan group. The level of SBP in the benidipine plus candesartan group was lower than that by either drug alone. In addition, benidipine alone and benidipine plus candesartan inhibited the glomerular sclerosis and the impairment of relaxant responses in the arteries. These results demonstrate that benidipine is more effective than candesartan in lowering blood pressure and preventing the impairment of kidney and vascular function in salt-sensitive hypertensive rats. In addition, the results suggest that combination therapy with benidipine and an ARB decreases blood pressure more effectively than either drug alone and may be useful for the treatment of hypertension. (Hypertens Res 2003; 26: 569–576)

Key Words: calcium channel blocker, angiotensin II type 1 receptor blocker, hypertension, combination therapy, endothelium

Introduction

The ultimate goal of antihypertensive therapy is to prevent the hypertension-related end-organ damage leading to dysfunction in organ systems, especially in the cardiovascular system. A positive correlation has been established between blood pressure and the risk of cardiovascular events (1). In order to achieve the targeted reduction in blood pressure, most hypertensive patients require the coadministration of two or more drugs from different therapeutic classes (2).

Recently, the use of angiotensin II type 1 receptor blockers (ARBs) has been increasing as a first-line treatment for hypertension. Calcium channel blockers (CCBs) are also widely used as antihypertensive drugs without serious adverse effects (3). The effectiveness of ARBs depends on the activity of the renin-angiotensin system (RAS). In fact, in the absence of RAS activation, ARBs have been shown to exert only minor effects on blood pressure in a salt-sensitive rat model of hypertension (4). In contrast, CCBs lower blood
pressure in a RAS-independent manner. In Dahl salt-sensitive (DS) hypertensive rats, a model of RAS-independent hypertension, the dihydropyridine CCBs showed potent hypotensive effects (5, 6). Furthermore, the dihydropyridine CCB benidipine plus ARB candesartan improved the renal function induced in DS hypertensive rats more effectively than either drug alone (7). However, the details of the combined effects of benidipine and an ARB in salt-sensitive hypertension remain unclear. In this study, we examined the effect of benidipine, candesartan, and the combination of these drugs on blood pressure, pathological changes in the kidney, and renal and vascular function in DS hypertensive rats.

Methods

Animals

Male DS rats at 7 weeks of age (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu, Japan) were used. The rats were kept at 19–25°C under a 12-h light–dark cycle, and they had free access to tap water and commercial chow (FR-2; Funabashi Farms, Chiba, Japan). All animals received humane care in compliance with the “Guiding Principles for the Care and Use of Laboratory Animals” formulated by the Japanese Pharmacological Society, and the protocol was approved by the Bioethical Committee of the Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.

Drugs

Benidipine hydrochloride (benidipine; Kyowa Hakko Kogyo, Tokyo, Japan) and candesartan cilexetil (candesartan) synthesized at Kyowa Hakko Kogyo were suspended in 0.5% w/v methylcellulose 400cP (Wako Pure Chemical, Tokyo, Japan) and then precontracted with L-phenylephrine (PE, 3 μmol/l). Relaxant responses to acetylcholine (ACh, 1 to 1,000 nmol/l) or sodium nitroprusside (SNP, 1 to 1,000 nmol/l) were obtained and expressed as a percentage of the contraction to PE. In another series of experiments, the effects of ACh were assessed after preincubation of some vascular rings with the nitric oxide synthase (NOS) inhibitor Nω-nitro-l-arginine methyl ester (L-NAME, 100 μmol/l) for 60 min.

Experimental Procedures

A total of 56 DS rats aged 7 weeks were divided into 7 groups (8 rats/group). Group 1 (normal) rats were fed a normal salt diet (FR-2 containing 0.19% NaCl; Funabashi Farms) and groups 2–7 rats were fed a high salt diet (FR-2 containing 8% NaCl; Funabashi Farms). The rats fed the high salt diet were orally given vehicle (control), 1 mg/kg/day of benidipine (B1), 3 mg/kg/day of benidipine (B3), 1 mg/kg/day of candesartan (C1), 3 mg/kg/day of candesartan (C3), or 3 mg/kg/day of benidipine plus 3 mg/kg/day of candesartan (B3 + C3) for 6 weeks. At 5 days before and 3 and 6 weeks after the start of the repeated dosing, urine was collected for 24 h and systolic blood pressure (SBP) was measured by the tail-cuff method (Ps-600; Riken Development, Tokyo, Japan) 3 to 5 h after drug administration. The concentration of albumin in the collected urine was measured with an autoanalyzer (AU600; Olympus, Tokyo, Japan). Urinary albumin excretion (mg/kg/day, U-Alb) was calculated from the urinary albumin concentration and urine volume. After 6 weeks of the drug treatment, the rats were weighed and anesthetized with ether, and then the hearts and left kidneys were removed and weighed. In addition, the superior mesenteric arteries were excised. The kidneys and the vessels in the normal, control, B3, C3, and B3 + C3 groups were prepared for histopathological examination and for vascular reactivity experiments, respectively.

Vascular Reactivity

The excised vessels were immediately placed in gassed (95% O2 + 5% CO2) and warmed (37°C) Krebs-Henseleit solution of the following composition (mmol/l): NaCl 119, KCl 4.7, MgSO4·7H2O 1.2, CaCl2·2H2O 1.8, KH2PO4 1.2, NaHCO3 24.9, glucose 11.1. Under a microscope, the mesenteric arteries were cleaned of adherent tissue and cut into rings of 1–2 mm length.

The vascular rings were attached to two metal holders and placed in an organ bath filled with a 20 ml Krebs-Henseleit solution. Changes in isometric tension were measured using an isometric force transducer (TB-611T; Nihon Kohden, Tokyo, Japan) connected to an amplifier (AP-621G; Nihon Kohden) and recorded on a recorder (LR8100, LR4210 or TYPE 3066; Yokogawa, Tokyo, Japan). The rings were equilibrated for 90 min under resting tensions of 1.96 mN, and then precontracted with l-phenylephrine (PE, 3 μmol/l). Relaxant responses to acetylcholine (ACh, 1 to 1,000 nmol/l) or sodium nitroprusside (SNP, 1 to 1,000 nmol/l) were obtained and expressed as a percentage of the contraction to PE. In another series of experiments, the effects of ACh were assessed after preincubation of some vascular rings with the nitric oxide synthase (NOS) inhibitor Nω-nitro-l-arginine methyl ester (L-NAME, 100 μmol/l) for 60 min.

Histological Examination

The left kidneys were immediately cut into transverse sections around the renal pelvis at a thickness of approximately 5 mm and immersed in 10 vol% buffered formalin solution for fixation. After fixation, paraffin sections were made by the standard method, then stained with periodic acid-Schiff (PAS) for light microscopic observation. The specimens were observed under a light microscope (BX60; Olympus). Histopathological scoring was performed according to the method of Uehara et al. (8).

The glomerulosclerosis scores were obtained as follows. A minimum of 100 glomeruli in each specimen were examined. The severity of the lesions was graded from 0 to 4 +
with the value in the control group. The arterial injury score was obtained by multiplying the degree of damage (0 to 4) by the percentage of arteries displaying the corresponding infiltration and thrombus formation. The arterial injury score was obtained by multiplying the degree of damage (0 to 3) by the percentage of glomeruli displaying the corresponding lesions.

The severity of tubular lesions was graded from 0 to 4+: 0, no lesions; 1+, less than 25% involvement; 2+, 26% to 50%; 3+, 51% to 75%; and 4+, more than 76%. The injury score was assessed by Wilcoxon’s rank sum test for two groups comparison or the Kruskal-Wallis test followed by Steel’s test for multiple group comparison. A difference was considered statistically significant at \( p < 0.05 \).

### Statistical Analysis

All values are expressed as the means ± SE. Statistical analysis was performed using SAS software (version 6.12; SAS Institute, Inc., Cary, USA). Intergroup differences were assessed by Wilcoxon’s rank sum test for two groups comparison or the Kruskal-Wallis test followed by Steel’s test for multiple group comparison. A difference was considered statistically significant at \( p < 0.05 \).

### Results

#### Blood Pressure, Body Weight, Heart Weight, and Kidney Weight

SBP significantly increased in DS rats fed with the high salt diet (control rats) for 3 and 6 weeks as compared with rats fed with a standard chow (normal rats) (Fig. 1). Both benidipine and candesartan inhibited the rise in SBP in the high salt-fed DS rats at 3 weeks of treatment. Benidipine at 3 mg/kg showed similar hypotensive effects throughout treatment. In the 1 mg/kg benidipine and the 1 and 3 mg/kg candesartan groups, on the other hand, SBP continued to rise over the 6 weeks of the treatment period. The antihypertensive effect of benidipine at 3 mg/kg was more potent than that of candesartan at 3 mg/kg. The combination of benidipine and candesartan inhibited the rise of SBP. The level of SBP was not affected by any of the drugs (Table 1). Both kidney

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**Table 1. Effects of Benidipine Combined with Candesartan on Body Weight (BW), Heart Weight (HW) and Kidney Weight (KW) in Dahl Salt-Sensitive Hypertensive Rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg/day)</th>
<th>BW (g)</th>
<th>HW/BW</th>
<th>KW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>—</td>
<td>403 ± 7†</td>
<td>0.297 ± 0.005***</td>
<td>0.334 ± 0.004***</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>375 ± 7</td>
<td>0.420 ± 0.012</td>
<td>0.472 ± 0.009</td>
</tr>
<tr>
<td>Benidipine</td>
<td>1</td>
<td>376 ± 7</td>
<td>0.400 ± 0.007</td>
<td>0.452 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>3 (B3)</td>
<td>391 ± 6</td>
<td>0.358 ± 0.006**</td>
<td>0.435 ± 0.008*</td>
</tr>
<tr>
<td>Candesartan</td>
<td>1</td>
<td>382 ± 4</td>
<td>0.417 ± 0.009</td>
<td>0.465 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>3 (C3)</td>
<td>379 ± 2</td>
<td>0.394 ± 0.008</td>
<td>0.468 ± 0.012</td>
</tr>
<tr>
<td>Combination</td>
<td>B3 + C3</td>
<td>388 ± 7</td>
<td>0.360 ± 0.007**</td>
<td>0.437 ± 0.015*</td>
</tr>
</tbody>
</table>

Dahl salt-sensitive rats were fed a high-salt diet and treated with drugs for 6 weeks from the age of 7 weeks. Each value represents the means ± SE. * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \) compared with the control group. † \( p < 0.05 \) compared with the C3-treated group.

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**Fig. 1. Effects of benidipine combined with candesartan on systolic blood pressure in Dahl salt-sensitive rats.** Dahl salt-sensitive rats in all but the normal groups were fed a high-salt diet and given the drugs or vehicle orally for 6 weeks from the age of 7 weeks. Results are expressed as the means ± SE of 8 rats. ** \( p < 0.01 \), *** \( p < 0.001 \) compared with the value in the control group. # \( p < 0.05 \), ## \( p < 0.001 \) compared with the value in the B3 group. *** \( p < 0.001 \) compared with the value in the C3 group.

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The severity of arterial injury was graded from 0 to 3+: 0, no lesions; 1+, medial and intimal hyperplasia or necrosis and lumen narrowing; 2+, medial necrosis with infiltration of inflammatory cells; and 3+, medial and intimal hyperplasia and/or medial necrosis accompanied by inflammatory cell infiltration and thrombus formation. The arterial injury score was obtained by multiplying the degree of damage (0 to 3+) by the percentage of arteries displaying the corresponding degree of severity.

The severity of tubular lesions was graded from 0 to 4+: 0, no lesions; 1+, very mild focal dilatation of tubules; 2+, larger number of dilated tubules with widening of the interstitium; 3+, fairly extensive dilatation of tubules with cystic formation and widening of the interstitium; and 4+, complete atrophy of tubules.
weight and heart weight were higher in the control than in the normal rats. The kidney-to-body weight and heart-to-body weight ratios were significantly reduced by benidipine (3 mg/kg) and benidipine plus candesartan, but not by candesartan alone.

**Albuminuria**

Urinary albumin excretion in the control rats was significantly increased as compared with that in the normal rats (Fig. 2). Benidipine at 3 mg/kg significantly inhibited the increased urinary albumin excretion at 6 weeks of treatment. Although candesartan alone did not lower the albuminuria, the level of albuminuria in the B3 + C3 group was lower than that in the B3 group.

**Histological Examination**

In the control rats, glomerulosclerosis, arterial injuries, and tubular lesions were prominently observed in the kidney (Fig. 3). Benidipine significantly improved all lesions in the hypertensive DS rats. Candesartan tended to improve the lesions, but the effects were not significant. The combination of benidipine and candesartan significantly reduced the glomerulosclerosis and tubular lesion scores, and this reduction was equivalent to that by benidipine alone. The arterial injury score in the group treated with the drug combination was lower than the score in either single-drug group (Table 2).

**Vascular Relaxation**

In mesenteric arterial rings from the control rats, the endothelium-dependent relaxations induced by ACh were markedly impaired as compared with those in the normal rats (Fig. 4). Benidipine, but not candesartan, significantly improved the endothelium-dependent relaxations induced by ACh at 30−1,000 nmol/l. The combination of benidipine and candesartan also significantly improved the relaxations in-
Preincubation with the NOS inhibitor L-NAME inhibited ACh (1–30 nmol/l)-induced relaxations in the mesenteric arteries (Fig. 5). However, higher concentrations of ACh (100–1,000 nmol/l) caused relaxations even in the presence of L-NAME. The L-NAME-resistant relaxations were also impaired in the control rats as compared with those in the normal rats. Benidipine, as well as benidipine plus candesartan, improved the relaxant responses to ACh. Candesartan alone had no effect on the L-NAME-resistant relaxation.

Endothelium-independent relaxations induced by the NO donor SNP were smaller in mesenteric arterial rings from the control rats than in those from the normal rats (Fig. 6). Benidipine, candesartan, and benidipine plus candesartan improved the endothelium-independent relaxations. The relaxant effects of benidipine and benidipine plus candesartan were significantly greater than those of candesartan alone.

**Discussion**

DS rats are a suitable model for human salt-sensitive hypertension and have been used to examine the effects of antihypertensive drugs on survival and target organ injuries (9). In
DS rats, a high-salt diet causes vascular hypertrophy and varying degrees of renal injury-including arterial injury, glomerular sclerosis, and dilated tubular damage with interstitial fibrosis. In recent years, hypertensive patients have frequently been treated with a combination therapy of antihypertensive drugs. The present study was conducted to determine whether a combination of benidipine, which is a slow-onset and long-acting CCB (13), and an ARB would have beneficial effects on blood pressure, pathological changes in the kidney, or vascular functions in DS rats. The results revealed that benidipine prevented renal and vascular dysfunctions as well as the development of hypertension in DS rats. In addition, the antihypertensive effect of benidipine combined with the ARB candesartan was more potent than the effect of either drug alone. Furthermore, the combination effectively prevented the vascular dysfunction.

The treatment of hypertensive DS rats with benidipine prevented albuminuria and reduced glomerulosclerosis, renal arterial injury, and tubular damage. In DS rats, the amelioration of renal injuries by benidipine has been attributed to both antihypertensive and non-hemodynamic mechanisms (8). In general, L-type CCBs preferentially dilate preglomerular blood vessels, resulting in hyperfiltration that has been recognized as an important inducer of glomerular injury (11, 12). In contrast, benidipine dilates both glomerular efferent arterioles and glomerular afferent arterioles (13). In addition to the antihypertensive effect, the unique renal hemodynamic action of benidipine may contribute to the renoprotective effect in DS rats. The exact mechanism for the effects of benidipine on renal function remains unknown. Mibeferadil and efonidipine, which block both L-type and T-type calcium channels, have been shown to dilate both afferent and efferent arterioles, while L-type-specific CCBs, including nifedipine, dilate only afferent arterioles (12, 14). Cilnidipine, which blocks both L-type and N-type calcium channels, also dilates both arterioles (15). The blocking action of benidipine on some calcium channels—but not L-type channels—may contribute to the unique effects of benidipine on the renal microcirculation (16). Nakamura et al. (17) reported that benidipine reduced the glomerular expression of transforming growth factor-β and α-smooth muscle actin through a hypotension-independent mechanism and had a renoprotective effect in glomerulonephritic rats. This suggests that a direct action of benidipine on the kidney was involved in the present renoprotective effects of benidipine at 3 mg/kg. However, further studies will be needed to clarify the mechanism of the renoprotective effects of benidipine.

In the present study, the hypotensive effect of candesartan on blood pressure was weak in DS rats. Candesartan tended to reduce albuminuria and the morphological changes, but the effects were not significant. The doses of candesartan used in this study have been shown to be sufficient to exert antihypertensive effects in spontaneously hypertensive rats (18). The weak hypotensive effect of candesartan in this study was due to the low activity of RAS in salt-fed DS rats (4). Benidipine lowered blood pressure in a RAS-independent manner, and prevented the development of hypertension-induced organ damage in DS rats. These results suggest that benidipine is superior to candesartan for the treatment of salt-sensitive hypertension.

The responses of vascular smooth muscle cells to various relaxing factors produced in endothelial cells, including nitric oxide (NO), prostacyclin, and hyperpolarizing factor(s) (EDHF), are known to contribute to endothelium-dependent relaxation (19). Impaired endothelium-dependent vascular relaxation has been described in human essential hypertension as well as in animal models of hypertension (19). It is well established that salt-sensitive hypertension is associated with impaired endothelial function (20). In the present study, treatment of DS rats with a high-salt diet reduced not only endothelium-dependent relaxation to ACh but also endothelium-independent relaxation to SNP. In addition, the L-NAME-resistant relaxation to ACh was impaired in the mesenteric arteries.

All the drug treatments significantly prevented the dysfunction of relaxant responses to SNP to a similar extent. In contrast, both benidipine alone and benidipine plus candesartan significantly improved the endothelium-dependent relaxations, but candesartan alone did not. Furthermore, the im-
The order of potency of benidipine, candesartan, and the combination in the endothelium-dependent relaxations corresponds to that in the decreases in SBP. Therefore, the antihypertensive action of these drugs is considered to be responsible for the endothelial protective action (21). However, benidipine has been found to reduce vascular endothelial damage in various experimental models, such as splanchnic artery occlusion and reperfusion (22), the endothelial dysfunction induced by vitamin D3 and nicotine (23), and endothelial injury induced by sodium citrate (24). The protective effects of benidipine against endothelial injury may be at least partly independent of the calcium channel blockade in vascular smooth muscle cells (24). In a study on renovascular hypertensive rats, Kobayashi et al. (25) reported that benidipine increased NO production and endothelial NOS (eNOS) mRNA expression not only by lessening endothelial damage via a reduction in blood pressure levels, but also by stimulating NOS activity and eNOS mRNA. In addition, they (26) reported that a subdepressor dose of benidipine improved the wall-to-lumen ratio and perivascular fibrosis in the coronary arterioles in DS hypertensive rats. These findings and the present results suggest that an additional mechanism distinct from the antihypertensive action contributes to the vasoprotective effect of benidipine.

Candesartan significantly prevented the dysfunction of relaxant responses to SNP, but not to ACh. The lack of a protective effect on endothelial function may be attributed to the weak antihypertensive action of candesartan. Tamura et al. (27) reported that salt-mediated hypertension stimulated the aortic fibronectin gene with activation of the tissue local RAS, resulting in hypertensive vascular hypertrophy. In addition, angiotensin II stimulates the production of oxygen radicals through angiotensin II type 1 receptors in arteries (28). The vasoprotective action of candesartan may be due to the inhibition of the vascular RAS activity in the hypertensive DS rats.

In the present study, the L-NAME resistant relaxation was preserved in the benidipine and benidipine plus candesartan groups but not in the control and candesartan groups, indicating that benidipine prevented the impairment of endothelial function in the mesenteric artery. As discussed by Ghisdal et al. (29), EDHF is an important mediator of the endothelium-dependent and NO-independent relaxation in the mesenteric artery. Benidipine may improve the relaxation mediated by EDHF, resulting in the improvement of endothelial function. The L-NAME resistant relaxation in the combination group was smaller than that in the benidipine group. The combination treatment improved the endothelial-dependent relaxation more effectively than benidipine alone, probably due to the increase in NO-dependent components.

This might have led to a compensatory decrease in L-NAME resistant (NO-independent) relaxation in the combination group. However, the exact mechanism of the vasoprotective effects remains unknown, and further investigations are necessary.

In the treatment of hypertensive patients, combination therapy with different types of antihypertensive drugs is recommended if monotherapy fails to achieve the target blood pressure. The combination of benidipine and candesartan inhibited the increase in blood pressure more effectively than either drug alone, and tended to be more efficacious than benidipine alone in decreasing the albuminuria and renal arterial injuries. The antihypertensive effect of benidipine plus candesartan may be associated with the reduction in renal and/or vascular injuries. Some studies have reported that RAS activation contributes to the renal injuries in DS rats (7, 30). Losartan, an ARB, reduces cerebral and renal vascular lesions without affecting blood pressure in DS rats, suggesting that ARBs involve hypertension-independent mechanisms in their amelioration of organ injury (31). Renal and vascular dysfunctions are due to not only hypertension but also the RAS activation, and thus the combination of benidipine and candesartan would be useful for the treatment of salt-sensitive hypertension. However, further studies will be needed to clarify the significance of this combination therapy in hypertension.

In conclusion, the present study demonstrated that benidipine is more effective than candesartan in preventing the impairment of renal function and of vasorelaxation in salt-sensitive hypertensive rats, and that combination therapy with benidipine and an ARB could be more useful in the treatment of hypertension, as compared with either monotherapy alone.

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