Azelnidipine ameliorates Ang II-induced damage


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Azelnidipine exerts renoprotective effects by improvement of renal microcirculation in angiotensin II infusion rats

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**Abstract**

**Background.** Hypoxia-induced tubulointerstitial injury caused by loss of peritubular capillary (PTC) blood flow may be associated with progressive renal disease. Therefore, the maintenance of blood flow in PTCs may protect against loss of renal function. A long-acting calcium channel blocker, azelnidipine, has been shown to be useful in the treatment of progressive renal disease. However, its mechanism of action remains unclear. The aim of the present study was to elucidate whether azelnidipine maintains PTC blood flow and to compare it to nifedipine in its ability to improve tubulointerstitial injury caused by angiotensin II (AII) infusion in rats.

**Methods.** PTC blood flow was initially monitored using a pencil-lens interval microscope before and after intravenous AII (30 ng/kg/min) infusion with or without azelnidipine (10 µg/kg/min). Next, Wistar rats were treated with chronic infusion of AII (500 ng/kg/min) via an osmotic minipump with or without azelnidipine (3 mg/kg/day, orally) or nifedipine (60 mg/kg/day, orally) for 14 days, and tubulointerstitial damage (PTC loss, interstitial fibrosis, tubular atrophy) was examined.

**Results.** PTC blood flow was reduced after AII infusion but improved after a bolus injection of azelnidipine. Tubulointerstitial damage observed in chronically AII-treated kidneys was associated with hypoxic conditions, as indicated by the measurement of hypoxia biomarkers (intracellular hypoxyprobe-1 adducts). These tubulointerstitial injuries in AII-infused rats were more effectively reduced by azelnidipine than by nifedipine. The area showing hypoxic conditions in the kidney was also more reduced with azelnidipine than nifedipine treatment.

**Conclusions.** Azelnidipine may increase PTC blood flow and improve renal hypoxia and tubulointerstitial injury induced by AII infusion.

**Keywords:** calcium channel blocker; hypoxia; peritubular capillary; tubulointerstitial injury

**Introduction**

Tubulointerstitial injury is an important risk factor for progressive renal damage [1,2]. It is reported that ischaemia, i.e. a decrease in tissue oxygen concentration caused by loss of peritubular capillaries (PTCs) and a decrease in blood flow in PTCs, is extensively involved in the pathogenesis of tubulointerstitial injury [3–5]. Therefore, the maintenance of blood flow in PTCs and the resultant improvement of tissue hypoxia may prevent progression of renal damage.

Calcium channel blockers (CCBs) are the most commonly used antihypertensive agents. Their renoprotective effects have been demonstrated in many studies using experimental models of renal damage, especially those of hypertensive renal damage [6–8]. Recent large-scale clinical studies, such as the Lipid-Lowering Treatment to Prevent Heart Attack Trial and international Nifedipine GITS study: Intervention as a Goal in Hypertension Treatment, have shown that long-acting dihydropyridine CCBs effectively suppress progression of renal damage [9,10]. Several hypotheses have been proposed for the mechanism of such renoprotective effects of CCBs; one of these proposed mechanisms is the improvement of renal haemodynamics.
by CCBs. CCBs are reported to not only decrease blood pressure but also increase renal plasma flow (RPF) [11,12].

End-stage renal failure can result from various factors, such as diabetic nephropathy and chronic glomerulonephritis. Previous reports have revealed that the renin–angiotensin (RA) axis is involved in the common mechanism of progressive renal damage. Large-scale clinical studies have also suggested that RA inhibitors, such as angiotensin II (AII)-receptor antagonists, have renoprotective effects that are independent of their antihypertensive effects [13]. All exerts its constrictive effect more intensely on efferent arterioles than on afferent arterioles, causing an increase in intraglomerular pressure (glomerular hypertension). It is reported that some dihydropyridine CCBs reduce RA-induced constriction of efferent arterioles and thereby reduce intraglomerular pressure [14,15]. On the other hand, there has been no report on the effect of CCBs on blood flow in PTCs.

Azelnidipine is a long-acting dihydropyridine CCB developed and marketed in Japan. The drug has been shown to not only have a potent antihypertensive effect but also a cardioprotective effect [16]. Its renoprotective effect has also been demonstrated in several experimental models [17,18] and in patients with hypertension [19]. In the present study, we investigated the renoprotective effects of azelnidipine via its improvement of renal haemodynamics. We demonstrated that azelnidipine exerts its renoprotective effect by not only decreasing blood pressure but also increasing blood flow in PTCs and improving tubulointerstitial hypoxia and injury.

Subjects and methods

Acute effect of azelnidipine in AII-infused rat

Analysis of renal microcirculation. All experiments were performed with the approval (No. 08–004) of the Ethics Review Committee for Animal Experimentation of the Kawasaki Medical School (Kurashiki, Japan). Male Wistar rats (Charles river laboratories Japan Inc., Kanagawa, Japan) weighing 200–250 g were used in this study. The rats were anaesthetized with isoflurane. Then a catheter was inserted and held in place in the left jugular vein. A continuous infusion of saline (0.05 mL/min) was administered through the catheter. Another catheter was inserted and held in place in the left carotid artery for measurement of blood pressure. Monitoring of blood flow in PTCs and observation of glomeruli were performed using a needle-probe Charge-Coupled Device Videomicroscope (CCDV), based on reports by Yamamoto et al. [20–22]. This system (VMS-1210; Nihon Kohden, Tokyo, Japan) consisted of a needle-type probe, a camera body with a CCD sensor, a square index lens and an optical fibre guide for illumination. The needle-type probe (4.5 mm in diameter) contained 18 optical fibres. The CCD had an image sensor with 680 × 480 pixels, and provided digitized images and a video recorder (EVO-9850; Sony, Tokyo, Japan). The spatial resolution of this CCD system is 0.87 µm. PTC blood flow was recorded with the CCDV placed in direct contact with the exposed surface of the kidney. Images of glomeruli were taken using the CCDV, whose tip was inserted into the kidney through a small incision (0.5–1 mm) made on the exposed surface of the kidney. Bleeding was minimal during the procedure and usually discontinued within 10 min. The probe was moved gently to gain clear images of glomeruli. Sequential images of renal microvessels were captured with a freeze-frame modality. The diameters of afferent and efferent arterioles were measured using the Image-I software (http://rsbweb.nih.gov/ij/), and the flow rate of red blood cells (RBCs) was analysed using blood velocity analysis software. Medex (JMC Co., Ltd., Kyoto, Japan). A vessel segment ~15 µm in length was scanned, and the mean RBC velocity was determined by averaging at least five measurements. The specific protocol was as follows. Firstly, blood flow in PTCs or glomeruli was recorded under continuous infusion of saline. Then, the infusion was changed from saline to AII, which was then continuously infused (30 ng/kg/min) for 10 min, and blood flow in PTCs or glomeruli was recorded. Under continuous infusion of AII, azelnidipine (Sankyo Co., Ltd., Tokyo, Japan) at 10 µg/kg/min was administered through the jugular vein, and blood flow in PTCs or glomeruli was recorded 10 min after azelnidipine administration. The dosage of azelnidipine was determined to achieve enough blood pressure drops as previously described [23].

Chronic effect of azelnidipine in AII-infused rat

Experimental animal models. Male Wistar rats (Charles river laboratories Japan Inc.) weighing 210–230 g were used. An osmotic minipump (Alzet model 2001; Alza Pharmaceuticals, Palo Alto, CA, USA) was placed under the skin of the rats, and either AII (500 ng/kg/min, n = 18) or saline (Cont., n = 6) was administered for 14 days. The rats that received AII were further divided into three groups based on whether they received no treatment (AII, n = 6), daily oral azelnidipine at 3 mg/kg/day (AII + Azl, n = 6) or nifedipine at 60 mg/kg/day (AII + Nif, n = 6). The dosage of azelnidipine was determined by referring to previous reports [24]. The dosage of nifedipine was determined by preliminary experiment to achieve same blood pressure with azelnidipine treatment. Twenty-four-hour urine collection was performed 14 days after the completion of the administration of either AII or saline. On the same day, rats were sacrificed under anaesthesia, and blood samples and both kidneys were collected. Before the sacrifice, whole kidney renal blood flow (RBF) was measured using a V-shaped (size 0.5 V) non-cannulating flow probe connected to an ultrasonic transit-time flowmeter (Transonic Systems, Inc., Ithaca, NY, USA). In this technique, the left renal artery was dissected away from the perinephritic fat, and a flow probe (ø 1 mm) was placed around the renal artery near the hilum of the kidney. The probe was connected via a flowmeter to a computer, and RBF was recorded. After the animals were sacrificed, portions of the removed kidneys were immersed and fixed in 4% paraformaldehyde and then embedded in paraffin, while other portions were frozen-fixed using Tissue Tee.

Physiological and biochemical measurements. Body weight and blood pressure were measured just before sacrifice. Blood pressure was measured in pre-warmed rats by the tail-cuff method (BP-98A; Softron Co., Ltd., Tokyo, Japan). To collect urine samples, rats were placed in metabolic cages for 24 h and were provided tap water but no food. After the final 24-h fasting in metabolic cages, blood samples were obtained from rats via an 18-gauge needle inserted into the left ventricle after the rats were killed. Kidney was removed and the kidney weight was measured. The blood urea nitrogen (BUN) level was measured using urease UV. Serum and urinary creatinine levels were also measured using enzymatic methods, and the urinary protein excretion (UPE) level was measured by an enzyme immunoassay.

Histopathological examination of kidneys. Paraffin-embedded sections of kidneys (approximately 4-µm thick) were deparaffinized and stained by Masson staining. From these sections, the percentage of the area with tubulointerstitial injuries (infiltration of inflammatory cells, tubular dilatation/atrophy, interstitial fibrosis and tubular cast formation) was scored (grade 0 = no area of damage, grade 1 ≤ 10%, grade 2 = 10–25%, grade 3 = 25–50%, grade 4 = 50–75% and grade 5 = 75–100%). The grades were assessed by examining 10 fields in six samples from each animal [25]. For the detection of tubulointerstitial damage, immunohistochemical staining for vimentin was performed. Frozen tissue sections were incubated in methanol with 3% hydrogen peroxide to block endogenous peroxidase activity and then incubated with a mouse anti-vimentin monoclonal antibody (Sigma-Aldrich Japan K.K., Tokyo, Japan) diluted 1:100 at 4 °C overnight. For negative controls, a mouse IgG1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used at equivalent concentrations. The primary antibody was detected using the Histofine Simple Stain MAX-PO (MULTI) kit (Nichirei Co., Tokyo, Japan) and 3,3′-diaminobenzidine (Sigma-Aldrich Japan K.K.). The percent area of vimentin positive areas in the renal cortex, excluding the glomeruli and small arteries, was measured using the Image-I software.

Examination of PTC volume. PTCs were detected by staining for RECA-1, a vascular endothelial marker for rats. Frozen sections of kidneys (~4-µm thick) were fixed with acetone, to which an anti-RECA-1 monoclonal antibody (Cosmo Bio, Tokyo, Japan) diluted 1:50 was applied

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and incubated at 4°C overnight. Histofine Simple Stain MAX-PO was then dropped onto the sections, which were then incubated for 30 min and thereafter colour was developed with 0.02% 3,3′-diaminobenzidine (Sigma-Aldrich Japan K.K.). The percent area of PTCs in the renal cortex, excluding the glomeruli, was measured using the Image-J software.

Detection of tissue ischaemia. Renal tissue ischaemia was detected using pimonidazole (hypoxyprobe-1; CHEMICON International, Inc., Temecula, CA, USA). Briefly, pimonidazole hydrochloride (60 mg/kg) was administered through the caudal vein 2 h before euthanasia, and paraffin-embedded sections of the kidneys were prepared. The sections were deparaffinized and anti-pimonidazole monoclonal antibody diluted 1:100 was applied and incubated at 4°C overnight. Histofine Simple Stain MAX-PO was then dropped onto the sections, which were then incubated for 30 min and thereafter colour was developed with 0.02% 3,3′-diaminobenzidine. The percentage of areas that stained positive for pimonidazole in the renal cortex, excluding the glomeruli, was measured using the Image-J software.

Statistical analysis
All results were expressed as mean ± standard error. Statistical data processing was performed using the StatView software (StatView SE+ Graphics Version 4.0, Abacus Concepts, SAS Institute, Inc., Cary, NC, USA). Differences between the groups were analysed by the two-tailed unpaired Student’s t-test, Welch’s t-test or Mann–Whitney’s U-test. The values of \( P < 0.05 \) were considered significant.

Results
Evaluation of renal microcirculation in rats receiving acute AII-infusion
The effects of azelnidipine on AII-induced altered renal microcirculation were evaluated by measuring systolic blood pressure (SBP), blood flow in PTCs and the diameters of afferent and efferent arterioles. A marked increase in SBP was observed after AII administration (139 ± 5 mmHg post-AII versus 101 ± 4 mmHg at baseline; \( P < 0.05 \); Figure 1D), and this increase was significantly reduced by azelnidipine administration (118 ± 7 mmHg; \( P < 0.05 \) versus post-AII; Figure 1D).

Fig. 1. Effect of azelnidipine on changes to PTC microcirculation induced by acute AII infusion. Typical images of PTC haemodynamics at baseline (A), after AII (30 ng/kg/min) infusion (B) and after azelnidipine (10 µg/kg/min) administration (C) recorded using a CCD videomicroscope. Scale bar = 50 µm. (D) Systolic blood pressure changes. (E) RBC velocity in PTCs. \( n = 6 \) in each group. \( * P < 0.05 \) versus baseline; † \( P < 0.05 \) versus AII infusion.

Fig. 2. Effects of azelnidipine on glomerular microcirculation changes induced by acute AII infusion. Typical images of glomerular haemodynamics at baseline (A) recorded using a CCD videomicroscope. Glo, glomerulus; A, afferent artery; E, efferent artery. Scale bar = 50 µm. (B) RBC velocity in PTCs at baseline, after AII (30 ng/kg/min) infusion and after azelnidipine (10 µg/kg/min) administration. (C) Afferent arteriolar diameter. (D) Efferent arteriolar diameter. \( n = 6 \) in each group. \( * P < 0.05 \) versus baseline; † \( P < 0.05 \) versus AII infusion.

Functional data and morphological evaluation in rats receiving chronic AII infusion
Physiological parameters 14 days after the subcutaneous administration of AII are shown in Table 1. An increase in the mean blood pressure was observed in rats
receiving subcutaneous administration of AII (159.4 ± 11.3 mmHg, *P* < 0.05 versus control). The blood pressure in rats treated with azelnidipine or nifedipine decreased significantly compared with those that received AII alone (116.1 ± 3.5 or 110.4 ± 6.4 mmHg, respectively, *P* < 0.05 versus AII). No significant differences were found in body weight among the four groups. The kidney weight was increased by chronic AII infusion, but there was no difference among the four groups. Excretion of urinary protein was significantly increased in rats that received AII and was suppressed in those treated with azelnidipine or nifedipine (42.0 ± 6.1 in AII versus 7.0 ± 1.7 in AII plus azelnidipine, 11.4 ± 5.3 mg/day in AII plus nifedipine, *P* < 0.05). The urinary protein excretion was much lowered by treatment with azelnidipine than by treatment with nifedipine. BUN was markedly increased by AII administration, which was improved by azelnidipine or nifedipine treatment (18.3 ± 0.8 in AII versus 13.8 ± 1.5 in AII plus azelnidipine, 25.8 ± 1.0 mg/dL in AII plus nifedipine, *P* < 0.05). BUN was also considerably lower in rats treated with azelnidipine than in those treated with nifedipine. Creatinine clearance was markedly reduced by AII administration and improved by azelnidipine treatment (0.77 ± 0.09 in AII versus 1.23 ± 0.10 mL/min/100 g BW in AII plus azelnidipine, *P* < 0.05), but not by nifedipine treatment (0.93 ± 0.13 mL/min/100 g BW). RBF was reduced by AII administration (5.0 ± 0.3 mL/min/g kidney weight, *P* < 0.05 versus control). RBF was increased in both the azelnidipine treatment group and the nifedipine treatment group, but no statistically significant differences were observed in whole-kidney RBF between two groups.

To investigate whether azelnidipine treatment can improve AII-induced renal damage, we evaluated damage to renal cortical tissue by Masson staining. While the rats that did not receive AII (control group) showed almost no tubulointerstitial injuries (data not shown), some of the rats that received AII showed various types of tissue damage, such as tubular dilatation/atrophy and perivascular fibrosis (Figure 3A). The formation of these tubulointerstitial injuries was suppressed by azelnidipine treatment (Figure 3B). When the tubulointerstitial injuries were scored and semiquantitatively evaluated, it was also found that azelnidipine treatment significantly reduced AII-induced tubulointerstitial injuries (1.57 ± 0.18 in AII versus 0.55 ± 0.22 in AII + azelnidipine, *P* < 0.05; Figure 3D). Nifedipine treatment also prevented AII-induced interstitial injury, but less than azelnidipine treatment (1.03 ± 0.15 in AII + nifedipine, *P* < 0.05 versus AII + azelnidipine; Figure 3C and D). Meanwhile, few glomerular lesions such as glomerular sclerosis were found in all groups of rats.

We also evaluated renal cortical tissue damage by vimentin staining. There were few vimentin-positive areas except in the glomeruli and small arteries of the control rats (data not shown). The vimentin-positive area was enhanced by chronic AII infusion (Figure 4A). However, azelnidipine treatment limited the enhancement of AII-induced tubular damage in the renal cortex (Figure 4B).
Azelnidipine ameliorates Ang II-induced damage.

**Fig. 4.** Effects of azelnidipine or nifedipine on tubular damage induced by chronic AII infusion. Tubular vimentin was used as a marker of tubular damage. Immunohistochemical micrographs of renal cortex stained with anti-vimentin antibody from rats that received chronic AII (500 ng/kg/min) infusion alone (A), with azelnidipine (3 mg/kg/day) treatment (B) or with nifedipine (60 mg/kg/day) treatment (C). Scale bar = 100 µm. (D) Quantitation of vimentin-positive area in the renal cortex. n = 6 in each group. *P < 0.05 versus control rats; †P < 0.05 versus AII-infused rats; ‡P < 0.05 versus nifedipine treated, AII-infused rats.

Vimentin-positive area was markedly increased in rats that received AII rather than saline (control group) (9.3 ± 0.3% versus 1.8 ± 0.4%, P < 0.05; Figure 4D) and was significantly reduced in rats treated with azelnidipine (3.0 ± 0.3%, P < 0.05 versus AII; Figure 4D). Nifedipine treatment also decreased AII-induced tubular damage (Figure 4C), but this effect was smaller than that with azelnidipine treatment (4.5 ± 0.6%, P < 0.05 versus AII + azelnidipine; Figure 4D).

**Evaluation of renal hypoxia by pimonidazole protein adducts**

To determine whether the effects of azelnidipine on AII-induced tubulointerstitial injuries are mediated by improvement in blood flow in PTCs, a decrease in oxygen concentration in the renal cortex was detected using pimonidazole. In the control group, the only pimonidazole-positive cells were renal tubular epithelial cells in the corticomedullary junction and outer medulla, with few positive cells in the cortex (data not shown). In contrast, an apparent increase in pimonidazole-positive tubular epithelial cells in the cortex was observed in rats that received AII (Figure 5A). The AII-induced increase in pimonidazole-positive cells was suppressed by nifedipine treatment (Figure 5C), and even more so by azelnidipine treatment. Figure 5D shows a chart comparing percent pimonidazole-positive areas in the renal cortex in each group. The pimonidazole-positive area was markedly increased in rats that received AII compared with the control group (10.0 ± 6.3% versus 65.1 ± 5.4%, P < 0.05). In contrast, the pimonidazole-positive area in the renal cortex was significantly reduced in rats treated with nifedipine compared with those that received AII alone (41.0 ± 7.0%, P < 0.05 versus AII) and even more reduced in rats treated with azelnidipine (21.0 ± 8.1%, P < 0.05 versus AII and AII + nifedipine).

**Determination of PTC volume by RECA-1 staining**

PTC staining with an anti-RECA-1 antibody was performed to evaluate the effects of azelnidipine on the PTC volume. In the control group, PTCs were found evenly in the interstitium of the renal cortex (data not shown). In contrast, decreases in PTC volume were observed in rats that received AII (Figure 6A). The percent area of RECA-positive areas in the renal cortex was also significantly reduced in rats that received AII compared with the control group (31.2 ± 4.0% in control versus 17.6 ± 2.0% in AII, P < 0.05; Figure 6D). These results suggest a decrease in total vascular bed blood and flow in PTCs in rats that received AII. The RBC velocity in the PTCs, as measured by CCDV, was decreased to 50.0 ± 3.0% in AII-infused rats (Figure 6E). On the other hand, changes in the PTC volume observed in rats infused with AII were significantly suppressed in those treated with azelnidipine and nifedipine (Figure 6B and C). The percent area of RECA-positive areas in the renal cortex was significantly increased...
in rats treated with azelnidipine or nifedipine compared with those that received AII alone (28.5 ± 4.2% or 24.1 ± 4.5%, respectively, *P* < 0.05 versus AII; Figure 6D). The RBC velocity in the PTCs was also increased to 63.3 ± 3.0% with nifedipine, and even more increased with azelnidipine (88.4 ± 7.0%; *P* < 0.05 versus AII + nifedipine, Figure 6E).

**Discussion**

We directly monitored the haemodynamics of PTCs and glomeruli in the superficial layer of the renal cortex using a CCDV. This approach enables monitoring of haemodynamics under physiological conditions and can be used to evaluate changes in microcirculation in glomeruli and PTCs [20–22]. The present study revealed that (1) AII constricts both afferent and efferent arterioles and thereby reduces downstream blood flow in PTCs, and (2) azelnidipine partially suppresses AII-induced constriction of afferent and efferent arterioles and thereby increases blood flow in PTCs. It has already been reported that AII constricts not only efferent but also afferent arterioles and reduces RBF in a dose-dependent manner [20]. However, there have been no reports, to our knowledge, of changes in blood flow in PTCs as monitored directly on visualized images. The method used in the present study has enabled direct, in vivo monitoring of blood flow in PTCs and revealed a decrease in blood flow in PTCs following AII administration.

AII is known to be involved in the pathogenesis of renal impairments through various effects. High-dose, chronic administration of AII induces severe tubulointerstitial injuries in rats; however, the degree of glomerular injuries is known to be milder than that of tubulointerstitial injuries [26,27]. Moreover, AII has been shown to induce morphological changes in tubulointerstitial cells [26,27] and infiltration of inflammatory cells [26]. The results of the present study suggest that AII induces tubulointerstitial injuries by decreasing blood flow in PTCs. Animal models of renal damage induced by chronic administration of AII also exhibited tubulointerstitial injuries and the spread of tissue hypoxia in the cortex, as shown by pimonidazole staining. These results suggest that AII reduces blood flow in PTCs and thereby causes a decrease in blood flow and tissue hypoxia in the renal cortex, resulting in tubulointerstitial injuries.

Oizumi et al. have reported an increase in RPF in spontaneously hypertensive rats (SHRs) treated with azelnidipine at 3 mg/kg/day for 15 weeks [28]. Similarly, Yagil et al. compared changes in RPF induced by intravenous administration of azelnidipine with those induced by nicardipine, with blood pressure controlled at comparable levels, and reported a significant increase in RPF induced by azelnidipine [17]. In this study, azelnidipine also increased glomerular filtration rate (GFR), but the degree of increase in GFR was lower than that of increase in RPF. In a study by Kanazawa et al. using 5/6 nephrectomized SHR, azelnidipine was comparable to angiotensin-converting enzyme inhibitors in exerting an inhibitory effect on urinary protein excretion [18]. The results from these reports suggest that azelnidipine dilates both afferent and efferent arterioles. Dihydropyridine CCBs generally block only the L-type calcium channel, which is present in afferent arterioles but not in efferent arterioles. It has thus been believed that dihydropyridine CCBs dilate only afferent arterioles. Because nifedipine acts exclusively on L-type calcium channels, it may predominantly dilate afferent arterioles and cause glomerular hypertension [29]. However, it has recently been reported that some long-acting dihydropyridine CCBs also dilate efferent arterioles [30]. Azelnidipine is a new CCB and thus its dilating effect on afferent and efferent arterioles has not been established to date. It has been reported that azelnidipine provides renal sympathoinhibitory effects [23,31]. Activation of renal sympathetic nerves decrease RBF and increase preglomerular and postglomerular resistance [32].

Thus, the vasodilatory action of azelnidipine in afferent and efferent arterioles may be mediated at least in part by the inhibition of the sympathetic nervous system.
Azelnidipine ameliorates Ang II-induced damage

Azelnidipine suppressed Ang II-induced tubulointerstitial injuries and reduced tissue hypoxia in the renal cortex. The renoprotective effect of CCBs is mediated by their improving effect on the glomerular microcirculation, as well as by their antihypertensive effect [27]; however, no reports have so far demonstrated that CCBs exert their renoprotective effects by increasing blood flow in PTCs. Kondo et al. reported that azelnidipine attenuates Ang II-induced peritubular ischaemia, which may be involved in its beneficial effects on renal injury [33]. In the previous report, azelnidipine, unlike other dihydropyridine CCBs, was shown to slightly decrease heart rate in clinical settings [34]. Moreover, azelnidipine decreased urinary protein excretion in patients with hypertension much more than didamlodipine [19]. These effects may result from its anti-sympathetic and anti-inflammatory effects. Given the present finding that azelnidipine increases blood flow in PTCs, as confirmed using a CCDV, this agent may improve tubulointerstitial ischaemia by correcting interstitial blood flow altered by Ang II.

PTCs diverging from glomerular efferent arterioles form a vascular network in the renal cortex. Thus, blood flow in PTCs is closely linked with changes in glomerular blood flow. Furthermore, damage in PTCs is not only involved in the pathogenesis of tubulointerstitial injuries but is also an important prognostic factor for renal function [1–3]. Maintenance of blood flow in PTCs is thus essential for prevention of progressive renal damage. In the present study, a decrease in the PTC volume induced by chronic administration of Ang II was observed in the regions of interstitial injuries, and the lumens of PTCs were also narrowed following Ang II administration. These findings were consistent with the findings of PTCs obtained using a CCDV. Taken together, the decrease in the area of PTCs was assumed to be a result of the narrowing of their lumens due to a decrease in blood flow. In addition, rats treated with azelnidipine retained normal lumens, as also confirmed using a CCDV. We thus speculated that azelnidipine maintains blood flow in PTCs by improving microcirculation.

Our observations using a CCDV were limited to the outer layer of the kidney. In the kidney, renal glomerular arterioles are morphologically heterogeneous, and the responses of intracellular calcium concentration to Ang II differ among cell types [35,36]. Ang II controls the vascular tone of pre- and post-glomerular arterioles, and thereby controls glomerular filtration. In the outer cortex, Ang II-induced smaller intracellular calcium concentration increases in thin efferent arterioles than in afferent arterioles. In the inner cortex, two subpopulations of juxtaglomerular efferent arterioles, muscular ones that terminate as vasa rectae and thin ones that terminate as PTCs, have been described. They display functional heterogeneity with regard to the Ang II response. The response to Ang II was lower in thin than in the muscular efferent arterioles but did not differ from that obtained with corresponding afferent arterioles. The glomeruli and PTCs observed using a CCDV were almost in the outer cortex, so the data from the CCDV do not reflect the PTCs of the whole kidney.

The RBF reduced by Ang II administration was increased by both azelnidipine treatment and nifedipine treatment to the same degree. However, the PTC volume and RBC velocity in the PTCs were higher in the azelnidipine group than in the nifedipine group. These data indicate that azelnidipine treatment improved cortical blood flow especially. The data from pimonidazole staining showing that azelnidipine reduced the cortical hypoxic area in the Ang II-infused kidney also support this conclusion. The blood flow near the cortex is supplied through efferent arteries of the cortical glomeruli. Azelnidipine may improve cortical blood flow by dilating these arteries, thereby increasing the PTC blood supply.

Our RBF values in healthy control animals were slightly lower than normal (∼7.0 mL/min). RBF in the rat varies greatly depending on the anaesthetic used and the plane of anaesthesia. Hypothermia is also a common cause of lower than expected flow measurements. In our experiment, RBF was measured just before sacrifice. So, we did not maintain the body temperature with a heat pad, which may have resulted in the lower RBF values than expected.

Tubular hypoxia and hypoxia-induced tubular damage may be improved by an increase in the number of PTCs. However, it is difficult to precisely measure the PTC number. The PTC volume that we measured using RECA staining is dependent on tubular blood flow. This measurement of PTC volume does not reflect the exact number of PTCs. Instead of measuring PTC number, we checked VEGF mRNA expression, an indicator of angiogenesis. The VEGF mRNA expression was increased by Ang II infusion and decreased by azelnidipine or nifedipine treatment, and the VEGF expression was much higher in the nifedipine group than the azelnidipine group (supplemental data). Therefore, the increase in PTC volume and RBC velocity in PTCs by azelnidipine was not induced by increased angiogenesis.

In conclusion, we have demonstrated that azelnidipine maintains blood flow in PTCs by suppressing Ang II-induced constriction of afferent and efferent arterioles and thereby suppresses tubulointerstitial injuries caused by ischaemia. Currently, CCBs are widely used for the treatment of hypertension. The present study suggests that azelnidipine improves not only glomerular blood flow but also renal interstitial microcirculation.

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Conflict of interest statement. None declared.

Supplementary data

Supplementary data are available online at http://ndt.oxfordjournals.org.

References


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