Combination Therapy with an Angiotensin-Converting Enzyme (ACE) Inhibitor and a Calcium Antagonist: beyond the Renoprotective Effects of ACE Inhibitor Monotherapy in a Spontaneous Hypertensive Rat with Renal Ablation

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To assess the renal benefits of combined angiotensin-converting enzyme inhibition and calcium antagonism, we studied the antihypertensive and renoprotective effects of temocapril (TMP) alone or in combination with azelnidipine (AZN) in a spontaneously hypertensive rat (SHR) remnant kidney model of chronic renal failure. Male 5/6-nephrectomized SHR/Izumo rats were randomly assigned to receive vehicle (control group), TMP (TMP group; 10 mg·kg⁻¹·day⁻¹), AZN (AZN group; 3 mg·kg⁻¹·day⁻¹), or both (TMP + AZN group) orally for 12 weeks. Systolic blood pressure (SBP) and urinary excretion of albumin (UalbV) were measured every 2 weeks. At the end of the experiment, serum creatinine (Scr), heart weight (HW), and blood urea nitrogen (BUN) levels were measured and the remnant kidneys were examined to determine the index of glomerular sclerosis (IGS). SBP and UalbV in the control group increased progressively throughout the experimental period. TMP, AZN, and TMP + AZN blocked the development of hypertension. TMP + AZN did not enhance the antihypertensive effects of either TMP or AZN used singly. TMP, AZN, and TMP + AZN all significantly decreased the UalbV, Scr, BUN, and HW/BW ratio. The level of UalbV and the HW/BW ratio in the TMP + AZN group were significantly lower than those in the TMP and AZN groups, and the level of Scr in the TMP + AZN group was significantly lower than that in the TMP group. TMP, AZN, and TMP + AZN all significantly protected against an increase in the IGS. The IGS in the TMP + AZN group was significantly lower than that in the TMP and AZN groups. These results indicate that both TMP and AZN have antihypertensive and renoprotective effects in this model. They also suggest that simultaneous administration of TMP and AZN provides greater renoprotective effects than TMP alone.


Key Words: chronic renal failure, SHR, combination therapy, angiotensin-converting enzyme inhibitor, calcium antagonist

Introduction
Systemic hypertension is a well-known cause of progressive renal injury in both humans and experimental animals (1, 2). In rats, a reduction of the functioning renal mass increases the systemic blood pressure and causes progressive renal failure. The increased filtration rate per glomerulus has been
suggested to be responsible for the progressive deterioration of renal function (3, 4).

Antihypertensive therapy reduces the rate of decline of renal function in established progressive renal disease (5, 6). Recent evidence suggests that the ability of antihypertensive therapy to protect the kidneys relates largely to the glomerular hemodynamic consequences of therapy (7). Not all antihypertensive regimens afford equal renal protection, and agents that control glomerular hypertension may provide maximal protection to the kidney at risk for progressive glomerular injury (7).

Angiotensin-converting enzyme inhibitors (ACEIs) have been shown to be protective against hyperfiltration damage in remnant kidney nephrons in chronic renal failure (7, 8), and a meta-analysis indicated that ACEIs have the twin beneficial effects of reducing proteinuria and preserving renal function (6).

On the other hand, calcium antagonists have been shown to reduce glomerular injury in a rat remnant kidney model of chronic renal failure (9), and to be effective antihypertensive agents for treating hypertensive patients with chronic renal failure (10, 11).

The results of clinical trials suggest that ACEIs and calcium antagonists may have comparable renal protective effects (12–14). These beneficial effects of treatment with ACEIs and calcium antagonists raise the question of whether any additional benefit can be derived from combination therapy with both classes of agents.

Temocapril (TMP) (Sankyo Co., Tokyo, Japan) is a long-acting ACEI without a sulfhydryl group, and is the ester prodrug of the pharmacologically active diacid metabolite, temocaprilat. TMP is excreted mainly into the feces with a drug of the pharmacologically active diacid metabolite, acting ACEI without a sulfhydryl group, and is the ester prodrug of the pharmacologically active diacid metabolite, temocaprilat. TMP is excreted mainly into the feces with a prolonged duration of action. The efficacy of AZN as an antihypertensive agent has been shown in experimental models (15–18).

Despite the above findings, the effects of combination therapy with TMP and AZN on progressive albuminuria and glomerular sclerosis in a spontaneously hypertensive rat (SHR) remnant kidney model of chronic renal failure have not been studied. Therefore, to assess the combined effects of these novel agents, we studied the antihypertensive and renoprotective effects of AZN and TMP alone and in combination in SHR with renal ablation.

**Methods**

Thirty-six male SHR/Izumo rats (Funabashi Farm, Chiba, Japan), 5 weeks old, were subjected to 5/6-nephrectomy by removal of the left kidney and infarction of two-thirds of the right kidney. This subtotal nephrectomy was performed under ether anesthesia. The right kidney was exposed via a flank incision, and the two poles of the right kidney were excised by encircling them with loops of ligatures and then tightening the loops, and then the incision was closed. Two weeks later, the left kidney was removed in total after exposure via a flank incision, and then the flank incision was closed. Throughout the study, rats were housed in a metabolic cage designed to prevent feces-urine contact (model ST; Sugiyamagen, Tokyo), in a humidity- and temperature-controlled room (55 ± 10% and 22 ± 2°C, respectively) with a 12-h light/dark cycle. The rats were fed a regular diet (Funabashi F2 (0.19 wt% sodium, 0.25 wt% potassium and 20.8 wt% protein); Funabashi Farm (Funabashi, Japan), and had free access to tap water.

One week after ablation, when the rats were 8 weeks old, baseline measurements of body weight (BW), systolic blood pressure (SBP), urine volume (UV) and urinary excretion of albumin (UalbV) were made. The rats were then randomly assigned to one of four groups. The control group (n = 9) was given vehicle alone. The TMP group (n = 9) received TMP 10 mg/kg/day. The AZN group (n = 9) received AZN 3 mg/kg/day. And the TMP + AZN group (n = 9) received a combination of both TMP 10 mg/kg/day and AZN 3 mg/kg/day. TMP and AZN were mixed with 0.5% carboxymethyl cellulose before administration and administered by daily gavage at 9 AM for 12 weeks. Oral administration of TMP at 10 mg/kg/day for 21 weeks lowered systemic blood pressure of conscious SHR significantly from week 1 and throughout the rest of the 21-week observation period (19). Single oral administration of AZN at 3 mg/kg produced a long-lasting decrease of systemic blood pressure in conscious SHR (18).

Twelve weeks after the initiation of treatment, the rats were killed by decapitation and trunk blood was collected in polyethylene tubes for the determination of serum creatinine (Scr) and blood urea nitrogen (BUN). The heart was removed and weighed, and the heart weight (HW)/BW ratio was calculated. The remnant kidneys were removed and porc.

The index of glomerular sclerosis (IGS) was calculated using the following formula (21–23):

\[
IGS = \frac{1 \cdot N_4 + 2 \cdot N_3 + 3 \cdot N_2 + 4 \cdot N_1}{N_4 + N_3 + N_2 + N_1},
\]
Fig. 1. Sequential systolic blood pressure (SBP) values. □, control (vehicle alone); ○, TMP (temocapril alone); △, AZN (azelnidipine alone); ◊, TMP + AZN (both temocapril and azelnidipine). Values are expressed as the means SEM. TMP, AZN, and TMP + AZN each induced a similar and significant reduction in SBP compared with the control group (repeated measures analysis of variance (ANOVA): p < 0.001 TMP vs. control; p < 0.001 AZN vs. control; p < 0.001 TMP + AZN vs. control). *p < 0.0001 TMP vs. control; †p < 0.0001 AZN vs. control; ‡p < 0.0001 TMP + AZN vs. control.

Fig. 2. Urinary excretion of albumin (UalbV) values. □, control (vehicle alone); ○, TMP (temocapril alone); △, AZN (azelnidipine alone); ◊, TMP+AZN (both temocapril and azelnidipine). Values are expressed as the means SEM. TMP, AZN, and TMP + AZN decreased the level of UalbV significantly compared with that in the control group (repeated measures analysis of variance (ANOVA): p < 0.001 TMP vs. control; p < 0.001 AZN vs. control; p < 0.0001 TMP + AZN vs. control). The level of UalbV in the TMP + AZN group was significantly lower than that in the TMP or AZN groups (repeated measures ANOVA: p < 0.05 vs. TMP; p < 0.05 vs. AZN). * p < 0.05, † p < 0.01, ‡ p < 0.001, †† p < 0.0001 compared to values in the control group. *p < 0.05 compared to the values in the TMP group. * p < 0.05, † p < 0.01, ‡ p < 0.001, ‡‡ p < 0.0001 compared to values in the AZN group.

where N is the number of glomeruli at each grade of sclerosis.

SBP was monitored every 2 weeks in conscious rats by the indirect tail-cuff method (UEDA UR 1000; Ueda Industries Co., Tokyo, Japan) without anesthesia between 1 PM and 3 PM. The SBP measured using this method correlates well with that measured using a direct method (24). UV and BW were measured gravimetrically, and urine was collected every 2 weeks and immediately stored at -20°C until use. For the determination of UalbV, we used an enzyme-linked immunosorbent assay (NEPHRAT; Exocell, Inc., Philadelphia, USA). BUN and Scr were measured using a standard autoanalysis technique (Synchron CX-3; Clinical Systems, Beckman Coulter Inc., Fullerton, USA).

Values are expressed as the means SEM. With respect to BUN, Scr, and the HW/BW ratio, comparisons between groups were performed using the unpaired Student’s t-test. For SBP and UalbV, comparisons between groups were performed by analysis of variance (ANOVA) with repeated measures over the duration of the study. Statistically significant differences on each day were assessed between groups by the unpaired Student’s t-test. Values of p < 0.05 were considered to indicate statistical significance. With respect to the IGS, comparisons between groups were performed using the non-parametric Mann-Whitney’s U test. The statistical analysis was performed using STATVIEW 5.0 software (Abacus Concepts Inc., Berkeley, USA).

The study conformed to the principles for the use of live animals as outlined in the Declaration of Helsinki and those of the ethical committee of Tohoku University Graduate School of Medicine.

Results

The SBPs of the rats during the 12-week experimental period are shown in Fig. 1. The SBP in the control group increased progressively throughout the experimental period. TMP, AZN, and TMP + AZN each induced a similar and significant decrease in SBP compared with the control group (repeated measures ANOVA, p < 0.001). Twelve weeks after the initiation of treatment, the SBPs were 265 ± 7, 156 ± 3, 160 ± 7, and 154 ± 1 mmHg in the control, TMP, AZN, and TMP + AZN groups, respectively (n = 9 each).

The values of UalbV of the rats during the 12-week experimental period are shown in Fig. 2. UalbV in the control group was increased progressively throughout the experimental period. TMP, AZN, and TMP + AZN each induced a similar and significant decrease in SBP compared with the control group (repeated measures ANOVA: p < 0.001). Furthermore, the level of UalbV in the TMP + AZN group was significantly lower than that in the TMP or AZN groups (repeated measures ANOVA: p < 0.05 vs. TMP + AZN: p < 0.05 TMP vs. TMP + AZN; p < 0.01 AZN vs. TMP + AZN).

The levels of Scr in the TMP, AZN, and TMP + AZN groups were significantly lower than that in the control group...
(unpaired t-test: \( p < 0.05 \) TMP vs. control; \( p < 0.05 \) AZN vs. control; \( p < 0.01 \) TMP + AZN vs. control) (Fig. 3a). Furthermore, the level of Scr in the TMP + AZN group was significantly lower than that in the TMP group (unpaired t-test; \( p < 0.01 \)) (Fig. 3a).

The BUN values in the TMP, AZN, and TMP + AZN groups were significantly lower than that in the control group (unpaired t-test: \( p < 0.01 \) TMP vs. control; \( p < 0.05 \) AZN vs. control; \( p < 0.01 \) TMP + AZN vs. control) (Fig. 3b).

The HW/BW ratios in the TMP, AZN, and TMP + AZN groups were significantly lower than that in the control group (unpaired t-test: \( p < 0.01 \) TMP vs. control; \( p < 0.05 \) AZN vs. control; \( p < 0.01 \) TMP + AZN vs. control) (Fig. 3c). Further-

In addition, the IGS in the TMP + AZN group was significantly lower than that in the TMP or AZN group (non-parametric Mann-Whitney’s \( U \) test: \( p < 0.0001 \) TMP vs. control; \( p < 0.05 \) AZN vs. control) (Fig. 4). In addition, the IGS in the TMP + AZN group was significantly lower than that in the TMP or AZN group (non-parametric Mann-Whitney’s \( U \) test: \( p < 0.001 \) TMP + AZN vs. TMP; \( p < 0.05 \) TMP + AZN vs. AZN) (Fig. 4).

Discussion

In the present study, a model combining SHR with reduced renal mass was chosen because the functional and structural nephropathy is known to develop faster in this model than in a normotensive model with reduced renal mass (25). In the intact SHR, the glomerulus is protected from high systemic blood pressure by relative afferent arteriolar vasoconstriction. Despite severe systemic hypertension, the superficial nephrons of the SHR exhibit relatively low values for glomerular capillary plasma flow rate, and normal values for glomerular capillary pressure and single-nephron glomerular filtration rate, with relative resistance to focal and segmental glomerular sclerosis (2). However, renal ablation in the SHR results in lowering of afferent arteriolar resistance in the remaining kidney, allowing transmission of systemic hypertension and elevation of glomerular capillary pressure (2). Therefore, this hemodynamic alteration is associated with a sharp increase in values for proteinuria and acceleration of

![Fig. 3. (a) Serum creatinine (Scr), (b) blood urea nitrogen (BUN) and (c) heart weight (HW)/body weight (BW) ratio. Rats received vehicle alone (control), temocapril alone (TMP), azelnidipine alone (AZN), or both TMP and AZN (TMP + AZN). Values are expressed as the means ± SEM. TMP, AZN, and TMP + AZN decreased Scr significantly compared with the control group (unpaired t-test: *\( p < 0.05 \) vs. control; **\( p < 0.01 \) vs. control). Scr in the TMP + AZN group was significantly lower than that in the TMP group (unpaired t-test: \( p < 0.01 \) vs. TMP). TMP, AZN, and TMP + AZN decreased BUN significantly compared with the control group (unpaired t-test: *\( p < 0.05 \) vs. control; **\( p < 0.01 \) vs. control). The HW/BW ratios in the TMP, AZN, and TMP + AZN groups were significantly lower than that in the control group (unpaired t-test: \( p < 0.01 \) vs. control; **\( p < 0.001 \) vs. control). The HW/BW ratio in the TMP + AZN group was significantly lower than that in the TMP or AZN groups (unpaired t-test: \( p < 0.05 \) vs. TMP; \( p < 0.01 \) vs. AZN).]
In the present study, untreated control rats demonstrated progressive albuminuria, which is consistent with the development of hypertension, as well as glomerular structural lesions characterized by mesangial expansion and segmental sclerosis.

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**Fig. 4.** *Index of glomerular sclerosis (IGS).* Rats received vehicle alone (control), temocapril alone (TMP), azenlidipine alone (AZN), or both TMP and AZN (TMP + AZN). Values are expressed as the means ± SEM. The IGS value in the TMP, AZN, and TMP + AZN groups were significantly lower than that in the control group (non-parametric Mann-Whitney’s U test: *p* < 0.05 vs. control; **p** < 0.001 vs. control). The IGS value in the TMP + AZN group was significantly lower than that in the TMP or AZN group (non-parametric Mann-Whitney’s U test: ⋅ p < 0.001 vs. TMP; ⋅ p < 0.05 vs. AZN).

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In addition to efferent vasoconstriction, angiotensin II have many other actions in the kidney, including effects on mesangial cell contraction and macromolecular uptake through the mesangium (30), glomerular permeability to pro-
er, TMP + AZN did not enhance the antihypertensive effects of either TMP or AZN used singly. The reason why the combination did not produce an additional significant reduction in systemic blood pressure is unclear. TMP might have little additive effect on systemic blood pressure in this model with severe hypertension already on treatment with AZN, in particular when the AZN is maximally effective.

In conclusion, the antihypertensive and renoprotective effects of TMP and AZN were demonstrated in an SHR remnant kidney model of chronic renal failure. Moreover, combination treatment with an ACEI and a calcium antagonist had additional renoprotective effects in this rat model. Although ACEIs and calcium antagonists have been thought to be the best antihypertensive drugs for treating hypertension with chronic renal failure, the combined use of an ACEI and a calcium antagonist to protect against the progression of chronic renal failure might yield greater benefits than antihypertensive therapy with either component alone.

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References


