Design and synthesis of ^{68}Ga-labeled DOTA-conjugated Sstr2-targeting small molecules for imaging neuroendocrine tumor

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Background: Somatostatin receptor subtype 2 (Sstr2) which is overexpressed in neuroendocrine tumors represents an attractive imaging marker and therapeutic target. Radioligands derived from Sstr2-targeting peptides such as DOTATATE have been labeled with therapeutic (Lu-177 and Y-90) radionuclides to treat neuroendocrine tumors. However, most patients do not respond to the treatment of Lu-177 labeled DOTATATE (also called lutathera). This is because peptide-derived radiotherapeutics are prone to degradation in vivo [1], leading to suboptimal tumor accumulation and therapeutic efficacy. In this study, we aimed to design and synthesize imaging agents derived from an Sstr2-targeting small-molecule pharmacophore which might be more stable against in vivo degradation.

Methods: The selected pharmacophore is based on the 3,6-diaryl-4-aminoalkoxyquinoline scaffold reported by Wolkenberg SE et al, which showed very high binding affinity to Sstr2 (Ki = 0.025 nM) [2]. Multi-step synthetic approach was developed to prepare the N-Boc protected Sstr2-targeting pharmacophore. Its phenolic group was coupled with 4-pentyn-1-ol to provide the alkynyl group for click reaction. Three DOTA-conjugated Sstr2-targeting ligands based on the same pharmacophore but with a different charged linker were synthesized on solid phase starting with Fmoc-Lys(ivDde)-Wang resin. Fmoc was removed with piperidine and azidoacetic was coupled to the α-amino group, followed by subsequent coupling of the alkyne-conjugated pharmacophore via the Cu(I)-catalyzed click reaction. The ivDde protecting group was then removed with hydrazine. The radiometal chelator DOTA was directly coupled to the side-chain amino group (for Z06112) or coupled after further elongation with Arg (for Z06111) or Arg-Arg (for Z06110) to provide derivatives with -1, 0, and +1 charge linker, respectively, between DOTA and the Sstr2-targeting pharmacophore. The peptides were cleaved from resin and purified by HPLC. Ga-68 labeling was performed in acetate buffer (pH 4) using Ga-68 GaCl3 with microwave heating for 1 min. Imaging and biodistribution studies were carried out at 1-h post-injection in mice bearing Sstr2-expressing AR42J rat pancreatic cancer xenografts.

Results: The alkyne-conjugated N-Boc protected Sstr2-targeting pharmacophore was synthesized in a total of 11 reaction steps starting from the coupling of 3-chloro-4-iodoaniline and 3-benzyloxyphenylboronic acid. The DOTA-conjugated Z06112, Z06111 and Z06110 were successfully prepared on solid phase and purified by HPLC after cleavage with trifluoroacetic acid. Their identities were confirmed by MS analysis. Z06112, Z06111 and Z06110 were labeled with Ga-68 in acetate buffer. After HPLC purification, the average isolated radiochemical yields (decay-corrected) of Ga-68 labeled Z06112, Z06111 and Z06110 were 68, 51 and 57%, respectively, and their radiochemical purities were > 98%. Imaging studies showed that all Ga-68 labeled tracers were excreted through both renal and hepatobiliary pathways as urinary bladders and livers were clearly visualized in PET images. Higher blood retention was observed for both Ga-68 labeled Z06112 and Z06111. No tumor visualization was achieved using Ga-68 labeled Z06110, whereas Ga-68 labeled Z06112 and Z06111 enabled clear tumor visualization in PET images. Biodistribution data were consistent with the observations from PET images. The average tumor uptake values of Ga-68 labeled Z06112, Z06111 and Z06110 were 3.52, 3.26 and 0.77 %ID/g (percent of injected dose per gram of tissue), respectively. The average blood uptake values of Ga-68 labeled Z06112, Z06111 and Z06110 were 14.2, 7.78 and 3.13 %ID/g, respectively. The average tumor-to-muscle contrast ratios for Ga-68 labeled Z06112, Z06111 and Z06110 were 4.87, 8.53 and 2.60, respectively.

Conclusions: We synthesized three Ga-68 labeled tracers derived from an Sstr2-targeting small-molecule pharmacophore, and successfully evaluated their potential for imaging using a preclinical tumor model. Two of the tracers (Ga-68 labeled Z06112 and Z06111) enabled tumor visualization and showed good tumor-to-muscle contrast ratios. Further investigations on the cause of high blood retention of these tracers are needed to improve their tumor-to-background contrast rations and imaging potentials.

References:

1. Lubberink M et al. J Nucl Med 2020; published ahead of print: http://jnm.snmjournals.org/content/early/2020/01/16/jnumed.119.237818.short?rss=1

2. Wolkenberg SE et al. J Med Chem 2011; 54: 2351-2358.