Non-Invasive Near-Infrared Fluorescence Quantitative Tomography (FMT ™) of the Effects of PDE4 Inhibitor Therapy in an LPS Murine Model of COPD *In Vivo*

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1 Abstract

Chronic bronchitis and emphysema, conditions collectively known as chronic obstructive pulmonary disease (COPD) are the fourth leading cause of death in the United States after heart disease, cancer, and stroke. COPD has been associated with the presence of chronic inflammation, generally neutrophils, that leads to long term airway obstruction and irreversible, destructive remodeling of lung tissue. An animal model for COPD-like lung inflammation can be established in mice by intranasal challenge with lipopolysaccharide (LPS), an endotoxin from gram-negative bacteria. Current assessments of disease progression rely on invasive measures of pulmonary function and cell infiltration and are therefore limited to terminal assessment. The ability to non-invasively monitor and quantify the underlying inflammatory processes in LPS-induced lung inflammation would provide a significant advance in characterizing longitudinal disease processes and the effects of targeted therapeutics. We used near-infrared (NIR) fluorescence imaging agents, in combination with fluorescence molecular tomography (FMT ™) imaging (FMT 2500™, PerkinElmer) to non-invasively quantify pulmonary neutrophil protease activity and associated edema in the LPS-induced lung inflammation model. In these studies, BALB/c mice received intranasal instillation of LPS followed by intravenous injection with either the cathepsin-activable fluorescent agent, ProSense® 680 (PerkinElmer), to assess cathepsin activity in activated neutrophils or with AngioSense® 680 (PerkinElmer) to measure vascular leak in the lungs. This imaging approach provided a robust quantified measurement of two different biological processes. The quantified fluorescence increased correspondingly with dose-dependent effects of LPS on bronchoalveolar lavage (BAL) neutrophil counts. The phosphodiesterase IV (PDE4) inhibitor, Rolipram, when given intraperitoneally at the time of LPS administration, dramatically decreased both neutrophil recruitment and ProSense 680 fluorescence (90%) in the lungs as well as revealing a measurable decrease in the volume of the lung affected by LPS-induced inflammation.

2 Methods and Materials

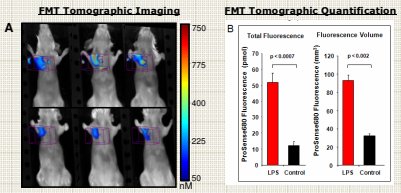
Experimental Animals: Specific pathogen-free female BALB/c mice (6-8 weeks of age, 18–20 g) were obtained from Charles River (Wilmington, MA) and housed in a controlled environment (72°F; 12:12-h light-dark cycle) under specific-pathogen free conditions with water and and food provided ad libitum.

LPS Inflammation Protocol: LPS-induced lung inflammation is a simple model for cellular processes occurring in Chronic Obstructive Pulmonary Disease (COPD). On day 0, mice received intranasal injections of either 100 µg LPS solubilized in phosphate buffered saline (PBS) or PBS alone. Diseased and control mice were injected with ProSense680 or AngioSense 680 on day 1, 4 hrs after the intranasal administration of LPS. Mice were anesthetized, depilated to minimize interference with fluorescent signal, and imaged at 7 or 24 hrs, for AngioSense 680 and ProSense 680, respectively.

BAL Cell Analysis: BAL cell analysis in asthma and control mice was performed 24h following probe injection. Mice were killed by CO2 inhalation, and a midline neck incision was made to cannulate the trachea. The lungs were washed three times with 0.8 ml of lavage buffer PBS containing 1% fetal calf serum and 2% Paraformaldehyde (Poly Scientific), the recovered BAL cell suspension was centrifuged at 300 x g for 10 minutes at 4°C and cells resuspended in 0.5 ml of lavage buffer. Cells were counted on a hemocytometer, and cytospin preparations of 2 x 10^5 cells were used to establish differential cell subpopulation counts (Hemacolor staining system, EMD chemicals, Inc).

***In Vivo* Tomographic Imaging and Analysis:** Anesthetized mice were placed in the imaging cassette one at a time, and the cassette depth was adjusted to a thickness of 13 mm. The imaging cassette was placed into the imaging chamber of the FMT 2500™ in vivo imaging system (PerkinElmer) where a low-powered NIR laser diode transilluminated the thorax region, with signal detection occurring via a thermoelectrically cooled CCD camera placed on the opposite side of the imaged animal. Appropriate optical filters allowed collection of both fluorescence and excitation datasets and the multiple source-detector fluorescence projections were normalized to the paired collection of laser excitation data. The entire image acquisition sequence took approximately 3–4 min per mouse.

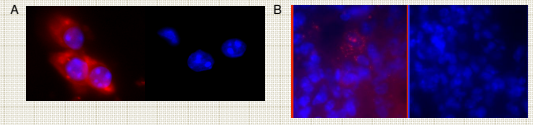
3 Fluorescent Tomographic (FMT) Imaging of a Cathepsin-Activatable Agent in LPS-induced Lung Inflammation



FMT imaging **(A)** shows precise fluorescence localization of activated ProSense 680. Non-invasive tomographic imaging not only detected disease-related protease activity, but also allowed the accurate quantification of the internal distribution of this fluorescence in the lung. FMT measured a 5-fold increase in ProSense 680 fluorescence in the lungs of COPD mice (> 55 pmol/lung) as compared to those of control mice (< 10-15 pmol/lung)(B, left panel).

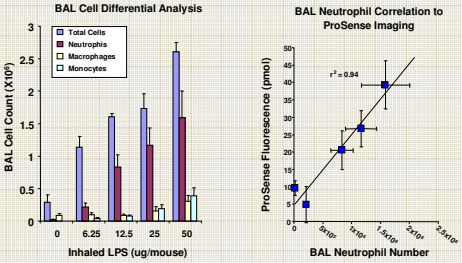
In addition, the FMT software precisely quantified the volume of the fluorescence within the lung regions of the mice (B, right panel) to provide a measure of affected lung volume, showing approximately a 3-fold increase in COPD animals as compared to controls (~100 mm3 in asthma vs 30 mm3 in controls). Both the total fluorescence quantification (pmol) and the volume measurement showed high statistical differences in comparing asthma and control mice ($p<0.0007$ and $p<0.002$, respectively).

4 BAL Neutrophils Internalize and Activate a Cathepsin-Activatable Imaging Agent



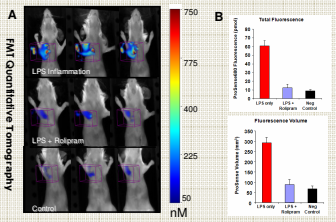
Fluorescent microscopy of the BAL cells isolated from LPS and control mice that received intravenous ProSense injection confirmed that the neutrophils were the predominant cell activating ProSense fluorescence in the airways **(A)**. Tissue sections from the lungs of LPS-treated mice demonstrated multiple areas of fluorescence localized to the lung parenchyma and alveoli as compared to the minimal fluorescence detectable in control lungs **(B)**.

5 LPS Dose Effect on Lung Inflammation and FMT Imaging



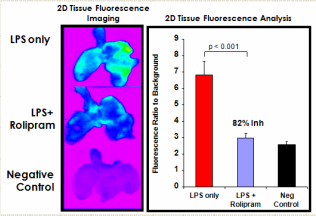
An LPS dose-dependent influx of neutrophils to the lung was measured and compared to the in vivo FMT quantification of lung ProSense Fluorescence. An excellent correlation between ProSense lung fluorescence BAL neutrophilia was determined.

6 PDE4 Inhibition Decreases Pulmonary Inflammation and ProSense 680 Activation



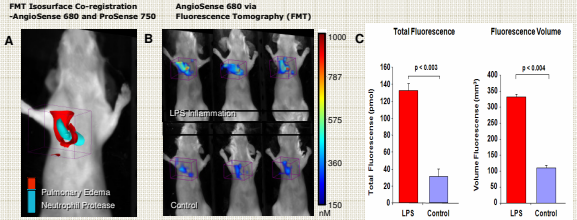
PDE4 inhibitors such as Rolipram have proven potential as anti-inflammatory drugs especially in airway diseases, they suppress the release of inflammatory signals like cytokines and have a high therapeutic potential as a non-steroidal disease controller in COPD and Asthma. To evaluate whether the effect of the PDE4 inhibitor on COPD treatment could be detected and measured non-invasively by FMT, mice were treated with Rolipram (2 mg/kg given IP 24, 12, and 0.5 hrs prior to LPS). Controls received injections of PBS. All mice were injected with ProSense 680 4hrs after intranasal LPS. FMT showed high fluorescence signal in untreated asthmatic mice (A, top pannel) compared to Rolipram-treated mice (A, bottom panel). The quantification of the therapeutic response in treated LPS-induced lung inflammation showed 92% inhibition of total ProSense 680 signal (B left) and 86% inhibition of fluorescence volume (B right), with excellent statistical significance ($p<0.002$ and $p<0.006$), respectively.

7 Rolipram Effect on Lung Tissue Fluorescence



Tissue 2D imaging of ProSense fluorescence was measured *ex vivo* to verify the dramatic effects of Rolipram treatment on LPS lung inflammation and on non-invasive FMT quantification. Image analysis to generate tissue fluorescence ratios to background yielded a highly significant ($p < 0.001$) inhibition of ProSense tissue fluorescence by Rolipram treatment.

8 Imaging LPS-Induced Pulmonary Edema Using Tomographic Imaging of a Vascular Imaging Agent



To assess the existence of edema caused by the inflammation of LPS in the airways, we used FMT imaging in combination with a vascular imaging agent, AngioSense 680. We performed multiplex imaging to compare the pattern of distribution (represented as colored, volumetric surface renderings) of AngioSense 680 to the highly characterized distribution that we have established with ProSense 750 imaging of neutrophilia (A). As expected, the sites of neutrophilia were spatially distinct from regions showing leakage of the vascular probe due to pulmonary edema; edema signal surrounded regions of more centralized neutrophilia. Representative mice are shown (n = 5 per group) to portray the consistent edema signal induced by LPS as compared to background signal in control animals (B). FMT quantified a 5-fold increase in total AngioSense 680 fluorescence in the lungs of LPS mice (> 130 pmol/lung) indicating increased vascular leak in the inflamed lungs as compared to those of control mice (< 20-30 pmol/lung and $p<0.003$) (C, left). A 3.5-fold increase in the tomographically-determined fluorescence volume in inflammatory lung edema (>400 mm^3) as compared to control lungs (<120 mm^3 and $p<0.004$) (C, Right).

7 Summary

We demonstrated the ability of the FMT 2500™ in vivo imaging system in combination with two different imaging agents, AngioSense® and ProSense®, to non-invasively visualize and quantify both pulmonary edema and neutrophil protease activity respectively, induced by LPS exposure. The consistency of the quantitative tomography imaging results and its excellent correlation with BAL neutrophil counts show that it is a robust tool for quantifying lung pathology in mouse pulmonary inflammation models. The efficacy of a PDE4 inhibitor was accurately and rapidly determined using tomographic imaging of neutrophil protease activity, revealing near-complete inhibition of lung ProSense 680 fluorescence, in striking agreement with an independent measure of lung neutrophilia. FMT imaging in COPD research, utilizing new and existing imaging agents, provides useful, non-invasive tools for understanding and quantifying pulmonary inflammation and therapeutic efficacy *in vivo*.

8 References

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