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Influence of 99mTc-chelation at N-terminal and/or C-terminal on receptor binding affinity of NGR peptides

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Objective: The NGR peptide has high affinity towards aminopeptidase receptors (APN or CD13 receptors) upregulated in tumor angiogenic blood vessels as well as in melanoma, ovarian, prostate, lung, and breast tumors. This study aimed at determining the influence of modification of NGR peptide at either N- or C-terminal on the receptor binding affinity. Tridentate ligand scaffolds were introduced at N- or C-terminal of NGR peptide via click chemistry for radiolabeling with 99mTc(CO)3-precursor. In vitro and in vivo evaluation of N- and C-terminal radiometalated peptides was performed to determine the effect on receptor binding affinity.
Methodology: The N- and C-terminal azide-functionalized NGR peptides, K(N3)c(CNGRC)G-CONH2 (1a) and c(CNGRC)K(N3)G-CONH2 (2a) were synthesized manually by standard Fmoc solid phase peptide synthesis protocol. For N-terminal modification CNGRCG sequence was first assembled on the solid phase followed by coupling of Fmoc-Lys(N3)-OH at the N-terminus. The C-terminal modification of NGR peptide was performed by loading Fmoc-Gly-OH as the first amino acid on the solid phase followed by Fmoc-Lys(N3)-OH. Further CNGRC sequence was assembled and disulphide bridge was formed by cyclization of cysteine sulphides. Subsequent to cleavage from the solid phase and purification by semi-preparative HPLC, the azide peptides were subjected to click reaction with propargyl glycine in solution phase to synthesize peptide constructs K(Pra-Tz)c(CNGRC)G-CONH2 (1b) and c(CNGRC)K(Tz-Pra)G-CONH2 (2b) respectively, containing a tridentate chelating unit for radiolabeling with [99mTc(CO)3]+ core. Peptide constructs were characterized by mass spectroscopy. The radiotracers, 99mTc(CO)3-K(Pra-Tz)c(CNGRC)G-CONH2 (1c) & 99mTc(CO)3-c(CNGRC)K(Tz-Pra)G-CONH2 (2c) were analyzed by HPLC and evaluated for in vitro receptor affinity in murine melanoma B16F10 cells and in vivo pharmacokinetic behavior was determined in C57BL/6 mice bearing melanoma tumor.
Results and discussion: The N- and C-terminal azide-NGR peptides (1a and 2a) synthesized manually by solid phase peptide synthesis were obtained in an overall yield of 27% and 31% respectively; with >98% purity. Radiometalation of peptide constructs 1b and 2b resulted in formation of neutral 99mTc(CO)3-NGR complexes, 1c and 2c respectively with >95% radiochemical purity. The C-terminal modified peptide exhibited higher binding affinity towards B16F10 cells in comparison to N-terminal modified peptide (IC50 values 1b: 136 ± 2.3 nM; 2b: 65 ± 1.7 nM). The C-terminal construct 2c exhibited higher uptake in murine melanoma B16F10 cells during in vitro studies. In vivo tumor uptake of 2c was higher than of N-terminal modified peptide construct 1c at 2 h p.i. (2.4 ± 0.5 vs 1.9 ± 0.3% ID/g respectively). Blocking studies carried out by co-injection of cNGR peptide led to ~50% reduction in the tumor uptake at 2 h p.i. suggesting receptor-mediated uptake of radiotracers. Both the radiotracers exhibited rapid urinary excretion and cleared from all the major organs (heart, lungs, spleen, stomach and blood) at 4 h p.i. (<1% ID/g).
Conclusion: The N- and C-terminal modified peptide constructs could be radiometalated with 99mTc(CO)3 core in good radiochemical yield (>95%). However higher in vitro cell uptake and in vivo tumor uptake was observed for the C-terminal construct illustrating the preferred conjugation of drugs, dyes, radiometals etc. at C-terminus of NGR peptide.

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