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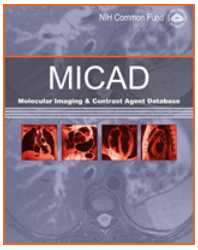

Bombesin peptide conjugated–cross-linked iron oxide-Cy5.5

BN-CLIO-Cy5.5

The MICAD Research Team

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Chemical name:	Bombesin peptide conjugated–cross-linked iron oxide-Cy5.5	
Abbreviated name:	BN-CLIO-Cy5.5	
Synonym:	(FITC)BCDDDGQRLGNQWAVGHLM-CLIO(Cy5.5), BN-CLIO(Cy5.5)	
Agent Category:	Peptide	
Target:	Gastrin-releasing peptide receptor	
Target Category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI), Optical (NIRF)	
Source of signal:	Iron oxide, Cy5.5, FITC	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is not available in PubChem.

Background

[PubMed]

One of the leading causes of cancer death in the United States of America is pancreatic ductal adenocarcinoma (PDAC). Late diagnosis of this tumor and its strong drug resistance are factors that lead to the low survival rate of PDAC patients (1). Limitations in diagnostic techniques and lack of specific symptoms often account for the late diagnosis of PDAC. It will be of tremendous help to patients to develop a technique for early diagnosis of PDAC.

The design of molecules or peptides that specifically target the overexpressed or upregulated receptors on cancerous cells is an important strategy in the development of therapeutic and diagnostic drugs. This strategy, however, does not work well in PDAC because many of these receptors also express in normal pancreas. An inverse strategy, targeting the receptors on normal cells instead of those on tumor cells, was thus developed by Montet et al. (1) to overcome this difficulty. It has been shown that targeting the normal cells instead of the tumor cells enhances the visualization of tumors in liver or lymph nodes by molecular resonance imaging (MRI) (2, 3).

Bombesin (BN)-like peptide is an analog of human gastrin-releasing peptide (GRP) that binds to GRP receptors (GRP-R). BN peptides educe such biological responses as: secretion of adrenal, pituitary, and gastrointestinal hormones; gastric acid secretion; modulation of neuronal firing rate; and regulation of smooth muscle contraction (4). BN-related peptide receptors can be divided into four subtypes: GRP-R (BB2, BRS-2),

neuromedin B receptor (NMB-R, BB1, BRS-1), the orphan receptor bb3-R (BRS-3), and the amphibian receptor bb4-R. Several human cancers, including prostate, breast, lung, and pancreatic cancers, express receptors for BN-like peptides. The BN-like peptides have been radiolabeled with different radionuclei for *in vivo* imaging of various cancers (5-8). However, it was reported that BN peptide-binding receptors were present in normal pancreas but not in PDAC (9). This provides a great potential to develop the inverse strategy for detecting PDAC at an early stage. The BN binding receptor was reported to be able to internalize ligands, and consequently could provide great potential for accumulation of nanoparticles for detection (10-12). A BN receptor-targeted peptide was therefore used to conjugate with CLIO-Cy5.5, a nanoparticle that consists of a cross-linked superparamagnetic iron oxide nanoparticle (CLIO) and a near infrared (NIR) fluorochrome (Cy5.5) and thus possesses both MRI and NIR fluorescence (NIRF) imaging modalities.

BN-CLIO-Cy5.5 was prepared by conjugating the fluorescein (FITC)-labeled BN peptide (FITC-BCDDDGQRLGNQWAVGHLM) with the nanoparticle CLIO-Cy5.5. It serves as a contrast agent for MRI and, in the meantime, provides a NIRF image.

Synthesis

[PubMed]

The synthesis of FITC-BCDDDGQRLGNQWAVGHLM-CLIO-Cy5.5 (BN-CLIO-CY5.5) and FITC-BCDDDGQMLGNHLAVGQWR (ScrBN-CLIO-Cy5.5, control peptide) was described by Montet et al. (1). In brief, the peptides were synthesized using solid-phase peptide synthesis *via* 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry. The N-terminus beta-alanine (B) was used to conjugate with FITC and the thiol group of cysteine was utilized to conjugate the peptide with CLIO-Cy5.5 nanoparticles. CLIO-NH_2 nanoparticles (produced by coating iron oxide nanoparticles with dextran, followed by cross-linking with epichlorohydrin, and then activated by reacting with ammonia (13, 14)) were mixed with Cy5.5 solution to produce 5.1 dyes per nanoparticle. Purified CLIO(NH_2)-Cy5.5 was further activated by reacting with succinimidyl iodoacetate. Activated CLIO-Cy5.5 was then conjugated with a BN peptide through cysteine. The size of the nanoparticle was determined to be 35 nm using laser light scattering method, with an average of 50 peptides per iron oxide particle.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Normal mouse pancreas tissue was used to prove the binding of BN-CLIO-Cy5.5 with the BN receptor (1). The tissues were treated with BN-CLIO-Cy5.5 or ScrBN-CLIO-Cy5.5; BN-CLIO-Cy5.5 generated a darker stain than ScrBN-CLIO-Cy5.5 with a fluorescein hapten visualization method (15). The result was verified using a fluorescein hapten assay (15) to quantify the amount of fluorescein that bound to the BN receptors. There was 3.5-fold more BN-CLIO-Cy5.5 bound to the tissue specimen than ScrBN-CLIO-Cy5.5.

A tissue microarray consisting of 5 normal human pancreas specimens and 13 pancreatic ductal adenocarcinomas was used to assess the method (1). It appeared that the normal pancreas tissue showed a much darker stain than the ductal adenocarcinoma specimens. The result from mouse and human pancreas tissue showed that BN-CLIO-Cy5.5 appeared to bind to normal pancreas and not to PDAC.

Animal Studies

Rodents

[PubMed]

Normal, female, athymic nude mice were used to determine the nanoparticle distribution. The tissue specificity of BN-CLIO-Cy5.5 was determined by comparing the uptake of the nanoparticle in the pancreas and the uptake of ScrBN-CLIO-Cy5.5, a control with similar size and function but different peptide sequence (1). Pancreas injected with BN-CLIO-Cy5.5 had 3-fold higher fluorescence intensity than pancreas injected with Scr-BN-Cy5.5. Both BN-CLIO-Cy5.5 and Scr-BN-Cy5.5 are taken up by Kupffer cells in the liver in a non–receptor-dependent fashion. It appeared that receptor-mediated targeting of BN-CLIO-Cy5.5 occurred in pancreas, whereas high non–peptide-dependent uptake occurred in liver and spleen. The distribution of BN-CLIO-Cy5.5 within the pancreas bearing a tumor was examined *ex vivo* by fluorescence microscopy. It appeared that the nanoparticle accumulated to the greatest extent at the border of the tumor.

Montet et al. also reported that BN-CLIO-Cy5.5 can improve the MRI contrast in normal pancreas and tumor (1). Pancreatic tumor cell line MIA-PaCa2 was implanted directly in the pancreatic tail of female athymic nude mice 6 days before the image was taken. BN-CLIO-Cy5.5 was introduced through tail-vein injection and images were taken before and 24 h after the administration of the agent. The tumor was barely visible in the precontrast image, but it became clearly identifiable after the darkening of normal pancreas. The T2 values for tumors were 62 ± 6 ms pre-injection and 62 ± 5 ms post-injection. The T2 values for normal pancreas decreased from 46 ± 5 ms pre-injection to 39 ± 5 ms post-injection.

Other Non-Primate Mammals

[PubMed]

No relevant publication is currently available.

Non-Human Primates

[PubMed]

No relevant publication is currently available.

Human Studies

[PubMed]

No relevant publication is currently available.

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