Effects of angiotensin type 1 receptor blockade on arginine and ADMA synthesis and metabolic pathways in fawn-hooded hypertensive rats

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Abstract

Background. The fawn-hooded hypertensive (FHH) rat develops spontaneous glomerulosclerosis that is ameliorated by inhibition of the angiotensin II type 1 receptor (AT-1). Since kidney damage is associated with nitric oxide (NO) deficiency, we investigated how AT-1 antagonism influenced nitric oxide synthase (NOS), as well as NOS substrate [L-arginine (L-Arg)] and inhibitor [asymmetric dimethylarginine (ADMA)]. L-Arg is synthesized by renal argininosuccinate synthase/argininosuccinate lyase (ASS/ASL) and then either consumed within the kidney by arginase II or NOS or released into the circulation. L-Arg is then taken up from plasma into cells where it can be utilized by NOS and other pathways. The competitive inhibitor of NOS, ADMA, is degraded by dimethylarginine dimethylaminohydrolase (DDAH).

Methods and results. Male FHH rats were put on a 40% casein diet for 13 weeks, and some received AT-1 antagonist which reduced blood pressure and kidney weight and prevented glomerulosclerosis and hyperfiltration. The AT-1 antagonist reduced the expression of DDAH2, increased DDAH1 and increased total DDAH activity in the kidney cortex, although there was no change in plasma or kidney cortex ADMA levels. The AT-1 antagonist caused no change in the expression of renal ASS/ASL, but reduced renal and aortic arginase expression and renal arginase activity, which could explain the increased plasma L-Arg. In separate studies, 1 week of AT-1 blockade in young FHH rats had no effect on any of these parameters.

Conclusion. Thus, the net result of AT-1 antagonist was an improved L-Arg to ADMA ratio due to the prevention of renal and vascular arginase activation which favours increased NO production. Since 1 week of AT-1 blockade in the absence of kidney damage was without effect on arginases, this suggests that the reduction in arginase activity is secondary to the prevention of structural damage rather than a direct immediate effect of AT-1 antagonism.

Keywords: arginase; DDAH; irbesartan; kidney disease; olmesartan

Introduction

Nitric oxide (NO) is essential to normal cardiovascular and renal function. There is a net NO deficiency in patients and animals with advanced chronic and end-stage kidney disease (CKD and ESKD) [1, 2]. NO is produced by conversion of L-arginine (L-Arg) and oxygen to L-citrulline and NO by nitric oxide synthase (NOS). Much of the L-Arg required for NO synthesis is produced from L-citrulline by the argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) enzymes, and the kidney provides most of the circulating arginine in the body [3]. A deficiency in renal L-Arg production may result in decreased substrate availability for NOS and hence decreased NO production. NOS substrate depletion can also occur when arginases are activated, diverting L-Arg away from NOS as seen in several disease models [4]. Any L-Arg that is available for NO synthesis must compete with endogenous inhibitors, such as asymmetric dimethylarginine (ADMA) for NOS [5]. Therefore, the balance between L-Arg and ADMA is also an important determinant of NO-generating ability. ADMA is made by protein arginine methylation by protein arginine methyltransferase (PRMT1) followed by proteolysis and is removed mainly by hydrolysis by dimethylarginine dimethylaminohydrolase (DDAH). The kidney is an important site of ADMA breakdown [6].

The fawn-hooded hypertensive (FHH) rat provides a genetic model for spontaneous glomerulosclerosis that has been linked to several renal failure genes [7]. NO deficiency may contribute to the glomerular damage since chronic NOS inhibition accelerates the development of glomerulosclerosis in the FHH rat [8] and perinatal exposure to a NO
donor attenuates hypertension and glomerular injury [9]. Angiotensin II (ANGII) antagonism slows the progression of many types of CKD, including injury in the FHH rat [10–14]. In this study, we tested the hypothesis that angiotensin type 1 receptor (AT-1) blockade would attenuate hypertension and renal injury and thereby improve the L-Arg to ADMA ratio thus preserving NO production.

Materials and methods
All experiments were performed using male FHH rats (purchased at 5 weeks old; Hilltop Lab Animals, Scottsdale, PA) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Florida Institutional Animal Care and Use Committee. Rats were housed in conventional cages and given free access to normal chow and water. Urine was collected overnight (16 h) in metabolic cages at 6 weeks of age. In the first series, all animals were switched to a 40% casein diet (MP Biomedicals, Solon, OH) and separated into two groups (n = 10/group): high protein diet alone (HP) and HP plus irbesartan (AT-1 antagonist) in the diet [7–100 mg/kg body weight (BW)/day; HP+Irb]. All rats were maintained on the respective diet for 13 weeks, and BW and urinary protein excretion (UpV, by Bradford method; Bio-Rad, Hercules, CA) were measured at Weeks 2, 4, 7, 10 and 13. At Week 13, rats were anaesthetized with isoflurane and mean arterial pressure (MAP) was measured by an abdominal aortic puncture, blood was collected and plasma was stored at −80°C. The organs were then perfused with PBS; the left kidney was removed, weighed and fixed in 10% formalin for histology. The aorta, liver and right kidney were harvested and flash frozen in liquid nitrogen and stored at −80°C.

In the second series, 6-week-old FHH rats were placed on a low-nitrate but nutritionally complete diet (MP Biomedicals, Solon, OH) and separated into two groups (n = 5/group): vehicle-treated FHH (Veh) and olmesartan-treated FHH (Olm). Olmesartan, an AT-1 blocker, was delivered via the drinking water at 2.5 mg/kg BW/day. In pilot studies, this dose of olmesartan normalized blood pressure (BP; measured by telemetry) in rats with 5/6 renal mass reduction treated for 6 weeks vs untreated rats (116 ± 8 vs 170 ± 8 mmHg; P < 0.001; unpublished data). Olmesartan (25 mg) was dissolved in a 0.1% NaHCO3–5% ethanol solution, and vehicle-treated rats drank the same 0.1% NaHCO3–ethanol solution and were placed in metabolic cages overnight for the collection of urine. Then, rats were anaesthetized with isoflurane, and MAP was measured by an abdominal aortic puncture; blood was collected and plasma was stored at −80°C. The organs were then perfused with PBS, the left kidney was removed and weighed and fixed in 10% formalin for histology. The aorta, liver and right kidney were harvested and flash frozen in liquid nitrogen and stored at −80°C.

Renal function
Urine collected overnight in metabolic cages on Week 13 and plasma collected at sacrifice were analysed for creatinine by HPLC, using the method of Tsikas et al. [15] with modifications as described by us previously [16].

L-Arg and ADMA measurements
The concentrations of L-Arg and ADMA in plasma and kidney cortex homogenates were measured using reverse-phase HPLC, using the Waters AccQ-Fluor fluorescent reagent kit using an adaptation of the method of Hereztyn et al. [17] as described by us previously [16].

Citrulline
Renal cortex homogenate, plasma and urine citrulline concentrations were measured by a colorimetric assay described previously [18].

Plasma and tissue oxidative stress measures
Plasma and tissue indices of oxidative stress were measured using an OXItek thiobarbituric acid reactive substances (TBARS) assay kit (ZeptoMetrix, Buffalo, NY) and an Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes, Eugene, OR).

NOx measurements
Kidney cortex homogenates were prepared as previously described [19]. Total NO content from NOx = NO+ + NO2− was measured in kidney cortex homogenates and urine by Griess reaction [20].

Western blot
Sample preparation for western blot analysis was made as described previously [19]. Measurements were conducted on kidney cortex, aorta and liver homogenates. Rabbit polyclonal antibodies (developed by Dr Masataka Mori, Kumamoto University, Kumamoto) [21] against ASS and ASL were used at 1:2000 dilutions, 1 h incubation. Rabbit polyclonal arginase II antibody (Santa Cruz) was used at a dilution of 1:3000. A goat anti-rabbit IgG-HRP secondary antibody (Bio-Rad, 1:3000 dilution, 1 h incubation) was used for detection. Chicken arginase I antibody (gift from Dr Sidney Morris) was used at 1:10 000 and a goat anti-chicken IgG-HRP secondary antibody was used for detection. For PRMT1, we used a rabbit antibody (Upstate, 1:2000 dilution, overnight incubation) and a goat anti-rabbit antibody secondary. For DDAH, we used a goat anti-rabbit DDAH1 antibody (Santa Cruz, 1:500 dilution, overnight incubation) and a goat anti-rabbit DDAH2 antibody (Santa Cruz, 1:100 dilution, overnight incubation), followed by a secondary donkey anti-goat antibody (Santa Cruz, 1:2000 dilution, 1 h incubation). Bands of interest were visualized using SuperSignal West Pico reagent (Pierce, Rockford, IL) and quantified using the VersaDoc imaging system and Quantity One Analysis software (Bio-Rad) as integrated optical density (IOD) after subtraction of background. The IOD was factored for Ponceau red staining to correct for any variations in total protein loading and was also factored for an internal positive control (rat liver for ASS, ASL and arginase I; rat kidney cortex for arginase II, DDAH1/2 and PRMT1). The protein abundance was represented as IOD/positive control/total protein by Ponceau red, relative to the HP group (set to 100).

Arginase activity assay
Arginase activity in the liver and kidney cortex was determined by measuring the rate of urea production in the homogenate using α-isonitrosopropionophenone (9% in absolute ethanol) as previously described [22]. Tissue were homogenized in 0.5 mL of lysis buffer containing 50 mM Tris–HCl (pH 7.5), 0.1 mM EDTA, 0.1 mM EGTA and protease inhibitors (Inhibitor Cocktail Set III, Calbiochem). Homogenates were incubated with L-Arg (0.5 M; pH 9.7) at 37°C for 60 min. The hydrolysis of L-Arg by arginase was stopped by adding 750 mL of an acid solution mixture (H2SO4, H3PO4, H2O, 1:3:7).

DDAH activity assay
DDAH activity was measured by a colorimetric assay measuring the rate of citrulline production as previously described [18]. Kidney cortex samples were homogenized in sodium phosphate buffer and pre-incubated with urease for 15 min. Then, 100 µl (2 mg total protein) of homogenate were incubated with 1 mM ADMA for 45 min at 37°C. After deproteinization, supernatant was incubated with colour mixture at 60°C for 110 min. The absorbance was measured by spectrophotometry at 466 nm. DDAH activity was represented as micromoles of citrulline formation per gram of protein per minute at 37°C.

Histology
The left kidney was cut along the transverse axis, fixed in 10% formalin and paraffin embedded. Five-micrometre sections were stained with periodic acid–Schiff (Sigma, St Louis, MO) followed by haematoxylin as the secondary stain. Up to 100 glomeruli were scored blindly based on the following scale: 0 = healthy glomeruli, +1 = <25% damage, +2 = 25–75% damage, +3 = >75% damage. A glomerulosclerosis index score (GSI) was calculated using the following equation: (n of +1) + (2 of +2) + 3(# of +3) + 4(# of +4)/total glomeruli observed [23].

Statistics
Repeated-measures ANOVA with Bonferroni post hoc analysis were used to compare BW and UpV progression between groups. Student’s t-test with a two-tailed P-value was used to compare between treated and untreated groups.
Results

HP and HP+Irb FHH rats had similar weight gain up to 7 weeks on the high protein diet, but by 10 weeks of treatment, the irbesartan-treated rats weighed slightly less than the untreated rats (Figure 1A). Baseline UpV was similar in both groups (Figure 1B) and increased in untreated rats over the 13 weeks of high protein intake by a maximum of ~2.5-fold compared to the baseline. In contrast, irbesartan-treated rats on high protein diet showed no increase throughout the treatment period (Figure 1B). The total percent of sclerotic glomeruli was higher in untreated (~18 ± 2%) compared to irbesartan-treated rats (~8 ± 1%), and the severity of injury was lower in the irbesartan-treated rats (Figure 1C; Table 1). Irbesartan treatment also reduced BP and kidney weight (KW; Table 1). The GFR was ~50% higher in the untreated rats than in those receiving irbesartan (Table 1).

Kidney cortex NO\textsubscript{x} was significantly increased with irbesartan treatment (Table 2), suggestive of increased renal NO production. The rats were not fasted or given a low nitrate diet (since the high protein diet was an integral part of the CKD model), thus urine NO\textsubscript{x} values could not be used to assess total NO production [24]. We measured plasma hydrogen peroxide (10.1 ± 2.4 vs 5.8 ± 0.9 µM) and TBARS in plasma (20.6 ± 2.2 vs 16.3 ± 1.5 µM) and kidney cortex homogenates (0.22 ± 0.01 vs 0.20 ± 0.01 pmol/mg protein) as indices of oxidative stress (values are untreated vs irbesartan-treated). There was no difference in any of these measurements between the two groups.

Plasma L-Arg concentration was significantly higher in irbesartan-treated rats, but there was no difference in kidney cortex L-Arg levels (Table 2). The abundance of the enzymes responsible for renal and hepatic L-Arg synthesis (ASS and ASL) was similar between the two groups (Figure 1). The abundance of renal cortex arginase II and aortic arginase I was reduced with irbesartan treatment, while hepatic expression of arginase I was unchanged (Figure 3A). Arginase activity corresponded with protein expression and showed reduced activity in the renal cortex with irbesartan treatment (Figure 3B) and no difference in activity in the liver between groups (Figure 3C). There was insufficient tissue to measure arginase activity in the aorta.

There was no difference in the abundance of any of these proteins in the aorta (Figure 4D).

Table 1. Functional data taken at time of sacrifice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GSI</th>
<th>MAP (mmHg)</th>
<th>KW (g)</th>
<th>Total CCr (mL/min/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>10</td>
<td>0.41 ± 0.04</td>
<td>140 ± 6</td>
<td>3.9 ± 0.1</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>HP+Irb</td>
<td>10</td>
<td>0.16 ± 0.01***</td>
<td>116 ± 4*</td>
<td>3.0 ± 0.1**</td>
<td>0.6 ± 0.1***</td>
</tr>
</tbody>
</table>

GSI, MAP measured by aortic puncture, total KW and creatinine clearance (CCr) factored for BW. Data are shown as mean ± SE. Significance was determined by t-test between treatment groups.

[*P < 0.05.
**P < 0.01.
***P < 0.001.*]
A separate group of young (6 weeks old) FHH rats was treated with the AT-1 antagonist olmesartan to determine the effect of AT-1 blockade itself on L-Arg levels and NO availability in the absence of renal injury. In these rats, there was no difference in BP (106 ± 4 vs 100 ± 2 mmHg), BW (192 ± 16 g vs 207 ± 7 g) or KW (1.1 ± 0.1 vs 1.2 ± 0.1 g) between vehicle-treated and AT-1 antagonist-treated rats. In addition, the kidneys of these young rats showed minimal structural damage and no difference between vehicle-treated and olmesartan-treated rats. In contrast to other models of CKD, plasma citrulline was not elevated and that abundance of renal cortex ASS and ASL was similar to levels observed in the irbesartan-treated FHH rats with preserved kidney structure. This suggests that renal L-Arg synthesis is not inevitably reduced with CKD but may depend on the type and/or severity of the injury process. It is likely that, in the FHH rat, the increase in GFR and hence increase in citrulline delivery offsets the mild level of structural damage. Nevertheless, we also find that irbesartan treatment and preservation of kidney function in FHH rats is associated with an increase in plasma L-Arg, suggesting increased L-Arg availability.

The major novel findings of this study are that protection of kidney structure and function with AT-1 blockade in the 19-week-old FHH rat resulted in reduced arginase protein and activity in the kidney cortex. Although the abundance of the renal L-Arg-synthesizing enzymes was unchanged, the plasma concentration of L-Arg was increased with AT-1 antagonism in association with a reduction in renal arginase activity. Plasma ADMA concentrations were not changed even though renal DDAH activity and DDAH1 expression were increased. The increased plasma L-Arg and unchanged ADMA with AT-1 blockade caused an increased plasma L-Arg to ADMA ratio, which should favour NO production.

The FHH rat develops early hypertension and renal injury that is exacerbated by intake of a high protein diet. Several genes contribute to the increased susceptibility to renal disease [7] and autoregulatory failure [25,26] that lead to glomerular hypertension and susceptibility to glomerular injury [27,28]. The prevention of hyperfiltration by AT-1 blockade seen in the present study suggests that GFR was restored to normal by correction of this autoregulatory failure. Blockade of ANGII with ACE inhibitors or AT-1 antagonists reduces glomerular BP and preserves glomerular structure and function [14]. ANGII inhibition with ACE inhibitors initiated early and later in the course of the disease protects the FHH kidney from glomerular damage [10,12]. In the present study, we used AT-1 blockade to attenuate renal damage in FHH to test the hypotheses that L-Arg availability would be preserved and ADMA would be reduced vs untreated FHH.

The kidney is the major site of production of the L-Arg that enters the circulation. L-Arg is produced via citrulline uptake and conversion by ASS and ASL [4], and we have observed marked reductions in renal L-Arg synthesis in the 5/6 ablation infarction model of CKD due to both reduced citrulline uptake and reduced ASS/ASL abundance [29]. In the present study using the FHH rat, we find that, in contrast to other models of CKD, plasma citrulline was not elevated and that abundance of renal cortex ASS and ASL was similar to levels observed in the irbesartan-treated FHH rats with preserved kidney structure. This suggests that renal L-Arg synthesis is not inevitably reduced with CKD but may depend on the type and/or severity of the injury process. It is likely that, in the FHH rat, the increase in GFR and hence increase in citrulline delivery offsets the mild level of structural damage. Nevertheless, we also find that irbesartan treatment and preservation of kidney function in FHH rats are associated with an increase in plasma L-Arg, suggesting increased L-Arg availability.

L-Arg is a promiscuous substrate used by several metabolic enzymes, including arginase. Arginase can consume considerable quantities of L-Arg [4], and arginase abundance and activity increase with vascular injury. Arginase contributes to vascular remodelling after arterial injury [30], and elevated arginase I expression increases rat aortic smooth muscle cell proliferation via polyamine production [31]. Vascular arginases are increased in several types of experimental hypertension including DOCA salt-treated rats, Dahl salt-sensitive rats, the spontaneously hypertensive rat and the 5/6 renal ablation infarction model [32]. In the present study, we find that AT-1 blockade (which reduces BP) also leads to reduction in aortic arginase abundance, presumably because of protection against hypertension and vascular damage.

Activation of vascular arginase is likely to exacerbate hypertension due to both increasing vascular stiffness.

### Table 2. Citrulline, L-Arg and ADMA concentrations and the L-Arg to ADMA ratio (L-Arg:ADMA) in the plasma and kidney cortex NO, concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Citrulline (μM)</th>
<th>L-Arg (μM)</th>
<th>ADMA (μM)</th>
<th>L-Arg:ADMA</th>
<th>NOx (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>66 ± 3</td>
<td>40 ± 4</td>
<td>0.18 ± 0.01</td>
<td>214 ± 14</td>
<td>NA</td>
</tr>
<tr>
<td>HP+Irb</td>
<td>64 ± 3</td>
<td>62 ± 8*</td>
<td>0.18 ± 0.02</td>
<td>295 ± 28*</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SE. Significance was determined by t-test between treatment groups. NA, not available.

*P < 0.05.
and reducing endothelial NO production, secondary to substrate depletion [33]. Arginase knockout mice have higher aortic and endothelial NO production compared to their wild-type littermates [33], and overexpression of arginase in cultured endothelial cells decreases NO production [34]. There is little information on renal arginase activity in hypertension and CKD, but the present study demonstrates that protection from injury and hypertension (with AT-1 blockade) lowers renal cortex arginase activity and abundance but has no effect on liver arginase. There was insufficient tissue to measure aortic arginase activity, but given the fall in abundance with antihypertensive treatment, it is likely that there is also lower vascular arginase activity in the irbesartan-treated FHH rats. This would lead to reduced L-Arg utilization, as evidenced by the increase in plasma L-Arg in the irbesartan-treated FHH.

It is not clear how AT-1 blockade lowers renal and vascular arginase in the FHH. We hypothesize that the action is secondary to the prevention of hypertension and CKD, although it could be that ANGII has specific actions to trigger arginases, as suggested by the recent observation that arginase-1 expression increased by about 70% after 14 days of ANGII infusion and was prevented by AT-1 blockade [35]. To address this, we determined whether endogenous AT-1 antagonism, in the absence of BP-lowering or renoprotective effects, alters arginase abundance or activity in young FHH rats. We observed no change in renal or hepatic arginases or plasma L-Arg, suggesting that the effects observed in the high protein-fed older FHH rats with kidney damage and high BP were secondary to the antihypertensive and renoprotective effects of AT-1 antagonism.

Many factors contribute to the regulation of NO production in addition to L-Arg availability. The endogenous NOS inhibitor ADMA competes with L-Arg for NOS [5] to inhibit NO production. Elevated plasma ADMA causes endothelial dysfunction and is associated with increased cardiovascular morbidity and mortality in many diseases [36–38]. In ESKD, there is a clear association between adverse cardiovascular events and the plasma level of ADMA [39], although there is considerable variability and some controversy about plasma ADMA levels in patients with CKD [36]. While loss of renal clearance plays some role in the increased plasma ADMA in renal disease, the primary method of ADMA removal is by catabolism via the DDAH enzymes. Since the kidney contains a high density of DDAH [6] and because plasma ADMA often increases with renal disease, it is thought that renal DDAH activity significantly contributes to ADMA removal [40]. While we observed opposing effects of irbesartan treatment on DDAH abundance in the kidney (DDAH1 increased and DDAH2 decreased), we found that total DDAH activity was increased by irbesartan treatment in the FHH. The mechanisms responsible for the increase in renal DDAH activity remain unknown, and the lack of concordance between enzyme activity and abundance underscores the need to bet-

**Fig. 2.** L-Arg synthesizing enzyme abundance, ASS (A) and ASL (B) in renal cortex and liver (representative blot shown from kidney cortex; data is presented as mean ± SE; *P < 0.05, **P < 0.01, ***P < 0.001).**

**Fig. 3.** Tissue arginase enzyme abundance (A) and activity in renal cortex (B) and liver (C) (representative blot shown from kidney cortex; data is presented as mean ± SE; *P < 0.05, **P < 0.01, ***P < 0.001).
understand the post-translational regulation of the DDAH isoforms.

Despite the moderate renal injury observed in the present study, we find that plasma ADMA is low in the untreated FHH, at a value similar to the normal Sprague Dawley rat [19]. The plasma ADMA level remains unchanged in the FHH treated with irbesartan, despite an increase in renal DDAH activity, but the plasma L-Arg:ADMA ratio increases due to the increased plasma L-Arg. While plasma ADMA is also low in untreated older FHH (27 weeks), the plasma L-Arg:ADMA ratio is lower than in other strains due to lower L-Arg levels, consistent with our findings [41].

We suggest that, as CKD progresses, NO deficiency develops, which is probably caused by many factors including L-Arg depletion because of arginase activation. In the case of the FHH, Weichert and colleagues have reported that an increase in macula densa neuronal NOS precedes glomerulosclerosis in the FHH [42]. In fact, they suggest that a local increase in NO production may be a primary event that is instrumental in initiating the injury both by
impairing autoregulation of glomerular BP and perhaps by stimulation of renin release from granular cells. As shown in the present study, however, by the time structural damage is developing (at age 19 weeks), renal cortical NO production has begun to decline.

Conclusion

We have shown that the spontaneous kidney injury and hypertension developed by the FHH rat is independent of ADMA. We have shown a novel benefit to AT-1 blockade in the treatment of this model of hypertension and CKD which involves reductions in tissue arginase activity and abundance leading to an improved L-Arg to ADMA ratio.

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Conflict of interest statement

None declared.

References

Amelioration of nephropathy with apoA-1 mimetic peptide in apoE-deficient mice

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Abstract

Background. There is mounting evidence that dyslipidaemia may contribute to development and progression of renal disease. For instance, hyperlipidaemia in apolipoprotein E-deficient (apoE−/−) mice is associated with glomerular inflammation, mesangial expansion and foam cell formation. ApoA-1 mimetic peptides are potent antioxidant and anti-inflammatory compounds which are highly effective in ameliorating atherosclerosis and inflammation in experimental animals. Given the central role of oxidative stress and inflammation in progression of renal disease, we hypothesized that apoA-1 mimetic peptide, D-4F, may attenuate renal lesions in apoE−/− mice.

Methods. Twenty-five-month-old female apoE−/− mice were treated with D-4F (300 µg/mL in drinking water) or placebo for 6 weeks. Kidneys were harvested and examined for histological and biochemical characteristics.

Results. Compared with the control mice, apoE−/− mice showed significant proteinuria, tubulointerstitial inflammation, mesangial expansion, foam cell formation and up-regulation of oxidative [NAD(P)H oxidase subunits] and inflammatory [NF-κB, MCP-1, PAI-1 and COX-2] enzymes. Mice treated with D-4F showed a significant reduction in proteinuria, tubulointerstitial inflammation, mesangial expansion and foam cell formation. D-4F significantly reduced the mRNA expression of CAT1, CAT2, arginase-1, and DDAH2 in preglomerular vessels from angiotensin II hypertensive rats.

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