GLUT Family Transporter Involvement in Cellular Uptake of IRDye® 800CW 2-Deoxyglucose

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ABSTRACT

Malignant neoplasms exhibit a higher rate of glucose metabolism when compared to normal cells. IRDye® 800CW 2-DG was produced to exploit its use as a metabolic marker to paint tumors exhibiting elevated levels of glycolysis. Data presented here characterizes the probes actions *in vitro*. Confocal fluorescent microscopy confirmed the presence of the labeled agent in the cell cytoplasm. Although the exact mechanism for uptake is unknown, competition assays with increasing concentrations of unlabeled 2-deoxyglucose or glucose effectively blocked IRDye 800CW 2-DG uptake, suggesting GLUT family transporters are involved. In addition, treatment with phorbol-12-myristate-13-acetate, a compound known to translocate intracellular vesicles containing GLUT1 to the cell surface, increased IRDye 800CW 2-DG uptake by 50% in differentiated 3T3-L1 adipocytes. Tissue section analysis of a variety of organs confirmed the liver, responsible for glycolysis, exhibited the highest retention of signal compared to the other tissues evaluated. These data provide further evidence that IRDye 800CW 2-DG is gaining access to the inner cell environment and that GLUT family transporters are likely playing a key role in that uptake.

INTRODUCTION

Cancer cells characteristically exhibit an elevated rate of glycolysis to fuel the growth of tumors. Early studies using [^{14}C]deoxyglucose demonstrated cellular uptake by GLUT transporters (GLUTs) and phosphorylation for glycolytic processing (1). Because the structure of glucose analogs, such as deoxyglucose, prohibits isomerization by the next enzyme in the pathway, it accumulates in the cell. Positron emission tomography (PET) exploits these characteristics with the use of ^{18}FDG (2-fluoro-2-deoxyglucose) in clinical imaging to visualize tumor growth and location. Although PET is sensitive and quantitative, for pre-clinical studies, ^{18}FDG is impractical due to the specialized instrumentation required and short half-life of the isotope. Alternatively, near infrared (NIR) fluorophores emit in the 650-900 nm range of the spectrum where tissue absorption coefficients and non-specific fluorescence are relatively low. By effectively reducing the background fluorescence, sensitivity and depth of penetration are increased, improving image quality. IRDye 800CW has been shown to yield high signal-to-background ratios and to be effective in several *in vivo* tumor targeting studies (2-4). Our goal was to develop an alternative fluorescent analogue of 2-deoxyglucose and test its tumor detection efficacy *in vitro* and *in vivo*. Due to the relative size contribution of IRDye 800CW (~1100 Daltons) with respect to the targeting moiety, 2-deoxyglucose, it was important to conduct in-depth evaluation of the probes actions for any aberrant behavior. To that end, data presented here include binding, blocking, and stimulation assays which confirm that the overall contribution of the dye molecule on the probes actions is minimal, and confirms that GLUT family transporters are playing a role in the probes uptake. Furthermore, deconvolution microscopy pinpoints the labeled agent in the cytoplasm; however, the molecular mechanism by which IRDye 800CW 2-DG enters the cell is unknown.

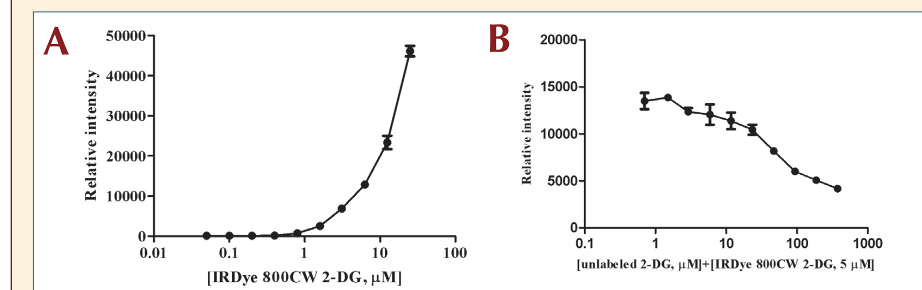


Figure 1. Immunofluorescent cell-based assays. **A)** A dose dependent increase in fluorescence was noted with A431 cells when incubated with increasing concentrations of IRDye 800CW 2-DG (0.1-50 μM). **B)** Incubation with increasing concentrations of unlabeled 2-DG (0.8-375 mM) prior to IRDye 800CW 2-DG (5μM) treatment showed a reduction in fluorescent signal.

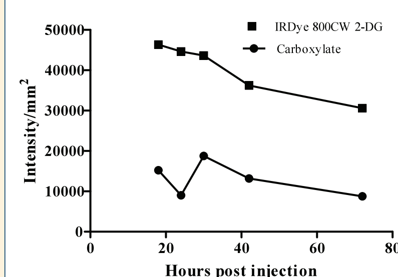
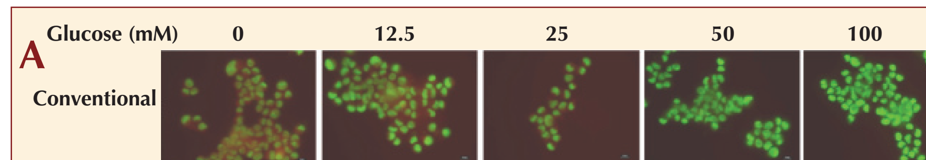


Figure 2. Specificity comparison of IRDye 800CW 2-DG and IRDye 800CW carboxylate (non-reactive form) in mice. The NIR fluorophore is a major component of IRDye 800CW 2-DG. Therefore it was important to measure the relative difference in clearance profiles for IRDye 800CW 2-DG and IRDye 800CW carboxylate. Mice bearing subcutaneous prostate carcinoma xenografts were given an intravenous injection of 20 nmol IRDye 800CW 2-DG or IRDye 800CW carboxylate. Images were collected with the Pearl™ Imager at intervals between 18-72 h post-injection. Signal intensities corrected for background revealed ~4-fold increase in tumor retention of IRDye 800CW 2-DG compared to the IRDye 800CW carboxylate dye-only control.



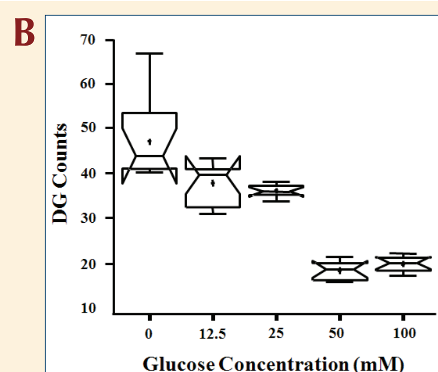
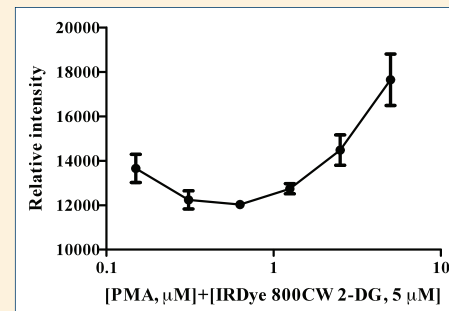


Figure 3. Fluorescent microscopy of glucose challenge. **A)** MDA-MB-231 breast cancer cells were incubated with increasing concentrations of glucose (0-100 mM) prior to the addition of IRDye 800CW 2-DG (50 μM). Microscopy images show IRDye 800CW 2-DG signal in red and a cell nuclear stain in green.

B) Statistical notched box-and-whisker plot showed a significant reduction in IRDye 800CW 2-DG uptake at 50 mM glucose addition ($P<0.05$). These data support the involvement of the GLUT family transporters for binding of IRDye 800CW 2-DG.

Figure 4. Stimulation of IRDye 800CW 2-DG binding. 3T3-L1 adipocytes received a pre-treatment of phorbol ester (0.08-5 μM), effectively translocating GLUT family glucose transporters to the cell surface from intracellular vesicles. Following the pre-treatment, IRDye 800CW 2-DG (5 μM) was added. The data analysis showed an increase in probe binding by 50%.



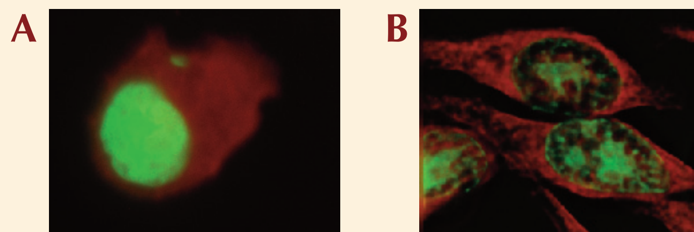


Figure 5. Analysis of cells by deconvolution microscopy (63X). A431 epidermoid **(A)** and SW620 **(B)** colon carcinoma cells were incubated with IRDye 800CW 2-DG (red) and a nuclear stain (SYTOX® Green; green) prior to collecting images. Deconvolved images of both tumor cell lines confirm internalization of the optical agent.

Figure 6. Multiple tumor models were characterized. Mice bearing ~0.5 cm subcutaneous tumors from a prostate, epidermoid, or colon carcinoma cell lines were injected with various concentrations of IRDye 800CW 2-DG. Signal intensities per area (mm²) varied depending on tumor type.

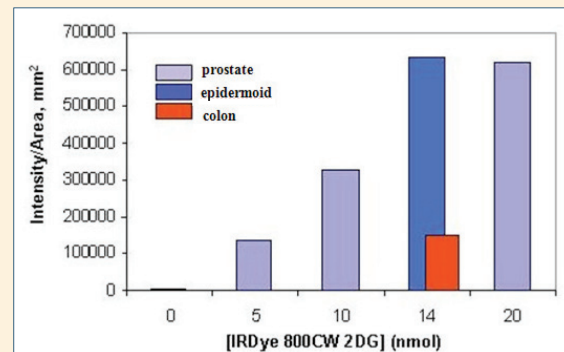
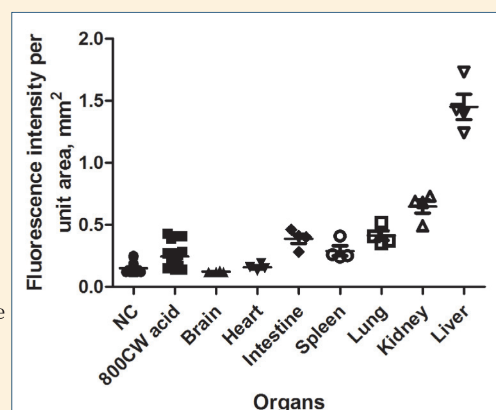


Figure 7. Fluorescent signal evaluation for organ sections. Organ sections from animals receiving PBS (NC), IRDye 800CW carboxylate (800CW acid), or IRDye 800CW 2-DG were evaluated for 800 nm signal. All specimans were cryopreserved, sectioned, and scanned on the Odyssey® Imaging System (LI-COR Biosciences). Area-weighted fluorescence to autofluorescence was measured. Negative control and 800CW acid organs show very little signal, while IRDye 800CW 2-DG kidney and liver sections showed the highest retention of the probe. The kidney signal reflects the route of clearance for the unbound probe, while the liver, having an increased level of GLUT family transporters, exhibited a 4-fold increase in signal retention over the other organs evaluated.



CONCLUSIONS

We have characterized a general tumor targeting agent for noninvasive NIR optical imaging in mice. Although the actual molecular mechanism for the uptake of IRDye 800CW 2-DG is unknown, we demonstrated that IRDye 800CW 2-DG uptake is mediated by GLUT family proteins and therefore can be considered a metabolic optical imaging agent with broad applicability in pre-clinical testing for drug development, and in the evaluation of mouse cancer models.

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