Non-Invasive Quantitative Tomography of Disease Progression and Therapeutic Response in a Mouse Model of Asthma In Vivo

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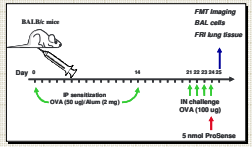
1 Abstract

Asthma is a chronic inflammatory disease of the airways characterized by a T cell-driven eosinophil and mast cell inflammation that leads to intermittent reversible airway obstruction. Asthma is a rapidly growing public health problem affecting 300 million people worldwide and it continues to be a major cause of morbidity. Due to the prevalence of asthma, and the complexity of this multifactorial and multicellular disease, it has become important to establish relevant animal models to quantify the inflammation of the disease and to better aid in the discovery of new therapeutic agents. The capacity to non-invasively visualize and quantify specific biological processes of asthma in mouse pulmonary models in real time would provide a significant advance in characterizing disease processes and the effects of targeted therapeutics. We report the utility of a near-infrared (NIR) fluorescent imaging agent, ProSense® 680 (PerkinElmer), in combination with fluorescence molecular tomography (FMT™) for the non-invasive quantitative imaging of mouse lung inflammation in an ovalbumin (OVA)-induced chronic asthma model. BALB/c mice were intraperitoneally sensitized with OVA-Alum at day 0 and 14, followed by daily intranasal instillation of OVA in PBS from day 21 to 24. Dexamethasone therapy, or the vehicle control, was given intraperitoneally four hours before each intranasal inhalation of OVA from day 21 to 24. Twenty-four hours prior to imaging, the mice were injected via tail vein with 5 nmoles of the cathepsin-activatable fluorescent agent, ProSense 680. Fluorescence molecular imaging (FMT 2500™, PerkinElmer) revealed in vivo cysteine protease activity within the lung associated with the inflammatory eosinophilia and the response to treatment with Dexamethasone. Results were correlated with in vitro laboratory tests (Bronchoalveolar lavage cell analysis and immuno-histochemistry) and revealed good correlation between these measures and ProSense 680 imaging. We have demonstrated the ability of FMT imaging to non-invasively visualize and quantify inflammation in the lung.

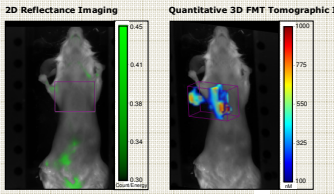
2 Experimental Design

Ovalbumin (OVA)-induced Asthma in BALB/c Mice



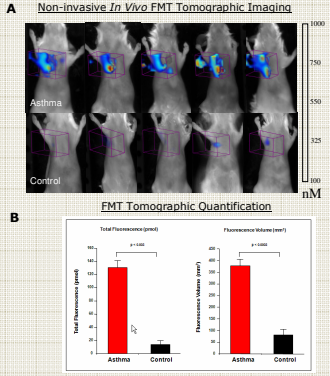
BALB/c mice were immunized on day 0 and day 14 by intraperitoneal injections of 50 µg OVA (grade V; Sigma) together with 2 g aluminum hydroxide (Sigma, St.Louis, MO) in phosphate buffered saline (PBS). Control mice received intraperitoneal injections of PBS. From day 21 to day 24, both immunized and control mice were given daily intranasal administration of 100 µg OVA grade VII; Sigma) solubilized in PBS.

3 Benefits of FMT (Fluorescence Quantitative Tomography) in In Vivo Asthma Imaging



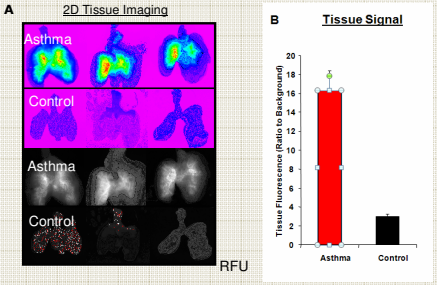
To assess the relative benefit of quantitative fluorescence tomographic imaging, as compared to the standard 2D fluorescence approach, we imaged OVA-induced pulmonary inflammation using both types of imaging technology with ProSense 680, an agent that is activated predominantly in vivo by a family of lysosomal cathepsin proteases. The intravenous injection of ProSense 680 allows the detection of activated macrophages, eosinophils, and neutrophils. 2D imaging of asthmatic mice (left) revealed only low levels of fluorescent signal predominantly in the lower abdomen, attributed to bladder and intestine, and showed little or no fluorescence in the animals' lung regions. In contrast, imaging using fluorescence molecular tomography (FMT 2500) of the thorax region of asthmatic mice (right) revealed deep tissue fluorescence associated with the disease process. The signal provided a clear 3-dimensional image indicating the presence of widespread lung protease activity with apparent intrapulmonary heterogeneity of signal.

4 FMT Tomographic Images Show Clear Difference in Asthmatic and Control Mice



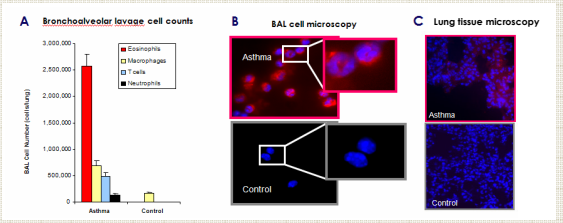
FMT imaging of multiple animals (n= 5 asthmatic mice and 5 controls) (A) shows a consistent pattern and extent of fluorescence, with control animals showing only basal levels of fluorescence. Importantly, non-invasive tomographic imaging not only detected disease-related protease activity rendering striking deep tissue images, but also allowed the accurate quantification of this fluorescence (B). FMT measured a 10-fold increase in ProSense 680 fluorescence in the lungs of asthmatic mice (> 120 pmol/lung) as compared to those of control mice (< 10-15 pmol/lung)(B, left panel). In addition, the FMT 2500 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. A 5-fold increase in asthmatic animals was illustrated as compared to controls (~375 mm3 in asthma vs 75 mm3 in controls). Both the total fluorescence quantification (pmol) and the volume measurement showed high statistical differences in comparing asthma and the control mice ($p<0.003$ and $p<0.0003$, respectively).

5 *Ex Vivo* Imaging of Ovalbumin-Induced Asthma (OVA)

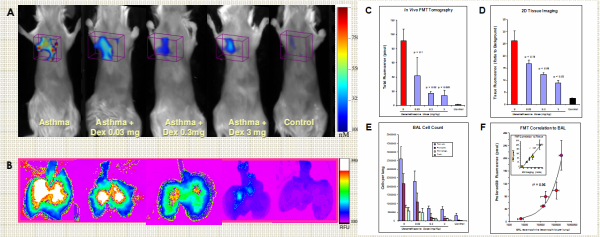


2D imaging of ex vivo tissue confirmed that the fluorescent signal originated specifically from the lung, consistent with the results from non-invasive FMT in vivo imaging. Excised lungs from sacrificed mice showed wide-spread fluorescence throughout the lung while control lungs showed minimal fluorescence (A, color panels). Grayscale versions of the images (A, grayscale panels) further reveal a very clear distribution of activated ProSense 680 in the inflamed bronchial tree of asthmatic lungs as compared to controls. Measurement of the ratio of fluorescence intensity in asthmatic lungs yielded a signal intensity 4-5 fold higher than that in the lungs of control mice (B).

6 Bronchoalveolar Lavage (BAL) Cell Assessment by Fluorescent Microscopy



BAL cell differential analysis indicates the expected recruitment of a predominant and large number of eosinophils (> 60% of the total cells) infiltrating the airways of asthmatic mice with no detectable eosinophils in control mice (A). Fluorescent microscopy of the BAL cells isolated from asthmatic and control mice that received intravenous ProSense 680 injection further established that the eosinophils were the predominant cell activating ProSense 680 fluorescence in the airways (B). BAL mononuclear cells in the same mice showed little or no fluorescence and the small number of collected BAL cells from control mice, predominantly macrophages, showed minimal fluorescence (B). Tissue sections from the lungs of asthmatic mice demonstrated multiple areas of fluorescence localized to the lung parenchyma as compared to the minimal fluorescence detectable in the control lungs (C).



To evaluate whether the effect of a conventional asthma treatment could be detected and measured non-invasively by FMT imaging, mice were treated with dexamethasone and imaged with the FMT 2500. FMT showed high fluorescence signal in untreated asthmatic mice and a decreased signal in dexamethasone-treated mice in a dose-dependent fashion. 55%, 65%, and 80% inhibition was shown at 0.03, 0.3, and 3 mg/mouse dose respectively (A, C). 2D imaging showed definite effects of treatment on ProSense 680 lung signal (B,D) with the signal remaining fairly widespread at the lowest treatment dose. FMT imaging provided a non-invasive tool that correlated extremely well with terminal BAL cell harvest and eosinophil counts (E, F).

7 Summary

We have demonstrated the ability of the FMT 2500TM in vivo imaging system and ProSense 680 to non-invasively visualize and quantify inflammation in the lung in a robust and validated manner. The consistency of the quantitative tomography, as well as its excellent correlation with BAL assessment of eosinophilia, provide a powerful toolkit for quantifying the therapeutic efficacy of dexamethasone treatment. Utilizing new and existing imaging agents, FMT imaging in asthma research provides useful, non-invasive tools for understanding pulmonary inflammation and for developing new therapeutics in vivo.

8 References

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