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BRIEF ARTICLE

Imaging Pulmonary Foreign Body Reaction Using [^{125}I]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 [^{125}I]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 [^{125}I]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 [^{125}I]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.

Key words: Foreign body, Iodo-DPA-713, Granulomatous inflammation

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 Biosafety, Radiation Safety, and Animal Care and Use Committees.

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Four-to-six-week-old female C57BL/6 (Charles River Laboratory) mice were injected intratracheally through a small neck incision with 80 µl 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-µl 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 µl volume of DPA-713-IRDye800CW was combined with a 100 µl 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 µm 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

[^{125}I]iodo-DPA-713 imaging of dextran-treated mice and un-inoculated, control mice is shown in Fig. 1. Pulmonary infiltrates consistent with lesions were clearly visible in the dextran-treated mice (Fig. 1a). [^{125}I]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 [^{125}I]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

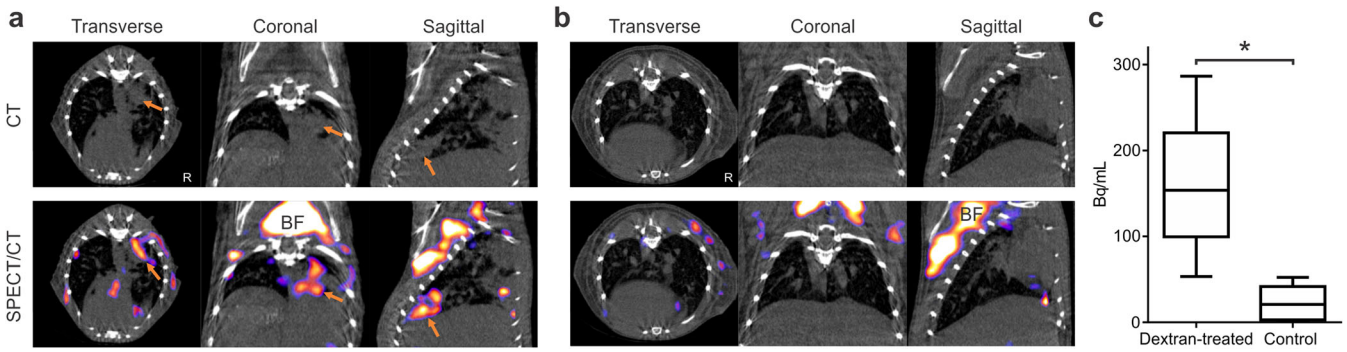

Fig. 1. [^{125}I]iodo-DPA-713 SPECT/CT in a model of foreign body lesions. Transverse, coronal, and sagittal sections from pulmonary imaging performed on dextran-treated mice with foreign body reaction. **a** Pulmonary infiltrates on CT demonstrating foreign body lesions are clearly visible in affected 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.

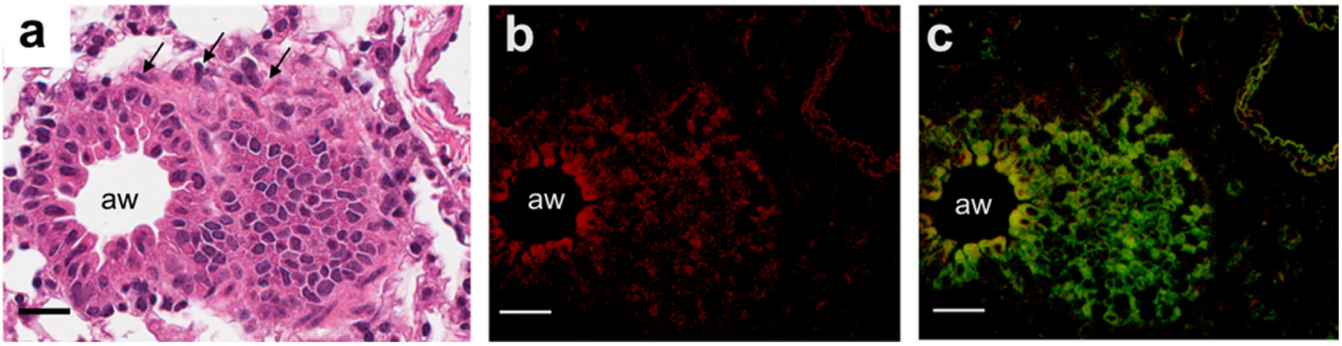

Fig. 2. **a** H&E-stained lung section from a representative dextran-treated mouse associated with an inflamed airway and adjacent parenchymal tissue (arrows). **b** Showing DPA-713-IRDye680LT. **c** Showing co-localized CD68+ macrophages (green) and DPA-713-IRDye680LT (red). Scale bar = 25 μm. aw airway.

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 [^{125}I]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]. [^{125}I]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.

[^{125}I]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.

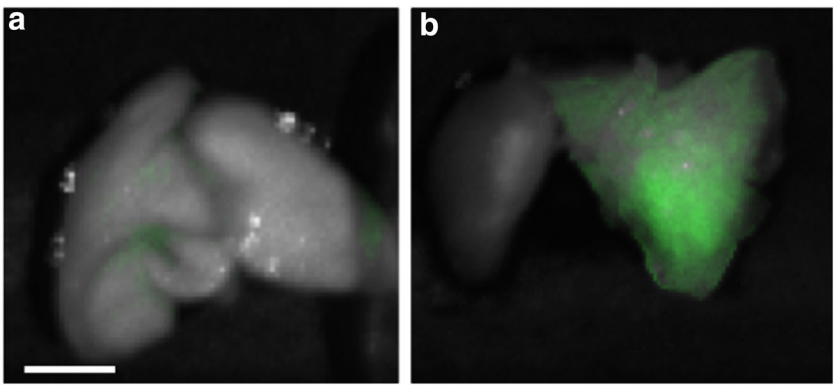

Fig. 3. Near-infrared fluorescence imaging of DPA-713-IRDye800CW of lungs from **a** control and **b** fluorescence tracer uptake (green) observed in the dextran-treated lung with foreign body reaction. Scale bar = 1 cm.

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 [^{124}I]iodo-DPA-713 using PET has recently been described in healthy human subjects [13], making [^{124}I]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.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

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