cypate-D: -(+)-glucosamine (cyp-GlcN), and D: -(+)-glucosamine-cypate-D: -(+)-glucosamine (cyp-2GlcN)

cyp-GlcN and cyp-2GlcN

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Created: August 8, 2012; Updated: September 13, 2012.

Chemical name:	cypate-D: -(+)-glucosamine (cyp-GlcN), and D: -(+)-gluosamine-cypate-D: -(+)-glucosamine (cyp-2GlcN)	
Abbreviated name:	cyp-GlcN and cyp-2GlcN	
Synonym:		
Agent Category:	Compound	
Target:	Glucose transporter protein 1 (GLUT1)	
Target Category:	Transporter	
Method of detection:	Near-infrared fluorescence (NIRF) imaging; optical imaging	
Source of signal / contrast:	Cypate	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Increased nutritional requirements are a characteristic feature of neoplastic tumor cells, and because these lesions do not rely on mitochondrial oxidation for energy, most of the energy in these cells is generated through glycolysis (1). To keep up with the high nutritional and energy needs of a malignant tumor, the transformed cells in the lesion

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NLM Citation: Chopra A. cypate-D: -(+)-glucosamine (cyp-GlcN), and D: -(+)-glucosamine-cypate-D: -(+)-glucosamine (cyp-2GlcN). 2012 Aug 8 [Updated 2012 Sep 13]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

show a 20- to 30-fold higher rate of glucose uptake and glycolysis compared with normal cells. As a consequence, to meet the increased demand for glucose, the facultative glucose transport proteins (GLUTs; there are 14 known isoforms of these proteins, designated GLUT1 through GLUT14) that facilitate glucose uptake are upregulated on the plasma membranes of the tumor cells (2). In general, GLUT1 and GLUT3 are able to transport glucosamine (GlcN) in addition to glucose and are overexpressed in the different cancer types, including prostate cancer. In addition, an elevated expression of these transporters correlates with the malignant potential of the lesions and indicates a poor prognosis for the patient (2). Among the different glucose transporters, GLUT2 and GLUT4 can also transport GlcN, and both of these isoforms are transcriptionally repressed by the p53 protein, a tumor suppressor protein that regulates the cell cycle and promotes apoptosis (3). GLUT1 and GLUT4 exhibit a similar affinity for glucose and GlcN, but GLUT2 has an approximate 20-fold higher affinity for GlcN than for glucose (3). The PC3 human prostate cancer cell line does not express the p53 protein, and an increased expression of GLUT1 has been observed in these cells (3). Therefore, an imaging probe derived from GlcN would probably accumulate rapidly in tumors generated from the PC3 cells (3).

Currently, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]-FDG), a glucose analog, is commonly used with positron emission tomography (PET) to detect, stage, and monitor cancers after therapy [PubMed]. A major limitation of using [^{18}F]-FDG to detect and monitor cancers is that this probe often generates false positive results (4). The main disadvantages of imaging with PET are that this technique involves the use of radionuclides that have a very short half-life and that the radiolabeled compounds used for this procedure have very high specific activities, which may expose the patient to an abnormal dose of radiation. As an alternative, there is much interest to develop probes that contain cyanine fluorophores, such as the cypate dye (cyp), because these fluorophores are visible in the near-infrared (NIR) wavelength of light and can be used for the noninvasive optical imaging of targeted tissues. The advantages of using cyp are that the compound has very low autofluorescence, absorption, and scattering within the excitation and emission ranges of the wavelength and that photons emitted from the dye can travel several centimeters through the tissue (3). Korotcov et al. investigated the *in vitro* and *in vivo* applications of cypate conjugated either to a single molecule of GlcN (cypate-D-(+)-GlcN; cyp-GlcN) or to two molecules of GlcN (D-(+)-glucosamine-cypate-D-(+)-glucosamine; cyp-2GlcN) to visualize luciferase-expressing PC3 cell (PC3-luc) xexograft tumors in mice (3). The biodistribution of cyp-GlcN and cyp-2GlcN was also studied in these animals.

Related Resource Links

Related chapters in MICAD

Glucose transporters in Online Mendelian Inheritance in Man (OMIM) database

GLUT2 in Gene database; Gene ID: 6514

Synthesis

[PubMed]

The synthesis and purification of cyp-GlcN and cyp-2GlcN have been described by Ye et al. (5). The final yields of the compounds after the synthesis were >80%. The identity and purity (not reported) of the compounds were confirmed with electrospray mass spectrometry, nuclear magnetic resonance, and high-performance liquid chromatography. The number of cyp molecules per GlcN was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The cellular uptake of cyp, cyp-GlcN, and cyp-2GlcN was investigated by exposing PC3-luc cells to the probes in the presence or absence (cell growth medium alone; controls) of 1 mM D-GlcN, 5 mM D-GlcN, 5 mM L-glucose (L-Glc), 25 mM L-Glc, 5 mM D-glucose (D-Glc), 25 mM D-Glc, or 25 mM *N*-acetyl-D-glucoseamine (GlcNAc) (3). Fluorescence microscopy showed that the dye alone and the dye conjugates were distributed in the entire cytoplasm, and no probe was visible in the nucleus or the periphery of the cells. The uptake of the fluorescent agents was reported to be cyp-2GlcN > cyp-GlcN > cyp, both in the presence or absence of the competing carbohydrates ($P > 0.05$). This indicated that the GLUT-mediated transport of cyp, cyp-GlcN, and cyp-2GlcN into the cells was either not completely inhibited by the presence of excess concentrations of the sugars or the dye-conjugated GlcNs were not transported into the cells through the GLUT system. The investigators suggested that more studies were necessary to delineate the mechanism of cyp, cyp-GlcN, and cyp-2GlcN uptake in the PC3 cells.

Animal Studies

Rodents

[PubMed]

The *ex vivo* biodistribution of cyp, cyp-GlcN, and cyp-2GlcN was investigated in mice bearing PC3-luc cell tumors (3). The animals (number per group not reported) were injected with the probes (10 nmol) through the tail vein, and whole-body dynamic fluorescence images of the rodents were acquired at time points ranging from 10 min postinjection (p.i.) to 20–30 days p.i. as described elsewhere (3). Signal intensities from the images were reported as photons per second per centimeter square per steradian ($p/s/cm^2/sr$). The tumor/muscle (T/M) ratios for cyp-2GlcN, cyp-GlcN, and cyp were 7.8, 3.8, and 2.0, respectively, at 24 h p.i., indicating that the uptake of cyp-2GlcN in the tumor cells was higher than that of either cyp-GlcN or cyp. With cyp-2GlcN, the T/M ratio was ~3.7 at 20 d p.i., indicating that this derivative of cyp had a long retention time in the tumors. However, the retention of cyp-2GlcN in the kidneys and the liver of the mice (~10^8 $p/s/cm^2/sr$ in each organ) was higher than that of either cyp or cyp-GlcN (~7.1

p/s/cm^2/sr in each organ/probe), even at 500 h p.i. This indicated that the dye and its carbohydrate conjugates were excreted through the urinary and biliary systems in the rodents.

To determine the *in vivo* tumor uptake specificity of cyp-2GlcN, mice bearing PC3-luc cell tumors (n = 6 animals/group) were given an intraperitoneal injection of either D-Glc (2 g/kg body weight (BW)), D-GlcN (0.5 g/kg BW), or GlcNAc (2 g/kg BW) 30 min before the administration of cyp-2GlcN through the tail vein (3). Fluorescence images of the rodents acquired at 24 h p.i. showed that pretreatment of the animals with D-Glc did not alter the uptake of cyp-2GlcN in the various organs, including tumors, of the animals as observed earlier in the *in vitro* study. The *in vivo* uptake of cyp-2GlcN was enhanced by the pretreatment with D-GlcN, suggesting that this carbohydrate activated the transport of GlcN and its derivatives into the different organs and tumors in the mice. Only GlcNAc inhibited the uptake of cyp-2GlcN in the various tissues and the tumors of the animals. This observation was in contrast to the results obtained during the *in vitro* study. The investigators suggested that the *in vivo* inhibition of cyp-2GlcN by GlcNAc was probably due to the higher concentration of GlcNAc (2 g/kg BW) used in this study compared with the concentration used for the *in vitro* experiments (10 nmol).

In another study, the mice (n = 3 animals/group) were injected with cyp, cyp-GlcN, or cyp-2GlcN as before, and the fluorescence intensities of the PC3 cell xenograft tumors were compared with those of the surrounding tissues for up to 10 h p.i. (cyp), 24 h p.i. (cyp-GlcN), and 45 h p.i. (cyp-2GlcN) (3). The maximum fluorescence intensities of the tumors with cyp, cyp-GlcN, or cyp-2GlcN were 4.7×10^7 p/s/cm^2/sr, 1.35×10^8 p/s/cm^2/sr, and 9.6×10^9 p/s/cm^2/sr at 1 min p.i., 30 min p.i., and 60 min p.i., respectively. In addition, the residence half-lives of cyp, cyp-GlcN, and cyp-2GlcN in the tumors were 15 min, 1.7 h, and 15 h, respectively. This indicated that addition of the GlcN moieties to cyp increased the retention time of the fluorescence probe in the lesions.

To determine the targeting specificity of cyp-2GlcN to the PC3-luc xenograft tumors, the mice (number of animals not reported) were administered 10 nmol of cyp-GlcN or cyp-2GlcN through the tail vein, and whole-body fluorescence images of the rodents were acquired for up to 30 days p.i (3). Between the two NIRF probes, cyp-2GlcN had a higher tumor specificity and retention time compared with the surrounding tissues. With cyp-2GlcN, the maximum fluorescence intensity of the tumor was observed at 24 h p.i., and the lesion was visible for up to 30 days p.i. Fluorescence was evident in the liver and kidneys of the mice for up to 24 h p.i., and the signal from these organs was observed to gradually decrease up to 96 h p.i.

From these studies, the investigators concluded that cyp-GlcN and cyp-2GlcN were suitable to detect prostate cancer cell derived tumors in mice (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.

NIH Support

Supported in part by grant RCMI 2G12RR003048 from the National Institutes of Health and the National Center for Research Resources.

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