Published in final edited form as:

Mol Imaging Biol. 2019 April ; 21(2): 228–231. doi:10.1007/s11307-018-1249-0.

Imaging Pulmonary Foreign Body Reaction Using [¹²⁵I]iodo-DPA-713 SPECT/CT in Mice

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Abstract

Purpose: Foreign body reactions elicit granulomatous inflammation composed of reactive macrophages. We hypothesized that [¹²⁵I]iodo-DPA-713 single-photon emission computed tomography (SPECT), a low-molecular-weight pyrazolopyrimidine ligand selectively trapped by phagocytes, could be used to detect foreign body reactions in a murine model.

Procedures: C57BL/6 mice intratracheally inoculated with dextran beads, which developed foreign body lesions, were imaged after injection of [¹²⁵I]iodo-DPA-713 or DPA-713-IRDye800CW using SPECT and optical imaging, respectively.

Results: Foreign body lesions were clearly observed in the lungs of the dextran-treated mice on computer tomography imaging and demonstrated significantly higher [¹²⁵I]iodo-DPA-713 uptake compared with control animals ($p < 0.01$). *Ex vivo* studies demonstrated granulomatous reactions in the lungs of dextran-treated mice and localization of DPA-713-IRDye800CW at the diseased sites confirming the imaging findings.

Conclusion: Radioiodinated DPA-713 may be used as a noninvasive biomarker for the detection of pulmonary foreign body reactions.

Keywords

Foreign body; Iodo-DPA-713; Granulomatous inflammation

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11307-018-1249-0>) contains supplementary material, which is available to authorized users.

Introduction

Translocator protein (TSPO) is an 18-kDa trans-mitochondrial membrane channel for the transport of cholesterol and other endogenous ligands [1], and is upregulated in reactive glial and immune cells [2]. We have previously demonstrated that [125 I]iodo-DPA-713, a low-molecular-weight pyrazolopyrimidine ligand that is trapped by phagocytes, can noninvasively monitor macrophage-associated pulmonary inflammation [3, 4]. Given that foreign body reactions elicit granulomatous inflammation composed of activated macrophages [5], in this study, we assessed the ability of [125 I]iodo-DPA-713 single-photon emission computed tomography/x-ray computed tomography (SPECT/CT) to visualize pulmonary foreign body reactions in a mouse model.

Materials and Methods

All protocols were approved by the Johns Hopkins Bio-safety, Radiation Safety, and Animal Care and Use Committees.

Four-to-six-week-old female C57BL/6 (Charles River Laboratory) mice were injected intratracheally through a small neck incision with 80 03BCI of a dextran bead (Sephadex® G-50, Sigma-Aldrich) suspension at 1×10^6 /ml with the use of a 20-gauge needle. Seven animals were inoculated over two different experiments.

[125 I]iodo-DPA-713 was synthesized as described previously [6] and specific activity ranged from 70.3 to 77.7 GBq/mmol (1900–2100 Ci/mmol). The radiotracer was formulated in 10 % ethanol in phosphate buffer saline (PBS), pH 7.4, and was injected as a 100- μ l intravenous bolus through the tail vein 48-h post-dextran beads inoculation. Four healthy mice were also injected as controls. DPA-713-IRDye800CW was formulated in 10 % dimethyl sulfoxide in PBS, pH 7.5, as previously described [3, 7, 8]. A 100 μ l volume of DPA-713-IRDye800CW was combined with a 100 μ l volume of [125 I]iodo-DPA-713, formulated in 100 % PBS, pH 7.4 prior to bolus tail vein injection.

Mice were imaged 24 h post-injection using a NanoSPECT/CT (Bioscan) small animal imager as described previously [4]. Our prior studies have demonstrated that imaging at 24 h post-tracer injection provides adequate time for radiotracer trapping in reactive macrophages and washout from other TSPO-expressing and non-target tissues [3, 9]. SPECT images were reconstructed and co-registered with computed CT images using VivoQuant 3.0 (inviCRO). Volumes of interest (VOIs) were drawn by one individual using the CT images as a reference and independently validated by another individual in the research team.

Four mice from the dextran-treated group were co-injected with DPA-713-IRDye800CW and [125 I]iodo-DPA-713. Mice underwent a 24-h uptake period prior to sacrifice, when the lungs were harvested, fixed in formalin for 1 h, and imaged using a Pearl Impulse Imager (LI-COR Biosciences). Images were acquired using a 790/800-nm band pass filter as well as a white light photograph and were displayed using the manufacturer's software (Pearl Impulse Software v. 2.0).

Tissues from dextran-treated mice injected with DPA-713-IRDye680LT only were collected, fixed in neutral-buffered formalin for 48-h, grossed, and embedded in paraffin prior to sectioning to 4 μ m onto charged glass slides. The slides were then stained with mouse anti-CD68 (Abcam, ab955, 1:67) antibody using methods described previously [3]. The slides were then washed and probed with goat anti-mouse secondary antibody-fluorescein conjugate (Abcam, ab97022, 1:250). The slides were also exposed to Hoechst 33342 dye (Invitrogen, H3570, 1:1000 in PBS) to stain the nuclei. Slides were visualized using a Nikon 80i upright epifluorescence microscope equipped with a Nikon DS-Qi1Mc darkfield CCD camera and excited by a Nikon Intensilight C-HGFI lamp. All images were recorded and processed using Nikon Imaging Software Elements.

Results

[¹²⁵I]iodo-DPA-713 imaging of dextran-treated mice and uninoculated, control mice is shown in Fig. 1. Pulmonary infiltrates consistent with lesions were clearly visible in the dextran-treated mice (Fig. 1a). [¹²⁵I]iodo-DPA-713 uptake was noted in the dextran-treated mice and co-localized well with the foreign body lesions visualized on CT (Fig. 1a), with little to no uptake in the lungs of control mice (Fig. 1b). SPECT/CT imaging with [¹²⁵I]iodo-DPA-713 demonstrated higher uptake (6.86 times higher, $p < 0.01$) in the pulmonary lesions of dextran-treated mice compared with the negative control animals (Fig. 1c, two-tailed t test $p < 0.01$).

Foreign body granulomas are rich in activated macrophages (Fig. 2). Inspection of stained macrophages reveals substantial co-localization of DPA-713-IRDye680LT uptake and CD68 uptake (Suppl. Fig. 1, see Electronic Supplementary Material). Near-infrared fluorescence revealed focal uptake of DPA-713-IRDye800CW conjugate in dextran-treated lungs, while lungs from control animals exhibited no uptake of conjugate (Fig. 3). This demonstrates the specific retention of DPA-713 by macrophages in this type of lesions.

Discussion

Here, we have described the visualization and quantification of macrophage-specific inflammation using [¹²⁵I]iodo-DPA-713 SPECT/CT imaging and DPA-713-IRDye800CW near-infrared fluorescence and within a murine foreign body model using dextran beads. Our data demonstrate that DPA-713 is selectively trapped within dextran-treated lungs and reports on reactive macrophages within lesions after a 24-h uptake period. Indeed, the radiotracer and fluorescent tracer uptake patterns share the same distribution.

Currently, high-resolution CT is the standard of care technique for suspected foreign body reactions, classical findings include nodular opacities, a diffuse ground glass pattern, adenopathy, and emphysema, but these characteristics are nonspecific for foreign body reactions [10]. Gallium-67 scanning has been used to assess these lesions but is not helpful. Although diffuse pulmonary uptake has been described, those findings did not correlate with clinical symptoms or radiographic abnormalities [11]. Reactive macrophages and giant cells are the hallmark of foreign body-associated inflammation [5]. DPA-713 is a ligand with high affinity for TSPO receptor [12] which is upregulated in activated glial and immune cells [2].

[¹²⁵I]iodo-DPA-713 was first introduced in 2009 by Wang et al. [4] for imaging TSPO expression and is also employed as an imaging tool for peripheral macrophage-associated inflammation.

[¹²⁵I]iodo-DPA-713 SPECT/CT revealed significant differences ($p < 0.01$) in radiotracer uptake between dextran-treated mice and controls (Fig. 1). Qualitative imaging of macrophage-associated inflammation in inoculated lung tissue using DPA-713-IRDye800CW near-infrared fluorescence imaging showed uptake of the fluorophore in the lesions (Figs. 2 and 3). Subsequent co-staining with anti-CD68 demonstrated that accumulation of DPA-713-IRDye800CW was confined to CD68-expressing macrophages after 24 h of *in vivo* uptake (ESM Suppl. Fig. 1). One limitation of this approach is that iodo-DPA-713 is not specific for pulmonary foreign body reactions and thus may not be able to differentiate it from other pulmonary diseases mediated by activated macrophages. Similarly, while iodo-DPA-713 is an excellent marker of granulomatous inflammation, they cannot be differentiated from other disease processes (*e.g.*, oncologic) mediated by CD68-expressing phagocytic cells.

Iodo-DPA-713 can be labeled with various radioisotopes including I-124, which is suitable for positron-emission tomography (PET) imaging and I-123, which is suitable for SPECT imaging in humans. Human dosimetry and biodistribution of [¹²⁴I]iodo-DPA-713 using PET has recently been described in healthy human subjects [13], making [¹²⁴I]iodo-DPA-713 an excellent clinical candidate for diagnostic imaging of foreign body-associated inflammation compared with the current imaging techniques.

Conclusion

Iodo-DPA-713 can be used to image macrophage-specific inflammation in a murine foreign body model. Because of its cell-type specificity, radioiodinated DPA-713 may be used as a noninvasive biomarker for the detection of foreign body reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding This study was funded by the National Institutes of Health (NIH) Director's Transformative Research Award R01-EB020539 (S.K.J.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Fig. 1. [^{125}I]iodo-DPA-713 SPECT/CT in a model of foreign body lesions. Transverse, coronal, and sagittal sections from pulmonary imaging performed on dextran-treated mice with foreign body reaction. **a** Pulmonary infiltrates on CT demonstrating foreign body lesions are clearly visible in affected lungs (arrows), **b** but are absent in control mice. [^{125}I]iodo-DPA-713 pulmonary uptake is significantly higher (6.86 times higher, $p < 0.01$) and co-localizes with lesions identified by CT in dextran-treated mice compared to **b** untreated control mice. **c** Lesion-specific uptake is represented as box plots where whiskers show the upper and lower limits of range. $n = 7$ animals. BF brown fat, R right.

Fig. 2.

a H&E-stained lung section from a representative dextran-treated mouse associated with an inflamed airway and adjacent parenchymal tissue (arrows). **b** Showing DPA-713-IRDye680LT. **c** Showing co-localized CD68+ macrophages (green) and DPA-713-IRDye680LT (red). Scale bar = 25 μ m. aw airway.

Fig. 3. Near-infrared fluorescence imaging of DPA-713-IRDye800CW of lungs from **a** control and **b** fluorescence tracer uptake (green) observed in the dextran-treated lung with foreign body reaction. Scale bar = 1 cm.