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RHENIUM (Re) AND TECHNETIUM (Tc)-99M OXOCOMPLEXES OF NEUROTENSIN(8-13)

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SUMMARY

Radio-labelled Neurotensin (NT) analogues have applications as potential tumour imaging agents. In this study, the $N_2N'S$ chelator dimethylGly-Ser-Cys (acetoamidomethyl)-Gly (RP414) was attached to the N-terminus of four different NT(8-13) analogues. DimethylGly-Ser-Cys(acetoamidomethyl)-Gly (RP414) co-ordinates strongly to MO^{3+} (M = Tc or Re) making this chelator ideal for labelling NT with either Tc-99m or Re. One step labelling at room temperature with Tc-99m was performed using stannous gluconate at pH 5. Labelling yields > 98% were obtained within 1 hour. Re (V) oxo complexes were synthesised in a two-steps synthesis including deprotection of the chelator using mercuric acetate followed by complexation with Re using bisethylenediamino dioxorhenium (V)chloride ($ReO_2(en)_2Cl$). All Re(V)oxo-NT analogues showed *in vitro* half-lives in plasma of between 20 and 30 minutes. Inhibition of the binding of ^{3}H-NT on HT29 colon adeno carcinoma cells yielded K_i values of 1.0 nM for NT(1-13) and 0.8 nM, 5.5 nM and 4.0 nM for the Re(V)oxo complexes of RP414-Arg-Arg-NT(10-13), RP414-Lys-Arg-NT(10-13) and RP414-Lys-Lys-NT(10-13) respectively.

Keywords: Neurotensin, Rhenium, Technetium, *in vitro.*

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INTRODUCTION

NT is a linear tridecapeptide (pGlu-Leu-Tyr-Gln-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH) (Figure 1) being first isolated from bovine hypothalamus and small intestine [1-2].

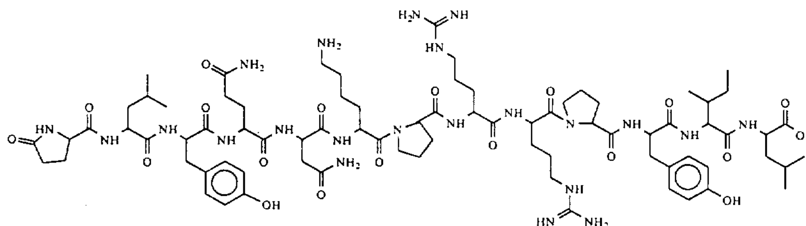

Figure 1 . Structure of Neurotensin

Functional characterisation revealed the involvement of NT in intracellular communication in the central nervous system and the gut. More recently, NT has been defined as a potential growth factor in different human cancer cell lines and tumours. NT receptor (NTR) expression is seen in 75% of all ductal pancreatic tumours [3] and in many cases of small cell lung carcinomas. Consequently, radio-labelled NT may act as an attractive vector for tumour targeting, a strategy already successfully explored for the somatostatin analogue Octreotide that has become a routine radiopharmaceutical in nuclear medicine. Radio-labelled peptides are seen as powerful alternatives to monoclonal antibodies showing slow blood clearance, an important drawback in scintigraphy of target specific tumours. Our group has already reported on the development and the pre-clinical evaluation of different radio-labelled analogues of the biological active C-terminal hexapeptide of NT, NT(8-13) [4-8]. The advantages of using ^{99m}Tc in diagnostic nuclear medicine are well known and numerous techniques have been developed for labelling biological molecules with ^{99m}Tc. Moreover, as Re possesses similar chemical properties to those of ^{99m}Tc, it can be provided as a non-radioactive alternative to working with ^{99m}Tc-radioisotopes. This study reports on the development of the first Tc-99m and Re labelled NT(8-13) analogues using dimethylglycine-L-serine-L-cysteine (aceto amido methyl (ACM))-glycine (RP414) as a bifunctional chelator (Figure 2).

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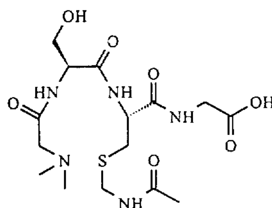

Figure 2. Structure of dimethylGly-Ser-Cys(ACM)-Gly (RP414)

RESULTS AND DISCUSSION

RP414 ® (Resolution Pharmaceuticals, ON-Canada) has been described as an effective chelator for labelling biomolecules with Tc-99m and Re [9]. RP414 was prepared via a solid phase peptide synthesis method on an Applied Biosystems Inc. Model 433A peptide synthesiser using Sasrin resin and Fmoc protected amino acids. The cysteine thiolate was protected with an acetoamidomethyl (ACM) group. The amino acids of NT(8-13) were linked sequentially to the chelator after removing Fmoc protection groups with 15% of piperidine in 1N 2-Methyl-pyrrolidone (NMP). Four different NT(8-13) analogues with either Arg or Lys in position 8 and 9 were synthesised. Amino acid residues were activated with 0.45M O-(1H-Benzotriazol-1yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 0.45M 1-hydrobenzotriazole (HOBT) in dimethyl formamide (DMF) in the presence of di-isopropyl ethylamine (DIEA). Peptides were cleaved off the sasrin resin using 95% aqueous trifluoroacetic acid. The sasrin resin was removed by filtration. HPLC purification and lyophilization followed precipitation of the crude peptide in tert-butyl methyl ether at 0°C. The following NT(8-13) analogues were synthesised and purified: RP414-Arg-Lys-Pro-Tyr-Ile-Leu (RP497), RP414-Arg-Arg-Pro-Tyr-Ile-Leu (RP498), RP414-Lys-Arg-Pro-Tyr-Ile-Leu (RP499) and RP414-Lys-Lys-Pro-Tyr-Ile-Leu (RP500). Quality control was performed with HPLC, electrospray-MS and amino acid analysis. Analogues were kept at –70°C.

Complexation with non-radioactive Re was performed on 8.5×10^{-5} mol (100 mg) of peptide dissolved in 5ml of 30% acetic acid. First, the acetoamidomethyl protection group was removed by adding 16×10^{-5} mol of mercuric acetate (50mg) to the solution. Argon gas was bubbled through the reaction mixture for 1 minute. The reaction mixture was kept at room temperature for 8h. H_2S gas was bubbled through the solution for 5 minutes, causing black HgS to precipitate. The precipitate was removed using a Centriprep 10 concentrator (cut off: 10,000 Dalton). The filtrate was frozen

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and lyophilised overnight. The resulting residue was used immediately for Re-complexation using $ReO_2(en)_2Cl$ (in house: 3.5 mmol potassium perrhenate ($KRe(V)O_4$, Aldrich) and 12 mmol triphenyl phosphine ($(C_6H_5)_3P$, Aldrich) are stirred and warmed in 10ml of ethanol (Merck) containing 1ml of contentrated HCl for 8h under Argon followed by the addition of 2ml of ethylene diamine (Merck) and filtration after 20minutes). The peptide was dissolved in 3ml of bidistillated water and 0.1 mmol (40mg) of the Re starting material was dissolved in 2ml of distilled water. The two solutions were combined to give a light green solution. The pH of the solution was adjusted to 5.5-6 using 1M NaOH. The solution was heated at 50-55°C for 6-8h leading to a yellow solution and later on a red one. After the reaction, the mixture was frozen in liquid N_2 followed by overnight lyophilisation. The dry Re-complexed peptide was further purified by HPLC, electrospray-MS and analytical HPLC were performed as quality control.

Labelling with Tc-99m was performed using 0.2 μmol of peptide (200μg) dissolved in 200μl of saline. 400MBq of $Na[^{99m}TcO_4]$ were added to the solution, followed by tin(II) chloride (0.2μmoles) and sodium gluconate (6μmoles). The pH was adjusted to 4.5-5 by adding 20μl of 0.1N NaOH giving a total reaction volume of 420μl. The solution was left at room temperature for 1hour. During the reaction, the acetoamidomethyl protection group was displaced from the cysteine thiolate. The radiochemical purity was evaluated by HPLC (Vydac RP18 for Proteins and Peptides 4.6mm*250mm) utilising a 0-70% gradient (A: H_2O/TFA 100/0.1 B: ACN/TFA 100/0.1) over 40 minutes. Radiochemical yields of 98% were obtained. The Re and ^{99m}Tc complexes of NT(8-13) were co-injected into the HPLC showing identical capacity factors (k', where k' is calculated as $(t_R - t_0) / t_0$ (10)). Figure 3 shows the UV and radiometric plots of the HPLC co-injection of ^{99m}TcO and ReO (dotted line) complexed RP414-Arg-Arg-NT(10-13) analogues showing k' values of 4.9 for both analogues.

In vitro plasma stability testing was performed on all ReO and ^{99m}TcO-NT(8-13) analogues. Biological half-lives between 20 and 30 minutes were observed for all analogues whereas parent NT was shown to have *in vitro* plasma half lives of 10 minutes. As expected from the enzymatic activity of different metallo-endopeptidases [11], HPLC analysis revealed 1 major metabolite, the Re or ^{99m}Tc complex of RP414-Arg or RP414-Lys.

Preliminary binding experiments with all RP414-NT(8-13) analogues were performed using HT29 colon adenocarcinoma cells (Universiteit Gent, Belgium and Janssen Research Foundation Beerse, Belgium). The competition of the binding of 1nM of ^3H-NT (NEN, Belgium) was undertaken. Cells were incubated in a modified

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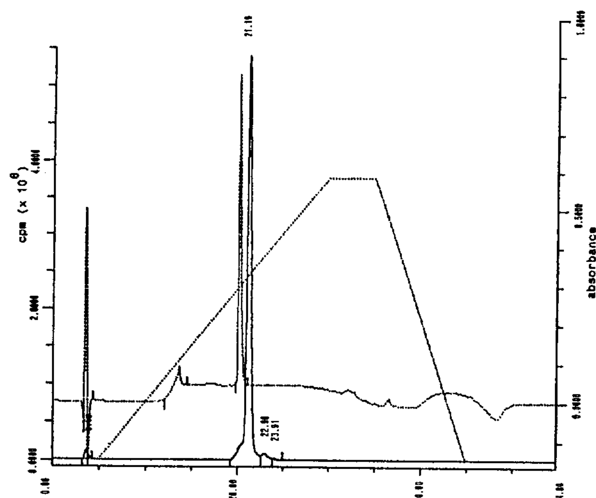

Figure 3. HPLC co-injection of ReO-RP414-Arg-Arg-NT(10-13) (Dotted line) and ^{99m}TcO-RP414-Arg-Arg-NT(10-13).

Krebs-Ringer Hepes buffer (KRB) (111mM NaCl, 4mM KCl, 2.5mM $CaCl_2$, 1.2mM $MgSO_4$, 1.2mM KH_2PO_4, 20mM Hepes, 0.1% Glucose, 1mM EDTA and 0.1mM Bovine Serum Albumine) at pH 7.4 at a final concentration of 5×10^6 cells per ml. For binding experiments, 2×10^6 cells were incubated with 50µl of ^{3}H-NT (1nM in KRB) together with 50µl of competitor (10^{-5}M to10^{-11}M in KRB). Incubation at 25°C for 30minutes was followed by rapid filtration under reduced pressure through pre-soaked (2h in pH 7.4 buffer added 1µM of NT) Whatman GF/B glass fiber filters. Filters were rinsed twice with 2ml of Krebs Ringer Hepes buffer without serum. Filters were placed in plastic scintillation vials containing 2ml of Instagel Gold MV Scintillation fluid. Counting was performed in a Packard Scintillation spectrometer. Inhibition constants (K_i) were calculated using the Cheng and Prusoff equation (K_i = $IC_{50}\times K_d/(K_d+L)$) with K_d = 3.6nM (dissociation constant obtained from equilibrium binding experiments) and L = 1nM (the concentration of ^{3}H-NT). K_i values for all (ReO)-RP414 analogues are shown in Table 1.

CONCLUSION

RP414 is a bifunctional chelator which enables NT(8-13) analogues to be labelled with either Re or ^{99m}Tc-labelling without affecting receptor affinity. ^{99m}Tc

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Analogue	**K_i *(nM)**
NT(1-13)	1.0 (0.5)
RP414-Arg-Arg-NT(10-13)	1.5 (0.5)
RP414-Arg-Lys-NT(10-13)	2.4 (1.0)
RP414-Lys-Arg-NT(10-13)	3.9 (1.4)
RP414-Lys-Lys-NT(10-13)	2.4 (1.0)
ReO-RP414-Arg-Arg-NT(10-13)	0.8 (0.5)
ReO-RP414-Arg-Lys-NT(10-13)	3.0 (0.8)
ReO-RP414-Lys-Arg-NT(10-13)	5.5 (0.8)
ReO-RP414-Lys-Lys-NT(10-13)	4.0 (1.1)

* Means of four different experiments (standard deviations)

Table1. Competitive binding of 1nM of ^{3}H-NT on 2×10^6 HT29 cells : Ki-values.

labelled RP414-NT(8-13) might find application as a potential new ligand for tumour targetting in combination with SPET. Labelling RP414-NT(8-13) analogues with Re186 or Re188 could lead to the development of a new potential radiopharmacon for radiotherapy of NT receptor positive tumours.

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REFERENCES

[1] Carraway R., S.E. Leeman. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J. Biol. Chem.* 248: 6854-6861, 1973.

[2] Kitabgi P., R. Carraway, S. E. Leeman. Isolation of a tridecapeptide from bovine intestinal tissue and its partial characterization as neurotensin. *J. Biol. Chem.* 251: 7053-7058,1976.

[3] Reubi J.C., B. Waser, H. Friess. Neurotensin receptors : a new marker for human ductal pancreatic adenocarcinoma. *Gut*, 42(4) : 546-50, 1998.

[4] Terriere D., K. Chavatte, R. Boumon, D. Tourwé, J. Mertens. Radiosynthesis of a new 111In-Neurotensin analogue . *Eur. J. Nuc. Med*, 30.6, 902, 1997.

[5] Chavatte K., D. Terriere, W. Gommeren. Evaluation of new stabilised Neurotensin (8-13) analogues for SPET. *Abstract book of the conference on achievements and prospects of new radiotracers (Cost Action B) FZ Jülich D*, C1, 1997.

[6] Tourwé D., J. Mertens, M. Ceusters. Synthesis of metabolically stabilised peptides and their radiolabelling : new ^{111}In- and *I-Neurotensin analogues. *Tumor Targeting*, 3 , 41-45, 1998.

[7] Terriere D., K. Chavatte, M. Ceusters. Radiosynthesis of new radio Neurotensin (8-13) analogues. *J. Lab. Comp & Pharm*. XLI – 1, 19-27, 1998.

[8] Chavatte K., D. Terriere, W. Gommeren. Labelling and Evaluation of New Neurotenisin (8-13) analogues for SPET. *International Symposium on Modern Trends in Radiopharmaceuticals in Diagnosis and Therapy, Lisbon P, IAEA98 Book for Extended Synopses and congress proceedings*, 1998, in press.

[9] Wong E., T. Fauconnier, S. Bennett. Rhenium (V) and Technetium (V) oxo complexes of an N_2N'S peptidic chelator : Evidence of interconversion between the syn and anti conformations. *Inorg. Chem.*, 36 : 5799 – 5608, 1997.

[10] Snyder L.R. and J.J. Kirkland. Introduction to Modern Liquid Chromatography, 2nd edition, *Wiley-& Sons, Inc. New York* : 23-24, 1979.

[11] Lee Y.C., L.O. Uttenthal, H.A. Smith and S. R. Bloom. In vitro Degradation of Neurotensin in Human Plasma. *Peptides*, 7, 383-387, 1986.

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