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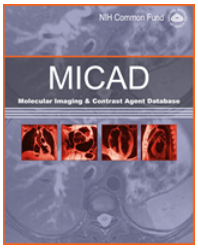

^{99m}Tc-Labeled, PEGylated (N$^{\alpha}$His)Ac-β^{3}hLys-βAla-βAla-Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Cha^{13}-Nle^{14}-NH$_2$

^{99m}Tc-PEG$_x$-Lys-BN

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Chemical name:	^{99m}Tc-Labeled, PEGylated (N$^{\alpha}$His)Ac-β^{3}hLys-βAla-βAla-Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Cha^{13}-Nle^{14}-NH$_2$	
Abbreviated name:	^{99m}Tc-PEG$_x$-Lys-BN (x = 5, 10, and 20 kDa)	
Synonym:	^{99m}Tc-PEG$_5$-Lys-BN, ^{99m}Tc-PEG$_{10}$-Lys-BN, and ^{99m}Tc-PEG$_{20}$-Lys-BN	
Agent Category:	Peptides	
Target:	Gastrin-releasing peptide receptors (GRPR)	
Target Category:	Receptors	
Method of detection:	Single-photon emission computed tomography (SPECT) or planar imaging	
Source of signal / contrast:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure available

Background

[PubMed]

The ^{99m}Tc-labeled, PEGylated (N$^{\alpha}$His)Ac-β^{3}hLys-βAla-βAla-Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Cha^{13}-Nle^{14}-NH$_2$ (Lys-BN), abbreviated as ^{99m}Tc-PEG$_x$-Lys-BN (x = 5, 10, and 20 kDa), are PEGylated bombesin (BN) analogs that were synthesized by Dapp et al. for imaging of tumors that overexpress gastrin-releasing peptide receptors (GRPR) (1).

Engineered proteins and peptides are widely applied in the development of molecular imaging agents; however, they exhibit some unfavorable pharmacokinetic properties when used *in vivo*, such as rapid clearance, immunogenicity, and poor stability (e.g., aggregation, degradation, deamination, oxidation, etc.) (2, 3). As a technique to overcome these limits of proteins and peptides, PEGylation has been extensively studied recently, and the number of agents newly developed with PEGylation is increasing continuously (3, 4). PEGylation is defined as the covalent attachment of poly(ethylene glycol) (PEG) chains to bioactive substances (3). PEG

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possesses three key properties: great flexibility due to the absence of bulky substituents along the chain; high hydration of the polymeric backbone; and a high degree of safety, with toxicity only at very high doses (1, 3, 4). Furthermore, PEG can be coupled with virtually any exposed surface and even some buried amino acids in a protein, and this coupling can be achieved at the N- or C-terminus, the cysteines located far from the receptor-binding site, or the incorporated unnatural amino acids. PEG increases the blood circulation of a given protein by increasing its hydrodynamic volume, prevents its immunogenicity, reduces its aggregation, and increases its thermal stability. However, a reduction in biological potency is common after PEGylation because of the steric entanglement of polymer chains during the protein/receptor recognition process (3). This reduction is also related to the PEGylation methods and PEG selected. The properties of PEG vary significantly with molecular weight and concentration.

Radiolabeled BN analogs are promising radiotracers for tumor imaging and therapy by targeting GRPR (5-7). However, the low *in vivo* stability of BN analogs limits their clinical application (8, 9). Dapp et al. prepared a series of PEGylated BN(7-14) analogs and evaluated their properties *in vitro* and *in vivo* (1). PEGylation was performed with linear PEG molecules of various sizes (5 kDa (PEG_5), 10 kDa (PEG_{10}), and 20 kDa (PEG_{20})) through the ε-amino group of a β^3hLys-βAla-βAla spacer between the BN sequence and the (N^{α}His)Ac chelator. *In vitro* results showed that PEGylation did not affect the binding affinity of BN analogs, but it did slow their binding kinetics (1). *In vivo* results showed that PEGylation increased the stability of the analogs, improved their pharmacokinetics, and enhanced the tumor retention.

Related Resource Links:

Bombesin-based imaging agents in MICAD

Bombesin-based clinical trials

Nucleotide and protein sequences of GRPR

Synthesis

[PubMed]

The BN peptide Lys-BN was synthesized with peptide solid-phase synthesis (1). Methoxypolyethylene glycol succinimidyl esters (MeO-PEG-NHS; 5, 10, and 20 kDa) were commercially available. To PEGylate the BN peptides, MeO-PEG-NHS was mixed with each BN analog and incubated for 1 h at room temperature. More MeO-PEG-NHS was added after 30 min and again after 45 min. The solvents were removed by evaporation, and the final product was dissolved in water before labeling with ^{99m}Tc. The yields of PEG_x-Lys-BN analogs were 85%, 74%, and 46% for PEG_5, PEG_{10}, and PEG_{20}, respectively. The molecular weight was 1,441.9 for Lys-BN, 6,335.1 for PEG_5-Lys-BN, 10,945.4 for PEG_{10}-Lys-BN, and 22,709.5 for PEG_{20}-Lys-BN.

To label ^{99m}Tc, a solution of Na[$^{99m}TcO_4$], sodium boranocarbonate, borax, potassium sodium tartrate tetrahydrate, and sodium carbonate was heated for 40 s at 150°C. After adjusting the pH to 6.5, the solution was mixed with the PEGylation analogs and heated for 2 min at 90°C. The non-PEGylated Lys-BN was similarly labeled with ^{99m}Tc. High-performance liquid chromatography was performed to separate the non-PEGylated from the PEGylated ^{99m}Tc-labeled peptides, and the ^{99m}Tc-labeled from the unlabeled peptides on the basis of the difference of their retention times. The radiochemical purities and specific radioactivities for both ^{99m}Tc-Lys-BN and ^{99m}Tc-PEG-Lys-BN analogs were >95% and 5.02 TBq/μmol (135.7 Ci/μmol), respectively (1). The radiochemical yields for the analogs were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The octanol/phosphate-buffered saline partition coefficients (log D) were determined at pH 7.4, and the log D values were −0.74 ± 0.06, −1.61 ± 0.30, −1.05 ± 0.16, and −1.08 ± 0.25 for ^{99m}Tc-Lys-BN, ^{99m}Tc-PEG$_5$-Lys-BN, ^{99m}Tc-PEG$_{10}$-Lys-BN, and ^{99m}Tc-PEG$_{20}$-Lys-BN, respectively, showing that PEGylation resulted in increased hydrophilicity of the analogs (1).

The *in vitro* stability was evaluated after 24 h of incubation with human plasma (1). The analog ^{99m}Tc-Lys-BN was rapidly degraded, with only 16.6 ± 2.3% intact at 24 h. In contrast, all PEGylated BN analogs remained intact. The half-life of ^{99m}Tc-Lys-BN was 11.4 h, whereas the half-lives of the PEGylated BN analogs were >24 h. The cell binding assay confirmed these results (1). With the analogs obtained after incubation with human plasma for 24 h, the specific binding of the ^{99m}Tc-Lys-BN to PC-3 cells was reduced to 17.2 ± 1.9% in comparison to the specific binding without incubation in human plasma. The percentages of the PEGylated BN analogs after 24 h of incubation with human plasma were still 115.5 ± 18.3%, 96.0 ± 3.2%, and 99.9 ± 4.9% for ^{99m}Tc-PEG$_5$-Lys-BN, ^{99m}Tc-PEG$_{10}$-Lys-BN, and ^{99m}Tc-PEG$_{20}$-Lys-BN, respectively.

Saturation binding assays were performed with PC-3 cells to evaluate the binding kinetics of the ^{99m}Tc-labeled analogs (1). The dissociation constants (K_d) for ^{99m}Tc-PEG-Lys-BN analogs were 0.89 ± 0.32, 0.53 ± 0.04, and 0.63 ± 0.20 nM for PEG$_5$, PEG$_{10}$, and PEG$_{20}$, respectively. These values were in the same range as the K_d value of Lys-BN, which was 0.65 ± 0.35 nM. Compared to the non-PEGylated ^{99m}Tc-Lys-BN, the PEGylated forms had slower binding kinetics.

On cell internalization, all analogs showed specific, time-dependent cell uptake (1). ^{99m}Tc-Lys-BN internalized rapidly, reached its maximum within the first 30 min of incubation (~30% per 10^6 cells), and remained virtually constant for about 2 h. All PEGylated analogs showed lower internalization into PC-3 cells, and longer incubation was required to reach the plateau (between 4 h and 24 h). The size of the PEG entity had an influence on the amount of internalized fraction. After incubation for 4 h, the internalization rates were 2.9 ± 0.9%, 1.7 ± 1.4%, and 1.2 ± 1.2% for ^{99m}Tc-PEG$_5$-Lys-BN, ^{99m}Tc-PEG$_{10}$-Lys-BN, and ^{99m}Tc-PEG$_{20}$-Lys-BN, respectively.

On cell externalization, ^{99m}Tc-Lys-BN was externalized quickly, with 73.37 ± 1.30% of the internalized activity released within the first 2.5 h, and only 21.44 ± 2.09% of the internalized fraction remained in the cells after 24 h. In contrast, the externalization of PEGylated analogs was slower, with 80.2 ± 3.7% (^{99m}Tc-PEG$_5$-Lys-BN), 42.8 ± 0.9% (^{99m}Tc-PEG$_{10}$-Lys-BN), and 54.2 ± 12.1% (^{99m}Tc-PEG$_{20}$-Lys-BN) of the internalized fraction remaining in the cells after 24 h (1).

Animal Studies

Rodents

[PubMed]

The effect of PEGylation on *in vivo* stability was tested in Balb/c mice after injection of the radiolabeled BN analogs (10–20 MBq (0.27–0.54 mCi)) (n = 2 mice/time point) (1). The non-PEGylated ^{99m}Tc-Lys-BN was rapidly metabolized in mice, with only 5.28 ± 0.06% intact at 5 min and no intact ^{99m}Tc-Lys-BN at 30 min after injection in blood. ^{99m}Tc-PEG$_5$-Lys-BN showed higher stability *in vivo* than ^{99m}Tc-Lys-BN, with 13.20 ± 1.59% intact at 5 min and 4.38 ± 0.60% intact at 30 min after injection. No data were reported for ^{99m}Tc-PEG$_{10}$-Lys-BN and ^{99m}Tc-PEG$_{20}$-Lys-BN.

The effect of PEGylation on biodistribution was tested in mice bearing PC-3 tumor xenografts after injection of 0.5–3.5 MBq (13.51–94.59 μCi) of the analogs (Table 1) (1). Mice (n = 3–6/group) were euthanized at 1, 4, and 24 h after injection. The radioactivity in each tissue was determined and expressed as a percentage of injected dose per gram of tissue (% ID/g).

The blood clearance of ^{99m}Tc-Lys-BN was fast. Conjugation with PEG_5 did not affect the blood clearance of the BN analog, whereas conjugation with PEG_{10} and especially PEG_{20} led to longer blood circulation times and slower clearance rates ($P < 0.01$) (1).

The highest tumor uptake values for ^{99m}Tc-Lys-BN, ^{99m}Tc-PEG_{10}-Lys-BN, and ^{99m}Tc-PEG_5-Lys-BN were found 1 h after injection (2.80%, 1.79%, and 3.91% ID/g, respectively), with the uptake of ^{99m}Tc-PEG_5-Lys-BN being significantly higher than that of ^{99m}Tc-Lys-BN ($P < 0.05$). The highest tumor uptake for ^{99m}Tc-PEG_{20}-Lys-BN, however, was found 4 h after injection (4.86% ID/g). The tumor washout was slower for the PEGylated analogs; only 0.53% ID/g of the ^{99m}Tc-Lys-BN remained in the tumor, whereas 1.73%, 1.14%, and 4.12% ID/g were found at 24 h after injection for the PEG_5, PEG_{10} and PEG_{20} analogs, respectively.

^{99m}Tc-PEG_5-Lys-BN showed the highest tumor/nontarget ratios at all time points, especially at 24 h after injection. Compared to the non-PEGylated ^{99m}Tc-Lys-BN, PEG_{10} did not lead to improved tumor/nontarget ratios, while PEG_{20} improved tumor/kidney ratios (2- and 4-fold increase at 4 h and 24 h after injection, respectively) and tumor/liver ratios (2- and 3-fold increase at the same time points, respectively).

In the GRPR-expressing pancreas, all analogs showed highest uptake at 1 h after injection, but there was no statistical difference in uptake between PEGylated and non-PEGylated analogs. In the GRPR-expressing colon, the uptake was different for the PEGylated and non-PEGylated analogs. ^{99m}Tc-PEG_5-Lys-BN and ^{99m}Tc-PEG_{10}-Lys-BN showed significantly lower colon uptake, while ^{99m}Tc-PEG_{20}-Lys-BN and ^{99m}Tc-Lys-BN showed comparable uptake.

In the liver, the highest uptake was observed with ^{99m}Tc-Lys-BN at 1 h after injection (3.92% ID/g); however, its clearance was relatively fast, with only 0.56% ID/g of the radioactivity remaining at 24 h after injection. ^{99m}Tc-PEG_5-Lys-BN exhibited a significantly lower liver uptake at all time points (<1% ID/g). ^{99m}Tc-PEG_{10}-Lys-BN showed lower liver uptake at early time points but slow clearance. There was no difference in the liver uptake between ^{99m}Tc-PEG_{20}-Lys-BN and ^{99m}Tc-Lys-BN.

Accumulation in the kidney and washout from the kidneys were similar for both ^{99m}Tc-Lys-BN and ^{99m}Tc-PEG_5-Lys-BN. Despite a slightly lower accumulation of ^{99m}Tc-PEG_{10}-Lys-BN in the kidneys (4.10% ID/g at 1 h after injection), the accumulated radioactivity was cleared slowly, with 2.16% ID/g found at 24 h after injection. ^{99m}Tc-PEG_{20}-Lys-BN showed the highest kidney uptake at all time points and the slowest clearance from kidney.

Table 1: Influence of PEGylation on the biodistribution of BN analogs

Organ	^{99m}Tc-Lys-BN	^{99m}Tc-PEG_5-Lys-BN	^{99m}Tc-PEG10-Lys-BN	^{99m}Tc-PEG_{20}-Lys-BN
Blood	0.96 ± 0.30	1.00 ± 0.08	3.70 ± 0.26	14.46 ± 1.27
Kidneys	5.79 ± 0.95	5.09 ± 1.83	4.10 ± 0.26	7.73 ± 0.48
Pancreas	13.75 ± 2.32	12.92 ± 0.53	9.43 ± 1.94	12.42 ± 1.23
Colon	4.94 ± 0.65	2.04 ± 0.68	2.27 ± 0.27	4.35 ± 0.86
Liver	3.92 ± 0.39	0.64 ± 0.06	2.03 ± 0.22	3.61 ± 0.36
Tumor	2.80 ±0.28	3.91 ± 0.44	1.79 ± 0.39	2.79 ± 0.34

*Data were obtained at 1 h after injection of analogs (n = 3-4)

Blocking studies were performed at 1 h after co-injection of unlabeled BN(1-14) (100 μg) and radiolabeled analogs (n = 3 mice) (1). The uptake in the GRPR-expressing tissues, such as the pancreas, colon, and tumor, was markedly reduced. The uptake of the non-PEGylated BN analog was inhibited the most, while the inhibition was less effective for the PEGylated analogs in a size-dependent manner. The pancreas and colon uptake values were reduced by 54%–95% and 50%–86%, respectively, and no inhibition was found for ^{99m}Tc-PEG_{20}-Lys-BN. The

tumor uptake was significantly inhibited for ^{99m}Tc-Lys-BN and ^{99m}Tc-PEG$_5$-Lys-BN (70% and 58% reduction, respectively). However, no significant differences were found in the tumor uptake of ^{99m}Tc-PEG$_{10}$-Lys-BN and ^{99m}Tc-PEG$_{20}$-Lys-BN for the blocked and the unblocked groups.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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