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Tetraamine-derived bifunctional chelators for 99mTc labeling: synthesis, bioconjugation and evaluation as targeted SPECT imaging probes for GRPr positive tumors

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Introduction: Owing to its optimal nuclear properties, ready availability, low cost and favorable dosimetry, ^{99m}Tc continues to be the ideal radioisotope for medical imaging applications. Most of the bifunctional chelators reported for ^{99m}Tc complexation, suffer from tedious labeling protocols and undesired physicochemical properties. On the other hand, bifunctional chelators based on tetraamine framework exhibit facile complexation at ambient temperature with $Tc(V)O_2$ forming monocationic species with high *in vivo* stability and significant hydrophilicity leading to favorable pharmacokinetics.[1] Although, many tetraamine based conjugates have been studied for the cell surface receptor targeted imaging of tumors, none of the reports exemplify the straightforward synthesis of tetraamine based bifunctional chelators.[2,3]

Methods: Four different tetraamine based chelators (N4) were synthesized and characterized. A bombesin antagonist (BB-ANT), D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH_2, which specifically targets tumors expressing gastrin-releasing peptide receptors (GRPr), was synthesized on solid phase and conjugated to the chelator. The antagonistic property of the conjugate was determined by immunofluorescence-internalization assays. The labeling of N4-BB-ANT was performed with ^{99m}Tc which was eluted as Na[^{99m}Tc]TcO_4 from a ^{99}Mo/^{99m}Tc generator. The radiolabeled conjugate was evaluated *in vitro* and *in vivo* in tumor-bearing nude mice, using the PC-3 cell line expressing GRP-receptors. Scintigraphy study was performed using a clinical SPECT/CT camera.

Results: Tetraamine chelators containing carboxylic acid, amine, alcohol and azide functions were synthesized by straightforward methods. Bioconjugation to the bombesin antagonist peptide was carried out easily on the solid phase. The labeling of N4-BB-ANT was performed at room temperature (pH 11.5) achieving a radiolabeling yield of >97% at specific activity of 37 GBq/µmol. An IC_{50} value of 2.4±0.8 nM was obtained confirming high affinity of the conjugate to GRPr. The immunofluorescence assays confirmed strong antagonist properties of the conjugate. The cell assays showed substantially high receptor mediated uptake of ^{99m}Tc-N4-BB-ANT by PC-3 cells (41.4±0.4% bound and 13.5±0.1% internalized at 4h). *In vivo* pharmacokinetic studies of ^{99m}Tc-N4-BB-ANT with nude mice showed high and specific uptake in PC-3 xenografts and also in other GRPr positive organs such as pancreas and intestine. The tumor uptake was 22.5±2.6% ID/g at 1 h p.i and increased to 29.9±4.0% ID/g at 4 h p.i. At 24 h p.i., radioactivity was cleared from all the organs including pancreas and intestine with exceptionally high retention only in the tumor (15.1±0.9% ID/g) reaching a remarkable tumor to kidney ratio of 10.7. The SPECT/CT images acquired at 12 h p.i. of ^{99m}Tc-N4-BB-ANT are in accordance with the biodistribution data with obvious tumor localization, clear background and negligible radioactivity in the abdomen.

Conclusions: A series of tetraamine based chelators are reported for the facile conjugation of targeting vectors such as peptides and consecutive labeling with ^{99m}Tc. The ^{99m}Tc-N4-BB-ANT thus developed is found to be a highly potent bombesin antagonist targeting GRPr positive tumors. The exceptionally high and specific tumor uptake and favourable pharmacokinetics of ^{99m}Tc-N4-BB-ANT as revealed by preclinical studies warrant its potential candidature for clinical translation.

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References:

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