Eligibility Criteria

Inclusion Criteria

- Healthy man or woman, any race or ethnicity, age 19 – 44 years old
- Screening FEV_1 and FVC > 90% of predicted
- Screening oxygen saturation by pulse oximetry $\geq$97% on room air
- Capable of lying still and supine within the PET/CT scanner for ~1.5 hours
- Capable of following instructions for breathing protocol during CT portion of PET/CT
- Able and willing to give informed consent
- BMI < 35

Exclusion Criteria

- Pregnancy (confirmed by qualitative serum hCG pregnancy test)
- Lactation
- Active menstruation
- History of cardiopulmonary disease
- Currently taking any prescription medications
- History of tobacco use or illicit drug use within the past year
- Presence of implanted electronic medical device
- Enrollment in another research study of an investigational drug
- Known allergy to both trimethoprim/sulfamethoxazole and amoxicillin
- Known allergy to drugs routinely used during bronchoscopy (e.g. midazolam and fentanyl)

Methods

Bronchoscopy and BAL

The bronchoscopy and BAL procedures were performed as previously described (*1, 2*). Briefly, meperidine 50 mg and hydroxyzine 25 mg was given intramuscularly followed by midazolam and fentanyl intravenously for sedation. Lidocaine was administered via aerosol and subglottic instillation at the time of bronchoscopy. The bronchoscope was wedged into a segment of the right middle lobe (usually the lateral segment). Subsequently, a 5-Fr balloon-tipped catheter was threaded through the bronchoscope and the balloon inflated to occlude the target segment. Endotoxin (4 ng/kg in 2 ml sterile water) was then instilled as previously described (*2*). For BAL, the bronchoscope was again wedged into the endotoxin-challenged segment. Three sequential 50-ml volumes of warmed sterile saline (37ºC) were instilled in the suction channel of the bronchoscope and recovered by gentle aspiration as previously described (*2*). Vital sign monitoring was performed throughout the procedure.

Immunohistochemistry

Cells from the BAL were gauze-filtered, spun down and resuspended in PBS. The number and viability of the cells was determined by trypan blue exclusion on a hemacytometer. Thin-layer cell preparations (cytospins) of 3 x 10^5 BAL cells were created by cytocentrifuge. These slides were then air dried and fixed in 100% methanol. The slides were stored at 4^0C until ready for staining. One slide was stained with Hema 3 (Fisher Scientific #123-869) to determine the percentage of macrophages and neutrophils. Separate slides were stained with one of two different polyclonal rabbit anti-human iNOS antibodies for fluorescence microscopy (Millipore #AB5384 that binds at the C-terminus, 1:200 dilution, or Santa Cruz #sc-8310, clone H-174, that binds the N-terminus, 1:50 dilution). Each slide was rehydrated with PBS and blocked with 5% goat serum for 30 minutes. Each iNOS antibody was applied on separate slides overnight at

4°C in a humidified chamber. After washing thrice with PBS, a fluorescent-labeled goat anti-rabbit secondary conjugated antibody (Alexafluor 488, Cell Signalling #4412S, 1:500 dilution) was applied at room temperature for 1 hour. The slides were washed 3 times with PBS and mounted with Vectashield containing DAPI (#H-1200, Vector Laboratories) to stain the nuclei. Images were captured on a Leica DM 5000B microscope using the excitation 480/emission 570 filter with a Retiga 20000R Fast 1395 camera and Q capture Pro51 software (QImaging, Surrey, BC). Separate cell preparations were also processed in the absence of the primary antibody to assess for nonspecific binding.

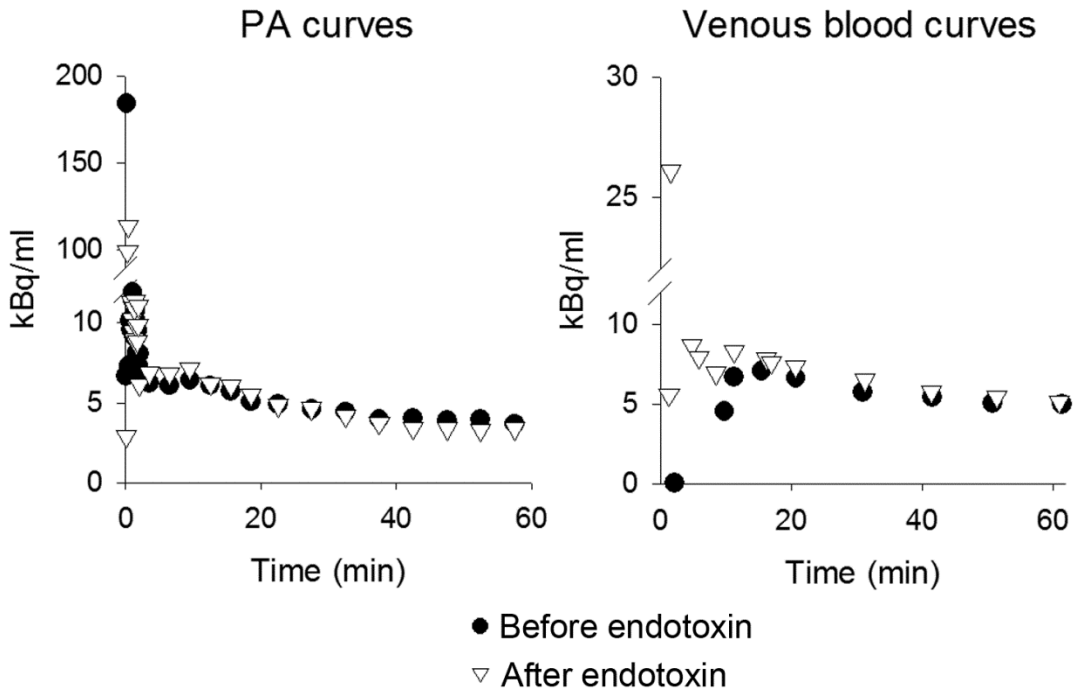
Image Analysis

The DICOM PET and CT image files were imported into Integrated Research Workflow 4.0 (Siemens) for analysis and coregistered. The baseline PET and CT images were then aligned to the post-endotoxin PET and CT images by first visually aligning the airways, mediastinal contours, and chest wall of the baseline CT to the post-endotoxin CT. The transformation matrix defining this CT-to-CT alignment was then applied to align the baseline PET to the post-endotoxin CT. Volumes of interest (VOIs) were placed on the areas of infiltrate in the right middle lobe and in an equivalent region of lung on the left at the same anterior-posterior level and approximately same distance from the mediastinum on the post-endotoxin CT images using standard lung windows (center -500 HU, width 1500 HU). In some cases, because of the location of the heart, the left lung VOI was located more cranially than that of the right middle lobe VOI. The VOIs on the post-endotoxin CT were then used to extract the time-activity curves in the right and left lungs on both the baseline and post-endotoxin PET scans. The same VOIs were also used to determine the mean HU on the baseline CT after alignment with the post-endotoxin CT. A VOI was also placed over the main pulmonary artery as the reference region for the Logan plot analysis (*3*), which determined the distribution volume ratio (DVR) of [^{18}F]NOS for the lung VOIs.

Metabolism analysis

The plasma was separated from whole blood samples taken at 5, 15, 30, and 60 min after tracer injection. The plasma was then added to acetonitrile and spun down to remove the protein pellet. The supernatant, along with non-radioactive FNOS, was loaded onto an HPLC system for analysis (Column: Alltech EPS C18 250×4.6 mm 5μ; mobile phase: 30% acetonitrile/70% 0.05 M phosphorus buffer pH = 6.5; flow rate: 1.2 mL/min; UV: 272 nm). Eighteen total fractions were collected (1 tube/min) and counted on a Beckman 6000 gamma counter. The available parent compound in the plasma was then expressed as a percent of the total activity as analyzed by HPLC.

Supplemental Figure 1



References

1. Chen DL, Bedient TJ, Kozlowski J, et al. [18F]fluorodeoxyglucose positron emission tomography for lung antiinflammatory response evaluation. *Am J Respir Crit Care Med.* Sep 15 2009;180(6):533-539.

2. 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 (1985).* May 2006;100(5):1602-1609.

3. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab.* Sep 1996;16(5):834-840.