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Preclinical Evaluation of [^{18}F]F-[^{nat}Lu]Lu-DOTA-rhCCK-18, the First ^{18}F-Labeled Radiohybrid-Based Minigastrin Derivative with high Target Affinity and Tumor Accumulation

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Objectives: In order to enable ^{18}F- and ^{177}Lu-labeling within the same molecule, we previously introduced a silicon-based fluoride acceptor (SiFA) into the hexa-D-glutamate chain of DOTA-PP-F11N (DOTA-(D-Glu)$_6$-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH$_2$) via the side chain of a D-2,3-diaminopropionic acid (D-Dap), which led to a decelerated activity clearance and thus improved tumor retention despite a noticeably lower CCK-2R affinity (~5-fold higher IC_{50} values). However, activity uptake in the kidneys was massively elevated. Hence, in this study we aimed to maintain high activity levels in the tumor while reducing kidney retention. Therefore, we substituted the (*R*)-DOTAGA- by a DOTA moiety to reduce the negative charges in direct proximity to the SiFA building block.

Methods: Synthesis of all compounds was carried out via standard Fmoc-based solid-phase peptide synthesis (SPPS). ^{177}Lu-labeling studies were performed at 90°C within 15 min (1.0 M sodium acetate buffer, *pH* = 5.5, 0.1 M sodium ascorbate). ^{18}F-Labeling was carried out

at 60°C for 5 min, followed by cartridge purification. Cell-based experiments (IC_{50} determination, internalization studies) were performed on AR42J cells. Lipophilicity (depicted as *n*-octanol/PBS distribution coefficient; log$D_{7.4}$) was investigated. Biodistribution studies (n = 4) as well as μSPECT/CT imaging (n = 1) were carried out at 1 and 24 h post-injection (p.i.) in AR42J tumor-bearing CB17-SCID mice.

Results: SPPS yielded 4–8% RP-HPLC-purified labeling precursor. ^{177}Lu-labeling proceeded quantitatively in high radiochemical purities (RCP, >95%) and molar activities (A_m = 40 GBq/μmol). ^{18}F-labeling resulted in radiochemical yields (RCY) of 10–30% and molar activities of A_m ~85 GBq/μmol. Simple substitution of a (*R*)-DOTAGA by a DOTA moiety led to a noticeably enhanced CCK-2R affinity (~4-fold improved IC_{50} values) for almost all ^{nat}Lu-labeled compounds, pointing to the negative influence of the additional negative charge at the chelator moiety. Concomitantly, the most affine (IC_{50}: 4.7 ± 0.6 nM) compound, [^{19}F]F-[^{177}Lu-]Lu-DOTA-rhCCK-18 ([^{177}Lu]Lu-DOTA-D-Dap([^{19}F]F-SiFA)-(D-Glu)$_8$-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2) also revealed the highest internalization values at 6 h (~250% of reference ligand [^{177}Lu]Lu-DOTA-PP-F11N) as well as an favorable lipophilicity (log$D_{7.4}$: − 2.69 ± 0.06). *In vivo* at 24 h p.i., [^{19}F]F-[^{177}Lu]Lu-DOTA-rhCCK-18 revealed 13-fold increased activity levels in the tumor as compared to [^{177}Lu]Lu-DOTA-PP-F11N (25.4 ± 4.7 *versus* 1.9 ± 0.8%ID/g), but also massively elevated retention levels in the kidneys (134 ± 18 *versus* 3.1 ± 0.5%ID/g). At 1 h p.i., high activity levels were found for [^{19}F]F-[^{177}Lu]Lu-DOTA-rhCCK-18 in the tumor (24.1 ± 4.2%ID/g) and the kidneys (97.2 ± 14.0% ID/g), whereas overall non-tumor organ uptake was low. μSPECT/CT imaging studies at 1 h p.i. with the chemically identical [^{18}F]F-[^{nat}Lu]Lu-DOTA-rhCCK-18 underlined these results, rendering this compound a valuable asset for imaging of medullary thyroid carcinoma (MTC) when ^{18}F-labeled.

Conclusion: Substitution of a (*R*)-DOTAGA by a DOTA moiety in rhCCK ligands led to a noticeably increased CCK-2R affinity and receptor-mediated internalization, which resulted in distinctly improved activity levels in the tumor for [^{19}F]F-[^{177}Lu]Lu-DOTA-rhCCK-18 at 1 and 24 h p.i. However, elevated kidney retention might be a limiting factor when ^{177}Lu-labeled. Nevertheless, the high kidney uptake is of lesser concern when the rhCCK ligand is ^{18}F-labeled, which is why [^{18}F]F-[^{nat}Lu]Lu-DOTA-rhCCK-18 might surpass the detection rate of currently clinically applied imaging agents for MTC such as ^{68}Ga- or ^{111}In-labeled CCK-2R targeted compounds.

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