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Regression of superficial glomerular podocyte injury in type 2 diabetic rats with overt albuminuria: effect of angiotensin II blockade

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Abstract

Objective—Clinical studies indicate that the remission, regression or both of nephrotic-range albuminuria are exerted by angiotensin II receptor blockers (ARBs) in diabetes. The current study was performed to test the hypothesis that these effects of ARBs are associated with regression of glomerular podocyte injury.

Methods—We examined the effects of an ARB, olmesartan, on glomerular podocyte injury in type 2 diabetic Otsuka–Long–Evans–Tokushima–Fatty rats with overt albuminuria.

Results—At baseline (55-week-old), diabetic Otsuka–Long–Evans–Tokushima–Fatty rats showed severe albuminuria with desmin-positive areas (an index of podocyte injury) in both superficial and juxtamedullary glomeruli, and podocyte injury was much greater in juxtamedullary than in superficial glomeruli. At 75-week-old, Otsuka–Long–Evans–Tokushima–Fatty rats had developed more severe albuminuria and superficial glomerular podocyte injury, whereas juxtamedullary glomerular podocyte injury did not advance further. Olmesartan (10 mg/kg per day) decreased albuminuria and superficial glomerular desmin staining to levels that were lower than those at baseline, whereas advanced juxtamedullary glomerular podocyte injury was not changed.

Conclusion—The current study demonstrates for the first time that juxtamedullary glomerular podocyte injury reaches a severe condition at an earlier time than superficial glomerular podocyte injury during the progression of overt albuminuria in type 2 diabetic rats. Our data also support the hypothesis that the antialbuminuric effects of ARBs are associated with regression of superficial glomerular podocyte injury in type 2 diabetes with nephrotic-range albuminuria.

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There are no conflicts of interest.

Keywords

angiotensin II receptor blockers; juxtamedullary glomeruli; olmesartan; Otsuka-Long-Evans-Tokushima-Fatty rats; podocyte; superficial glomeruli

Introduction

It has been suggested that once patients with diabetes develops overt albuminuria, nephropathy inevitably progresses to end-stage renal disease (ESRD) [1]. However, a growing body of evidence indicates that remission and regression of albuminuria can be achieved in patients with diabetic nephropathy by intensive multidrug treatments to control blood pressure (BP) and glucose levels [2–5]. In particular, large-scale clinical trials [6,7] have revealed that the antialbuminuric effects of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II (AngII) receptor blockers (ARBs) are beyond their blood-lowering and hemodynamic effects, suggesting that the intrarenal renin–angiotensin system (RAS) exerts an essential contribution to the progression of albuminuria in diabetic nephropathy. Furthermore, long-term observational studies have also demonstrated that remission of nephrotic-range albuminuria is associated with substantial reductions in the risks of progressing to ESRD and cardiovascular events, greatly improving the survival rate of type 2 diabetic patients [8]. On the basis of these clinical observations, most national guideline groups [9–12] now recommend the use of ACEIs or ARBs in preference to other antihypertensive agents for hypertensive patients with diabetic nephropathy. However, the mechanisms by which treatment with ACEIs or ARBs leads to remission and regression of overt albuminuria have remained unclear.

Diabetic nephropathy is morphologically characterized by the thickness of the glomerular basement membrane and mesangial matrix expansion [13,14]. However, scoring of these glomerular changes shows variability, particularly in patients with type 2 diabetic nephropathy, because pathological progression of glomerular injury often occurs in a heterogeneous manner [15–17]. Although preclinical studies [18,19] indicate that juxtamedullary glomerular injury is more severe than superficial glomerular injury in some experimental models, clinical studies have limitations in terms of morphological evaluation because the renal needle biopsy technique usually only enables us to investigate glomerular injury in the superficial but not in the juxtamedullary cortical area. Thus, it is possible that there is a problem in the way we currently evaluate morphological changes without clearly distinguishing between superficial and juxtamedullary glomerular injury during the progression of diabetic nephropathy.

There is increasing evidence indicating that glomerular podocyte (glomerular visceral epithelial cells) abnormalities, including functional changes, loss and injury, are cardinal features of diabetic nephropathy, and are essentially involved in the progression of albuminuria, glomerular sclerosis and tubulointerstitial injury [20–22]. However, the heterogeneity between superficial and juxtamedullary glomerular podocyte injury has not been determined. Therefore, the current study was conducted to determine whether glomerular podocytes are damaged in a heterogeneous manner depending on their location during the progression of overt albuminuria in type 2 diabetic Otsuka–Long–Evans–Tokushima–Fatty (OLETF) rats, which exhibit pathological features of renal injury similar to those of human type 2 diabetes [23,24]. Studies [23,25,26] were also performed to determine whether the antialbuminuric effects of an ARB, olmesartan, are associated with remission, regression or both of glomerular podocyte injury.

Methods

Animals

All experimental procedures were performed according to the guidelines for the care and use of animals established by the Kagawa University Medical School and Tulane University Health Sciences Center. A total of 60 male 4-week-old OLETF rats and 24 age-matched Long-Evans Tokushima Otsuka (LETO) rats (genetic control for OLETF rats) were supplied by Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). From 20 weeks of age, SBP was measured in conscious rats by tail-cuff plethysmography (BP-98A; Softron Co., Tokyo, Japan). Twenty-four-hour urine samples were collected using metabolic cages every 5–15 weeks. Postprandial blood glucose (PPBG) levels were also measured every 5–15 weeks from the tail vein with a glucose analyzer (Sanwa-Kagaku, Co. Ltd., Aichi, Japan). At 55 weeks of age, we excluded the OLETF rats with PPBG less than 150 mg/dl in two consecutive measurements and/or with proteinuria less than 150 mg/day in two consecutive measurements or were not obese (owing to injuries, for example).

After obtaining basal measurements, 12 LETO and 12 OLETF rats were killed at 55 weeks of age. The remaining LETO rats ($n=12$) were treated with vehicle (0.5% methyl cellulose; Nacalai Tesque Inc., Kyoto, Japan), whereas OLETF rats were randomly divided into groups for treatment with olmesartan [10 mg/kg per day; oral(ly) (p.o.), $n=10$], triple therapy [hydralazine 15 mg/kg per day, reserpine 0.3 mg/kg per day and hydrochlorothiazide 6 mg/kg per day (HRH); p.o., $n=11$] or vehicle ($n=12$). The doses of olmesartan and HRH were determined on the basis of previous studies [23,25,26] on rats. These animals continued to receive their treatment and were killed at 75 weeks of age.

After decapitation, trunk blood was collected. Then, the right renal artery was clamped and the right kidney was removed. Half of the right kidney was homogenized in cold methanol and processed to measure the AngII content [23–27], whereas the other half of the kidney was snap-frozen in liquid nitrogen and stored at -80°C . Then, retrograde perfusion of left kidney with isotonic saline was performed as previously described [24].

Histological examination

The left kidney was fixed with 10% formalin (pH 7.4), embedded in paraffin, sectioned into 3- μm -thick slices and stained with periodic acid–Schiff (PAS), and the extent of glomerular sclerotic change was semiquantitatively evaluated by an automatic image analysis system using PAS-stained sections, as previously described [26]. The severity of podocyte injury was evaluated by immunohistochemistry for desmin, as previously described [24]. The ratio of the affected lesions in each glomerulus or microscopic field was calculated using Image-Pro Plus software (Media Cybernetics, Silver Spring, Maryland, USA). For each glomerulus, the area of affected lesions where the intensity exceeded a threshold level calculated according to the background signal was measured automatically by the software, and then divided by the total area. A total of 30 superficial and 15 juxtamedullary glomeruli were examined for each rat, and the average percentage of affected lesions was calculated for each rat.

Real-time reverse-transcription PCR

The mRNA levels of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), collagen I, transforming growth factor-beta (TGF- β) and connective tissue growth factor (CTGF) in renal cortical tissues were analyzed by real-time reverse-transcription PCR (RT-PCR) using a LightCycler FastStart DNA Master SYBR Green I kit (Roche Applied Science, Indianapolis, Indiana, USA), as previously described [23,24]. All data are expressed as the relative differences between OLETF and LETO rats after normalization to the corresponding GAPDH expression.

Other analytical procedures

Urinary protein, plasma creatinine, total cholesterol (TC), triglycerides and nonesterified free fatty acid (FA) were determined using commercially available assay kits (all kits from Wako Co., Osaka, Japan). Urinary albumin was determined using an ELISA kit (Shibayagi Co. Ltd., Gunma, Japan). The AngII content was measured by a radioimmunoassay, as previously described [23–27].

Statistical analysis

Values are presented as means $\pm$ SE. Statistical comparisons of the differences between the values were performed using one-way or two-way analysis of variance combined with the Newman–Keuls post-hoc test. Values of P less than 0.05 were considered statistically significant.

Results

General characteristics

Figure 1 shows the temporal profiles of PPBG and SBP in LETO and OLETF rats in which values were obtained in continuously monitored animals from 20 to 75 weeks of age. Vehicle-treated OLETF rats showed higher PPBG levels than vehicle-treated LETO rats throughout the observation period (Fig. 1a). OLETF rats treated with olmesartan or HRH (triple therapy with hydralazine, reserpine and hydrochlorothiazide) showed similar PPBG levels to vehicle-treated OLETF rats. During the observation period, SBP remained unchanged in vehicle-treated LETO rats, whereas vehicle-treated OLETF rats showed hypertension. Treatment of OLETF rats with olmesartan or HRH resulted in a similar SBP reduction (Fig. 1b). At 55 weeks of age, the plasma TC, triglyceride and nonesterified free FA levels in vehicle-treated OLETF rats were significantly higher than those in vehicle-treated LETO rats (242 ± 21 vs. 92 ± 7 mg/dl, 420 ± 39 vs. 89 ± 10 mg/dl and 0.68 ± 0.08 vs. 0.37 ± 0.02 mEq/l, respectively, all $P < 0.05$). In vehicle-treated OLETF rats, these levels increased further at 75 weeks of age (324 ± 35 mg/dl, 505 ± 49 mg/dl and 0.89 ± 0.09 mEq/l, respectively, all $P < 0.05$). On the contrary, the plasma TC, triglyceride and nonesterified free FA levels in vehicle-treated LETO rats did not differ between 55 and 75 weeks. In OLETF rats, olmesartan and HRH did not affect these levels (data not shown). The body weight of vehicle-treated OLETF rats was higher than that of vehicle-treated LETO rats during the observation period, and none of the treatments affected the body weight of OLETF rats (data not shown).

Urinary albumin excretion rate and plasma creatinine levels

Figure 2(a) shows the temporal profiles of urinary albumin excretion rate ($U_{\text{albumin}}V$) in LETO and OLETF rats in which values were obtained in continuously monitored animals from 20 to 75 weeks of age. Figure 2(b) shows the percentage changes in $U_{\text{albumin}}V$ observed between before (55-week-old) and after treatment (75-week-old). After 20 weeks of age, vehicle-treated OLETF rats developed albuminuria with age (33 ± 7 , 179 ± 24 and 330 ± 38 mg/day at 20, 55 and 75 weeks of age). Treatment with olmesartan significantly decreased $U_{\text{albumin}}V$. At 75 weeks of age, the $U_{\text{albumin}}V$ in olmesartan-treated OLETF rats (72 ± 20 mg/day) was much less than before treatment at 55 weeks of age. Treatment with HRH also attenuated the progression of $U_{\text{albumin}}V$ in OLETF rats (252 ± 27 mg/day at 75 weeks of age). However, the effects of HRH on $U_{\text{albumin}}V$ were much less than those of olmesartan, as shown in Fig. 2(a and b).

LETO and OLETF rats showed similar plasma creatinine levels during the observation period, suggesting that renal function is maintained in OLETF rats at this age. Olmesartan and HRH did not affect the plasma creatinine levels in OLETF rats (data not shown).

Glomerular sclerotic change (periodic acid–Schiff-positive area)

The extent of glomerular sclerotic change was semi-quantitatively evaluated by an automatic image analysis system using PAS-stained sections, as previously described [26]. There were no significant findings, except for minor changes due to aging in the glomeruli of LETO rats at 55 and 75 weeks of age. However, mesangial expansion, which was accompanied by an accumulation of extracellular matrix and capillary wall thickening, occurred in superficial and juxtamedullary glomeruli of 55-week-old OLETF rats (Fig. 3a). Semiquantitative analyses confirmed that the PAS-positive area was significantly larger in juxtamedullary glomeruli than in superficial glomeruli (22 ± 3 vs. $12 \pm 3\%$, Fig. 3b) in OLETF rats at this age (Fig. 3b). At 75 weeks of age, OLETF rats showed additional superficial glomerular sclerotic changes, whereas the juxtamedullary glomerular sclerotic changes seemed to be similar between 55 and 75 weeks of age (Fig. 3a). The PAS-positive area in superficial glomeruli was larger in 75-week-old than in 55-week-old OLETF rats, suggesting that the superficial glomerular sclerotic changes had advanced between 55 and 75 weeks of age. However, the PAS-positive area in juxtamedullary glomeruli was not significantly different between 55-week-old and 75-week-old OLETF rats (Fig. 3b). These data suggest that the juxtamedullary glomerular sclerotic changes did not advance between 55 and 75 weeks of age.

At 75 weeks of age, the superficial glomerular PAS-positive area was significantly smaller in olmesartan-treated OLETF rats than in vehicle-treated animals (14 ± 2 vs. $21 \pm 3\%$, Fig. 3b). However, the PAS-positive areas in superficial glomeruli were not different between the 75-week-old olmesartan-treated OLETF rats and OLETF rats before treatment at 55-weeks-old. These data suggest that treatment with olmesartan causes a remission, but not regression, of superficial glomerular sclerotic changes. There was no significant difference in juxtamedullary glomerular PAS-positive area between pretreated 55-week-old OLETF rats and vehicle-treated or olmesartan-treated 75-week-old OLETF rats, suggesting that the advanced juxtamedullary glomerular sclerotic changes in 55-week-old rats were not affected by treatment with olmesartan. HRH did not affect the superficial and juxtamedullary glomerular PAS-positive area in OLETF rats (Fig. 3b).

Podocyte injury (desmin-positive area)

The severity of podocyte injury was evaluated by immunohistochemistry for desmin, as previously described [24]. The desmin-positive area in the superficial and juxtamedullary glomeruli was greater in OLETF rats than in LETO rats at 55 weeks of age (Fig. 4a). Furthermore, the desmin-positive area in juxtamedullary glomeruli was much larger than in superficial glomeruli (9.9 ± 1.2 vs. $2.0 \pm 0.2\%$, Fig. 4b) in OLETF rats at this age. At 75 weeks of age, OLETF rats showed greater superficial glomerular podocyte injury (desmin-positive area, $2.8 \pm 0.2\%$), suggesting that superficial glomerular podocyte injury had advanced between 55 and 75 weeks of age. However, the juxtamedullary glomerular desmin-positive area was not different between 55-week-old and 75-week-old OLETF rats (Fig. 4b). These data suggest that juxtamedullary glomerular podocyte injury did not advance between 55 and 75 weeks of age.

The superficial glomerular desmin-positive area was much smaller in 75-week-old olmesartan-treated OLETF rats ($1.2 \pm 0.1\%$) than in vehicle-treated animals of same age (Fig. 4b). Furthermore, the desmin-positive area in superficial glomeruli of olmesartan-treated 75-week-old OLETF rats was significantly smaller than that before treatment at 55 weeks of age. These results suggest that treatment with olmesartan causes a regression of superficial glomerular podocyte injury. There was no significant difference in the juxtamedullary glomerular desmin-positive area between untreated 55-week-old OLETF rats and vehicle-treated or olmesartan-treated 75-week-old OLETF rats, suggesting that the advanced juxtamedullary glomerular

podocyte changes were not altered by treatment with olmesartan. HRH did not affect the superficial and juxtamedullary glomerular desmin-positive area in OLETF rats (Fig. 4b).

Angiotensin II content in the kidney

At 55 weeks of age, the AngII content in the kidney was higher in OLETF rats (257 ± 30 fmol/g) than in LETO rats (131 ± 16 fmol/g). Augmentation of the renal AngII content in OLETF rats was also observed at 75 weeks of age (238 ± 22 fmol/g). However, the renal AngII content was not different between 55-week-old and 75-week-old OLETF rats. Treatment with olmesartan prevented the augmentation of the renal AngII content in OLETF rats (109 ± 4 fmol/g). Renal AngII content did not differ between vehicle-treated and HRH-treated OLETF rats at 75 weeks of age (Fig. 5a).

Collagen I, transforming growth factor-beta and connective tissue growth factor mRNA levels in renal cortical tissues

At 55 and 75 weeks of age, the mRNA levels of collagen I, TGF- β and CTGF in renal tissues of OLETF rats were significantly higher than those of LETO rats. The collagen I mRNA levels in 75-week-old OLETF rats was significantly higher than in 55-week-old OLETF rats, whereas the TGF- β and CTGF mRNA levels were not different. In OLETF rats, treatment with olmesartan significantly decreased the mRNA levels of collagen I, TGF- β and CTGF, but their levels did not differ from those in pretreated OLETF rats (55-week-old). On the contrary, treatment with hydralazine did not alter the mRNA levels of collagen I, TGF- β and CTGF (Fig. 5b–d).

Discussion

The current study demonstrates that juxtamedullary glomerular podocyte injury reaches a severe condition more rapidly than superficial glomerular podocyte injury in type 2 diabetic rats with overt albuminuria. Furthermore, treatment with the ARB olmesartan resulted in a marked reduction in albuminuria with a regression of superficial glomerular podocyte injury. On the contrary, severely damaged juxtamedullary glomerular podocyte was not affected by ARB treatment. To the best of our knowledge, these data indicate, for the first time, that remission, regression or both of nephritic-range albuminuria induced by treatment with ARBs is accompanied by the regression of superficial glomerular podocyte injury in type 2 diabetes.

It has been shown that proteinuria is caused by changes in the glomerular filtration barrier, which is composed of the glomerular endothelium, glomerular basement membrane and podocytes [20]. In particular, the contribution of podocyte abnormalities to the progression of proteinuria has been indicated by recent studies [20–22]. In the current study, we found that juxtamedullary glomerular podocyte injury initially becomes severe during the development of albuminuria in type 2 diabetic rats. Kim *et al.* [28] showed that the expression of nephrin, which is a functional molecule in slit diaphragms, which are located between each two adjacent foot processes of podocytes and plays a critical role in albuminuria in diabetes [20,21], was significantly reduced in large glomeruli compared with small glomeruli in streptozotocin-induced diabetic rats. Although the heterogeneity of nephrin expression between superficial and juxtamedullary glomeruli was not investigated in the earlier studies, the data are consistent with the concept that glomerular podocyte injury does not occur in a homogenous manner during the progression of albuminuria in diabetes.

In the current study, treatment with an ARB markedly reduced albuminuria with a regression of superficial glomerular podocyte injury in type 2 diabetic rats. These data are in agreement with results obtained by Suzuki *et al.* [29], showing that treatment with an ARB ameliorates proteinuria by preventing podocyte injury in rats treated with an antibody against nephrin. On

the contrary, the current study showed that remission and regression of superficial glomerular podocyte injury was not observed with HRH treatment in OLETF rats, although treatment with an olmesartan and HRH showed similar antihypertensive effects. Thus, the protective effect of an ARB against podocyte injury cannot be explained simply by its BP-lowering effect. Hoffmann *et al.* [30] showed that transgenic rats overexpressing AngII type 1 receptor in podocytes developed proteinuria without a BP change. These data are consistent with the concept derived from recent in-vitro observations [31,32] that local activation of the RAS plays an important role in the progression of podocyte injury. The moderate effects of HRH on the progression of albuminuria might be simply associated with the reduction in BP, as suggested by recent clinical studies [33,34].

Glomerular sclerosis, which is characterized by mesangial matrix expansion, is observed in advanced diabetic nephropathy [13,14,16]. Consistent with previous observations [23], the 55-week-old OLETF rats exhibited overt albuminuria and significant glomerular sclerotic changes with maintained plasma creatinine levels. Similar to the findings for podocyte injury, sclerotic change is more severe in the juxtamedullary glomeruli than in the superficial glomeruli at 55 weeks of age. Furthermore, OLETF rats developed superficial glomerular sclerotic changes age-dependently, whereas juxtamedullary glomerular sclerotic changes did not advance further. Treatment with an ARB failed to significantly regress superficial or juxtamedullary glomerular sclerotic changes. The mechanisms for the discrepancies of these effects of an ARB on podocyte injury or albuminuria and glomerular sclerotic changes are not clear. However, these data support the concept that the development of albuminuria is associated with glomerular podocyte injury, but is not simply associated with the progression of glomerular sclerotic changes in type 2 diabetic nephropathy with overt albuminuria. The effects of an ARB on glomerular sclerosis may be dependent on complex interactions between several factors that mediate mesangial matrix expansion. Several cell culture studies have indicated that high glucose-induced mesangial cellular injury cannot be completely blocked by ARBs [35,36]. Fioretto *et al.* [37] reported that moderate glomerular sclerosis in type 1 diabetic nephropathy was regressed 10 years after pancreatic transplantation, suggesting the importance of blood glucose control for the regression of glomerular sclerosis. More recently, Teles *et al.* [13] quantitatively determined the fractional mesangial area and showed that treatment with an ARB plus insulin caused a regression of mesangial expansion in streptozotocin-treated hyperglycemic rats. These data agree with the concept based on clinical observations [2–4] that combined strict control of BP by AngII blockade and of blood glucose levels are essential for the remission and regression of diabetic nephropathy including glomerular sclerosis. However, it should be mentioned that our semiquantitative method for assessing glomerular sclerotic changes by measuring the PAS-positive staining area in glomeruli [26] is not always representative of glomerular sclerosis. Therefore, more careful assessment of glomerular sclerosis is necessary in future studies.

Renal AngII levels are regulated independently [27,38] and augmented from the early to advanced stage of renal injury in type 1 and type 2 diabetic rats [24,39]. Consistent with previous observations [23], an augmentation of renal AngII levels was observed in OLETF rats at 55 weeks of age. The current study also showed that augmentation of AngII levels continued in OLETF rats until 75 weeks of age. Furthermore, augmentation of renal AngII levels was prevented by an ARB in OLETF rats, suggesting that some of the renoprotective effects of ARBs are accompanied by reduced renal AngII levels. We previously demonstrated that ARBs decrease renal AngII levels by preventing AngII type-1 receptor-mediated AngII uptake and intrarenal production of AngII by overexpression of angiotensinogen [25,27,38]. However, the current study cannot determine the precise mechanisms responsible for the olmesartan-induced reductions in renal AngII levels in OLETF rats with nephrotic-range albuminuria. Furthermore, the intrarenal distribution of the augmented AngII in OLETF rats was not investigated in the current study. Similarly, although we observed BP-independent

effects of an ARB on reducing the gene expression of renal cortical collagen I, TGF- β and CTGF, we cannot determine the contribution of these effects of an ARB to its preventive effects on glomerular injury because the distribution of collagen I, TGF- β and CTGF expression was not investigated. Further studies are needed to measure the AngII levels and gene expression of collagen I, TGF- β and CTGF in isolated superficial and juxtamedullary glomeruli during the progression of type 2 diabetic nephropathy.

In conclusion, the current study demonstrates that juxtamedullary glomerular podocyte injury reaches a severe condition more rapidly than superficial glomerular podocyte injury in type 2 diabetic rats with overt albuminuria. Our results also support the hypothesis that antiproteinuric effects of ARBs are associated with regression of superficial glomerular podocyte injury, whereas severely damaged juxtamedullary glomerular podocyte injury is not affected by ARB treatment. Thus, AngII blockade offers an effective strategy for initiating regression of overt proteinuria and glomerular podocyte injury in type 2 diabetes, but that regression of advanced glomerular podocyte injury may be hard to achieve. Nevertheless, more careful assessments of the histopathology and of the RAS in both superficial and juxtamedullary podocytes are required.

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Abbreviations

ACEI	angiotensin-converting enzyme inhibitor
AngII	angiotensin II
ARB	angiotensin II receptor blocker
CTGF	connective tissue growth factor
ESRD	end-stage renal disease
LETO	Long–Evans Tokushima Otsuka
OLETF	Otsuka–Long–Evans–Tokushima–Fatty
PAS	periodic acid–Schiff
PPBG	postprandial blood glucose
RAS	renin–angiotensin system
RT-PCR	reverse transcription PCR
TGF- β	transforming growth factor-beta
U _{albumin} V	urinary excretion rate of albumin

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Fig. 1.

(a) Postprandial blood glucose and (b) SBP profiles. The vehicle-treated type 2 diabetic OLETF rats develop diabetes and hypertension. In these animals, treatment with olmesartan or a triple therapy (HRH) does not affect PPBG, but decreases SBP to levels that are similar to those of vehicle-treated control LETO rats. * $P < 0.05$ vs. the pretreatment value at 55 weeks of age. HRH, hydralazine, reserpine and hydrochlorothiazide; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka–Long–Evans–Tokushima–Fatty; PPBG, postprandial blood glucose.

Fig. 2.

(a) Urinary excretion rate of albumin and (b) the percentage changes in urinary excretion rate of albumin profiles before (55 weeks of age) and after treatments (75 weeks of age). Vehicle-treated OLETF rats develop proteinuria. Treatment with olmesartan significantly decreases U_{albumin} V. The U_{albumin} V is much lower in olmesartan-treated OLETF rats at 75 weeks of age than before treatment at 55 weeks of age. Treatment with HRH attenuates the progression of U_{albumin} V; however, the effects of HRH on U_{albumin} V are much weaker than those of olmesartan. * $P < 0.05$ vs. vehicle-treated LETO rats. † $P < 0.05$; OLETF rats + vehicle vs. OLETF rats + olmesartan or HRH at 75 weeks of age. HRH, hydralazine, reserpine and hydrochlorothiazide; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka–Long–Evans–Tokushima-Fatty; PPBG, postprandial blood glucose; U_{albumin} V, urinary excretion rate of albumin.

Fig. 3.

(a) Photomicrographs of glomeruli stained with periodic acid–Schiff (original magnification $\times 200$) and (b) their quantitative analysis. In 55-week-old diabetic OLETF rats, mesangial expansion is accompanied by an accumulation of extracellular matrix and glomerular capillary wall thickening in both the superficial and juxtamedullary glomeruli. The PAS-positive area is larger in the juxtamedullary glomeruli than in the superficial glomeruli in OLETF rats. At 75 weeks of age, OLETF rats show a further increase in the PAS-positive area in superficial glomeruli, but not in the juxtamedullary glomeruli. Superficial glomerular PAS-positive area in olmesartan-treated OLETF rats is significantly smaller than in vehicle-treated animals, but there is no significant difference in the juxtamedullary glomerular PAS-positive area between vehicle-treated or olmesartan-treated OLETF rats. HRH does not affect the superficial or juxtamedullary glomerular PAS-positive area in OLETF rats. * $P < 0.05$ vs. vehicle-treated LETO rats at each age. $^{\dagger}P < 0.05$; OLETF rats + vehicle vs. OLETF rats + olmesartan or HRH at 75 weeks of age. HRH, hydralazine, reserpine and hydrochlorothiazide; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka–Long–Evans–Tokushima–Fatty; PAS, periodic acid–Schiff.

Fig. 4.

(a) Photomicrographs of glomeruli stained with desmin (original magnification $\times 200$) and (b) their quantitative analyses data. The desmin-positive area is larger in both the superficial and juxtamedullary glomeruli in OLETF rats than in LETO rats at 55 weeks of age. Furthermore, the desmin-positive area in juxtamedullary glomeruli is much larger than in superficial glomeruli in OLETF rats. At 75 weeks of age, OLETF rats show further progression in superficial glomerular podocyte injury, whereas the juxtamedullary glomerular desmin-positive area is not different between 55 and 75 weeks of age. Superficial glomerular desmin-positive area in 75-week-old olmesartan-treated OLETF rats is much smaller than in vehicle-treated animals. Furthermore, the desmin-positive area is significantly smaller in the superficial glomeruli of olmesartan-treated 75-week-old OLETF rats than that before treatment (55 weeks of age), but there is no significant difference in juxtamedullary glomerular desmin-positive area between these groups of rats. On the contrary, HRH does not affect the superficial or juxtamedullary glomerular desmin-positive area in OLETF rats. * $P < 0.05$ vs. vehicle-treated LETO rats at each age. $^{\dagger}P < 0.05$; OLETF rats + vehicle vs. OLETF rats + olmesartan or HRH at 75 weeks of age. HRH, hydralazine, reserpine and hydrochlorothiazide; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka–Long–Evans–Tokushima–Fatty.

Fig. 5.

Renal angiotensin II contents (a), renal cortical mRNA levels of collagen I (b), transforming growth factor (c) and connective tissue growth factor (d). As compared with vehicle-treated LETO rats ($n=12$ at 55 and 75 weeks of age, respectively), vehicle-treated OLETF rats show augmented renal AngII levels and mRNA levels of collagen I, TGF- β and CTGF ($n=12$ at 55 and 75 weeks of age, respectively). In OLETF rats, treatment with olmesartan ($n=10$) reduces these levels, whereas treatment with HRH ($n=11$) does not. The mRNA levels are expressed as the relative differences between OLETF and LETO rats after normalization to GAPDH expression. * $P < 0.05$ vs. LETO rats. † $P < 0.05$; OLETF rats + vehicle vs. OLETF rats + olmesartan or HRH at 75 weeks of age. AngII, angiotensin II; CTGF, connective tissue growth factor; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; HRH, hydralazine, reserpine and hydrochlorothiazide; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka-Long-Evans-Tokushima-Fatty; TGF- β , transforming growth factor-beta.