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To the Editor:

Cigarette Smoking Acutely Decreases Serum Levels of the Chronic Obstructive Pulmonary Disease Biomarker sRAGE

Chronic obstructive pulmonary disease (COPD) is caused by a combination of genetic susceptibility and chronic exposure to inhaled noxious gases such as cigarette smoke. To monitor progression and classification of this complex disease, reliable biomarkers are needed. One of the most promising biomarkers for COPD, especially reflecting the presence and progression of emphysema, is sRAGE (soluble receptor for advanced glycation end-products) (1).

The RAGE receptor is a multiligand pattern recognition receptor that is mainly expressed by type I alveolar epithelial cells. RAGE activation induces NF-κB-mediated proinflammatory responses and is involved in alveolar tissue damage (2). RAGE signaling is inhibited by the endogenous decoy receptor sRAGE, which binds freely circulating RAGE ligands and prevents homodimerization of RAGE, which is necessary for activation. Production of sRAGE takes place by alternative splicing or proteolytic cleavage. In patients with COPD, expression of RAGE was found to be increased in lung tissue, whereas circulating concentrations of sRAGE were decreased (1, 3). The cause of the lower circulating sRAGE levels in patients with COPD is currently unknown. Furthermore, little is known about the effect of smoking on circulating levels of sRAGE. In the current study, we investigated the effect of smoking on serum sRAGE levels, which should be taken into account when investigating sRAGE as a biomarker for COPD.

Serum samples from patients with COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage I, n = 25; GOLD stage II, n = 12; GOLD stage III, n = 20; and GOLD stage IV, n = 16) (ClinicalTrials.gov Identifier: NCT00807469) (4). Serum samples were collected from the young and old healthy control subjects and matched patients with COPD after they went at least 2 days without smoking, and 2 hours after they smoked three cigarettes within 1 hour. Exhaled carbon monoxide was analyzed using a Micro+ Smokerlyzer at baseline to determine whether the individuals had not smoked recently, and after smoking to determine whether they had inhaled a sufficient amount of cigarette smoke. Serum sRAGE levels were measured using a simplified immunoprecipitation in 96-well ELISA format–coupled liquid chromatography mass spectrometry assay (5), a novel and fully validated method to measure sRAGE with high specificity and selectively, and a commercially available sRAGE ELISA (Human RAGE DuoSet DY1145; R&D Systems). All study protocols were approved by the Medical Ethics Committee of the University Medical Center Groningen, Groningen, the Netherlands, and all subjects provided written informed consent. Furthermore, all clinical procedures were performed according to the standards set by the latest Declaration of Helsinki.

Patients with COPD of all stages of severity had significantly lower serum sRAGE levels than control subjects (Figure 1A). Furthermore, patients with severe COPD (GOLD stage IV) had lower circulating sRAGE levels than patients with mild (GOLD stage I) COPD. The serum sRAGE levels were independent of age, as the levels in young (<40 yr old) control subjects were similar to those observed in old (>40 yr old) control subjects. Next, we investigated the acute effect of smoking...
on serum sRAGE levels, comparing samples taken after at least 2 days without smoking and 2 hours after smoking three cigarettes within 1 hour. Here, we observed that smoking significantly decreased serum sRAGE levels within 2 hours after smoking in young control subjects, old control subjects, and patients with COPD (Figure 1B). The median decrease in serum sRAGE levels upon smoking three cigarettes was between 15% and 25%, with some individuals showing a more than 50% decrease (Figure 2A). To confirm our results, we remeasured all samples using a commercially available ELISA, which provided similar results (Figure 2A). Next, three healthy individuals provided serum samples at several time points after smoking three cigarettes within 1 hour. Here, it was shown that the decrease in sRAGE occurred within 1 hour, reached its maximum after approximately 8 hours, and was not fully restored after 48 hours (Figure 2B). Interestingly, when serum sRAGE levels were investigated in active smokers and never smokers, no differences were observed (data not shown), suggesting an acute and temporary effect of smoking on serum sRAGE levels. Several previous studies found no differences in serum sRAGE between smokers and nonsmokers (6, 7), whereas other studies showed decreased serum sRAGE levels in smokers (8, 9), which

Figure 1. Serum levels of sRAGE (soluble receptor for advanced glycation end-products) are decreased in patients with chronic obstructive pulmonary disease (COPD) and further decreased by smoking, independently of disease status. The levels of sRAGE were measured in serum of patients with COPD and healthy control subjects using immunoprecipitation in 96-well ELISA format–coupled liquid chromatography mass spectrometry. (A) sRAGE levels were measured in serum from young (18–40 yr, n = 49) and old (>40 yr, n = 26) control subjects and patients with COPD of different severity (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage I, n = 38; GOLD stage II, n = 12; GOLD stage III, n = 20; GOLD stage IV, n = 16). Data are shown as individual measurements and median and interquartile range. Significance was tested using a Mann-Whitney U test, **P < 0.01 between indicated conditions, ###P < 0.001 compared with the control (old) group. (B) sRAGE levels were measured in serum from young (18–40 yr, n = 49) and old (>40 yr, n = 26) control subjects and patients with COPD (n = 13) who were age- and smoking history–matched to the old control group. All samples were taken at baseline after at least 2 days without smoking (light gray symbols) or 2 hours after smoking three cigarettes within 1 hour (dark gray symbols). Significance was tested using a Wilcoxon signed-rank test, ***P < 0.001.

Figure 2. Smoking three cigarettes immediately decreases serum sRAGE (soluble receptor for advanced glycation end-products) levels for up to 48 hours. (A) The relative change in serum sRAGE levels in patients with chronic obstructive pulmonary disease (n = 13) and control subjects without airway obstruction (n = 75) induced by smoking three cigarettes within 1 hour, measured by immunoprecipitation in 96-well ELISA format–coupled liquid chromatography mass spectrometry (IPE-LC-MS) and the DuoSet ELISA kit from R&D Systems. (B) Absolute and relative serum sRAGE levels in three healthy individuals before and after smoking three cigarettes within 1 hour, measured by ELISA. Blood samples were taken 1, 2, 4, 8, 24, and 48 hours after smoking. Data are shown as mean ± SEM.
may be the consequence of recent smoking within the smokers group.

Considering the fact that the decrease in serum sRAGE levels takes place within 1 hour, it is likely that this decrease is caused by either binding or acute breakdown of sRAGE. Although future studies are required to elucidate the mechanism behind the acute smoke-induced decrease in serum sRAGE levels, one explanation could be that sRAGE binds to circulating leukocytes, which are removed during the process of isolating serum from whole blood. In a preliminary study, we observed that smoking three cigarettes within 1 hour increased the percentage of circulating neutrophils (before smoking: 79.94% ± 0.8%; after smoking: 83.48 ± 0.51%; n = 81; P < 0.0001) and CD11b expression on circulating leukocytes (mean fluorescence intensity before smoking: 362.44 ± 25.33; mean fluorescence intensity after smoking: 488.57 ± 48.7; n = 81; P = 0.0116) within 2 hours. The latter observation may be particularly relevant because the polymorphonuclear leukocyte–specific β2-integrin complex CD18/CD11b is known to bind RAGE via the I-domain of CD11b and thus may contribute to the observed decrease in sRAGE levels, although other receptors may also be involved (10).

In conclusion, we showed that patients with COPD had lower serum sRAGE levels than age- and smoking history–matched control subjects. Furthermore, we showed that smoking immediately and severely decreased serum sRAGE levels. This decrease may influence the discriminative value of sRAGE as a biomarker for COPD. Monitoring or controlling smoking behavior before blood sampling may decrease preanalytical variation and increase the validity of serum sRAGE as a biomarker for COPD.

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

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References


Concerns over Airway Pressure Release Ventilation Management in Children with Acute Respiratory Distress Syndrome

To the Editor:

I read with interest the article by Lalgudi Ganesan and colleagues (1). It is the first prospective, randomized controlled trial in pediatrics comparing airway pressure release ventilation (APRV) to low–tidal volume ventilation (LoTV), and its importance cannot be overstated. There are several limitations, however, to generalizing the findings:

1. Disparate disease type. The authors state “the trend toward harm persisted despite adjustment for [higher severity of lung disease] on multivariable analysis,” but the APRV group not only had more severe acute respiratory distress syndrome but also had different types of acute respiratory distress syndrome, as noted in the percentages of primary versus secondary lung injury causes. Furthermore, the greater lung severity may have had an exponential effect on outcomes if APRV was mismanaged (see points 3, 4, and 5).
2. Disparate ages. Ventilating a 2-month-old is very different from ventilating a 2-year-old, and is different still from ventilating...