ORIGINAL ARTICLE

A Pilot Study Linking Endothelial Injury in Lungs and Kidneys in Chronic Obstructive Pulmonary Disease

Francesca Polverino[1,2,3*], Maria E. Laucho-Contreras[4*], Hans Petersen[2], Vanesa Bijol[5], Lynette M. Sholl[5], Mary E. Choi[6], Miguel Divo[1,2], Victor Pinto-Plata[1,2], Alfredo Chetta[3], Yohannes Tesfaigzi[2], Bartolomé R. Celli[1,2], and Caroline A. Owen[1,2]

[1]Pulmonary Division and [5]Pathology Department, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; [2]Lovelace Respiratory Research Institute, Albuquerque, New Mexico; [3]University of Parma, Parma, Italy; [4]Universidad Central de Venezuela, Caracas, Venezuela; and [6]Nephrology Division, Weill Cornell Medical College, New York, New York

ORCID IDs: 0000-0001-9686-5698 (F.P.); 0000-0003-4377-3591 (C.A.O.).

Abstract

Rationale: Patients with chronic obstructive pulmonary disease (COPD) frequently have albuminuria (indicative of renal endothelial cell injury) associated with hypoxemia.

Objectives: To determine whether (*1*) cigarette smoke (CS)-induced pulmonary and renal endothelial cell injury explains the association between albuminuria and COPD, (*2*) CS-induced albuminuria is linked to increases in the oxidative stress–advanced glycation end products (AGEs) receptor for AGEs (RAGE) pathway, and (*3*) enalapril (which has antioxidant properties) limits the progression of pulmonary and renal injury by reducing activation of the AGEs–RAGE pathway in endothelial cells in both organs.

Methods: In 26 patients with COPD, 24 ever-smokers without COPD, 32 nonsmokers who underwent a renal biopsy or nephrectomy, and in CS-exposed mice, we assessed pathologic and ultrastructural renal lesions, and measured urinary albumin/creatinine ratios, tissue oxidative stress levels, and AGEs and RAGE levels in pulmonary and renal endothelial cells. The efficacy of enalapril on pulmonary and renal lesions was assessed in CS-exposed mice.

Measurements and Main Results: Patients with COPD and/or CS-exposed mice had chronic renal injury, increased urinary albumin/creatinine ratios, and increased tissue oxidative stress and AGEs-RAGE levels in pulmonary and renal endothelial cells. Treating mice with enalapril attenuated CS-induced increases in urinary albumin/creatinine ratios, tissue oxidative stress levels, endothelial cell AGEs and RAGE levels, pulmonary and renal cell apoptosis, and the progression of chronic renal and pulmonary lesions.

Conclusions: Patients with COPD and/or CS-exposed mice have pulmonary and renal endothelial cell injury linked to increased endothelial cell AGEs and RAGE levels. Albuminuria could identify patients with COPD in whom angiotensin-converting enzyme inhibitor therapy improves renal and lung function by reducing endothelial injury.

Keywords: kidney; endothelium; cigarette smoke; comorbidities; COPD

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*These authors contributed equally to the study.

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Correspondence and requests for reprints should be addressed to Caroline A. Owen, M.D., Ph.D., Brigham and Women's Hospital, Harvard Institute of Medicine Building, Room 855B, 77 Avenue Louis Pasteur, Boston, MA 02115. E-mail: cowen@bwh.harvard.edu

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At a Glance Commentary

Scientific Knowledge on the Subject: A subset of patients with chronic obstructive pulmonary disease (COPD) has persistent albuminuria indicative of chronic renal endothelial injury. Albuminuria severity correlates directly with the degree of hypoxemia in patients with COPD. Although chronic renal failure is known to be more common in patients with COPD than control subjects, it is unclear whether COPD is associated with chronic renal pathologies.

What This Study Adds to the Field: Chronic injury to the glomeruli, renal tubules, interstitium, and vessels along with double contouring around capillaries (indicative of repetitive renal endothelial injury) is more severe in patients with COPD than control subjects without COPD. Patients with COPD have lower estimated glomerular filtration rates than control subjects. Wild-type mice exposed chronically to cigarette smoke (CS) develop albuminuria, and renal podocyte and glomerular injury. Increases in the oxidative stress–advanced glycation end products (AGEs) and the receptor for AGEs pathway occur in pulmonary and renal endothelial cells in patients with COPD and CS-exposed mice. Treating mice that have CS-induced pulmonary and renal injury with an angiotensin-converting enzyme inhibitor (enalapril, which has antioxidant properties) limited the progression of both renal and pulmonary disease, and reduced activation of the oxidative stress–AGEs–receptor for AGE pathway in CS-exposed mice. These results provide a rationale for testing the therapeutic efficacy of angiotensin-converting enzyme inhibitors in human patients with COPD with endothelial injury and dysfunction as evidenced by microalbuminuria.

Chronic obstructive pulmonary disease (COPD), a major cause of mortality (1), is associated with comorbidities, most importantly cardiovascular disease (2, 3). Albuminuria, a marker of endothelial dysfunction/injury and inflammation (4), is associated with worse cardiovascular outcomes in patients with diabetes mellitus (5), hypertension (6), and the general population (7). Approximately 24% of patients with COPD (vs. 4% of control subjects) have persistent albuminuria (8, 9). Albuminuria severity correlates with degree of hypoxemia in stable patients with COPD and during exacerbations (8, 9), and is associated with systemic inflammation (10) and increased mortality risk independent of cardiovascular comorbidities (11).

Several studies have suggested a link between COPD and renal dysfunction. In a cohort of smokers screened for lung cancer, there was an association between emphysema severity and the estimated glomerular filtration rate (eGFR) (12). Patients with COPD have a higher prevalence of renal failure than age-matched control subjects without COPD (13). A study of more than 900,000 subjects followed for 10 years reported a two-fold increase in the risk of dying from renal failure in smokers versus nonsmokers after adjusting for potential confounders (14). These results support the notion that the link between COPD and renal dysfunction is caused by the vascular effects of cigarette smoke (CS). However, no prior studies have assessed whether COPD is associated with structural renal lesions.

We tested the hypothesis that CS exposure causes simultaneous injury to the lungs and kidneys by increasing tissue oxidative stress levels leading to injury to both the pulmonary and renal endothelium (and other pulmonary and renal cells) in humans and mice. This hypothesis could explain the albuminuria developing in some patients with COPD. We also tested the hypothesis that CS-induced pulmonary and renal endothelial cell (EC) injury is associated with increased EC levels of advanced glycation end-products (AGEs) and the receptor for AGEs (RAGE). The rationale for this second hypothesis is that oxidative stress leads to the generation of AGEs, which activate the RAGE (15), and both AGEs and RAGE have been

Table 1. Demographic and Clinical Characteristics of the Nephrectomy Cohort

	NSC (*n* = 13)	SC (*n* = 12)	COPD* (*n* = 5)	*P* Value
Males, %	46	75	60	NS
Age, yr‡	66 ± 3	65 ± 3	70 ± 6	NS
Pack-yr of smoking†,‡	0 (0)	24 ± 18	51 ± 21	<0.001
Current smokers, %	0	8	40	NS
FEV_1, % of predicted‡	97 ± 17	92 ± 16	56 ± 21	<0.001§
FEV_1/FVC, %‡	78 ± 5	80 ± 6	57 ± 15	0.002§
Hypertension, %	61	50	40	NS
Cardiovascular disease, %	23	17	20	NS
Obesity, %	0	8	0	NS

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; NS = no significant difference; NSC = nonsmoker control subjects; SC = smoker control subjects.
NSC were all never-smokers. SC were defined as subjects who had 10 or more pack-year smoking history. Current smokers were defined as active smokers at the time of the nephrectomy, or had stopped smoking less than or equal to 1 year before the nephrectomy. Statistical analyses included one-way analysis of variance tests for continuous variables (age, FEV_1% predicted, and pack-yr) followed by pairwise comparisons using Student's *t* tests or Mann-Whitney *U* tests. Categorical variables were analyzed using χ^2 tests or Fisher exact tests for small sample sizes. Pairwise comparisons showed no significant differences in the percentage of males, the ages, the proportion of current smokers, or the presence of hypertension, cardiovascular disease, or obesity between the COPD and the SC, the COPD, and the NSC, or the SC and the NSC groups.
*All patients with COPD had FEV_1/FVC less than 0.7, whereas SC and NSC had FEV_1/FCV greater than or equal to 0.7.
†The pack-year smoking histories of the patients with COPD and SC groups were significantly different from those of NSC by design as assessed using a one-way analysis of variance followed by pairwise Student's *t* tests; *P* less than or equal to 0.001 for both comparisons. The pack-year smoking histories of the patients with COPD and SC were not significantly different ($P = 0.07$).
‡The results for age, FEV_1% predicted, FEV1/FVC %, and pack-year smoking history are expressed as mean ± SEM.
§The FEV_1% predicted and the FEV_1/FVC in the COPD group were significantly different from those of the SC and the NSC groups by design ($P < 0.001$ for both comparisons). The FEV_1% predicted and the FEV_1/FVC in the SC group were not significantly different from that of the NSC group ($P > 0.3$ for this comparisons).

linked to COPD pathogenesis in humans and mice (16, 17). Also, in diabetic nephropathy, increased renal EC levels of AGEs and RAGE are linked to both endothelial dysfunction/injury and albuminuria (18, 19).

We also assessed whether increased urinary albumin/creatinine ratios (UACRs) occur in a subset of a longitudinal cohort of smokers, and whether UACRs correlate with rate of decline in FEV_1 in these subjects. In mice, we investigated whether CS also increases UACRs, and the increased UACRs are associated with increased renal and pulmonary oxidative stress levels leading to increased AGEs and RAGE levels in pulmonary and renal ECs. We also assesses whether these changes in mice are associated with the development of pulmonary and renal endothelial injury and chronic end-organ lesions. Angiotensin-converting enzyme inhibitors (ACEi), such as enalapril, which have antioxidant properties (20, 21), are used to treat patients with microalbuminuria secondary to diabetes mellitus and hypertension. Thus, we also assessed whether treating CS-exposed mice with enalapril ameliorates the pulmonary and renal lesions induced by CS by reducing AGEs and RAGE levels in ECs, and generalized EC injury.

Some of the results of these studies have been previously reported in the form of an abstract (22).

Table 2. Demographic and Clinical Characteristics of the Biopsy Cohort

	NSC ($n = 19$)	SC ($n = 12$)	COPD ($n = 21$)	*P* Value
Males, %	63	50	62	NS
Age, yr‡	63 ± 9	69 ± 12	68 ± 8	NS
Pack-yr of smoking*,‡	1.5 ± 3	27 ± 18	40 ± 27	<0.001
Current smokers, %	0	17	19	NS
FEV_1, % of predicted†,‡	99 ± 17	91 ± 12	56 ± 20	<0.001
FEV_1/FVC, % of predicted†,‡	82 ± 4	78 ± 5	60 ± 9	<0.001
Hypertension, %	53	67	71	NS
Cardiovascular disease, %	10.5	25	14.3	NS
Obesity, %	5	8	5	NS

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; NS = no significant difference; NSC = nonsmoker control subject; SC = smoker control subject.
The demographic and clinical characteristics of NSC, SC, and patients with COPD that underwent a renal biopsy are shown along with the percentages of subjects with comorbid diseases. All subjects were non-Hispanic white persons except for one subject in the COPD group and one subject in the NSC group who were both Hispanic white persons. Information about race was not available for two subjects. All patients with COPD had FEV_1/FVC less than 0.7, whereas SC and NSC had FEV_1/FCV greater than or equal to 0.7. NSC included never-smokers (76%) and smokers (24%) that had a less than 10 pack-year history and had stopped smoking for greater than 2 years before the renal biopsy. The mean ± SD pack-years of the smokers in the NSC group was 5.6 ± 2.7. SC were defined as subjects that had 10 or more pack-year smoking history. Current smokers were defined as active smokers at the time of the biopsy, or had stopped smoking less than or equal to 1 year before the biopsy. Statistical analyses included one-way analysis of variance tests for continuous variables (age, FEV_1% predicted, and pack-yr) followed by pairwise comparisons using Student's *t* tests or Mann-Whitney *U* tests. The χ^2 test was used to analyze categorical variables. Pairwise comparisons showed no significant differences in percentage of males, the ages, the percentages of current smokers, or the presence of hypertension, cardiovascular diseases, or obesity between COPD and SC, COPD and NSC, and SC and NSC. The percentage of current smokers in the COPD versus SC and NSC groups was not significantly different ($P = 0.7$ and $P = 0.1$, respectively). The percentage of current smokers in the SC versus NSC was not significantly different ($P = 0.3$).
*The pack-year smoking histories of the COPD and SC groups were significantly different from those of the NSC by design as assessed using a one-way analysis of variance followed by pairwise Student's *t* test ($P < 0.001$ for both comparisons). The pack-year smoking histories of the COPD and SC groups were not significantly different ($P = 0.2$).
†The FEV_1% predicted and the FEV_1/FVC in the COPD group were significantly different from that of the SC and NSC by design ($P < 0.001$ for both of these comparisons).
‡The results for age, FEV_1% predicted, FEV_1/FVC %, and pack-year smoking history are expressed as mean ± SEM.

Methods

Additional details are provided in the online supplement.

Human Studies

All human studies were approved by local institutional review boards.

Pathologic analysis of kidneys from patients with COPD versus control subjects. We analyzed the medical records of 6,736 patients who underwent a nephrectomy (from 2008 to 2015) (Table 1) or a renal biopsy (from 2010 to 2015) (Table 2) at Brigham and Women's Hospital. Most subjects underwent a nephrectomy for a primary renal tumor (*see* Table E1 in the online supplement). Suspected nephritis or glomerulonephritis were the primary indications for renal biopsies in the subjects that underwent a kidney biopsy (*see* Table E2). Exclusion criteria for both cohorts were age less than 50 years; acute kidney injury before the procedure; and physician-diagnosed diabetes mellitus, autoimmune diseases, vasculitis, and restrictive lung diseases. We selected only subjects that had spirometry available (*see* Figure E1). In both cohorts, COPD subjects were restricted to those with a greater than or equal to 20-pack-year smoking history and spirometrically confirmed airflow obstruction (FEV_1/FVC, <0.7). In both cohorts, smoker control subjects (SC) were defined as having greater than or equal to 10-pack-year smoking history and FEV_1/FVC greater than or equal to 70%. Current smokers were defined as active smokers at the time the samples were obtained, or those that had stopped smoking less than or equal to 1 year before the samples were obtained. Nonsmoker control subjects (NSC) were all never-smokers in the nephrectomy cohort. NSC in the renal biopsy cohort included never-smokers (76%) and smokers (24%) who had less than a 10-pack-year history and had stopped smoking for more than 2 years before the renal biopsy.

Estimated GFR. The eGFR was estimated in the nephrectomy and renal biopsy cohorts using equations described in the online supplement.

Macroscopic and ultrastructural analysis of renal sections. These were evaluated by clinical pathologists blinded to the experimental condition as part of the patients' clinical care. In the nephrectomy cohort, the percentage of glomeruli with glomerulosclerosis, the percentage of kidney with fibrotic interstitium and tubular atrophy, and the arterial/arteriolar sclerosis scores were analyzed. In the renal biopsy cohort, renal sections were examined by electron microscopy to evaluate ultrastructural changes in the glomerular capillaries. A renal pathologist

(V.B.) blinded to the experimental condition used a scoring system to measure glomerular capillary wall remodeling (indicative of repetitive renal EC injury).

Immunostaining of renal and pulmonary sections for AGEs and RAGE in ECs. Lung tissue sections were available from subjects that underwent an open resection for a lung nodule or a localized carcinoma (*see* Table E1). None of the subjects studied were taking ACEi or angiotensin receptor blockers. Renal tissue sections were available from subjects that underwent an open resection for localized carcinoma (*see* Table E2). Tissue sections from patients with COPD and control subjects were double immunostained for AGEs and RAGE in ECs in both organs.

UACRs in ever-smokers. First-void morning urine samples were obtained at the baseline visit from smokers recruited to the Lovelace Smokers Cohort (LSC), an observational study of 2,400 subjects followed for more than 5 years with three or more spirometries (23, 24). UACRs were measured in 148 subjects randomly selected from this cohort over a broad range of rate of decline in FEV_1.

Animal Studies

All experiments were approved by the Harvard University Institutional Animal Care and Use Committee.

CS exposures. C57BL/6 strain wild-type (WT) mice were exposed to air or CS using a Teague TE-10z device (Woodland, CA) for 2 hours daily, 6 days-a-week for up to 24 weeks (25).

Enalapril treatments. Saline or enalapril (25 mg/kg body weight) solutions were delivered by the intraperitoneal route to mice 6 days-a-week beginning at the mid-point of 4-week (acute) or 24-week (chronic) CS exposures. Urine was collected weekly. In the chronic study, we measured (*1*) chronic renal injury as renal glomerular diameter to assess glomerulosclerosis, and podocyte width (a readout of podocyte injury); (*2*) chronic COPD-like lung lesions (emphysema as alveolar cord length [25], and small airway fibrosis as extracellular matrix deposition around small airways [25]); and (*3*) pulmonary and renal endothelial injury with terminal deoxynucleotidyl transferase (TUNEL) staining in pulmonary and renal ECs. In the acute study, we measured (*1*) lung inflammation as bronchoalveolar leukocyte counts (26), (*2*) UACRs, (*3*) tissue oxidative stress levels (27), (*4*) AGE levels in lungs and kidneys, and (*5*) AGEs and RAGE staining in pulmonary and renal ECs.

Statistical Analysis

Data were analyzed using one-way analysis of variance, Holm-Sidak, or Dunn methods for multiple group comparisons, followed by pairwise testing using Sigmaplot. Data that are not normally distributed are presented as median and interquartile range (IQR) and pairwise testing was performed using the Mann-Whitney *U* test. Normally distributed data are presented as mean and SEM and pairwise testing was performed using the Student's *t* test. Correlations were calculated using the Pearson Product-Moment correlation test. Categorical variables were analyzed using χ^2 tests or Fisher exact tests for small sample sizes. *P* less than 0.05 was considered significant.

Results

Human Renal Pathology Analyses

Tables 1 and 2 show the demographic and clinical characteristics of the patients that underwent a nephrectomy or a renal biopsy, respectively. Among patients with COPD in the biopsy cohort, 57.1% had Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I–II and 42.9% had GOLD stage III–IV COPD. Among both cohorts, 61.5% of patients with COPD had GOLD stage I–II COPD and 38.5% had GOLD stage III–IV COPD. Patients with COPD did not differ from SC in age, sex ratios, race, ethnicity, pack-year smoking histories, or current smoking status. A similar proportion of patients with COPD and control subjects in both cohorts had hypertension and cardiovascular disease (defined as physician-diagnosed

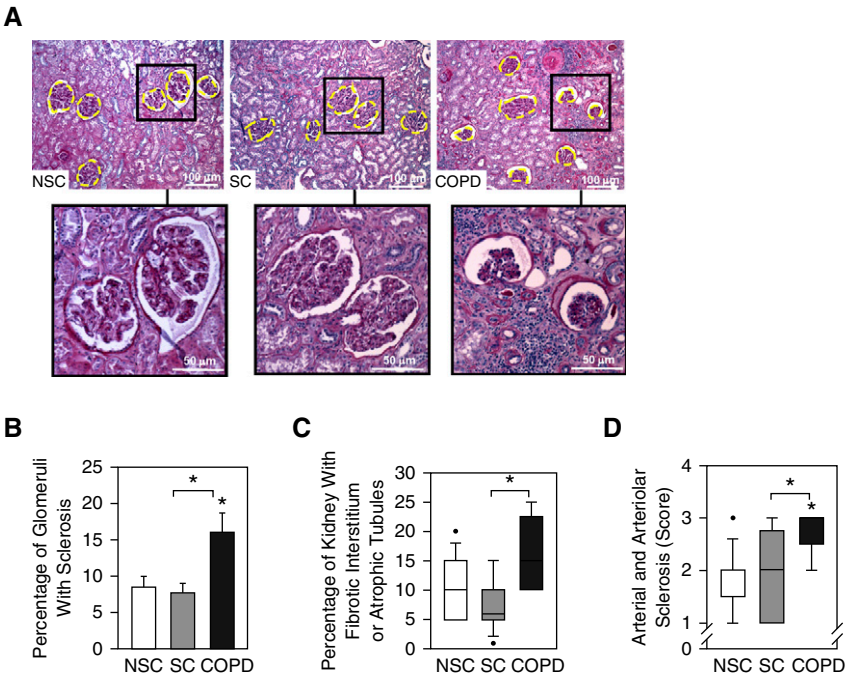

Figure 1. Patients with chronic obstructive pulmonary disease (COPD) have renal injury. (*A*) Representative images of trichrome-stained renal sections from patients with COPD, smoker control subjects (SC), and nonsmoker control subjects (NSC) in the nephrectomy cohort. The patient with COPD has hypoperfused glomeruli with a very widened Bowman space and a small shriveled tuft. The *dotted yellow lines* trace the glomerular perimeters. The *insets* show glomeruli at a higher magnification. (*B*) The percentages of glomeruli having glomerular sclerosis in each group. (*C*) The percentages of the renal interstitium having fibrosis, and renal tubules that were atrophied. (*D*) The arterial and arteriolar sclerosis score in each group. In *D*, scores of *1*, *2*, and *3* represent none or mild, moderate, and severe sclerosis, respectively. *Bar graphs* show mean + SEM, *boxes* in box plots show the median values and 25th and 75th percentiles, and *error bars* show the 10th and 90th percentiles. The *dots* shown in the graphs represent outliers. Student's *t* test (*B*) or a Mann-Whitney *U* test (*C* and *D*) were used to perform the statistical analyses. *$P < 0.05$ versus nonsmoker control subjects or the group indicated. In *B–D*, 5–13 subjects/group were studied.

coronary artery disease, congestive heart failure, myocardial infarction, or cardiomyopathy).

Figure 1A shows representative images of Masson trichrome–stained renal sections from patients with COPD and control subjects in the nephrectomy cohort. The patients with COPD had a higher percentage of sclerotic glomeruli and more global glomerulosclerosis (Figures 1A and 1B) and tubulointerstitial fibrosis (Figure 1C) (median, 15%; IQR, 10–22.5%) than SC (median, 6%; IQR, 1–15%) and NSC (median, 10%; IQR, 5–15%). Patients with COPD also had greater vascular sclerosis (Figure 1D) (median score, 3; IQR, 2.5–3) than SC (median score, 2; IQR, 1–2.7) and NSC (median score, 2; IQR, 1.5–2). Most patients with COPD had evidence of repetitive renal EC injury with glomerular capillary wall remodeling visible as double contours in the glomerular basement membranes. Figure 2A shows representative images of absent, rare, and frequent double contours occurring in NSC, SC, and COPD kidneys, respectively. NSC had no or few double contours, most SC had a few double contours, but 57% of patients with COPD had frequent double contours (Figure 2B). Patients with COPD had a higher double contour frequency score (median, 2.7; IQR, 2–3) than either SC (median, 2; IQR, 1.2–2) or NSC (median, 2; IQR, 1–2) (Figure 2C). In the nephrectomy cohort, 80% of the patients with COPD, 25% of SC, and 8% of NSC had at least moderately severe chronic renal injury as defined by glomerular and/or tubular and/or interstitial pathologies. In the biopsy cohort, 50% of patients with COPD had severe double contours compared with 17% of SC, and 0% of NSC.

Relationships between eGFR, FEV_1, and Double Contour Frequency

When the biopsy and nephrectomy cohorts were combined, the eGFR was significantly lower in patients with COPD than either the SC or NSC (*see* Figure E2A). None of the subjects studied had evidence of acute kidney injury before the procedure as determined by Kidney Diseases Improving Global Outcome criteria. The eGFR had a significant positive correlation with the FEV_1% predicted (*see* Figure E2B). Subjects with the highest double contour frequency score (which were mostly patients with COPD) had significantly lower FEV_1% predicted values and lower eGFRs than patients with intermediate or low double contour frequency scores (*see* Figures E2C and E2D).

UACRs in Smokers and Rate of FEV_1 Decline

Albumin was detected in urine samples from most smokers in the LSC (*see* Figure E3). However, UACRs did not correlate with rate of FEV_1 decline in this cohort of smokers.

Animal studies. CS exposure increased UACRs in WT mice from baseline (median, 110; IQR, 87–128 μg/mg) as early as after 4 weeks of CS exposure (median, 250; IQR, 230–663 μg/mg) and remained elevated after 24 weeks of CS exposure (median, 368; IQR, 307–534 μg/mg) (Figure 3A). After 24 weeks of CS exposure, WT mice had smaller glomeruli than air-exposed mice (Figure 3B) and pronounced widening of podocyte foot processes (Figure 3C), an invariable feature of glomerular diseases associated with proteinuria (28, 29). CS exposure did not induce ultrastructural lesions in the renal tubules in mice (not shown). As expected, CS-exposed mice developed emphysema and small airway fibrosis (Figures 3D and 3E).

Enalapril Reduces Chronic Renal and Pulmonary Disease and Endothelial Injury

Mice exposed to CS for 24 weeks had more TUNEL-positive ECs in pulmonary small vessels than air-exposed mice and more TUNEL-positive ECs in renal capillaries (median, 4; IQR, 3.0–8.0 fold change vs. air) than in air-exposed mice (Figure 4A). CS-exposed mice had higher AGEs levels (Figure 4B) in homogenates of lungs (median, 5.4; IQR, 3–6.6 fold change vs. air) than air-exposed mice. CS-exposed mice also had higher AGEs levels in homogenates of kidneys (median, 4.2; IQR, 3.4–9 fold change vs. air) than air-exposed mice. CS-exposed mice had higher RAGE staining in pulmonary ECs (median, 7.8; IQR, 5.4–29.6 fold change vs. air-exposed mice). CS-exposed mice also had higher

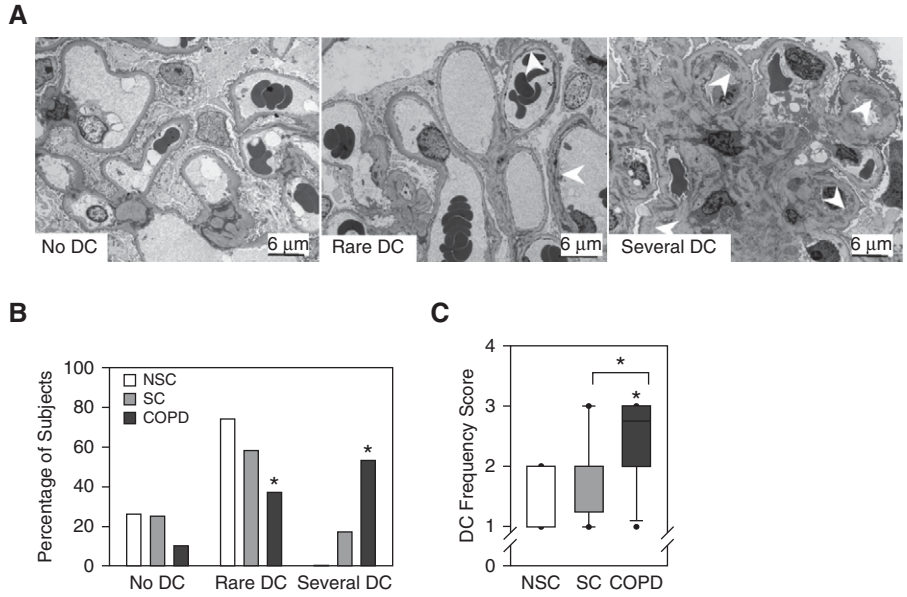

Figure 2. Patients with chronic obstructive pulmonary disease (COPD) have repetitive renal endothelial cell injury. (*A*) Representative electron photomicrographs of glomerular capillary tufts with no, rare, or several double contours (DC) indicated by *arrowheads*. DC are capillary wall remodeling pathologies indicative of repetitive cycles of endothelial cell injury followed by remodeling. (*B*) The mean percentage of subjects having no, rare, or several DC around renal capillaries as assessed using electron microscopy. (*C*) DC frequency in COPD, smoker control subjects (SC), and nonsmoker control subjects (NSC). Scores of *1*, *2*, and *3* represent no, rare, and several DC, respectively. *Bar graphs* show means, *boxes* in box plots show the median values and 25th and 75th percentiles, and *error bars* show the 10th and 90th percentiles. The *dots* shown in the graph represent outliers. Student's *t* test (*B*) or a Mann-Whitney *U* test (*C*) were used to analyze the results. $^*P < 0.05$ versus nonsmoker control subjects or the group indicated. In *B* and *C*, 12–21 subjects were studied per group.

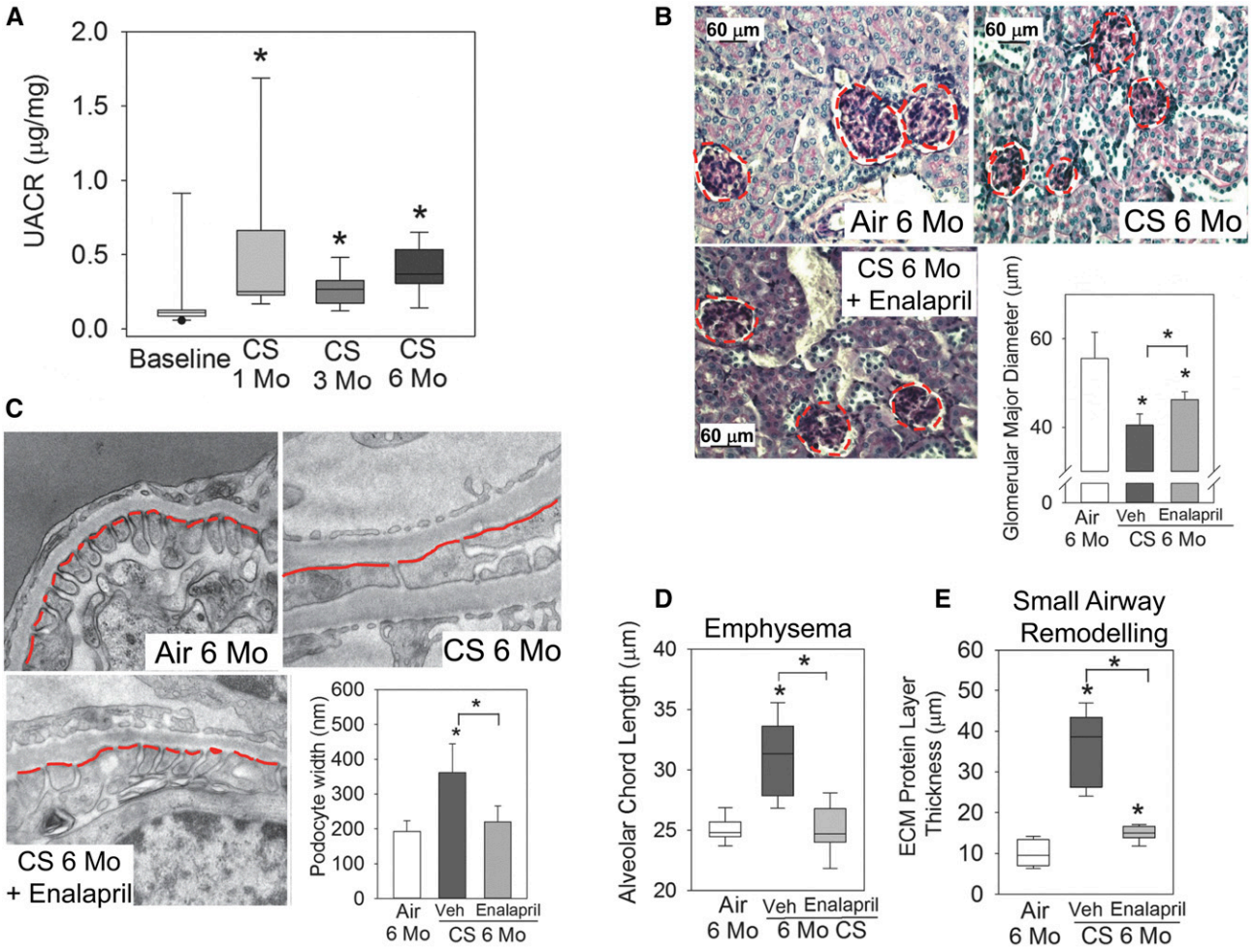

Figure 3. Chronic cigarette smoke (CS) exposure increases urinary albumin/creatinine ratios and induces chronic renal and pulmonary lesions in wild-type mice. (*A*) UACRs measured in urine samples from C57BL/6 wild-type mice. The *dot* shown in the plot represents an outlier. (*B*) Images of glomeruli in hematoxylin and eosin–stained renal sections from mice exposed to air for 6 months or mice exposed to CS for 6 months and treated with saline or enalapril 6 days a week beginning at the 12-week time point and continued for the second 12 weeks of the CS exposures. The *dotted red lines* trace the glomerular perimeters. The images are representative of 3–4 mice/group. The *bar graph* shows the mean + SEM glomerular major diameters. (*C*) Electron microscopy images of podocytes from air-exposed mice or CS-exposed mice treated with saline or enalapril as outlined above. The *red lines* trace the widths of the base of the podocytes. The *bar graph* shows mean + SEM podocyte widths. Airspace enlargement (*D*) and small airway fibrosis (*E*) were quantified in the three experimental groups. *Boxes* in box plots show the median values and 25th and 75th percentiles, and *error bars* show the 10th and 90th percentiles. Data were analyzed using Mann-Whitney *U* test (*A*, *D*, and *E*) or Student's *t* test (*B* and *C*). In *A*–*C*, 3–4 mice were studied per group, and in *D* and *E*, 7–16 mice were studied per group. (*A*–*E*) $^*P < 0.05$ versus the air-exposed group or versus the group indicated. ECM = extracellular matrix; UACR = urinary albumin/creatinine ratio; Veh = vehicle.

RAGE staining in renal ECs than air-exposed mice (Figure 4C; *see* Figure E4), and the greatest RAGE staining was present in glomerular ECs (*see* Figure E4B).

Enalapril therapy initiated at the mid-point of 24-week CS exposures reduced the progression of endothelial injury (assessed with TUNEL staining) in lungs and kidneys (enalapril median, 2; IQR, 1.9–2.6 fold change vs. air) (Figure 4A), glomerular shrinkage (Figure 3B), ultrastructural podocyte injury (Figure 3C), emphysema development (enalapril median, 25.1; IQR, 2.1–0.8 μm) (Figure 3D), and small airway fibrosis (enalapril median, 15.0; IQR, 13.8–16.6 μm) (Figure 3E). Enalapril therapy limited the CS-induced increases in pulmonary AGEs levels (enalapril median, 2.3; IQR, 1.2–4.3 fold change vs. air-exposed mice) (Figure 4B). Enalapril also reduced the CS-induced increases in renal AGEs levels (enalapril median, 2.3; IQR, 1.4–2.4 fold change vs. air-exposed mice) (Figure 4B), and RAGE staining in pulmonary ECs (enalapril median, 0.5; IQR, 0.2–0.8 fold change vs. air-exposed mice) (Figure 4C) and renal ECs (Figure 4C; *see* Figure E4).

Enalapril Reduces Acute Renal and Pulmonary Lesions and Endothelial Injury

Enalapril therapy that was initiated at the mid-point of 4-week (acute) CS exposures

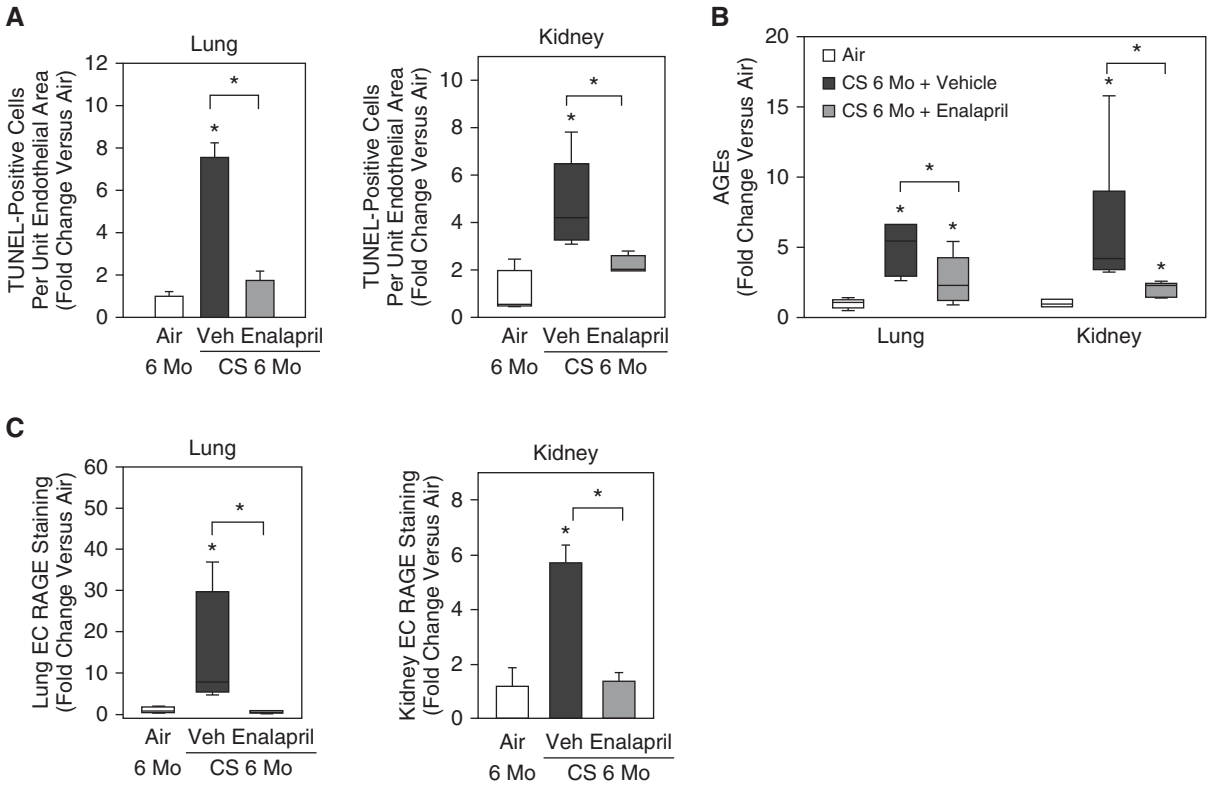

Figure 4. Chronic cigarette smoke (CS) exposure induces injury to pulmonary and renal endothelial cells (ECs) in mice and increases renal EC advanced glycation end-products (AGEs) and receptor for AGEs (RAGE) staining, which are abrogated by enalapril therapy. C57BL/6 wild-type mice were exposed to air or CS for 24 weeks. In CS-exposed mice, enalapril or vehicle therapy was initiated at the 12-week time point as described in the legend to Figure 3. (*A*) Terminal deoxynucleotidyl transferase (TUNEL)-positive ECs (identified by staining lung and kidney sections with a green color to detect TUNEL-positive cells and a red color to detect von Willebrand factor–positive ECs) were counted in 3–5 mice/group. (*B*) Tissue AGEs levels measured in homogenates of lungs and kidneys from 5–8 mice/group. (*C*) RAGE immunostaining levels in von Willebrand factor–positive ECs in sections of lungs or kidneys from 3–4 mice/group. *Bar graphs* show mean + SEM, *boxes* in box plots show the median values and 25th and 75th percentiles, and *error bars* show the 10th and 90th percentiles. Data were analyzed using Mann-Whitney *U* test (*A* and *B*, *right*; *C*, *left*) or Student's *t* test (*A*, *left*; *C*, *right*). $^*P \leq 0.05$ versus air-exposed group or the group indicated. Veh = vehicle.

reduced pulmonary inflammation (Figure 5A) and UACRs (enalapril median, 0.2, IQR, 0.1–0.3 μg/mg) when compared with saline-treated mice (Figure 5B). Enalapril therapy also reduced tissue oxidative stress levels in lungs (enalapril median, 0.95; IQR, 0.4–1.3 fold change vs. air-exposed mice) (Figure 5C), and kidneys (enalapril median, 0.7; IQR, 0–1.7 fold change vs. air-exposed mice) (Figure 5C), AGEs levels in homogenates of lungs (enalapril median, 3.2; IQR, 1–5.4 fold change vs. air-exposed mice) (Figure 5D) and kidneys (enalapril median, 0.8; IQR, 0.7–3.5 fold change vs. air-exposed mice) (Figure 5D), and active caspase-3 levels in lungs and kidneys (*see* Figure E5). Enalapril therapy also reduced RAGE staining in ECs in lungs and kidneys (Figure 5E).

In CS-exposed mice, enalapril therapy increased angiotensin converting enzyme I (ACE-I) levels in lungs and increased (rather than decreased) serum angiotensin-II (Ang-II) levels when compared with saline-treated mice (*see* Figure E6). Ang-II was not detectable in renal samples in any experimental condition.

Immunostaining for AGEs and RAGE in Human Pulmonary and Renal ECs

We evaluated whether AGEs and RAGE levels are elevated in ECs in lungs and kidneys from patients with COPD and in ECs in these organs from CS-exposed mice. Tables E2A and E2B show the clinical characteristics of the human subjects studied. Pulmonary and renal sections from patients with COPD had greater AGEs and RAGE immunostaining in ECs (identified by costaining sections for von Willebrand factor) than SC and NSC (Figures 6A and 6B). None of the subjects studied were taking ACEi.

Discussion

We show that chronic renal lesions (with injury to glomeruli, and the renal tubules and interstitium) are more frequent in patients with COPD than control subjects. Studies of samples from humans and CS-exposed mice provide evidence that pulmonary and renal/endothelial injury in small vessels (likely mediated by increases in the oxidative stress–AGEs–RAGE pathway) may explain the coincident pulmonary and renal lesions detected in patients with COPD. To our knowledge, this is the first time that chronic

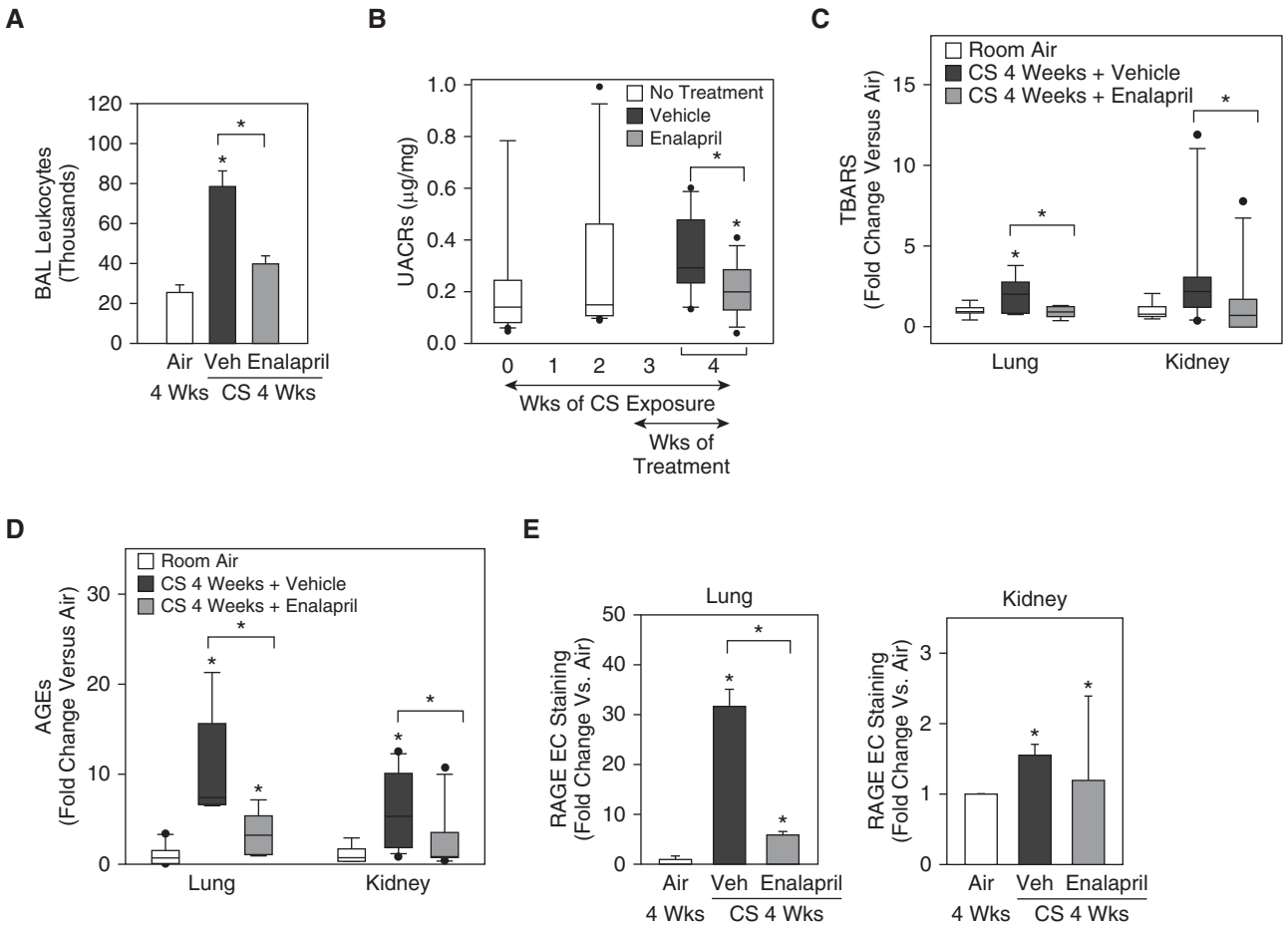

Figure 5. Enalapril therapy reduces pulmonary inflammation, urinary albumin/creatinine ratios (UACRs), tissue oxidative stress and advanced glycation end-products (AGEs) levels, and endothelial cell (EC) receptor for AGEs (RAGE) staining in the kidneys and lungs of mice exposed acutely to cigarette smoke (CS). C57BL/6 wild-type mice were exposed to air or CS for 4 weeks. In CS-exposed mice, enalapril therapy versus vehicle (6 d/wk) was initiated beginning at the mid-point of the 4-week CS exposures. (*A*) Total leukocyte counts in bronchoalveolar lavage samples from 5–7 mice/group. (*B*) UACRs in 10–13 mice/group. (*C*) Oxidative stress levels measured as tiobarbituric acid reactive substances (TBARS) in homogenates of lungs and kidneys from 5–11 mice/group. (*D*) Tissue AGEs levels in homogenates of lungs and kidneys from 5–17 mice/group. (*E*) RAGE immunostaining levels in von Willebrand factor–positive ECs in sections of lungs (*left*) or kidneys (*right*) from 3–4 mice/group. *Bar graphs* show mean + SEM, *boxes* in box plots show the median values and 25th and 75th percentiles, and *error bars* show the 10th and 90th percentiles. Data were analyzed using Student's *t* test (*A* and *E*) or Mann Whitney *U* test (*B–D*). (*A–E*) $^*P \leq 0.05$ versus air-exposed group or the group indicated. Two-tailed tests were used for all analyses. (*E*, *right*) $^*P = 0.06$ was obtained when comparing the enalapril-treated versus the vehicle-treated groups. BAL = bronchoalveolar lavage; Veh = vehicle.

pulmonary and renal lesions have been described in synchrony and linked to CS exposure. Therapeutic intervention with an ACEi limited the progression of both renal and pulmonary disease in CS-exposed mice, suggesting that ACEi might limit disease progression in human patients with COPD with endothelial dysfunction/injury as evidenced by microalbuminuria.

COPD, Smoking, and Renal and Pulmonary Injury

The association between human COPD and renal dysfunction has been suspected but no prior study has assessed whether patients with COPD have chronic renal lesions to explain the links between albuminuria, renal function, smoking, and COPD. An increased odds ratio for renal failure was reported in patients with COPD versus age-matched control subjects (13), and COPD prevalence was inversely related to renal function in vascular surgery patients (30). An indirect association between high-resolution computed tomography–determined emphysema (but not airway dimension) and decreased eGFR was reported in smokers screened for lung cancer (12). Persistent microalbuminuria was reported in 24% of patients with COPD versus 4% of age-matched control subjects, correlated with the degree of hypoxemia (9), and increased during COPD exacerbations (8). The presence of microalbuminuria was associated with increased risk of death in patients with COPD (11), and current smoking is linked to death from renal failure in subjects without COPD (14). However, none of these prior studies investigated whether patients with COPD have renal pathologies to explain these associations or the mechanism involved.

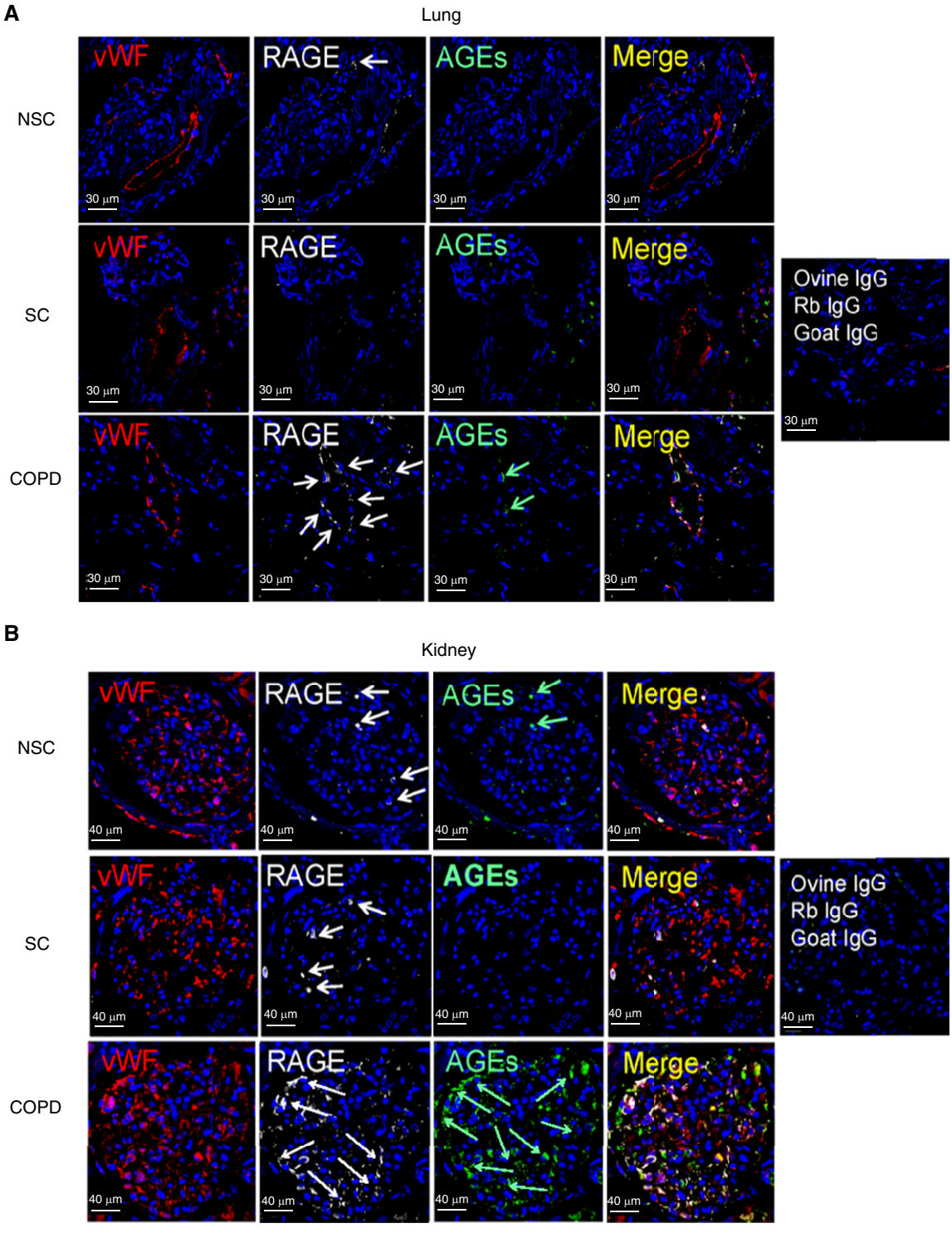

Figure 6. Advanced glycation end-products (AGEs) and receptor for AGEs (RAGE) levels are increased in pulmonary and renal endothelial cells in patients with chronic obstructive pulmonary disease (COPD). Confocal images of triple-color immunofluorescence staining of sections of lungs (*A*) or kidneys (glomeruli, in *B*) from patients with COPD, smoker control subjects (SC), and nonsmoker control subjects (NSC). Endothelial cells in the sections were labeled with a *red* fluorophore for von Willebrand factor (vWF). Sections were also stained with a *green* fluorophore for AGEs and *gray* color for RAGE, and nuclei were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (*blue*). The merged images show colocalized staining in *yellow*. *White arrows* indicate RAGE staining and *green arrows* indicate AGEs staining in endothelial cells. The far right panels show a section of a lung (*A*) or a kidney (*B*) sample from a patient with COPD stained with isotype-matched nonimmune control antibodies. The images shown are representative of 4–5 subjects/group. Rb = rabbit.

The presence of renal endothelial injury in patients with COPD is supported by the pathologic and ultrastructural abnormalities in glomeruli, and the presence of more frequent double contours (a marker of repetitive renal endothelial injury [31]) in kidneys from patients with COPD versus SC. Chronic glomerular injury in humans can induce secondary injury to the tubules and interstitial fibrosis (32), and we also detected tubular and interstitial lesions in kidneys from patients with COPD that were greater than those detected in SC. The renal lesions detected in patients with COPD could not be explained by differences in age, pack-year smoking history, current smoker status, or comorbidities between the patients with COPD and control subjects. African American race is associated with an increased prevalence of chronic kidney disease (33). However, it is noteworthy that all of the subjects studied herein were non-Hispanic white persons except for two Hispanic white persons in the biopsy cohort. Thus, it is unlikely that our findings were related to race or ethnicity differences between the study groups.

The eGFR was lower in patients with COPD than control subjects, independent of their smoking history, and eGFR correlated directly with FEV_1. Patients with the highest double contour frequency score had lower eGFRs and lower FEV_1 percent predicted values than patients with rare or no double contours, thereby linking renal EC injury to pulmonary and renal injury. However, we cannot determine whether there is a causal link between the pulmonary and renal lesions. Likely, CS exposure leads to generalized endothelial injury affecting both the lungs and kidneys leading to coincident, chronic injury to both organs. It is possible that in patients with COPD, changes caused by or linked to COPD *per se* (such as hypoxemia, chronic systemic inflammation, and increased oxidative stress levels) contribute to the progression of renal injury by increasing the severity of renal endothelial injury (34). To investigate this possibility, additional studies are needed in larger COPD cohorts with a range of COPD severities.

In CS-exposed mice, endothelial injury was detected in the lungs and kidneys and associated with increased UACRs, podocyte and glomerular injury, and COPD-like lung lesions. Podocytes are specialized ECs attached to glomerular capillaries by numerous interdigitating and elongated foot processes. The width of the podocyte foot processes is small in health and widening is an early, uniform response of podocytes to injury and is associated with albuminuria (29, 35).

Few prior studies have linked pulmonary endothelial injury to COPD (36, 37), and none have linked renal endothelial injury to COPD. Ever-smokers were reported to have more severe myointimal hyperplasia of small arteries, arteriolar hyalinosis, and glomerular sclerosis than never-smokers (38), but patients with COPD were not included in this prior study. Herein, there were no significant differences in double contour frequency or other measures of renal injury between SC and NSC, but our sample sizes were small and additional studies of larger cohorts are needed to determine whether SC also have significant chronic renal lesions. Although the reason for the differences in renal lesions in SC in the two studies is not clear, Lhotta and colleagues (38) included smokers with diabetes mellitus, whereas we excluded subjects with diabetes mellitus from our study.

In a subset of a cohort of smokers (78% of whom did not have without COPD at baseline), UACRs reached microalbuminuria thresholds (30 vs. 20 mg/g in women vs. men, respectively) only in two male smokers studied and UACRs were not related to rate of decline in FEV_1. Likely, endothelial dysfunction/injury of greater severity or duration is needed for emphysema and chronic renal injury to develop in smokers. Rate of FEV_1 decline in the LSC could have reflected increases in small airway disease rather than emphysema development as various COPD cohort studies have shown that loss of FEV_1 in early disease relates primarily to small airway disease (39, 40). Additional longitudinal studies of smokers are needed to further explore associations between albuminuria and the development of pulmonary and renal lesions.

Potential Mechanisms Linking Renal and Pulmonary Endothelial Injury

Prior studies of renal injury in diabetic mice (41) led us to investigate whether CS increases tissue oxidative stress levels to promote the generation of AGEs (42), thereby causing RAGE activation, which, in turn, increases RAGE expression (43). Oxidative stress, AGEs, and RAGE levels were increased in pulmonary and renal tissue homogenates in CS-exposed mice. Immunostaining studies showed that the increases in AGEs and RAGE levels occurred mostly in glomerular and pulmonary ECs in patients with COPD and CS-exposed mice. Increases in AGEs and RAGE levels have been linked to COPD previously (16, 17) but not to endothelial dysfunction/injury and microalbuminuria. Interestingly, soluble RAGE (generated by proteolytic shedding of RAGE from cell surfaces) functions as a decoy receptor blocking the binding of RAGE ligands to transmembrane RAGE (44). Reduced plasma soluble RAGE levels are linked to the presence of emphysema in humans (45). Single-nucleotide polymorphisms in the RAGE (*AGER*) locus are linked to COPD development (46, 47). CS-exposed $AGER^{-/-}$ mice are protected from emphysema development (17). Thus, reducing RAGE expression or signaling could be an important therapeutic approach for patients with COPD.

Therapeutic Implications

The presence of microalbuminuria is an indication for ACEi therapy in patients with hypertension and diabetes mellitus (6). However, the efficacy of ACEi in patients with COPD with albuminuria has not been tested even though its potential use is supported by observational studies associating ACEi use with lower rates of FEV_1 decline in smokers (48) and a trend to lower mortality in patients with COPD on oxygen therapy (49). ACEi inhibit ACE-I (a metalloproteinase, expressed by pulmonary ECs [50]), which cleaves the C-terminal dipeptide from inactive serum angiotensin-I generating biologically active Ang-II (51). However, in animal models of diabetes mellitus, ACEi attenuate tissue injury by reducing endothelial injury via their antioxidant properties (20, 52). ACEi reduce the production of reactive carbonyl precursors for AGEs and chelate transition metals, and inhibit various oxidative steps thus reducing AGE generation (53).

Herein, enalapril therapy in CS-exposed mice reduced the progression of chronic COPD-like lung and renal lesions and these changes were associated with reductions in tissue oxidative stress levels, and AGEs and RAGE staining in ECs in

both organs. Serum and pulmonary levels of Ang-II (the product of ACE-I) were reduced in CS-exposed mice and (surprisingly) not reduced further by enalapril. Enalapril increased serum ACE-I levels likely by reducing pulmonary endothelial injury because ACE-I is expressed highly by pulmonary ECs (50). These results suggest that enalapril mediates its disease-modifying effects in mice by inhibiting activation of the oxidative stress–AGEs–RAGE–endothelial injury pathway rather than by inhibiting ACE-I. In humans, although the ACEi, fosinopril, did not improve quadriceps function or exercise performance in patients with COPD selected for having weak quadriceps (54), enalapril augmented the beneficial effects of pulmonary rehabilitation in improving exercise capacity in patients with moderately severe COPD (55). However, microalbuminuria and Ang-II levels were not measured in these clinical trials. We hypothesize that patients having microalbuminuria are the subset that responds best to ACEi therapy.

Study Limitations

This study has several limitations. First, UACRs were not measured in the patients whose kidney samples were included in the electron microscopy studies and a large number of kidney samples from subjects that may have had COPD could not be analyzed because spirometry had not been performed to confirm the diagnosis of COPD. Thus, a selection bias is likely, and we may not have identified all possible confounders because only a small percentage of patients undergoing major surgery in our hospital had physician-diagnosed COPD or had preoperative pulmonary function testing performed. This issue reflects the common problem of underdiagnosis of COPD in smokers both in hospital and primary care settings (56). Although all patients selected met strict spirometric criteria for COPD, our results need to be confirmed in additional larger COPD cohorts in the future. Second, we acknowledge that our human studies are correlative, but similar perturbations in the oxidative stress–AGEs–RAGE–endothelial injury pathway were induced by exposing mice to CS.

Third, the sample size in the human nephrectomy cohort in which the pathologic assessment for renal glomerular, tubular, and interstitial abnormalities was performed was too small to correlate renal injury with COPD severity, which would have linked the oxidative stress–AGEs–RAGE–endothelial injury pathway to COPD progression. Fourth, there were no African American subjects in our nephrectomy or biopsy cohorts. This issue limits the generalizability of our findings, particularly given the high risk for chronic kidney disease in African Americans (33). Finally, only one ACEi was studied, and its choice was one of convenience because enalapril is delivered once daily to mice, and widely used in humans. Future studies will determine whether other ACEi have similar effects.

Conclusions

Patients with COPD and CS-exposed mice have EC injury associated with increases in tissue oxidative stress–AGEs–RAGE–endothelial injury pathway in lungs and kidneys. Enalapril reduces the progression of CS-induced pulmonary and renal injury in mice, and these changes are associated with reduced activation of the tissue oxidative stress levels–AGEs–RAGE–endothelial injury pathway in both organs. Our results provide a rationale for clinical trials testing the efficacy of ACEi in limiting the progression of COPD in patients with albuminuria. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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