VIA MEDICA

Nuclear Medicine Review 2004
Vol. 7, No. 2, 2004, pp. 203–218

ISSN 1506–9680

Chosen abstracts of 12th European Symposium on Radiopharmacy and Radiopharmaceuticals

1

OVERLOOKING THE SITUATION OF NUCLEAR MEDICINE IN CENTRAL AND EASTERN EUROPE

P. Lass

Department of Nuclear Medicine, Medical University, Gdańsk, Poland

This paper reviews the situation of nuclear medicine in Central and Eastern Europe, i.e. a group of 15 countries lying east of Oder River — Trieste line and west of present/planned EU eastern border. Together they count 106.6 millions of inhabitants, i.e. about a quarter of inhabitants of European Economic Space. Its nuclear medicine, however, represents less than 10% of European nuclear medicine manpower and equipment.
In those countries there are at least 245 nuclear medicine departments with 661 nuclear medicine specialists and 376 gammacameras. There are 6 PET units operable, 1 manufacturer of gammacameras, 6 radiopharmaceuticals manufacturers, 2 nuclear medicine scientific journals. The biggest and best devefoped nuclear medicine communities are in Czech Republic and Hungary, in absolute numbers to some extent also Poland.
The scientific input of those countries to European science is moderate, about 10% when measured by EANM congresses abstracts, 5% when measured by the number of papers in MEDLINE indexed journals. Mean European bibliometric parameters are approached only by Hungary and — to some extent — Czech Republic.
This survey indicates the need of International cooperation to improve the level of nuclear medicine in those countries for reaching the European standards, but also the tremendous potential which could be used for a benefit of European nuclear medicine.

2

P53 INDEPENDENT RADIATION-INDUCED BYSTANDER EFFECTS INDUCED BY RADIOPHARMACEUTICALS LABELLED WITH AUGER EMITTERS

M. Boyd, K.W. Tan, J. Dorrens, S. Ross, M.R. Zalutsky, R.J. Mairs

Introduction: Targeted radiotherapy is the selective irradiation of tumour cells by radionuclides conjugated to tumour seeking molecules. Gene therapy can expand the tumour types accessible to this type of therapy. We recently introduced the noradrenaline transporter gene (NAT) into tumour cells endowing them with the capacity for uptake of radiolabelled MIBG. Current cancer gene therapy strategies are however limited by low gene transfer efficiencies so cancer gene therapy strategies must have a component of collateral celt kilt to neighbouring cells. Utilising the radiation-induced biological bystander effect (RIBBE) to its full potential could increase therapeutic efficacy. WE adapted media transfer methodology to quantify the contribution of RIBBE to celt kill in NAT transfected cells after exposure to the emitter [1311]MIBG, the Cl-emitter [211At]MABG or the Auger emitter [1231]MIBG. The role of p53 in mediating RIBBE was investigated.
Material and methods: NAT transfected human cancer cells and their P53 null variants were irradiated using a 60Co source or by incubation with radiopharmaceutical. An amended media transfer protocol was employed to assess the magnitude of RIBBE contributing to celt kill from different targeted radiation qualities.
Results: Dose-dependent RIBBE were identified in human cancer cell lines following X-ray external beatu radiation. RIBBE were P53 independent. Treatment with [1311]MIBG, [1231]MIBG and [211At]MABG showed a substantial impact of RIBBE on cell clonogenic survival. Cell kill due to RIBBE in cells never exposed to radiation was equal to that afforded by radiopharmaceutical treatment . The level of RIBBE correlated with dose of radiopharmaceutical.
Discussion: The large RIBBE observed with radiopharmaceutical treatment of NAT transfected cancer cells and the lack of requirement for functional P53, implies the feasibility of utilising this strategy to enhance the efficacy of combined gene therapy and targeted radiotherapy.

3

CHANGES IN THE HEMATOLOGICAL-, BIOCHEMICAL PARAMETERS, IN THYROIDAL FUNCTION AND IN MICRONUCLEUS FREQUENCY IN DOGS AFTER DIFFERENT RE-188 RADIOPHARMACEUTICAL APPLICATION

L. Balogh[1], D. Máthé[1], G. Andócs[1], A. Polyák[1], R. Király[1], L. Kőrösi[1], G. Bognár[1], J. Thuróczy[2], J. Szilágyi[2], G.A. Jánoki[1]

[1]"Fodor József" National Center for Public Health, National "Frederic Joliot Curie" Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary, [2]Szt István University, Veterinary Faculty, Department of Gynaecology and Reproduction, Budapest, Hungary

Introduction: Rhenium-188 became one of the more and more frequently used radionuclid from the last decade. The labelled ligands deliver a significant whole body internat dose to different critical organs and the free form of isotope (Re-perrenate) behaves as a NaI Symporter (NIS) substrate, similarly to 99mTc-pertechnetate and the iodides. On that base the control of possible side effects is crutial after systemie or local application of 188Re-labelled radiopharmaceuticals.
Material and methods: Different Re-188 labelled ligands (188Re-(tin-)colloid, 188Re-HEDP, 188Re-DMSA(V)) and generator eluted 188Re-perrhenate were applied in a 600–1200 MBq/10 bwkg dose intravenously for 16, and locally (intraarticularly and intraoperatively via arteria hepatica) for 8 other healthy Beagle dogs. Moreover 9 spontanaeously occuring canine tumors were treated with 188Re-labelled radiopharmaceuticals. Hematological- (WBC, RBC, PLT, Ht ...), biochemical parameters (AST, ALT, ALKP, CARB, CREA), thyroxin (T4) concentrations were measured, TRH-stimulation tests and micronucleus tests were performed before-, 1 day-, 1 week-, 3 weeks and 2 months after radiopharmaceutical application. Differences of data obtained at different time were evaluated paired Student t-test and Wilcoxon matched pairs test. Internat dosimetry data were calcuiated based on whole-body gamma camera imaging (5 min, 10 min, 30 min, 1 h, 2 h, 4 h, 24 h, 48 h) ROI data using Mirdose v 3.1 computer program.
Results: All the 188Re-labelled radiopharmaceuticals showed a high labelling efficiency (96%) immediatelly after labelling and the labet stayed stable(> 90%) incubated in physiological salina and canine sera and synovial fluid until 48 h at 37°C. All the evaluated initial parameters of healthy Beagles were within the normal range but some of the tumor bearing dogs showed increased WBC, ALKP, AST or decreased RBC and Ht values. Intravenously applied 188Re-perrhenate caused significantly ($p = 0.01$) lower thyroxin values and resistance of TRH stimulation and increased micronucleus occurancy 1 week, 3 weeks and 2 months after application however no alterations were detected 1 day after perrhenate injection. One day, and 1 week after intravenously applying 188Re-(tin-)colloid, 188Re-HEDP, and 188Re-DMSA(V) we recognized significantly lower Ht, PLT and RBC values but these changes proved to be transient from the third week. Systematically and intrahepatically injected 188Re-(tin-)colloid caused a significant increase in AST, ALT and ALKP values that were normalized 1 week, 3 weeks and 2 months after treatment. No changes were detected in the evaluated parameters after intraarticularly applied 188Re-(tin-)colloid.
Conclusion: Intravenously applied high-dose 188Re-perrhenate without perchlorate prevention lead to hypothyreosis and increased micronucleus occurancy. In contrast of that 188Re-labelled radiopharmaceuticals with high and stable labelling could be applied in a safe manner even in high-dose but monitoring the critical organ parameters is essential to prevent the side effects.

4

TRACER-LEVEL CHEMISTRY OF THE ^{188}RE N GROUP FIRST CLINICAL APPLICATION TO THE TREATMENT OF HEPATOCELLULAR CARCINOMA

A. Boschi, L. Uccelli, M. Giganti, H. Turner, A. Martindale, E. Cazzola, M, Pasquali, P. Colamussi, A. Duatti

Laboratory of Nuclear Medicine, Department of Radiological Sciences, University of Ferrara, Italy, Fremantle Hospital and University of Western Australia, Fremantle WA, Australia

Introduction: Recently, we proposed a novel procedure for the efficient reduction of generator-produced [$^{188}ReO_4$]- based on the combined action of oxalate and Sn^{2+} ions [1]. We describe here the successful application of this method to the production of the first class of complexes containing a terminal ^{188}Re N group, and their use for the labeling of the iodinated oil "lipiodol" employed in the treatment of hepatocellular carcinoma (HCC).
Material and methods: The ^{188}Re N group was obtained Prom a freeze-dried kit formulation containing 2.0 mg of N-methyl, S-methyl dithiocarbazate (DTC), 0.4 mg of $SnCl_2$ $2H_2O$ and 28.0 mg of sodium oxalate ($Na_2C_2O_4$). After addition of 0.1 mL of glacial acetic acid and 1.0 mL of a [$^{188}ReO_4$)- solution (activity range 0.1 12.0 GBq), the vial was kept at room temperature for 15 min to yield the ^{188}Re N group with high radiochemical purity (98 1.5%). The ^{188}Re N group produced in this way, was successively reacted with 15.0 mg of sodium diethyldithiocarbamate (NaDEDC), in the presence of a carbonate buffer (0.5 M, pH = 9.0), at 70°C for 15 min to afford the bis-substited complex [^{188}Re(N)$(DEDC)_2$] with a final yield = 95 2.2%. Labeling of lipiodol was achieved through selective and quantitative (> 98%) extraction of lipophilic [^{188}Re(N)$(DEDC)_2$ finto this hydrophobic material. In situ administration of labeled lipiodol in HCC patients was performed through the hepatic artery using a catheter.
Results: Efficient preparation of ^{188}Relipiodol was obtained through dissolution of the radioactive complex ^{188}ReN-DEDC finto this hydrophobic material. Administration to HCC patients showed selective localization in tumours immediately following intrahepatic arterial injection. CT/SPECT imaging confirmed retention of ^{188}Re-lipiodol in the hepatoma with minimal gut uptake and no lung activity over 24 hours.
Discussion/Conclusion: ^{188}Re-lipiodol prepared using the novel kit formulation is stable in-vivo and may provide safe and effective therapy of unresectable hepatocellular carcinoma.

[1] Boschi A, Bolzati C, Uccelli L, Duatti A. Nucl Med Biol 30: 381–387.

5

ASTATINE-211: PRODUCTION, AND PROTEIN-LABELLING

G.J. Meyer, H.J. Helmeke, P. Gielow, K.H. Matzke, E. Pötter, W.H. Knapp

Klinik für Nuklearmedizin der Medizinischen Hochschule Hannover

Aim: First experiences with 211At as an α-therapeutic radionuclide [1] encouraged us to improve the production of 211At with a new target, and to develop methods for the labelling of more specific — therapeutic agents, like small peptides and antibodies.
Material and methods: We developed a horizontal target, which is irradiated by a 15° downward β-beam at our MC-35 cyclotron. It consists of a 2 cm × 4 cm ceramic-holder onto which Bi is deposited at a thickness of ca. 100Nm. This allows a quick evaporation of 211At by heating in a quartz oven. 211At can be trapped in 1 ml of cooled aqueous or organic solvents. Reaction of 211At with p-tri-butyl-stannyl-benzoic-acid-succinimidyl-ester yielded p-211At-succinimidylbenzoate [2], which after purification and identification was reacted with human IGG. All reactions were verified by analogue reactions with radioiodine, and were analyzed by DC and HPLC. In-vitro stability of 211At- IGG in serum was analyzed by gel-electrophoreses.
Results: The target tolerates a beam of at least 25NA. 211At production reaches > 90% of the theoretical yield. With a beam energy of 27.5 MeV on target, the production capacity reaches 24 MBq/NAh, leading to > 500 MBq after a 1-hour 20 NA irradiation. Recovery from the target by heating (20–900°C) under a 5 ml/min flux of nitrogen takes 30 minutes. The recovery yield in 1 ml of trapping medium is 85% for 0.1 m aqu.sulphite, 80% for H_2O, and 90% for $CHCl_3$. Preparation of p-211 At-succinimidylbenzoate was not yet optimized, and resulted in variable yields of 20–60%. Further reaction of the precursor with human-IGG was nearly quantitative with yields > 85%. The in-vitro stability of 211At-IGG in human serum at 37°C was determined by gel-electrophoresis over 14 hrs. The overall stability of the 211At-antibody bond was > 80%.
Conclusions: We developed a new target for 211At-production, which allows production and recovery of 211At in amounts suitable for therapy applications. In order to label antibodies and proteins we prepared p-211At-succinimidylbenzoate, which gave good labelling yields in test-labellings of human IGG. 211At-IGG was proven to be sufficiently stable in serum over a period of 14 hours.

[1] Petrich T et al. Eur J Nucl Med 2002; 29: 842–854.
[2] Zalutzki MR, Vaidyanathan G. Curr Pharm Des 2000; 6: 1433–1455.

6

IN VIVO ENHANCEMENT OF ANTICANCER ACTIVITY BY THE COMBINATION OF CHEMICAL AND AUGER ELECTRONS EFFECTS OBTAINED WITH $PTCl_2[^{125}I]$HISTAMINE COMPLEX

P. Garnuszek, I. Licinska

Department of Radiopharmaceuticals, The National Institute of Public Health, Warsaw, Poland

The in vivo chemotoxicity and radiotoxicity of dichloroplatinum(II)-histamine labelled with iodine-125 were investigated to ascertain the potential of new radioplatinum coordination complexes as antineoplastic agents.
Material and methods: Two murine models of transplantable cancers, i.e. mammary adenocarcinoma (MA) and colon adenocarcinoma (C38) in C3H and C57BL6 mice, respectively, were used for the experiments. The groups of animals were treated every 3–4 days with low doses of Pt(II)Cl2Histamine (C3H/MA — 1/6 of MTD, and C57BL6/C38 — 1/3 of MTD), or with the ^{125}I-labelled complex (0.5–1 MBq/ /animal each time). The solution of 15% DMF in saline was applied for the control groups. Anticancer activity was evaluated based on the variations of the relative tumour volumes of the treated mice and that of the control mice (*t*-test), and by the comparison of the median survival times (log-rank statistic).
Results: In the both tumour models, treatment with $PtCl_2$Hist and $PtCl_2[^{125}I]$Hist preparations revealed inhibiting activity on tumour growth and size in comparison to the control groups. However, significant and enhanced anti-cancer activities were observed for the radioactive complexes. The tumour growth delay factors (GDF) observed for C3H/MA model were 0.29 and 0.68 for $PtCl_2$Hist and $PtCl_2[^{125}I]$Hist, respectively. Relatively higher GDFs were observed for the colon cancer model (0.84 and 0.90, respectively), what resulted most probably due to a higher single doses being applied. A significant prolongation of the survival times of treated animals has been observed. The median survival time (read from K-M curves) for C57BL6/C38 mice treated with $PtCl_2$Hist complex was 67% longer comparing to the control groups, whereas more than 120% (value undefined) for the group treated with the radioactive complex.
Conclusion: The significant enhancement of anti-cancer activity by concomitant combination of the therapeutic factors i.e. cytotoxic/cytostatic activity of the platinum(II)-histamine and the Auger electrons effects generated by the attached I-125 radionuclide has been found.

7

MARIE SKLODOWSKA-CURIE — SCIENTIST, HUMAN, CITIZEN

J. Liniecki

Lodz, Poland

Marie Sklodowska-Curie (…) was an outstanding scientist. She was awarded twice the Nobel Prize for her research in physics and chemistry she was at Sorbonne the first women with PhD in science and a first women professor of physics Her contribution to knowledge of natural radioactivity was fundamental both form the theoretical and practical application view points. Her research and practical use of radon sources of great activity created technical background for discovery of artificial., radioactivity by Irene and Frederic Jolio-Curie. These discoveries were among the very important ones that lead later to utilisation of artificial radionuclides in all branches of science and technology. This applied also to medicine and eventual emergence of nuclear medicine.
Marie Curie contributed greatly to practical application of radiation in medicine, mostly in form of brachytherapy. This because the first method in addition to surgery, that brought obvious therapeutic benefit in oncology. She contributed also to establishment of oncological institutes in Paris, Warsaw and urany other places where fundamental and applied research was associated with clinical activity. Marie Curie trained large number of specialists from various countries in handling and use of radium sources for oncological therapy.
Marie Sklodowska-Curie was also a great citizen of France, Poland and the world. She was a champion of education and promotion of science and advocate of proper utilisation of young talents in research. Last not least she was also considered one of the leading moral authorities of her times.

8

CAN ^{99m}TC-EDDA/HYNIC-TATE REPLACE OTHER MARKERS IN DETECTION OF SOMATOSTATIN RECEPTOR-POSITIVE NEUROENDOCRINE TUMOURS?

R. Mikołajczak[1], B. Janota[1], E. Zakrzewska[1], A. Hubalewska[2], Fröss K.[2], A. Staszczak[2], B. Huszno[2]

[1]Radioisotope Centre POLATOM Otwock-Swierk, Poland, [2]Nuclear Medicine Unit, Chair and Department of Endocrinology, Medical College Jagiellonian University, Kraków, Poland

Introduction: The aim of this study was to assess the clinical usefulness of the ^{99m}Tc-labelled somatostatin analogue HYNIC-d-Phe[1]-Tyr[3]-octreotate (HYNIC-TATE) in pre- and intraoperative diagnosis of the TESR (tumour expressing somatostatin receptor).
Material and methods: Since 2002, 78 patients (27 men, 51 women, mean age 45.5 ± 16) with neuroendocrine tumour (NT) (37 carcinoids, 11 pheochromocytomas, 10 medullary ca, 12 insulomas, 8 other) have been examined using HYNIC-TATE. SPET and Whole Body Scanning were performed in all patients; ^{111}In-Octreoscan or ^{131}I-MI BG scans were done in justified cases. ^{99m}Tc-HYNIC-TATE (740 MBq) scintigraphy was performed 10 min, 4 and 24 hours after i.v. injection. ITLC-SG and SepPak C-18 were used for RCP assessment.
Results: Maximal tumour accumulation was found 4 hours after injection. In 18 out of 19 patients with histopatologicaly proved carcinoid suspected of recurrence, the metastatic lesions were detected, 1scan was negative. In 18 patients suspected of carcinoid, in 3 the disease was confirmed, accidentally meningioma, ca medullare and thymoma were found. In 11 patients suspected of pheochromocytoma 5 scans were positive (histopathology: 4 pheochromocytomas and 1 lymphoma). In 10 patients with diagnosed medullary ca, the metastases were found in 7cases. Islet celt tumours were suspected in 12 patients — 5 scans were positive (2 insulinomas, 1 non-classified NT, 1 lymphoma, 1 Crohn disease, and 1 case to be proved). In 6 patients ectopic hormone production was searched for — 2 scans were positive and the patients have been qualified for the surgery. The scintigraphy was undiagnosable (carcinoids) in ventricular location of the tumour.
Conclusions: 99mTc-HYNIC TATE scintigraphy were comparable to 111In-Octreoscan and 131I-MIBG wich higher target to background ratio (4.4:1 *vs.* 2.8:1) and more metastatic lesions detected using this tracer. ^{99m}Tc-HYNIC-TATE can replace ^{111}In-Octreoscan and ^{131}I-MIBG in diagnosis of the TESR (without gastric location of the tumour), especially for staging and re-staging assessment.

9

GA-67 LABELLED DOTA-DERIVATISED PEPTIDE-LIGANDS

G.J. Meyer, P. Gielow, M. Hofmann, W.H. Knapp

Klinik fur Nuklearmedizin, Medizinische Hochschule Hannover

Introduction: Ga-67 labelled peptides may become a new class of therapeutic radiopharmaceuticals, based on the high micro-dosimetric effect of Auger-electrons produced by the electron-capture decay of Ga-67. Since the specificity of peptides can be optimized to a very high degree, the synergistic effects of high target specificity and micro-dosimetric efficacy may lead to an improvement in the therapeutic strategy towards cancers which propagate via micro metastases.
Material and methods: For triple loaded cations like Ga^{3+}, DOTA (1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tretaaceticacid) has proven to be a very suitable chelator, which can be attached to peptide-ligands without loss of specificity. Since the concentrations of peptide receptors are usually in the order of nMol/l, higher than μmol amounts of peptide ligands tend to override the specific targeting by unspecific distribution. At these small concentration levels other cations need to be rigorously excluded. This necessitates the prepurification, concentration, and deacidification of the Ga-67. Furthermore alt handling must be carried out in small volumes in order to maximize the concentration of reactants and to improve the labelling yield. Handling of multi-GBq amounts of Ga-67 furthermore necessitates the use of semi-automated preparation techniques.
Results: The DOTA derivatised peptide Tyr-Octreotide (TOC), was labelled at 100 nmol amounts with a concentrated and highly purified solution of Ga-67 ($< 200\,\mu$l) at pH 4.5 within 4 min at 90°C. For the adjustment of the pH we used 800 N1 1 m HEPES-solution as a non-ionic buffer. Purification of the end product was achieved with RP18 mini-cartridges within 10 min. Quality control was carried out by DC and HPLC. The overall concentration-, labelling-, and purification-procedure took less than 50 min and resulted in 50–60% overall radiochemical yield with a radiochemical purity > 97%.

10

NOVEL SYNTHESIS OF 4-[^{18}F]-FLUOROBENZENECARBOHYDRAZIDE-FOLATE AND INITIAL BIOLOGICAL EVALUATION

I. Al Jammaz, B. Al-Otaibi, S. Okarvi, J. Amartey

Cyclotron and Radiopharmaceuticals Department, King Faisal Specialist Hospital and Research Centre, Riyadh. Kingdom of Saudi Arabia

Membrane-folic acid receptors are responsible for cellular accumulation of folate and folate analogs that overexpressed on various cancers, including breast, cervical, ovarian, colorectal and renal cancers.
However, these receptors are highly restricted in most normal tissues. *In-vitro* and *in-vivo* studies of the Tc-99m, Ga-67 and In-111 radiolabeled folate-conjugates have been revealed to bind folate-receptors with high affinity and were taken up by tumor cells by folate-receptor-mediated endocytosis. These have demonstrated their potential as radiopharmaceuticals for tumors detection and visualization. With the increased use of positron emission tomography, there has been great interest in the development of positron emitters radiopharmaceuticals for earlier detection and characterization of cancer; molecular assessment of treatment effects and more fundamental understanding of the disease process. As part of an on-going research effort to develop prosthetic precursors for radiofluorination of peptides, we have synthesized 4-[^{18}F]-fluorobenzenecarbohydrazide-folate using two different synthetic approaches. The synthetic approaches for preparation of 4-[^{18}F]-fluorobenzenecarbohydrazide-folate (^{18}F-folate, Figure 1) entailed several sequence of reactions. The key precursor 4-N,N,N-trimethylammonium ethylbenzoate triflate was treated using catalyzed nucleophlic no-carrier-added radiofluoride produced by the ^{18}O(p, n) ^{18}F nuclear reaction on ^{18}O-enriched (95% water and Kryptofix 222 as nucleophilic catalyst in anhydrous acetonitrile at 95°C. The resulted ethyl 4-[^{18}F]-fluorobenzoate in the first approach, was reacted with hydrazine at 95°C followed by reaction with N-hydroxysuccinimide-folate (NHS-folat) in DMSO at 60°C to give the folate conjugate ^{18}F-folate in a quantitative yield. However, the resulted ethyl 4-[^{18}F]-fluorobenzoate was converted to the corresponding acid in the second approach then activated with O-(N-succinimidyl) N, N, N, 'N'-tetramethyluronium tetrafluoroborate (TSTU) to form the [^{18}F]-N-succinimidyl-4-fluorobenzoate (SFB). This prosthetic intermediate was used to label the hydrazide-folate in DMSO at 60°C to give the folate conjugate ^{18}F-folate in also quantitative yield. Work up of this product by C-18 Sep-Pak column gave radiochemically and chemically pure ^{18}F-folate as assessed by HPLC in less than 70 and in 110 minutes for first and second synthetic approaches respectively. The first synthetic approach in comparison with the second appears to be advantageous in the synthesis of ^{18}F-folate conjugate in less laborious way and shorter time. *In vitro* and *in vivo* characterization of this radiofabeled folate conjugate is currently in progress.

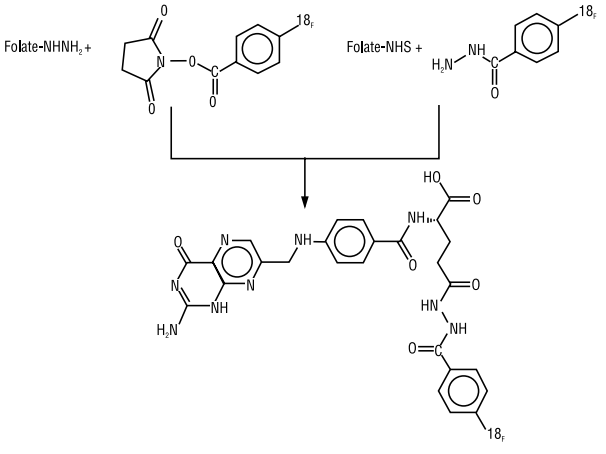


Figure 1. Synthetic approaches for preparation of 4-[^{18}F]-fluorobenzenecarbo--hydrazide-folate (^{18}F-folate).

11

BIODISTRIBUTION OF THE NK1 RECEPTOR TRACER [^{18}F]SPA-RQ IN GUINEA PIG

T. Grönroos[1], S. Forsback[1], P. Marjamäki[1], M. Haaparanta[1], O. Eskola[1], J. Bergman[1], C. Ryan[2], T.G. Hamill[2], R.E. Gibson[2], H.D. Burns[2], O. Solin[2]

Turku Pet Centre, Preclinical Imaging Unit, Turku, Finland, [2]Merck Research Laboratories, West Point, PA, USA

Introduction: The purpose of this study was to evaluate [18F]SPA-RQ (Merck, USA) in a gwinea pig (GP) animal model. This tracer is a high affinity, selective ligand for the NK1-receptor.
Material and methods: Thecompound was labelled with fluorine-18 to a high specific radioactivity (> 400 GBq/μmol) (Solin et al. [2004] Mol Imaging Biol, in press). Anesthetized animals (n = 30) were injected with the tracer i.v. Fourteen animals were pre-treated with a potent, brain penetrant NK1 receptor antagonist. The animals were sacrificed 15, 60, 180 or 360 min after tracer injection. Animals were then dissected and parts of organs were counted and weighted. The % ID/g tissue of tracer was determined from this data as a function of time. The spatial distribution of 18F-radioactivity from brain slices (20 μm) was determinated with digital autoradiography and analyzed for uptake of radioactivity in frontal caudate putamen, cortex, cerebellum as well as other structures.
Results: The tracer shows specific binding in structures of GP brain compatible with the known location of NK1-receptors. In animals pre-treated with the NK1 antagonist the uptake was inhibited to the level of that of cerebellum in all brain structures. The kinetics of the specific uptake in caudate showed promise for the tracer to be useful in human studies. Specific binding was seen in several organs, notably intestine, lung and pancreas. The uptake in bone increased with time, indicating some defluorination of the tracer.
Conclusion: Initial experimental screening in gwinea pigs ex vivo indicated a high signal/noise ratio in caudate as well as appropriate distribution and pharmacology for this tracer.

12

SYNTHESES OF ^{18}F-LABELLED ACYCLIC PURINE AND PYRIMIDINE NUCLEOSIDES INTENDED FOR MONITORING GENE EXPRESSION

B. Noll, St. Noll, M. Grote

Forschungszentrum Rossendorf, Dresden, Germany

Introduction: Radiolabelled ganciclovir analogues have shown promise as imaging agents to detect herpes simplex virus 1 thymidine kinase (HSV-1 tk) expression finto tumours with positron emission tomography (PET). To search improved PET radiotracers for gene therapy monitoring in addition to [^{18}F]FHBG 1 and [^{18}F]FHPG 2 a series of novel acyclic nucleosides were synthesized (Figure 1).

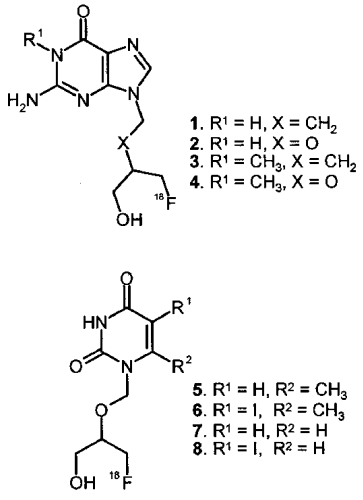


Figure 1. Synthesized 18F-labelled acyclonucleosides.

Material and methods: The tracers were produced by nucleophilic substitution of the corresponding precursors with a K[^{18}F]Fkryptofix 2.2.2 complex and subsequent cleavage of methoxytrityl protecting groups under acidic conditions followed by RP-HPLC separation. The radiochemical yield of the ^{18}F-tracers amount to 5–15% (decay corrected) after a synthesis time of 85–95 min, the radiochemical purity was > 98% and the average specific activity was 19 GBq/umol at the end of synthesis.
Results: The labelling procedures of [^{18}F]FHBG 1, [^{18}F]FHPG 2 and of the new tracers 3–8 were studied in detail. The precursors and the reference substances were synthesized in an extensive multistep procedure [1]. To evaluate the behavior of the new fluorinated compounds 3–5 and their related hydroxylated analogues against the viral thymidine kinase, screening tests with the isolated enzyme HSV-1 TK were performed. Cell uptake in transfected (HT-29 tk + and MC38 tk +) and non-transfected cells (HT-29, MC38) depends on the celt types and on the nucleobases used.
Discussion/Conclusion: Labelling procedures of all precursors were optimised concerning the reaction time, radiochemical yields and purities. Modification of the lipophilicity of [18F]FHBG 1 by introduction of a methyl group finto the N1-position to get N1-Methyl-9-[(4-[^{18}F]fluoro-3-hydroxymethyl)butyl]-guanine [^{18}F]MFHBG 3 does not cause a decreased celt uptake.

[1] Grote M, Noll S, Noll B, Johannsen B, Kraus W. Can J Chem 2004; 82: 513–523.

13

PRODUCTION AND QUALITY CONTROL OF ^{82}RB^{m} FOR HEART PET SCANS

P. Rowshanfarzad, A.R. Jalilian, M. Kiyomarsi, M. Sabet

Cyclotron/Nuclear Medicine Department, Research Center for Agriculture & Medicine, Karaj, Iran

Introduction: ^{82}Rb^{m} radioisotope with a half life of 6.5 hours, can be used in PET imaging due to its decay to stable ^{82}Kr as a result of competition between positron emission (3β+: 26%, $E_{\beta+}$ = 0.80 MeV) and electron capture (E.C.:74%) phenomena. ^{82}Rb^{m} is widely used in PET imaging for the investigation of heart ischemic diseases, coronary stenosis, and noninvasive myocardial imaging [1–3]. The possibility of ^{82}Rb^{m} production was studied in our cyclotron according to the increasing importance of PET radioisotopes in nuclear medicine.
Material and methods: ^{82}Kr gas (isotopic purity of 30%) was bombarded by protons in a special stainless steel compartment with a titanium window. ALICE [4] nuclear code showed that the best proton beam energy was 15 MeV (Figure 1).

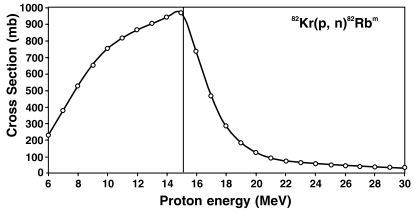


Figure 1. Result of ALICE code for ^{82}Kr(p, n)^{82}Rb^{m} reaction.

The bombardment was performed with a proton current intensity of 10 uA and a total current of 5.5 μAh. After washing the compartment and extraction of ^{82}Rb^{m}, the activity of the final product was 35.04 mCi. The production yield was 6.37 mCi/μAh according to the nuclear reaction ^{82}Kr(p, n)^{82}Rb^{m} (6.5 h) ^{82}Kr. Quality control was performed in 3 steps: 1 — Gamma spectroscopy by HPGe detector showed a radio-nuclide purity of 93% (Figure 2);

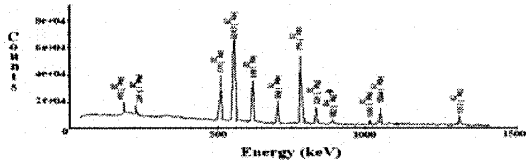


Figure 2. Gamma spectroscopy scheme of final ^{82}Rbm product.

2 — Due to the possibility of presence of Ti and Fe (originated from target window and walls), chemical purity of the product was checked by precise colorimetric methods. The results were better than the U.S. P. standard limits; 3 — Radiochemical purity was controlled by paper chromatography to detect RbOH in the final product. The result was checked by gamma spectrometry based on the 511 keV peak and was in agreement with the U.S.P. standard (Rf = 0.8). In order to convert the product to an injectable form, the following steps were performed:
1 — The product was dried slowly and 0.5 ml normal saline was added per 1 mCi activity of ^{82}Rbm for its biocompatibility;
2 — pH was adjusted between 4–7 by addition of small amounts of 0.01 N-HCl;
3 — The solution was passed through a 0.22 μ antimicrobial filter (Cathivex) and was then autoclaved;
4 — Microbial-fungal test showed no colonial growth during one month;
5 — Pyrogen test was performed using commercial LAL kit.
Results: Primary images were taken after the injection of 300 uCi ^{82}Rb^{m} Chloride to healthy rats by a dual head coincidence SPECT. The rat was studied for 4 hours during which, images were taken every 30 minutes. During the first 30 minutes, the activity was spread in organs. Later, the activity gradually reduced in head, neck and chest, but was concentrated in the heart. After 3 hours, the activity was obviously increased in the heart (Figure 3).

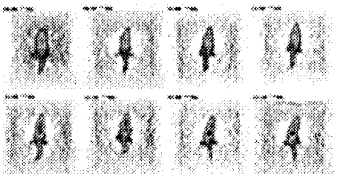


Figure 3. Primary whole body coincidence image of a rat after the injection of 300 μCi of sterile ^{82}Rb^{m} solution.

Conclusion: As a result of the bombardment of ^{82}Kr with 15 MeV protons, 35.04 mCi activity was finally achieved. The nuclear reaction efficiency was 6.37 mCi/μAh. *In vivo* studies showed that 3.5 hours after injection, the activity reached its maximum in rat heart which may be attributed to the presence of a large number of Na/K pumps in heart muscles. This method can be used for the production of large amounts of ^{82}Rb^{m} for PET imaging, according to the optimal results obtained and the importance of ^{82}Rb^{m} as a PET radioisotope.

[1] Shea MJ, Wilson RA et al. Use of short and long-lived rubidium Traces for the study of Transient ischemia. J Nucl Med 1987; 28: 989–997.
[2] Stoll HP, Huwer H et al. Experimental validation of a new coronary guide wire labeled with rubidium 81/krypton 81 m for continuous assessment of myocardial blood flow. J Nucl Cardiol 2000; 7 (3): 255–262.
[3] Cherry SR, Carnochan P et al. Quantitative in vivo measurements of tumor perfusion using rubidium-81 and positron emission tomography. J Nucl Med 1990; 31 (8): 1307–1315.
[4] Blann M, Bislinghoff J. Code Alice. Lawrence Livermore National Laboratory, Internat Report 1991; UCID-19614.

14

RADIOPHARMACY EDUCATION AND LEGISLATION IN A NON-EU COUNTRY: TURKEY

A. Soylu

Ankara University, Faculty of Medicine, Nuclear Medicine Department, Ankara, Turkey

There are more than 130 radiopharmacy laboratories in nuclear medicine departments of universities, state hospitals or private practice in Turkey. Two cyclotrons facilities producing F^{18}-FDG and one center producing Mo99/Tc99m generators, Tl-201 chloride solution and I-131 capsule/solutions are worth considering. A large number of people are working in this area but not all of them are properly educated. The present status and future plans of radiopharmacy education in Turkey in relation to the standard certification program in EU will be discussed in this presentation. The Turkish Guidelines on Radiopharmaceuticals. came into act in 1993 making registration of all radiopharmaceuticals produced in or imported to Turkey compulsory. But still regulations are needed for "in house" preparations and clinical trials involving radiopharmaceuticals. There is a countinous modification of available legislation for harmonization to EU which holds for this area also. The status of some kits used in Turkey which are produced by EU countries but do not have a general license in Europe also need special consideration.

15

^{99m}TC MIXED-LIGAND COMPLEXES WITH HETEROFUNCTIONALIZED PHOSPHINES WITH HIGH AFFINITY FOR 5-HT_{1A} RECEPTOR

C. Fernandes[1], J. Correia[1], L. Gano[1], R. Bergmann[2], H. Spies[2], S. Seifert[2], I. Santos[1]

[1]ITN, Química, Sacavém, Portugal, [2]Institute of Bioinorganic & Radiopharmaceutical Chemistry, Forschunszentrum Rossendorf, Dresden, Germany

Introduction: Visualization of serotonin receptors in the brain, particularly the 5-HT_{1A} sub-type, is of great interest since these receptors are implicated in various neuropsychiatric diseases. The so-called "3 + 1" approach, has been thoroughly investigated over the past few years for the design of specific radiopharmaceuticals of rhenium and technetium. However, one of the drawbacks of these "3 + 1" complexes is their low stability *in vivo,* due to the substitution of the monodentate co-ligand by gluthathione (GSH), forming hydrophilic complexes of the type ^{99m}Tc(SES/GS) (E = S, NR). It was also shown that this instability was strongly dependent on the nature of the tridentate and monodentate co-ligand. The aim of this work was the preparation of novel ^{99m}Tc mixed-ligand complexes for imaging 5-HT_{1A} receptors with an improved *in vivo* stability.
Material and methods: We synthesized different thiolated arylpiperazine derivatives (HSL1–HSL4) to prepare mixed-ligand "3 +1" complexes of general formula [M(O)(3-PNS)(1-SL))] [M = ^{99m}Tc (1–4); Re (1a–4a)] using the H_2PNS phosphine as tridentate ligand. The ^{99m}Tc-complexes obtained were identified and their stability at different experimental conditions (saline, PBS, rat plasma and 1 mM and 10 mM GSH solutions) studied by HPLC. In order to determine the effect of chemical modifications in the monodentate ligand, binding affinity for the 5-HT_{1A} and 5-HT_{2A}, receptors was determined. Biodistribution and *in vivo* stability in mice was also assessed.
Results: After purification by HPLC, the radiochemical purity of the ^{99m}Tc complexes is higher than 95% and all are stable in saline, PBS and in rat plasma. No exchange with glutathione was observed. One of the complexes has high affinity and selectivity for the 5-HT_{1A} receptors (IC50 for 5-HT_{1A} 2.35 0.02 nM; competitor 5-HT_{2A} 372 11 nM). Biodistribution studies indicated a poor brain uptake and high *in vivo* stability.
Conclusion: H_2PNS ligand allows the preparation of stable "3 + 1" oxotechnetium(V) complexes with high affinity and selectivity for 5-HT_{1A} receptor.

16

PREPARATION AND CHARACTERISATION OF ^{125}I-LABELLED TYROSYL-PENTOSAN POLYSULPHATE DERIVATIVE

R. Braddock[1], O. Denney[1], B.L. Ellis[2], M.C. Prescott[2], S. Dealler[3], D.S. Pepper[4], H.L. Sharma[1]

[1]Department of Imaging Science and Biomedical Engineering, University of Manchester, Manchester, [2]Department of Nuclear Medicine, Manchester Royal Infirmary, Manchester, [3]Royal Lancaster Infirmary, [4]Scottish National Blood Transfusion Service, Liberton, Edinburgh

Introduction: Pentosan polysulphate (PPS) is being investigated as a prophylactic agent against new variant Creutzfeldt-Jakob disease (nvCJD). This transmissible neurodegenerative disease may be present in a proportion of the UK population, who have been infected through the food chain. The aim of the study was to prepare and characterise ^{125}I-tyrosyl-PPS (*tyr*-PPS) with a view to investigating the *in-vivo* pharmacokinetics in mice and humans.
Material and methods: A tyrosyl-PPS derivative was radioiodinated using the chloramine-T method and purified by gel-filtration with a Sephadex G-15 column and the percentage labelling efficiency and specific activity were calculated. Several parameters were modified to determine optimum conditions for radioiodination. These included the bed height of the column, radioactive concentration and reaction time. The molecular size distribution of ^{125}I-*tyr*-PPS was compared with that of underivatised PPS by gel filtration using a Sephadex G-25 column, eluted with 0.9% NaCl solution. Characterisation of the ^{125}I-*tyr*-PPS was also investigated by ion-exchange chromatography on DEAE-resin using a 0–3M NaCl linear ionic gradient. The stability of the radiolabelled compound was determined at different storage temperatures and in synthetic gastric juice.
Results: *Tyr-PPS* was radioiodinated with 76% yield and high specific activity (76 Ci/g) using the chloramine-T method. Optimum radioiodination conditions .were achieved using a 25 cm column and 30 s incubation time. The molecular size distribution of the ^{125}I-*tyr*-PPS determined by gel-filtration was found to be similar to that of the underivatised PPS. Ion-exchange chromatography showed that ^{125}I-*tyr*-PPS and underivatised PPS had similar charge properties. Stability studies showed no significant breakdown of the product on incubation with simulated gastric juice or after 24 h storage at –20°C.
Discussion/Conclusion: ^{125}I-*tyr*-PPS was produced in high yield and successfully characterized showing a similar size distribution and affinity for DEAE-resin anion exchange to underivatised PPS. We conclude that ^{125}I-*tyr*-PPS would be suitable for pharmacokinetic studies in an animal model.

17

OPTIMALIZATION OF CONDITIONS FOR COMPLEXATION OF ^{111}IN WITH NOVEL BIFUNCTIONAL MONO(PHOSPHINATE) ANALOG OF DOTA

M. Fŏrsterová[1, 2], J. Zimová[1, 3], P. Hermann[2], I. Lukeš[2], F. Melichar[1]

[1]Radiopharmaceutical Department, Nuclear Physics Institute, ASCR, Rez near Prague, Czech Republic, [2]Department of Inorganic Chemistry, Universita Karlova (Charles University), Prague, Czech Republic, Department of Analytical Chemistry, Institute of Chemical Technology, Prague, Czech Republic

Introduction: The bifunctional polyazaligands such as DTPA or DOTA are used in radioimmunoterapy and radioimunodiagnosis. A key parameter for these applications is a fast and efficient complexation of a suitable radioisotope. Acyclic DTPA forms complexes immediately; complexation with macrocyclic DOTA is much slower. However, the macrocycles are better from all other aspects (e.g. kinetic and thermodynamic stability). In general, the most suitable central ions for DOTA-like ligands are trivalent lanthanides and indium. The ^{111}In isotope was chosen for this study, because it has convenient radio-properties. Our DOTA derivative contains three acetate and one phosphinate pendant arms with *p*-aminobenzyl molety on phosphorus atom (DO3A-P^{ABn}) and it displays a faster complexation in comparison with DOTA.
Material and methods: DO3A-P^{ABn} was synthesized on Department of Inorganic Chemistry (Charles University). $^{111}InCl_3$ (in 0.05 M HCl) was purchased from Amersham Health. All experiments were realized in NH_4OAc buffer with carrier added indium. The reactions were followed with radio-TLC on silikagel plates (Merck).
Results: The complexation proceeds very quickly. The requested level (> 95%) of complexation is reached within 15 minutes even at the room temperature. The careful pH control is very important, as the solution of the radioactive metal is highly acidic. Previously published buffer solutions were not optimal.
Discussion/Conclusion: The complexation rate of this ligand containing the phosphinate group is satisfactory for use in nuclear medicine. We can expect that other structurally related phosphorus acid ligands would exhibit the same properties as well.

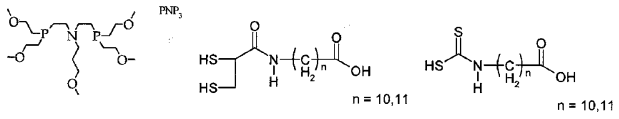
18

ASYMMETRICAL NITRIDO TECHNETIUM-99M HETEROCOMPLEXES FOR THE STUDY OF HEART METABOLISM

E. Cazzola[1], Ch.M. Jung[2], L. Uccelli[1], A. Boschi[1], P. Micol[1], M. Walther[2], H. Spies[2], H.J. Pietzsch[2], A. Duatti[1]

[1]Laboratory of Nuclear Medicine, Italy, [2]Froschungszentrum Rossendorf, Germany

Introduction: Under physiological conditions long chain fatty acids, (Fa) serve as a major energy source for the normoxic myocadium. During ischemia, however, the extraction of Fa is reduced and glycolysis is enhanced [1]. Thus, an alteration of fatty acids oxidation is considered to be a sensitive marker of ischemia and myocardial damage. Because fatty acids are a major energy source in the myocardium, the radiolabelled fatty acid analogs have been introduced to assess myocardial cellular function. In this work are present and compared some new heterocomplexes containing fatty acids that present different systems of coordination and with different lenght of chain; all the complexes are coordinated to the [99mTc (N)PNP]2+[2] fragment, for the metabolic heart study.



Material and metods: The diphosphine ligand PNP3 [3], Fatty acids dithiole FaDtn (n = 10, 11) [4] and Fatty acids dithiocarbammate (n = 10, 11) [5] ligands were prepared according to literature.

Labelling with FaDtn, FaDtcn (n = 10, 11) and fragment [99mTcNPNP3]2+ Na99mTcO4 (0.250 mL, 50.0 MBq- 4.0 GBq) was mixed in a vial containing 5.0 mg of SDH, 0.1 mg of SnC12 2H2O (suspended in 0.10 mL of saline), and 1.0 mL of ethanol. The mixture was kept at room temperature for 30 min. To the resulting solution, 1.0 mg of PNP3 (dissolved in 0.30 mL of ethanol) was added and kept for 30 min at room temperature. Finally 1.0 mg of Fan (n = 10, 11) dissolved in 0.2 mL of solution of EtOH, was added and kept at 60 min for 1 h. The isolated working heart experiment in guinea pig have been performed according to literature [6].
Results: Each compound [99mTc(N)(PNP3)FaDtn] (n = 10, 11) and [99mTc(N)(PNP3)FaDtCn]+ (n = 10, 11) was labelled according to the method shown before and the radiochemical yield determined by TLC was > 90%. After purification for remove the free Fa, the compounds were studied by Isolated working heart experiment[6]. In the Figure 1 are shown the ventricular extraction values of new asymmetrical nitrido complexes in comparison with the 99mTc-SESTAMIBI.

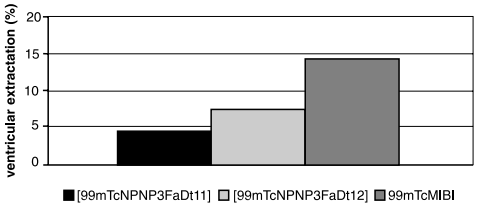


Figure 1. Comparison between 99mTcNPNP3FaDtn and 99mTcMIBI

Discussion/Conclusion: First results showed the presence of a correlation between the length of the carbon atoms chain and the heart uptake. With the [99mTcNPNP3FaDt11] the heart uptake value is 4.4%, while with the complex [99mTcNPNP3DT12] we have an heart uptake of 7.6%. The complexes studies with FaDtcn are in progress.

[1] Tamaki N, Morita K, Kuge Y, Tsukamoto E. J Nucl Med 2000; 41: 1525–1534.
[2] Bolzati C, Boschi A, Uccelli L, Duatti A. JACS 2002; 124: 11468–11479.
[3] Marchi A, Martelli L, Rossi R, Magon L, Uccelli L, Bertolasi V, Ferretti V. J Chem Soc Dalton Trans 1993: 1281–1286.
[4] Jung CM, Pietzsch H-J, Spies H, Kropp J. Eur J Inorg Chem 2002; 1219–1225.
[5] Castillo M, Criado JJ, Macias B, Vaquero MV. Inorg Chim Acta 1986; 124: 127–132.
[6] Depresse C. Nucl Med Biol 1998; 25: 711–713.

19

[2+1] MIXED LIGAND CONCEPT: NEW $^{185/187}$RE AND ^{99m}TC(CO)3(SS)(P) COMPLEXES

M. Riondato[1], D. Camporese[1], M. Bello[2], D. Martin Vila[3], J. Suades[3], U. Mazzi[1]

[1]Department of Pharmacological Sciences, University of Padova, Italy, [2]Department of Physics, University of Padova, Italy, 3Department of Inorganic Chemistry, Autonoma University of Barcelona, Spain

Introduction: The concept of drug targeting can be applied to tumours that exhibit biochemical difference from normal tissue. Because of the over-expression of receptors in mapy solid tumours and other diseases, biomolecules (peptides i.e.) with high affinity for a target can be labelled with a radionuclide for the development of site-directed diagnostic and therapeutic radiopharmaceuticals. 1, 2 Actually the common approaches to produce 99mTc-labelled peptides are based in hynic method or by means of tetradentate bifunctional ligands. But thanks the fundamental contribution of Alberto R, Shibli R and Schubiger PA with the introduction of novel radioactive metal aquaions, fac-[99mTc(OH2)(CO)3]+ and fac [188Re(OH2)(CO)3]+ + (99mTc, γ-emitter and 188Re, β-emitter), the labelling research has been intensely stimulated. 3, 4 Furthermore the [M(CO)3]+ moiety allows the use of a variety of ligand systems so urany different chelating sets have been tested.7 Despite a lot of interesting goals urany research groups are still looking for an ideal building block with optimised *in vitro* and *in vivo* behaviour. 5, 6 In the last few years coordination studies have been principally focused in the development of tridentate BFCA to obtain tri coordinated complexes. This fashion generally exhibits more stability in biological system than mono- or bidentate set, which can permit interactions with the biomolecular donor groups present in the media. But tricoordination can also be achieved by using a mixture of two ligands, one bidentate L2 and one monodentate L1 and this method is still not well developed ([2+1] mixed ligand approach). 8 In the present work we propose a new suitable chelating system for the stabilization of [M(CO)3]+moiety with the [2+1] mixed ligand approach, based on a simple dithiocarbamate as bidentate ligand and a class of diphenylphosphino derivatives with growing side chains as monodentate ligands.
Material and method: All the complexes were obtained by stochiometric reactions in good yealds and purity (with mdtc and ligands in methanol), after purification with flash column chromatography. Complexes were widety characterized by 1 H, 13C, 31 P NMR, FT-IR spectroscopies, elementar analysis, ESI-MS and crystallographic analysis (two complexes). The labelled compounds were characterized by RA-HPLC chromatography, and the chemical structures were identified for comparison with the "cold" homologues complexes HCLC UV-traces.
Results and discussion: The coordination chemistry of the phosphine derivatives with sodium N,N-dimethyldithiocarbamate (mdtc) was studied toward the "cold" rhenium synthon [Re(C03)Br3](NEt4)2. For comparison we afforded the same reaction by adding a mixture of mdtc and ligand to the rhenium precursor in a methanolic solution, in order to evaluate a possible application for the production of a [2+1] mixed ligand kit. We obtanined the same complexes with mdtc as bidentate and phosphine as monodentate ligand. Labelling studies with 99mTc tricarbonyl species were carried nut to perform the reaction at tracer level with high yields. The stability of a labelled compound was tested successfully in rat serum, and finally biodistribution experiments after intravenous administration in C57BU6 healthy mgle mice were complied by means of a YAP-camera device, demonstrating a fast clearance from the blond by hepatobiliary pathways. This *in vivo* behaviour was confirmed after sacrifice of the mouse (120 minutes postinjection) and *ex vivo* results.

Smith CJ, Kannan R. Cancer Res 2003; 63: 4082–4088.
La Bella R, Shubiger PA. Nucl Med Bio 2002; 29: 553–560.
Alberto R, Schubiger PA. Eur J Nucl Med 2002; 29 (11).
Jurisson SS, Lydon JF. Chem Rev 1999; 99: 2205–2218.
Alberto R, Schibli R, Waibel R, Abram U, Schubiger AP. Coordination Chemistry Reviews 1999; 190–192, 901–919.
Volkert WA, Hoffman TJ. Chem Rev 1999; 99: 2269–2292.
Schibli R, Schwarzbach R, Alberto R, Ortner K, Schmalle H, Dumas C, Egli A, Schubiger PA. Bioconjugate Chem 2002; 13: 750–756.
Alberto R, Welch MJ. Eur J Nucl Med Mol Imaging 2003; 30: 1299–1302.

20

SYNTHESIS AND BIOLOGICAL EVALUATION OF A NEW TYPE OF TECHNETIUMLABELLED FATTY ACIDS FOR MYOCARDIAL METABOLISM IMAGING

M. Walther[1], C.M. Jung[1], R. Bergmann[1], J. Pietzsch[1], K. Rode[1], S. Stehr[2], A. Heintz[2], G. Wunderlich[2], W. Kraus[3], H.J. Pietzsch[1], J. Kropp[2], A. Deussen[2], H. Spies[1]

[1]Forschungszentrum Rossendorf, Dresden, [2]TU Dresden, Medizinische Fakultat Carl Gustav Carus, [3]Bundesanstalt fur Materialforschung und prufung, Berlin, Germany

Introduction: In an effort to develop technetium-labelled fatty acid analogues for myocardial metabolism imaging rhenium model complexes and their 99mTc analogues were synthesized according to the "4+1" mixed-ligand approach and investigated *in vitro* and *in vivo*.
Material and methods: The rhenium model complexes were completely characterised by NMR, IR, MS, EA and the geometrical impact of the chelate unit on the integrity of the fatty acid head structure was determined by single crystal X-ray analyses. To estimate the diagnostic value of the 99mTc-labelled fatty acids the compounds were investigated using the isolated constant-flow-perfused guinea pig heart model (see presentation A. Heintz et al), in celt-uptake experiments and in biodistribution studies using mate Wistar rats (5–6 weeks old, body weight 151 ± 15 g).
Results: The new fatty acid tracers contain the metal Gore in the oxidation states +3, well-wrapped in a trigonal-bipyramidal coordination moiety which is attached at the omega-position of a fatty acid chain. This structural feature is considered to be a good imitate of the well-established iodinated phenyl fatty acids. The formation of the rhenium models was accomplished by ligand exchange reactions using different pre-formed rhenium precursors.
Noticable heart uptake of the 99mTC tracers being in the order of 2% ID/g 5' p.i. and accompanied by a good heart to blond ratio of 8.6 confirms the remarkably results of the perfused heart experiments. A significant time-dependent celt uptake in HepG2 cells is shown for finro representatives.
Conclusion: Further species such as mice and guinea pigs will be involved to characterise in *vivo* patterns of those derivatives, that show high extraction rates in the isolated constant-flow-perfused guinea pig heart model. While the tracers are superior to other described Tc-fatty acid imitates with regard to good heart to blond ratios, heart to liver ratio has to be improved. For this, chemical modifications will be performed at the chelating part as well as at the alkyl chain.

21

^{177}LU POTENTIAL THERAPEUTIC RADIOPHARMACEUTICALS: PREPARATION AND QUALITY CONTROL OF ^{177}LU-EDTMP AND ^{177}LU-DOTA-TYR3-OCTREOTATE COM PLEXES

M.M. Ishfaq, B. Khan, N. Hussain, M. Jehangir

Isotope Production Division, Pakistan Institute of Nuclear Science & Technology, P.O. Nilore, Islamabad, Pakistan

Reactor produced ^{177}Lu is emerging as an important radionuclide for cancer therapy since it decays with half life of 6.71 d by the emission of particles with E of 498 keV (78.6), 384 keV (9.1 %) and 176 keV (12.2) to stable ^{177}Hf. It also emits gamma rays of 208 keV (11%) and 113 keV (6.4%) suitable for imaging. Recently, a somatostatin analogue, EDTMP has proven to be effective radiotherapeutic agent in the treatment of metastatic bone cancer psin due to its selective skeletal uptake, high Jesion affinity and low toxicity. DOTA-Tyr3-Octreotate has proven very successful in tumour reduction in patients with metastatic neuroendocrine tumours. In this paper, we describe the studies on the optimization of the production of ^{177}Lu radionuclide in PARR-I (Pakistan Atomic Research Reactor) using natural lutetium and isotopically enriched ^{176}Lu (68.9%) targets for different time intervals. Natural and enriched liquid $Lu(NO_3)_3$ are irradiated to optimize the production yield of ^{177}Lu. The results showed that low specific activity of 96 mCi/mg of ^{177}Lu was obtained by irradiating natural Lu targets in PARR-I reactor for 48 hours whereas irradiation of enriched ^{176}Lu targets for the same time produced high specific activity of 2.5 Ci/mg of ^{177}Lu. However, the specific activity may be increased to 5.5 Ci/ mg by irradiating liquid $Lu(NO_3)_3$ in the reactor for a period of 96 hours. The irradiation data indicate that specific activity of liquids targets (5.5 Ci/mg) is higher than solid targets (4.6 Ci/mg). Studies on the preparation of ^{177}Lu-EDTMP complex was investigated w.r.t. pH, temperature and incubation period to get a complex of high yieid (> 99%), which showed excellent stability of more than 10 days. The preparation of ^{177}Lu-DOTA--Tyr3-Octreotate complex under different experimental variables is also described in order to determine the optimal preparative conditions. The data regarding the effect of pH and incubation period on the labelling yields of ^{177}Lu-DOTA-Tyr3-Octreotate complex indicated optimum labelling yield at pH 4 to pH 7 with incubation period of more than 25 minutes at 80°C. Radiochemical purity of ^{177}Lu-DOTA-Tyr3-Octreotate was determined by radio TLC with C18 plates developed in 70:30 MeOH: 10%NH_4OAc. Under these conditions ^{177}Lu-DOTA-Tyr3-Octreotate complex appears at the R_f 0.8 while ^{177}Lu-acetate stays at origin. Stability of ^{177}Lu-DOTA-Tyra-Octreotate complex was also studied in the above acetate buffer and in the saline for 4, 16, 48 hours intervals and the quality control of the complex was performed using the above radio TLC technique. The results showed that the complex was stable in both acetate buffer and in saline for the period of 48 hours. The animal study of ^{177}Lu--DOTA-Tyra-Octreotate complex using rats is also described.

22

PROSTHETIC LABELED INTERLEUKIN-8 ([$^{123/131}$I]-IL-8): BIOLOGICAL BEHAVIOR IN A MOUSE INFECTION MODEL

J.K. Amartey, C. Esguerra, B. Al-Otaibi, I. Al-Jammaz, M. Al-Qahtani, R.S. Parhar

Cyclotron and Radiopharmaceuticals Department, King Faisal Specialist Hospital and Research Centre, Kingdom of Saudi Arabia

Introduction: Numerous molecular entities with diverse structures have been radiolabeled and investigated as potential infection and inflammation imaging agents. However, none of these molecules have gained the acceptance of gallium citrate or radiolabeled autologous white blood cells (WBCs). The cytokines and chemokines are candidate molecules that when developed as radiotracers could be used for such an application because of their role in these processes. We have radioiodinated interleukin-8 (IL-8) using two different methods and tested the biological behavior of the products in mice.
Material and methods: The N-Succinimidyl-5-trimethylstannylpyridine-3-carboxylate activated (PyATE) precursor was synthesized using published methods with minor modifications. The PyATE was radioiodinated by iododestannylation. The radioiodinated N-succinimidyl activated ester ([$^{123/131}$I]-SIPC) was conjugated to the peptide and purified by chromatography. The same peptide was radioiodinated using the classical electrophilic method. The radioiodinated tracers were then evaluated in normal and *E. coli* infected mice.
Results: The radioiododestannylation reaction yield was excellent, however the conjugation reaction yield was low. The conjugation yield was moderate as a result of competing hydrolytic reaction. As expected the direct radioiodinated material displayed extensive in vivo deiodination. The use of pyridine based-prosthetic label yielded a product with better kinetics than the direct radioiodination method and showed a better target to non-target ratio.
Conclusion: The *in vivo* stability of the iodopyridine carboxylate prosthetic group was demonstrated by low thyroid uptake of radioactivity. Nonetheless this method is not suited for labeling bioactive peptides such as the title peptide because of the very high specific activity required to prevent cytotoxic effects in human application.

23

PURIFICATION AND CONCENTRATION OF THE SBGA-SOLUTIONS FROM COMMERCIAL GENERATOR FOR PEPTIDE LABELLING

A. Bruskin, A. Sevast'yanova, G. Kodina, V. Andronov, G. Myasoedova

SSC-Institute of Biophysics, Russia and GEOKhi, RAS

Introduction: The radionuclide Ga-68 is rather promising due to the posibility of the production from generator directly in clinic. The most interesting is the application of 68Ga for a labelling of biomolecules, that demands minimum amount of substrate and, correspondingly, maximum specific activity of nuclide solution. The aims of the present work were the comparative study of the method of 68Ga eluate concentration and labelling of the modified peptides.
Material and methods: The commercial 68Ge/68Ga generator (20 mCi of 68Ge, manufactured by ZAO „Cyclotron", Obninsk, Russia) was used. Elution was carried out by 0.1 M HCl. Anion-exchange resins PolyorgsR (Russia) were investigated in static and dynamic modes. DTPA-Octreotide (PharmSintez, Russia) was chosen as a model peptide for labelling.
Results: During operation time (more 1 year) the decrease of elution efficiency (from 70 up to 45 %%) was observed, thus the position of a maximum on the elution curve practically has not changed. The breakthrough of mather nuclide always was 0.003–0.0006%%. The modification of eluation system with using of peristaltic pump (more convenient in operation), has not caused worsning of eluate. Gallium-68 is practically completely sorbed on all investigated anion-exchangers from concetration solutions (5–8 M) of hydrochloric acid at 10–15 min and is desorbted at pH 1–2. Study of those processes in dynamic conditions with mini-cartrige has allowed to choose sorbent which will increase the specific activity of Ga-68 more than 20 times at simultaneous decreasing of Ge-68 content more than in 10 times. The investigation of the factors affecting on a labelling of model peptide has allowed to find conditions, in which 68Ga-DTPA-Octreotide is obtained with a yield about 90% without heating.
Discussion/Conclusion: The efficiency of concentration procedure is depends of a nature of function groupes in used sorbents. The pyrazol-containing sorbents have shown better results. The concentrated pure solution of gallium-68 can be applied for labelling of DTPA-modified peptides and for other medical application.

24

A ONE-YEAR EXPERIENCE WITH AN IBA CYCLONE 18/9 CYCLOTRON

G. Tosi, L. Leonardi, K. Marzo, A. Chiti

Nuclear Medicine, Health Physics — Istituto Clinico Humanitas, Rozzano, Italy

Introduction: We analyzed the first year of experience in running a dedicated medical cyclotron for the production of PET radiopharmaceuticals.
Materials and methods: The IBA Cyclone 18/9 is a fixed energy cyclotron able to accelerate H-ions up to 18 MeV and D-up to 9 MeV of energy. The machine has 8 exit ports which can host 8 different targets. In this first year we only used 2 liquid targets: 1 small volume (SV) with a 0.55 ml silver cavity and 1 large volume (LV) with a 1.7 ml titanium cavity. Both targets are loaded with 180-enriched water to produce 18F-fluoride.
Results: We run the cyclotron for a total of 270 18F production, 88 with the LV target and 182 with the SV target. The functional parameters for both targets are showed in Table 1.

Table 1.

^{18}F Large volume	Current	Time [min]	μA *h	Production (Ci)	Yield [mCi/μA]	Pressure [bar]
Mean	31.4	94	49.6	2.1	45.3	29.5
Range	25–48	37–140	19–79	0.8–4.2	18.4–82.9	0–7
^{18}F Small volume						
Mean	17.3	56.5	16.7	0.85	56.0	22.1
Range	7–26	10–115	3–37	0.1–1.5	22–140	7–34

Conclusions: Our first year experience indicates the SV target has a better yield than the LV target. This is in accordance with the different metal used for the target cavity, as the titanium is known to have a lower yield than silver. In our experience, the most important variable for the final yield is the pressure inside the target, which seems to be independent from the ratio (target current / target + collimator current) and from the foils and strippers status.

25

FUSION OF MRI AND SPECT WITH ^{131}I ALPHA-METHYLTYROSINE IMAGES COMPARED WITH 1H-MRS USED FOR EVALUATION OF MALIGNANT BRAIN TUMORS RECURRENCES

P. Grzelak[1], M. Górska-Chrząstek[2], W. Gajewicz[1], R. Mikołajczak[3], E. Zakrzewska[3], L. Stefańczyk[1], J. Kuśmierek[2]

[1]Department of Radiology Medical University of Lodz, [2]Department of Nuclear Medicine Medical University of Lodz, [3]"POLATOM" Isotope Centre, Świerk, Poland

Purpose: The most essential modalities for morphological imaging of brain are magnetic resonance imaging (MRI) and, for metabolic imaging, single photon emission tomography (SPECT). By using image fusion of different modalities, the location of areas exhibiting functional changes can be more easily identified. In our studies ^{131}I alpha-methyltyrosine, a radiopharmaceutical manufactured in Poland, has been used. This ^{131}I labeled compound is much less expensive that ^{123}I labeled analogue; use of ^{131}I although delivering higher dose, was found justified in patients with already diagnosed malignancy. The study compares the results of ^{131}I alpha-methyltyrosine SPECT (IMT-SPECT) with MRI in detection of brain gliomas tumor. We used fusion images as the method of planning H1-Magnetic Resonance Spectroscopy (H1-MRS) and verifying the metabolic content of the regions.
Material and methods: We investigated 27 patients. SPECT scintigraphy of the brain was performed using a double head gamma camera (ELSCINT), 15 min after intravenous administration of the IMT at activity of 74 MBq. MRI has been performed using a 1.5 T Magnetom Vision Plus (Siemens) unit. Fusion was made in modern technique of three-dimensional superimposing on the PC workstation. For accurate spatial data Pitting of the image sequences we used isotope markers in specific head points during SPECT. Areas of the biggest activity appointed in this way were used as brie data for planning 1 H-MRS.
Results: In 19 patients the MRI imaging disclosed presence a polymorphic focus suggesting a tumor recurrence, in 8 subjects the result was equivocal due to presence of post surgery and irradiation squeal. In all 19 patients with a positive relapse in the MRI and in 4 with the equivocal image there was an enhanced IMT uptake in SPECT image. In 4 patients the scintigraphy yielded a negative result. SPECT/MRI image fusion enabled a topographic localization of the area with a peak activity, in most cases corresponding to the solid tumor visualized in MRI In patients with positive SPECT results 1 H-MRS done in the determined areas revealed spectra typical for malignancy.
Conclusion: The SPECT study using ^{131}I alpha-methyltyrosine enables confirmation of the presence of neoplastic tissue that may correspond to the site of tumor glioma recurrence. In equivocal MRI images the scintigraphy may disclose or exclude recurrence. It seems also important that ^{131}I IMT yields images of good quality. The fused SPECT/MRI images let to determine areas of tumor metabolic activity, being extremely useful data in planning spectroscopy.

26

IODINE [131 I] LABELLED GELATIN MICROSPHERES — LABELLING, STABILITY AND BIODISTRIBUTION AFTER PERORAL ADMINISTRATION

E. Janevik-Ivanovska[1], K. Mladenovska[2], R. Slavevska-Raicki[2], K. Goracinova[2], O. Vaskova[1]

[1]Institute of Pathophysiology and Nuclear Medicine,Medical Faculty, Skopje, Macedonia, [2]Institute of Pharmaceutical Technology, Faculty of Pharmacy, Skopje, Macedonia

Aim: A number of potential delivery systems, including sustained-antigen releasing microparticulated carriers have been used for targeting the Peyer's patches and protecting the antigen of interest from the harsh environment of the GIT. Although intracellular uptake, intracellular/paracellular uptake and uptake via the M-ells and Peyer's patches are the three possible mechanisms of gastro-intestinal pathway seems to be via the M-cells and Peyer's patches. Gelatin microspheres have already showed the stronę adjuvant effect by inducing IgA secretion at the genito-urinary mucosa after peroral application to mice.
Material and methods: Certain variations in the process parameters (emulsification time, surfactant concentration) were performed in order to prepare BSA-loaded gelatin microspheres with high loading efficacy and particle size ranging from 1 to 10 m. The mathematical modeling of drug release in the presence of collagenase showed a biphasic release pattern, where the rate constant for the initial time release confirmed the influence of the particle size and/or enzymatic degradation rate on drug release rate. Iodination of loaded gelatin microspheres with iodine-131 was performed using Chloramine-T method and quality control was checked using Paper Chromatography (PC) and Instant Thin Layer Chromatography (ITLC) in methanol/water (3:1) as a solvent. Stability studies of radiolabelled [131I] BSA loaded gelatin microspheres were performed after labelling and one and eight days after in the same condition.
Results: The percentage of the obtained complex after labelling was more than 90% and the free iodine less than 10%.The labelled product was stable without changing the percent of labeling eight days stored at +4°C. Visual examination of the product was directly through a lead glass screen and pH value was always in the optimal range of 6.5–8.5. Biodistribution studies of radiolabelled [131I] BSA loaded gelatin microspheres were carried out on BALB/c mice after peroral administration. To two groups, the radiolabelled [131 I] BSA gelatin microspheres with different mean particie size, 1.196 ± 1.961 and 7.028 ± 1.231 m were administered orally. To the control group, a solution of [131 I] BSA was also orally administered. Biodistribution was followed periodically within 15 days as a percent of total radioactivity present in stornach, smali intestine with Peyer's patches and mesentery, colon with Peyer's patches, appendix and mesentery, liver, spleen, blood, kidney, lungs and heart.
Conclusions: Our results data confirmed that uptake in mice into Peyer's patches and passage to the liver and spleen via the mesentery lymph supply and nodes, increased with decreasing particle size.

27

[^{66}GA]OXINE COMPLEX: PREPARATION AND STABILITY AS A POSSIBLE PET RADIOPHARMACEUTICAL

A.R. Jalilian, P. Rowshanfarzad

Cyclotron/Nuclear Medicine Department, Research Center for Agriculture & Medicine, Karaj, Iran

Introduction: ^{66}Ga(T1/2 = 9.49 h, E: 833, 1039.5 keV; +: 56.5% ; E.C: 43.5%) [1] is an intermediate-lived radionuclide and has been proposed for PET imaging [2–6]. ^{66}Ga has been used for the radiolabeling of monoclonal antibodies [1, 7] and blond cells [8]. Tris(8-quinolinolato)Ga(III) complex (Ga-oxine) has been proved to have suppressive effects on the viability of A549 human malignant lung adenocarcinoma cells [9]. Due to the increasing importance of PET, ^{66}Ga complex formation conditions with oxine was optimized, in order to develop [^{66}Ga]oxine.
Material and methods: ^{66}Ga was prepared via the ^{66}Zn(p, n) ^{66}Ga reaction by 15 MeV proton bombardment of an electroplated enriched 0.04 (g/cm^2) ^{66}Zn-target (6° angle) with a current intensity of 180 A for 67 min (200 Ah) in a 30 MeV cyclotron and was separated by cation exchange resin in the form of [^{66}Ga]$GaCl_3$ using a NCA method described previously with slight modifications [10–13]. The resultant activity of ^{66}Ga was 2.23 Ci (E.O.B.) and the production yield was 11.2 mCi/ Ah. Gamma spectroscopy by HPGe detector showed a radio-nuclide purity higher than 97% (Figure 1).

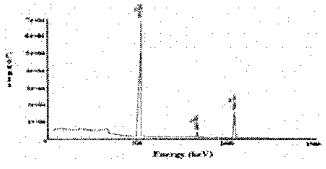


Figure 1. Gamma spectroscopy scheme of final product The presence of copper and zinc ions was checked by polarography.

Radiochemical purity was checked by polymer-backed silica gel layer chromatography. RTLC showed a major and distinct .radio peak at the Rf of 0.8. The radiochemical yields were determined by RTLC (> 97%): [^{66}Ga]$GaCl_3$ mixture was evaporated under a nitrogen flow followed by reconstitution with phosphate buffer solution (pH = 5, 0.4 ml). 300 l of 0.14 mg/ml ethanolic oxine solution was added to the residue and kept at different temperatures. The final solution was passed through a 0.22 filter and pH was adjusted. Pyrogen test was performed by a commercial LAL kit. Microbial-fungal tests showed a suitable pharmaceutical sterility [14, 15]. The ratio of free radiogallium to [^{66}Ga]oxine was checked by RTLC. The patterns for [^{66}Ga]$GaCl_3$ and [^{66}Ga]oxine did not change during 24 hours.
Results: The complex formation was optimized for pH, temperature, time, and the amount of oxine. At a random temperature, the best pH for the labeling was 5 (Figure 2).

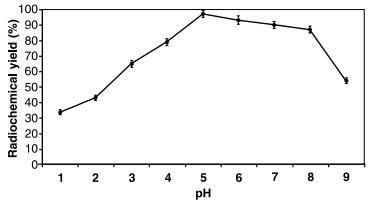


Figure 2. Effect of pH on radiochemical yield of [^{66}Ga]oxine at 25°C, n = 5, SE < 3%.

At the optimum temperature and pH, the maximum yield was reached within 15 minutes. Increasing the ratio of oxine to radioactivity increased the yield, presumably due to more available chelate (Figure 3).

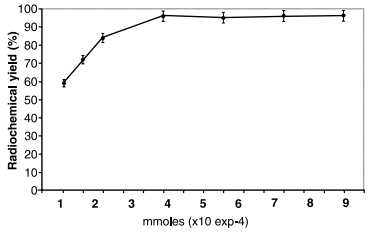


Figure 3. Effect of the amount of oxine used in the reaction on radiochemical yield of [^{66}Ga]oxine at 25°C, n = 5, SE < 3%.

Heating the reaction mixture to 50°C did not increase the yield. Further heating reduced the radiochemical yield due to the decomposition of oxine andlor the product. The thermal stability of [^{66}Ga]oxine was so excellent, that autoclaving made no change in the amount of free gallium present.
Conclusion: Total labeling and formulation of [^{66}Ga]oxine took about 15 minutes, with a yield of 97%. A suitable specific activity was ormed via insertion of [^{66}Ga]gallium cation. No unlabeled and/or labeled by-products were observed upon TLC or HPLC analysis of the final preparations. The radio-labeled complex was stable in aqueous solutions at least for 24 hours. No significant amount of other radioactive species was detected by HPLC 24 hours after labeling. HPLC and TLC showed that radiochemical purity of the [^{66}Ga]labeled components was higher than 95% with a specific activity of 896 mCi/ml.

28

RADIOSYNTHESIS OF FLUORINE-18 RADIOTRACERS WITH HPLC PURIFICATION ON A COMMERCIAL [^{18}F]FDG AUTOMATED SYNTHESIS MODULE

M. Cleij[1], C. Mosdzianowski[2], R. Smith[1], O. Golovko[1], P. Burke[1], F.I. Aigbirhio[1], J.C. Clark[1]

[1]Wolfson Brain Imaging Centre, Department of Clinical Neuroscience, University of Cambridge, Addenbrookes Hospital, Cambridge, United Kingdom,
[2]GEMS Benelux s.a., ANS (Loncin), Belgium

Introduction: The GE TRACERIab MX-FDG Synthesiser (previously the Coincidence FDG Synthesizer) is a high yielding, rapid and reliable system for the preparation of [18F]FDG from cyclotron-produced [^{18}F]fluoride. Implementation towards GMP is enhanced with the radiosynthesis been performed on disposable one-use kits. With it's use of the generic [^{18}F]radiofluorination method based on the generation of the nucleophilic [^{18}F]fluoride-K+-aminopolyether-2,2,2 system and with programmable hardware it was considered feasible to use this module for the synthesis of other fluorine-18 tracers.
Material and methods: Additional fluorine-18 tracers preparations were then implemented on the module with the following key modifications of the disposable kit, reagents and synthesis program: [^{18}F)FLT: removal of tC18 cartridges, hydrolysis with 1 N HCl, precursor N-(2, 4-Dimethoxybenzyl)-5'-O-dimethoxytrityl-3'-O-nosylthymidine, reaction at 130°C for 5 min [^{18}F]FMISO: hydrolysis with 1N HCl, precursor 1-(2'-Nitro-1' -imidazolyl)-2-O-tetrahydropyranyl-3-O-tosyl-propanediol, reaction at 100°C for 6 min. Purification of the radiotracer by radio-HPLC was performed by the transfer of 10m of the final reaction solution through neutral alumina ([^{18}F]FLT) or alumina and C18 ([^{18}F]FMISO) cartridges on to a preparative HPLC module with external software control for acquisition of radio-detector signal and collection of product fraction. HPLC Method: Phenomenex Luna 5 C-18 100Å 250 × 10 mm, water/ethanol, 90/10 v/v, 3 ml/min RT = [^{18}F)FLT, 22 mins; [^{18}F] FMISO, 15 min.
Results and Conclusion: With little modifications to the disposable kit we have implemented the preparation of the radiotracers [^{18}F]FLT and [^{18}F]FMISO on the TRACER-lab MX-FDG Synthesiser with good radiochemical yields (FLT, 25%; FMISO 44%, decay corrected) and high radiochemical purities (> 95%). Products, which have been confirmed to be sterile and pyrogen free, are now been used for human and animal PET studies.

29

PRE-CLINICAL INVESTIGATIONS OF α-METHYLTYROSINE LABELED WITH IODINE-131 OR IODINE-123 (IMT-^{131}I, IMT-^{123}I)

E. Zakrzewska, R. Mikołajczak, A. Markiewicz, A. Korsak

Radioisotope Centre "POLATOM", Otwock-Świerk, Poland

Introduction: The aminoacid a-methylotyrosine (IMT) radiolabelled with the iodine-131 or iodine-123 has been used in the diagnostics of recurrent brain tumours, in the planning of re-operation and/or external radiotherapy. The aim of our work was to indicate that both radiopharmaceuticals IMT-^{131}I and IMT-^{123}I can be prepared in a reproducible manner, retaining high in vitro and in vivo stability and thus can be a subject of preliminary clinical investigation. Although the iodine-123 is more suitable for imaging, due to its very high cost it is not often used for economical reasons while cheaper iodine-131 may be an alternative.
Material and methods: For radiolabelling of IMT-$^{123/131}$I the electrophilic substitution reaction has been applied in the presence of iodogen. The walls of the reaction vial were covered with a film of iodogen and then 300 μg of L-α-methylotyrosine dissolved in the boric buffer (pH = 8.0) was added followed by iodine-131 or iodine-123 (111–3700 MBq) in carbonate buffer (pH =8.5). After 10 min the reaction mixture was transferred on the Sepahadex DEAE A-25 column and the column eluted with water. For quality control of the labelling yield and radiochemical purity of the iodinated compounds the methods of HPLC and electrophoresis were employed. The investigations of biological distribution were carried out on Swiss mice.
Results: Altogether 18 batches of IMT-^{131}I and 6 batches of IMT-^{123}I were prepared. The radiolabelling yield of IMT-^{123}I was at the level of 85–92%, and for IMT-^{131}I at the level of 92–98%. The radiochemical purity of both iodinated compounds was in the range of 99.5–99.9%. The preparation is not harmful in the dose of 4200 MBq/70 kg.
Conclusions: The final parameters of $^{123/131}$I — IMT were as follows — solution in 0.9% NaCl for injection, specific activity — 10–36.6 mCi/mg (370–1357 MBq/mg), radioactive concentration 95%, radionuclidic purity 98%. Both radiopharmaceuticals $^{123/131}$I-IMT were pre-clinically tested and their usability confirmed.

30

USE OF THE ^{131}I-LABELED MONOCLONAL ANTI-BETA3 ANTIBODY FOR DIAGNOSIS OF TUMOR NEOANGIOGENESIS

M. Bilski[1], E. Lisiak[1], R. Mikołajczak[3], U. Karczmarczyk[3], J. Pietrzykowski[2], E. Nowosielska[1], M.K. Janiak[1]

[1]Military Institute of Hygiene and Epidemiology, Warsaw, Poland, [2]Central Hospital The Ministry of Defence, Military Institute of Health Service, Warsaw, Poland, [3]Research and Development Department, Radioisotope Centre "POLATOM", Świerk, Otwock, Poland

Introduction: Anty-angiogenesis treatment is recently drawing more and more attention. In consequence, some diagnostic methods enabling the estimation of the progress of anty-angiogenic therapy should be developed. Among potential candidates, which could serve as a specific tracer for diagnostic purposes, is a monoclonal antibody (MoAb) directed against subunit β_3 of integrin $\alpha_v\beta_3$ (CD61, 2C9.G2 produced by BD Bioscience). Conjugating this MoAb with short living isotopes emitting gamma radiation enables the imaging of radiopharmaceutical disposition using detectors such as a gamma-camera.
Material and methods: For estimation of the applicability of the anti-CD61 MoAb (2C9.G2) to visualization of tumor blond vessels in vivo, the antibody was conjugated with iodine ^{131}I. For this purpose standard chloramine T method was used. The radiochemical purity of MoAb-^{131}I measured at 1 hour after the iodination was completed exceeded 99%. Imaging of the distribution of the conjugates in the transplanted syngeneic tumors as murine models of angiogenesis was carried nut implementing visualization techniques used in nuclear medicine. The biodistribution of the conjugates in the body of mice was evaluated using the same model of angiogenesis as in imaging studies. Results are expressed as the percent of injected dose in one gram of tissue (%ID/g), each value represents the mean and SD of three animals.
Results: The results of preliminary studies showed that conjugat MoAb anty-β_3-^{131}I given intravenously, accumulates in engrafted subcutaneously Lewi's lunę carcinoma, keeps steady for 144 hours, and demonstrates high tumor/background ratio (16/1 for tumor/muscle). Additionally, the biodistribution reveals predominantly urinary excretion. This data was confirmed in scintigraphic studies, which show a good visualization of neoangiogenesis in tumor bearing mice.
Conclusion: In conclusion, we anticipate that using MoAb anty-CD61 as a radiotracer could be the basis for elaboration of a non-invasive diagnostic method allowing to display tumor neovasculature in vivo, to monitor tumor growth and to estimate the anty-angiogenic therapy progression in early and late stages of disease.

31

LABELING OF AMPICILLIN SODIUM WITH ^{99m}TC FOR IMAGING INFECTION

O. Orumlu[1], M. Asikoglu[1], F. Gamze Durak[1], K. Koseoglu[2], H. Ozkilic[2]

[1]Ege University, Faculty of Pharmacy, Department of Radiopharmacy, Izmir, Turkey, [2]Ege University, Faculty of Medicine, Department of Nuclear Medicine, Izmir, Turkey

Introduction: Ampicillin sodium ((6R)-6-(-D-Phenylglycylamino)penicillanic acid) (ABPC) is a -lactam antibiotic that is active agent against both gram (+) and gram (–) bacteria and is widely used for treatment of infections. Radiolabeled antibiotics are being used for diagnosis of infections. Ampicillin sodium is a penicillin antibiotic was radiolabeled with technetium-99m and evaluated as a potential infection imaging agent.
Material and methods: Ampicillin sodium was labeled by a direct method. Stannous chloride was used as reducing agent. Quality controls were performed by thin-layer chromatography by silica gel plates and three different developing media: 1) acetone, 2) butanol:ethanol:water, (35:35:30), 3) butanol:pyridine: water (35:35:30). ^{99m}Tc-Ampicillin (^{99m}Tc-ABPC) was given intravenously to rats and rabbits which are infected with *Staphylacoccus aureus* and *Eschericia coli* and 1 hour later scintigraphic images were obtained by gamma camera.
Results: The radiochemical purity of the compound was higher than 95%. According to scintigraphic studies the compound was located at infection site.
Conclusion: There are several radiolabeled antibiotics for diagnosis of infection. ^{99m}Tc-ABPC is a suitable agent for scintigraphic detection of infection in the animals infected with *Staphylacoccus aureus* and *Eschericia coli* and further studies are in progress.

32

LABELING OF ZOLEDRONIC ACID WITH ^{99m}-TECHNETIUM FOR BONE IMAGING AND BIODISTRIBUTION STUDIES IN RABBITS

F. Gamze Durak[1], M. Asikoglu[1], O. Orumlu[1], K. Koseoglu[2], H. Ozkilic[2]

[1]Ege University, Faculty of Pharmacy, Department of Radiopharmacy, Izmir, Turkey, [2]Ege University, Faculty of Medicine, Department of Nuclear Medicine, Izmir, Turkey

Introduction: Zoledronic acid is a new generation biphosphonate strongly inhibit bone resoption. Biphosphonates have high affinity for calcium phosphonates and hydroxyapatite. They have been chosen agents for bone imaging because of their structure. The most commonly used agent for skeletal scintigraphy in nuclear medicine is a radiolabeled biphosphonate, ^{99m}Tc-MDR. For this reason it is important to evaluate the new generation biphosphonates that have better pharmacokinetic parameters as bone scanning agents when they labeled with ^{99m}Tc. Zoledronic acid is a new generation biphosphonate strongly inhibit bone resoption. The aim of the present study is to label zoledronic acid and evaluate its in vitro stability and its biological behaviors in animal modela and compare it with ^{99m}Tc-MDP.
Material and methods: Zoledronic acid was labeled with ^{99m}Tc by direct method. Labelling was achieved in the presence of stannous chloride as reducing agent. After labeling with ^{99m}Tc, chromotographic, stability and animal biodistribution studies were performed. Labeling efficiency, radiochemical purity and stability of the radiolabeled complex was determined by Paper Chromatography. For *in vivo* biodistribution studies ^{99m}Tc-Zoledronic acid and ^{99m}Tc-MDP were adminestered intravenously to rabbits and images were obtained at 1 h and 2 h by using a gamma camera.
Results: For Paper Chromatography acetone and saline were used as solvents to detect $^{99m}TcO_4$- and ^{99m}Tc-reduced-hydrolized. According to the chromatography results, the labeling efficiency was found greater than %95 without significant changes until 6 h post labeling at room temperature. The in vivo distribution of ^{99m}Tc-Zoledronic acid was studied over a 2 h period of time. The bone-soft tissue ratios from biodistribution after injection was for ^{99m}Tc-Zoledronic acid 2.85 after 1 h and 4.43 after 2 h, and for ^{99m}Tc-MDP 3.45 after 1 h and 4.49 after 2 h.
Conclusion: ^{99m}Tc-Zoledronic acid can be used as a bone scanning agent in nuclear medicine.

33

RECOMBINANT HTSH RADIOLABELLED WITH TECHNETIUM-^{99m}, A NEW PROMISING RADIOPHARMACEUTICAL FOR THE DIAGNOSIS OF METASTASES IN DIFFERENTIATED THYROID CANCER — PRELIMINARY STUDIES

E. Byszewska-Szpocińska, U. Karczmarczyk, R. Mikołajczak

Radioisotope Centre "POLATOM", Otwock-Świerk, Poland

Introduction: The imaging of TSH receptors using technetium-99m labelled TSH (rhTSH) might be useful to follow-up differentiated thyroid cancer (DTC) (after ablation by iodine-131 therapy) because currently used whole body scan (WBS) technique is very often characterized by poor sensitivity. The aim of our preliminary studies was to prepare tracer — rhTSH labelled with technetium-99m with high specific activity, good radiochemical purity and stability *in vitro*.
Material and methods: Recombinant human thyrotropin (rhTSH, Thyrogen) was labelled with technetium-99m using direct method with the addition of $SnCl_2$ and indirect — using the bifunctional chelating agent HYNIC. Purified HYNIC-rhTSH complex was labelled with technetium-99m in presence of $SnCl_2$ and tricine as co-ligand. Radiochemical purity of the obtained tracers and their *in vitro* stability in human serum was studied using chromatography on BioSep-SEC-S 2000 column in HPLC system and ITLC paper chromatography. Preliminary pharmacokinetic studies were performed in healthy Swiss mice and Wistar rats.
Results: Obtained tracers ^{99m}Tc-rhTSH and ^{99m}Tc-HYNIC-rhTSH were almost 100% pure (unbound ^{99m}Tc-pertechnetate was not detected) and specific activity of 125 mCi/mg. ^{99m}Tc-HYNIC-rhTSH was very stable in serum up to 3 hours. The thyroid uptake of the tracers tested in mice and rats was stable and at the even level over the period of 3 hours while in the same time fast blond clearance was observed.
Conclusions: After labelling with technetium-99m the obtained rhTSH complexes presented high radiochemical purity and in vitro stability in serum. The retention of radioactivity in thyroid showed that affinity to specific receptors was tint affected by the radiolabelling process. These initial data require confirmation in cell and animal studies.

34

IN VITRO AND *IN VIVO* COMPARISON OF ^{99m}TC-LABELLED-UBI 29-41 BY FOUR DIFFERENT METHODS

J.L. Crudo[1], N. Nevares[1], A.M. Zapata[1], M.E. Bronzi[2], A.E. Sorge[2], E.R. Obenaus[1], S.G. de Castiglia[1]

[1]División Radiofarmacos, Centro Atómico Ezeiza, Comisión Nacional de Energia Atómica, Buenos Aires, Argentina, [2]Sección Bacteriologia, Instituto Roffo, Buenos Aires, Argentina

Introduction: The aim of the present study was to investigate how affect four different 99mTc labelling methods the *in vitro* and *in vivo* behaviour of the UBI 29-41 and to select the most appropriate ^{99m}Tc-labelled UBI 29-41 for detection of *Staphylococcus* aureus infected sites in mice.
Material and methods: The UBI 29-41 was labelled with ^{99m}TC by two direct methods: a) using stannous pyrophosphate and BHIC4 and b) using $SnCl_2$ and NaOH, and by two indirect methods previous conjugation with BFCs: c) NHS-MAG_3 and d) NHS-HYNIC using tricine as coligand. HPLC studies, stability in saline, cysteine challenge and *in vitro* binding to 107 CFU of S.a. were done. Biodistribution studies in S.a. infected mice were carried out and infected thigh/normal thigh ratios (ITINT) were calculated.
Results: Radiochemical purities were higher than 87%, stability in PBS for 24 h was higher than 95% and the percentage of total activity transchelated to cysteine was lower than 10%. *In vitro* binding to bacteria showed big differences: the highest value was 41.4% of total activity for ^{99m}Tc-UBI 29-41 (method a) and the lowest value was 12.5% for ^{99m}Tc-MAG_3-UBI 29-41. IT/NT ratios of each compound were a) 2.56, b) 1.50, c) 1.60 and d) 1.76 respectively.
Discussion and conclusion: Our results showed that the direct labelling approach (method a) applied to UBI 29-41 gave the best complex for S.a. infection detection in mice, due its high IT/NT ratio and easy labelling procedure compared with indirect labelling methods.

35

^{99m}TC-PYROPHOSPHATE AS POTENTIAL IMAGING AGENT FOR BACTERIAL INFECTION

D.Dj. Djokić[1], D.Lj. Janković[1], S.V. Pavlović[2]

[1]Laboratory of radioisotopes, Institute of Nuclear Sciences «Vinča», Belgrade, Serbia and Montenegro, [2]Nuclear Medicine Institute, Clinical Centre of Serbia, Belgrade, Serbia and Montenegro

Introduction: Various radiolabelled compounds have been explored in nuclear medicine diagnostic for visualisation of bacterial infection or sterile inflamatory processes. The main goal of these investigations was to find out some compound labelled with technetium which had also capability to distinguish infection from sterile inflammation. Among the labelled compounds, white blond cells labelled with indium-111 or with technetium-99m, through ^{99m}Tc-HMPAO, could be useful. Recently, a new radiopharmaceutical ^{99m}Tc-ciprofloxacin has been developed. ^{99m}Tc-Sn complex of pyrophosphate was proposed for skeletal imaging in 1972. Since that time, ^{99m}Tc-PYP has been used for visualisation of acute myocardial infarction as well as for *in vivo* labelling of red blond cells for radionuclide ventriculography and blond pool scintigraphy. In this study the possibilities for use of ^{99m}Tc-PYP as potential specific agent for bacterial infection and sterile inflammation-imaging were investigated. *In vitro* binding study results of ^{99m}Tc-PYP to bacteria, as well as *in vivo* study in infected animals and human with known infection or inflammation, were presented. Material and methods: ^{99m}Tc-PYP mixed with an aliquot of bacteria suspension (S. aureus ATCC 25923 suspensions, 2 × 108 organisms/ml) was incubated and after centrifugation, the radioactivity in the bacteria pellet was measured. The results were expressed as the percent uptake of radioactivity bound to the viable bacteria in regard to total radioactivity. Wistar rats were used in all animal studies of ^{99m}Tc-PYP. The radiopharmaceutical was injected via the tail vein 24 or 48 h after infection. One or four hours after i.v. application of ^{99m}Tc-PYP, the animals were sacrificed and the radioactivity per organ of interest (or per g), was measured. In clinical study ^{99m}Tc-PYP was used for early (30 min) and late (3 hours) sequential whole body scintigraphy in two patients with known infection or inflammation.
Results/Discussion: The *in vitro* binding results have shown that uptake of ^{99m}Tc-PYP by S. aureus was higher than 30%. The *in vivo* investigation results on rats have shown some increase of radioactivity in infected muscles (TINT > 2.5) and high bone uptake (5.4 6.9 % ID/g). Scintigraphic study in a patient with pleuropneumonia and chronic rheumatoid arthritis has shown that ^{99m}Tc-PYP accumulated in pulmonary and bone lesions. Lesion to non-lesion ratios (L/NL), expressed as a ratio of total counts in infected/inflamed region and identical region in the contralateral normal tissue, for early sequential whole body scintigraphy were 1.48 for lung and 6.03 for bone.

36

^{99m}TC LABELLED RITUXIMAB: A NEW NON-HODGKIN'S LYMPHOMA IMAGING AGENT

T. Gmeiner Stopar[1], S.J. Matherz[2], S.Hojker1, J. Fettich[1]

[1]University Medical Centre Ljubljana, Department for Nuclear Medicine, Slovenia, [2]Cancer Research UK, Department of Nuclear Medicine, St Bartholomew's Hospital, London, United Kingdom

Introduction: This study was performed to explore the possibility of labelling Rituximab with 99mTc for use as an imaging agent suitable for early detection, staging and subsequent remission assessment of non-Hodgkin's lymphoma. Rituximab (Mabthera) is a chimeric mouse/human monoclonal antibody, highly specific to the CD20 antigen expressed by > 90% of B-cell lymphomas.
Material and methods: Mabthera was purified by ultrafiltration using a Centricon YM-10 tube and labelled with 99mTc by photo-activation method. Aliquotes of 5 mg/ml Rituximab and Amerscan Medronate II kit (in 0.1 M PB) was irradiated for 30 min at 302 nm, aliquoted in nitrogen-filled vials and stored at –80°C. Each aliquot was labelled with sodium (99mTc) pertechnetate and incubated at RT for 1 h before RCP determination by TLC and HPLC. Free thiol groups on reduced Rituximab were quantified using 5-iodoacetamidofluorscein (5-IAF). 80 l of 1 mg/ml 5-IAF in methanol was added to reduced Rituximab, pre-incubated with EDTA, and incubated for 120 min at 37°C. The Rituximab-IAF complex was separated using PD-10 column in PBS. Absorbance were measured at 280 and 495 nm. For calculation of the number of free thiol groups/Rituximab molecule calibration curves from a series of standard solutions of 5-IAF (at 280 and 495 nm) and an extinction coefficient of 1.4 for Rituximab (at 280 nm) were used. The immunoreacitve fraction (IRF) of the 99mTc-rituximab was determined using Lineweaver-Burk analysis in a series of 6 double dilutions of Raji cells. To estimate non-specific binding unlabelled Rituximab was added.
Results: RCP over 95% was determined in aliquots stored at –80°C over 4 weeks. On average 4.11 free thiol groups/photoreduced Rituximab were determined. The IRF of 25.92% requires further investigation.
Conclusion: 99mTc labelled Rituximab is a promising imaging agent suitable for early detection, staging and subsequent remission assessment of NHL.

37

MYOCARDIAL EXTRACTION OF A NEW TYPE OF TECHNETIUM-LABELED FATTY ACIDS

A. Heintz[1], S.N. Stehr[1], G. Wunderlich1, M. Walther[2], C.M. Jung[2], R. Bergmann[2], J. Pietzsch[2], K. Rode[2], W. Kraus[3], H.-J. Pietzsch[2], J. Kropp[1], H. Spies[2], A. Deussen[1]

[1]TU Dresden, Medizinische Fakultat Carl Gustav Carus, [2]Forschungszentrum Rossendorf, Dresden, [3]Bundesanstalt fur Materialforschung und -prufung, Berlin, Germany

Introduction: Newly developed technetium-labeled fatty acid analogues are promising agents for myocardial metabolism imaging. We tested the myocardial extraction of 99mTc analogues of rhenium model complexes synthesized according to the "4 + 1" mixed-ligand approach (see presentation M. Walther et al.) in a guinea pig heart LANGENDORFF model.
Material and methods: Firstly 30 min of steady state perfusion with 10 ml/min of normotherm Krebs-Henseleit-Buffer enriched with 0.1% albumin was performed. Isolated guinea pig hearts were then perfused with fatty acid tracers for 180 seconds in a 1:100 ratio. The venous effluate was collected in intervals of 15 s for 225 s following the start of tracer infusion. Perfusion was stopped, the heart was dissected into atria and ventricle compartments and y-activity was measured. Eight new (C11, C12, C15, C10S, C11S, C12S, C14S, C16S) and four known control substances (1231-IPPA, 1231-BMIPP, 99mTc-MIBI, 99mTc-DTPA) were tested in this model (n = 3–5).

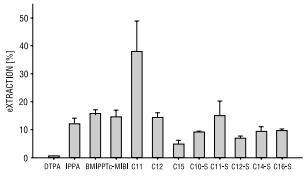


Figure 1.

Results: Results are summarized in Figure 1. All eight substances showed acceptable extraction rates. Especially experiments with the C11 (39.8%), but also C12 (14.8%) and C11 S (17.1%) fatty acid analogues showed excellent results.
Conclusion: When compared with extraction rates for IPPA determined with the same set-up, extraction for the C11 and C11 S compounds is 3 fold and 1.3 fold, respectively. These results confirm our hypothesis that the "4+1" Tc(III) chelate unit is a promising tool for the Tc-labelling of fatty acids. Further experiments with different species are planned to elucidate exact myocardial uptake mechanisms.

38

INTRODUCTION OF ^{99m}TC- EDDA/HYNIC-TOC: PRECLINICAL EVALUATION

S. Kuzmanovska, O. Vaskova, T. Tripunovski, M. Zdraveska-Kocovska, E. Janevik-Ivanovska

Institute of Pathophysiology and Nuclear Medicine, Medical Faculty, Skopje, Macedonia

Introduction: The use of ^{99m}Tc-labeled somatostatin analogues has been a valuable diagnostic tool in nuclear oncology for over a decade. Therefore, our goal was to introduce an „in house" production of ^{99m}Tc-EDDA/HYNIC-TOC, within the project activities supported by the IAEA. Initially, we tried to optimize the labeling protocol concerning our own facilities and perform a basic quality control, before proceeding the clinical application.
Material and methods: The conjugated peptide (HYNIC-TOC) was provided by Polatom and wet labeled according a given procedure. The incubation step was performed using two labeling conditions. The radiochemical purity was assessed on ITLC-SG plates (Merck, 5553) in different solvents. The biological distribution was performed on Wistar rats, both with crude and Seppak purified radiopharmaceutical.
Results: The fraction of free pertechnetate in crude RF ranged from 0.5–0.9%, ^{99m}Tc-RH were between 1.6 and 3.0%, while for the ^{99m}Tc-non peptide bound impurities we found 7.6–23.5%. The biodistribution experiments with crude and purified RF showed certain differences.
Conclusion: According to the results presented, we couldn't omit the Seppak purification of the wet labeled product. Further activities will be undertaken towards production of a small batch kit.

39

PREPARATION AND PRELIMINARY EVALUATION OF THE LIPOSOME--ENCAPSULATED 99MTC-DITHIZONE

M. Maurin, P. Garnuszek

Department of Radiofarmaceuticals, National Institute of Public Health, Warsaw, Poland

Introduction: Research on radioisotope markers for viable pancreatic islets cells resulted in development of conjugate of 2,2'-dicarboxy-dithizone and 131 I-Histamine. Conjugate has properties of binding to zinc(II) ions and shows a specific distribution in pancreas in vivo. Nevertheless, cumulated activity was not satisfactory to use it in scynthygraphic methods. The aim of the research was focused on enlarging the retention of complex in pancreatic cells by applying liposomes as an isotope drug carrier. In the research Tc-99m radioisotope was used for obtaining radioactive dithizone, because of its wide use in scynthygraphic imaging and good accessibility.
Material and methods: During the study the method of labeling dithizone with Tc-99m has been developed using of NaBH4 as a reducing agent. Liposomes with positive and modified membrane (Cationic-MPEG) were formed from L-alpha-phosphatidylcholine, stearylamine, cholesterol and DSPE-MEG. The Tc-99m dicarboxo-dithizone was encapsulated by incubation 30min. at 550C. The yield of the complex entrapment into liposome was monitored by TLC techniques. The encapsulated fraction of the complex was isolated on Sephadex G-25M column. Biodistribution studies were carried on star rats and C3H/mammae adenocarcinoma tumor-bearing mice.
Results: The yield of incorporation 99mTc dithizone finto liposomes was 40%. Column purification resulted in pure encapsulated Tc-99m dithizone (ca 100%). Biodistribution studies of entrapped 99mTc-dithizone showed greater accumulation in pancreas (most activity located in spleen 13.1%ID/g) then that of 99mTc-ditizone without carrier: Additional biodistribution study of 99mTc dithizone-cationic-MPEG in C3H tumor bearing mice reveled a great uptake of radioactivity in tumor tissue (6.34%ID/g), calculated T/M — 14.
Discussion/Conclusions: Performed study resulted in establishing method of labeling dicarboxy-dithizone with Tc-99m as well as its incorporation into modified Cationic-MPEG liposomes. Biodistribution in rats has shown improved accumulation of the radioactive dithiozone, however still not satisfactory for scinthygraphy of viable pancreatic islets. Therefore, further modifications of a liposome membrane are needed. Moreover, this study has shown the perspective usefulness of a lipisome-encapsulated 99mTc-dithizone for tumor scinthygraphy.

40

PRODUCTION OF ^{99m}TC-ANTIMONY TRISULFIDE COLLOID FOR LYMPHOSCINTIGRAPHY

G.A. Shabani, H. Hamzeh, R. Najafi

Radioisotope Department NRC, Atomic Energy Organization of Iran, Tehran, Iran

The lymphatic system provides one of the main paths for the spread (metastasis) of cancer from one part of body to another. Hogdkin's diseases, lymphocytic leukemia, various metastatic disease and urany lymphnode disorders can be assessed by lymphoscintigraphy. Radionuclide lymphoscintigraphy has been used for urany years to define lymphatic drinage of melanoma. The most common radiopharmaceuticals used for lymphoscintigraphy are Tc-99m-SC, Tc-99m-antimony sulfide colloid and Tc-99m-HSA-nanocolloid. Preparation of Tc-99m-antimony sulfide colloid has been chosen among other colloids.
Material and methods: For antimony colloid preparation, hydrogen sulfide gas was passed through DW until saturation. Antimony potassium tartrate is then added to the solution to form Sb_2S_3 colloid. The colloid was stabilized with P.V.P. Excess H_2S was removed by bubbling with nitrogen. The preparation was filtered through a 0.22 Nur membrane filter and aliquots containing 1.017 mg Sb_2S_3 were dispensed finto kit vials. Labeling was accomplished by adding $^{99m}TCO^-$ and HCl to the vial then heating it at 100°C in boiling water bath for 10 min. The pH was adjusted by adding a phosphate buffer.
Results: The radiochemical purity of Tc-99m-antimony trisulfide colloid by ITLC--SG/normal saline was more than 95%. The amount of Sb in reaction vial was 0.729 mg. Conclusion: The study demonstrated that our formulation of antimony trisulfide which has 0.036 mg (Sb) in 0.2 ml of injection per patient (total volume after labeling with Tc-99m was 4 ml).

41

^{99m}TC HYNIC- IgG CONJUGATE. INDIRECT LABELLING IN PRESENCE OF EDTA AS COLIGAND AND STABILIZATION BY METAL DIVALENT CATIONS

J. Michalik, U. Karczmarczyk, A. Markiewicz

Radioisotope Centre "POLATOM", Otwock-Świerk, Poland

Introduction: ^{99m}Tc human gammaglobulin has Been demonstrated to be useful radiopharmaceutical in imaging inflammation lesions. In our work the indirect labelling of hIgG has been investigated.
Material and methods: A conjugate: HYNIC-IgG was synthesized according to Abrams M.J. et al. J Nucl Med 1990; 31: 2022–2028. The radionuclide ^{99m}Tc has been introduced into protein molecule by indirect method incorporation in phosphate buffer, pH = 7.4, in presence of stannous chloride as reducing agent for sodium pertechnetate and EDTA as coligand for ^{99m}Tc. Stability of labelled IgG-HYNIC derivative in serum in presence of copper, cobalt, iron and magnesium was analyzed by HPLC method (BioSEP SEC 4000, eluent: 0.1 mol/L phosphate buffer). Inflammation lesions were induced in Balb/3 mice thigh muscle by injection of 0.2 ml turpentine oil.
Results: The efficiency of ^{99m}Tc hIgG labelling (pH = 7.4, temp. 37°C) was strictly dependant on the presence of coligands. HYNIC-IgG derivative in EDTA presence gave the radiochemical purity value above 95%. Stability of HYNIC(EDTA)IgG — derivative labelled with technetium 99mTc decreased rapidly during incubation in serum at 37°C in time. During 4 h incubation in serum almost 80% of radiotracer present in IgG molecules has been dissociated. Better stability of ^{99m}Tc-HYNIC(EDTA)IgG conjugate in circulating serum for at least six hours was achieved by adding to the reaction mixture divalent metal cations using the following compounds: $MgCl_2$, $CoSO_4$, $Cu(NO_3)_2$ and $FeCl_2$ in equimolar ratio to EDTA. Scintigraphy of ssmTc gammaglobulin in mouse inflammatory lesions showed the tracer accumulation 6 times higher after 24 hours than in non-treated muscle.
Conclusions: Human IgG-HYNIC conjugate labelled with technetium ^{99m}Tc by indirect method can be used as basic compound in formulation for infection/inflammation scintigraphy agent.

42

EVALUATION OF ^{90}Y-COLLOID RADIOPHARMACEUTICALS FOR RADIOSYNOVIORTHESIS IN HEALTHY RABBITS

D. Mathe, L. Balogh, A. Polyak, L. Korosi, G. Kocsy, A. Kerekes, G.A. Janoki

National Center of Public Health — National Frederic Joliot-Curie Institute of Radiobiology and Radiohygiene, Budapest, Hungary

Introduction: Leakage and biodistribution of radioactivity after intra-articular injection finto the knee joint has been studied in healthy rabbit knee joints. Two colloidal systems for ^{90}Y have been studied, ^{90}Y citrate and ^{90}Y silicate.
Material and methods: Leakage studies have been performed at 6, 24, 48 and 120 hours post injection with 6 mate animals per time point for both preparations. Knee joint injection was done with 100–500 NCi of activity in 100 Nl volume. Correctness of injection was checked in all animals by gamma camera. The remaining activities in the knees were determined by measuring bremsttrahlung in a NaI(Tl) scintillation counter. Biodistribution studies were performed with ^{90}Y-citrate colloid, at 6 h, 24 h, 5 days, 8 days and 13 days post injection, in 6 male rabbits for each time point. Activity present in the skeleton, liver, spleen, inguinal lymph node, blood, kidney, testes, and injected knee was measured. Residence times and dosimetric data were calculated.
Results: Results of the leakage studies are summarized in Table 1 for ^{90}Y-silicate and Table 2 for ^{90}Y-citrate.

Table 1.

^{90}Y-silicate in knee joint		
Time oint	Mean	SD
6 h	100.95	8.67
24 h	87.33	12.98
48 h	84.36	11.42
120 h	78.83	11.08

Table 2.

^{90}Y-citrate in knee joint		
Time oint	Mean	SD
6 h	96.05	2.04
24 h	94.19	1.53
48 h	92.84	3.22
120 h	91.12	2.32

Results of the biodistribution studies showed mean activity values below 2% of ID in the whole skeleton and 0.5% of ID in the liver for early time points, and no detectable activity except in the knee joints at late time points.
Discussion: This extensive study has shown that both preparations reside mostly in the knee, the citrate colloid showing better retention. The estimated radiation burden to other organs is negligible after intraarticular injection of ^{90}Y-citrate colloid.

43

RHENIUM-188 SOLUTION OBTAINED FROM THE STATIONARY ^{188}W/^{188}RE GENERATOR

U. Karczmarczyk, K. Chrustowski, Z. Zelek, K. Sawlewicz

Radioisotope Centre "POLATOM", Świerk-Otwock, Poland

Introduction: Rhenium-188 is a generator produced radioisotope emitting both beta ($E_{\beta max}$ = 2.1 MeV) and gamma ($E\gamma$ = 155 keV, 15%) radiation with physical half-life of 16.98 h. Beta particles provide radiation suitable for destroying tumour cells with little or no damage to adjacent organs. During last decade the interest in using ^{188}Re for radiotherapy as well as for brachytherapy in the treatment of coronary vessels was growing rapidly. The technology of manufacturing the sodium perrhenate- ^{188}Re solution has been developed at the Radioisotope Centre POLATOM.
Material and methods: ^{188}Re is the product of tungsten-188 decay. The ^{188}W was adsorbed on the alumina column which was conditioned with 0.9% NaCl and 32% HCl. The daughter radionuclide ^{188}Re was eluted from the column using 0.3 M acetic acid. The obtained solution was purified and concentrated in the chromatographic system consisting of cation exchanger AG-50W-X12, on which the sodium ions were adsorbed and anionic column Sep-Pak QMA Light, on which the perrhenate ions were concentrated. Sodium perrhenate-^{188}Re was eluted in 1–3 ml of saline.
Results: The developed method enabled preparation of the carrier-free solution of sodium perrhenate-^{188}Re with activity up to 180 GBq in 1 to 3 ml volume. Radiochemical purity of ^{188}Re solution was > 99.9%. The highest activity of ^{188}Re was obtained 3 days after the previous elution. It was shown that ^{188}Re could be efficiently used for labelling of HEDP, radiopharmaceutical applied for palliative therapy of bone metastases.
Conclusion: The developed method for preparation of ^{188}Re in the form of perrhenate solution using the stationary ^{188}W/^{188}Re generator approach allows obtaining portions of ^{188}Re solution with required activity and radioactive concentration, which can be further on transported to the clinics and used for patient treatment.

44

CARRIER-FREE ^{90}Y — PRECURSOR FOR RADIOLABELLING OF RADIOTHERAPEUTIC RECEPTOR TARGETING AGENTS

A. Markiewicz, I. Sasinowska, A. Korsak, A. Muklanowicz, W. Piecyk

Radioisotope Centre "POLATOM", Otwock-Świerk, Poland

Introduction: The carrier-free ^{90}Y manufactured as a precursor for peptides labelling should conform to the qualitative and quantitative specification which assure its pharmaceutical usefulness. The parameters having significant influence on the quality is radionuclidic purity (Sr-90 content) and the content of chemical impurities, such as metallic cations, which may decrease the labeling yield of biological substances.
Material and methods: The carrier-free 90Y was obtained as chloride solution at the Radioisotope Centre POLATOM. For identification the liquid scintillation methods was used. The content of ^{90}Sr was determined by chromatographic extraction on the Sr-Resin (Eichrom) column followed by LSC measurement. The concentration of chemical impurities was determined by the optical emission spectrometry (ICP-OES). Radioactive concentration of ^{90}Y was measured in ionization chamber. Peptide DOTATATE used for verification of labeling yield was prepared in the dry-kit form (1.25 mCi/g, 1800 mCi/ mol). The radiochemical purity of ^{90}Y-DOTATATAE preparation was determined by HPLC method.
Results: In tested batches of ^{90}Y the content of ^{90}Sr was well bellow 2.5 10^{-4}%. The radioactive concentration was about 20 GBq/mL. The content of chemical impurities was below 1.0 g/mL for As, Cu and Ni, 5.0 g/mL for Pb and 10.0 g/mL for Fe and Zn. The complex of DOTATATE with 90Y was obtained with high radiochemical purity, over 99%. The ^{90}Y-DOTATATE preparation was stable at temperature 4–10°C over at least 72 hours (RCP > 98%).
Conclusions: The methods used for quality control of ^{90}Y enable the determination of critical parameters for assessment of $^{90}YCl_3$ solution. The carrier-free ^{90}Y manufactured at RC POLATOM conforms to the following specification: content of ^{90}Sr below 2.5 10^{-4} g/mL, Pb < 5.0 g/mL, Fe, Zn < 10.0 g/mL. The suitability of ^{90}Y precursor to label DOTATATE at patient therapeutic dose level has been proved.

45

COMPARATIVE *IN VITRO* EVALUATION OF DOTATATE LABELLED WITH ^{177}LU OR ^{90}Y, RADIOPHARMACEUTICAL FOR RECEPTOR MEDIATED RADIOTHERAPY

D. Pawlak, A. Korsak, R. Mikołajczak

Radioisotope Centre "POLATOM", Świerk-Otwock, Poland

Introduction: Rapid growth of clinical applications of peptide receptor radioimmunotherapy (PRRT) using somatostatin analogues labelled with beta/gamma-radiation emitters prompted us to establish the laboratory conditions for cell lines studies and to investigate in vitro the peptide DOTATATE (DOTA-Phe1,Tyr3-octreotate) labelled with ^{90}Y or 177Lu, both produced at Radioisotope Centre POLATOM.
Material and methods: DOTATATE (PiChem, Austria), the radionuclides ^{90}Y (carrier-free) and ^{177}Lu (about 8 Ci/mg Lu) obtained as chloride solutions (POLATOM) were used for labelling carried out in acetic buffer with addition of ascorbic acid at pH = = 4.5–5.3 followed by 30 min incubation at 90°C. The radiochemical purity of radiolabelled peptides was determined by TLC, HPLC and Sep-Pak separation. Serum stability was tested at 37°C over 24 hours after labelling. The internalisation and receptor affinity studies (non specific binding using Sandostatin or cold peptide) were carried out on live AR42J.
Results: The complexes of DOTATATE with ^{90}Y and ^{177}Lu were obtained with high radiochemical purity exceeding 98%. No significant differences between the stability of ^{90}Y or ^{177}Lu labelled peptide were observed (RCP values were: after 4 hours 99.91% and 99.40%, after 24 hours 99.08% and 99.62% respectively). Both complexes were stable in human serum. The tracers were rapidly internalizing to the AR42J cells (about 80% for ^{90}Y-DOTATATE and ^{177}Lu-DOTATATE during 60–90 minutes, while the binding to the receptors on cell surface is about 20%). The non-specific binding was low and equal to about 1%. The somatostatin receptor affinity, IC50, was found 40 nM.
Conclusions: The results of in vitro evaluation of ^{90}Y-DOTATATE and ^{177}Lu-DOTATATE did not show any significant differences in their in vitro behavior, high labelling yields as well as stability of both radiotracers confirm their suitability for therapeutic applications. The study was done within the IAEA co-ordinated research project "Comparative Evaluation of Therapeutic Radiopharmaceuticals".

46

MEASUREMENTS OF COLLOID PARTICLE SIZE IN THERAPEUTICAL RADIOPHARMACEUTICALS

A. Polyák, L. Balogh, D. Máthé, G.A. Jánoki

NCPH — „F.J.C." National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

Introduction: Localization and pharmacokinetics of therapeutical colloid radiopharmaceuticals mainly depends on their particle sizes. In our experiments we used laser scattering method for determination the sizes of different colloids. Samples tested were 90Y-Citrate colloid, 90Y-Silicate colloid, 188Re-(Tin-)Colloid and 166Ho-Fytate colloid. Radiochemical stability was also tested in vitro and ex vivo in synovial fluid.
Material and methods: The analytical instrument used in our experiments named DynaPro is a product of the Proteinsolutions Inc. (USA). The sample is illuminated by a semi-conductor laser of ~830 nm wavelength. The light scattered by the sample in the cell is collected and guided via a fiber optic Gable to an actively quenched, solid state Single Photon Counting Module (SPCM). The photons are then converted to electrical pulses and correlated. The DynaPro analyses the time scale of the scattered light intensity fluctuations by a mathematical process called autocorrelation. The translational diffusion coefficient (D) of the molecules in the sample cell is determined from the decay of the intensity autocorrelation data. The hydrodynamic radius (R) of the sample is then derived from D, using the Stokes-Einstein equation. In our experiments paralelly with the samples calibration standard was measured, it was 160 nm polystyrene standard (Bangs Laboratories Inc., Serial NO: 5692). Radiochemical stability measurements were in different time points by ITLC.
Results: Low sample volume (50 l) needed for determination, which offers possibility for serial measurements of labelled and inactive colloid materials. The calibration found more than 95% exactness of the polystyrene standard measurements. Particie fraction of 90Y-Citrate colloid ranged between 1 and 7 μm (mean diameter: 3.5 μm) and in case of 90Y-Silicate colloid particles ranged between 0.1 and 4.5 um (mean diameter: 1 μm). 188Re-(Tin-)Colloid mean diameter was around 4.5 μm. We also examined the stability of the colloid products. These radiopharmaceuticals showed high particle and radiochemical stability (> 99%) in vitro and in synovial fluid. Ideal measurement range of DynaPro is ranged between 1 and 5000 nm. Outside this range particie size measurement needs further evaluation.
Conclusion: Because of pharmacokinetics of therapeutical radiocolloids depends on their particle sizes the measurements by laser scattering method can be a very important element of radiopharmaceutical research.

47

A KIT-PREPARATION FOR LABELING OF ^{188}RE HSA-MICROSPHERES FOR THERAPEUTIC USE IN NUCLEAR MEDICINE

G. Wunderlich[1], A. Drews[2], J. Kotzerke[1]

[1]University Hospital, Department of Nuclear Medicine, Dresden, Germany, [2]Rotop Pharmaka GmbH, Dresden, Germany

Introduction: Intra-arterial infusion of labeled particles is an effective method for local endoradiotherapy of tumors. In this study, we present data on a efficient radiolabeling of biodegradablehuman serum albumin (HSA) microspheres with the short-lived beta-emitter 188Re by means of a sufficient kit-preparation.
Material and methods: One labeling-kit consists of three vials containing 9.3 mg 2.5 Dihydroxybenzoic acid and 11.4 mg Stannous-(II)-chloride dihydrate, and 10 mg HSA-microspheres (1.2–2.0 106 microspheres) to be dissolved in water. The desired quantity of sodium [188Re] perrhenate in saline available from the aluminia-based 188W/188Re generator system (Oak Ridge National laboratory, USA) should be transferred to the HSA-microspheres. For the labeling reaction the vial is placed on a heat-shaker heated at 95°C and shaked for one hour. After setting a physiological pH of 5–7 the preparation is ready for injection.
Results: The kit-preparation allows a simple and reliable quantitative labeling (> 97%) within 75 min with minimal handling of high 188Re radioactivity and small radiation risk for laboratory staff. After labeling the microspheres remained in a narrow grainsize distribution (mean diameter = 22 μm). The 188Re bound to the particles was found to be stable in vitro. The biological half life was > 250 h and demonstrated sufficient in vivo stability after i.v. injection in Wistar rats.
Conclusion: A simple and reliable kit preparation of 188Re HSA microspheres is available for intra-arterial infusion of particles for tumor therapy as for possible application in radiation synovectomy. The quantitative labeling with minima) handling of 188Re lowers the radiation burden for laboratory staff especially for high radioactivity.

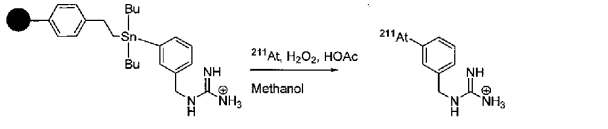
48

A KIT METHOD FOR THE HIGH LEVEL SYNTHESIS OF [^{211}AT]MABG

G. Vaidyanathan[1], D.J. Affleck[1], K.L Alston[1], J.W. Babich[2], D.H. Hunter[3], M.R. Zalutksy

[1]Department of Radiology, Duke University Medical Center, Durham, North Carolina, USA, [2]Molecular Insight Pharmaceuticals, Inc., Cambridge, Massachusetts, USA, [3]Department of Chemistry, University of Western Ontario, Canada

Introduction: Meta-iodobenzylguanidine labeled with ^{211}At will be potentially useful in the treatment of micrometastatic neuroblastomas. Earlier we have developed a method for the synthesis of *meta*[^{211}At]astatobenzylguanidine ([^{211}At]MABG) from a silicon precursor by solution-phase chemistry[1].



1 *Meta*-[^{211}At]astatobenzylguanidine ([^{211}At]MABG)

Figure 1. Synthesis of [^{211}At]MABG from a solid-supported tin precursor

The goal of this study was to develop a kit method with which high doses of [211At]MABG useful for clinical applications can be prepared.
Material and methods: A solid-supported tin precursor [1] was synthesized as reported [2] (Figure 1). Temporal effect on the [^{211}At)MABG yield was studied by treatment of 5 mg of 1 at room temperature with 50 Nl of a solution of ^{211}At in methanol (7–11 MBq) and 10 Nl of a 17:10 (v/v) H_2O_2 (30% w/v):HOAc. For high level synthesis, 10–12 mg of the resin 1 was treated with ^{211}At activity (ranging from 40 to 300 MBq; Mean 189 MBq) in 100 μl of methanol and 20 ul of H_20_2-HOAc mixture. The reaction mixture was stirred gently for 10 min at room temperature and diluted with 10 ml of water. [^{211}At)MABG was isolated by solid-phase extraction and reconstituted in appropriate buffer for biological applications.
Results: With smaller amounts of ^{211}At, radiochemical yields of 50.2 ± 6.3%, 57.4 ± 6.3% and 50.6 ± 3.7% were obtained during 5, 15 and 30 min, respectively. The radiochemical yield from the 5 reactions conducted with higher amounts of ^{211}At was 56.4 ± 9.8%; a maximum of 185 MBq of [^{211}At)MABG has been synthesized. HPLC indicated just one radioactive peak corresponding to MIBG and no UV peaks were detected.
Discussion/Conclusion: The feasibility of [^{211}At]MABG synthesis using a resin-bound tin precursor has been demonstrated. With this method, it was possible to synthesize up to 185 MBq of [^{211}At)MABG in a pure form without the use of HPLC and in a shorter duration than that needed for solution-phase synthesis.

Vaidyanathan G, Zalutky MR. Bioconjugate Chem 1992; 3: 499–503.
Hunter DH, Zhu, X J. Labelled Cpd Radiopharm 1999; 42: 653–661.

49

PREPARATION AND TESTING OF LIPOPROTEIN MACROMOLECULES AND LIPOSOMES FOR SCINTIGRAPHIC DETECTION OF ATHEROSCLEROTIC AND TUMOUR CELLS

Z. Bozóky, L. Fülöp, D. Bálogh, A. Máthé, G. Polyák, A. Jánoki

Department of Applications of Isotopes, Budapest, Hungary

Introduction: There are stronę need for non-invasive techniques in directly imaging atherosclerotic plaques and tumour lesions for early detections. 99mTc(Technetium)--labelled lipoproteins and lipoproteins mimie liposomes can be used as radiotracer because it acts as intracellularly trapped ligands providing an scintigraphic measurement of lipoprotein and liposomes uptake by tissues. Liposomes can be preparated with similar features to lipoproteins. The radiolabeling of LDL and liposomes were tested in atherosclerosis and in cancer.
Material and methods: Rabbits fed a diet containing 2% cholesterol for 60 days to develop hyperlipidemia and atheromatous aortic plaques and to detect with 99m Tc labelled LDL and liposomes on the basis of scintigraphy. In nude mice developed human tumor cells were also investigated using 99mTc labelled LDL and liposomes. Preparative density gradient centrifugation methods were applied for the isolation of the major lipoprotein density classes i.e. VLDL, IDL, LDL, HDL. Analytical ultracentrifugation methods were developed for the investigation of native and modified lipoproteins. Liposomes were manufactured from lipoproteins and from natura) phospholipids.
Results: Gamma camera in vivo scintigraphy of rabbits revealed visible signal corresponding to atherosclerotic plaques of aorta and carotid arteries in rabbits with 99mTc labelled LDL and liposomes. In nude mice developed human tumor cells and in dogs spontaneous developed tumours were detected on the basis of 99mTc--labelled LDL and of liposomes with similar features to lipoproteins with in vivo scintigraphy.
Discussion/Conclusion: Radiolabelled LDL and liposomes with similar features to lipoproteins offer the promising approach to identify the local metabolic fate of these compounds in vascular tissue and tumour tissues, and should be good tracer for the scintigraphic studies of atherosclerosis and cancer. Liposomes from natural and synthetic lipids which closely mimic the metabolic behaviour of lipoproteins can be used in animal models to targeted plaques and tumor cells.

50

PROGNOSTIC VALUE OF ^{99m}TC-EDDA-TRICINE-HYNIC-TYR--OCTREODTIDE IN PATIENTS WITH ADVANCED LIVER CANCER

M. Chianelli, G. Forlini, A. Signore, R. Mikołajczak, S. Taccogna, A. Crescenzi, G. Bizzarri, C.M. Pacella, Z. Rossi, V. Todino

Department of Diagnostic Imaging, Unit of Liver Focal Lesions, Unit of Pathologic Anatomy, Regina Apostolorum Hospital, Unit of Nuclear Medicine, Department of Clinical Seiences, University of Rome "La Sapienza"

Aim: To evaluate the potential of 99mTc-EDDA-tricine-HYNIC-(tir)octreotide (99mTc--HYNIC-TOC) to detect pathologic expression of somatostatin receptor by hepatocarcinomas in order to identify patient who might beneft from the use of long-acting somatostatin analogue (octreotide).
Material and methods: Twenty patients (6 females and 14 females, mean age 68.5 yrs) with advanced hepatocarcinoma were recruited. All patients had a life expectancy greater than 3 months and were previously treated with LTA or chemoembolisation and were not amenable to further treatment. All patients underwent confirmatory liver biopsy, abdominal CT, and measurament of alpha-pheto-protein. Somatostatin receptor scintigraphy (SRS) was performed 3 hours after the injection of 99mTc-HYNIC-TOC (370 MBq). Liver scan was performed 20 minutes after the injection of 99mTc-albumin nanocolloids (185 MBq). Tomographic images of the liver were obtained for both scintigraphy using the same acquisition parameters and compared. A SRS was considered positive if uptake of 99mTc-HYNIC-TOC was seen in an area of no colloids uptake corresponding to a Jesion detectable on a CT scan. Four patients positive to SRS were treated with long-acting octreotide (30 mg/ /month i.m.).
Results: Fourteen out of twenty patients (70%) showed a significant uptake of 99mTc--HYNIC-TOC. No correlation with -Pheto-protein was found neither with the Edmonson severity score. Patients treated with long-acting octreotide showed stabilisation of the disease or partial response in a follow up of six months. All patients who werse not treated with long-acting octreotide showed progression of the disease. No side effects wers observed following SRS.
Conclusion: SRS with 99mTc-HYNIC-TOC showed pathological uptake in most patients with advanced, untreatable, liver cancer. In this preliminary study our results suggest that patients positive to 99mTc-HYNIC-TOC scitingraphy benefited from treatment with long-acting somatostatin analogue. The in vitro study of SR on hepatocarcinomas is in progress to correlate the *in vivo* results.

51

FINDINGS IN THE BRAIN SCINTIGRAPHY WITH OLFACTORY ACTIVATION (OA)

S. Saferstein, H. Garcia del Rio, O.J. Degrossi, M. del C. Alak, L. Copat, L. Ruano

Inst. Argen. Diagnóstico Tratamiento, Buenos Aires, Argentina

Anosmia is a neurological symptom through which the p. is unable to perceive smells. This symptom can settle as a consequence of a Head Trauma (HT). MRI and TC are useful to confirm this pathology when there is structural damage. However, ps. Without evidenced by MRI and CT of the frontal lobe show up claiming to sufler from anosmia. This challenge led us to use brain SPECT with HMPAO 99mTc with OA technique as a mean to demonstrate the existence of real anosmia. In 10 normal volunteers (N) and 12 patients. HT, 7 with anosmia, SPECT in basal state were performed; and on a second day with OA. All of them had previously a MRI study. The basal SPECT were carried out according with traditional techniques and the second ones making use of aromatic substances. The uptakes values of HMPAO 99mTc was determined in the following areas: frontal (F), temporal (T), parietals (P) and occipito-cerebelum (C) in basal state and during the OA. The results expressed an increase of the uptake values in the F as a sign of olfactory activity. The 10 N showed a normal basal SPECT and a positive response to OA by an uptake increment in F. The 5 HT without anosmia and normal MRI had a norma) basal SPECT and a positive response in F. From the 7 HT with anosmia, 5 presented pathologic MRI, abnormal basal SPECT and negative SPECT with OA; whereas the 2 remaining showed normal MRI, positive basal SPECT and positive SPECT with OA.
Conclusion: from the obtained results we think that SPECT with OA test shows the response to the activation of the olfactory tract. Only in 2 HT with anosmia but who shoed normal MRI, the SPECT and OA study were normal; this is why we presume that they were simulating. The importance of this study is that, if confirmed in a large number of ps. would signify the possibility to establish the validity of the existence of anosmia in a p. using brain SPECT with OA. This possibility is also relevant when is comes to legat demands and may bring about a solution to establishing whether a person suffers from anosmia or not (detecting simulated anosmia). It can help as well as a test to verify the qualifications needed for certain professions which require a keen sense of smell.

52

USEFULNESS OF ^{99m}TC-EDDA/HYNIC-TOC IN DIFFERENTIAL DIAGNOSIS OF SOLITARY PULMONARY NODULES

A. Płachcińska[1], R. Mikołajczak[2], B. Janota[2], J.A. Michalski[4], K. Rzeszutek[4], J. Kuśmierek[1]

[1]Department of Nuclear Medicine, Medical University, Łódź, Poland, [2]Radioisotope Centre "POLATOM", Otwock-Świerk, Poland, [3]Ward of Thoracic Surgery, Copernicus Hospital, Łódź, Poland, [4]Centre for Treatment of Pulmonary Diseases, Łódź, Poland

Introduction: Differentiation of solitary pulmonary nodules (SPN) to malignant and benign tumours is a serious diagnostic problem. The aim of the study was the assessment of clinical usefulness of scintigraphy with ^{99m}Tc-EDDA/HYNIC-TOC for purposes of differential diagnosis of SPNs.
Material and methods: 50 consecutive patients with solitary pulmonary nodules (SPN) on chest radiographs were studied scintigraphically, after administration of a somatostatin analogue ^{99m}Tc-EDDA/HYNIC-TOC.The activity amounted to 740–925 MBq and a SPECT technique was applied. Verification of the nodule etiology was based on histology or cytology and in some patients on bacteriology. As additional criterion for nodule benignity its stable size in chest radiograph for at least 3 years was accepted.
Results: In 31 patients malignant etiologies of nodules were found. The diagnoses included: 11 adenocarcinomas, 6 squamous cell carcinomas, 2 large celt carcinomas, 6 non-small cell lung cancers (NSCLC) of unspecified more detailed morphology, 2 small cell lung cancers (SCLC), 2 typical carcinoids and 2 metastatic tumours: leiomyosarcoma and malignant melanoma. In 19 patients the following benign tumours were diagnosed: 6 tuberculomas, 2 other granulomas, 4 hamartomas, 2 non-specific inflammatory infiltrate, 1 abscess, 1 peripheral carcinoid of morphological characteristics of a benign tumour, 1 ectopic lesion of thyroid tissue and 2 benign tumours of unspecitfied etiology with stable size over 3 and 5 years. Positive scintigraphic results were obtained in 28 out of 31 patients (90%) with malignant SPNs; among these were 26 out of 27 (96%) cases with primary pulmonary carcinoma. The remaining 2 false negative cases included metastatic tumours: liposarcoma and melanoma. Among 19 benign lesions 15 (79%) did not accumulate the radiopharmaceutical. The remaining 4 tumours visible on scintigrams included: 1 tuberculoma, 1 hamartoma, 1 abscess and 1 case of non-established diagnosis (with stable size over 3 years).
Conclusion: Scintigraphy with ^{99m}Tc-EDDA/HYNIC-TOC appears to be an effective procedure for differentiation between malignant and benign SPNs.

53

EXPERIMENTAL ANIMAL MODEL IN DETECTION OF DEEP VENOUS THROMBOSIS USING RADIOLABELLED TIROFIBAN-GPIIB/IIIA INHIBITOR

E. Janevik-Ivanovska[1], O. Vaskova1, I. Djorgoski[2], B. Andonovski[1], V. Milenkov[3], G. Jánoki[4]

[1]Institute of Pathophysiology and Nuclear Medicine, Faculty of Medicine, Skopje, Macedonia, [2]Institute of Biology, Faculty of Natural and Mathematics Science, Skopje, Macedonia, [3]Institute of Blood Transfusion, Skopje, Macedonia, [4]NFJC Research Institute of Radiobiology and Radiohygiene, Budapest, Hungary

Detection of acute deep vein thrombosis (DVT) based on the bimolecular behaviour of components of the clotting process including the platelets and their specific expressed receptors have been suggested as a new approach in nuclear medicine. For this reason, the new venue has focused on the use of small peptide or peptidomimetic ligands with high specificity for GPIIb/IIIa adhesion molecule receptors (of the integrin family), leading to platelet aggregation and his incorporation of a convenient radionuclide for imaging purposes. The aim of our study was to introduce Tirofiban, non-peptide tyrosine derivate, as a specific imaging agent to GPIIb/IIIa receptor in the case when we have activated platelets during the process of the platelet aggregation and thrombus formation in the experimental animal model and to evaluate his radiochemical and biological behavior.
Material and methods: The experimental model of DVT is induced in the femoral vein of mgle Wister rats, by a combination of venous stasis and hypercoagulability. The thrombi developed in the rats were visualized using Tirofiban radiolabelled with technetium--99m[99mTc] and iodine-125[125 I]. Iodine-125[I] — Tirofiban labelling was performed using Iodo-gen method and the quality control of the labelled product was checked, using Thin Layer Chromatography (TLC) in 1 mol/L HCl as a solvent. Technetium 99m [99mTc]--Tirofiban labelling was in the presence of reducing agent [Sn^{2+}]. The quality control was done by Paper Chromatography (PC) and Instant Thin Layer Chromatography (ITLC) using two solvents — methylethylketone and 0.9% NaCl. Planar images were obtained 30 min, 2 and 24 hours after i.v. application of 2–6 $\times$ 10^6 cpm in 50–100 m technetium 99m [99mTc]--Tirofiban or 1.6–2.1 $\times$ 10^6 cpm in 50 m of iodine 125 [125I]-Tirofiban in rat's tail vein. The sensitivity and specifificity were determined using ratio "left leg positive for DVT" and "right leg negative for DVT". By using ROI technique and biodistribution studies of sacarified animals we quantified left thrombotic/right nothromotic leg ratio.
Results: The percentage of the obtained complex after labeiling with both of used products was more than 95% and he was stable without changing the percent of labeling 2 hours at room temperature. For in vivo studies, the obtain ratio "left leg positive for DVT" and "right leg negative for DVT" was 1.76 after 30 min, 1.99 after 2 hours and 2.06 after 24 hours. These values were considered as positive in the detection of acute DVT.
Conclusions: Animal models provide convenient screening tools for radiolabeled products before a radiopharmaceutical is further developed in clinical trials. Our results obtained from experimental studies showed that radiolabeled Tirofiban could be helpful in the further clinical investigation in the patients with acute deep venous thrombosis and that he has some diagnostic potential in nuclear medicine.

54

MICROBIOLOGICAL MONITORING OF RADIOPHARMACEUTICALS PREPARATION

M. Zitko Krhin, T. Gmeiner Stopar, S. Hojker

Introduction: Since radiopharmaceuticals are released for use before sterility testing is finished, microbiological monitoring of aseptic conditions should be carried out regulary in order to estimate the risk for microbiological contamination of the final product.
Materials and methods: In our department preparation of radiopharmaceuticals from technetium-99m generators and kits is done in a grade A environment/grade B background. Microbiological control of air (two hours exposure of settle plates during preparation), surfaces (24–30 cm^2 area swabs after preparation), personnel (glove prints) and sterility testing of radiopharmaceuticals is performed regularly from 2001 according to Ph Eur IV. Acceptability limits considering EU GGMP recomendations are used.
Results:

Air

Place	Number of exposures	Colony forming unit (CFU)/settle plate
Grade A	22	1 positive (*Stahylococcus haemolicus*)
Grade B	7	Negative

Surfaces

Place	Number of swabs	CFU/swab
Grade A	25	Negative
Grade B	23	2 positive (*Staphylococcus hominis, Conebacterium species)*
Generator	24	1 positive (*Bacillus spp*)
Waste — container	22	2 positive (*Staphylococcus epidermidis*, *Bacillus spp.*)
Air lock	20	2 positive (*Staphylococcus epidermidis*, *Bacillus spp.*)

Personnel

	Number of glove prints	CFU/glove print
Right hand	7	6 positive (*Staphylococcus epidermidis, Staphylococcus warneri, Staphylococcus haemolyticus, Staphylococcus hominis, Bacillus cereus*)
Left hand	7	5 positive (*Corynebacterium species, Staphylococcus haemolyticus, Staphylococcus epidermidis, Staphylococcus hominis, Corynebacterium species*)

Sterility testing

Number of samples tested	Results
397	Negative

Conclusions: Hands are the most critical part for microbiological contamination, probably due to handling of unsterile lead containers and shields during preparation. To improve the conditions, repeated desinfection of hands during preparation was introduced. Results of microbiological monitoring during longer time period will be used to set our own acceptability limits, alert and action levels.

55

STUDIES ON THE EFFECTS OF DIFFERENT SYRINGES AND PERSONNEL APPLYING RADIOPHARMACEUTICALS IN THE CLINIC ON THE RESIDUEL ACTIVITY

D. Keskintepe[1, 2], A.Y. Ozer[1]

[1]Hacettepe University, Faculty of Pharmacy, Radiopharmacy Department, Ankara, Turkey, [2]Ankara University, Faculty of Medicine, Nuclear Medicine Department, Ankara, Turkey

Introduction: The type of syringes and the volume of the patient doses of radiopharmaceuticals cause high residuel radioactivity in the syringes during application to patients. Therefore the investigation of the effects of syringes type and differences in volume and personnel on residuel activity and adsorption are aimed.
Material and methods: Two types of syringes were chosen and the residuel activity left in 0.5 and 1.0 mL syringes after the application of different $Tc0^-_4$ activities was investigated. Commercial radiopharmaceuticals injected to the patients by nurses and technicians. Then, radiopharmacist withdrew these radiopharmaceuticals to the syringes and emptied in-vitro and amounts of residuel activity were determined. The change of residuel activity was evaluated depending on the syringe, radiopharmaceutical kit and applicant types.
Results: The residuel activity reduced by 35–45% in the syringes with fiat plungers as the decrease in the amount of radioactivity decreased when the volume was doubled but the radioactivity dose remained the same. The highest values were 15––38% in syringes with an elostomeric tip on the plunger. When the injection of radiopharmaceutical kits were examined the residuel activities resulting from personnel application reached up to 10–22% where as the application by radiopharmacist reduced this result.
Discussion: The residuel radioactivity was decreased in both types of syringes by keeping the radioactivity dose the same and doubling the volume. The residuel activity was lower in syringes with elastomeric tip due to smaller dead volume. Radiopharmaceuticals must not be prepared with very small volumes in syringes.
Conclusion: Injection of radiopharmaceuticals needs special care. It is concluded that less care during injection or the preparation of radiopharmaceutical in syringes will cause decrease in the accuracy of the radioactive dose received by the patient and the residuel radioactivity in the syringes will cause and increase in the enviromental contamination.