

ABSTRACT

Optical imaging is a fast, sensitive, and cost-effective way to image and track molecules in small animals. The LI-COR® Pearl® Impulse imager has been used with near-infrared probes to quickly acquire optimized images from various animal models. The aim of this study is to use the Pearl Impulse to detect intravenously injected near-infrared fluorescent probes of Parkinson's disease (PD), Alzheimer's disease (AD), and dermatitis in mouse models. For PD and AD probe, PSVue™ 794 from Molecular Targeting Technologies was used to visualize cellular damage in the brain. PSVue 794 is a near-infrared fluorescent probe for detection of apoptotic cells, bacteria, and other anionic membranes. PSVue 794 binds strongly to negatively charged bacterial cell walls, necrotic regions in various tumors, and phosphatidylserine (PS) residues exposed on the surface of apoptotic cells. For the dermatitis model probe, IRDye® 800CW 2-DG from LI-COR was used to visualize areas of increased glycolysis.

We demonstrated that PSVue 794 distributes almost immediately to the brain, with a 24-hour signal in PD mice approximately 9-fold higher than with a non-targeting optical probe. In 13 month-old AD mice, brain signal was approximately 40% higher than in age-matched control mice. Optical signal for IRDye 800CW 2-DG in the dermatitis model was 7-fold higher in a hapten-challenged ear, compared to a vehicle-challenged ear. These results suggest that PSVue 794 is highly brain penetrable and may be used as an imaging probe in various nervous system models, and possibly other disease models, and that IRDye 800CW 2-DG can be used to visualize inflammatory environments. Further studies should explore the use of optical imaging to assess therapeutic interventions in these various disease models.

MATERIALS AND METHODS

Probes

For brain imaging, PSVue™ 794 was provided by Molecular Targeting

Technologies, Inc. (MTTI, West Chester, PA). A control probe without a targeting moiety was also provided by MTTI. For imaging peripheral inflammation, IRDye® 800CW 2-DG Optical Probe was provided by LI-COR® Biosciences (Lincoln, NE), along with non-reactive control dye IRDye 800CW Carboxylate.

Parkinson's Model

MPTP is a potent and selective nigrostriatal dopaminergic neurotoxin that produces many of the neuropathological features of idiopathic Parkinson's disease (PD) in humans, nonhuman primates, and mice. In mice, MPTP produces nigrostriatal dopaminergic degeneration and locomotor impairment. Pharmacological agents that increase dopaminergic function or that block the neurotoxicity of MPTP also attenuate MPTP-associated locomotor dysfunction and have been useful in the clinic for treating Parkinson's disease. Moreover, MPTP-mediated toxicity may have a relationship to the mechanisms associated with dopaminergic loss in the disease, indicating that this model may also be potentially useful for identifying agents that slow or reduce nigrostriatal dopaminergic loss.

Animals. Male C57Bl/6 mice 8-10 weeks of age were used for these studies. Animals were kept on a standard 12 hr light cycle and given access to water and standard mouse chow *ad libitum*.

MPTP Administration. MPTP was formulated in phosphate buffered saline and administered to mice three times at a dose of 20 mg/kg (2 mg/mL delivered at 10 mL/Kg) at two hr intervals (final dose of MPTP = 60 mg/kg).

Alzheimer's Disease APP/PS-1 Gene-Targeted Mice

A number of animal models (mainly rodent) have been developed for Alzheimer's Disease (AD) typically based on over-expression of wild-type or mutant human transgenes for amyloid precursor protein (APP) or presenilin. More recently, however, a rodent model was developed in which gene targeting was used to introduce human familial AD mutations to the endogenous murine APP and presenilin genes. These

double gene-targeted mice (APPNLh/PS-1P264L) demonstrate many of the fundamental biochemical, histological, neurophysiological and behavioral abnormalities of AD.

The therapeutic significance of this model is two-fold: 1) This is the first genetic animal model of amyloid deposition produced in the absence of APP overexpression; and 2) This is the only mouse model of AD that expresses exclusively human A β , with no production of endogenous (murine) A β .

Contact Dermatitis

Contact hypersensitivity (CHS), a form of delayed-type hypersensitivity, is characterized by an immune response in a host when a pre-sensitized antigen comes in contact with the skin. This exposure typically results in swelling and redness of the sensitized area, as well as a T cell mediated immune response, including an increase in localized inflammatory cytokines. The animal model of CHS used in these studies involves pre-sensitization of animals to a chemical hapten (DNFB) followed by a subsequent ear challenge several days later. Drugs that reduce inflammation or modulate this immune response have shown efficacy in immune-mediated dermatitis diseases as well as acute allergic reactions.

Animals. Male ICR:CD-1 mice 8-10 weeks of age were used for this study. Mice were kept on a standard 12 hr light cycle, and given free access to water and standard mouse chow.

Procedure. Following a 7 day acclimation period, 6-wk old mice were anaesthetized and backs shaved and sensitized with 0.5% 2, 4-dinitrofluorobenzene (DNFB) in acetone/olive oil (4:1). For 2 consecutive days, DNFB solution was administered onto the shaved area and allowed to evaporate. Five days after sensitization, 0.2% DNFB was applied to the dorsal surface of right ear; left ear was painted with acetone.

Probe Preparation, Injection, and Visualization

PSVue™ reagents are a family of fluorescent probes containing a

bis(zinc²⁺-dipicolylamine) group (Zn-DPA), a motif that has been found to bind with high affinity to surfaces enriched with anionic phospholipids, especially phosphatidylserine (PS) exposed on cell membranes. The fluorescent part of the probe is a reporter element that provides a means of detecting the probe once it is bound to the membrane of interest. For *in vivo* imaging, PSVue was prepared by addition of an equal volume of 4.2 mM zinc nitrate solution provided with the probe. The solution was placed in a 40°C water bath for 15 minutes and injected via tail vein at 5 mg/kg. A control probe with no zinc binding domain was used as a negative control. Immediately prior and at various time points after injection, mice were anesthetized with isoflurane, depilated on top of the head to expose skin, and scanned with LI-COR Pearl® Impulse Imager software.

IRDye 800CW 2-DG optical probe was reconstituted in 1 ml sterile PBS for a final concentration of 0.1 nmol/ μ l and injected via tail vein at 10 nmol/mouse. Immediately prior and at various time points after injection, mice were anesthetized with isoflurane and scanned in the LI-COR Pearl Impulse imager. Image analysis was performed using LI-COR Pearl Imager software.

CONCLUSIONS

- PSVue 794 is a brain-penetrable fluorescent dye.
- PSVue 794 compared to control dye is specifically retained in the brains of Alzheimer's and Parkinson mice.
- IRDye 800CW 2-DG optical probe can be used to visualize areas of inflammation in a mouse contact dermatitis model.
- Fluorescence imaging can be a valuable tool for non-invasive evaluation of disease severity in multiple therapeutic areas, including CNS and inflammation.
- These results suggest that fluorescence imaging may be used to non-invasively assess pre-clinical therapeutic interventions in a number of disease models.

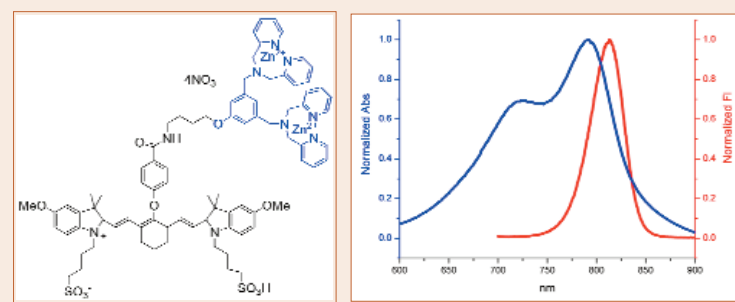


Figure 1. PSVue 794 structure and Absorption and Fluorescence Emission Spectra (5 μ M solution; abs. max = 794 nm; fl. em. max = 810 nm).

Figure 2 illustrates the 3-component assembly process that results in high affinity association of PSVue with phosphatidylserine (PS)-rich membranes. Under physiologic concentrations of Zn²⁺, the predominant coordination complex is the mono-zinc species (species A). The binding of species A to the anionic PS-exposed membrane (species B), promotes binding of the second Zn²⁺ with subsequent binding to the membrane, forming a bivalently-bound species C.

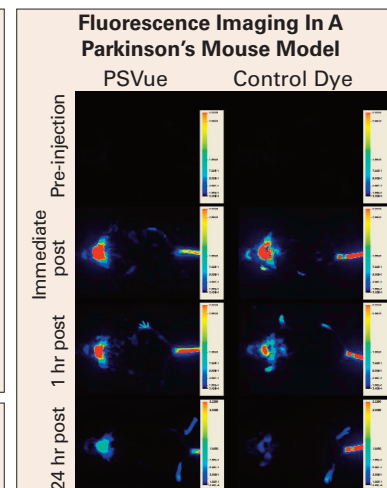
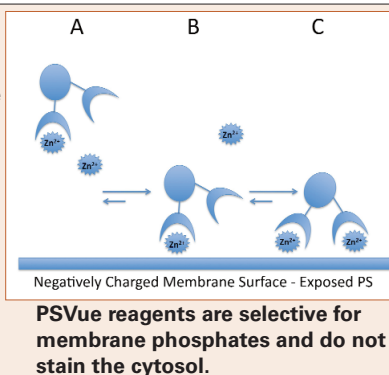


Figure 3. C57Bl/6 mice were treated with MPTP to selectively destroy dopaminergic neurons. Mice were then injected with PSVue or control dye, proprietary agents from MTTI and imaged on a LI-COR Pearl Imager. Immediately after injection, both dyes stained intensely in the brain. By 24 hours post-injection, the control dye has cleared the brain; however, the PSVue still shows intense brain staining. Note: PSVue is commercially available from MTTI under exclusive license from University of Notre Dame.

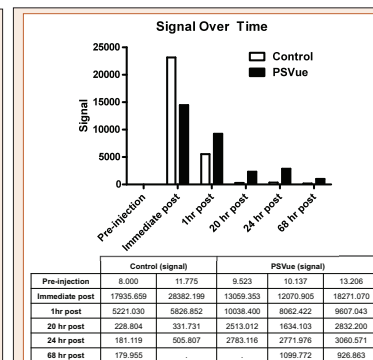


Figure 4. Fluorescence imaging in a Parkinson's mouse model.

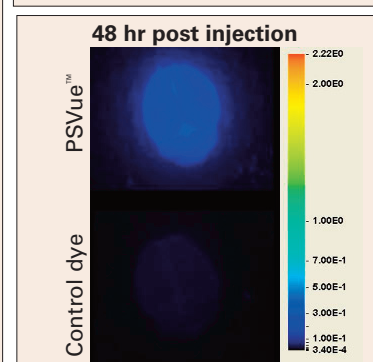


Figure 5. Whole brain imaging, Parkinson's model.

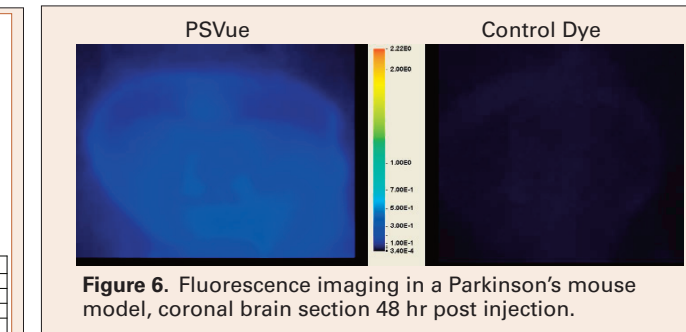


Figure 6. Fluorescence imaging in a Parkinson's mouse model, coronal brain section 48 hr post injection.

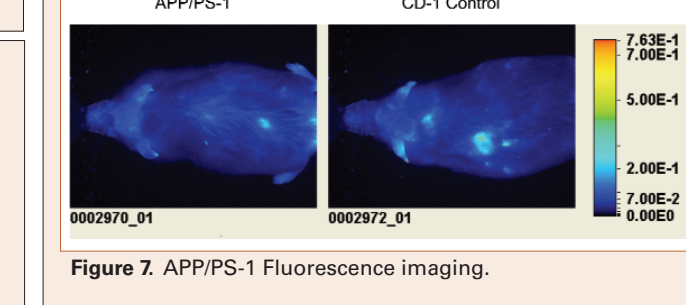


Figure 7. APP/PS-1 Fluorescence imaging.

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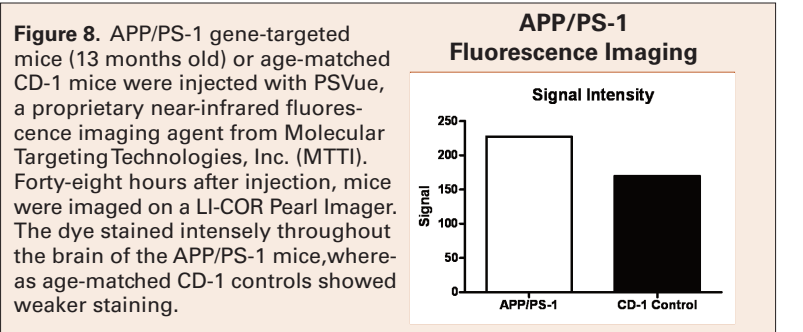


Figure 8. APP/PS-1 gene-targeted mice (13 months old) or age-matched CD-1 mice were injected with PSVue, a proprietary near-infrared fluorescence imaging agent from Molecular Targeting Technologies, Inc. (MTTI). Forty-eight hours after injection, mice were imaged on a LI-COR Pearl Imager. The dye stained intensely throughout the brain of the APP/PS-1 mice, whereas age-matched CD-1 controls showed weaker staining.

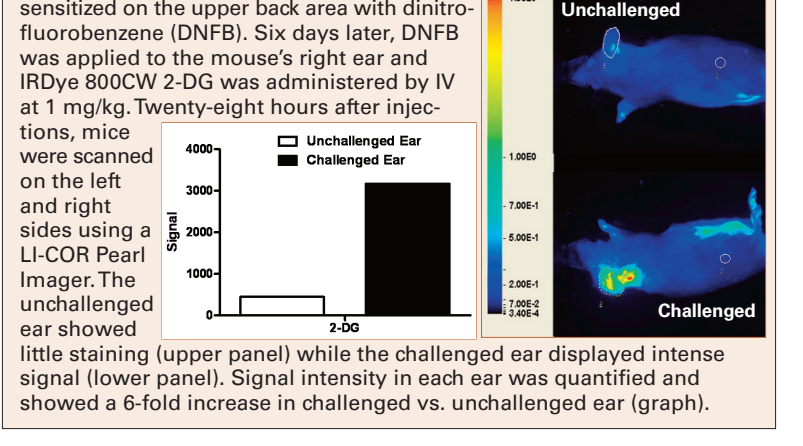


Figure 9. ICR:CD-1 mice were shaved and sensitized on the upper back area with dinitrofluorobenzene (DNFB). Six days later, DNFB was applied to the mouse's right ear and IRDye 800CW 2-DG was administered by IV at 1 mg/kg. Twenty-eight hours after injections, mice were scanned on the left and right sides using a LI-COR Pearl Imager. The unchallenged ear showed little staining (upper panel) while the challenged ear displayed intense signal (lower panel). Signal intensity in each ear was quantified and showed a 6-fold increase in challenged vs. unchallenged ear (graph).