Angiotensin II Type 1 Receptor Blockade Inhibits the Development and Progression of HIV-Associated Nephropathy in a Mouse Model

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HIV-associated nephropathy (HIVAN) is characterized by a collapsed glomerular capillary tuft with hyperplasia and hypertrophy of podocytes. Recently generated were conditional transgenic mice (podocin/Vpr) that express one of the HIV-1 accessory genes, vpr, selectively in podocytes using podocin promoter and Tet-on system. These transgenic mice developed renal injury similar to HIVAN when treated with doxycycline for 8 to 12 wk. This study demonstrated that nephron reduction by heminephrectomy markedly enhanced phenotypic changes of podocytes and led to severe FSGS within 4 wk. Nephrotic-range proteinuria was observed already at 2 wk, together with dedifferentiation and dysregulation of podocytes, indicated by decreased expression of nephrin, synaptopodin, and Wilms’ tumor 1 protein and increased expression of Ki-67. The acceleration of phenotypic changes of podocytes, proteinuria, and subsequent glomerulosclerosis by heminephrectomy was almost completely inhibited by angiotensin II type 1 receptor (AT1R) blocker olmesartan. In contrast, the renoprotective effect of the calcium channel antagonist azelnidipine was minimal, although it lowered systemic BP to the same level as olmesartan, demonstrating that the inhibitory effect of AT1R blocker was independent of systemic BP. Olmesartan also reduced proteinuria and prevented glomerulosclerosis even by the delayed treatment, which was initiated after the podocyte injury appeared. These data suggest that nephron reduction exaggerates podocyte injury and subsequent glomerulosclerosis, possibly through glomerular hypertension, in the mouse model of HIVAN. AT1R blockade could be beneficial in the treatment of HIVAN by ameliorating podocyte injury by avoiding the vicious cycle of nephron reduction and glomerular hypertension.


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transgenic mice in which HIV-1 genes, including vif, vpr, nef, tat, and rev, were expressed selectively in podocytes by nefhrin promoter. The transgenic mice developed collapsing glomerulopathy after 4 wk of age. Shigehara et al. (20) generated inducible transgenic mice using the podocin promoter and Tet-on system (T. Shigehara et al., unpublished observations, 2004). In the mice, one of the HIV-1 accessory genes, vpr, is induced selectively in podocytes by the administration of doxycycline. After 8 to 12 wk of doxycycline administration, these mice developed proteinuria, glomerulosclerosis, and tubulointerstitial changes similar to HIVAN (T. Shigehara et al., unpublished observations, 2004). These two recent engineered transgenic mice clearly demonstrate that podocyte injury by the direct expression of HIV-1 genes is sufficient to develop renal lesions similar to HIVAN at least in mice. Of note, other factors are necessary for the development of the full picture of HIVAN. Genetic factors are considered to be important in both animal models and human HIVAN (4,9,19).

Although HIV-1 genes in podocytes are shown to be responsible for the development of HIVAN, the mechanisms by which HIV-1 infection in podocytes leads to collapsing FSGS and ESRD are not well understood. Nephron reduction by heminephrectomy is known to enhance glomerular injury in several animal models (21–27). In this study, using a podocyte-specific transgenic HIVAN mouse line, we demonstrated that removal of one kidney dramatically accelerated podocyte injury and led to severe glomerulosclerosis. Moreover, we showed that angiotensin II (AngII) type 1 receptor (AT1R) blocker (ARB) almost completely inhibited the development and progression of podocyte injury and subsequent glomerulosclerosis in these mice.

Materials and Methods

Transgenic Mice

We used dual-transgenic mice that bear the podocin/rtTA construct and the tetO/Vpr construct; after administration of doxycycline, these mice express Vpr selectively in the podocytes (12; T. Shigehara et al., unpublished observations, 2004) (Figure 1). We cross-bred podocin/rtTA transgenic mice with tetO/Vpr mice to generate bitransgenic mice (termed here podocin/Vpr mice for simplicity). Briefly, the tetO/Vpr transgenic mice were generated as follows. HIV-1 vpr gene was amplified by PCR from the infectious HIV-1 clone pNL4–3 (Genbank AF324493) with the following primers: TPR1-ATCCCCCGGGATGGAAACAAGCCCCAGAA-3′ and TPR2 5′-ATAGCGGATCCTCTAGGAATCAGTG ACTGACTGCGT-3′. After the digestion of the PCR product with BamHI and SacII, the fragment was ligated into the Tet-responsive promoter-enhancer region directs expression from the cytomegalovirus (CMV) promoter (PCMV) of the vpr gene.

Histologic Analysis

Kidneys were fixed in 4% paraformaldehyde and embedded in paraffin. Three-micrometer sections were stained with periodic acid–Schiff. For immunohistochemical analysis, 3-µm sections were deparaffinized, treated with 0.3% H2O2 to block endogenous peroxidase, and microwaved at 500 W for 10 min in 10 mmol/L citric acid to retrieve antigens. The sections then were incubated overnight at 4°C with anti-Wilms’ tumor 1 protein (WT-1; Santa Cruz Biotechnology, Santa Cruz, CA), synaptotodin (PROGEN, Heidelberg, Germany), and Ki-67 (Lab Vision, Fremont, CA) antibodies. After washing with PBS, sections then were incubated with the biotinylated secondary antibody (Vector Laboratories, Burlingame, CA). The VECTASTAIN ABC reagent (Vector Laboratories) was used for signal amplification and detected with diamino benzidine (Nichirei, Tokyo, Japan). Slides were counterstained with methyl green.

For immunofluorescence analysis, kidneys were fixed for 4% formaldehyde and embedded in paraffin. Three-micrometer sections were stained with periodic acid–Schiff. For immunohistochemical analysis, 3-µm sections were deparaﬃnized, treated with 0.3% H2O2 to block endogenous peroxidase, and microwaved at 500 W for 10 min in 10 mmol/L citric acid to retrieve antigens. The sections then were incubated overnight at 4°C with anti-Wilms’ tumor 1 protein (WT-1; Santa Cruz Biotechnology, Santa Cruz, CA), synaptotodin (PROGEN, Heidelberg, Germany), and Ki-67 (Lab Vision, Fremont, CA) antibodies. After washing with PBS, sections then were incubated with the biotinylated secondary antibody (Vector Laboratories, Burlingame, CA). The VECTASTAIN ABC reagent (Vector Laboratories) was used for signal amplification and detected with diamino benzidine (Nichirei, Tokyo, Japan). Slides were counterstained with methyl green.

Urinary and Serum Analysis

Individual mice were placed in metabolic cages for 24-h urine collections at 0, 2, and 4 wk after initiation of doxycycline therapy. Urinary albumin concentration was determined by competitive ELISA kit (Albuwell M; Exocell, Philadelphia, PA). Serum creatinine, urea nitrogen, total protein, albumin, total cholesterol, and triglyceride were assessed by a Hitachi 7180 autoanalyzer (Hitachi High-Technologies Corp., Tokyo, Japan).

Figure 1. Vpr expression in podocin/Vpr mice. A 2.5-kb fragment of the NPHS2 (podocin) promoter-enhancer region directs the expression of reverse tetracycline-controlled transcriptional activator (rtTA) in podocytes. In the presence of tetracycline or derivative, such as doxycycline, rtTA binds to tetracycline-response operon promoter element (tetO) and initiates transcription from the cytomegalovirus (CMV) promoter (PCMV) of the vpr gene.

Podocin promoter

rtTA

Doxycycline

rtTA

tetO

PCMV

Vpr

vpr

Figure 1. Vpr expression in podocin/Vpr mice. A 2.5-kb fragment of the NPHS2 (podocin) promoter-enhancer region directs the expression of reverse tetracycline-controlled transcriptional activator (rtTA) in podocytes. In the presence of tetracycline or derivative, such as doxycycline, rtTA binds to tetracycline-response operon promoter element (tetO) and initiates transcription from the cytomegalovirus (CMV) promoter (PCMV) of the vpr gene.

Experimental Protocol

Podocin/Vpr mice and wild-type mice that weighed 20 to 25 g were heminephrectomized 1 wk before the administration of doxycycline (Sigma-Aldrich, St. Louis, MO). Doxycycline was administered in drinking water (2 mg/ml) for 4 wk to induce Vpr expression in podocytes. Mice received an ARB, olmesartan medoxomil (Sankyo Co. Ltd., Tokyo, Japan), or a dihydropyridine calcium channel antagonist, azelnidipine (Sankyo Co., Ltd.), mixed with a powder diet for 4 wk simultaneously with doxycycline. In the delayed-treatment olmesartan group, the drug was started 2 wk after the initiation of doxycycline and continued for 2 wk. Systolic BP (SBP) was measured by tail-cuff plethysmography in conscious mice using a UR-1000 device (Ueda Avancer Corp., Tokyo, Japan).
followed by incubation with FITC-conjugated anti-guinea pig IgG antibody (Molecular Probes, Eugene, OR).

Detection of Vpr mRNA

Mouse kidneys were snap-frozen in liquid nitrogen, and total RNA was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA). cDNA was synthesized using SuperScript III RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA). For quantification of the Vpr expression, real-time PCR was performed on the ABI PRIZM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) using the QuantiTect SYBR Green PCR Kit (Qiagen) and primers that amplify a 109-bp fragment of the HIV-1 vpr transgene. The sequences of the primers were as follows: forward 5’-GATACTTGGGACGGTGGA-3’ and reverse 5’-TCCTGTFTGAGTAAAGCCTTA-3’. We ran the reaction for 40 cycles of 15 s at 95°C and 1 min at 60°C after the initial 10 min at 95°C. Glyceraldehydes-3-phosphate dehydrogenase RNA was used as an endogenous control for PCR quantitation.

Scoring of Glomerulosclerosis

Glomerular damage was quantified by grading sclerosis/hyalinosis in the glomeruli on periodic acid-Schiff–stained sections by an observer who was masked to sample identity, using a score of 0 to 4 in each glomerulus. The percentage of sclerosis or hyalinosis area was scored in each glomerulus; 0, no lesion; 1, <25%; 2, 25 to <50%; 3, 50 to 75%; 4, >75% of the glomerular tuft, respectively. More than 50 sequential glomeruli from each mouse were evaluated, and the average of glomerular sclerosis/hyalinosis was calculated.

Scoring of Podocyte Markers

To evaluate the expression of WT-1 and Ki-67 staining in podocytes, we examined the number of positive cells in each glomerulus. For Ki-67 staining, the number of positive cells was counted among cells that were located within Bowman’s space or cells on glomerular basement membrane. Apparent parietal epithelial cells were excluded. The average of positive cell numbers in one glomerulus was...
calculated by counting more than 50 sequential glomeruli from each mouse. For the evaluation of expression of synaptopodin and nephrin, we used a semiquantitative grading system: 0, no stain; 1, weak staining; 2, intermediate staining; and 3, strong staining. More than 50 sequential glomeruli from each mouse were evaluated, and the average was calculated.

Statistical Analyses

Data were expressed as means ± SD. Differences between experimental groups were evaluated by ANOVA or by the Mann-Whitney test. P < 0.05 was regarded as statistically significant.

Results

Heminephrectomy Accelerates HIVAN

Bitransgenic podocin/Vpr mice that were treated with doxycycline showed histologic features that were concordant with those that are seen in human patients with HIVAN. The earliest changes were seen after 2 to 4 wk of doxycycline treatment, including albuminuria and podocyte hypertrophy and hyperplasia. There was marked progression in renal injury after 8 to 16 wk. In some experimental animal models, heminephrectomy accelerates renal injury (21–27). To test the hypothesis that nephron reduction enhances podocyte injury and accelerates collapsing glomerulopathy, we heminephrectomized podocin/Vpr mice 1 wk before the administration of doxycycline (Figure 2A). Heminephrectomized podocin/Vpr mice that were treated with doxycycline (heminephrectomized podocin/Vpr mice) showed heavy albuminuria and mild glomerulosclerosis, represented by mesangial matrix expansion with occlusion of capillary lumens, at 2 wk after the initiation of doxycycline administration, whereas modest albuminuria and scant glomerulosclerosis were observed in podocin/Vpr mice without nephrectomy and intact podocin/Vpr mice at that time point (Figure 2, B and C). At 4 wk, the amount of urinary albumin excretion was increased further and severe glomerulosclerosis was observed in accelerated podocin/Vpr HIVAN mice, when original podocin/Vpr HIVAN mice started to show mild to moderate albuminuria and mild morphologic changes. In heminephrectomized podocin/Vpr mice, severe glomerulosclerosis was associated with hypertrophy and hyperplasia of glomerular visceral epithelial cells and pseudocrescent formation (Figure 3, B through D). These changes also were seen in intact podocin/Vpr mice, in that about half of the glomeruli showed segmental or global sclerosis at 16 wk after the initiation of doxycycline administration (T. Shigehara et al., unpublished observations, 2004) but less intense and restricted at 4 wk. Tubulointerstitial changes, such as dilated tubules and interstitial mononuclear infiltrates, also were seen. Magnifications: ×100 in A and B; ×400 in C and D.

ARB, Not a Calcium Channel Antagonist, Inhibits the Development of HIVAN

The renin-angiotensin system is known to be involved in the development and progression of renal injury (28–31). We hypothesized that AngII, acting via the AT1R, enhances podocyte injury and leads to severe glomerular damage under nephron...
reduction in HIVAN. To clarify the role of AT1R in heminephrectomized podocin/Vpr mice, we administered an ARB, olmesartan, simultaneously with doxycycline (Figure 4A). As shown in Figure 4, B and C, olmesartan inhibited the amount of urinary albumin excretion and the degree of glomerulosclerosis in a dosage-dependent manner. Olmesartan at a dosage of 10 mg/kg per d almost completely inhibited the albuminuria and histologic changes as well as serologic abnormalities (Table 2). We selected 10 mg/kg per d olmesartan for subsequent experiments. Olmesartan did not decrease the expression of Vpr, as assessed by real-time PCR (Figure 4E).

Systemic BP is an important contributing factor in the progression of glomerular injury. Olmesartan lowered the SBP during disease progression, whereas untreated heminephrecto-
Table 2. Serologic data of untreated and olmesartan-treated micea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Olmesartan</th>
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<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>4.3 ± 0.5</td>
<td>5.5 ± 1.3b</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.1 ± 0.2</td>
<td>3.1 ± 0.3b</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.36 ± 0.20</td>
<td>0.14 ± 0.09b</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>138.3 ± 58.9</td>
<td>62.3 ± 14.8b</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>492.2 ± 179.0</td>
<td>135.8 ± 26.2b</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>226.0 ± 110.1</td>
<td>55.0 ± 18.4b</td>
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aMice received doxycycline, underwent heminephrectomy, and were studied 4 wk later. Data are means ± SD.

b$P < 0.05$ versus untreated.

Figure 5. Effect of azelnidipine on SBP, albuminuria, and glomerulosclerosis in heminephrectomized podocin/Vpr mice. (A) Experimental protocol. (B) SBP. Azelnidipine ($n = 5$) lowered SBP to the same levels of olmesartan-treated mice ($n = 7$) during disease progression, compared with untreated mice ($n = 9$). (C) Urinary albumin excretion. The same level of albuminuria was observed in azelnidipine-treated mice ($n = 5$) compared with untreated mice ($n = 9$), whereas olmesartan-treated mice ($n = 9$) showed almost no albuminuria. (D) Glomerular sclerosis/hyalinosis score at 4 wk. Azelnidipine ($n = 7$) mildly suppressed glomerulosclerosis compared with untreated mice ($n = 9$), whereas olmesartan ($n = 9$) almost completely inhibited it. *NS versus olmesartan-treated mice; **$P < 0.005$ versus untreated mice; ***$P < 0.05$ versus untreated mice. §§NS versus untreated mice; §§§$P < 0.0001$ versus olmesartan-treated mice.
mized podocin/Vpr mice showed elevated SBP (Figure 4D). To elucidate whether protective effect of olmesartan was due to lowering systemic BP, we used a long-acting dihydropyridine calcium channel antagonist, azelnidipine (Figure 5A). Azelnidipine at a dosage of 100 mg/kg per d lowered the SBP to the same level as with olmesartan at 10 mg/kg per d at 2 wk (Figure 5B). However, urinary albumin excretion was not suppressed compared with untreated heminephrectomized podocin/Vpr HIVAN mice, whereas almost no albuminuria was detected in olmesartan-treated mice (Figure 5C). At 4 wk, SBP tended to increase in azelnidipine-treated mice but was significantly lower compared with untreated mice. However, the same level of urinary albumin excretion was observed in both azelnidipine-treated mice and untreated transgenic mice. Extensive glomerulosclerosis was observed in azelnidipine-treated mice compared with olmesartan-treated mice, although partial and significant suppression was observed compared with untreated mice (Figure 5D). These data strongly suggest that inhibitory effect of olmesartan on glomerular injury in part is independent of the suppression of systemic BP.

ARB Inhibits Dedifferentiation and Dysregulation of Podocytes

We next examined phenotypic changes of podocytes and the effect of AT1R blockade in podocin/Vpr mice. Dedifferentiation and dysregulation of podocytes similar to human HIVAN was observed in intact podocin/Vpr mice (T. Shigehara et al., unpublished observations, 2004). We examined the expression of synaptopodin, nephrin, WT-1, and Ki-67 by immunostaining in heminephrectomized podocin/Vpr mice. Expression of synaptopodin, nephrin, and WT-1 was observed before doxycycline administration. Untreated heminephrectomized podocin/Vpr mice showed reduced or absent expression of these proteins, whereas Ki-67 expression was increased (Figure 6). These changes were observed already at 2 wk and become more prominent at 4 wk (Figure 7). In contrast, olmesartan treatment inhibited these phenotypic changes (Figures 6 and 7). The ab-

![Figure 6. Immunostaining of heminephrectomized podocin/Vpr mice. Immunohistochemical staining for Wilms’ tumor-1 (WT-1; A through C), synaptopodin (D through F), and Ki-67 (J through L). Immunofluorescent staining for nephrin (G through I). Untreated mice at 4 wk showed decreased expression of WT-1, synaptopodin, and nephrin but increased expression of Ki-67 compared with 0 wk. In contrast, olmesartan inhibited these changes. For Ki-67 staining, the number of positive cells was counted among cells that were located within Bowman’s space or cells on glomerular basement membrane (K, arrows).](image-url)
The presence of synaptopodin expression indicates dedifferentiation (because they are markers of the mature podocyte), whereas the absence of WT-1 is a sign of podocyte dysregulation (because WT-1 is present in both immature podocytes in the developing kidney and in mature podocytes). Increased expression of Ki-67 also suggests dedifferentiation of podocytes, because they are markers of cells in cell cycle for proliferation.

**Delayed Treatment with ARB Also Inhibits Renal Injury**

Finally, we asked whether the therapeutic approach was effective even when ARB was started when renal injury already was apparent, indicated by massive albuminuria and mild glomerulosclerosis with dedifferentiated podocytes. Therefore, we started olmesartan at 2 wk after the initiation of doxycycline. Olmesartan effectively reduced SBP and prevented albuminuria and glomerulosclerosis at 4 wk (Figure 8). Delayed olmesartan treatment significantly preserved WT-1, synaptopodin, and nephrin expression in podocytes compared with untreated mice (Figure 9). The increase of Ki-67–positive cells was suppressed significantly in olmesartan-treated mice.

**Discussion**

Growing evidence highlights the importance of podocytes in the development and progression of various forms of renal injury.
In HIVAN, characteristic features are glomerular capillary tuft collapse and podocyte hyperplasia and hypertrophy (5–8). Podocytes are terminally differentiated, nonreplicating cells (34). However, in collapsing glomerulopathy, as seen in HIVAN, dedifferentiation and dysregulation of podocytes occurs (7,37). Podocytes lose their differentiated markers, such as synaptopodin, glomerular epithelial protein-1 (GLEPP-1), and podocalyxin, and express the proliferation marker such as proliferating cell nuclear antigen, Ki-67, and cyclin A (37–39). Recently, two podocyte-specific transgenic mouse models demonstrated that selective expression of HIV-1 genes, vpr alone or a combination of vif, vpr, nef, tat, and rev, in podocytes is sufficient to induce HIVAN, indicating that podocytes play a central role in the development of HIVAN (19; T. Shigehara et al., unpublished observations, 2004).

In this study, we used podocyte-specific Vpr transgenic mice and examined the effect of nephron reduction. Vpr is one of six HIV regulatory and accessory proteins. It is a 14-kD basic protein that is present in the virion and is required for viral replication in macrophages but not for replication in lymphocytes. Vpr has multiple effects on host cells, including induction of G2 cell-cycle arrest (40), reducing the mitochondrial membrane potential (41), and alteration in cytokine production (42,43). The first major finding is that nephron reduction by heminephrectomy significantly enhanced phenotypic changes of podocytes and subsequent glomerular injury in the HIVAN mouse model. In podocin/Vpr mice without nephrectomy, >50% of mice developed proteinuria after 6 wk of doxycycline treatment, and nearly all developed proteinuria by 20 wk.

Figure 8. Effect of delayed treatment by olmesartan on heminephrectomized podocin/Vpr mice. (A) Experimental protocol. (B) Urinary albumin excretion. Olmesartan-treated mice (n = 7) showed significant reduction of albuminuria at 4 wk compared with untreated mice (n = 9). (C) Glomerular sclerosis/hyalinosis at 4 wk. Olmesartan (n = 6) prevented glomerulosclerosis. (D) SBP. *P < 0.001 versus untreated.
earliest morphologic changes were seen at 4 wk, and a marked progression was observed after 8 to 16 wk of doxycycline treatment in the original mice (T. Shigehara et al., unpublished observations, 2004). In heminephrectomized podocin/Vpr mice, proteinuria appeared at 2 wk, and podocytes showed aggressive morphologic changes, such as hyperplasia and hypertrophy, within 4 wk after the initiation of doxycycline. Extensive loss of maturity markers, such as synaptopodin and nephrin, was observed already at 2 wk, before widespread glomerulosclerosis. Dysregulation of podocytes also was demonstrated by disappearance of WT-1. In contrast, the podocytes increased Ki-67 staining, demonstrating that they reenter cell cycle. Although these changes were observed in intact podocin/Vpr mice, the speed and the magnitude of the changes were much greater in an accelerated model. These data demonstrated that in the setting of podocyte Vpr expression, heminephrectomy exaggerates podocyte dedifferentiation and dysregulation and leads to severe glomerulosclerosis.

Clinically, HIVAN has a propensity to progress to ESRD. No prospective, randomized, controlled studies that evaluated treatment for HIVAN have been published. From available retrospective studies, treatment options include steroids, angiotensin-converting enzyme (ACE) inhibitors, and antiretroviral therapy (3). Data from a retrospective cohort study of patients with HIVAN suggested that the use of antiretroviral therapy was associated with slower progression of renal replacement therapy (44). Recently, Schwartz et al. (3) demonstrated that highly active antiretroviral therapy reduces the rate of progression from AIDS to ESRD by approximately 40%. The effect of ACE inhibition (ACEi) on HIVAN progression also has been studied. Kimmel et al. (45) reported that the use of captopril was associated with enhanced renal survival of HIVAN in a retrospective, case-control study. Wei et al. (46) also reported that initiation of ACEi before severe renal insufficiency offered sustainable renoprotective benefits in a single-center prospective cohort study. Although the beneficial effect of ACEi suggested that inhibition of the renin-angiotensin system is important in the treatment of HIVAN, no data are available concerning the treatment of patients with ARB.

The second major finding in our study is that AT1R blockade prevents podocyte injury in HIVAN mice. An ARB, olmesartan, almost completely inhibited the development and progression of renal injury in mice with accelerated HIVAN. Although we do not yet understand the molecular pathways by which Vpr expression induces severe podocyte injury, our data demonstrated that ARB was effective in preventing the development and progression of HIVAN in a mouse model by amelioration of podocyte injury in the setting of nephron reduction. In olmesartan-treated mice, not only urinary albumin excretion and glomerulosclerosis but also dedifferentiation and dysregulation of podocytes were improved. How might ARB protect podocytes? In general, there are several favorable effects of

Figure 9. Semiquantitative analysis for WT-1 (A), synaptopodin (B), nephrin (C), and Ki-67 (D) in heminephrectomized podocin/Vpr mice in delayed treatment with olmesartan. WT-1, synaptopodin, and nephrin staining was preserved in mice with delayed treatment by olmesartan (n = 5), compared with untreated mice (n = 5). The number of Ki-67–positive cells that were within Bowman’s space or on glomerular basement membrane was decreased significantly in olmesartan-treated mice.
ARB in preventing renal injury, such as lowering systemic BP, lowering glomerular capillary pressure, and antifibrotic properties (30,31,47). Heminephrectomy increases glomerular capillary hydrostatic pressure (48,49). Therefore, increased glomerular pressure may enhance hyperplasia and hypertrophy of Vpr-expressing podocytes and lead to heavy albuminuria, and ARB may offer protection by suppressing intraglomerular pressure. In contrast, a calcium channel antagonist, azelnidipine, did not prevent albuminuria at all, although it lowered systemic BP to the same level with an ARB, indicating that the effect of ARB was independent of systemic BP. Despite an almost complete absence of histologic lesions in ARB-treated mice, the blood urea nitrogen levels in heminephrectomized Vpr mice showed inappropriate increase compared with the creatinine levels. The increased blood urea nitrogen/creatinine ratio also was observed in heminephrectomized wild-type mice that were treated with ARB (data not shown), suggesting that ARB induced significant renal hemodynamic changes. Although heminephrectomy is an experimental model, in the case of human HIVAN, a vicious cycle of nephron reduction, glomerular hypertension, and progressive podocyte injury may exist, leading in some cases to rapid deterioration of renal function. Lowering glomerular hypertension by AT1R blockade could break this vicious cycle and protect HIV-infected patients from podocyte and glomerular injury.

Another plausible and fascinating but unproved explanation is that, in addition to decreasing intraglomerular pressure, ARB may preserve directly the phenotype of Vpr-expressing podocytes through blocking AT1R signaling in those cells. Recently, several lines of evidence demonstrated that AngII directly affects podocytes (50–53). AngII is reported to affect podocyte membrane voltage and conductance properties (50), expression of heparan sulfate proteoglycans (52), production of α3(IV) collagen (53), and cytoskeleton redistribution and the shedding of nephrin (51). Moreover, transgenic rats that express AT1R in podocytes, using the nephrin promoter, develop albuminuria and podocyte damage (54). It is interesting that mechanical strain increases both production of AngII and expression of AT1R in cultured podocytes (55). For clarification of the direct effect of AngII on podocyte injury, further experiments are required, such as to examine the expression of AT1R on podocytes and the level of intraglomerular AngII in HIVAN mice, as well as the effect of AngII on phenotypic changes of Vpr-expressing cultured podocytes.

Of note, there are limitations to fitting this mouse model to human HIVAN. First, Vpr expression has not been detected in human HIVAN yet, although the existence of the other HIV mRNA or DNA, such as nef, gag, and env, was demonstrated successfully (15). Second, the histologic changes of our mouse model have some differences compared with human collapsing HIVAN. Our model showed proliferation of parietal epithelial cells, vacuolization of podocytes, hyalinosis in capillary lumen, and mild mesangial increase rather than collapse. These changes also were observed to some extent in the original podocin/Vpr mice by the doxycycline administration without nephrectomy (T. Shigehara et al., unpublished observations, 2004), so the protective effect of ARB on human HIVAN should be determined carefully.

Conclusion

We have demonstrated that podocyte-specific expression of Vpr, one of the HIV regulatory and accessory proteins, in combination with heminephrectomy induced rapid and severe morphologic changes of podocytes and glomerulosclerosis. The acceleration of podocyte injury probably is due to glomerular hypertension, because the ARB olmesartan but not calcium channel blocker nearly completely inhibited the development and the progression of the disease. These data suggest that ARB could be beneficial in the treatment of HIVAN to reduce podocyte injury by avoiding the vicious cycle of nephron reduction and glomerular hypertension.

Acknowledgments

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Disclosures

None.

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