The pleiotropic actions of rosuvastatin confer renal benefits in the diabetic Apo-E knockout mouse

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Giunti S, Calkin AC, Forbes JM, Allen TJ, Thomas MC, Cooper ME, Jandeleit-Dahm KA. The pleiotropic actions of rosuvastatin confer renal benefits in the diabetic Apo-E knockout mouse. Am J Physiol Renal Physiol 299: F528–F535, 2010. First published June 16, 2010; doi:10.1152/ajprenal.00127.2010.—Diabetic nephropathy is a leading cause of end-stage renal disease. Statins may exert renoprotective effects independently of lipid-lowering properties. We investigated the pleiotropic effects of rosuvastatin on renal structure and function in streptozotocin diabetic apolipoprotein-E knockout (Apo-E−/−) mice, a model of progressive nephropathy in which dyslipidemia is resistant to statin treatment. These effects were compared with those observed with conventional renin-angiotensin system blockade (candesartan) or combined treatment. Nondiabetic and diabetic Apo-E−/− mice were randomized to no treatment or treatment with candesartan (2.5 mg/kg), rosuvastatin (5 mg/kg), or their combination per gavage for 20 wk. Urine and blood samples were collected for assessment of albuminuria, creatinine clearance, plasma lipids, glucose, and glycated hemoglobin. Renal sclerosis was analyzed on paraffin-embedded kidney sections stained with periodic acid-Schiff. Renal expression of collagen IV, fibronectin and advanced glycation end products (AGEs), receptor for advanced glycation and products (RAGE), NADPH oxidase 4 (NOX4), and nitrotyrosine was assessed by real-time PCR and/or immunohistochemistry. Diabetes-induced albuminuria was not affected by rosuvastatin and combination treatment but was prevented by candesartan. Diabetes resulted in increased creatinine clearance, which was not modified by the treatments. Rosuvastatin and/or candesartan prevented diabetes-associated renal extracellular matrix accumulation. Rosuvastatin reduced accumulation of AGEs and expression of RAGE, NOX4, and nitrotyrosine. In conclusion, in the diabetic Apo-E−/− mouse, rosuvastatin confers renal benefits that are independent of lipid lowering and equivalent or greater to those observed with candesartan. The combination treatment is not superior to monotherapies.

MATERIALS AND METHODS

Animal model. Six-week-old male Apo-E−/− mice (backcrossed 20 times to a C57BL/6 background; Animal Resource Centre, Canning Vale, WA, Australia, and maintained in the Precinct Animal Centre, Melbourne, Australia) were used in this study. The Apo-E gene deletion in mice results in chronic hypercholesterolemia that is not responsive to HMG-CoA reductase inhibitors. The animal studies were approved by the Animal Welfare Committee of the Baker IDI Heart and Diabetes Institute and Alfred Hospital in accordance with the guidelines laid down by the National Health and Medical Research Council of Australia.

Animal groups and experimental design. Apo-E−/− mice were rendered diabetic via intraperitoneal injection of streptozotocin (55 mg·kg−1·day−1; MP Biomedicals, Eschwege, Germany) for 5 days. Animals with >10% glycated hemoglobin 10 wk after the induction of diabetes were included in the study as diabetic. Apo-E−/− mice sham-injected with sodium citrate buffer were followed concurrently and used as nondiabetic control animals. Diabetic animals were randomized to receive no treatment or the ARB candesartan (2.5 mg·kg−1·day−1·gavage), the HMG-CoA reductase inhibitor rosuvastatin (5 mg·kg−1·day−1), or their combination by gavage for a 20-wk period (n = 6–8/group). None of the animals with diabetes required supplemental insulin to maintain body weight or prevent ketosis. During the study, mice had unrestricted access to standard chow and water.

After 20 wk of experimental diabetes, mice were killed with an intraperitoneal injection of Euthal (10 mg/kg; Delvet Limited, Seven Hills, Australia) followed by exsanguination by cardiac puncture. The kidneys were rapidly dissected out, weighed, and frozen or processed to paraffin for subsequent analysis.

Measurement of physiological and biochemical parameters. Before being killed, mice were placed in individual metabolic cages (Iffa Credo, L’Arbresle, France) for a 24-h period. Urine was collected for subsequent analysis. Blood glucose was measured serially using a glucometer (Accutrend; Boehringer Mannheim Biochemicals, Manheim, Germany). Glycated hemoglobin was measured by HPLC (CLC330 GHb Analyzer; Primus, Kansas City, MO) in whole blood obtained at the time of death. Plasma glucose, total low-density lipoprotein (LDL)/high-density lipoprotein cholesterol, and triglycerides were
Table 1. Characteristics of nondiabetic and diabetic apo-E KO mice untreated or treated with candesartan, rosuvastatin, or the combination

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Apo-E KO</th>
<th>Diabetic Apo-E KO</th>
<th>Diabetic Apo-E KO + C</th>
<th>Diabetic Apo-E KO + R</th>
<th>Diabetic Apo-E KO + C + R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt at diabetes induction 7 wk, g</td>
<td>21.3 ± 0.3</td>
<td>20.2 ± 0.3</td>
<td>19.8 ± 0.4</td>
<td>20.4 ± 0.6</td>
<td>19.3 ± 0.3</td>
</tr>
<tr>
<td>Body wt 10 wk, g</td>
<td>28.6 ± 0.4</td>
<td>24.5 ± 0.6†</td>
<td>24.5 ± 1.2†</td>
<td>23.5 ± 0.7†</td>
<td>25.0 ± 0.7†</td>
</tr>
<tr>
<td>Body wt 20 wk, g</td>
<td>30.8 ± 0.5</td>
<td>24.5 ± 0.3†</td>
<td>24.3 ± 1.7†</td>
<td>24.3 ± 0.6†</td>
<td>25.7 ± 0.5†</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>98 ± 4</td>
<td>108 ± 2†</td>
<td>99 ± 4‡</td>
<td>106 ± 3</td>
<td>104 ± 2</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>12.4 ± 0.8</td>
<td>29.1 ± 1.3†</td>
<td>27.2 ± 1.1†</td>
<td>27.2 ± 3.2†</td>
<td>28.6 ± 1.9†</td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>5.3 ± 0.3</td>
<td>14.9 ± 0.6†</td>
<td>15.1 ± 0.5†</td>
<td>15.3 ± 0.8†</td>
<td>14.7 ± 0.9†</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>11.2 ± 0.4</td>
<td>25.0 ± 1.5†</td>
<td>22.9 ± 1.7†</td>
<td>27.1 ± 2.2†</td>
<td>25.2 ± 1.6†</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.3 ± 0.3</td>
<td>3.3 ± 0.4‡</td>
<td>2.3 ± 0.4</td>
<td>3.3 ± 0.6‡</td>
<td>2.7 ± 0.3‡</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>2.6 ± 0.2</td>
<td>4.0 ± 0.2‡</td>
<td>4.2 ± 0.5†</td>
<td>4.1 ± 0.6†</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/l</td>
<td>7.9 ± 0.2</td>
<td>19.6 ± 1.4†</td>
<td>17.7 ± 1.2†</td>
<td>21.4 ± 1.6†</td>
<td>20.5 ± 1.6†</td>
</tr>
<tr>
<td>Mean urinary albumin excretion, µg/day*</td>
<td>126 (106–155)</td>
<td>256 (232–283)†</td>
<td>153 (130–181)†</td>
<td>209 (178–248)</td>
<td>218 (204–234)</td>
</tr>
<tr>
<td>Creatinine clearance, ml·min⁻¹·m²</td>
<td>34 ± 1</td>
<td>62 ± 4†</td>
<td>68 ± 3†</td>
<td>61 ± 3†</td>
<td>62 ± 8†</td>
</tr>
</tbody>
</table>

Data shows mean ± SE unless otherwise indicated. Apo-E, apolipoprotein; KO, knockout; C, candesartan; R, rosuvastatin; C+R, combination of candesartan and rosuvastatin; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *Data show geometric mean (tolerance interval). †P < 0.05 vs. nondiabetic Apo-E KO mice. ‡P < 0.01 vs. diabetic untreated mice.

measured in samples obtained at the time of death, using an automated system (Abbott Architect ci8200; Abbott Laboratories).

Estimation of systolic blood pressure. Systolic blood pressure was assessed using the computerized noninvasive tail-cuff method. Mice were familiarized with the equipment to ensure accurate measurements. Readings were taken by an experienced technician on conscious mice at the conclusion of the dose phase.

Assessment of renal functional parameters. The urinary albumin concentration was measured in samples obtained at the time of death, using an automated device (Abbott Architect ci8200; Abbott Laboratories). Urine and serum creatinine concentration was measured by a mouse albumin ELISA quantitation kit (Bethyl Laboratories, Montgomery, TX). Urine and serum creatinine were measured by HPLC, as previously described (1) according to the Animal Models of Diabetic Complications Consortium guidelines (www.amdcc.org/shared/showFile.aspx?doctypeid=21). Creatinine clearance was estimated as the ratio of daily urinary creatinine excretion to plasma creatinine concentration and expressed as milliliters per minute per square meter surface area.

Evaluation of renal sclerosis indexes. Three-micrometer kidney sections were stained with periodic acid-Schiff (PAS). Glomerulosclerotic injury was graded based on the severity of glomerular damage, including mesangial matrix expansion, hyalinosis with focal adhesion, capillary dilation, and tubular and interstitial sclerosis, as previously described (11). Twenty glomeruli per kidney were assessed in a blinded fashion.

Fig. 1. A: representative images of glomerular and tubulointerstitial injury, as assessed by periodic acid-Schiff (PAS) staining, in nondiabetic (N), diabetic (D), and diabetic apolipoprotein-E knockout (Apo-E −/−) mice treated with candesartan (D + C), rosuvastatin (D + R), and the combination (D + C + R) for 20 wk (×400 magnification). B and C: results of glomerular and tubulointerstitial sclerosis index assessment. Data are means ± SE. †P < 0.01 vs. nondiabetic Apo-E −/− mice. ‡P < 0.01 vs. diabetic Apo-E −/− mice.
The tubulointerstitial area was estimated at the corticomedullary junction using a point counting system, as previously described (1). Six ×200 power fields were analyzed per kidney. Results were expressed as the percentage of tubulointerstitial space within the area assessed.

**Immunohistochemistry.** Renal paraffin sections (4 μm) were stained for collagen type IV, fibronectin, advanced glycation end products (AGEs), receptor for advanced glycation (RAGE), and nitrotyrosine. The primary antibodies included a polyclonal goat anti-collagen type IV antibody (Southern Biotechnology, Birmingham, AL), a polyclonal rabbit anti-fibronectin antibody (Sigma-Aldrich), a polyclonal rabbit anti-AGE antibody (AGE 4G9, which recognizes the AGE CML), a polyclonal goat anti-RAGE antibody (Chemicon, Temecula, CA), and a polyclonal rabbit anti-nitrotyrosine antibody (Chemicon International). Briefly, sections for AGES and RAGE were dewaxed, hydrated, and quenched with 3% H2O2 in Tris-buffered saline (TBS). This was followed by incubation in Protein Blocking Agent (Lipshaw-Immunon, Pittsburgh, PA) for 60 min at room temperature. The sections were then incubated with anti-AGEs and anti-RAGE antibodies overnight at 4°C in a humid atmosphere. Sections for collagen IV, fibronectin, and nitrotyrosine were dewaxed, hydrated, and quenched with 3% H2O2 in TBS. In addition, sections were digested with 0.4% pepsin (Sigma Chemical) in 0.01 M HCl at 37°C for 10 min. Subsequently, sections were incubated with the primary antibody anti-collagen IV, anti-fibronectin, and anti-nitrotyrosine overnight at 4°C followed by avidin/biotin blocking. Thereafter, biotinylated anti-rabbit Ig (Vector Laboratories) for AGES, fibronectin, and nitrotyrosine and biotinylated anti-goat Ig (Vector Laboratories) for collagen type IV and RAGE were applied as the secondary antibodies, followed by horseradish peroxidase-conjugated streptavidin (VECTASTAIN Elite ABC Staining Kit; Vector Laboratories). Peroxidase conjugates were subsequently visualized using 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical) in 0.08% H2O2/TBS. Finally, sections were counterstained with Mayer’s hematoxylin, dehydrated, and mounted. All sections were examined under light microscopy (Olympus BX-50; Olympus Optical) and digitized with a high-resolution camera. For the quantification of the proportional area of staining, 20 views (×400) and 6 views (×200) were analyzed in the renal cortex and in the tubulointerstitium, respectively (Optimas 6.2-Video Pro-32; Bedford Park, SA, Australia). All assessments were performed in a blinded manner.

**Quantitative RT-PCR.** Total RNA was extracted after homogenization of kidneys (Polytron PT-MR2100; Kinematica) in TRIzolR Reagent (Invitrogen Life Technologies). Contaminating DNA was removed after treatment with DNA-free DNase according to the manufacturer’s specifications (Ambion, Austin, TX). Finally, DNA-free RNA was reverse transcribed into cDNA using the Superscript First Strand Synthesis System (Life Technologies BRL, Grand Island, NY).

Gene expression of collagen IV, fibronectin, RAGE, and NADPH oxidase 4 (NOX4) was analyzed by quantitative RT-PCR using the Taqman system based on real-time detection of accumulated fluorescence (ABI Prism 7500; Perkin-Elmer, Foster City, CA). Fluorescence for each cycle was analyzed quantitatively, and gene expression was normalized relative to the expression of the housekeeping gene 18S ribosomal RNA (18S rRNA Taqman Control Reagent kit) that was multiplexed together with the gene of interest. Probes and primers were designed using a Primer Express program and were purchased from Applied Biosystems (ABI, Foster City, CA). Probes and primers were as follows: collagen IV: forward primer GGGGTACACAGTCAGACCAT, reverse primer GGCGGTACACAGTCAGACCAT, and probe 6- FAM CAGTGCCAGAATAGCCGATCCACAGTGA, and probe 6- FAM CCCCCTCAGCGTCTA; RAGE: forward primer GCTGACAA, reverse primer ACATTCGGCAGGTATGGTCTTG, and probe 6- FAM CCCCGTCAGGCTTA; fibronectin: forward primer ACCATGGCTTTAGGCGGGAATAGCCGATCCACAGTGA, and probe 6- FAM CCCCCTCAGCGTCTA; and NOX4: forward primer GGCGGTACACAGTCAGACCAT, reverse primer GGCGGTACACAGTCAGACCAT, and probe 6- FAM CCCCCTCAGCGTCTA.
TAGCTGGTGGTCAGAACA, reverse primer CCCCTTACAGCT-TAGCACAAGTG and probe 6- FAM CACAGCCGGATTG; NOX4: forward primer AAAAATATCACAACCTGAATTCCGAGACT, reverse primer TGGGTCCACACGCAGAAAACTC, and probe 6- FAM CATTTTGCTATTTTCAAA.

Amplifications were performed with the following time course: 50°C for 2 min and 95°C for 10 min and 40 cycles of 94°C for 20 s and at 60°C for 1 min. Results were expressed relative to nondiabetic Apo-E−/− mice.

Statistical analysis. Data were analyzed by ANOVA. Comparison of group means was performed by Fisher’s least-significant difference method. Analyses were performed using Statview V (Brainpower, Calabasas, CA). Data are shown as means ± SE, except for urinary albumin excretion, which was not normally distributed; therefore, it is shown as geometric mean. P < 0.05 was considered statistically significant.

RESULTS

Metabolic and blood pressure parameters. The induction of diabetes was associated with a sustained increase in circulating glucose levels and a threefold increase in glycated hemoglobin. This was not affected by treatment with rosuvastatin or candesartan separately, or with their combined use (Table 1). Diabetic Apo-E−/− mice had significantly lower body weights than nondiabetic animals after a study period of 10 and 20 wk. Body weight was not affected by any of the treatments, either as monotherapy or in combination (Table 1).

The induction of diabetes was also associated with a sustained increase in plasma total and LDL-cholesterol and in triglycerides compared with nondiabetic mice. Lipid levels were not affected by rosuvastatin. This is consistent with the resistance of Apo-E−/− mice to the lipid-lowering effects of statins (24) (Table 1).

Lower systolic blood pressure was observed in diabetic mice treated with candesartan. Rosuvastatin had no effect on blood pressure (Table 1).

Albuminuria and creatinine clearance. A significant increase in albuminuria was observed in diabetic compared with nondiabetic mice, consistent with the development of nephropathy (Table 1). Rosuvastatin alone or in combination had no significant effect on diabetes-associated albuminuria while candesartan alone reduced albuminuria in diabetic mice (Table 1).

The induction of diabetes was also associated with a sustained increase in creatinine clearance (hyperfiltration) that was not modified by candesartan, rosuvastatin, and their combination (Table 1).

Renal sclerosis. Twenty weeks of diabetes in Apo-E−/− mice resulted in increased glomerular and tubulointerstitial sclerosis, as assessed semiquantitatively by PAS staining (11). This was prevented by treatment with rosuvastatin. Treatment with candesartan also reduced glomerulo- and tubulosclerosis, consistent with its known renoprotective actions. However, the

Fig. 3. Glomerular fibronectin expression as assessed by immunohistochemistry and real-time PCR. A: representative images of fibronectin staining in nondiabetic, diabetic, and diabetic Apo-E−/− mice treated with candesartan, rosuvastatin, and the combination (∼×400 magnification). Kidney paraffin sections from both nondiabetic and diabetic Apo-E−/− mice untreated or treated with candesartan, rosuvastatin, or the combination for 20 wk were stained for fibronectin as described in MATERIALS AND METHODS. B: quantification of glomerular proportional area of staining for fibronectin. Data are means ± SE. †P < 0.01 vs. nondiabetic Apo-E−/− mice. ‡P < 0.01 vs. diabetic Apo-E−/− mice. C: quantitative RT-PCR analysis of fibronectin mRNA expression in total renal cortex from both nondiabetic, diabetic Apo-E−/− mice untreated, or treated with candesartan, rosuvastatin, or the combination for 20 wk. *P < 0.05 vs. nondiabetic Apo-E−/− mice. #P < 0.05 vs. diabetic Apo-E−/− mice.
combination of rosuvastatin and candesartan showed no additional advantage over either agent alone (Fig. 1).

Renal sclerosis in diabetic mice was associated with increased expression of matrix proteins, collagen IV (Fig. 2), and fibronectin (Fig. 3). Again, these changes were prevented by treatment with rosuvastatin or candesartan, but the combination of rosuvastatin and candesartan showed no additional advantage over either agent alone (Figs. 2 and 3). Diabetes also resulted in a significant increase in renal cortical collagen IV and fibronectin mRNA expression, which was prevented only by rosuvastatin treatment (Figs. 2 and 3).

**AGEs and RAGE.** The accumulation of AGEs and the activation of RAGE are thought to be important pathogenic mediators of renal damage in diabetes (17). In our study, there was a significant increase in renal AGE accumulation and both RAGE mRNA and protein expression following 20 wk of diabetes. These increases were prevented by treatment with rosuvastatin alone and in combination with candesartan (Figs. 4 and 5). Treatment with candesartan alone also reduced tubular AGE accumulation but did not reduce renal RAGE expression.

**Oxidative stress.** Oxidative stress also plays a key role in the pathogenesis of diabetic nephropathy. Glomerular staining for nitrotyrosine, an index of peroxynitrite formation, was increased in diabetic mice. Again, this increase was prevented in diabetic mice treated with rosuvastatin alone and in combination with candesartan. Candesartan alone had no effect on peroxidation (Fig. 6B).

Oxidative stress in diabetes is partly mediated by the increased expression and activity of NADPH oxidase (7). In the current study, renal cortical mRNA expression of the NADPH oxidase subunit NOX4 was significantly increased in diabetic Apo-E−/− mice compared with nondiabetic controls. Again, this increase was prevented in diabetic mice treated with rosuvastatin alone and in combination with candesartan. Candesartan alone had no effect (Fig. 6C).

**DISCUSSION**

Statins have a number of pleiotropic, lipid-independent actions that may render this class of compounds useful for renoprotection. In this study, we show that treatment with rosuvastatin attenuated the diabetes-associated increase in renal fibrosis, and this renoprotection appears to involve the modulation of key pathways that have been implicated in the pathogenesis of diabetic nephropathy, including oxidative stress and the AGE/RAGE axis. These effects are consistent with previous observations in experimental models of both type 1 (2, 9, 13) and type 2 (6, 10) diabetes. However, we clearly show here that these benefits are independent of any effects on circulating lipid levels and equivalent or more effective than conventional renoprotective therapy with the angiotensin receptor blocker candesartan.

The potential mechanisms by which statins exert their pleiotropic actions continue to be hotly debated. Some benefits may occur via inhibition of the mevalonic acid pathway, which

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**Fig. 4.** Renal expression of advanced glycation end products (AGEs) as assessed by immunohistochemistry. Kidney paraffin sections from both nondiabetic and diabetic Apo-E−/− mice untreated or treated with candesartan, rosuvastatin, or the combination for 20 wk were stained for AGEs as described in MATERIALS AND METHODS. A: representative images of AGE staining in nondiabetic, diabetic, and diabetic Apo-E−/− mice treated with candesartan, rosuvastatin, and the combination for 20 wk (×200 magnification). Kidney paraffin sections from both nondiabetic and diabetic Apo-E−/− mice untreated or treated with candesartan, rosuvastatin, or the combination for 20 wk were stained for AGEs as described in MATERIALS AND METHODS. B and C: quantification of both glomerular and tubulointerstitial proportional areas of staining for AGEs. Data are means ± SE. †P < 0.01 vs. nondiabetic Apo-E−/− mice. ‡P < 0.01 vs. diabetic Apo-E−/− mice.
leads to inactivation of signaling proteins, including Ras and Rho GTPases, that regulate important processes, such as mesangial proliferation, inflammation, tissue remodeling, and oxidative stress (8, 10). Direct antioxidant effects have also been ascribed to agents of this class. Consistent with this hypothesis, rosuvastatin attenuated renal oxidative stress associated with diabetes, as indicated by reduced nitrotyrosine staining. In addition, rosuvastatin reduced the induction of NOX4, a key subunit of NADPH oxidase that is widely distributed in the kidney and is considered to play a role in diabetic nephropathy (6, 7).

In our study, rosuvastatin had no significant effect on albuminuria or hyperfiltration, despite preventing glomerulosclerosis and other markers of renal injury. This is consistent with data from a recent meta-analysis that found that statins do not significantly modify albuminuria in individuals with low (abnormal) levels of albumin excretion (4). It is possible that these agents can reduce renal structural injury without a major effect on renal functional parameters such as albuminuria, as has been reported with other interventions such as transforming growth factor-β blockade (25).

It has also been suggested that high-dose rosuvastatin may increase albumin excretion because of inhibition of tubular protein uptake (16, 22). Therefore, this may represent a novel mechanism to explain a tubuloprotective action of rosuvastatin. It should be appreciated that a previous study in a rat streptozotocin model of diabetic nephropathy suggested that simvastatin treatment may have a modest effect on glomerular injury (14), albeit the effect did not reach statistical significance. Thus, one cannot exclude that the renoprotective effects conferred by various statins represent a class effect rather than the effect of individual statins.

Blockade of the RAS is the most widely employed renoprotective intervention to slow the progression of diabetic renal disease. In some clinical studies, but not all, statins have been shown to reduce albuminuria and other markers of renal damage in individuals with established kidney disease on RAS blockers (21). Previous experimental studies in streptozotocin-induced diabetic rats have sometimes shown reduced albuminuria (14) or amelioration of glomerular filtration rate (9) with ACE inhibitor/statin combination therapy, although interpretation of both results is potentially confounded by the effects of lipid lowering. In our model, in the absence of lipid lowering, the addition of rosuvastatin had a number of benefits over candesartan alone, including reduced oxidative stress and RAGE expression. However, no superiority of the combination treatment was observed on renal structural injury in our study, consistent with previous reports (9, 14), suggesting that both drugs may modulate similar downstream pathways such as advanced glycation examined in this study, thereby leading to reduced renal fibrosis.

AGEs play an important role in both the development and progression of diabetic kidney disease and exert their effects through both receptor-dependent and -independent mechanisms (18). Accordingly, targeting of AGEs and modulation of AGE receptor expression has been proposed as a potential therapeutic approach for diabetic nephropathy (17). In the present study, rosuvastatin was able to reduce renal AGE accumulation and RAGE mRNA and protein expression. Be-

Fig. 5. Renal expression of receptor for advanced glycation (RAGE) as assessed by real-time PCR and immunohistochemistry. A: quantitative RT-PCR analysis of RAGE mRNA expression in total renal cortex from nondiabetic, diabetic, and diabetic Apo-E−/− mice treated with candesartan, rosuvastatin, or the combination for 20 wk. *P < 0.05 vs. nondiabetic Apo-E−/− mice. †P < 0.05 vs. diabetic Apo-E−/− mice. B: representative images of RAGE staining in nondiabetic, diabetic, and diabetic Apo-E−/− mice treated with candesartan, rosuvastatin, and the combination for 20 wk (×200 magnification). Kidney paraffin sections from both nondiabetic and diabetic Apo-E−/− mice untreated or treated with candesartan, rosuvastatin, or the combination for 20 wk were stained for RAGE as described in MATERIALS AND METHODS. C and D: quantification of both glomerular and tubulointerstitial proportional areas of staining for RAGE. Data are means ± SE. †P < 0.01 vs. nondiabetic Apo-E−/− mice. ‡P < 0.01 vs. diabetic Apo-E−/− mice. &P < 0.01 vs. diabetic Apo-E−/− mice treated with candesartan.
cause dyslipidemia may also drive AGE accumulation in diabetes, statins may have substantial benefits with respect to activation of the AGE/RAGE axis. In addition, we show for the first time that rosuvastatin is also able to impact on the AGE/RAGE pathway independent of lipid lowering. How this may occur is unclear. However, decreased intrarenal oxidative stress, less absorption of AGE-modified proteins, reduced inflammation, and the subsequent induction of RAGE through reduced isoprenylation of Ras and Rho GTPases may all have a role.

One must be cautious in extrapolating findings from experimental models to the clinical context. Unfortunately, there is no ideal model of human diabetic nephropathy, with most models showing only early renal functional and structural damage without the development of end-stage renal failure. The diabetic Apo-E−/− mouse develops more advanced renal disease than wild-type mice on the same C57BL/6 background but still has the limitation of not developing renal failure. Although not observed at this stage, long-term follow up of renal function in diabetic Apo-E−/− mice has been reported to be associated with a degree of renal impairment (23), with this phenomenon generally preceded by hyperfiltration, a phenomenon widely described not only in various models of diabetic nephropathy, but also in humans.

In conclusion, the HMG-CoA reductase inhibitor rosuvastatin confers renal benefits in the diabetic hyperlipidaemic Apo-E−/− mouse, in the absence of lipid lowering. The beneficial renal effects conferred by rosuvastatin were equivalent or greater to those observed with the ARB candesartan. Although lipid lowering is an important clinical outcome and confers its own renoprotective actions, better understanding of these lipid-independent pleiotropic actions has the potential to lead to the development of more effective renoprotective agents for use in the clinical setting.

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DISCLOSURES

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