Development of Radioactively Labelled Cancer Seeking Biomolecules for Targeted Radiotherapy

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INTRODUCTION

The main objective of this project is the labelling and quality control of biomolecules with radionuclides beta emmiters with high purity and stability. Techniques and methodology for labelling with ^{188}Re, ^{153}Sm, and ^{125}I as well as quality controls were done according the recomendations of the first research coordination meeting of this CRP. Also some usefull chelators reported in the literature were synthetized in our laboratory.

Experimental Results

Labelling and Quality Control of Biomolecules With ^{188}Re

For this purpose $^{188}W/^{188}Re$ generators were received from MAP, Finland and evaluated until six month after calibration, considering the quality parameters for its use in therapy. The results indicate that elution yield was 72±4% for 10/98 generator and 70±1% for 06/99 generator, elution profile showed the maximum of activity in the first three mililliters (88% of total activity). The pH was according with specifications and chromatography in saline and methylethylketone (MEK) as solvents on Whatman N°1 as support showed a radiochemical purity (RQ.P) higher than 99%. Level of radionuclidic impurities was investigated with a multichannel analyzer coupled with a NaI(Tl) 3'' detector with an efficiency of 1,76% for photons of 290 keV and 8,8% for photons of 155 keV. The presence of low amounts of ^{60}Co ($2,3x10^{-4}$ uCi/mL of eluate; $1,6x10^{-5}$%) and ^{134}Cs ($2,5x10^{-5}$ to $1,4x10^{-4}$ uCi/mL of eluate; $4,3x10^{-6}$ to $5.9x10^{-6}$%) were detected altough these values remains under the levels admited according to the generator specifications. The presence of ^{188}W ranged from 5,2 to $9,6x10^{-3}$ %. Chemical controls showed that levels of Al^{3+} were in all cases lower than 5 ppm.

Lanreotide provided by the IAEA, a peptide with excellent characteristics for uptake by somastotatin receptors was labelled by direct method with ^{188}Re in the form of perrenate obtained from a generator previously eluted two days earlier. Different methodologies were used. Of them the most promising in our laboratory conditions was with the use of SnF_2 as reducing agent and HEDP as transchelating agent at pH 2-3. For molar ratios HEDP/lanreotide and SnF_2/lanreotide of 260 and 40 respectively, the labelling yield determined by different chromatography systems were more than 90%. Sep-pak (C18) purification allow the separation of $^{188}ReO_4^-$ and ^{188}Re colloid with good yield. Stability of the purified peptide at room temperature revealed that after 24 hours the RQ.P is in the order of 90%. In the case of non purified peptide the decrease of RQ.P is to about 80%. Specific activity of labelled lanreotide was about $1,74x10^{14}$ Bq/mol.

During the expertise of Dr. Chinol, experiences for labelling IgG bovine (Sigma Chemical Co.) were also carried out. The immunoglobulin was reduced with 2-mercaptoethanol (equal volume of 10% solution) by incubation at room temperature during 30 minutes and purification by PD-10 column (Sephadex G-25) eluting with acetate saline buffer 0,05N pH: 5,3. Reduced IgG (40ug/mL) was added to 1 mL $^{188}ReO_4^-$ (OAK Ridge) in the presence of $SnCl_2.H_2O$ (17umoles) in a final pH=3,5. Different incubation conditions were tested but ITLC-SG chromatography control systems indicated very low yield of labelling (less than 20%) and very poor stability of the labelled compound.

Labelling and Quality Control of Biomolecules with ^{153}Sm

Samarium chloride ($^{153}SmCl_3$) in HCl 1N with specific activity: 159,94 mCi/mg Sm^{3+}, was received from IPEN, Perú, in the scope of ARCAL programme. The different biomolecules investigated were:

a) Biotin: Based on the strategy of administration of biotin conjugated Mabs followed by avidin and final addition of biotin labelled with a beta emiter, the labelling of EDTA-Biotin (EB1) (gift from Silvia Castiglia, CNEA, Argentina) was investigated. Different ratios of reagents, incubation conditions and thin layer chromatography as well as HPLC (radiometric and UV detection at 280 nm) were evaluated.

The labelling yield for heating at 80°C during 10 minutes was determined as:

Molar ratio EB1:^{153}Sm	1,4:1	10:1	20:1
% Labelled molecule	51±2	82±8	92±2

The influence of temperature for molar ratio EB1: ^{153}Sm 20:1 and 10 minutes incubation time was:

Temperature (°C)	20	37	80	100
% Labelled molecule	86±4	88±2	92±2	88±2

ITLC-SG chromatography in the different solvent systems assay: a) ethanol:amoniac:water (3:1:5), b) methanol:amoniac:water (10:1:20) and c) saline allowed to resolve the labelling yield of ^{153}Sm-EB1. HPLC in TSK-Gel column, Type G3000SW$_{XL}$ 7,8 mm x 300 mm with isocratic program, flow 1 mL/min with phosphate buffer 0,1M, pH 6.9, sodium azide 0,01% with and without EDTA 0,002M permits to resolve the species ^{153}Sm-acetate from ^{153}Sm-EB1 and complex ^{153}Sm-EB1-avidine.

Considering all the conditions analyzed, the best yield for the labelling of ^{153}Sm-EB1 was obtained at 80°C incubation temperature during 10 minutes in a molar ratio of EB1-^{153}Sm 20:1. Up to 5 mCi of ^{153}Sm was used in the labellings. The incorporation of ^{153}Sm does not affect the binding site for avidin-biotin complex.

b) Anti-Tn Mab 83D4 (IgM) provided by LOBBM, Faculty of Medicine and IgG bovine previously conjugated to cDTPAa.

For that purpose the synthesis of cDTPA was peformed using as starting material DTPA according with Hnatowich procedure (J.Nucl.Med,1985, 26: 503). The reaction yield was more than 80% and the decomposition point of the product was 172,3 – 179,7°C. Conjugation to Immunoglobulins was done as follows: 5 mg of cDTPAa were disolved in 10 mL Chloroform, different volumes of this solution were added to 47 or 100uL of Mab83D4 1,1 mg/mL (1,2uM) to achieve a molar ratio of cDTPAa to Mab83D4 20:1, 100:1 and 2500:1. For preparation of IgG conjugate, the solution of cDTPAa in CHCL3 was added to 1mg of IgG bovine in100uL (69 mM). In both cases pH reaction was adjusted to 8,3 by adding $NaHCO_3$ 0,1M and incubated 30' at room temperature. After this, purification was done by gel filtration on PD10 column (Sephadex G-25) eluting with acetate buffer 0,2M, pH 5,5 and

measuring protein concentration by Lowry method. For IgG the protein peak was eluted in one 0,5 mL fraction after 3,5 mL of eluent were passed trough the column and corresponded to a mass of 0,5 mg of IgG (recovery 50%). HPLC profile on I-250 Waters (protein separation) column eluting with PBS 0,1M with EDTA 0,002M showed a main peak with Tr 3,69 min.

Labelling was attempted by mixing equal volumes of ^{153}Sm-acetate and protein solution obtained with the different conjugation conditions. Evaluation of labelling yield was done by ITLC in NaCl 0,9%, Methanol 85%, trichloroacetic acid (TCA) 10% and gel filtration in PD10 column. Low yields (less than 10%) were obtained in all cases.

Labelling andQuality Control of Biomolecules With ^{125}I

Breast and other kind of tumours have the characteristic of an aberrant glycosilation in the cancerous cells with important expression of Tn (N-acetylgalactosamine) structures. Mab83D4 (15 ug; $1.7x10^{-11}$ mol) was labelled with 227 to 540 uCi ^{125}I (CIS, France; 100mCi/mL; 17.400 Ci/g without reducing agents) by means of chloramine-T limiting method and evaluated in vitro against Tn structures immobilized to solid phase in order to verify its capability to recognize tham after labelling. The yield was higher than 50%, purification was done by gel filtration (PD-10) obtaining a product with RC.P higher than 90%. Specific activity was 368 Bq/fmol according to an incorporation of 5 atoms of ^{125}I per molecule of Mab. Binding studies with coated tubes with aOSM (5ug/mL), a Tn rich mucin, were done giving 28 % B/T. Biodistribution studies were performed in rats with breast tumours induced with N-nitroso methyl urea as well as in normal rats.

Biodistribution pattern

	Tumour 2 hs	Tumour 24 hs	Normal 2hs
%Activity in tumour	5.65	1.19	---
% Act/g in tumour	4.50	1.08	---
% Act/g in muscle	0.60	0.26	0.30
% Act/g in blood	4.64	1.24	4.80
% Act/g in thyroid	1.90	0.45	1.22
Tumour/muscle ratio	7.5	4.2	

Synthesis of TETA

Direct labelling of peptides or other biomolecules is not always possible so it its needed the coupling of a chelating molecule capable to be labelled with the radionuclide of interest. Due to the adequate properties of 1,4,8,11-tetraazacyclodecane-N,N',N'',N'''-tetraacetic acid (TETA). Its synthesis and control was performed according technique for DOTA synthesis reported by Desreux (Inorg. Chem., 1980, 19:1319-1324) and the advise of Dr. Varvarighou, Greece. Briefly 1g of 1,4,8,11-tetracyclo-tetradecane (cyclam) was suspended in cold water. In a three mouth balon, a solution of 2,2g of chloroacetic acetic was prepared (10% excess respect to cyclam), and neutralized with NaOH 10M in an ice bath. Cyclam is added to the balon and then heated at 80°C with stirring during 24 hs mantaining pH range between 9 and 10. After this time, mixture is cooled and acidified with HCl 12N until pH:2,5. A white precipitate is formed. Purification was carried out by cationic exchange (Dowex). The obtained precipitate is dissolved in NaOH and incubated for 1 hour with stirring in a beaker with ten-fold resin excess. Solution is then filtered and evaporated in a rotavapor. After this the product is dryed in phosphorus penthoxid desicator and analyzed by melting point IR and NMR spectra.

The synthesis of TETA was performed with 95% yield obtaining a product with melting point of 211-214°C. IR spectra showed an intense band in 1735cm^{-1} and 3413cm^{-1} corresponding to the functional groups C=O and O-H respectively. NMR peaks correspond to the structure proposed with four carboxylic groups.

Discussion and Conclusions

During this period, it could be possible to carry out different techniques and methodology for labelling molecules with three radionuclides with potential value in therapy, gaining experience in laboratory work as well as increasing national interdisciplinary collaboration, regional co-operation and expertise from other participants of the CRP. Also, synthesis of some chelating agents like cDTPAa and TETA was succesfully achieved. The preliminary results obtained in labelling ^{188}Re-lanreotide, ^{153}Sm-EB1 and ^{125}I-Mab83D4 encourage further work in this field.

Other Activities

Participation of Dr. H. Balter and Dr. S.Verdera in the laboratory work done at CNEA Argentina during in the expertise of Dr. M.Chinol by invitation of Dr. S.Castiglia. Labelling of biotin with ^{111}In and radiochemical and biological controls were performed.

List of Publications

- 153Sm-biotin as a potential radiotherapy radiopharmaceutical. B. Souto, H. Balter, G. Rodríguez, A. López, Z. Goncalvez, J. Berbejillo, S. Verdera. **Nucl. Med. Comm.** 1999, 20, 969 (abstract).
- Interacciones proteína carbohidrato: estudio de la estructura Tn. Tesis de doctorado en Química, Henia Balter, Montevideo, Uruguay, 1999.

Description of Future Tasks

Development and improvement of direct and indirect radiolabelling methods of peptides with ^{188}Re.
Standardisation of quality control methods such as ITLC, HPLC, PAA-SDS.
Biodistribution studies in tumour bearing experimental animals.
Stability studies *in-vitro* and *in-vivo*.
Start up of studies to conjugated small peptides such as lanreotide with prosthetic groups eg TETA and DOTA to be labelled.

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J. Berbejillo, A. López, Z. Goncalvez