

Co-complexation by histidine residues improves tumour targeting of ^{99m}Tc -HYNIC peptide conjugates

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Introduction A common method of technetium-99m (^{99m}Tc) peptide attachment involves the use of a hydrazinonicotinic acid (HYNIC) bifunctional ligand, which acts as a linker between radioisotope and peptide. A coligand is needed to complete the ^{99m}Tc -HYNIC complex, and ethylenediaminodiacetic acid (EDDA) is commonly used due to the resulting high complex stability (1). This research is focused upon the further development of a tumour imaging ^{99m}Tc -HYNIC-peptide by testing the hypothesis that a histidine within the peptide sequence could act as an additional monodentate ligand, coordinate with the ^{99m}Tc atom, and thereby increase the stability of the ^{99m}Tc -EDDA-HYNIC complex, thus improving the biodistribution profile. Two peptides were synthesised with and without histidine in the sequence using a gastrin analogue termed 'Nanogastrin' (NG), which binds to the CCK-2 receptor over-expressed on certain tumours.

Methods The peptides were labelled with Tc-99m using EDDA as coligand and their stability was tested using HPLC and size exclusion chromatography. The ^{99m}Tc -ligand binding affinity and internalisation were tested in vitro upon AR42J cells, and the biodistribution was tested by using imaging and biodistribution studies in AR42J-bearing CD1 nude mice xenografts at 4 h post injection.

Results Both peptide derivatives showed high complex stability upon dilution in PBS and plasma, >98% and >97% radiopurity respectively. Competition assays showed high CCK-2 receptor affinity for all conjugates under study (>10 nM). The percentage of total activity receptor bound and the internalisation of the ^{99m}Tc -EDDA-HYNIC-($^2\text{gly}^3\text{his}$)NG peptide was consistently higher than ^{99m}Tc -EDDA-HYNIC-NG, see Graph 1/2. Biodistribution of both peptides revealed a rapid elimination from most organs and mainly renal excretion. At 4 h pi the peptide containing the histidine side chain showed a reduction in liver (0.38 ± 0.06 vs 0.53 ± 0.09 %ID/g) and intestinal retention (0.61 ± 0.25 vs 0.96 ± 0.36 %ID/g), and an increase in tumour retention (2.08 ± 0.34 vs 1.39 ± 0.13 %ID/g), see table-1. The imaging studies supported the biodistribution data, see image-1/-2. Co-injection of unlabelled NG showed that the majority of tumour uptake was receptor specific.

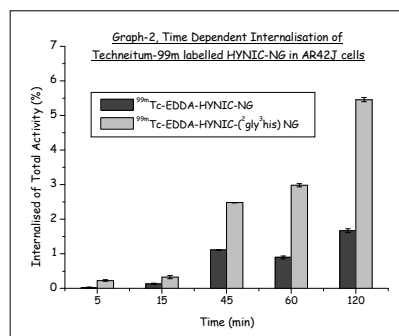
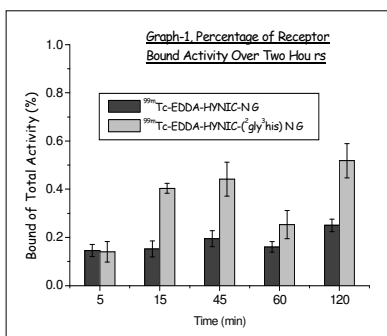
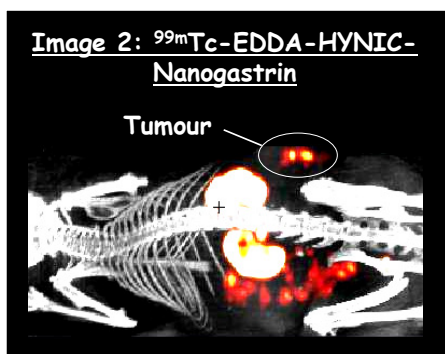
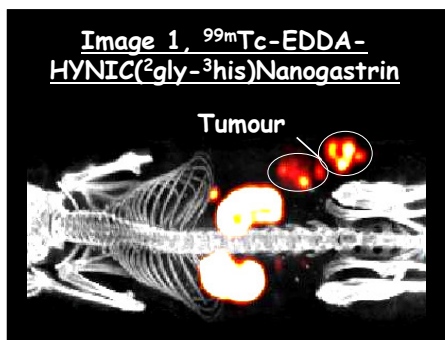


Table-1, ^{99m}Tc -HYNIC-EDDA-NG biodistribution in CD1 nude mice, (n=3) 2 d.p.

Post Injection	4 hr	4 hr (blocked)	4 hr	4 hr (blocked)
Ligand	NG	NG	(Gly-His)NG	(Gly-His)NG
Liver	0.53 ± 0.10	0.25 ± 0.05	0.38 ± 0.06	0.25 ± 0.03
Kidneys	9.48 ± 0.58	7.25 ± 1.97	9.03 ± 1.84	11.20 ± 0.93
Tumour	1.39 ± 0.13	0.53 ± 0.08	2.09 ± 0.34	0.73 ± 0.13
Blood	0.24 ± 0.05	0.12 ± 0.04	0.20 ± 0.04	0.18 ± 0.03
Stomach	0.76 ± 0.29	0.21 ± 0.15	0.53 ± 0.10	0.19 ± 0.04
Spleen	0.32 ± 0.08	0.10 ± 0.01	0.18 ± 0.03	0.10 ± 0.03
Intestine	0.96 ± 0.36	0.96 ± 0.44	0.61 ± 0.25	0.33 ± 0.05
muscle	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	0.08 ± 0.03
pancreas	0.16 ± 0.03	0.08 ± 0.13	0.20 ± 0.03	0.11 ± 0.01

Conclusion The insertion of a $^2\text{gly}^3\text{his}$ in to the nanogastrin sequence did not alter the stability of the technetium-99m-EDDA-HYNIC complex in vitro, but in vivo the ($^2\text{gly}^3\text{his}$)NG derivative showed a reduction in liver and intestinal uptake. This may be due to an increased complex stability or the alteration of the pharmacokinetics. In vitro cell studies showed an improvement in the tumour cell internalisation/retention, which translated in vivo to a higher percent of ID/g in the tumour.

References

1. Von Guggenberg E, Behe M, Behr TM, Saurer M, Seppi T, Decristoforo C. ^{99m}Tc -labeling and in vitro and in vivo evaluation of HYNIC- and (Nalpha-His)acetic acid-modified [D-Glu1]-minigastrin. *Bioconjugate Chem.* 2004 Jul-Aug;15(4):864-71.