Journal of Industrial and Engineering Chemistry xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Short communication

Near-infrared fluorescent sorbitol probe for tumor diagnosis *in vivo*

Sungsu Lee[a,1], Wonbong Lim[b,1], Danbi Jo[c], Jin Seok Jung[c], Subin Kim[c], Gayoung Jo[c], Jung-Joon Min[d], Eun Young Choi[e,**], Hoon Hyun[c,*]

[a] Department of Otolaryngology—Head and Neck Surgery, Chonnam National University Medical School, Gwangju 61469, South Korea
[b] Department of Premedical Program, School of Medicine, Chosun University, Gwangju 61452, South Korea
[c] Department of Biomedical Sciences, Chonnam National University Medical School, Gwangju 61469, South Korea
[d] Department of Nuclear Medicine, Chonnam National University Medical School, Gwangju 61469, South Korea
[e] Department of Biomedical Sciences, University of Ulsan College of Medicine, Seoul 05505, South Korea

ARTICLE INFO

Article history:
Received 2 January 2018
Received in revised form 18 January 2018
Accepted 2 February 2018
Available online xxx

Keywords:
Sorbitol
ZW800-1
Tumor targeting
Near-infrared fluorescence
Optical imaging

ABSTRACT

This study evaluated the near-infrared (NIR) fluorescent probe Sorbitol-ZW800-1 as tracer for targeted tumor imaging of multiple cancer types. Preliminarily, Sorbitol-ZW800-1 conjugate was validated *in vivo* on breast (MCF-7) cancer cell lines. Subsequently, the tracer was tested in xenograft mouse models with malignant breast (MDA-MB-231), lung (NCI-H460), and colorectal (HT-29) tumors, respectively. Importantly, the Sorbitol-ZW800-1 conjugate showed highly selective tumor uptake in various tumor mice models. Moreover, biodistribution for 24 h exhibited the significant fluorescence signals in tumors and kidneys. Therefore, sorbitol is a promising candidate as a potential cancer targeting agent for the accurate detection of cancer cells.

Introduction

Currently, imaging is one of the most fundamental steps in tumor diagnosis. It provides not only anatomical information but also pathophysiological insights into the disease. Non-invasive imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) are widely used in the clinic. However, they have both merits and limitations in terms of spatial resolution, penetration depth, sensitivity, specificity, cost and other aspects [1]. One common challenge associated with increasing the accuracy and efficiency of tumor detection is distinguishing the tumor from the surrounding normal tissues, i.e., the signal-to-background ratio (SBR).

Near-infrared (NIR) fluorescence imaging enables higher SBR. NIR fluorescence uses 650–900 nm wavelength, a range associated with extremely low non-specific tissue absorption [2–8]. Thus, the background signal is reduced resulting in excellent SBR compared with conventional fluorophores that operate at a wavelength of 300–500 nm. Therefore, NIR fluorescence imaging currently represents an emerging technique for *in vivo* imaging. ZW800-1 is a newly developed NIR fluorophore that shows zero net charge [9–12]. Compared with commonly used NIR fluorophores such as Indocyanine green (ICG) and Cy5.5 that have net negative charges, ZW800-1 showed even lower chances of non-specific tissue binding.

Sorbitol known as glucitol is a sugar alcohol that is naturally found in apples, pears, peaches and prunes [13]. It can also be obtained via glucose reduction by changing the aldehyde group in glucose to a hydroxyl group. Sorbitol is an isomer of mannitol, in which the hydroxyl group on carbon 2 is oriented differently. Sorbitol is used as a sweetener in syrups and chewing gum. It has been used as a laxative medicinally. Cancer cells show high utilization and consumption of glucose known as 'Warburg effect' [14], which has been used widely for cancer targeting. Since sorbitol is a derivative of glucose and possesses similar conformation, we hypothesized that it might also be used for tumor targeting. In this study, we prepared a Sorbitol-ZW800-1 conjugate and confirmed the tumor targetability in multiple tumor types to demonstrate the feasibility of sorbitol probe as a tumor tracer.

* Corresponding author at: 160 Baekseo-ro, Dong-gu, Gwangju 61469, South Korea.
** Corresponding author at: 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, South Korea.
E-mail addresses: choieun@ulsan.ac.kr (E.Y. Choi), hhyun@jnu.ac.kr (H. Hyun).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jiec.2018.02.004
1226-086X/

Results

In vivo *NIR fluorescence imaging of Sorbitol-ZW800-1 for tumor targetability*

Amino-sorbitol was conjugated with ZW800-1 NHS ester through amide bond formation by a condensation reaction, then Sorbitol-ZW800-1 conjugate could be trackable tumors *in vivo* under real-time NIR fluorescence imaging system (Fig. 1). We first injected the Sorbitol-ZW800-1 conjugate into MCF-7, a human breast invasive ductal carcinoma cell line, which is the most popularly used breast cancer model. At 4 h post-injection, the MCF-7 tumor showed a bright uptake of the Sorbitol-ZW800-1, and the tumor was clearly visualized by the NIR fluorescence imaging system (Fig. 2). ZW800-1 was reported to be almost completely washed out of the body as early as 4 h due to its zero net charge [9–11]. We found that the NIR fluorescence signal was very low with ZW800-1 alone. Thus, this high tumor uptake of Sorbitol-ZW800-1 conjugate can be attributed to sorbitol *per se*. Signals in the kidneys, where the molecule is excreted, were still detected at 4 h post-injection, suggesting that Sorbitol-ZW800-1 has increased the washout time compared with ZW800-1 alone. At 24 h post-injection, the tumor showed a robust signal of Sorbitol-ZW800-1 while the tracer conjugate was slowly diminished from the kidneys. We dissected the tumor and organs 24 h post-injection and Sorbitol-ZW800-1 conjugate was distributed on the surface of the MCF-7 tumor (Fig. 3). It was detected in the kidneys and slightly in the liver, but was undetectable elsewhere.

In vivo *validation of Sorbitol-ZW800-1 specificity in multiple tumor types*

High uptake of Sorbitol-ZW800-1 was seen in another most commonly used breast cancer cell line MDA-MB-231 (Fig. 4). Sorbitol-ZW800-1 was suitable for *in vivo* breast cancer detection with high SBR. We investigated whether the Sorbitol-ZW800-1 complex can be applied to other cancer cells as well. NCI-H460 is a well established non-small cell lung cancer cell line. HT-29 is also a popular colorectal cancer model. When Sorbitol-ZW800-1 was administered to the cancer xenograft models, tumors were brightly detected in a similar pattern at 24 h post-injection (Fig. 4). No other organs showed appreciable signals except for kidneys where the conjugate is excreted.

Discussion

For effective tumor detection, the tracer should contain a fluorochrome with a high SBR or increased tumor specificity. In terms of fluorochrome SBR characteristics, NIR fluorescence imaging is considered the most effective until now, compared to other fluorochromes. It uses a wavelength of 650–900 nm that is not emitted by normal tissues. A few trials used even the infrared region of 1000–2000 nm (short-wavelength infrared region). However, a versatile technology using this emitting system has yet to be established [15].

Targeted delivery to the cancer tissues is currently accomplished using three main strategies. First, antibodies against

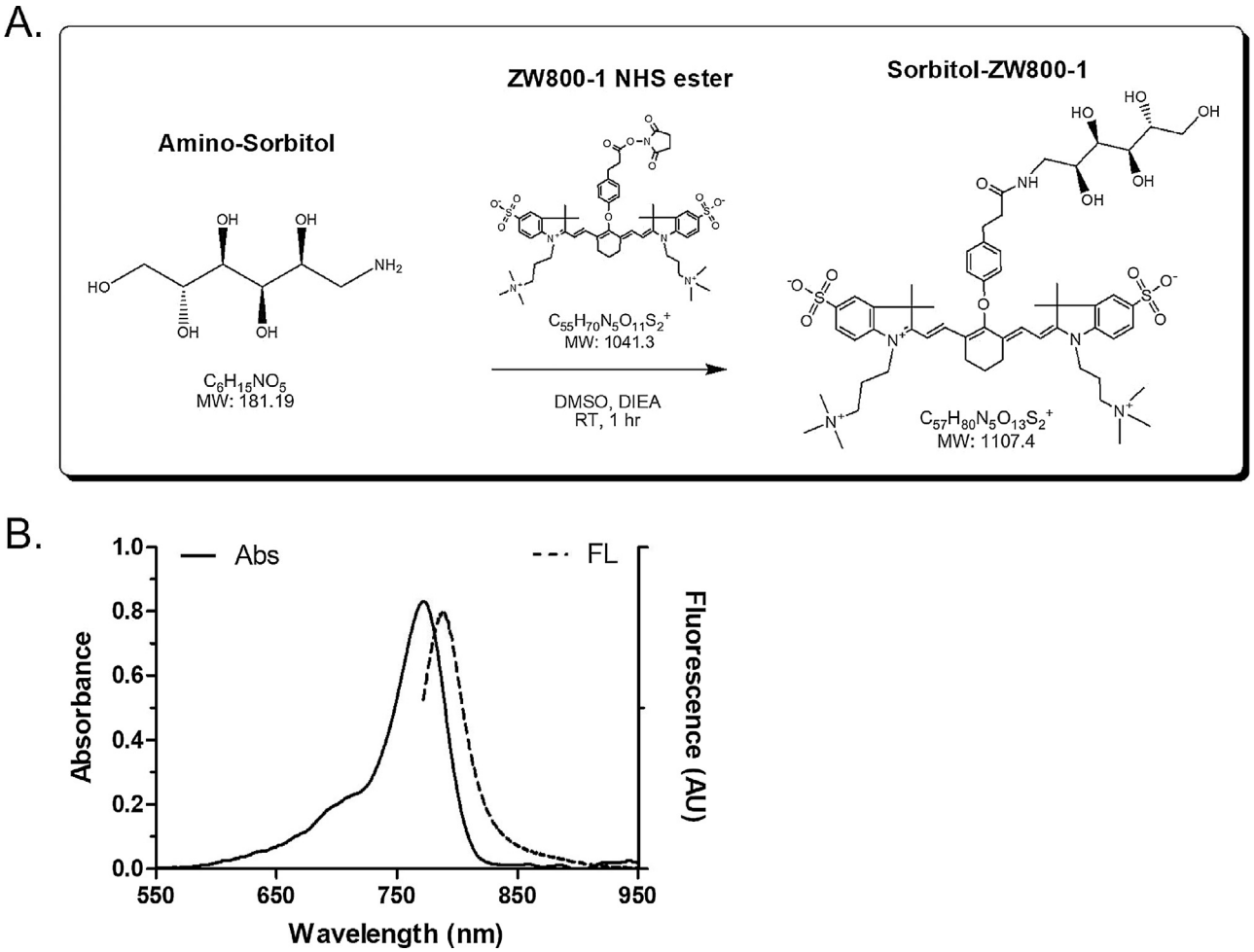

Fig. 1. Synthetic scheme (A) and optical spectra (B) of Sorbitol-ZW800-1 conjugate. Absorption and emission spectra were measured in 100% serum, pH 7.4.