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, tubulo-interstitial 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]
pathways. D-4F administration lowered proteinuria, improved renal histology and reversed up-regulation of inflammatory and oxidative pathways with only minimal changes in plasma lipid levels.

**Conclusions.** The apoE−/− mice develop proteinuria and glomerular and tubulo-interstitial injury which are associated with up-regulation of oxidative and inflammatory mediators in the kidney and are ameliorated by the administration of apoA-1 mimetic peptide. These observations point to the role of oxidative stress and inflammation in the pathogenesis of renal disease in hyperlipidaemic animals and perhaps humans.

**Keywords:** atherosclerosis; chronic kidney disease; hyperlipidaemia; inflammation; oxidative stress

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**Introduction**

Chronic kidney disease (CKD) results in profound dyslipidaemia, which stems largely from altered metabolism of high-density lipoprotein (HDL) and triglyceride-rich lipoproteins [1,2]. Conversely, there is mounting evidence that dyslipidaemia may contribute to the development and progression of chronic kidney disease [3–7]. For instance, several studies have shown a significant association between dyslipidaemia and deterioration of renal function in patients with pre-existing renal disease [8–10] and increased risk of renal dysfunction among individuals with elevated plasma triglycerides and reduced plasma HDL cholesterol [11] or those with type III hyperlipoproteinaemia [12].

Although the contribution of dyslipidaemia as a cardiovascular risk factor in the general population and patients with CKD is well established, its precise role in the development and progression of renal disease is unclear [5,13]. Apolipoprotein E gene inactivation (apoE−/−) in mice results in severe hyperlipidaemia, which is due to accumulation of atherogenic chylomicron and very-low-density lipoprotein (VLDL) remnants, resembling human type III hyperlipidaemia [14,15]. In fact, apoE−/− mice have been widely used as a model for studies of atherosclerosis [16], and several studies have shown glomerular inflammation, mesangial expansion, foam cell formation and endothelial activation in the kidneys of apoE−/− mice [17,18]. In man, renal involvement has been described in a patient with type III hyperlipoproteinaemia [16]. Therefore, apoE−/− mice provide a convenient animal model to study the effects of hyperlipidaemia on development and progression of kidney disease.

Chylomicron and VLDL remnants (which accumulate in the plasma of apoE−/− mice) are highly susceptible to oxidation. Binding and internalization of oxidized lipoproteins by mesangial cells and macrophages promote expression of adhesion molecules and monocyte chemotactic protein-1, inhibition of nitric oxide production, endothelial dysfunction and apoptosis [19,20]. These events culminate in oxidative stress, inflammation, atherosclerosis and other adverse outcomes [21,22]. In addition to facilitating clearance of the remnant lipoproteins by the liver [23], apoE is involved in cholesterol efflux from macrophages and peripheral tissues [24,25]. Furthermore, apoE plays an immune-modulatory role, and its deletion in mice leads to an inflammatory state [26]. Therefore, lipid deposition, oxidative stress and inflammation may contribute to the renal injury in this model.

Apolipoprotein A-1 (apoA-1) is the major lipoprotein constituent of HDL which mediates many of the anti-inflammatory and antioxidant properties of HDL. In addition, apoA-1 plays a critical role in HDL-induced efflux of excess cholesterol and phospholipid from peripheral tissues and their transport to the liver, a process known as reverse cholesterol transport. ApoA-1 mimetic peptides are an emerging class of therapeutic agents which utilize the antioxidant/anti-inflammatory properties and reverse cholesterol transport capacity of apoA-1 to treat atherosclerosis and inflammatory disorders. Among the existing products, the 18-amino acid peptide, 4F, has shown significant efficacy in the treatment of inflammation, oxidative stress and atherosclerosis in experimental animals. For instance, administration of 4F significantly improves HDL function in mice and monkeys [27] and improves vascular function and reduces endothelial damage in other rodents [30,31]. In addition, the use of apoA-1 mimetic peptides has been effective in a wide range of inflammatory conditions in experimental animals [32–34]. Finally, the administration of 4F ameliorates glomerulosclerosis, tubulo-interstitial injury and inflammation and reduces renal tissue lipid accumulation in the low-density lipoprotein (LDL) receptor-deficient mice fed a Western diet [35].

The present study was undertaken to explore the effect of 4F administration on the established renal injury and the associated oxidative stress and inflammation in apoE−/− mice. Accordingly, renal histology and kidney tissue expression of several pro-inflammatory, pro-fibrosis and pro-oxidant pathways were examined in the 24-week-old wild-type mice and untreated as well as 4F-treated apoE−/− mice.

**Materials and methods**

**Animals and experimental design**

All animal studies were conducted under a protocol approved by the Animal Care and Use Committee of the University of California, Los Angeles. Twenty-five-week-old female apoE−/− mice (Jackson Laboratory, Bar Harbor, ME) fed a normal chow diet (Harlan Teklad) were randomized to D-4F-treated (n = 6) and untreated (n = 6) groups. The treatment group was administered D-4F in the drinking water (300 µg/mL) for 6 weeks starting at week 19 of their age. The choice of the given D-4F dosage was based on earlier studies which demonstrated its efficacy in mice [35]. Sex- and age-matched C57BL/6 J mice (n = 6) were used as additional controls. At the conclusion of the study, under general anaesthesia, mice were euthanized by exsanguination, and plasma and kidney were isolated. Plasma samples were processed for lipid/lipoprotein analysis. A portion of the kidneys was fixed in 10% formalin for histological evaluation. The remaining tissue was immediately frozen in liquid nitrogen and stored at −70°C for further study. Urinary albumin and creatinine concentrations were measured using Nephart kit and Creatinine Companion kit purchased from Exocell, Inc. (Philadelphia, PA, USA). Serum cholesterol, triglyceride and creatinine were determined by AnTech Diagnostics (Irvine, CA, USA). A colorimetric assay was used to measure plasma blood urea nitrogen concentration using a kit obtained from Bioassay systems (Hayward, CA) following the manufacturer’s protocol. Ne-
phrat kit and serum cholesterol, triglyceride and urea concentrations were determined by AnTech Diagnostics (Irvine, CA, USA).

Tissue preparation

Kidney cortex was separated and homogenized in 10 mmol/L HEPES buffer, pH 7.4, containing 320 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L dithiothreitol (DTT), 10 mg/mL leupeptin, 2 mg/mL aprotinin and 1 mol/L phenylmethylsulfonyl fluoride (PMSE) at 0°C to 4°C. Homogenates were centrifuged at 12,000 g for 5 min at 4°C to remove tissue debris and nuclear fragments. The supernatant was used to perform the Western blot analyses. Total protein concentration was determined with the use of a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot analyses

All solutions, tubes and centrifuges were maintained at 0–4°C. The nuclear extract was prepared as described previously [36]. Briefly, 100 mg of kidney cortex was homogenized in 0.5 mL buffer A containing 10 mmol HEPES (pH 7.8), 10 mmol KCl, 2 mmol MgCl$_2$, 1 mL DTT, 0.1 mL EDTA, 0.1 mL PMSE, 1 µmol pepstatin and 1 mL P-aminobenzamidine with a tissue homogenizer for 20 s. Homogenates were kept on ice for 15 min, and then 125 µL of a 10% Nonidet p40 (NP 40) solution was added and mixed for 15 s, and the mixture was centrifuged for 2 min at 12,000 rpm. The supernatant containing cytosolic proteins was collected. The pellet nuclei were washed once with 200 µL of buffer A plus 25 µL of 10% NP 40, centrifuged, then suspended in 50 µL of buffer B (50 mmol HEPES, pH 7.8, 50 mmol KCl, 300 mmol NaCl, 0.1 mL EDTA, 1 mL DTT, 0.1 mL PMSE, 10% (v/v) glycerol), mixed for 20 min and centrifuged for 5 min at 12,000 rpm. The supernatant containing nuclear proteins was stored at −80°C. The protein concentrations in tissue homogenates and nuclear extracts were determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

Target proteins in the cytoplasmic and/or nuclear fractions of the kidney tissue were measured by Western blot analysis using the following antibodies: rabbit antibodies against rat NF-κB p65, NOX4, MCP-1, COX-2 and COX-1 antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antibodies against 12-lipoxygenase (12-LO) (Cayman chemical, Ann Arbor, MI), Cu, Zn-superoxide dismutase (SOD) and catalase (EMD Chemicals, Inc., Gibbstown, NJ), glutathione peroxidase (Gpx) and Mn-SOD (Millipore, Billerica, MA), phospho-IκBα (Cell Signaling Technology, Inc., Denver, CO), gp91phox and p47phox (BD biosciences, San Jose, CA) were purchased from the cited sources. Antibody against β-actin was purchased from Sigma Inc. (Saint Louis, MO).

Briefly, aliquots containing 50 µg proteins were fractionated on 8% polyacrylamide gels and then transferred to Hybond-ECL membrane (Amersham Life Science Inc.) at 200 V for 2 h and transferred to Hybond-ECL membrane (Amersham Life Science Inc.) at 200 V for 2 h. The membranes were fixed and stained, and the number of cells in six high-power fields was determined.

Histological and immuno-histological procedures

All histological and immuno-histological evaluations were performed blindly without previous knowledge of the experimental groups. Light microscopic studies were performed in formalin-fixed paraffin-embedded kidney sections stained with periodic acid–Schiff (PAS) and haematoxylin and eosin (H&E) staining. Severity of glomerulosclerosis and tubulo-interstitial injury was evaluated as described in previous communications [37,38]. Avidin–biotin–peroxidase methodology was used to identify lymphocytes (CD5-positive cells) and macrophages (ED1-positive cells) as described in previously [38]. Cellular infiltration was evaluated separately in the glomeruli (positive cells per glomerular cross section (gcs)) and in tubulo-interstitial areas (positive cells per square millimeter).

Monoclonal antibodies were used to identify lymphocytes (anti-CD5 clone MRXCO19; Biosource, Camarillo, CA, USA) and macrophages (anti ED1; Harlan Bioproducts, Indianapolis, IN, USA). Secondary rat anti-mouse and donkey anti-rabbit antibodies with minimal cross-reactivity to rat serum proteins were obtained from Accurate Chemical and Scientific Co. (Westbury, NY, USA).

Determination of inflammatory index

Inflammatory index was determined for LDL, and protective capacity for HDL was determined according to previous reports [27]. In this bioassay, LDL is added to cultured human aortic endothelial cells and undergoes oxidative modification. Oxidized LDL induces monocyte chemotactic protein 1 (MCP-1). The culture supernatant is tested for monocyte chemotactic activity (MCA) in a Neuroprobe chemotactic unit. In the absence of HDL, the MCA is high. In the presence of normal HDL, LDL oxidation and MCP-1 production are prevented. In the presence of dysfunctional HDL, however, the LDL oxidation is not prevented, and it is even amplified. To determine the inflammatory index, in brief, LDL and HDL were isolated from plasma using fast-protein liquid chromatography (FPLC) equipped with Superose 6B columns. Human aortic endothelial cells were isolated from trimmings of aorta during heart transplantation and were propagated in culture. Monocytes were isolated from the blood of healthy volunteers in a bank of donors at UCLA. LDL (at 100 µg/mL) and HDL (at 15 µg/mL) together or alone were added to endothelial cultures and incubated overnight. The supernatant was removed, diluted 20 folds and tested in the MCA assay. Following the monocyte migration, the filter membranes were fixed and stained, and the number of cells in six high-power fields was determined.

Data presentation and analysis

Data are expressed as mean ± SE unless indicated otherwise. ANOVA and Tukey post tests for multiple groups were used in statistical evaluation of the data using SPSS software version 12.0 (SPSS Inc, Chicago, IL). P values less than 0.05 were considered significant.

Results

General data

Data are summarized in Table 1. As expected, serum cholesterol and triglyceride concentrations were markedly elevated in the apoE−/− mice as compared with the control group. In contrast, HDL cholesterol and paraoxonase activity were significantly reduced, and urinary protein excretion was increased in apoE−/− mice. Treatment with 4F resulted in a significant reduction in the urinary protein excretion and a significant rise in plasma paraoxonase activity. However, 4F administration did not significantly change plasma cholesterol and only minimally but significantly raised HDL-cholesterol concentrations.

Table 1. Blood urea nitrogen (BUN), urine protein/creatinine concentration ratio (Prot/creat) and plasma total cholesterol (chol), triglyceride and HDL-cholesterol concentrations and paraoxonase activity in the wild-type (control) and untreated and D-4F-treated apoE−/− mice

<table>
<thead>
<tr>
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<th>Control</th>
<th>ApoE−/−</th>
<th>ApoE−/− + 4F</th>
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<tbody>
<tr>
<td>BUN (mg/dL)</td>
<td>25.5 ± 1.8</td>
<td>28.1 ± 0.1</td>
<td>26.1 ± 0.4</td>
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<tr>
<td>Urine Prot/creat</td>
<td>3.1 ± 0.3</td>
<td>4.7 ± 0.04*</td>
<td>2.4 ± 0.2***</td>
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<tr>
<td>chol (mg/dL)</td>
<td>87.5 ± 6.8</td>
<td>573.6 ± 26.6**</td>
<td>522.4 ± 28.8</td>
</tr>
<tr>
<td>triglyceride (mg/dL)</td>
<td>70.1 ± 9.0</td>
<td>166.4 ± 10.4**</td>
<td>150.6 ± 12.3</td>
</tr>
<tr>
<td>HDL-chol (mg/dL)</td>
<td>46.2 ± 4.2</td>
<td>27.8 ± 0.6*</td>
<td>32 ± 1.4***</td>
</tr>
<tr>
<td>PON activity</td>
<td>71.1 ± 0.3</td>
<td>31.4 ± 1.9**</td>
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n = 6 in each group.

*P < 0.05 ApoE−/− vs CTL mice.

**P < 0.005 ApoE−/− vs CTL mice.

***P < 0.05 D-4F-treated vs untreated apoE−/− mice.

****P < 0.005 D-4F-treated vs untreated apoE−/− mice.
Histological findings

The apoE−/− mice exhibited mesangial expansion and hypercellularity, occasional capillary microthrombi and foam cell accumulation (Figure 1). These abnormalities were associated with increased interstitial T-cell and macrophage infiltration (Figure 2). Treatment with 4F for 6 weeks significantly improved glomerular and interstitial macrophage and T-cell infiltration (Figure 3).

NAD(P)H oxidase and antioxidant enzymes

Data are shown in Figures 4 and 5. Compared with the wild-type mice, kidney tissue in the untreated apoE−/− mice exhibited significant up-regulation of the catalytic (NOX-4, gp91phox), and regulatory (p47phox) subunits of the superoxide-producing enzyme, NAD(P)H oxidase. Treatment with 4F significantly reduced p47phox abundance but failed to alter NOX-4 or gp91phox abundance. Despite up-regulation of re-

Fig. 1. Representative photomicrographs of glomeruli from an untreated apoE-deficient mouse showing capillary occlusion by microthrombi (arrowhead) and mesangiolysis with capillary dilatation (asterisk) (A) and mild mesangial expansion, collapsed glomerulus with foam cells (asterisks) and focal infiltration of inflammatory cells in the adjacent tubulo-interstitial region (arrowhead) (B). (PAS staining, original magnification ×400).

Fig. 2. Representative photomicrographs illustrating the contrasting features of renal biopsies of an untreated apoE-deficient mouse (A, B) and D-4F-treated apoE-deficient mouse (C, D). Infiltrating macrophages are present in A (arrows) and absent in C (immuno-peroxidase staining). Mesangial expansion and foam cells (asterisks) in B are absent in the glomerulus in D that shows normal appearance (PAS staining) (original magnification ×400).
active oxygen species (ROS)-producing NAD(P)H oxidase isoforms, Cu/Zn-SOD abundance in the kidney tissue was unchanged in the apoE−/− mice and was significantly increased with 4F therapy. Renal tissue catalase and EC-SOD abundance were similar among the apoE−/− and wild-type groups and were not affected by 4F therapy. Glutathione peroxidase abundance was significantly increased in the untreated apoE−/− group and was reduced by D-4F administration.

**Phospho-IkB, COX-2, MCP-1 and PAI-1 data**

Data are shown in Figures 6 and 7. Compared with the wild-type mice, kidney tissues from the untreated apoE−/− mice showed a significant increase in Phospho-IkB and a significant increase in nuclear translocation of p65 subunits of NF-κB. These observations point to activation of NF-κB, which is the general transcription factor for various inflammatory cytokines and chemokines. Treatment with 4F significantly attenuated but did not fully reverse NF-κB activation in the kidneys of the apoE−/− mice. Compared with the control group, kidney tissues in the untreated apoE−/− mice showed significant up-regulation of COX-2. Administration of 4F resulted in significant reduction of COX-2 abundance in the kidneys of apoE−/− mice. Likewise, kidney tissue MCP-1 and PAI-1 were significantly increased in the untreated apoE−/− compared with the wild-type mice and were lowered by 4F administration. Kidney tissue abundance of COX-1 and 12-LPO was similar among the wild-type and apoE−/− mice and was unaffected by 4F administration.

**LDL and HDL chemotactic activities**

Data are illustrated in Figure 8. LDL from the apoE-deficient mice showed significantly higher inflammatory index compared with those from wild-type mice (Figure 8), and HDL from apoE-deficient mice was significantly less protective against LDL oxidation by endothelial cells as compared with HDL from wild-type mice. This effect was more pronounced when autologous LDL (prepared from apoE-deficient ani-
mals vs standard LDL obtained from healthy human donors) was used in incubation with cultured endothelial cells. D-4F treatment resulted in significant improvements in the inflammatory index of LDL and in the protective capacity of HDL against LDL oxidation and MCP-1 induction (Figure 8).

**Discussion**

As expected, the apoE<sup>−/−</sup> mice employed in the present study exhibited severe hyperlipidaemia. This was associated with significant proteinuria, foam cell formation, occasional glomerular capillary thrombosis and significant glomerular and tubulo-interstitial macrophage and lymphocyte accumulation. These observations are consistent with the findings reported in earlier studies in this model [17,18]. Proteinuria and renal histological abnormalities were accompanied by marked up-regulation of oxidative and inflammatory pathways in the kidneys of the apoE<sup>−/−</sup> mice. Accordingly, kidneys in apoE<sup>−/−</sup> animals showed marked up-regulation of NOX-4, gp91phox and p47phox subunits of NAD(P)H oxidase isoforms which are the major

Fig. 5. Representative Western blots and group data depicting protein abundance of the CuZn-SOD, extracellular SOD, catalase and glutathione peroxidase (GPX) in the renal tissues of the wild-type (control) and untreated and D-4F-treated apoE<sup>−/−</sup> mice. n = 6 in each group. a: P < 0.05 vs control group, b: P < 0.05, D-4F-treated vs untreated apoE-deficient group.

Fig. 6. Representative Western blots and group data depicting protein abundance of phospho-IκB and nuclear p65 active subunit of NF-κB in the renal tissues of the wild-type (control) and untreated and D-4F-treated apoE<sup>−/−</sup> mice. n = 6 in each group. a: P < 0.05 vs control group, b: P < 0.05, D-4F-treated vs untreated apoE-deficient group.
Fig. 7. Representative Western blots and group data depicting protein abundance of MCP-1, PAI-1, COX-1, COX-2 and 12-lipoxygenase (12-LPO) in the renal tissues of the wild-type (control) and untreated and D-4F-treated apoE \(-/-\) mice. \(n = 6\) in each group. a: \(P < 0.05\) vs control group, b: \(P < 0.05\), D-4F-treated vs untreated apoE-deficient group.

Fig. 8. Bar graphs depicting LDL and HDL chemotactic activities in the wild-type and untreated- and D-4F-treated apoE \(-/-\) groups. Standard LDL and standard HDL were from healthy donors were used as controls. Mouse LDL and HDL were isolated from plasma by FPLC and inflammatory index determined in the as described in the Methods section. The values are presented as mean ± SD of migrated monocytes per high power field. The value for the standard LDL was taken as 1.0 and used as basis against which the inflammatory indices of the test samples were calculated. \(n = 6\) in each group.
source of superoxide (O$_2^−$) in the kidney and cardiovascular tissues. Despite up-regulation of the superoxide-production capacity, renal tissue abundance of Cu/Zn-SOD was reduced, and extracellular SOD abundance was unchanged in the apoE$^{−/−}$ mice kidneys. These events can contribute to oxidative stress and renal injury in the apoE$^{−/−}$ mice. Administration of 4F for 6 weeks resulted in partial but significant reduction of NADPH oxidase subunits and restoration of Cu/Zn-SOD. These observations point to the efficacy of this peptide in improving redox status of the kidney in the apoE$^{−/−}$ mice.

The apoE$^{−/−}$ mice exhibited a significant increase in the renal tissue abundance of MCP-1 which is a potent pro-inflammatory chemokine. This phenomenon can contribute to renal injury and dysfunction by promoting inflammation and leukocyte infiltration seen here as well as in earlier studies [17,18]. Similarly, PAI-1 expression was significantly increased in the kidneys of the apoE$^{−/−}$ animals. This can, in turn, contribute to thrombosis, glomerulosclerosis and interstitial fibrosis by inhibiting plasmin and matrix metalloproteinases. Up-regulation of MCP-1, PAI-1 and NAD(P)H oxidase and immune cell infiltration in the kidneys of apoE$^{−/−}$ mice were accompanied by activation of NF-κB as evidenced by elevation of Phospho-IκB and nuclear translocation of P65 active subunit of this transcription factor. NF-κB is the general transcription factor for numerous pro-inflammatory cytokines and chemokines, and as such its activation can account for the up-regulation of MCP-1 and PAI-1 and immune cell infiltration in the kidneys of the apoE$^{−/−}$ animals shown here and in previous studies [17,18]. Administration of 4F attenuated NF-κB activation, reduced MCP-1 and PAI-1 abundance and lowered glomerular and interstitial immune cell infiltration in the apoE$^{−/−}$ mice.

By-products of the main enzymes of the arachidonic acid metabolism play an important part in the pathogenesis of inflammation and oxidative stress as well as dysregulation of renal and systemic haemodynamics [39–43]. For instance, COX-2 can promote ROS production, inflammation and haemodynamic alterations in the kidney and other tissues [39–42,44–46]. Consequently, the observed up-regulation of renal tissue COX-2 abundance in our apoE$^{−/−}$ mice can contribute to renal injury and inflammation in these animals. Treatment with 4F significantly reduced COX-2 expression and mitigated oxidative stress and inflammation in the apoE$^{−/−}$ mice. This was accompanied by a significant attenuation of proteinuria and nephropathological abnormalities in these animals.

The results of the present study provide compelling evidence that severe persistent hyperlipidaemia in apoE$^{−/−}$ mice results in renal injury, which is marked by oxidative stress, inflammation, mesangial expansion and microvascular thrombosis. These observations support the potential role of long-standing hyperlipidaemia in the pathogenesis of renal disease. Administration of apoA-1 mimetic peptide, 4F, attenuated up-regulation of oxidative and inflammatory mediators, reversed proteinuria and ameliorated histological changes in the kidneys of apoE$^{−/−}$ mice. Since the treatment had only minimal impact on plasma lipid levels, these observations suggest that the associated renal disease in this model is largely mediated by oxidative stress and inflammation as opposed to a direct effect of elevated plasma lipid levels per se.

The apoA-1 mimetic peptides, which were originally designed to improve HDL function [27,28], have been found to possess profound antioxidant and anti-inflammatory properties [32–35]. In this context, 4F has been shown to significantly reduce LDL pro-inflammatory activity and enhanced HDL anti-inflammatory activity in human uraemic plasma [47]. Thus, the renal protective effect of 4F observed in apoE$^{−/−}$ mice appears to be mediated by its potent antioxidant/anti-inflammatory actions. In fact, in the present study, LDL from the apoE-deficient mice was less resistant to oxidative modification and induced a higher level of monocyte chemotactic activity by cultured endothelial cells when compared with LDL from the wild-type mice which is resistant to inflammatory pressure. Treatment of the animals with D-4F resulted in normalization of LDL resistance to oxidative modification. Similarly, HDL from the apoE-deficient mice was less protective against LDL oxidation when compared with the HDL from the wild-type animals. Treatment with D-4F normalized the protective capacity of HDL. Pro-inflammatory properties of LDL and HDL are primarily mediated by their oxidized lipid contents. In fact, in an earlier study, our coauthors have demonstrated that plasma lipoprotein fractions in apoE$^{−/−}$ mice contain large quantities of lipoperoxides which are dramatically reduced by D-4F administration [28]. Therefore, the observed improvement in the LDL and HDL inflammatory activities shown here can be in part due to the antioxidant properties of this peptide.

In conclusion, severe persistent hyperlipidaemia in apoE-deficient mice results in up-regulation of oxidative and inflammatory mediators in the kidney culminating in proteinuria and glomerular and tubulo-interstitial injury. Administration of apoA-1 mimetic peptide 4F attenuates up-regulation of oxidative and inflammatory mediators and improves proteinuria and renal histological abnormalities without altering plasma lipid profile. These observations suggest that the hyperlipidaemia-associated renal disease is primarily mediated by oxidative stress and inflammation as opposed to direct effect of elevated plasma lipid levels per se.

Acknowledgements. H.M. is supported by the NIH-NRSA Award No DK-082130. The results presented in this paper have not been published previously in whole or in part, except in abstract format.

Conflict of interest statement. M.N. and A.M.F. are principals in Bruin-Pharma Inc.

References
ApoE deficiency-induced nephropathy


Retinoic acid-inducible gene-I is induced by double-stranded RNA and regulates the expression of CC chemokine ligand (CCL) 5 in human mesangial cells

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Abstract

Background. Retinoic acid-inducible gene-I (RIG-I) is a putative RNA helicase involved in immune reactions against RNA viruses and various inflammatory and autoimmune diseases. The purpose of the present study was to investigate the role of RIG-I in glomerular diseases.

Methods. We treated human mesangial cells in culture with polyinosinic–polycytidylic acid (poly IC), which is an authentic double-stranded RNA, and analysed the expression of RIG-I, CC chemokine ligand 5 (CCL5) and interferon (IFN)-β by western blotting, reverse transcriptase–polymerase chain reaction (RT–PCR) or enzyme-linked immunosorbent assay (ELISA). To elucidate the poly IC-signalling pathway, we subjected the cells to RNA interference (RNAi) against RIG-I, IFN-β or Toll-like receptor (TLR) 3. Furthermore, we studied the effects of IFN-β receptor blocking and IFN-β overexpression.

Results. Poly IC induced the expression of RIG-I and CCL5 in human mesangial cells, and RNAi against RIG-I inhibited this poly IC-induced CCL5 expression. Poly IC-induced RIG-I expression was also inhibited by RNAi against IFN-β and by an antibody against the IFN-β receptor. IFN-β overexpression induced the expression of both RIG-I and CCL5. The knockdown of TLR3 abolished poly IC-induced RIG-I expression.

Conclusions. The TLR3/IFN-β/RIG-I/CCL5 signalling pathway may mediate immune and inflammatory responses against viral infection in mesangial cells, suggesting the role of this pathway in the aggravation of glomerulonephritis due to viral infection.

Keywords: CC chemokine ligand 5; interferon-β; mesangial cells; polyinosinic–polycytidylic acid; retinoic acid-inducible gene-I

Introduction

Viral infection may sometimes trigger the development of an inflammatory renal disease or worsening of pre-existing renal disease [1]. Mesangial cells produce a wide variety of pro-inflammatory molecules that play an important role in immune and inflammatory reactions in the kidney; however, the details of the anti-viral reactions of mesangial cells are not yet fully elucidated.

Retinoic acid-inducible gene-I (RIG-I) encodes a DExH box protein, which is an RNA helicase [2]. The DExH box family proteins regulate RNA metabolism and have various biological functions [3]. In particular, RIG-I may detect viral RNAs and mediate immune reactions against RNA viruses [4]. RIG-I is also suggested to be involved in immune and inflammatory responses in various physiological and disease conditions [5].

A double-stranded RNA (dsRNA) is generated during replication cycle of certain viruses. Polyinosinic–polycytidylic acid (poly IC) is an authentic dsRNA, and both viral dsRNA and poly IC are potent inducers of type I interferons (IFNs) and the downstream molecules of the innate immune pathway. Therefore, poly IC has been widely used to mimic viral infection in various cell types, including dendritic cells [6], T-cells [7] and mesangial cells [8–11].