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Synthesis of a new deoxyglucose derivative modified near-infrared fluorescent probe for tumor diagnosis

Chunlong Sun [a, b, 1, *], Wen Du [a, b, 1, **], Baoqin Wang [b], Zhigang Yao [b], Junhua Liu [a], Jun Wang [b], Wenjun Xie [b], Tao Wu [c], Yanhui Fan [b], Hongjun Yang [a]

[a] Shandong Provincial Key Laboratory of Eco-environmental Science for Yellow River Delta, Binzhou University, Binzhou 256600, China
[b] School of Bioengineering, Binzhou University, Binzhou 256600, China
[c] Shandong Provincial Engineering and Technology Research Center for Wild Plant Resources Development and Application of Yellow River Delta, School of Bioengineering, Binzhou University, Binzhou 256600, China

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ABSTRACT

Malignant neoplasms exhibit an elevated rate of glycolysis and a high demand for glucose over normal cells. This characteristic can be exploited for in vivo imaging and tumor targeting examined. In this manuscript, we describe the synthesis of near-infrared (NIR) fluorochrome IR-822-labeled 2-amino-2-deoxy-D-glucose (DG) for optical imaging of tumors in mice. NIR fluorescent dye IR-820 was subsequently conjugated with 3-Mercaptopropionic acid and 2-amino-2-deoxy-D-glucose to form IR-822-DG. The cell experiments and acute toxicity studies demonstrated the low toxicity of IR-822-DG to normal cells/tissues. The dynamic behavior and targeting ability of IR-822-DG in normal mice was investigated with a NIR fluorescence imaging system. The in vitro and in vivo tumor targeting capabilities of IR-822-DG were evaluated in tumor cells and tumor bearing mice, respectively. Results demonstrated that IR-822-DG actively and efficiently accumulated at the site of the tumor. The probe also exhibited good photostability and excellent cell membrane permeability. The study indicates the broad applicability of IR-822-DG for tumors diagnosis, especially in the glucose-related pathologies.

1. Introduction

Glucose is an indispensable energy source in the metabolic process and plays an important role in human physiology [1]. Malignant tumors exhibit an elevated rate of glycolysis and a high demand for glucose over normal cells, even under aerobic conditions [2–5]. The studies confirmed that glucose analogs, for example 2-amino-2-deoxy-D-glucose (DG), can be recognized and transported into the cells by GLUT1 on the cell membrane to form 2-deoxyglucose-6-phosphate by hexokinase phosphorylation [6]. Several groups also have demonstrated that DG and its derivatives, such as pyropheophorbide 2-deoxyglucosamide, polyvalent carbocyanine molecular beacons, and 2-NBDG, could be selectively delivered to and accumulated in tumors [7–11]. Because DG lacks a hydroxyl group, and isomerization by the next enzyme in the metabolic pathway is precluded, thereby inhibiting further metabolism of the 2-deoxyglucose-6-phosphate and leading to intracellular retention of the phosphorylated molecule [12,13]. Therefore, the uptake of 2-amino-2-deoxy-D-glucose by cells is used as a biomarker for tumor diagnosis.

Fluorescent optical imaging is a rapidly expanding methodology for noninvasive evaluation of disease and tumor progression, with application for drug and biomarker development [14]. The wavelength of NIR light for fluorescence imaging ranges from 650 to 900 nm, avoiding the interference of high absorption of intrinsic chromospheres. Moreover, NIR light penetrates deeper into tissue than visible light without any ionizing and radioactive damage [15].

Abbreviations: DG, 2-amino-2-deoxy-D-glucose; GLUT1, glucose transporter1; NIR, near-infrared; ICG, Indocyanine green; FDA, Food and Drug Administration; IR-822-DG, IR-822-labeled 2-amino-2-deoxy-D-glucose; 2-NBDG, 2-[*N*-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose; NHS, N-hydroxysuccinimide; DNEM, dulbecco's modified eagle medium.

* Corresponding author. Shandong Provincial Key Laboratory of Eco-environmental Science for Yellow River Delta and School of Bioengineering, Binzhou University, Binzhou 256600, China.
** Corresponding author. Shandong Provincial Key Laboratory of Eco-environmental Science for Yellow River Delta and School of Bioengineering, Binzhou University, Binzhou 256600, China.
E-mail addresses: sunchunlong2016@163.com (C. Sun), duwen6688@163.com (W. Du).
[1] These authors contribute equally.

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For these reasons, NIR fluorescent probes have found rapidly expanding applications in disease diagnosis and biomarker development [16,17]. Indocyanine green (ICG) is a widely used organic dye in optical imaging. This dye is approved for human use by the US FDA [18]. Indocyanine green is the only FDA approved agent that can be used for testing of hepatic function and fluorescence angiography in ophthalmology, has already been investigated as a potential contrast agent for the detection of tumors in both animal models and in humans since 1970 [19–24]. For this reason, many research groups have focused on the synthesis of ICG derivatives for in vivo imaging. Based on the structure of indol heptamethine, introducing reactive groups to the parent compounds or changing their structures can make fluorescent probes have different functions like labeling protein and tumor, which has become the hotspot in the field of fluorescence imaging of biological research [25].

We have designed and synthesized various ICG derivatives as NIR imaging probes for non-invasive in vivo imaging research. IR-820, a hydrophobic derivative of ICG synthesized in our lab, was used in our probe conjugation [14,15]. In this paper, we used IR-820 to label the linker (3-Mercaptopropionic acid) and 2-amino-2-deoxy-D-glucose for NIR tumor imaging. We studied the dynamic behavior of the material in nude mice and demonstrated the tumor targeting capability of IR-822-DG probe in mice bearing tumor cell lines. Herein, we describe IR-822-DG, a NIR fluorescent dye with high extinction coefficients, can provide good photostability with high extinction coefficients and show promising application in tumor targeting diagnosis.

2. Materials and methods

2.1. Apparatus and materials

The Mass Spectrometer (AGILENT, USA) was used to identify the products. ^{1}H-NMR data were recorded using an ACF-300 MHz spectrometer (BRUKER) at ambient temperature in DMSO-d_6, and referenced to tetramethylsilane as the internal standard. Fluorescence spectra were recorded on the spectrofluorometer (HITACHI, Japan). A UV-Vis Spectrophotometer (SHIMADZU, Japan) was used to perform the absorbance measurements. A Nikon confocal laser-scanning microscope (NIKON, Japan) with an objective lens was used to perform the florescence measurements. A NIR spectral system was used to in vitro acquire real-time fluorescence imaging of the probe's distribution in animal subjects. Briefly, it is mainly composed of an excitation laser (λ = 800 nm) and S2000 eight-channel optical spectrographotometer (Ocean Optics). All chemical reagents used in the study were certified analytical reagent grade (Shanghai Chemical Reagent Company, Shanghai), unless otherwise indicated.

Athymic nude mice were purchased from Charles River Laboratories (Shanghai, China). The animal experiments were carried out according to the protocol approved by the Ministry of Health in People's Republic of China (document no. 55, 2001) and the guidelines for the care and use of Laboratory Animals of China Pharmaceutical University.

2.2. Synthesis and characterization of IR-822-DG

Scheme 1 shows the synthetic route of designed heptamethine cyanine dye IR-822-DG. Compound 820 (1.80 mmol) and 3-Mercaptopropionic acid (1.80 mmol) were dissolved in DMF (100 ml). The solution was stirred under argon at 24 °C for 14 h. The resulting precipitate was isolated by vacuum filtration to give a crude solid that was recrystallized to yield compound IR-822 as a green solid (Yield, 47.3%). ^{1}H-NMR (300 MHz, DMSO-d_6), δ (ppm): 8.84 (d, 2H, J = 14.0 Hz), 8.31 (d, 2H, J = 8.4 Hz), 8.09–8.01 (m, 4H), 7.80 (d, 2H, J = 8.9 Hz), 7.65 (t, 2H, J = 7.5 Hz), 7.51 (t, 2H, J = 7.5 Hz), 6.41 (d, 2H, J = 13.7 Hz), 4.33 (br s, 4H), 3.07 (t, 2H, J = 6.6 Hz), 2.73 (br s, 4H), 2.65–2.55 (m, 6H), 1.98 (s, 12H), 1.89–1.77 (m, 10H). TOF-MS m/z: 895.4 [M-Na$^+$]$^-$. (The ^{1}H-NMR, MS spectra of compound IR-822; see Fig. S1).

IR-822 (0.9 mmol), N, N'-dicyclohexylcarbodiimide (0.45 mmol) and N-hydroxysuccinimide (NHS) (0.9 mmol) were dissolved in DMSO (30 ml). The solution was stirred under argon at 24 °C for 15 h, and then 2DG (0.9 mmol) was added and stirred for 15 h. DMSO was taken off by a rotary evaporator to give a crude solid. The solid was subjected to column chromatography on silica gel by using CH_2Cl_2/CH_3OH (4.5:1 v/v) as an eluent, affording sensor IR-822-DG (Yield, 12.3%) as a green solid. ^{1}H-NMR (300 MHz, DMSO-d_6), δ (ppm) 8.80 (d, 2H), 8.27 (d, 2H), 8.07 (m, 4H), 7.79 (d, 2H), 7.66 (t, 2H), 7.49 (t, 2H), 6.40 (d, 2H), 4.32 (br s, 4H), 3.47 (m, 4H), 3.20–3.00 (m, 2H), 2.72–2.60 (m, 6H), 2.55 (br s, 4H), 2.54 (br s, 4H), 2.08 (s, 2H), 1.97–1.86 (m, 12H), 1.81–1.71 (m, 12H). MS m/z: 1056.5 [M-Na$^+$]$^-$. (The ^{1}H-NMR, MS spectra of IR-822-DG; see Fig. S2).

2.3. Absorption analysis

Absorption spectra were obtained with the UV-Visible spectrophotometer at 25 °C. The probe IR-822-DG (1 ml, 0.01 mM) was added to the 1 ml color comparison tubes and studied through absorption spectroscopy in PBS, the mixture was equilibrated for 5 min before measurement. All performs were made in the presence of 0.10 M NaCl to maintain a constant ionic strength.

2.4. Fluorescence analysis

Fluorescence spectra were determined on the spectrofluorometer. The probe IR-822-DG (0.10 ml, 0.10 mM) was added to the 1 ml color comparison tubes and studied through fluorescence spectroscopy in PBS, the mixture was equilibrated for 5 min before measurement. The fluorescence intensity was measured at λ_{ex} = 847 nm. All performs were made in the presence of 0.10 M NaCl to maintain a constant ionic strength.

2.5. MTT assay

MTT assay was conducted to assess the cytotoxicity of IR-822-DG. L929 (mouse fibroblast cell line), MCF-7 (human breast adenocarcinoma cell line) and Colo 205 (human colon adenocarcinoma cell) (5000 cells per well) were seeded in 96-well plates for 24 h before treatment. After culture, medium in each wells were replaced with fresh medium containing different concentrations (0.65 μM–10.4 μM) of IR-822-DG. After the incubation period, the cells were washed three times with PBS before adding 20 μl MTT working solution (5 mg/ml in phosphate buffer solution), followed by incubation for 4 h at 37 °C with 5% (v/v) CO_2. After 4 h, the remaining MTT solution was removed, and 200 μl of DMSO was added to each well to dissolve the formazan crystals, followed by incubation at 37 °C with 5% CO_2 for 20 min. The optical density (OD) was measured at 640 nm with a multiwell plate reader. The cell viability was calculated by the following equation:

$$\text{Viable cells } (\%) = (\text{OD}_{\text{treated}}/\text{OD}_{\text{control}}) \times 100\%,$$

Where $\text{OD}_{\text{treated}}$ was obtained in the presence of IR-822-DG probe; $\text{OD}_{\text{control}}$ was obtained from the incubation medium.

2.6. Targeting ability of IR-822-DG in vitro tumor cells

MCF-7 (human breast adenocarcinoma cell line, CoBier