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Development and First-in-Man Study of a Novel Tetrapeptidic CCK-2R-Targeted Compound with Improved Metabolic Stability and Pharmacokinetics

Thomas Günther[1], Nadine Holzleitner[1], Georgine Wienand[2], Nicole Urzt-Urban[1], Constantin Lapa[3], Hans-Juergen Wester[1]
[1]*Technical University of Munich, Germany,* [2]*Augsburg, Germany,* [3]*University of Augsburg, Germany*

Objectives: In order to develop an improved CCK-2R ligand for imaging and radioligand therapy of medullary thyroid carcinoma (MTC), we synthesized and evaluated the novel ligand DOTA-CCK-66 (DOTA-γ-glu-PEG_3-Trp-(*N*-Me)Nle-Asp-1-Nal-NH_2). Its design is based on the four *C*-terminal amino acids of DOTA-MGS5 (DOTA-glu-Ala-Tyr-Gly-Trp-(*N*-Me)Nle-Asp-1-Nal-NH_2), a γ-glu-PEG_3 linker and a DOTA moiety for labeling with radiometals. As theranostic applications are desired, we carried out a comparative study on ^{64}Cu- ^{67}Ga- and ^{177}Lu-labeled DOTA-CCK-66 as well as DOTA-CCK-66.2 (glu instead of γ-glu) and the reference, DOTA-MGS5, by state-of-the-art experiments *in vitro* and *in vivo*.

Methods: ^{64}Cu- and ^{67}Ga-labeling was carried out at 90\xB0C within 15 min (1.0 M sodium acetate buffer, *pH* = 5.5, and 2.5 M HEPES buffer, respectively). ^{177}Lu-labeling was carried out at 90\xB0C within

15 min (1.0 M sodium acetate buffer, *pH* = 5.5, 0.1 M sodium ascorbate). CCK-2R affinity (*IC*50) was examined on AR42J cells. Studies on the metabolic stability were conducted *in vitro* (human serum, 37\xB0C, 72 \xB1 2 h) and *in vivo* (murine serum and urine, 30 min post-injection (p.i.)). Biodistribution studies and μSPECT/CT imaging at 1 and 24 h p.i. were carried out in AR42J tumor-bearing CB17-SCID mice.

Results: Synthesis via Fmoc-based solid-phase peptide synthesis yielded 5–10% reversed-phase liquid chromatography-purified labeling precursor. All labelings proceeded almost quantitatively. Irrespective of the nuclide (copper, gallium, lutetium) used, high CCK-2R affinity was determined for all three compounds (IC_{50}: 3.6–6.0 nM). Lipophilicity (expressed as *n*-octanol/phosphate-buffered saline solution distribution coefficient; log$D_{7.4}$) was similarly low for all three CCK-2R ligands, irrespective if ^{64}Cu- ^{67}Ga- or ^{177}Lu-labeled (log$D_{7.4}$: −3.0 to −2.2). The amount of intact tracer was high for both [^{177}Lu]Lu-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-CCK-66 *in vitro* in human serum (95–98%) and *in vivo* in murine serum (79–82%) but distinctly higher for the latter in murine urine (23.7 ± 9.2% *versus* 77.8 ± 2.3%).

[^{177}Lu]Lu-DOTA-MGS5 exhibited slightly increased activity levels in the tumor at 24 h p.i. than [^{177}Lu]Lu-DOTA-CCK-66 (11.0 ± 1.2%ID/g versus 8.6 ± 1.1%ID/g) but also elevated levels in all other organs, which is why overall tumor-to-background ratios were similar for both compounds. Beyond that, [^{67}Ga]Ga-DOTA-CCK-66 exhibited high tumor accumulation at 1 h p.i. (19.4 ± 3.5%ID/g) and low uptake in non-tumor organs (in %ID/g; blood: 0.61 ± 0.07, liver: 0.31 ± 0.02, pancreas: 0.23 ± 0.07, stomach: 1.81 ± 0.19, kidney: 2.51 ± 0.49). [^{68}Ga]Ga-DOTA-MGS5 displayed slightly higher tumor values (23.2 ± 4.7%ID/g) but also significantly higher activity levels in all non-tumor organs (in %ID/g; blood: 1.47 ± 0.82, liver: 1.02 ± 0.52, pancreas: 1.83 ± 0.42, stomach: 5.12 ± 1.13, kidney: 5.71 ± 1.38), which is why tumor-to-background ratios were noticeably higher for [^{67}Ga]Ga-DOTA-CCK-66 than for [^{68}Ga]Ga-DOTA-MGS5 [1]. μSPECT/CT studies confirmed the excellent biodistribution of [^{67}Ga]Ga-/[^{177}Lu]Lu-DOTA-CCK-66.

A proof-of-concept study using [^{68}Ga]Ga-DOTA-CCK-66 in a patient suffering from MTC revealed a favorable biodistribution of the tracer at 60 min p.i., which was highly accumulated in several MTC metastases.

Conclusion: Due to its simple design and thus improved metabolic stability, [$^{nat/67}$Ga]Ga-/[$^{nat/177}$Lu]Lu-DOTA-CCK-66 revealed favorable *in vitro* and *in vivo* data. In particular, similar (^{177}Lu-labeled) or even higher ($^{67/68}$Ga-labeled) tumor-to-background ratios were observed in all organs as compared to [^{177}Lu]Lu-/[^{68}Ga]Ga-DOTA-MGS5. A first-in-man study in a MTC patient using [^{68}Ga]Ga-DOTA-CCK-66 showed several metastases and an excellent biodistribution pattern, which is why further patient studies applying [^{68}Ga]Ga- and [^{177}Lu]Lu-DOTA-CCK-66 are warranted.

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Reference

1. Klingler et al., J Nucl Med. 2019;60:1010–1016.

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