Article

jraas

Journal of the Renin-Angiotensin-Aldosterone System
12(2) 65–74

DOI: 10.1177/1470320310387844
jra.sagepub.com
SAGE

Changes in renal vessels following the long-term administration of an angiotensin II receptor blocker in Zucker fatty rats

Kazushige Nakanishi[1], Yohko Nagai[1,2], Honglan Piao[3], Tatsuo Akimoto[1], Hirohito Kato[1], Nadezhda Yanakieva-Georgieva[3], Yukio Ishikawa[4], Kastunori Yoshihara[1], Kinji Ito[5], Nobuaki Yamanaka[2] and Takashi Oite[3]

Abstract

Introduction: The nephro-protective effects of angiotensin II receptor blockers (ARBs) are widely known; however, there are few reports of long-term effects focusing on the renal vessels. We studied afferent arteriolar changes induced by the long-term administration of an ARB.

Materials and Methods: Thirty-two 6-week-old male Zucker fatty rats (ZFRs) were divided into following four groups (n = 8 in each): ZFR Group and ZFR+High Group fed a standard or high-salt diet, respectively; ZFR+ARB Group and ZFR+High+ARB Group fed a standard or high-salt diet with ARB (Olmesartan, 5 mg/kg/day), respectively. Blood pressure, proteinuria, morphological examinations and glomerular haemodynamics *in vivo* were studied.

Results: Marked proliferative changes in the afferent arteriolar smooth muscle cells (SMCs) were frequently observed in the two groups given ARBs; in the ZFR+ARB group (77.3±10.3%) compared with the two groups without ARB (1.7%, $p < 0.005$; 1.2%, $p < 0.0005$) and 37.4±15.6% in the ZFR+High+ARB group. Proteinuria markedly decreased in the groups treated with ARBs, but the glomerular erythrocyte velocities showed no differences.

Conclusions: Our findings indicate that long-term ARB administration induced unusual proliferative changes in SMCs of afferent arterioles of ZFRs. These changes could narrow arteriolar lumens and reduce intraglomerular pressure, but they could cause also irreversible damage to the arterioles.

Keywords

Afferent arteriole, angiotensin II receptor blocker (ARB), Zucker fatty rat

Introduction

Angiotensin II type 1 receptor blockers (ARBs) are widely used as anti-hypertensive agents; moreover, they are known to have various organ-protecting effects even without lowering blood pressure.[1] The nephro-protective effects are well known. However, most studies in the effects of ARBs on the kidney were performed over a relatively short period,[2,3] and few studies have focused on the morphological changes in afferent arterioles,[4,5] which are the most important resistance vessels for glomerular haemodynamics. We attempted to clarify the changes induced by the long-term administration of ARBs on renal vessels, particularly the morphology of afferent arterioles, using Zucker fatty rats (ZFRs) under low- or high-salt conditions.

Materials and methods

Thirty-two 6-week-old male ZFRs were purchased from Charles River Japan. The rats were divided into four groups: the ZFR group (n = 8) was fed a standard NaCl diet (0.4%), the ZFR+High group(n = 8) was fed a high NaCl diet (4%), the ZFR+ARB group (n = 8) was fed a standard NaCl diet containing ARB (0.02% Olmesartan, 5mg/kg/day, Daiichi-Sankyo, Tokyo, Japan), and the ZFR+High+ARB group (n = 8) was fed a high NaCl diet containing ARB. The body weight of each group was 210–230 g. The rats were housed

[1] Department of General medicine and Emergency care, Faculty of Medicine, Toho University, Tokyo, Japan
[2] Tokyo Kidney Research Institute, Tokyo, Japan
[3] Department of Cellular Physiology, Institute of Nephrology, Niigata University, Graduate School of Medical and Dental Studies, Japan
[4] Department of Pathology, Faculty of Medicine, Toho University, Japan
[5] Department of History, Faculty of Letters, Hosei University, Japan

Corresponding author:
Kazushige Nakanishi, Department of General medicine and Emergency care, Faculty of Medicine, Toho University, 6-11-1, Omorinishi, Ota-ku, Tokyo 143-8541, Japan.
Email: kazunaka@med.toho-u.ac.jp

in a room at a temperature of 23±1°C, and a 12-hour light/dark cycle, and they were allowed free access to food and water. The experiments were carried out in accordance with the Animal Experimentation Guidelines of Toho University.

Blood pressure and biochemical measurement

Beginning at 6 weeks of age, body weight was recorded and systolic blood pressure (SBP) was measured every 3 weeks in all conscious rats using the indirect tail-cuff method (BP-98A; Softron, Tokyo, Japan) on a 37°C pre-heated cloth jacket for about 10 min. The mean of three such recordings were taken as the individual's SBP and heart rate. Each rat was transferred to a metabolic cage for the collection of a 24-hour urine sample. All urine for each rat was collected for the measurement of urinary protein concentration. At 12 weeks into their specified diet, they were anaesthetised with Inactin (100 mg/kg) after a 2-hour fasting period. Blood samples were obtained from the inferior vena cava and the animals were then sacrificed.

Erythrocyte velocity

Observations of microcirculation blood flow were made with an intravital microscope system (Nikon, Tokyo, Japan), equipped with a real-time confocal scanner unit (model CSU10) and image processing devices, as described previously.[6,7] To measure erythrocyte velocity, a batch of erythrocytes labelled with fluorescein isothiocyanate (FITC) was injected intravenously. Briefly, washed erythrocytes obtained from an experimental rat were incubated with a phosphate-buffered saline (PBS: 137 mmol/l NaCl, 6.4 mmol/l Na_2HPO_4, 2.7 mmol/L KCl, and 1.5 mmol/LKH_2PO_4 at pH 7.8) solution containing 1 mg/mL FITC (ICN Pharmaceutical, Inc., Cleveland, OH, USA). The labelled red blood cells (RBCs) were then washed twice with a saline solution containing 1% bovine serum albumin (BSA; Sigma Chemical Co.) to remove free dye. The final volume percentage of the labelled RBCs was adjusted to approximately 50% by adding an isotonic saline solution, and an aliquot of these suspensions was injected (1 ml/kg) into the tail vein of the each rat to measure erythrocyte velocity. The proportion of labelled RBCs compared with that of all RBCs was about 1% according to a single calculation. From the recorded images, erythrocyte velocity was calculated by frame-by-frame analysis, and more than five different areas were measured and averaged across at least five measurements.

Histological analysis

The rats were anaesthetised with Inactin (100 mg/kg), and the kidneys were removed just after the blood samples had been drawn. The left kidney was cut along the long axis and one half was used for light microscopic studies, and the other for immunofluorescence. The specimens for light microscopic examination were fixed with 10% neutral buffered formalin solution, and embedded in paraffin. Then they were sectioned at 2-μm-thick slices and stained with haematoxylin–eosin (HE) and periodic acid silver methenamine (PASM)–HE.

In the sections of the kidneys from each rat, morphological studies were performed by two experienced pathologists in a blinded trial. We tried to estimate the histopathological findings semi-quantitatively using PASM-HE sections. We took 20 microphotographs using a digital microscopic camera (Olympus BX61) at 100-fold magnification in each rat, in random areas that did not overlap. We counted the total number of glomeruli, and also assessed the frequency of global glomerulosclerosis and focal and segmental glomerulosclerosis (FSGS) in each microphotograph. FSGS was diagnosed and classified according to the working group criteria by D'agati et al.[8] We tried to estimate the glomerular hypertrophy by scoring the microphotographs containing enlarged glomeruli with diameters exceeding 150 μm (1: microphotographs filled with enlarged glomeruli; 0: filled with normal glomeruli; 0.5: mixed, with both enlarged and normal glomeruli). The mesangial expansion of glomeruli including an increase in mesangial matrix and/or mesangial cell proliferation was scored as follows: 0: not detectable; 1: mild; 2: moderate; 3: marked. The mesangial scores were calculated by multiplying each of the affected glomeruli by the degree of the mesangial expansion and these were then added together. The tubular atrophy, interstitial fibrosis and interstitial cell infiltration were estimated as the percentage of the affected area occupying each microphotograph. These were scored as follows: 0: none; 1: 0–30%; 2: 31–50%; 3: more than 51%. Interstitial cell infiltration was scored as follows: 1: mild; 2: moderate; 3: marked. The protein casts in the tubules were scored as follows: 0: none; 1: sparse; 2: frequent; 3: very frequent. These scores were summed in each group and analysed statistically. We counted the total number of the vertical and transverse cross sections of these arterioles and also enumerated the number of arterioles with more than three layers of smooth muscle cell (SMC) walls in each microphotograph, added them up, and statistically analysed them across the four groups. We excluded bifurcations, with just the initiation of preglomerular afferent or asymmetrically sectioned vessels, which were inadequate for examination. We examined the larger arteries for the changes in their walls.

Immunofluorescence

The specimens used for immunofluorescence analysis were divided immediately after nephrectomy and snap frozen at –90°C. They were stored at –80°C until examination and then were sliced into 3 μm sections using a cryostat and

subjected to direct immunofluorescence analysis using FITC-labelled rabbit anti-rat angiotensin II receptor 1 (Abcam, MA, USA), rabbit anti-rat inducible nitric oxide synthetase (iNOS: Abcam), rabbit anti-rat endothelial NOS (eNOS: Affinity BioReagents, Inc. CO, USA) and goat anti-rat renin (a gift from Professor Tadashi Inagami, Vanderbilt University, USA) antibodies. The biotinylated mouse monoclonal antibody OX7 (Abcam) together with tetramethylrhodamine isothiocyanate (TRITC)-labelled secondary antibodies were used as localisation markers for double staining and examined by confocal laser scanning microscopy (LSM 510, Carl Zeiss, Germany). The staining with each primary antibody was estimated semi-quantitatively (0: negative; 0.5: undeterminable; 1: positive; 3: strongly positive; 5: extremely strongly positive) in each observed glomerulus, and the scores were analysed statistically between the groups.

Statistical analysis

Values are presented as means ± SD. One-way repeated-measures analysis of variance (ANOVA) was performed for each group followed by the Kruskal–Wallis test to assess the significance of results. Values of $p < 0.05$ were considered to be significant. The Tukey test was used to assess differences between each group.

Results

Table 1 summarises the physiological and biochemical data at 12 weeks after the initiation of various diet regimens. Body weight tended to be higher in the ZFR group compared with the ZFR+ARB group. However, the ratio of kidney weight to body weight was not significantly different among the four groups. SBP was lower in the ZFR+ARB group than in the ZFR group, but there was no difference between the ZFR+High and ZFR+High+ARB groups. Urinary protein excretion was significantly decreased in the ZFR+ARB group compared with the ZFR group, but no significant difference was found between the ZFR+High and ZFR+High+ARB groups. Serum glucose levels were not statistically different among the four groups. Olmesartan significantly lowered SBP and urinary protein excretion in the normal salt group.

Figure 1 shows the change in erythrocyte velocity within glomeruli. There were no significant differences among the four groups (855±185, 747±175, 830±139 and 743±151 μm/s, respectively).

Histological findings

The total numbers of glomeruli observed in microphotographs of each rat showed no significant differences among the four groups (Table 2). Global glomerulosclerosis was rarely observed and its frequency among the four groups was not significantly different. FSGS, usually shown as 'FSGS (not otherwise specified) variant' in morphological classification, without hyalinosis, was rarely observed in the ZFR, ZFR+ARB or ZFR+High+ARB groups. In the ZFR+High group, it was seen more frequently, however the increase in its frequency was not statistically significant. The scores indicating hypertrophied glomeruli were significantly lower in the ZFR+ARB group compared with those of the ZFR+High group. The mesangial scores were significantly lower in the ZFR+ARB group than in the two groups that did not receive ARB (Figures 2–5 and Table 2). The scores of protein casts were significantly decreased in the ZFR+ARB group compared with those in the two groups that did not receive ARB. The tubulointerstitial changes showed no statistical differences among the four groups (Table 2).

Table 1. Physiological and biochemical changes

	ZFR	ZFR+High	ZFR+ARB	ZFR+High +ARB
Body Weight (g)	666±72	723±32	556±65 **p = 0.002	672±71
Kidney weight (g/Kg EW)	2.6±1.4	3±1.5	2.3±1.2	2.7±1.4
Systolic blood pressure (mmHg)	130±8.2	133±9.5	80±16.6 *p = 0.002 **p = 0.0009	116±128
Urinary protein (mg/day)	302±220	200±61	9.1±1.5 *p = 0.01	15.9±5.9
Serum glugose (mg/dl)	670±562	489±298	332±201	314±106

*significant difference from ZFR. **significant difference from ZFR+High. BW: body weight.

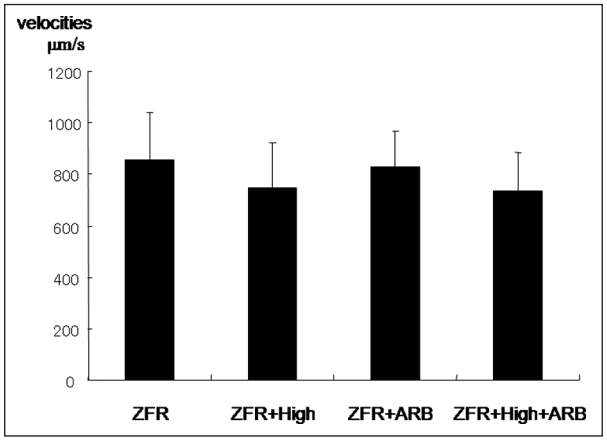

Figure 1. Erythrocyte velocities within glomeruli.

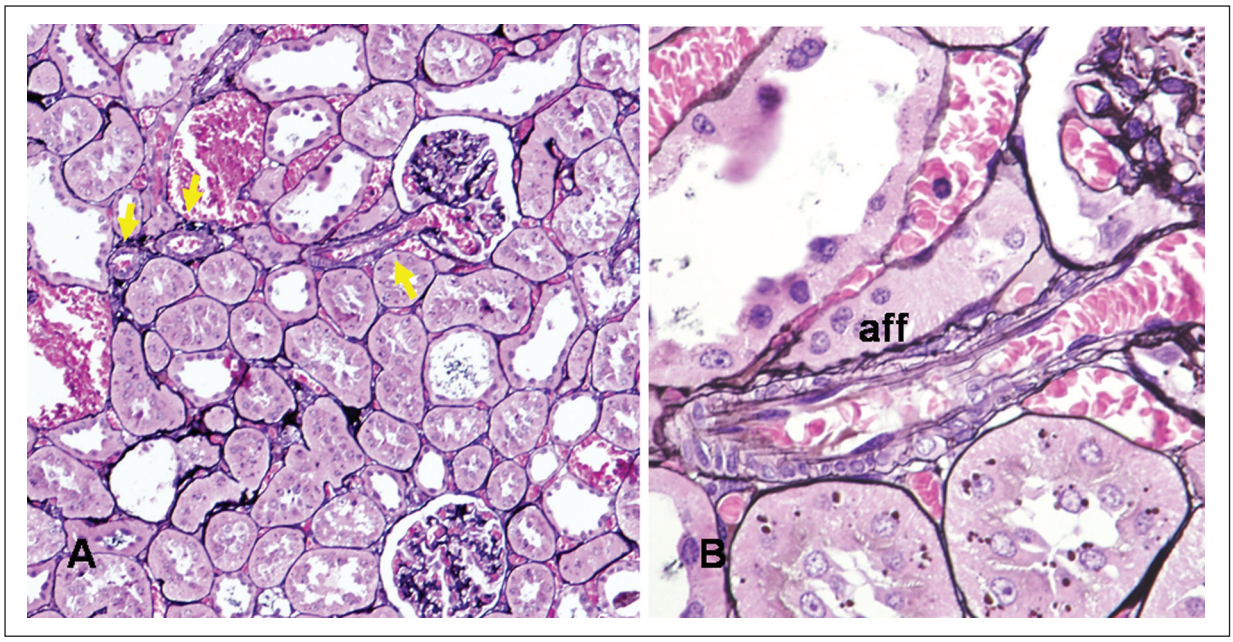

Figure 2. Stained with PASM-HE: the microphotographs of the kidney in the ZFR with normal diet. 2A: a microphotograph with low magnification (x100). Afferent arterioles (arrows) show no remarkable changes in their walls. The arteriolar smooth muscle cells are within two layers, show regularities in size, shape and arrangement. Mild global mesangial expansion is observed in one glomerulus. 2B: A glomerulus with intact afferent arteriole in higher magnification (x400). Aff: afferent arteriole.

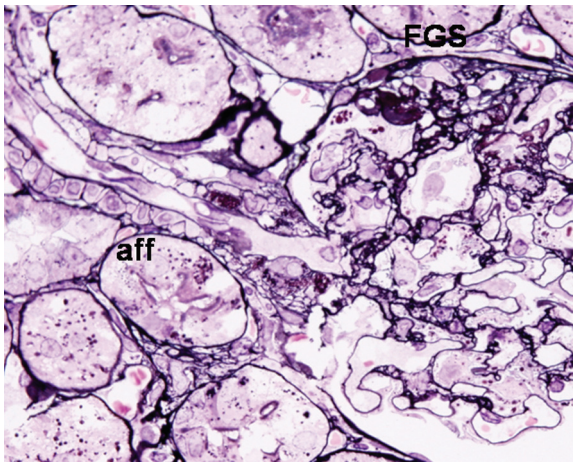

Figure 3. Stained with PASM-HE: the microphotograph of the kidney in the ZFR with high salt diet. A glomerular hypertrophy with segmental scrolosisig lesion is seen. The smooth muscle cells in the afferent arteriolar wall shows a slight irregularity in size and arrangement, but the wall is one layer and it is not thickened (x400). Aff: afferent arteriole, FGS: focal segmental glomeruloscrelosis.

The total numbers of interlobular arteries (IAs) observed in the microphotographs per kidney in each group showed no significant differences among the four groups. In the ZFR and ZFR+High groups, a few arteries showed a mild increase in the number of SMC layers in their walls. Some were associated with irregularly swollen endothelial cells. In the ZFR+ARB group, IAs that demonstrated proliferative changes in their SMC layers made up 54.1±36.3% of the observed IAs. Such findings were significantly more frequent in the ZFR+ARB group than in the two groups that did not receive ARB. In the ZFR+High+ARB group, thickened IAs accounted for 17.9±17.0 (0–47 in each) %, but the difference was not statistically significant (Table 3). Arteries bigger than IAs showed neither elastofibrosis of intima nor SMC thickening. A few arteries showed very slight irregularities in the arrangement of their SMCs.

The numbers of vertically and transversely sectioned arterioles observed in the microphotographs were not statistically different among the four groups. The total arteriolar number was not significantly different among the groups. In the two groups without ARBs, the vascular wall thickening, i.e. the increase of the SMC layers were not frequently observed, but SMC irregularities were sometimes seen. Mild to moderate endothelial cell swelling of the afferent arterioles were also occasionally observed (Figures 2 and 3, and Table 3).

Compared with the two groups described above, striking changes were observed in the afferent arterioles of the ZFR+ARB and ZFR+High+ARB groups. The arteriolar SMCs frequently showed a great increase in numbers of layers and cells, which showed marked irregularities in size and shape (Figures 4 and 5). The number of the arterioles with more than three SMC layers was significantly increased in the ZFR+ARB group (77.3±10.3% per rat in all observed arterioles) compared with those in the ZFR and ZFR+High groups ($p < 0.005$, $p < 0.0005$; Figure 6). In the

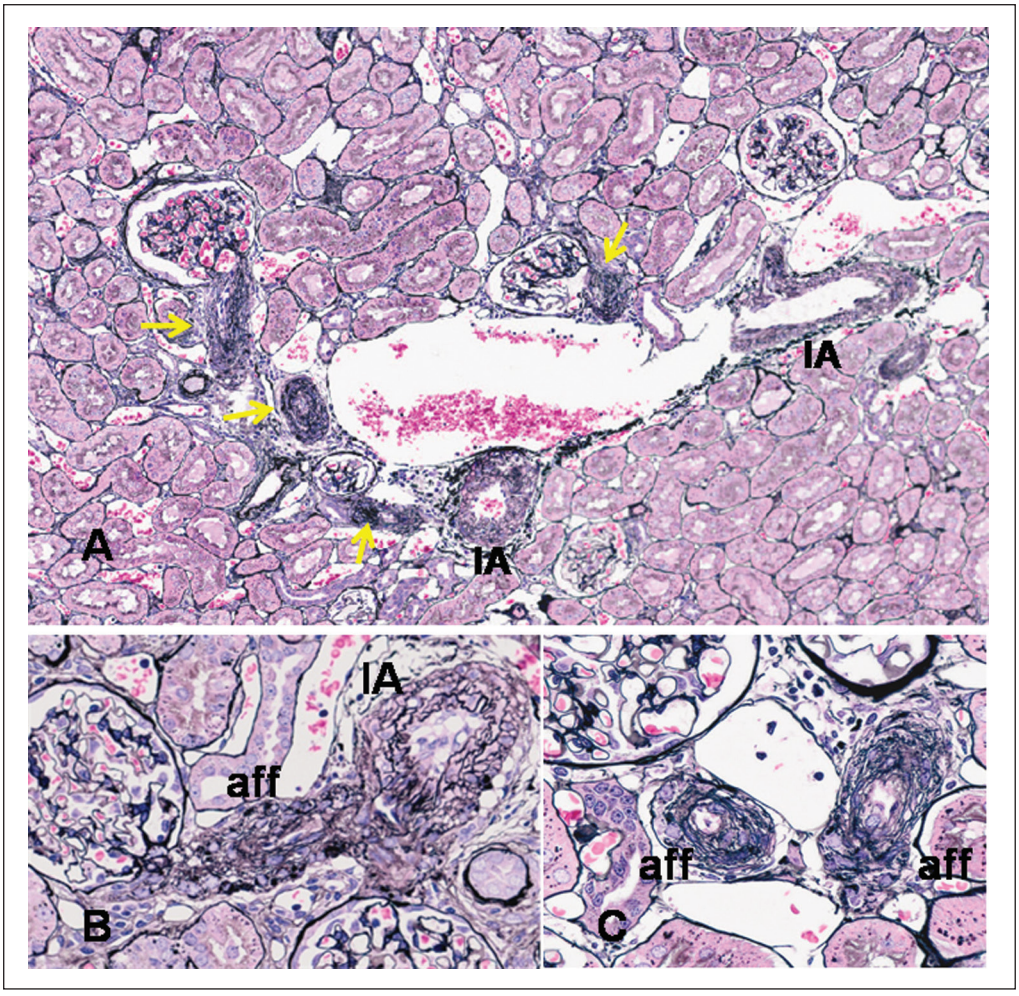

Figure 4. Stained with PASM-HE: the microphotographs of the kidney in the ZFR with normal diet and the treatment of Olmesartan. 4A: In lower magnification (x100), smooth muscle cells in afferent arterioles (arrows) show a vast increase in layers and numbers, marked irregularities in size, shape and arrangement. 4B: The proliferative changes extend from glomerular hilum to bifurcation of the interlobular arteriy. The number of granular cells increases extremely in the afferent arteriolar walls. 4C: The vertically cross-sectioned afferent arterioles show concentric multiplying smooth muscle cell layers in the walls and result in narrowing the lumens. Renin granules are evidently seen in arteriolar walls apart from hilum (x400). IA: interlobular artery, Aff: afferent arteriole.

ZFR+High+ARB group, it was also increased (37.4±15.6%; Figure 6), however, the difference was not statistically significant. In the proliferative arteriolar walls we observed neither necrotic lesions nor hyaline deposits. The marked SMC proliferation showed maximum thickening at approximately one third of the total length of afferent arterioles from the glomerulus and narrowed the arteriolar lumens (Figures 4 and 5).

Endothelial cell swelling was sometimes seen. The increased granular cells of afferent arterioles were frequently seen in the two groups treated with ARBs. The increased renin granules were clearly observed in PASM–HE stain in the markedly increased granular cells which were started at the preglomerular afferent, and sometimes extended into the middle of the afferent arterioles. The efferent arterioles showed fewer changes than the afferent and SMC layers were not increased. However, SMC irregularities were often seen in the two groups treated with ARBs.

Immnofluorescence studies

In the ZFR and ZFR+High groups, the renin staining was defined as undeterminable or positive in the preglomerular afferent arteriole. Semi-quantitative analysis showed very low scores in both groups. The ZFR+ARB and ZFR+High+ARB groups showed extremely positive (enlarged length and width as well as increased quantities) renin staining in the many preglomerular afferent arterioles (Figure 7). The scores of the two groups were strikingly higher compared with that of the two ZFR groups without ARB (Figure 8). Anti-AT-1 receptor, VEGF, eNOS and iNOS antibodies were weakly positive at the arterioles which were close to glomeruli in all four groups. There were no significant differences in the staining of these four antibodies among the four groups.

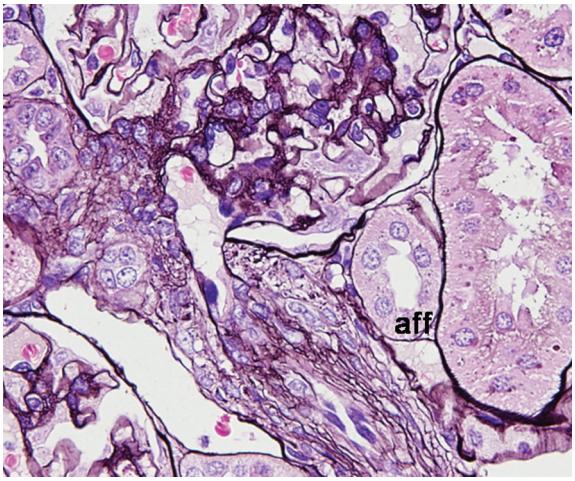

Figure 5. Stained with PASM-HE: the microphotograph of the kidney in the ZFR with high-salt diet and the treatment of Olmesartan. The smooth muscle cells in the afferent arteriolar wall proliferates same as Fig.4 (x400). Aff: afferent arteriole.

Discussion

In this study, we divided ZFRs into four groups to investigate the effect of an ARB. ARB treatment significantly inhibited weight gain in the ZFR+ARB group, clearly indicating that ARBs have an antiobesity effect on ZFRs.[9] Postprandial blood glucose levels showed some variation, but tended to be lower in the two groups treated with ARB than in the two groups that were not. The present study showed no difference in blood pressure between the ZFR and ZFR+High groups, which did not develop salt-sensitive hypertension. Previous studies reported varying results on the salt sensitivity of hypertension in ZFRs.[10-12] In this study, the ZFR+ARB group which received ARB under low-salt conditions showed a clear decrease in the blood pressure, whereas the ZFR+High+ARB group treated with ARB under high-salt conditions did not present a significant blood pressure reduction, indicating that the antihypertensive effect of ARB was markedly decreased under high-salt conditions. Considering the action mechanism of ARB, this is not surprising, and has been demonstrated by the previous study.[13] In high-salt conditions, blood volume increases and the renin–angiotensin–aldosterone (RAA) system is already depressed. Therefore, the blockade of RAA system by ARBs induces a smaller effect.

In the present study, as reported by many researchers,[14-16] we observed a marked reduction in urinary protein excretion and a significant decrease in mesangial expansion and glomerular hypertrophy as the nephro-protective effects of ARBs in the ZFR+ARB group. These effects were not significantly different in the ZFR+High+ARB group, indicating that the effects of ARBs were attenuated under high-salt conditions.

It is now generally recognised that in various kidney diseases and hypertensive states, intraglomerular haemodynamics, particularly glomerular hypertension, is an important factor causing proteinuria, glomerular structural changes and tissue damage which are common pathway of chronic renal dysfunction.[17-19] ARBs are known to dilate efferent arterioles more than afferent arterioles, and to

Table 2. Histological findings

	ZFR	ZFR+High	ZFR+ARB	ZFR+High+ARB
<glomerulus>				
total numbers	79.14±10.53	71.71±9.18	77.42±16.2	79.28±9.14
global sclerosis/total glomeruli (%)	0.14±0.38	0	1.83±4.86	0.1±0.50
FSGS/total glomeruli (%)	0.41±0.70	0.41±0.70	6.03±8.82	0.49±1.00
glomerular hypertrophy scores	0.43±0.18	0.50±0.08	0.15±0.11 **(p = 0.004)	0.4±0.18
mesangial expansion/total glomeruli (%)	26.42±12.05	16.95±6.06	5.30±1.49 *(p = 0.003)	13.61±8.30
mesangial scores	0.15±0.05	0.13±0.05	0.04±0.02 *(p = 0.005) **(p = 0.03)	0.10±0.05
<tubules>				
protein casts scores	0.37±0.23	0.5±0.37	0.03±0.05 *(p = 0.03) **(p = 0.007)	0.11±0.17
tubular atrophy scores	0.29±0.19	0.41±0.29	0.77±0.61	0.24±0.09
<interstitial tissue>				
fibrosis scores	0.29±0.24	0.38±0.26	0.76±0.68	0.22±0.14
cell infiltration scores	0.22±0.21	0.31±0.30	0.54±0.43	0.13±0.12

*significant difference from ZFR. **significant difference from ZFR+High. FSGS: focal segmental glomeruloscrelosis.

Table 3. Histological vascular changes

	ZFR	ZFR+High	ZFR+ARB	ZFR+High+ARB
<artery>				
total numbers	12.14±5.76	13.43±4.67	21.43±5.53	16.57±8.99
total numbers of IAs	10±4.54	10.42±3.73	16.57±4.31	13.57±3.55
IAs with increased smooth muscle cell layers (%)	4.58±7.83	2.04±5.39	54.07±36.33 *(p = 0.03) **(p = 0.006)	17.90±17.01
<arteriole>				
vertical sections	40.57±15.96	45±10.71	43.1±9.28	42.71±16.59
transverse sections	35±6.11	34.71±7.93	41±4.89	40.57±7.63
total number of arterioles	75.57±17.80	79.71±8.69	84.14±9.10	83.29±19.53
number of the arterioles with more than three layers of smooth muscle cell walls (%)	1.77±1.56	1.27±22.8	77.34±10.35 *(p = 0.003) **(0.0004)	37.4±15.6

*significant difference from ZFR. **significant difference from ZFR+High. IA: interlobular artery.

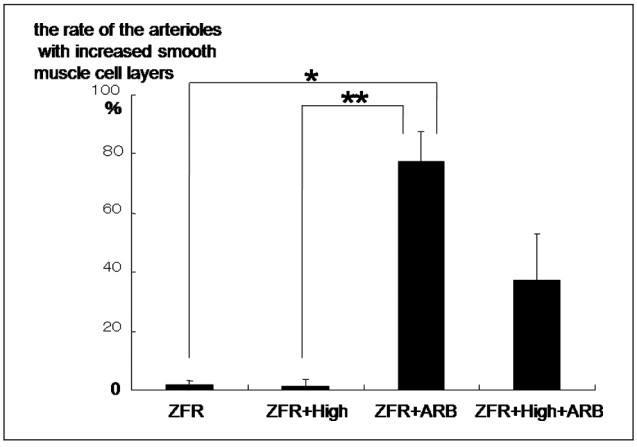

Figure 6. The rate of the arterioles with increased smooth muscle cell layers in the observed total arterioles. The numbers of the arterioles with more than three SMC layers in their walls are significantly increased in the ZFR+ARB group (77.34±10.35% per rat in all observed arterioles) compared to those in the ZFR and ZFR+High groups ($p<0.005$, $p<0.0005$). In the ZFR+High+ARB group, it was also increased (37.4±15.6%), however, the difference was not statistically significant.

improve glomerular hypertension because AT-1 receptors exist more abundantly in the former.[20] Reports on the nephro-protective effects of ARBs are diverse in methodology;[20-27] however, few studies have concentrated on the morphology of afferent and efferent arterioles.

In this study, we examined the morphology of rat renal tissue and noted a marked improvement in glomerular lesions and tubular protein casts in the ZFR+ARB group. Proliferation of renin-producing cells and marked increase of renin granules was very frequently observed in the two groups with ARB, which have already been reported as a result of the drug-induced RAA feedback mechanism.[4-6,28-31] In addition, we found a remarkable increase in the numbers and layers of afferent arteriolar SMCs and marked irregularities in the arrangement and morphology of SMCs. These proliferative changes in the afferent arteriolar walls are quite different from the changes that have been observed in other hypertensive, inflammatory or drug-induced vascular lesions. In the 1980s and 1990s, several patho-toxicological studies using rats and monkeys reported that the angiotensin-converting enzyme inhibitor (ACEI) captopril [29,30] and ARBs such as losartan potassium[5,31] commonly induced JGA hyperplasia and afferent arteriolar thickening;[4,5,29,31] however, those studies did not describe the arteriolar changes at a minute level.

The present study revealed unprecedented wall changes in a high percentage of afferent arterioles in the ARB-administered ZFR+ARB group (77.3±10.3%) and ZFR+High+ARB group (37.4±15.6%). Since ARB haves been reported to cause maximally a 13% increase in effective renal plasma flow,[32] it is likely that blood flow itself is increased down to the levels of renal arterioles. Extant research has claimed that the dilatation of efferent arterioles is an important factor, however; our results strongly suggest that the marked thickening of the wall in afferent arterioles is more important in the reduction of blood flow to glomeruli therefore improving glomerular hypertension. We measured the glomerular erythrocyte velocity which is an index of the glomerular blood flow velocity.[6] The mean velocities in the four groups showed no significant differences; moreover, most afferent arterioles in the two groups treated with ARBs were accompanied by markedly thickened walls and

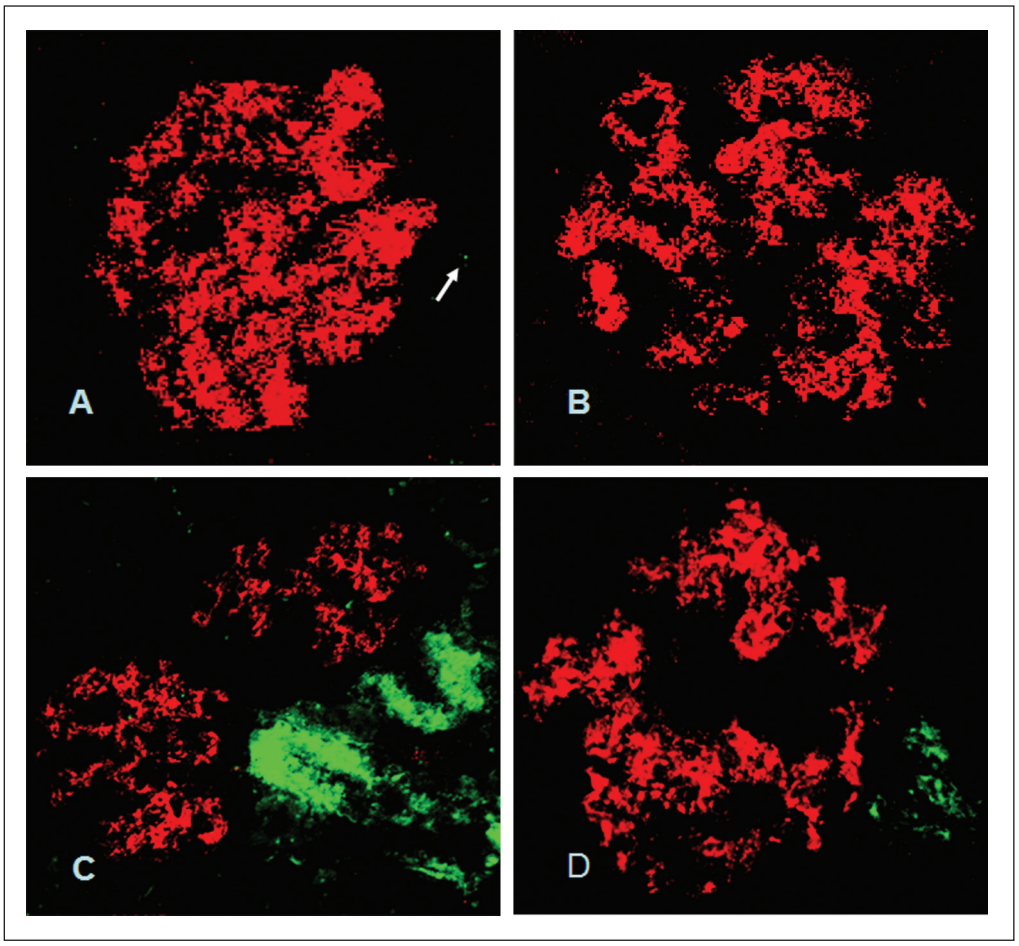

Figure 7. Laser scan microphotographs in the four groups. The frozen sections were first stained using FITC labeled renin antibody (green), and then the specimens were reacted with TRITC labeled OX-7 antibody (red). 7A: a glomerulus shows the markedly positive stained OX-7 antibody in expanded mesangial area, but weakly positive with renin antibody in the afferent arteriole (arrow, x400) in the ZFR group. 7B: a glomerulus shows marked mesangial expansion same as A, and negative renin antibody in the ZFR+High group (x400). 7C: two glomeruli show the extremely strong positive stained renin antibody in the ZFR+ARB group. The arterioles are thickened with renin producing cells along the arteriolar walls. Mesangial expansion is not observed (x100). 7D: A glomerulus shows mild mesangial expansion and strongly positive staining of renin in the ZFR+High+ARB group (x400).

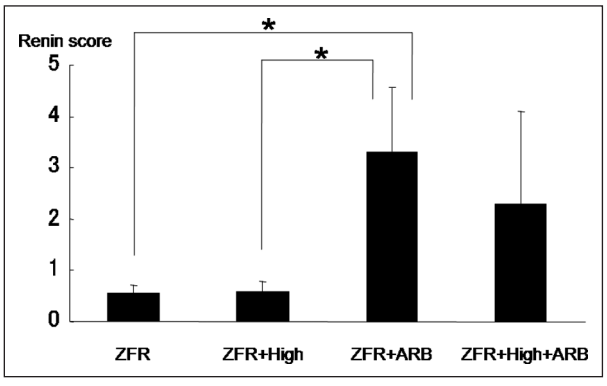

Figure 8. The scores of rennin: semi-quantitative analysis of renin antibody staining. The scores of the two groups treated with ARB were strikingly higher compared to the scores of the two groups that did not receive ARB.

strikingly narrowed lumens. These results indicated that ARB also reduced glomerular blood flow volume and improved glomerular hypertension compared with the two non-ARB groups with the same velocity of glomerular blood flow and morphologically normal afferent arterioles.

Increased renin-producing granular cells and the SMCs of afferent arterioles stem from vascular SMCs, and function with close communication,[7,33] but the factor causing SMCs to proliferate is unclear. In recent years, a pathway from AII to AIII and then a pathway to angiotensin IV leading toward cell proliferation and hypertrophy were discovered.[34,35] However, the angiotensin IV-mediated pathway is almost completely blocked by ACEI in rats, and the afferent arteriolar thickening was previously reported to be induced by ACEI as well;[28,29,36] therefore, their proliferation is unlikely to be mediated by angiotensin IV.

The possibility of renin itself being the growth factor must be considered. However, although studies reported that renin caused the proliferation of mesangial cells,[37,38] only one study reported that human prorenin/renin enhanced human vascular SMC proliferation and increased the expression of extracellular signal-regulated kinase (ERK), a growth factor for SMCs , independent of angiotensin II signalling pathways in culture.[39] In immunofluorescent studies, the expression of anti-renin antibody increased significantly in the renal tissue of the ZFR+ARB group. While the semiquantative analysis of tissue renin is not strictly accurate, it is clear there was a big difference between the two groups given ARB and the two groups without ARB shown in figure 8.

Other possible causes of these changes in the arterioles include the relatively large dose of ARB. However, its dose was estimated at 5 mg/kg/day, which is not an unusually high dose. ZFRs represent a special genetic model of obesity carrying a mutant gene; however, we also confirmed that similar morphological changes have occurred using spontaneous hypertensive rats and Wistar Kyoto rats in a preliminary study. Thus, they are unlikely to occur in this strain of rats alone. There are no reports of such changes in humans; however, hypertensive patients taking ARBs for a long period are unlikely to undergo renal biopsy, and such changes are easily overlooked, and dismissed as mere hypertensive thickening of the arterioles on HE stain.

The afferent arteriolar thickening had been reported with other angiotensin II receptor blockers, losartan[5] and DUP532,[31] and the proliferative changes in SMCs are suspected to be induced by a feedback system of RAA itself, which is common in any ARB. We consider that the effect of the thickening in the renal afferent arteriolar walls due to proliferation of SMCs must be induced not only by this specific ARB but also by other ARBs as a class effect.

Currently, ARB reigns supreme in the treatment of kidney disease, heart disease,[40] obesity and possibly to some extent in dementia,[41] as well as hypertension. However, a drug-induced blockade of the RAA system disrupts this biologically exact network. Further studies are needed to see if the unusual proliferative changes of the afferent arterioles in ZFRs induced by ARBs finally played a protective role for glomeruli against the increased pressure of blood flow, or whether it is a side effect leading to irreversible damage on afferent arterioles.

Acknowledgements

We are grateful to Harumi Tamura and Toshie Shimozeki (Department of Pathology, Toho University) for their excellent technical assistance. We would also like to thank Mitsuko Sato and Akemi Kaneko (Toho University) for performing blood pressure measurement, Dr Satoko Oda for her role in the taking confocal laser microphotographs (Department of Anatomy, Toho University) and Professor Chiaki Nishimura (Department of Informatics, Toho University) for his helpful suggestions during the statistical analysis.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Tominaga N, Robert A, Izuhara Y, Ohtomo S, Dan T, Chihara K, et al. Very high doses of valsartan provide renoprotection independently of blood pressure in a type 2 diabetic nephropathy rat model. *Nephrology* 2009; **14**: 581-587.
2. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S and Arner P; Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; 345: 870-878.
3. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. for the RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; 345: 861-869.
4. Jackson DG and Jones HB. Histopathological and ultrastructural changes in the juxtaglomerular apparatus of the rat following administration of ZENECA ZD6888 (2-ethyl-5,6,7,8-tetrahydro-4-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methoxy]quinoline), an angiotensin II antagonist. *Toxicol Pathol* 1995; 23: 7-15.
5. Owen RA, Molon-Noblot S, Hubert MF, Siegl PK, Eydelloth RS and Keenan KP. Juxtaglomerular cell hypertrophy and hyperplasia induced in rhesus monkeys by angiotensin II receptor antagonists. *Lab Invest* 1994; 71: 543-551.
6. Oyanagi-Tanaka Y, Yao J, Wada Y, Morioka T, Suzuki Y, Gejyo F, et al. Real-time observation of hemodynamic changes in glomerular aneurysms induced by anti-Thy-1 antibody. *Kidney Int* 2001; 59: 252–259.
7. Yao J, Oite T and Kitamura M. Gap junctional intercellular communication in the juxtaglomerular apparatus. *Am J Physiol Renal Physiol* 2009; 296; F939-F946.
8. D'agati V, Fogo AB, Bruijin JA and Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am J Kidney Dis* 2004; 43: 368-382.
9. Makita S, Abiko A, Naganuma Y, Moriai Y and Nakamura M. Effects of telmisartan on adiponectin levels and body weight in hypertensive patients with glucose intolerance. *Metabolism* 2008; 57: 1473-1478.
10. Pawioski CM. Kanagy NL, Mortensen LH and Fink GD. Obese Zucker rats are normal and increased sodium intake. *Hypertension* 1992; 19: I90-I95.
11. Reddy SR and Kotchen TA. Dietary sodium chloride increases blood pressure in obese Zucker rats. *Hypertension* 1992; 20: 389-393.
12. Riazi S, Madala-Halagappa VK, Dantas AP, Hu X and Ecelbarger CA. Sex differences in renal nitric oxide synthase, NAD(P)H oxidase, and blood pressure in obese Zucker rats. *Gend Med* 2007; 4: 214-229.
13. Endo S, Mori T, Yoneki Y, Nakamichi T, Hosoya T, Ogawa S, et al. Blockade of angiotensin II type-1 receptor increases

salt sensitivity in Sprague–Dawley rats. *Hypertension Res* 2009; 32: 513-519.
14. Russo D, Pisani A, Balletta MM, De Nicola L, Savino FA, Andreucci M, et al. Additive antiproteinuric effect of converting enzyme inhibitor and losartan in normotensive patients with IgA nephropathy. *Am J Kidney Dis* 1999; 33: 851-856.
15. Watanabe D and Tanabe A. Renoprotective effects of an angiotensin II receptor blocker in experimental model rats with hypertension and metabolic disorders. *Hypertens Res* 2009; 32: 807-815.
16. Liao J, Kobayashi M and Kanamuru Y. Effects of candesartan, an angiotensin II type 1 receptor blocker, on diabetic nephropathy in KK/Ta mice. *Nephrology* 2003; 16: 841-849.
17. Brenner BM, Lawler EV and Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int* 1996; 49: 1774-1777.
18. Neuringer JR and Brenner BM. Hemodynamic theory of progressive renal disease: a 10-year update in brief review. *Am J Kid Dis* 1993; 22: 98-104.
19. Neuringer JR and Brenner BM. Glomerular hypertension: cause and consequence of renal injury. *J Hypertens* 1992; 10: S91-S97.
20. Razga Z and Nyengaard JR. Up- and down-regulation of angiotensin II AT1-A and AT1-B receptors in afferent and efferent rat kidney arterioles. *J Renin Angiotensin Aldosterone Syst* 2008; 9: 196-201.
21. Roos MH, Eringa EC, van Rodijnen WF, van Lambalgen TA, Ter Wee PM and Tangelder GJ. Preglomerular and postglomerular basal diameter changes and reactivity to angiotensin II in obese rats. *Diabetes Obes Metab* 2008; 10: 898-905.
22. Naito M, Shenoy A, Aoyama I, Koopmeiners JS, Komers R, Schnaper HW, et al. High ambient glucose augments angiotensin II-induced proinflammatory gene mRNA expression in human mesangial cells: effects of valsartan and simvastatin. *Am J Nephrol* 2009; 30: 99-111.
23. Schiffrin EL. Vascular changes in hypertension in response to drug treatment: Effects of angiotensin receptor blockers. *Can J Cardiol* 2002; 18: 15A-18A.
24. Cianchetti S, Del Fiorentino A, Colognato R, Di Stefano R, Franzoni F and Pedrinelli R. Anti-inflammatory and anti-oxidant properties of telmisartan in cultured human umbilical vein endothelial cells. *Atherosclerosis* 2008; 198: 22-28.
25. Shao J, Nangaku M, Inagi R, Kato H, Miyata T, Matsusaka T, et al. Receptor-independent intracellular radical scavenging activity of an angiotensin II receptor blocker. *J Hypertens* 2007; 25: 21643-21649.
26. Yu Y, Fukuda N, Yao EH, Matsumoto T, Kobayashi N, Suzuki R, et al. Effects of an ARB on endothelial progenitor cell function and cardiovascular oxidation in hypertension. *Am J Hypertens* 2008; 21: 72-77.
27. Ferrario C. Effect of angiotensin receptor blockade on endothelial function: focus on olmesartan medoxomil. *Vasc Health Risk Manag* 2009; 5: 301-314.
28. Doughty SE, Ferrier RK, Hillan KJ and Jackson DG. The effects of ZENECA ZD8731, an angiotensin II antagonist, on renin expression by juxtaglomerular cells in the rat: comparison of protein and mRNA expression as detected by immunohistochemistry and in situ hybridization. *Toxicol Pathol* 1995; 23: 256-261.
29. Hashimoto K, Imai K, Yoshimura S and Ohtaki T. [Twelve month studies on the chronic toxicity of captopril in rats]. *J Toxicol Sci* 1981; 6: 215-246 (in Japanese).
30. Zaki FG, Keim GR, Takii Y and Inagami T. Hyperplasia of juxtaglomerular cells and renin localization in kidney of normotensive animals given captopril. Electron microscopic and immunohistochemical studies. *Ann Clin Lab Sci* 1982; 12: 200-215.
31. Owen RA, Molon-Noblot S, Hubert MF, Kindt MV, Keenan KP and Eydelloth RS. The morphology of juxtaglomerular cell hyperplasia and hypertrophy in normotensive rats and monkeys given an angiotensin II receptor antagonist. *Toxicol Pathol* 1995; 23: 606-619.
32. Gansevoort RT, Zeeuw D and Jong PE. Is the antiproteinuric effect of ACE inhibition mediated by interference in the renin-angiotensin system? *Kidney Int.* 1994; 45: 861-867.
33. Stephen M. Renal anatomy and Histology. In Jonette CL (Ed.), *Pathology of the Kidney* (6th Ed.) Philadelphia, PA: Lippincott Williams Wilkins, 2007; pp. 1-70.
34. Chai SY, Fernando R, Peck G, Ye SY, Mendelsohn FA, Jenkins TA, et al. The angiotensin IV/AT4 receptor *Cell Mol Life Sci* 2004; 61: 2728-2737.
35. Donna MR, Hanes S and Klassen DK. Antihypertensive drugs. In Brenner BM (Ed.), *Brenner & Rector's The Kidney* (8th Ed.). Saunders, 2008; pp. 1596-1645.
36. Boorman GA. *Vascular Lesions in Pathology of the Fischer Rat*. New York: Academic Press 1990, p. 151.
37. Huang Y, Noble NA, Zhang J, Xu C and Border WA. Renin-stimulated TGF-beta1 expression is regulated by a mitogen-activated protein kinase in mesangial cells. *Kidney Int* 2007; 72: 45-52.
38. He M, Zhang L, Shao Y, Wang X, Huang Y, Yao T, et al. Inhibition of renin/prorenin receptor attenuated mesangial cell proliferation and reduced associated fibrotic factor release. *Eur J Pharmacol* 2009; 606: 155-161.
39. Sakoda M, Ichihara A, Kaneshiro Y, Takemitsu T, Nakazato Y, Nabi AH, et al. (Pro)renin receptor-mediated activation of mitogen-activated protein kinases in human vascular smooth muscle cells. *Hypertens Res* 2007; 30: 1139-1146.
40. Weir MR. Effects of renin-angiotensin system inhibition on end-organ protection: can we do better? *Clin Ther* 2007; 29: 1803-1824.
41. Ostergren JB. Angiotensin receptor blockade with candesartan in heart failure: findings from the Candesartan in Heart failure—assessment of reduction in mortality and morbidity (CHARM) programme. *J Hypertens* 2006; 24: S3-S7.