[¹⁸F]Fluorodeoxyglucose Positron Emission Tomography for Lung Antiinflammatory Response Evaluation

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Rationale: Few noninvasive biomarkers for pulmonary inflammation are currently available that can assess the lung-specific response to antiinflammatory treatments. Positron emission tomography with [¹⁸F]fluorodeoxyglucose (FDG-PET) is a promising new method that can be used to quantify pulmonary neutrophilic inflammation.

Objectives: To evaluate the ability of FDG-PET to measure the pulmonary antiinflammatory effects of hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins) and recombinant human activated protein C (rhAPC) in a human model of experimentally-induced lung inflammation.

Methods: Eighteen healthy volunteers were randomized to receive placebo, lovastatin, or rhAPC before intrabronchial segmental endotoxin challenge. FDG-PET imaging was performed before and after endotoxin instillation. The rate of [¹⁸F]FDG uptake was calculated as the influx constant K_i by Patlak graphical analysis. Bronchoalveolar lavage (BAL) was performed to determine leukocyte concentrations for correlation with the PET imaging results.

Measurements and Main Results: There was a statistically significant decrease in K_i in the lovastatin-treated group that was not seen in the placebo-treated group, suggesting attenuation of inflammation by lovastatin treatment despite a small decrease in BAL total leukocyte and neutrophil counts that was not statistically significant. No significant decrease in K_i was observed in the rhAPC-treated group, correlating with a lack of change in BAL parameters and indicating no significant antiinflammatory effect with rhAPC.

Conclusions: FDG-PET imaging is a sensitive method for quantifying the lung-specific response to antiinflammatory therapies and may serve as an attractive platform for assessing the efficacy of novel antiinflammatory therapies at early phases in the drug development process.

Clinical trial registered with www.clinicaltrials.gov (NCT00741013).

Keywords: lovastatin; neutrophils; drug development; biomarker

Inappropriate airway inflammation contributes to pathologic lung injury in a number of acute and chronic airway diseases. Acute lung injury is characterized by an excessive and persistent neutrophilic response (1–3). Chronic inflammation also appears to contribute to the progression of cystic fibrosis and chronic

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Noninvasive tests that can accurately quantify lung-specific antiinflammatory properties of therapeutic agents are currently not available. Positron emission tomographic (PET) imaging with [¹⁸F]fluorodeoxyglucose (FDG-PET) is a sensitive, noninvasive method for quantifying neutrophilic pulmonary inflammation.

What This Study Adds to the Field

We show that FDG-PET can detect changes in levels of pulmonary inflammation, and also provide evidence of the antiinflammatory properties of lovastatin.

obstructive pulmonary disease (4–6), and neutrophilia appears to correlate with the severity of asthma (7–9). In addition, acute exacerbations in all of these lung diseases are often accompanied by an increase in neutrophilic lung inflammation or neutrophil products (10–13). As such, a number of antiinflammatory therapies have been investigated to ameliorate exacerbations and slow the progression of these diseases.

However, few noninvasive methods are available to assess the lung-specific inflammatory response in these conditions. Bronchoalveolar lavage (BAL) remains the “gold standard” for the assessment of lung inflammation; however, BAL is an invasive procedure and samples only a limited portion of the lung, which may not represent the total pulmonary inflammatory burden. Measurements of pulmonary function, such as the FEV₁, and induced sputum measures can be highly variable and are dependent on patient effort. Therefore, the development of a noninvasive, reproducible technique for assessing lung-specific inflammation would be highly valuable not only for determining baseline levels of pulmonary inflammation but also in assessing the efficacy of antiinflammatory treatments in these patients.

Positron emission tomographic (PET) imaging with [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG) is a promising technique that may serve as a more sensitive outcome measure for pulmonary inflammation. The advantages of PET imaging include its non-invasiveness, ease of quantification, and ability to assess the entire lung. Evidence to date suggests that neutrophils contribute primarily to the increased uptake of [¹⁸F]FDG in lung inflammation and that the FDG-PET signal correlates with the presence of activated neutrophils (14–17). Clinical studies have also demonstrated that FDG-PET imaging can be used to assess the neutrophilic inflammatory burden in the lungs in cystic fibrosis, pneumonia, and experimentally induced lung inflammation (16, 18, 19). These results together indicate that FDG-PET imaging can potentially be used to measure changes in pulmonary inflammation in response to antiinflammatory treatments.

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In addition, it would be highly desirable to identify a clinically relevant method of testing the efficacy of novel antiinflammatory therapies before embarking on large, expensive clinical trials in patients. We have shown that we can quantify by FDG-PET imaging low levels of inflammation induced by bronchoscopic instillation of endotoxin in humans (19), as initially described by O'Grady and colleagues (20). This model is directly relevant to cystic fibrosis and acute lung injury, given the exposure to gram-negative bacteria in these patients. Nick and colleagues have also demonstrated that the neutrophilic recruitment induced in this model can be attenuated by giving recombinant human activated protein C (rhAPC) (21). Therefore, the demonstration that FDG-PET imaging can assess changes in the segmental lung inflammatory response within hours or a few days after a pharmacologic intervention would make this model an attractive platform for obtaining information about the efficacy of novel antiinflammatory therapies early in the drug development process.

In this study, we sought to test the hypothesis that FDG-PET is sensitive enough to quantify the antiinflammatory effect of treatment with either lovastatin (which has been shown to have antiinflammatory properties [22]) or rhAPC before endotoxin instillation in healthy volunteers and that the change in [^{18}F]FDG uptake would correlate with changes in BAL measures of the lung inflammatory response.

METHODS

Study Design and Procedure Flow

We conducted a randomized, double-blinded, placebo-controlled trial with lovastatin and rhAPC treatment to evaluate the ability of FDG-PET to image pulmonary antiinflammatory response with the approval of the Washington University School of Medicine (St. Louis, MO) Human Studies Committee. All major procedures used in this study, except for the drug treatments, have been previously described and are illustrated in Figure 1 (19). After obtaining informed consent, healthy volunteers (age, 19–44 yr) with normal screening blood work, chest radiograph, electrocardiogram, and pulmonary function tests were admitted to the intensive research unit for a 3-day/2-night protocol. Drug treatment or placebo was administered after baseline FDG-PET imaging but before endotoxin (4 ng/kg) instillation in the lateral segment of the right middle lobe. Approximately 24 hours after endotoxin instillation, each subject underwent a second FDG-PET scan and BAL of the treated lung segment. See the online supplement for procedural details.

Treatments, Randomization, and Blinding

Endotoxin (*Escherichia coli* O113:H10K) was obtained from the National Institutes of Health Clinical Center (reference endotoxin) and instilled by bronchoscopy as described previously (19). Lovastatin (Sandoz, Holzkirchen, Germany) and rhAPC (Lilly, Indianapolis, IN) were purchased commercially. Lovastatin (80 mg/d in divided doses), rhAPC (24 $\mu\text{g/kg/hr}$, administered intravenously), or placebo was administered after randomization of patients according to a fixed randomization scheme generated from a random number table by the Moses-Oakford algorithm with variable block size and an allocation ratio of 1:1:1 (23). A dedicated research pharmacist at Barnes-Jewish Hospital performed all drug preparations, including placebo preparations, and randomization independently of the remaining research team. The study was double-blinded in that the study subject, clinical staff, and research staff performing the analysis were unaware of the treatment assignment. See the online supplement for details.

FDG-PET Image Acquisition and Data Analysis

PET images were obtained dynamically after injection of 362 ± 21 MBq (9.8 ± 0.6 mCi) of [^{18}F]FDG. All scans were coded so that imaging data could be analyzed by a technician blinded to treatment group. Quantification of [^{18}F]FDG uptake was the primary end point and was performed using a slight modification of previously described methods (19) to determine the influx constant K_i , calculated by Patlak graphical analysis (24, 25), and the intercept-corrected K_i (16) for both lungs. See the online supplement for details.

BAL Fluid Analysis

The first aliquot of BAL fluid (BAL1) was stored separately from the remainder of the BAL fluid (BAL2) collected. Cell counts were performed using standard methods (13, 19, 26). See the online supplement for details.

Statistical Analysis

To compare categorical variables between the three treatment groups, the chi-squared or Fisher's exact test was used. To compare continuous variables, one-way analysis of variance (ANOVA) with a post-hoc Scheffé procedure was used. To compare the K_i values before and after endotoxin for the various treatment groups, a repeated-measures ANOVA (mixed factorial ANOVA) for each lung was used. Post hoc comparison using the Scheffé procedure was performed to identify significant differences between the three treatment groups. For all tests, $P < 0.05$ was considered significant and analysis was performed with SPSS version 13.0 (SPSS Inc., Chicago, IL).

Figure 1. Study procedures and participant flow diagram: schedule of study procedures with associated volunteer participation flow. *Lovastatin or placebo was administered beginning on the evening of Day 1 every 4 hours, while recombinant human activated protein C (rhAPC) or placebo was administered as a continuous infusion beginning the morning of Day 2, 2 hours before endotoxin instillation.

tion. All drug treatments were stopped 2 hours before bronchoalveolar lavage on Day 3. [§]One volunteer had a drop in pulmonary function tests of greater than 20%. [‡]One volunteer was excluded for elevated liver function tests, the other for subsequent discovery of recent epidural placement. [†]Five screened volunteers declined to participate, and six did not meet inclusion criteria. ^{**}One volunteer withdrew consent before the second positron emission tomography with [^{18}F]fluorodeoxyglucose (FDG-PET) scan. BAL = bronchoalveolar lavage.

RESULTS

Participant Flow and Clinical Characteristics of the Treatment Cohorts

Figure 1 illustrates the patient flow for this study. Thirty-five normal volunteers were approached for participation. Thirteen were excluded: 6 declined to participate, and 7 did not meet the inclusion criteria. The remaining 22 were randomized to receive treatment with placebo ($n = 7$), lovastatin ($n = 8$), or rhAPC ($n = 7$) before intrabronchial instillation of endotoxin. Four subjects did not complete the study and were therefore excluded from analysis; one in the placebo arm did not complete the second bronchoscopy because of a drop in pulmonary function tests after endotoxin instillation, two in the lovastatin arm were excluded before study drug administration (one for increased liver function tests and one for a recent history of an epidural catheter), and one in the rhAPC arm withdrew consent and did not complete the second FDG-PET scan. Thus, 18 subjects (6 in each treatment arm) completed the trial and were analyzed for the primary outcome. The baseline demographic characteristics, pulmonary function tests, hematologic parameters, and serum chemistry values were within normal limits for all participants, and there were no statistical differences between the three treatment cohorts (Table 1).

Several studies have demonstrated that segmental endotoxin challenge is well tolerated without significant clinical adverse events (20, 21). Here again the subjects in the current trial tolerated the protocol without any serious complications. Twelve of the 18 participants reported at least one symptom: 6 with fever (5 requested acetaminophen), 5 with cough, 4 with headache (3 requested acetaminophen), 3 with nausea, 3 with chest pain, 2 with body aches, 2 with sore throat, and 1 with shortness of breath. All of these symptoms were transient. Two volunteers experienced transient vomiting, one of whom did not

request treatment; the other experienced complete resolution after a single dose of ondansetron.

As before, we observed clinically insignificant changes in vital signs and serum chemistry values after endotoxin. Compared with baseline values, for all 18 subjects there was a decrease in FVC (mean decrease, 5%; range, -16 to 6%) and FEV₁ (mean decrease, 5%; range, -18 to 16%). One subject in the placebo cohort had a transient decrease in postendotoxin FEV₁ of 31% and FVC of 26% that precluded completion of the trial. In this subject, repeat testing demonstrated daily improvement in lung function and at 5 days after endotoxin the FVC and FEV₁ improved to 16 and 8% of baseline, respectively.

There were no statistically significant differences between the three treatment cohorts with respect to frequency of symptoms, changes in vital signs, serum chemistry values, or pulmonary function tests. Interestingly, after endotoxin instillation, there was a statistically significant increase in the percentage of peripheral blood neutrophils and a decrease in lymphocytes in the lovastatin cohort compared with placebo. In the rhAPC-treated cohort there was a statistically significant, but clinically insignificant, increase in the international normalized ratio from 1.11 to 1.23 (Table 2).

FDG-PET Imaging Detects Changes in Endotoxin-induced Inflammation

Representative FDG-PET and subtraction images from each treatment group are shown in Figure 2. As easily visualized by use of a color scale on the subtraction images, the area of greatest difference between the baseline and postendotoxin scans is seen in the placebo image. No difference is visualized in the subtraction image for the lovastatin subject, and an intermediate difference is seen in the rhAPC group. Note that these differences are not easily identified without creating a subtraction image. Patlak plots generated from the PET

TABLE 1. BASELINE CHARACTERISTICS OF VOLUNTEERS WHO COMPLETED THE PROTOCOL

	Placebo ($n = 6$)	Lovastatin ($n = 6$)	rhAPC ($n = 6$)	<i>P</i> Value
Female, n (%)	2 (33)	2 (33)	1 (17)	1.00
Age, yr	30.0 $\pm$ 5.9	26.8 $\pm$ 5.6	29.7 $\pm$ 6.9	0.63
Race or ethnicity,* n (%)				0.38
White, non-Hispanic	5 (83)	2 (33)	4 (66)	
Black, non-Hispanic	0 (0)	3 (50)	1 (17)	
Hispanic or Asian	1 (17)	1 (17)	1 (17)	
Body mass index, kg/m ²	23.9 $\pm$ 3.0	26.7 $\pm$ 8.4	23.8 $\pm$ 4.7	0.61
Pulmonary function tests				
FVC, L	5.1 $\pm$ 1.3	4.2 $\pm$ 1.4	4.3 $\pm$ 1.3	0.42
FVC, % predicted	112.0 $\pm$ 15.0	105.5 $\pm$ 12.0	113.0 $\pm$ 7.5	0.51
FEV ₁ , L	4.2 $\pm$ 0.9	3.3 $\pm$ 1.1	3.5 $\pm$ 1.1	0.34
FEV ₁ , % predicted	108.7 $\pm$ 11.2	98.7 $\pm$ 6.9	110.0 $\pm$ 8.0	0.09
SaO ₂ , %	98 $\pm$ 1	98 $\pm$ 1	99 $\pm$ 1	0.69
Hematologic parameters				
White blood cells, $\times 10^3/\mu\text{L}$	5.6 $\pm$ 1.0	5.1 $\pm$ 0.9	5.4 $\pm$ 1.2	0.64
Hematocrit, %	39.7 $\pm$ 1.5	39.1 $\pm$ 7.0	37.8 $\pm$ 4.3	0.79
Platelets, $\times 10^3/\mu\text{L}$	236 $\pm$ 33	253 $\pm$ 46	298 $\pm$ 66	0.12
International normalized ratio	1.11 $\pm$ 0.05	1.10 $\pm$ 0.04	1.11 $\pm$ 0.04	0.85
Serum chemistry values				
Creatinine, mg/dL	0.9 $\pm$ 0.2	0.9 $\pm$ 0.1	0.8 $\pm$ 0.2	0.57
Bilirubin, mg/dL	0.5 $\pm$ 0.2	0.5 $\pm$ 0.3	0.3 $\pm$ 0.1	0.41
AST, IU/L	28 $\pm$ 6	22 $\pm$ 7	23 $\pm$ 4	0.23
ALT, IU/L	29 $\pm$ 11	21 $\pm$ 11	22 $\pm$ 10	0.40
Glucose, mg/dL	89 $\pm$ 9	81 $\pm$ 12	81 $\pm$ 13	0.42

Definition of abbreviations: ALT = alanine transaminase; AST = aspartate aminotransferase; IU = international unit; rhAPC = recombinant human activated protein C; SaO₂ = oxygen saturation in arterial blood as measured by pulse oximetry.

Values represent means $\pm$ SD unless otherwise indicated.

* Race and ethnicity were self-reported.

TABLE 2. CHANGE IN CLINICAL CHARACTERISTICS AFTER ENDOTOXIN ADMINISTRATION

	Placebo (n = 6)	Lovastatin (n = 6)	rhAPC (n = 6)	P Value
Change in vital signs*				
Temperature, °C	0.4 ± 0.4	0.7 ± 0.4	0.6 ± 0.3	0.51
Systolic blood pressure, mm Hg	-18 ± 5	-16 ± 16	-15 ± 4	0.88
Diastolic blood pressure, mm Hg	-13 ± 6	-11 ± 13	-10 ± 8	0.86
Mean arterial pressure, mm Hg	-14 ± 5	-13 ± 14	-12 ± 9	0.86
Heart rate, beats/min	30 ± 10	20 ± 6	30 ± 12	0.15
Respiratory rate, breaths/min	4 ± 4	2 ± 3	2 ± 3	0.58
Change in pulmonary function tests				
FVC, L	-0.23 ± 0.29	-0.15 ± 0.36	-0.22 ± 0.40	0.93
FVC, % predicted	-4.8 ± 6.7	-4.3 ± 9.8	-5.3 ± 8.87	0.98
FEV ₁ , L	-0.32 ± 0.29	-0.05 ± 0.36	-0.18 ± 0.29	0.37
FEV ₁ , % predicted	-7.8 ± 6.9	-3.8 ± 11.0	-5.3 ± 9.2	0.75
SpO ₂ , %	-2 ± 1	-3 ± 2	-4 ± 3	0.42
Change in hematologic parameters				
White blood cells, × 10 ³ /μl	1.5 ± 1.8	4.3 ± 2.4	3.0 ± 1.8	0.10
Neutrophils, %	5.3 ± 7.8	17.2 ± 6.9†	8.5 ± 8.0	0.04
Lymphocytes, %	-5.3 ± 3.2	-15.0 ± 2.2†	-7.3 ± 2.8	0.03
Monocytes, %	0.7 ± 1.4	-2.1 ± 0.7	-1.7 ± 0.6	0.12
Eosinophils, %	-0.4 ± 0.5	-0.2 ± 0.3	0.7 ± 0.3	0.13
Hematocrit, %	-0.8 ± 2.0	-1.4 ± 2.2	-2.4 ± 2.4	0.47
Platelets, × 10 ³ /μl	-30 ± 24	-26 ± 10	-49 ± 31	0.24
International normalized ratio	0.003 ± 0.06	0.02 ± 0.04	0.12 ± 0.05‡	0.002
Change in serum chemistry values				
Creatinine, mg/dl	0.03 ± 0.1	-0.03 ± 0.08	-0.05 ± 0.05	0.21
Bilirubin, mg/dl	-0.07 ± 0.2	-0.11 ± 0.26	-0.07 ± 0.08	0.89
AST, IU/L	-5 ± 7	3 ± 16	-5 ± 2	0.36
ALT, IU/L	-5 ± 5	-3 ± 5	-6 ± 4	0.49
Glucose, mg/dl	2.4 ± 5.9	5.9 ± 6.4	2.7 ± 5.5	0.55

Values represent means ± SD for postendotoxin measurement minus preendotoxin measurement.

* Represents maximal change from baseline.

† Significant difference compared with placebo.

‡ Significant difference compared with both placebo and lovastatin cohorts.

images in Figure 2 are shown in Figures 3A–3C. Comparison of the right lung, using a repeated-measures analytic approach, demonstrated that lovastatin treatment resulted in a statistically significant decrease in postendotoxin K_i compared with the placebo cohort (Figures 4A and 4B). Although rhAPC treatment also decreased the postendotoxin K_i relative to placebo, this difference was not statistically significant (Figure 4C). Comparison of each individual's change in K_i after endotoxin (the postendotoxin K_i value minus the preendotoxin K_i value)

demonstrated smaller increases in K_i in both treatment groups when compared with placebo, but this was statistically significant only in the lovastatin treatment group (Figure 4D). Identical analysis of a control region-of-interest in the unchallenged left lung did not demonstrate any statistically significant changes in [¹⁸F]FDG uptake between the treatment groups. Intercept correction of the K_i , as introduced by Jones and colleagues (16), did not change the interpretation of the PET data (data not shown).

Figure 2. Summed images from the last 20 minutes of dynamically acquired positron emission tomography (PET) images after injection of [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG), before and after bronchoscopic instillation of endotoxin (Etx), and subtraction images in a representative subject from each treatment group. Arrows point to location of maximal difference in the region of the Etx instillation in the right middle lobe. The greatest difference is seen in the placebo subject and easily visualized on the subtraction images, whereas this difference is not visualized in the lovastatin-treated subject and attenuated in the recombinant human activated protein C (rhAPC) subject. In addition, these differences are not readily apparent on the FDG-PET images alone. Lung fields are illustrated by the white regions of interest. The PET Scale represents pixel values in the Before and After Etx images (μCi/cm³). The Difference Scale represents difference between Before and After Etx pixel values (μCi/cm³). Black areas in the mediastinum (between lung fields) indicate overflow of counts in those

pixels in the region of the heart. Gray arrowheads in the rhAPC subject indicate a change in position of breast tissue between the two scans, leading to an apparent difference in uptake on the subtraction images.

Figure 3. Quantification of endotoxin-dependent [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) uptake. Representative Patlak plots of the right lobe region before (open circles) and after endotoxin (closed circles) for the placebo (A), lovastatin (B), and rhAPC (C) cohorts. The plots demonstrate a higher rate of uptake of [^{18}F]FDG as measured by the influx constant K_i (the slope of the linear regression line of the Patlak plots; solid line) in the subject treated with placebo compared with the subjects treated with lovastatin and rhAPC. Dashed lines represent the 95% confidence interval of the linear regression line.

Bronchoalveolar Lavage Characterization of Treatment Effect on Endotoxin-dependent Inflammation

To confirm the antiinflammatory effects of lovastatin or rhAPC, we quantified leukocytes in the first and second aliquots of BAL fluid. These results are summarized in Table 3. The lovastatin-treated group showed a decrease in total leukocytes and neutrophils in both BAL aliquots, but this was not statistically significant. In the rhAPC-treated group, the decrease in total cell and neutrophil counts was not as dramatic in BAL1 as seen in the lovastatin-treated group. The decrease in total cell and neutrophil counts in BAL2 for the rhAPC-treated group was similar to that seen in the lovastatin-treated group. These changes also were not statistically significant.

DISCUSSION

There is a growing body of literature supporting the use of FDG-PET imaging to assess the neutrophilic burden in a number of pulmonary diseases. FDG-PET has been used to quantify the levels of neutrophilic inflammation in patients with pneumonia, cystic fibrosis, and chronic obstructive pulmonary disease (16, 18, 27). Preclinical studies have also demonstrated a clear association between [^{18}F]FDG uptake and degree of neutrophil recruitment in models of pneumonia, acute lung injury, and ventilator-induced lung injury (14, 15, 17).

However, no studies to date have demonstrated the ability of FDG-PET to actually measure a change in the level of lung inflammation specifically as a result of antiinflammatory therapy. We have previously demonstrated that low levels of experimentally induced lung inflammation in healthy volunteers can be imaged by FDG-PET imaging (19). This study extends these observations, demonstrating that FDG-PET imaging can be applied in a human model of experimentally induced inflammation to assess the lung's response to antiinflammatory therapy and that this platform may be useful for testing efficacy in phase 1 studies of novel pharmacologic agents before embarking on expensive clinical trials in patients.

We and others have shown that the increased [^{18}F]FDG uptake seen in neutrophilic lung inflammation is due primarily to increased uptake of activated neutrophils (14, 15, 28), with possibly other cell types within the lung parenchyma contributing (28). Interestingly, in this study, the decrease in K_i in the lovastatin-treated group was nearly 50% of that of the placebo-treated group, whereas the decrease in cells and neutrophils in the BAL fluid overall was not nearly as prominent. There are several possible explanations. Lovastatin is known to interfere with the production of a number of inflammatory mediators and chemokines, thus decreasing neutrophil migration into the lungs (22, 29). The increase in peripheral blood neutrophil counts in the lovastatin-treated group suggests that, in this study, lovastatin did indeed interfere with the ability of neutrophils to

Figure 4. Effect of pharmacologic intervention on positron emission tomography (PET)-measured [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) uptake as an indicator of inflammation. (A–C) Changes in PET-measured the influx constant K_i before and after treatment with placebo, lovastatin, or recombinant human activated protein C (rhAPC), respectively, in both the right and left lungs. $n = 6$ for each group. Increases in K_i were noted in all groups after endotoxin treatment. Although K_i was lower in both treatment groups compared with placebo, the decrease was statistically significant only in the lovastatin group (* $P < 0.05$). (D) Mean change in K_i in both right and left lungs. Open columns = placebo; closed columns = lovastatin; gray columns = rhAPC. * $P < 0.05$ when compared with placebo. No changes in K_i were noted in the left lung in any treatment group.

TABLE 3. BRONCHOALVEOLAR LAVAGE CELL COUNTS AND NEUTROPHIL COUNTS

BAL Measure	Placebo	Lovastatin	rhAPC
BAL1			
Total cell count, cells/mm ³	545 ± 425	311 ± 237	520 ± 196
Neutrophils, %	73 ± 7	68 ± 12	62 ± 12
Lymphocytes, %	9 ± 6	11 ± 8	11 ± 8
Macrophages, %	16 ± 4	21 ± 9	26 ± 7
Neutrophil concentration, cells/mm ³	383 ± 285	232 ± 195	338 ± 191
BAL2			
Total cell count, cells/mm ³	1,905 ± 1,326	1,765 ± 804	1,788 ± 940
Neutrophils, %	65 ± 13	59 ± 16	55 ± 8
Lymphocytes, %	11 ± 9	12 ± 8	10 ± 2
Macrophages, %	23 ± 5	20 ± 17	33 ± 10
Neutrophil concentration, cells/mm ³	1,203 ± 869	1,006 ± 463	1,011 ± 658

Definition of abbreviations: BAL = bronchoalveolar lavage; BAL1 = first aliquot of BAL fluid; BAL2 = remainder of the BAL fluid.

Values are reported as means ± SD. No statistically significant differences were noted between groups.

migrate out of the vascular space in response to the endotoxin instillation. However, it is unknown whether lovastatin could cause a lower level of activation in the neutrophils that would lead to lower [¹⁸F]FDG uptake or whether neutrophil activation is an on-off phenomenon. We have demonstrated in endotoxin-treated mice that neutrophil depletion with vinblastine alone does not completely abrogate the PET signal and concluded that the residual [¹⁸F]FDG uptake was likely due to the endotoxin effect on the lung parenchymal cells (28). It is possible that lovastatin may have also decreased the [¹⁸F]FDG uptake in the lung parenchymal cells themselves, further lowering the PET signal, supporting the evidence that FDG-PET provides a measure of the total pulmonary inflammatory response. Finally, there still remains the question of how well cells collected by BAL represent the total neutrophilic burden in the lungs, because only neutrophils in the alveolar space can be sampled.

Interestingly, we did not see the same magnitude of decrease in the level of inflammation by BAL measures in the rhAPC-treated group when compared with the study performed by Nick and colleagues, using rhAPC treatment with the same experimental model (21). We showed at best a 16% decrease in neutrophils in BAL fluid compared with the placebo-treated group, in contrast to a nearly 60% decrease in BAL neutrophils in the previous study. This may be due to protocol differences, as we did not perform BAL until 24 hours after endotoxin instillation, whereas BAL was performed 16 hours after endotoxin instillation in the study by Nick and colleagues. However, despite these differences, our findings still confirm that FDG-PET is a useful marker of inflammation, as the K_i in the rhAPC-treated group was higher than that seen in the lovastatin-treated group but slightly lower than in the placebo-treated group, correlating with a similar pattern in total cell and neutrophil counts from the first BAL aliquot.

A separate, unique finding regarding this study is the evidence suggesting a pulmonary antiinflammatory effect with lovastatin treatment in this model of mild lung inflammation. The data summarized above—decreased neutrophil recruitment by BAL (although not statistically significant) with a more prominent significant decrease in [¹⁸F]FDG uptake relative to the change in neutrophil recruitment—suggest that the antiinflammatory effect of lovastatin may not be limited to interference with neutrophil recruitment alone. Although the small sample size certainly limited our ability to detect differences in the BAL cell counts as a marker of inflammation, the data together provide further

evidence that lovastatin may be an effective pulmonary antiinflammatory treatment. Further studies are warranted to elucidate the mechanisms behind the antiinflammatory effect of lovastatin and its relationship to glucose uptake.

In terms of quantifying the FDG-PET signal, we again chose to use K_i as the measure of increased [¹⁸F]FDG uptake. The intercept-corrected K_i method, initially proposed by Jones and colleagues, normalizes the rate of [¹⁸F]FDG uptake for differences in the volume of distribution (measured by the intercept calculated in the Patlak graphical analysis). As we have found in our previous reports, the intercept-corrected K_i was equivalent to using K_i alone in this study. Therefore, we continue to favor using K_i as the preferred method of quantification with this model.

Simple visual inspection of the images was judged to be inadequate for accurately identifying the region of inflammation given the levels of treatment response in this model. We used the creation of subtraction images to identify more precisely the location of the endotoxin instillation. In the future, it may be possible with combined PET and computed tomography (CT) scanners to use the CT images for placement of the regions of interest by delineating the actual anatomic segment that was challenged. However, these scanners introduce new challenges with respect to the effect of respiratory motion on the coregistration of the PET-CT images, which has important implications for the reliability of region of interest placement for small lung regions.

One of the primary limitations of this study was the sample size. However, we do not believe that the lack of statistical significance for the BAL fluid parameters detracts from the findings of the study, as discussed earlier. In addition, the threshold of inflammation that FDG-PET imaging can detect is still unknown. Although this study was not designed to address that question, we surmise from our data that FDG-PET imaging is sensitive enough to detect changes even in these low levels of induced inflammation if quantified by K_i . It is also unknown how generalizable the treatment effect on the mild focal lung inflammation in this model will be to diseases with more diffuse lung involvement such as cystic fibrosis. Finally, although FDG-PET provides a relatively rapid readout of changes in the level of inflammation in response to antiinflammatory therapy, it is still unknown whether this will correlate with other long-term outcomes, particularly when used in clinical trials.

Overall, we conclude from this study that FDG-PET imaging may be more sensitive in detecting changes in lung inflammation than BAL alone in this particular experimental model. These data not only provide additional evidence of the potential utility of FDG-PET as a noninvasive biomarker for neutrophilic inflammation in the lungs but also suggest that this platform may be useful for testing the clinical efficacy of novel antiinflammatory therapies in the early phases of drug development.

Conflict of Interest Statement: D.L.C. received up to \$1,000 from Boehringer Ingelheim as an honorarium for an invited discussant role at a conference and \$10,001 to \$50,000 from ParinGenix, Inc. in industry-sponsored grants. T.J.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.B.R. received \$1,001 to \$5,000 from Genentech and \$1,001 to \$5,000 from Corus in consultancy fees, \$1,001 to \$5,000 from the France Foundation in lecture fees, \$10,001 to \$50,000 from Novartis for a TPI clinical trial, \$10,001 to \$50,000 from Vertex for clinical trials, and \$10,001 to \$50,000 from PTC for clinical trials in industry-sponsored grants. W.I. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.W.F. received \$1,001 to \$5,000 from MedPro Communications, \$5,001 to \$10,000 from the France Foundation, and \$1,001 to \$5,000 from Genentech in lecture fees, has six U.S. patents relating to drug and gene delivery to the airway, but receiving no financial benefit, from Case Western Reserve University. B.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.A.M. does not have

a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.P.S. is deceased and is unable to provide a conflict of interest statement. M.J.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

- Lee WL, Downey GP. Neutrophil activation and acute lung injury. *Curr Opin Crit Care* 2001;7:1–7.
- Perl M, Lomas-Neira J, Chung CS, Ayala A. Epithelial cell apoptosis and neutrophil recruitment in acute lung injury—a unifying hypothesis? What we have learned from small interfering RNAs. *Mol Med* 2008;14:465–475.
- Martin TR. Neutrophils and lung injury: getting it right. *J Clin Invest* 2002;110:1603–1605.
- Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. *Chest* 2008;133:489–495.
- Petterson CA, Adler KB. Airways inflammation and COPD: epithelial–neutrophil interactions. *Chest* 2002;121(5 Suppl):142S–150S.
- Selby C, Drost E, Lannan S, Wraith PK, MacNee W. Neutrophil retention in the lungs of patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991;143:1359–1364.
- Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999;160:1532–1539.
- Zhang JY, Wenzel SE. Tissue and BAL based biomarkers in asthma. *Immunol Allergy Clin North Am* 2007;27:623–632; vi.
- Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma: persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* 1997;156:737–743.
- Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 1995;95:843–852.
- Fujimoto K, Yasuo M, Urushibata K, Hanaoka M, Koizumi T, Kubo K. Airway inflammation during stable and acutely exacerbated chronic obstructive pulmonary disease. *Eur Respir J* 2005;25:640–646.
- Ilumets H, Ryttilä PH, Sovijärvi AR, Tervahartiala T, Myllärniemi M, Sorsa TA, Kinnula VL. Transient elevation of neutrophil proteinases in induced sputum during COPD exacerbation. *Scand J Clin Lab Invest* 2008;9:1–6.
- Reid DW, Misso N, Aggarwal S, Thompson PJ, Walters EH. Oxidative stress and lipid-derived inflammatory mediators during acute exacerbations of cystic fibrosis. *Respirology* 2007;12:63–69.
- Chen DL, Schuster DP. Positron emission tomography with [¹⁸F]fluorodeoxyglucose to evaluate neutrophil kinetics during acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L834–L840.
- Jones H, Clark R, Rhodes C, Schofield J, Krausz T, Haslett C. *In vivo* measurement of neutrophil activity in experimental lung inflammation. *Am J Respir Crit Care Med* 1994;149:1635–1639.
- Jones H, Sriskandan S, Peters A, Pride N, Krausz T, Boobis A, Haslett C. Dissociation of neutrophil emigration and metabolic activity in lobar pneumonia and bronchiectasis. *Eur Respir J* 1997;10:795–803.
- Schroeder T, Vidal Melo MF, Musch G, Harris RS, Venegas JG, Winkler T. Modeling pulmonary kinetics of 2-deoxy-2-[¹⁸F]fluoro-D-glucose during acute lung injury. *Acad Radiol* 2008;15:763–775.
- Chen DL, Ferkol TW, Mintun MA, Pittman JE, Rosenbluth DB, Schuster DP. Quantifying pulmonary inflammation in cystic fibrosis with positron emission tomography. *Am J Respir Crit Care Med* 2006;173:1363–1369.
- Chen DL, Rosenbluth DB, Mintun MA, Schuster DP. FDG-PET imaging of pulmonary inflammation in healthy volunteers after airway instillation of endotoxin. *J Appl Physiol* 2006;100:1602–1609.
- O’Grady NP, Preas HL, Pugin J, Fiuza C, Tropea M, Reda D, Banks SM, Suffredini AF. Local inflammatory responses following bronchial endotoxin instillation in humans. *Am J Respir Crit Care Med* 2001;163:1591–1598.
- Nick JA, Coldren CD, Geraci MW, Poch KR, Fouty BW, O’Brien J, Gruber M, Zarini S, Murphy RC, Kuhn K, *et al.* Recombinant human activated protein C reduces human endotoxin-induced pulmonary inflammation via inhibition of neutrophil chemotaxis. *Blood* 2004;104:3878–3885.
- Hothersall E, McSharry C, Thomson NC. Potential therapeutic role for statins in respiratory disease. *Thorax* 2006;61:729–734.
- Meinert CL, Tonascia S. Clinical trials: design, conduct, and analysis. New York: Oxford University Press; 1986.
- Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data: generalizations. *J Cereb Blood Flow Metab* 1985;5:584–590.
- Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 1983;3:1–7.
- Mikols CL, Yan L, Norris JY, Russell TD, Khalifah AP, Hachem RR, Chakinala MM, Yusen RD, Castro M, Kuo E, *et al.* IL-12 p80 is an innate epithelial cell effector that mediates chronic allograft dysfunction. *Am J Respir Crit Care Med* 2006;174:461–470.
- Jones HA, Marino PS, Shakur BH, Morrell NW. *In vivo* assessment of lung inflammatory cell activity in patients with COPD and asthma. *Eur Respir J* 2003;21:567–573.
- Zhou Z, Kozlowski J, Goodrich AL, Markman N, Chen DL, Schuster DP. Molecular imaging of lung glucose uptake after endotoxin in mice. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L760–L768.
- Kuipers HF, van den Elsen PJ. Immunomodulation by statins: inhibition of cholesterol vs. isoprenoid biosynthesis. *Biomed Pharmacother* 2007;61:400–407.