

IRDye® 800CW 2-deoxyglucose, a near infrared metabolic optical imaging agent?

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ABSTRACT

Cancer cells are often characterized by a high metabolic rate exemplified by a dramatically elevated glucose uptake. This biological activity has been exploited for noninvasive imaging by positron emission tomography using glucose analogues such as ¹⁸F-2-deoxy-D-glucose to generate a tumor-localized signal. In this work, our goal was to adapt a similar methodology for optical imaging of tumors in mice. We selected a fluorophore with maximal excitation and emission wavelengths in the near infrared (NIR) spectral range (700-900 nm), where low absorption coefficients of tissues allow greater optical sensitivity, deeper tissue penetration, and low autofluorescence. The NIR fluorophore, IRDye® 800CW (excitation/emission of 778 nm/794 nm), was covalently coupled to 2-deoxyglucose (2DG). The resultant conjugate was evaluated first for specificity and sensitivity *in vitro*. Specificity of the agent was assessed using an In-Cell Western assay, in which concentration dependence of label uptake was established by fluorescence changes in a high throughput microplate format. Uptake of the labeled agent was specifically blocked in a dose-dependent manner by addition of unlabeled 2-deoxyglucose. Subsequent *in vivo* studies were conducted to optimize dosing, clearance, and optimal time post-injection for signal capture in nude mice. A research prototype imager, optimized to detect IRDye 800CW signal, was used to characterize the IRDye 800CW 2DG optical agent in subcutaneous tumors derived from either an epithelial carcinoma (A431 cells), colorectal carcinoma (SW620 cells), or prostate carcinomas (PC3M-LN4 and 22Rv1). In all cases, the tumors were clearly imaged with good signal-to-noise characteristics. This pilot demonstration suggests that IRDye 800CW 2DG will be a general optical imaging tool for studying tumor biology in mice.

INTRODUCTION

Small animal imaging has become a key tool for the investigation of tumor biology, and contrast agents are needed to delineate disease progression *in vivo*. Near infrared (NIR) fluorescent optical imaging agents provide high sensitivity and good tissue penetration due to the low absorption coefficients of tissue components in the NIR spectral region.

¹⁸F-2-deoxy-D-glucose (¹⁸FDG) has been routinely used for PET imaging as a tracer for identifying primary tumors and their metastases, in which rates of glycolysis are frequently elevated. Glucose taken up by tumor cells is rapidly phosphorylated and proceeds through glycolysis via an isomerization that requires a C2 hydroxyl. ¹⁸FDG lacks this moiety and is unable to progress further in glycolysis so it accumulates in the cell¹.

A number of groups have substituted fluorescent labels for ¹⁸F. O'Neil *et al.*² and Lloyd *et al.*⁴ demonstrated that N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino-labeled 2-deoxyglucose (2-NBDG) utilized GLUT family glucose transporters to enter breast cancer and smooth muscle cells, respectively. Cheng *et al.* tested Cy5.5-2DG with limited success, concluding that the compound was unlikely to enter through the GLUT transporter system due to the size of the fluorescent label relative to the glucose molecule⁴. However, uptake of fluorophore-labeled 2DG has been reported for vascular smooth muscle³, breast cancer², and glioma and melanoma tumors⁴. The entry mechanism is unknown, but specificity of the process suggests the labeled glucose analog binds GLUT proteins and is subsequently taken up during endocytic receptor recycling⁵.

We labeled 2DG with a near infrared fluorophore, IRDye 800CW, and tested its ability to act as a tumor targeting agent in mice. Specific staining of several different tumor cell lines was evaluated *in vitro* by dose dependence of fluorescent signal and competition with unlabeled 2DG. A prototype imager optimized for IRDye 800CW detection was then used to quantify the signal strength derived from this targeting agent in tumor-bearing nude mice. Although the exact mechanism of targeting is unknown, IRDye 800CW 2DG may be a useful agent for tumor imaging *in vivo*.

RESULTS

Labeling

- 2-amino-2-deoxy-D-glucose hydrochloride (2DG; Sigma-Aldrich, St. Louis) was labeled with IRDye 800CW NHS ester (LI-COR Biosciences) and purified by chromatography.

Figure 1. *In vitro* binding and competition assays

Confirmation of binding and specificity of NIR-conjugated 2DG was accomplished with an In-Cell Western (ICW) whole cell fluorescence microplate assay.

- Binding: Incubation of confluent A431 cell monolayers with increasing concentrations of IRDye 800CW 2DG (0-50 μ M) produced a dose-dependent increase in fluorescence.
- Competition: Inclusion of increasing concentrations of unlabeled 2DG (0-375 mM) with IRDye 800CW 2DG (5 μ M) reduced fluorescence, demonstrating specificity of the binding interaction.

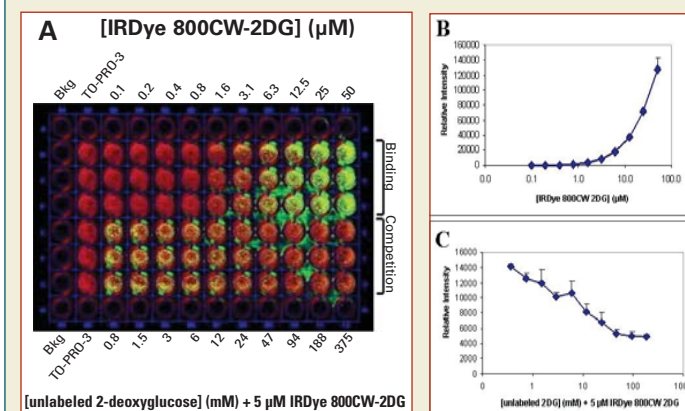


Figure 1. *In vitro* specificity of IRDye 800CW 2DG for A431 tumor cells. (A) Confluent monolayer cultures of A431 cells in a 96-well plate were incubated in triplicate with increasing concentrations of IRDye 800CW 2DG (0.1-50 μ M) in the top half of the microplate. The lower half shows the competition for binding between IRDye 800CW 2DG (5 μ M) and increasing concentrations of unlabeled 2DG (0.8-375 mM). Fluorescent signal from IRDye 800CW 2DG is displayed in green. TO-PRO-3 DNA staining (red) was used to normalize for variations in cell number. Graphical analyses for both binding (B) and competition (C) are presented.

Figure 2. *In vivo* dose determination

A preliminary study of the clearance characteristics of IRDye 800CW 2DG indicated that greater than 95% of the probe had cleared from nude mice by 24 hours post-IV injection, so this time point was used for subsequent imaging. Mice with subcutaneous tumors were then used to determine the optimal dose of the targeting agent for specific tumor detection.

- Administration of 10 – 20 nmol of the agent allowed the tumors to be clearly visualized at 24 hours post-injection.



Figure 2. Nude mice with subcutaneous tumors arising from a prostate tumor cell line were injected via the tail vein with increasing concentrations of IRDye 800CW 2DG; 5.0 nmol (A), 10 nmol (B), and 20 nmol (C). Images were taken 24 hours post-IV injection of the conjugate. Pseudocolor images depict the IRDye 800CW 2DG fluorescence intensity superimposed on a white light image. Higher intensity signal is shown in red, while lower signal intensity is shown in blue in this heat map display.

Figure 3: Broad-range tumor detection with IRDye 800CW 2DG

We tested the IRDye 800CW 2DG agent for its ability to target several different tumor types. Subcutaneous tumors were generated in nude mice by injection of prostate (22Rv1), epithelial (A431), and colon (SW620) carcinoma cell lines.

- All three tumor types were clearly visualized in this experiment.
- Signal intensity varied in different tumor types.



Figure 3. Nude mice bearing subcutaneous prostate (22Rv1), epithelial (A431), and colon (SW620) carcinomas were injected IV with 15 nmol IRDye 800CW 2DG. Images were acquired 24 hours post-injection and pseudocolored fluorescent signal was superimposed on a white light image. Higher intensity signal is in red, while lower signal intensity is in blue.

Figure 4: Organ section evaluation

Organs were recovered at 24 hours post-injection of either 1XPBS, unconjugated IRDye 800CW acid (10 nmol), or IRDye 800CW 2DG (10 nmol). Cryosectioned tissues were scanned on Odyssey® to evaluate deposition of the injected agent. The IRDye 800CW 2DG conjugate is being retained in the liver at a significantly higher level than is IRDye 800CW acid.



Figure 4. Liver, kidney, spleen/pancreas, lung, and brain tissue sections (5 micron) were prepared from nude mice at 24 hours post-injection of either 1XPBS, IRDye 800CW acid (10 nmol), or IRDye 800CW 2DG (10 nmol) and scanned on Odyssey. Scans were all normalized to the same intensity settings. Green represents probe signal and red represents tissue autofluorescence.

CONCLUSIONS

- Subcutaneous tumors were successfully imaged in nude mice 24 hours post-IV injection of 10-20 nmol IRDye 800CW 2DG.
- IRDye 800CW 2DG was effective for tumors derived from A431 (epidermoid), 22Rv1 (prostate), and SW620 (colon) cell lines. Uptake of the agent differed with tumor type.
- Cell-based assays were used to demonstrate dose-dependent binding of the agent to A431 cells. Binding could be competed by addition of excess unlabeled 2DG.
- In vivo* image analysis of subcutaneous prostate tumors in mice receiving either IRDye 800CW 2DG or IRDye 800CW acid (20 nmol) showed those mice receiving the labeled 2DG had a 4-fold increase in signal (data not shown)
- Differences in IRDye 800CW 2DG and IRDye 800CW acid retention were evident in the liver, the major organ responsible for glycolysis.
- The exact mechanism of IRDye 800CW 2DG uptake in these studies is not known, but may involve endocytotic glucose transporter recycling.
- Although the mechanism is yet to be determined, this study indicates that IRDye 800CW 2DG may be useful as a tumor targeting agent.

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