N'-Fluorescein-*N*''-[4-*O*-(β-D-glucopyranuronic acid)-3-difluoromethylphenyl]-*S*-methylthiourea (FITC-TrapG) and *N*'-(p-aminophenyl)thioether of IR-820-*N*''-[4-*O*-(β-D-glucopyranuronic acid)-3-difluoromethylphenyl]-*S*-methylthiourea (NIR-TrapG)

FITC-TrapG and NIR-TrapG

Arvind Chopra, PhD[1]

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Chemical name:	*N*'-Fluorescein-*N*''-[4-*O*-(β-D-glucopyranuronic acid)-3-difluoromethylphenyl]-*S*-methylthiourea (FITC-TrapG) and *N*'-(p-aminophenyl)thioether of IR-820-*N*''-[4-*O*-(β-D-glucopyranuronic acid)-3-difluoromethylphenyl]-*S*-methylthiourea (NIR-TrapG)	
Abbreviated name:	FITC-TrapG and NIR-TrapG	
Synonym:		
Agent Category:	Compounds	
Target:	β-Glucuronidase (βG; β-GUS)	
Target Category:	Enzyme	
Method of detection:	Optical imaging (near-infrared imaging for NIR-TrapG and fluorescence imaging for FITC-TrapG)	
Source of signal / contrast:	IR-820 (NIR) and fluorescein isothiocynate (FITC)	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	Structures not available in PubChem.

Background

[PubMed]

[1] National Center for Biotechnology Information, NLM, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

The β-glucuronidase (βG; EC 3.2.1.31) is a lysosomal enzyme that catalyzes the hydrolysis of β-glucuronic acid residues from the cell-surface glycosaminoglycans for normal reconstruction of the extracellular matrix (ECM) (1), and the enzyme is believed to participate in the processes of angiogenesis, cancer metastasis, and inflammation (2). The βG is known to activate prodrugs (PDs) for the treatment of cancer. βG also has been used to track the path of gene delivery vehicles, and there is evidence that it can be used as a biomarker to detect cancerous tumors (3). Normal tissues have low levels of βG in the ECM, but tissues under pathological stress, such as bacterial infection, fibrosis, or malignancy, show elevated levels of the enzyme (4). As a result of cell lysis, intracellular βG is released from the necrotic parts of neoplastic tumors, and its activity in these lesions has been utilized for the *in situ* activation of anti-cancer PDs to treat cancers (1). Because chemotherapeutic anti-cancer drugs are nonselectively toxic to healthy cells, they are generally of limited efficacy to the patient due to their undesirable side effects on the normal biological systems. The conversion of a toxic drug into a non-toxic PD that can be activated only under specific conditions (e.g., by enzyme catalysis or chemical hydrolysis) would facilitate drug activation only in tissues that provide the specialized microenvironment and improve its concentration and efficacy at the desired location in the body (5, 6). For example, glucuronide PDs (drugs that are linked to a glucuronic acid moiety with or without a linker) have been shown to have superior anti-tumor activity compared with the parent drugs because the activated drugs are released from the PDs by the βG activity in a site-specific manner (7, 8).

βG activity varies among individuals, and its expression or accumulation in tumor tissues may change depending on the type of neoplasm or location in the body (1, 9). Two [^{18}F]fluoride-labeled probes, 1-*O*-(4-(2-[^{18}F]fluoroethyl-carbamoyloxymethyl)-2-nitrophenyl)-*O*-β-D-glucopyronuronate ([^{18}F]-FEAnGA) (1, 4, 10) and its methyl ester (([^{18}F]-FEAnGA-Me) (11), were synthesized as PDs and used with positron emission tomography (PET) to detect and visualize βG activity in tumors and inflamed tissues in rodents. Although [^{18}F]-FEAnGA or [^{18}F]-FEAnGA-Me could detect the βG activity and distinguish cancerous tumors or inflamed tissues from the surrounding normal tissues, only modestly higher levels of radioactivity were observed to accumulate with either tracer in the tumorous or inflamed areas compared with the surrounding tissues. The investigators concluded that low accumulation of label in the lesions was probably due to rapid clearance of the radiochemicals from the body through the kidneys (1, 11). A nonfluorescent substrate, fluorescein di-β-D-glucuronide (FDGlcU), was developed to determine the expression of βG with optical imaging in the various tissues of mice (12). However, the signals generated with FDGlcU could be detected only in the superficial

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tissues of small animals (such as rodents), suggesting that the low depth of light penetration in tissues would limit its application for the visualization of deep-seated tumors in animals or humans (3). Therefore, two βG activity-based optical probes were developed and evaluated to detect the enzyme in deep tissues (e. g. liver) of mice (3). To generate the probes, difluoromethylphenol-glucuronide (TrapG) was linked to either a near-infrared (NIR) dye (IR-820, $\lambda_{ex}/\lambda_{em}$ = 490 nm/525 nm; NIR-TrapG) or a fluorescein isothiocyanate (FITC, $\lambda_{ex}/\lambda_{em}$ = 710 nm/835 nm; FITC-TrapG) moiety to visualize the enzyme activity in the NIR or the visible ranges of light, respectively. The mechanism of action of these probes has been explained and illustrated by Cheng et al. (3). Briefly, with NIR-Trap as the example, the βG hydrolyzes the glucuronyl bond in the NIR-TrapG molecule and produces a quinine methide intermediate that contains the NIR dye moiety. The intermediate complex is highly reactive toward nucleophilic side chains of amino acids (such as in lysine) in proteins that are close to the βG-expressing sites and results in crosslinking of the fluorochrome to these molecules. The signal generated from the fluorochrome can then be detected with an appropriate optical imaging system. The biodistribution of NIR-TrapG and FITC-TrapG was investigated in mice bearing CT26 cell tumors (control) or CT26/mβG cell tumors (these are derivatives of the CT26 cells and express βG on the cell membrane surface) (3). The probes were also evaluated for the detection of subcutaneous and deep tissue (hepatic) tumors in these animals.

Related Resource Links

Other prodrug chapters in MICAD

Homo sapiens β-GUS protein and mRNA sequences

Prodrug-related clinical trials

Synthesis

[PubMed]

The synthesis of NIR-TrapG and FITC-TrapG has been described by Cheng et al. (3). Briefly, FITC and the IR-820 dye were obtained from commercial sources and linked to a glucuronide residue through difluoromethylphenol (the trapping moiety) to obtain the NIR-TrapG and FITC-TrapG probes, respectively. The mass and structure of the final products were confirmed with liquid chromatography-mass spectrometry and nuclear magnetic resonance, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using an ELISA assay, increasing concentrations of FITC-TrapG were shown to be activated by purified βG enzyme or by intact CT26/mβG cells (3). The absorbance (at 405 nm) of the ELISA assay increased from ~0.1 unit in absence of FITC-TrapG to 2.0 units in presence of 40 μg/mL of the FITC conjugate. A similar trend was observed with the intact

CT26/mβG cells (absorbance at 405 nm was ~0.15 units without FITC-TrapG and increased to ~1.25 units in presence of 40 μg/mL of the FITC conjugate). Western blot analysis showed that activated FITC-TrapG could cross-link the nucleophile groups of both bovine serum albumin (BSA) and purified *Escherichia coli* βG (*e*βG) with FITC (3). It has been shown that FITC-TrapG did not label the BSA in absence of the *e*βG. These studies demonstrated that the probe is a suitable substrate for the *e*βG.

Animal Studies

Rodents

[PubMed]

FITC-TrapG and NIR-TrapG were evaluated for the detection of βG activity in subcutaneous CT26 cell and CT26/mβG tumors in the left and right flanks, respectively, of BALB/c nude mice (n = 3 animals) (3). The animals were injected intravenously with either ~0.833 μmol FITC-TrapG or ~0.12 μmol NIR-TrapG, and the fluorescence intensity (FI) values from the tumors and other organs were recorded at 24, 48, and 72 h postinjection (p.i.) with an acquisition time of 10 s at each time point. FI values of the tumors were calculated and reported as photons per $s^1 \cdot cm^2 \cdot sr^1$ as described by Cheng et al. (3). With FITC-TrapG, the FI values of the CT26/mβG cell tumors were 4.59-, 3.86-, and 2.86-fold higher than that of the control CT26 cell tumors at 24, 48, and 72 h p.i., respectively. Presence or absence of βG activity in the tumors was confirmed histologically as described by Cheng et al. (3). The FI values of the CT26/mβG cell tumors with NIR-TrapG at 24, 48, and 72 h p.i. were 4.25-, 4.92-, and 5.21-fold higher, respectively, than the control CT26 cell tumors. These results indicated that the NIR-TrapG probe was superior to the FITC-TrapG probe for detection of the βG activity because it generated higher a FI with the CT26/mβG tumors. Similar results were obtained from the biodistribution study (n = 3 mice) with both probes (3). Apart from accumulation in the CT26/mβG tumors, optical signals from the FITC-TrapG and the NIR-TrapG probes were detected in the liver, kidneys, intestines, and bladder of the animals. This indicated that the TrapG probe derivatives were eliminated from the body through the urinary and the hepatobiliary routes in these animals.

The FITC-TrapG and NIR-TrapG probes were also evaluated for the visualization of βG activity in CT26 cell (control) or CT26/mβG cell tumors transplanted under the capsule of the liver in mice (n = 3 animals/probe) (3). The animals were injected with FITC-TrapG or NIR-TrapG as before, and whole-body fluorescence signals were recorded from the animals under anesthesia at 24 p.i. After acquiring the images, the livers were extracted from the animals for *ex vivo* imaging to confirm the presence of the dyes in the organs. Whole-body images showed that NIR signals were detected in the livers of mice injected with the NIR-TrapG probe, whereas no FITC signals were observed in the livers of mice injected with the FITC-TrapG probe. However, FITC signals were visible in the livers only when *ex vivo* images of the organs were acquired.

From these studies, the investigators concluded that the NIR-TrapG probe was suitable for the noninvasive imaging of βG activity in deep tissues of rodents (3).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

References

1. Antunes I.F., Haisma H.J., Elsinga P.H., van Waarde A., Willemsen A.T., Dierckx R.A., de Vries E.F. In Vivo Evaluation Of 1-o-(4-(2-fluoroethyl-carbamoyloxymethyl)-2-nitrophenyl)-o-beta-d-glucopyronurona te: A Positron Emission Tomographic Tracer For Imaging beta-glucuronidase Activity In A Tumor/inflammation Rodent Model. Mol Imaging. 2012;11(1):77–87. PubMed PMID: 22418030.
2. Gandhi N.S., Freeman C., Parish C.R., Mancera R.L. Computational analyses of the catalytic and heparin-binding sites and their interactions with glycosaminoglycans in glycoside hydrolase family 79 endo-beta-D-glucuronidase (heparanase). Glycobiology. 2012;22(1):35–55. PubMed PMID: 21746763.
3. Cheng T.C., Roffler S.R., Tzou S.C., Chuang K.H., Su Y.C., Chuang C.H., Kao C.H., Chen C.S., Harn I.H., Liu K.Y., Cheng T.L., Leu Y.L. An activity-based near-infrared glucuronide trapping probe for imaging beta-glucuronidase expression in deep tissues. J Am Chem Soc. 2012;134(6):3103–10. PubMed PMID: 22239495.
4. Antunes I.F., Haisma H.J., Elsinga P.H., Dierckx R.A., de Vries E.F. Synthesis and evaluation of [18F]-FEAnGA as a PET Tracer for beta-glucuronidase activity. Bioconjug Chem. 2010;21(5):911–20. PubMed PMID: 20415436.
5. Antunes I.F., Haisma H.J., de Vries E.F. Tumor-specific activation of prodrugs: is there a role for nuclear medicine? Nucl Med Commun. 2008;29(10):845–6. PubMed PMID: 18769302.

6. Huttunen K.M., Raunio H., Rautio J. Prodrugs--from serendipity to rational design. Pharmacol Rev. 2011;63(3):750–71. PubMed PMID: 21737530.
7. Thomas M., Clarhaut J., Tranoy-Opalinski I., Gesson J.P., Roche J., Papot S. Synthesis and biological evaluation of glucuronide prodrugs of the histone deacetylase inhibitor CI-994 for application in selective cancer chemotherapy. Bioorg Med Chem. 2008;16(17):8109–16. PubMed PMID: 18692397.
8. Renoux B., Legigan T., Bensalma S., Chadeneau C., Muller J.M., Papot S. A new cyclopamine glucuronide prodrug with improved kinetics of drug release. Org Biomol Chem. 2011;9(24):8459–64. PubMed PMID: 22042246.
9. Tzou S.C., Roffler S., Chuang K.H., Yeh H.P., Kao C.H., Su Y.C., Cheng C.M., Tseng W.L., Shiea J., Harm I.H., Cheng K.W., Chen B.M., Hwang J.J., Cheng T.L., Wang H.E. Micro-PET imaging of beta-glucuronidase activity by the hydrophobic conversion of a glucuronide probe. Radiology. 2009;252(3):754–62. PubMed PMID: 19717754.
10. Antunes I.F., Doorduin J., Haisma H.J., Elsinga P.H., van Waarde A., Willemsen A.T., Dierckx R.A., de Vries E.F. 18F-FEAnGA for PET of beta-Glucuronidase Activity in Neuroinflammation. J Nucl Med. 2012;53(3):451–8. PubMed PMID: 22323774.
11. Antunes, I.F., H.J. Haisma, P.H. Elsinga, J.W. Sijbesma, A.V. Waarde, A.T. Willemsen, R.A. Dierckx, and E.F. de Vries, In vivo evaluation of [(18)F]FEAnGA-Me: a PET tracer for imaging beta-glucuronidase (beta-GUS) activity in a tumor/inflammation rodent model. Nucl Med Biol, 2012
12. Su Y.C., Chuang K.H., Wang Y.M., Cheng C.M., Lin S.R., Wang J.Y., Hwang J.J., Chen B.M., Chen K.C., Roffler S., Cheng T.L. Gene expression imaging by enzymatic catalysis of a fluorescent probe via membrane-anchored beta-glucuronidase. Gene Ther. 2007;14(7):565–74. PubMed PMID: 17235292.