PREPARATION OF COMPONENTS OF ^{99m}Tc-CO-MIBI AND THEIR BIODISTRIBUTION

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Key Words: Tricarbonyltechnetium, Biodistribution, Myocardium

The convenient synthesis of $[^{99m}Tc(CO)_3(OH_2)_3]^+$, developed by Alberto and his co-workers, has promoted the field of radiopharmacy. Our previous work has proved that ^{99m}Tc-CO-MIBI starting from $[^{99m}Tc(CO)_3(OH_2)_3]^+$ has excellent potential as a new myocardial imaging agent and it was a mixture with two main components. The aim of this study was to keep on exploring the chemical and biologic properties of the two components.

The ^{99m}Tc-CO-MIBI mixture was prepared using a two-step procedure involving the convenient preparation of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor followed by substitution of the water molecules by the MIBI (methoxyisobutylisonitrile) ligands. In the second step it has been found that there would be two main products represented by A and B with the pH of the precursor solution adjusted to different values. When the pH was moved from 5.0 to 8.5, the fraction of A would decrease gradually from 100% to 0 and the fraction of B was opposite to that of A. This was confirmed by a suitable HPLC method improved recently (Fig1).

The respective biodistribution results of A and B have been reported previously, but their separation was performed by HPLC. This method was not practical, so it was proposed that pure A and B could be prepared respectively by adjusting the pH of the precursor.

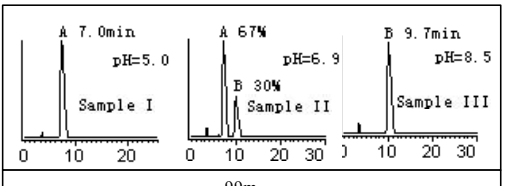

Fig1. HPLC of the ^{99m}Tc-CO-MIBI mixture

The biodistribution was performed in three groups presented in Fig1. According to Table1, the biodistribution indicates that there is still significant difference between A and B just like the previous results. The component B is comparable with $[^{99m}Tc(MIBI)_6]^+$ and even much better in the ratios of heart to liver. These results will help clinical research of ^{99m}Tc-CO-MIBI in the near future. The mechanism of pH action and the composition of the two components will be reported in detail.

Table1. Biodistribution in mice (%ID/g, mean±SE, n=3) and ratios of target to non-target

Sample	t/min	Blood	Heart	Liver	Lung	Kidney	Heart/Liver	Heart/Blood	Heart/Lung
I (A)	5	1.39+0.06	17.16+1.13	8.96+0.35	4.02+0.60	30.59+2.12	1.92	12.34	4.31
	15	0.67+0.03	15.72+0.53	8.50+0.45	3.72+0.24	24.08+5.54	1.86	23.60	4.24
	30	0.50±0.03	16.22±0.58	10.42±1.42	2.79±0.25	17.98±2.57	1.57	32.36	5.86
	60	0.41±0.02	15.61±1.57	10.30±0.63	2.44±0.38	14.92±2.62	1.51	38.23	6.56
II	5	1.69±0.07	18.96±1.49	13.96±1.25	5.34±0.47	49.28±4.60	1.38	10.88	3.56
	15	0.93±0.06	22.08±0.72	13.84±0.89	4.56±0.87	39.67±5.44	1.60	23.67	4.97
	30	0.74±0.02	23.37±1.12	12.26±1.82	3.77±1.01	30.30±4.12	1.93	31.49	6.42
	60	0.59±0.03	18.57±0.13	11.95±1.75	2.84±0.09	26.14±7.24	1.58	31.35	6.53
III (B)	5	1.22±0.58	18.45±4.45	11.21±2.42	5.91±0.77	71.82±16.38	1.66	17.41	3.10
	15	1.00±0.07	24.05±1.40	12.07±1.64	5.02±0.74	59.61±11.13	2.01	24.18	4.85
	30	0.61+0.06	22.29+2.86	10.02+2.85	4.86+1.00	34.78+0.26	2.33	36.53	4.68
	60	0.43+0.00	21.25+3.90	8.26+1.32	2.88+0.65	29.23+4.31	2.66	49.54	7.43

DOSE CALCULATION AND EXPERIMENTS FOR ^{188}RE LIQUID-FILLED BALLOON IN RADIATION THERAPY ON PREVENTING VASCULAR RESTENOSIS

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Keywords: Dose Evaluation, ^{188}Re, Preventing Vascular Restenosis

When conducting vascular radioactive therapy, it is needed to provide correct methods to evaluate the dose distribution. This study aims to fulfill domestic requirements on related human tests by exploring the research of the dose evaluation of ^{188}Re liquid-filled balloon when it was used in vascular restenosis therapy.

Method**:** The accomplished included the results comparisons of theory evaluation and experimental measurement by using two sets of 6 mm diameter vein phantoms, a set of 4 mm diameter vein phantom and a set of 3 mm diameter vein phantom. The Monte Carlo MCNP4C program was used for theory evaluation and the MD-55 GafChromic film was used in experimental measurement.

Results: The outcome showed that the average deviation between the results from theory evaluation and experimental measurement was about 3.7 % ~ 6.1 % which was within uncertainty range and proved to agree with each other. The calculation results showed that it was necessary to simulate the real size of the central catheter of the dilatation balloon to obtain the explicit dose distribution. On the other hand, the vein phantom simulation calculation showed that outside the dilatation balloon, the dose rate went down rapidly with the distance from the balloon surface got longer. The dose rate in the vein of smaller diameter went down faster than that in the vein of larger diameter. For the 6 mm-, 4 mm- and 3 mm-diameter dilatation balloons, the dose rates at the distance of 0.5 mm from the dilatation balloons were respectively 56.1 %, 52.4 % and 50.9 % of the surface dose rate of the dilatation balloon. When the distance from the balloon surface increased to be 3 mm, the dose rates were only 6.1 %, 5.5 % and 5.2 % respectively of the surface dose.

Conclusion: An easy dose evaluation interface program was established and proved that it could provide information such as ^{188}Re source irradiation time that could satisfy the description dose on vascular restenosis therapy.

THE SYNTHESIS AND CHARACTERIZATION OF Tc(I)/Re(I) DERIVATIVES OF INSULIN

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Keywords: Insulin, Tc/Re(I), Bioconjugate, Chelates

There are a number of strategies for radiolabeling insulin with ^{99m}Tc. Unfortunately existing methods are not without their limitations which include low labeling yields, unwanted reduction of disulfide bonds and the need to use elaborate HPLC protocols to isolate the final product. We, on the other hand, have been able to efficiently derivatize insulin regioselectively with a chelate that is designed to bind Tc(I) and Re(I). Our strategy avoids the previously encountered problems by using a chelate that: A) possess reasonably nucleophilic donor atoms and B) binds to Tc/Re in an oxidation state that is not particularly redox active.

Our methodology involves conjugating the recently reported single amino acid chelate (SAAC) and its Re and Tc complexes to A^1,B^{29}-DiBoc-insulin to give the corresponding B^1 derivatives (**Figure 1**). The target complexes can be isolated by simple chromatographic methods following the removal of the protecting groups. The presentation will cover the basic synthetic methodology, a comparison of pre and post-labeling methods, results of in vitro binding experiments and a discussion of the future applications of the system.

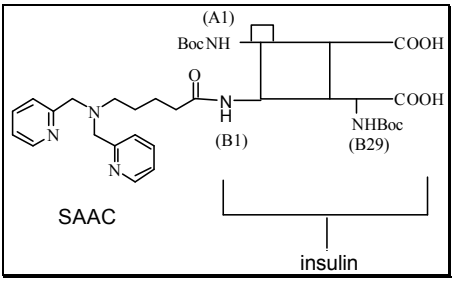

Figure 1

Tc-99M DIRECT LABELING OF ANNEXIN V FOR POTENTIAL APOPTOSIS IMAGING IN VIVO

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Key Words: Apoptosis imaging, ^{99m}Tc-Annexin V, Direct labeling

Apoptosis is associated with many diseases. It plays a critical role in the pathogenesis of a cerebral and myocardial ischemia, autoimmune and neurodegenerative diseases, organ and bone marrow transplant rejection, and tumor response to chemotherapy and radiation[1]. AnnexinV, a 32-Kda endogenous human protein, has a high affinity for membrane-bound phosphatidylserine that migrates to the surface of apoptosis cells. Annexin V with various fluorescent tags has been used both in vitro and in vivo as a marker of apoptosis. According to the literature[2], ^{99m}Tc-HYNIC-Annexin V (Apomate^{TM}) has been evaluated in human clinical trials for SPECT apoptosis imaging. Apoptosis imaging will plays an important role in nuclear medicine in the near future. The purpose of this research was to develop a faster and simpler method directly radiolabeling Annexin V with Tc-99m and improving imaging characteristics.

Preparation of ^{99m}Tc-Annexin V: the Tc-99m pertechnetate solution (100µl, 10-20mCi) was added to the Annexin V (20µg, Sigma) in saline followed by the addition of a stannous solution (100µg $SnCl_2.2H_2O$)in the present of citrate. The reaction was incubated at room temperature for about 10 min. The radiochemical purity was assessed by thin-layer chromatography (TLC) using 0.9% saline solution($Rf_{^{99m}Tc\text{-}Annexin\ V}$: 0.9-1.0;) and acetone ($Rf_{^{99m}Tc\text{-}Annexin\ V}$: 0.0-0.1) as a developing solvents separately and RP-HPLC with a C-18 reverse phase column and a gradient elution (0-5min: 100% acetonitrile;5- 6min 100% ~ 80 :20 = acetonitrile: H_2O;6~ 25min 80 : 20 = acetonitrile: H_2O; 25~ 26min 100% acetonitrile, the flow rate: 1.0ml/min)? HPLC retention time of ^{99m}Tc-Annexin V was at about 10.8min using both NaI detector and UV detector(254nm) [Fig1].

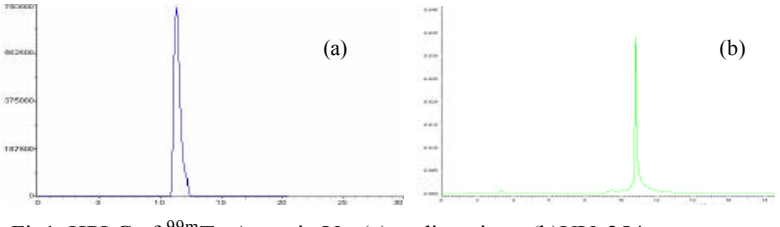

Fig1. HPLC of ^{99m}Tc-Annexin V (a) radioactive (b)UV 254nm

Radiolabeling efficiency and radiochemical purity were more than 95%. ^{99m}Tc-Annexin V solution was allowed to stay at room temperature for about 10 hrs, the radiolabeling efficiency and radiochemical purity has not decreased distinctly. The animal tests are in progressing.

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THE SYNTHESIS AND BIODSTRIBUTION OF 99mTc-HYNIC-HSA IN MICE AS A NEW BLOOD POOL IMAGING AGENT

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Key Words: blood pool imaging agent, HYNIC, 99mTc-HYNIC-HSA, bifunctional chelator

OBJECTIVE: Human Serum Albumin (HSA) is easy to label with Tc-99m but does not form a stable complex. To improve the labeling strength of HAS, several the bifunctional chelator such as DTPA, MA (mercaptoacetyl) and DMP (dimercaptopropionyl) were selected for coupling with HSA to label with Tc-99m [1 ,2]. In this paper, we used HYNIC (6-hydrazinonicotinamide) as a bifunctional chelator for Tc-99m labeling of HSA for development of a blood pool imaging agent.

METHODS: The precursor of the HYNIC was synthesized [3]. The HYNIC was coupled with the HSA and a BFCA-biomolecular compound was prepared. The HYNIC-HSA (10 mg) was labeled with Tc-99m at room temperature and PH 7.4 in PBS , The labeling was performed using EDTA (5 mg) as a co-ligand, stannous chloride (0.1 mg) as a reducing agent. In vivo biodistribution studies were performed in mice. The mice were injected in the tail vein with 0.1 ml (7.4×10^5 Bq) of the ^{99m}Tc-HYNIC-HSA, sacrificed at various time points injection, and specific tissues were counted.

RESULTS: The HYNIC was characterized by ^{1}H-NMR. Per HSA, 4 hydrazine groups were incorporated. The labeling efficiency of 99mTc-HYNIC-HSA was great than 90%. The labeled complex showed excellent stability within 4 hrs in vitro. The 99mTc-HYNIC-HSA with higher retention in blood at 10, 60 and 120 (min) are 35.98+/-4.09, 32.52+/-1.67 and 24.04+/-4.73 (ID%/g), respectively. It also showed the lower uptake in the heart, liver, lung and bone. The ratios of B/Li., B/H, B/Lu and B/Bo. at all time were over more than 3.6, 3, 3 and 7, respectively.

CONCLUSION: These studies demonstrated that 99mTc-HYNIC-HSA is a very promising new blood pool imaging agent.

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COMPARISON OF MYOCARDIAL PERFUSION SPECT USING DIFFERENT PROTOCOLS FOR THE DETECTION OF REVERSIBLE ISCHEMIA IN MALE DIABETIC PATIENTS

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Objectives: We prospectively compared (TeSPECT) and (STeRTlSPECT) with (TlSPECT) for detection of reversible ischemia.

Methods: Fourteen diabetic men with additional risk factors for coronary artery disease were taken for the study. The mean age was 52.43 8.13 years. All the patients underwent TlSPECT (stress redistribution protocol) and TeSPECT (one day stress rest protocol) on 2 separate days following a symptom limited treadmill exercise stress (standard Bruce protocol). The maximum heart rates achieved as well the duration of stress were kept similar for both the studies. The semiquantitative polar maps for perfusion generated using the Emory Cardiac Tool Box (ECTb) were given scores 0-4 after dividing it into 20 segments (0= normal, 1= equivocal, 2= moderately impaired, 3= severely impaired, 4=absent). Abnormal segments were then given a reversibility score according to the perfusion scores obtained by the redistribution / rest study. 0= normal, 1=partially reversible (stress-redistribution >1), 2=completely reversible (becoming normal in perfusion), 3= mild fixed defects (scores 1 or 2 without any improvement in perfusion), 4= severely fixed defects (scores 3 or 4 without any improvement in perfusion) and reverse redistribution (5=deterioration in perfusion). The STeRTlSPECT reversibility scores were analysed using stress TeSPECT perfusion scores and redistribution TlSPECT scores obtained from the respective studies.

Results: Out of the total 280 segments analysed, TeSPECT showed agreement in 60% (kappa value=0.505, Chi square for proportions p=0.129) whereas STeRTlSPECT showed agreement in 76% (kappa value=0.699, Chi square for proportions p=0.992). However when the studies were compared in individual patients, TeSPECT showed significant difference in 5 out of 14 (36%) patients whereas STeRTlSPECT showed significant difference only in one patient (7%).

Conclusion: Stress ^{99m}Tc Tetrofosmin - redistribution 201 Thallium dual isotope myocardial perfusion SPECT protocol shows more agreement with 201 Thallium Stress redistribution myocardial perfusion SPECT protocol compared to ^{99m}Tc Tetrofosmin stress rest one-day myocardial perfusion SPECT in the detection of reversible ischemia.

CHARACTERIZATION AND BIODISTRIBUTION OF A POTENTIAL BLOOD POOL AGENT: ^{99M}TC-TMCHI

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Keywords: Technetium-99m; Blood pool agent; TMCHI; Isonitrile; Biodistribution in mice

In our previous study we synthesized a isonitrile ligand 3,3,5-trimethyl cyclohexylisonitrile (TMCHI), and it was labeled with ^{99m}TcN core and the complex ^{99m}TcN-TMCHI showed promising properties for blood pool imaging [1]. In the present study, the isonitrile complex ^{99m}Tc-TMCHI is prepared by direct labeling method. The stability study indicated that ^{99m}Tc-TMCHI was stable over a period of 6h at room temperature. The partition coefficient value for the partition of ^{99m}Tc-TMCHI between 1-octanol and water was 912.53 12.88 (n=4).

The result of biodistribution in mice for ^{99m}Tc-TMCHI is shown in Table 1. ^{99m}Tc-TMCHI is well accumulated and maintained in blood with good blood-to-heart, blood-to-liver, blood-to-lung and blood-to-kidney ratios (Table 2). Compared with the known blood pool agent ^{99m}Tc-DMP-HSA, the ratios of blood-to-heart, blood-to-liver and blood-to-kidney are greatly improved. There is no appreciable difference between the blood-to-lung ratios of ^{99m}Tc-TMCHI and ^{99m}Tc-DMP-HSA. Based on its promising biological properties, the complex ^{99m}Tc-TMCHI maybe suitable for cardiac blood pool imaging. In addition, the complex ^{99m}Tc-TMCHI has other advantages over ^{99m}Tc-DMP-HSA, such as the synthesis of TMCHI ligand is much simpler than that of DMP-HSA and the stability of TMCHI ligand is also better than that of DMP-HSA.

On the basis of ^{99m}Tc-TMCHI provides excellent biological properties and the other advantages, we think it is worth to develop this complex for clinical use. Furthermore, a lyophilized kit for the preparation of ^{99m}Tc-TMCHI is easy to be obtained. The kit provides the convenience of the preparation of this complex for clinical use. Further studies on ^{99m}Tc-TMCHI are being conducted and will be reported in due course.

Table 1. The result of biodistribution in mice for ^{99m}Tc-TMCHI (x s, n=3)

Tissues	Post-injection time/min					
	%ID/g			%ID		
	5	30	60	5	30	60
Heart	7.45 0.55	6.61 0.53	6.50 1.20	0.74 0.03	0.63 0.11	0.56 0.13
Liver	9.32 0.78	13.53 0.86	18.73 4.03	9.19 0.26	13.30 1.10	16.8 2.87
Lung	24.50 5.57	19.10 1.30	26.90 2.40	4.88 0.91	4.00 0.78	5.47 0.91
Kidney	4.88 1.42	6.04 0.85	7.17 0.66	1.30 0.32	1.60 0.15	1.60 0.10
Brain	1.20 0.18	1.27 0.23	1.10 0.31	0.52 0.08	0.55 0.11	0.49 0.14
Blood	44.40 2.80	40.40 3.10	40.40 0.67	62.30 3.97	56.60 4.34	56.50 0.93

Table 2. The ratios of target to non-target for ^{99m}Tc-TMCHI and the comparison of it with ^{99m}Tc-DMP-HSA in mice (n=3)

Complexes	^{99m}Tc-TMCHI			^{99m}Tc-DMP-HSA[2]		
Post-injection time/min	5	30	60	10	30	60
R (Blood/Heart)	5.96	6.11	6.22	5.17	4.50	4.46
R (Blood/Lung)	1.81	2.12	1.50	2.80	4.15	3.89
R (Blood/Liver)	4.76	2.99	2.16	2.32	1.93	1.62
R (Blood/Kidney)	9.10	6.69	5.63	4.75	3.85	3.22

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IN VITRO AND *IN VIVO* EVALUATION OF ^{99M}TC-TMCHI FOR CARDIAC BLOOD POOL IMAGING

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Keywords: ^{99m}Tc-TMCHI; Cardiac blood pool imaging; Protein binding; Isonitrile; Blood retention in mice

In our previous studies, we prepared an isonitrile complex ^{99m}Tc-TMCHI (TMCHI=3,3,5-trimethyl cyclohexylisonitrile), and the result of biodistribution in mice showed that it has promising properties for blood pool imaging. In the present study, we evaluate the *in vitro* protein binding and blood retention in mice behavior of ^{99m}Tc-TMCHI as a potential cardiac blood pool imaging agent.

In vitro protein binding rate and the washout rate of ^{99m}Tc-TMCHI in BSA (Bovine Serum Albumin) and HSA (Human Serum Albumin) are shown in Table 1. The high protein binding rate in albumin (96.62% in BSA and 98.38% in HSA) of ^{99m}Tc-TMCHI indicated that this complex maybe has special binding characteristic with albumin, this leads to the complex accumulate in blood well at the early post-injection time. On the other hand, the low washout rate of ^{99m}Tc-TMCHI (44.93% in BSA and 58.85% in HSA) indicated that this complex should be retained in blood very well.

Table 1. The result of *in vitro* protein binding in albumin of ^{99m}Tc-TMCHI (n=4)

Albumin	The protein binding rate/%	The washout rate of activity/%
BSA	96.62	44.93
HSA	98.38	58.85

The result of the blood retention of the ^{99m}Tc-TMCHI in mice is shown in Fig. 1. On the ordinate the ratio between the percentage of the injected activities remaining in the blood at different post-injection time and the percentage of the injected activity in the blood at 2min post-injection (the initial activity). From Fig. 1 it is evident that ^{99m}Tc-TMCHI show a well retention in the blood. The blood activity of ^{99m}Tc-TMCHI decreased continuously during the 120min of the investigation. After 120min, there are still about 52% of the initial activity was retained within the blood. These properties of ^{99m}Tc-TMCHI measure up to the basic requirements of blood pool imaging agent.

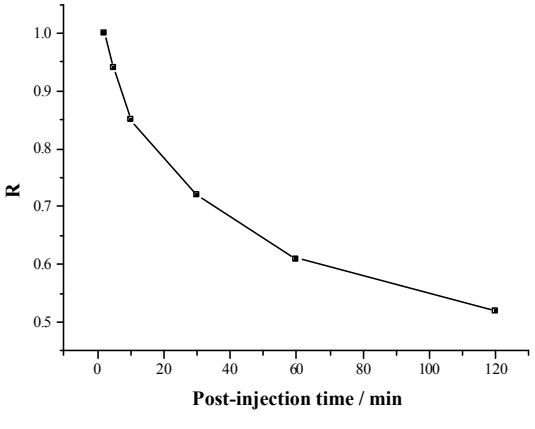

Fig. 1. Blood retention of ^{99m}Tc-TMCHI in mice (n=4)

In vitro and *in vivo* behavior of ^{99m}Tc-TMCHI show that ^{99m}Tc-TMCHI is suitable for cardiac blood pool imaging.

IN VITRO TUMOR UPTAKE OF ^{99M}TCN-MIBI AND ^{99M}TC-MIBI

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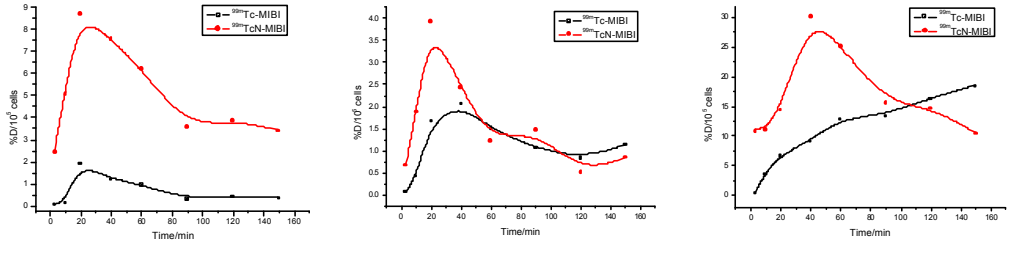
Keywords: ^{99m}TcN-MIBI; ^{99m}Tc-MIBI; Tumor imaging; Tumor cell; Radiopharmaceuticals

In this study, we have investigated the *in vitro* uptake of ^{99m}TcN-MIBI [1] and ^{99m}Tc-MIBI by colorectal cancer cell line CL-187, lung cancer cell line L-973 and breast cancer cell line MCF-7, respectively. The results (Fig.1) of uptake kinetics showed that ^{99m}TcN-MIBI have maximal accumulations between 20 and 40 min after incubation in all of these tumor cells, and the accumulation (%D) of ^{99m}TcN-MIBI was found to be obviously higher than that of ^{99m}Tc-MIBI, especially in colorectal cancer cell line CL-187. Uptake of ^{99m}TcN-MIBI decreased in the order MCF-7 CL-187 L-973. The maximal accumulation occurring at 40 min after incubation of ^{99m}TcN-MIBI in MCF-7 with the percentage dose about 30%D/10^5 cells. Influence of the culture conditions, such as cell concentration and incubation temperatures, on the accumulation were also investigated (Table 1 and 2), and the uptake of ^{99m}Tc-complexes by cells were effected significantly by the culture conditions.



a. CL-187 b. L-973 c. MCF-7

Fig.1 Uptake kinetics of ^{99m}Tc complexes in CL-187, L-973 and MCF-7 cell lines

Table 1 Influence of cell concentration and incubation time on ^{99m}Tc complexes in MCF-7 cell lines

Complexes	Incubation time/min	Percentage dose/%D			
		3.2×10^5 cells/mL	2.7×10^5 cells/mL	2.0×10^5 cells/mL	1.5×10^5 cells/mL
^{99m}Tc-MIBI	40	3.61	2.44	1.20	0.63
	120	4.28	4.38	2.55	1.52
^{99m}TcN-MIBI	40	7.87	8.16	11.43	4.67
	120	5.61	3.94	3.37	1.79

Table 2 Influence of incubation temperature on ^{99m}Tc complexes in MCF-7 cell lines

Complexes	Incubation time/min	Percentage dose/%	
		0~ 4 C	37 C
^{99m}Tc-MIBI	40	0.55	1.20
	120	0.35	2.55
^{99m}TcN-MIBI	40	10.04	11.43
	120	2.90	3.37

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LYMPHOSCINTIGRAPHIC EVALUATION OF HIGH SPECIFIC ACTIVITY ^{99m}Tc-ANTIMONY TRISULPHIDE COLLOID (ATC) IN RATS

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The aim of this study was to examine ^{99m}Tc-ATC: (i) uptake by rat lymph nodes; and (ii) interaction with leukocytes. Three ^{99m}Tc-ATC kits were prepared with different specific activities: low (0.08 GBq/ mol), moderate (0.77 GBq/ mol) and high (7.69 GBq/ mol). A dose (5 MBq) from each kit was administered in rat tails by subdermal injection, and then whole body images were taken after 30 minutes. From the images, % popliteal and inguinal lymph node counts were obtained. There was 1.2%, 2.1% and 3.6% uptake by the popliteal (right + left) nodes for low, moderate and high specific acitivity doses respectively. Also, the same level of uptake (0.5%) by the inguinal nodes was found for each specific activity dose used. In vitro experiments with peripheral leukocytes indicated <20% ^{99m}Tc-ATC was cell-associated, and the remainder as unbound. Leukocytes were incubated with ^{99m}Tc-ATC at 37^{o}C (control), 0-4^{o}C (hypothermia) or lipopolysaccharide/37^{o}C (LPS). Radiocolloid associated with neutrophils was unchanged for hypothermia versus control, and almost doubled for LPS versus control, implying that ^{99m}Tc-ATC is surface-bound rather than phagocytosed by these cells. In summary, higher specific activity ^{99m}Tc-ATC gave higher retention in the first draining popliteal lymph node, and an unchanged level by the second tier inguinal node. The retention mechanism may be related to: (i) physical entrapment of radiocolloid in the subcapsular sinuses within lymph nodes; and/or (ii) low binding by surface adsorption to leukocytes. With the sentinel node biopsy technique, a high specific activity radiocolloid could prove beneficial in locating the first draining node in complex node fields (ie: trunk), from its higher concentration of counts.

INVESTIGATION OF LIPOPHILICITY OF IDA DERIVATIVES FOR HEPATOBILIARY SCINTIGRAPHY

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Keywords: lipophilicity, partition coefficient, IDA derivatives

The lipophilicity of iminodiacetic acid (IDA) derivatives labeled with technetium-99m, radiopharmaceuticals for hepatobiliary scintigraphy, is important property that affects on biokinetics of these compounds. The parameter of the quantitative determination of the lipophilicity is the partition coefficient (log P). The partition coefficient was calculated using PACO softwer program for various theoretically possible halogenated IDA derivatives. 4-iodine-2.6-diethyl IDA derivative (DIETHYLIODIDA) with the highest calculated log P (3.8) was synthesized and labeled with Tc-99m. In this study lipophilicity and biological behavior of ^{99m}Tc-DIETHYLIODIDA has been checked experimentally and compared to commercial radiopharmaceutical ^{99m}Tc-BROMIDA (calculated logP=1.78).

Radiochemical purity was determined by ITLC method on SA and SG strips using 2 mol dm^{-3} NaCl and 80 % CH_3OH as mobile phases.

Partition coefficient was determined by extraction method with n-octanol. 0.5 ml of ^{99m} Tc-DIETHYLIODIDA and 1 ml of ^{99m}Tc-BROMIDA was extracted with different volumes of n-octanol for 30-180 min at 21 C. After extraction, organic phase was removed and the radioactivity of both aqueous and organic phases was measured.Biodistribution was studied on rats who 1 min after i.v. injection of bilirubine (45mg\kg) received i.v. injection of radiopharmaceuticals. The control groups received radiopharmaceuticals without bilirubine.in. The animals were sacrificed in various time intervals after injection of radiopharmaceuticals and the distribution of activity was measured in different organs.

Log P determined upon described conditions was –0.32 for ^{99m}Tc-BROMIDA and 1.05 for ^{99m}Tc-DIETHYLIODIDA. The correlation between higher log P and better hepatobiliary properties of ^{99m}Tc-IDA derivatives was determined. DIETHYLIODIDA (higher logP) has higher biliary excretion (30 % versus 20 % after 3.5 min) and faster hepatic clearance (liver 9.6% versus 14% after 15 min) than BROMIDA. More negative effect of bilirubine was noted for BROMIDA. Biliary excretion of BROMIDA and DIETHILIODIDA decreased about 12% and 30% (after 3.5min), 30% and 10% (after 15min), respectively. Renal excretion of BROMIDA increased about 66%.

Newly synthesised hepatobiliary imaging agent DIETHILIODIDA has better hepatobiliary properties than BROMIDA.

APPROACHES TO THE *IN VIVO* PET IMAGING OF T CELLS IN ADOPTIVE IMMUNOTHERAPY

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Keywords: Cu-64, antibody, CuPTSM, cell trafficking, T cell

The exquisite specificity of the immune system makes adoptive immunotherapy an attractive component for anti-tumor therapy. The ultimate goal is to use T cells to eliminate residual or metastatic tumors after surgery and/or chemotherapy. Mouse tumor models have been valuable in studying the anti-tumor immune response. Surprisingly; however there is very little information on T cell localization during and after tumor elimination. Here, we describe and compare two *in vivo* approaches to imaging T cell trafficking with microPET in order to understand this process.

The tumor model utilized in these studies is the previously described CMS5/DUC18 system.(1) The DUC18 is a T cell receptor (TCR) transgenic mouse line that expresses the and chains of TCR expressed on C18 $CD8^+$ T cell clone in a BALB/c background. C18 specifically recognizes a mutated ERK2 protein (tERK) when presented by a K^d MHC class I molecule. This complex is expressed on CMS5, a BALB/c derived fibrosarcoma cell line. DUC18 mice can specifically reject CMS5 tumor challenge; and *in vitro* stimulated DUC18 T cells can successfully eradicate CMS5 tumor that had grown in BALB/c hosts for eight days prior to the adoptive transfer.

Two possible approaches towards imaging the *in vivo* trafficking of the infused T cells have been examined; *in vitro* radiolabeling of activated DUC18 T cells with [^{64}Cu]PTSM prior to transfer (2), or radiolabeling of an antibody specific to the transferred DUC18 T cells. While initial efforts of *in vitro* labeling with [^{64}Cu]PTSM were successful, drawbacks to this methodology were soon found. The DUC18 T cells were found to be quite radiosensitive limiting the labeling to approximately 5 Ci/3 million cells. While initial influx of [^{64}Cu]PTSM was quite efficient, efflux was as rapid. These factors limited the utility of [^{64}Cu]PTSM labeled T cells for long term (> 24 hr) PET imaging, although the initial cell trafficking and localization were observed.

We utilized BALB/c-Thy1.1 congenic mice as tumor host, enabling us to specifically track the transferred DUC18 T cells by anti-Thy1.2, an antibody specific to the DUC18 T cells. The antibody was conjugated to DOTA using standard techniques (3), and then radiolabeled with copper-64 in high yield. This antibody was injected into mice with eight day old CMS5 tumors that had previously received DUC18 T cells. MicroPET imaging twelve hours after injection of the radiolabeled antibody clearly showed the location of the DUC18 T cells as they trafficked throughout the lymphatic system and accumulated around the tumor. This approach allows us to monitor mice at various stages of the ablation process and to determine that after ablation, the DUC18 T cells accumulate in a majority of the secondary lymphoid organs. Unfortunately this method is problematic for serial studies of the process, as each imaging study could further deplete the population of DUC18 T cells.

In conclusion, we have described two methods for radiolabeling T cells for *in vivo* PET imaging of T cell trafficking in adoptive immunotherapy. DUC18 T cell trafficking can be observed using either *in vitro* labeled cells, or with a radiolabeled antibody specific to DUC18 T cells. The *in vitro* labeling method works well for imaging the early trafficking after injection, while the labeled anti-Thy1.2 is effective for later imaging points.

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A REMOTE CONTROLLED SYSTEM FOR PHANTOM STUDIES WITH SHORT LIVED POSITRON EMITTERS

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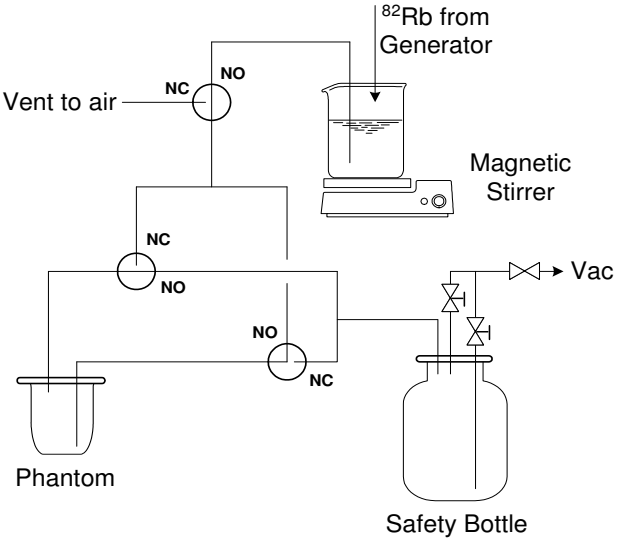
Keywords: PET, phantom, Rb-82, positron emitter

Phantom imaging studies are very important for assessing PET camera performance. Filling a phantom requires a high level of radioactivity with a high degree of uniformity to offset radioactive decay of the short-lived positron emitters. In order to facilitate phantom studies and reduce potentially excessive radiation exposure of staff members, we developed a remotely controlled system for quick and efficient filling and emptying of a PET phantom with short-lived positron emitters.

As shown in the Figure, the fluid transfer was facilitated by vacuum, which was controlled by a 2-way solenoid valve. For a cardiac phantom study, 50 mCi ^{82}Rb eluted from a ^{82}Rb generator was mixed with 600 ml of water using a magnetic stirrer. With the 2-way valve opened, the mixture was transferred into an empty cardiac phantom through three 3-way solenoid valves. The system was designed to simultaneously energize all three 3-way solenoid valves by a single switch. Rapid (< 1 min) and uniform filling and drainage of a phantom can be remotely controlled using this system. The technique offers multiple advantages and allows 3D-camera performance under extreme ranges of activity to be quickly evaluated when scanning in dynamic mode. Analysis of dead time, quantification linearity, resolution/contrast changes and effects of out of field activity and shielding is facilitated because of the wide range of activity the short-lived ^{82}Rb provides. Multiple experiments and changes to the phantom model are possible because of the quick decay of the positron emitter. Because the system is remotely controlled, the radiation exposure to personnel is greatly reduced. Besides ^{82}Rb, this system can be extended to phantom studies using other positron emitters, such as F-18, C-11, N-13, and O-15.



IN VIVO EVALUATION OF INDIUM-111-LABELED CROSS-BRIDGED TETRAAZAMACROCYCLIC LIGANDS

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Keywords: In-111, bifunctional chelator, biodistribution, macrocycle

Introduction. Cross-bridged TETA and DOTA derivatives (CB-TE2A (H_2**1**) and CB-DO2A (H_2**2**)) hold strong promise for use in copper radiopharmaceuticals for diagnostic imaging and targeted radiotherapy because of their high thermodynamic stability and kinetic inertness under biological conditions. In our previous studies, ^{64}Cu-**1** exhibited significantly higher resistance to in vivo transchelation than the corresponding TETA complex (1, 2). To extend the application of these cross-bridged ligands to other radionuclides, in this study we radiolabeled the ligands with ^{111}In, and evaluated their in vivo behavior in normal rats.

Methods. Indium-111 chloride (5 L, ~200 Ci, Mallinckrodt Inc., St. Louis MO) was added directly to each ligand solution (5 mM, 100 L in either pure ethanol or 0.1 M ammonium citrate, pH 8.2). The resulting solutions were incubated at 80 C for 4-5 h. The radiochemical purity was determined by reversed-phase C18 TLC. The eluant composition was MeOH:10% NH_4OAc (6:4) for developing ^{111}In-**1**, and MeOH:10% NH_4OAc (1:1) for developing ^{111}In-**2**. Under both conditions, $^{111}InCl_3$ remained at the origin. The biodistribution studies of ^{111}In-**1** and ^{111}In-**2** were carried out using mature female Sprague-Dawley rats (n = 4) as previously described (1).

Results and Discussion. Both H_2**1** and H_2**2** were successfully radiolabeled with ^{111}In. A high radiochemical purity (Rf 0.65, >98%) was obtained for labeling H_2**2** in 0.1M ammonium citrate buffer (pH 8.2), whereas the labeling of H_2**1** gave two species (Rf 0.54, ~80%; Rf ~0.88, ~20%). This phenomenon was observed in our previous studies with ^{64}Cu-**1** due to the possible formation of $^{64}Cu\mathbf{1}_2^{2-}$ (1). The biodistribution results for the two ^{111}In-labeled complexes showed similar *in vivo* behavior in normal Sprague-Dawley rats. At 1 h p.i., their uptake in blood and liver were similar (^{111}In-**1**: 2.040 0.190 %ID/blood, 0.530 0.053 %ID/liver; ^{111}In-**2**: 2.146 0.501 %ID/blood, 0.556 0.081 %ID/liver), while ^{111}In-**2** showed greater clearance than ^{111}In-**1** in these two organs (24-h p.i. ^{111}In-**2**: 0.0092 0.0.0004 %ID/blood, 0.179 0.015 %ID/liver; ^{111}In-**1**: 0.149 0.041 %ID/blood, 0.310 0.028 %ID/liver). Both complexes had relatively high kidney uptake at 1 h p.i. (^{111}In-**1**: 1.127 0.733 %ID/kidney; ^{111}In-**2**: 0.915 0.155 %ID/kidney) which cleared slowly (24-h p.i. ^{111}In-**1**: 0.240 0.034 %ID/kidney (^{64}Cu-**1**: 0.061 0.009 %ID/kidney, for comparison); ^{111}In-**2**: 0.261 0.030 %ID/kidney (^{64}Cu-**2**: 0.069 0.020 %ID/kidney, for comparison)) because of their positively-charged nature (3). Compared to their ^{64}Cu-labeled counterparts, the ^{111}In-labeled complexes similarly showed rapid clearance from major organs after 1 h p.i. Their clearance from the liver was even faster than was the case for the neutral ^{64}Cu-complexes at 2 and 4 h p.i., this is again probably because of the positive charge that renders them higher hydrophilicity. These results suggest that both ligands have potential to be used for labeling indium and gallium radionuclides to biomolecules for diagnostic imaging and targeted radiotherapy.

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^{94m}Tc AND ^{64}Cu LABELED ANNEXIN-V, POSITRON EMITTING RADIO-PHARMACEUTICALS TO STUDY APOPTOSIS

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Keywords: apoptosis, annexin-V, Fas, copper-64

Annexin V is a member of a structurally homologous group of proteins that show high affinity for phosphatidylserine (PS) containing membranes. This is an important feature, as the externalization of PS on the outer leaflet of the plasma membrane appears to be a general phenomenon in cells undergoing programmed cell death (apoptosis). Annexin V is therefore a sensitive marker of the early to intermediate phases of apoptosis.

Previous work with annexin V involved the conjugation with hydrazinonicotinamide (HYNIC) via the lysine residues, and subsequent coupling to ^{99m}Tc.(1) The goal of this project was to extend this work and label annexin with the PET isotopes ^{94m}Tc(2, 3) and ^{64}Cu.(4) ^{94m}Tc was attached using the method utilized for ^{99m}Tc,(5) while ^{64}Cu was attached via 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). One of the studies undertaken was the murine model of Fas-mediated apoptosis. This model was chosen as it had been previously examined with ^{99m}Tc-HYNIC-annexin and is a well described model of apoptosis *in vivo*.(1, 6)

This study was repeated with ^{94m}Tc-HYNIC-annexin and ^{64}Cu-DOTA-annexin with 7 week old female BALB/c mice. In these studies mice were pretreated with 10 µg of Fas one hour before injection of the radiopharmaceutical. To monitor changes Fas had on organ uptake, a group of mice were injected only with the radiopharmaceutical. 1 hr after administering the radiopharmaceutical the mice were sacrificed and the organs harvested and taken for scintillation well counting. In the mice pretreated with Fas the ^{94m}Tc conjugate showed a 340% increase in spleen uptake over control mice and a 388% decrease in kidney uptake. Similar values were obtained with the ^{64}Cu conjugate, which showed a 240% increase in spleen uptake and a 238% decrease in kidney uptake in mice pretreated with Fas. However no change in liver uptake was observed with either ^{94m}Tc or ^{64}Cu annexin. Blood metabolism studies with ^{64}Cu-DOTA-annexin in mice pretreated with Fas showed that after 1 hr only 3% of the total activity was bound to red blood cells, while it remained 65% intact.

The major difference between this study and the one previously reported with ^{99m}Tc-HYNIC-annexin is that after 1 hr the mice pretreated with Fas showed a 134% increase in liver uptake with ^{99m}Tc-HYNIC-annexin, while no change in liver uptake was observed with the ^{94m}Tc and ^{64}Cu conjugates. Changes in kidney and spleen uptake were also observed with ^{94m}Tc-HYNIC-annexin, but not as dramatic as those seen with the ^{94m}Tc and ^{64}Cu conjugates. The published data reports using 4-5 week old BALB/c mice, the sex was not given. In this study the mice used were 2-3 weeks older in order to obtain a similar weight to the mice used in the ^{99m}Tc study.

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INVESTIGATION INTO IMPROVED IMAGING AGENTS FOR CARDIOVASCULAR AND NEUROLOGIC HYPOXIA

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Keywords: copper-64, hypoxia, microPET imaging

The delineation of hypoxia in tissue has direct implications in the treatment of multiple disease states such as cancer and myocardial infarction. However, the definition of hypoxia differs in each disease state examined. For example radiobiological hypoxia is considered to define O_2 concentrations below 1000 pm, whereas cardiovascular hypoxia may have O_2 levels that are up to 100 times higher.(1) Studies have shown that the inflection point in cell uptake of ^{64}Cu-ATSM occurs at an O_2 level of between 1000-5000 ppm.(2) As a result ^{64}Cu-ATSM is suitable for tumor imaging. It is not however suitable for studying cardiovascular or neurological hypoxia as irreversible cell damage occurs at O_2 levels higher than those found in tumors. As a result new hypoxia imaging agents are being examined that would be more sensitive to hypoxic cells at these O_2 concentrations. This report describes our ongoing efforts to develop such ligands in an attempt to develop superior hypoxic imaging agents.

Two compounds of current interest are diacetyl bis(N^4-ethylthiosemicarbazone) (ATSE) and diacetyl bis(selenosemicarbazone) (ASS). The lipophilicities of these complexes were determined by octanol:PBS partition coefficient to be 2.34 ± 0.08 (^{64}Cu-ATSE) and 1.15 ± 0.15 (^{64}Cu-ASS). ^{64}Cu-ATSE and ^{64}Cu-ASS were screened for hypoxia selectivity using EMT-6 cells, as previously described.(2) In these experiments the cell suspension was equilibrated at 37°C for 1 hr with an atmosphere containing the desired O_2 concentration, 5% CO_2 and the balance N_2. Analysis of the data showed that both ^{64}Cu-ATSE and ^{64}Cu-ASS showed an increase in cell uptake at lower O_2 concentrations. The hypoxia selectivity, expressed as the $\log_{10}$[(% uptake in hypoxic cells)/(% uptake in normoxic cells)], was found to be 0.56 for ^{64}Cu-ATSE and 0.35 for ^{64}Cu-ASS. In an analogous study carried out with ^{64}Cu-ATSM, the hypoxia selectivity was calculated at 0.59. The inflection point of both ^{64}Cu-ATSE and ^{64}Cu-ASS was determined as being at an O_2 concentration of between 80000 and 100000 ppm. This puts both complexes in the range for studying cardiovascular and neurological hypoxia.

Rodent biodistribution at 5, 20, 40 min, 1 2, and 4 h were obtained with ^{64}Cu-ATSE and ^{64}Cu-ASS. In these experiments BALB/c mice bearing EMT6 tumors were injected with either ^{64}Cu-ATSE or ^{64}Cu-ASS. For both ^{64}Cu-ATSE and ^{64}Cu-ASS tumor uptake reached a maximum 20 minutes post-injection, 7.71 ± 0.46 and 10.33 ± 0.78 %ID/g respectively, and decreased slightly over 4 hours. The major difference in uptake, other than the liver, between ^{64}Cu-ATSE and ^{64}Cu-ASS was observed in the brain. ^{64}Cu-ATSE had rapid uptake followed by slow washout, while ^{64}Cu-ASS had substantially lower brain uptake that remained constant over 4 hours. Similar to ^{64}Cu-ATSM, both ^{64}Cu-ATSE and ^{64}Cu-ASS cleared through the liver and kidneys. However it should be noted that ATSE showed lower kidney and liver uptake than ^{64}Cu-ATSM at all time points examined, and as such could potentially be used to image liver metathesis. ^{64}Cu-ASS had similar kidney uptake as ^{64}Cu-ATSM, but liver uptake was dramatically higher.

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ADSORPTION OF SAMARIUM ETHYLENEDIAMINETETRAMETHYLENE PHOSPHONIC ACID TO HYDROXYAPATITE: AN *IN VITRO* MODEL FOR UPTAKE OF THE RADIOPHARMACEUTICAL IN BONE TISSUE

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Keywords: SmEDTMP, hydroxyapatite, adsorption, bone palliation

Samarium-153 (^{153}Sm) is a radiolanthanide used in nuclear medicine procedures. It is commonly used for the palliation of bone pain associated with metastatic bone cancer. In current palliative applications, radioactive samarium is introduced *in vivo* as a complex of ethylendiaminetetramethylene phosphonate (EDTMP). This ^{153}Sm-EDTMP complex (trade name Quadramet®) is known from clinical studies to rapidly localise in the skeleton, with the majority of the remainder being excreted *via* the bladder [1,2].

Recent work has focussed on the use of ^{153}Sm-EDTMP as a bone marrow ablation agent prior to bone marrow transplant in patients with marrow based haematological malignancies [3]. This treatment has been shown to effectively slow disease progression and is less toxic to organs such as the liver and kidneys, owing to its bone targeting behaviour.

Whilst it is known that ^{153}Sm-EDTMP accumulates in bone [1-4], the exact nature of the interaction between the agent and bone is not known. The ultrastructure of bone is complex, with microcrystalline hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] being embedded in a proteinaceous organic matrix. Inorganic hydroxyapatite (HAP) accounts for approximately 60 % of skeletal mass. This study uses HAP as an *in vitro* model of bone to examine the physicochemical behaviour of EDTMP and Sm-EDTMP in solution and in the presence of the mineral component of bone. Adsorption isotherms have been constructed describing the adsorption of EDTMP to HAP and the adsorption of Sm-EDTMP to HAP. The effect of the Sm-to-EDTMP ratio of the complex on adsorption is investigated. Analogous isotherms have been constructed with demineralised canine bone in place of HAP. Infrared spectroscopic studies and the isotherms suggest that chemisorption through interaction between the calcium of bone HAP and phosphonate groups of the Sm-EDTMP accounts for the uptake of the agent in bone *in vivo*.

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[^{11}C]DAA1106, [^{18}F]FMDAA1106 AND [^{18}F]FEDAA1106: RADIOSYNTHESIS AND EVALUATION AS PET TRACERS FOR PERIPHERAL BENZODIAZEPINE RECEPTORS (PBR)

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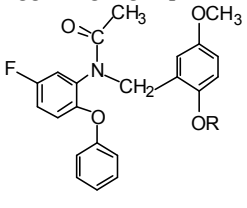
Keywords: Peripheral benzodiazepine receptors, [^{11}C]DAA1106, [^{18}F]FMDAA1106, [^{18}F]FEDAA1106, PET

Recent studies have shown that the density of peripheral benzodiazepine receptors (PBR) was increased following brain injury, and this increase was used as indication of neuronal damage or loss in several neurodegenerative disorders, such as Alzheimer's disease and epilepsy. As a result there has been great interest in developing a radioligand that could be used to visualize the distribution of PBR in primate brain using PET. In this work, we labeled DAA1106, a potent and selective ligand for PBR, with ^{11}C, synthesized two novel analogs of DAA1106: [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106, and evaluated these PET tracers for PBR with mouse, rat and monkey.[1,2)]

[^{11}C]DAA1106 was prepared by reacting the desmethyl precursor DAA1123 with [^{11}C]CH_3I and NaH in 72-85% radiochemical yield. The [^{18}F]fluoroalkylating intermediate [^{18}F]FCH_2I or [^{18}F]FCH_2CH_2Br was obtained by heating [^{18}F]F^- with CH_2I_2 or $TfOCH_2CH_2Br$ and purified by distillation. [^{18}F]FMDAA1106 and [^{18}F]FEDAA1106 were respectively synthesized by alkylation of DAA1123 with [^{18}F]FCH_2I and [^{18}F]FCH_2CH_2Br in the presence of NaH as a base in 80-92% yields. The radiochemical purities of the three tracers remained >95% after maintenance of the preparations at 25 C for 4 h, and they were stable for performing evaluation.

Binding studies of these compounds to PBR were performed using *in vitro* autoradiograph for rat brain sections. They displayed potent affinities (IC_{50}) for PBR: FEDAA1106, 0.77 nM; DAA1106, 1.62 nM; FMDAA1106, 1.71 nM. *Ex vivo* autoradiograms of the three tracers' binding sites in rat brains at 30 min postinjection revealed that significantly-high radioactivities were present in the olfactory bulb, the highest PBR density area in the rat brain. Other than the olfactory bulb, moderate radioactivity levels were observed in the cerebellum, whereas low uptakes were seen in the other brain regions. Metabolite analysis for the brain homogenate displayed that [^{11}C]DAA1106 and [^{18}F]FEDAA1106 were not metabolized, whereas [^{18}F]FMDAA1106 was decomposed in the brain at 30 min postinjection. PET studies on monkey were performed using [^{11}C]DAA1106 and [^{18}F]FEDAA1106. These two tracers showed high radioactivity accumulations in the occipital cortex, a high PBR density area in the monkey brain: [^{11}C]DAA1106, 0.02% dose/mL; [^{18}F]FEDAA1106, 0.03% dose/mL. Co-injection with DAA1106 and PBR-selective PK11195 reduced the radioactivity levels of [^{11}C]DAA1106 and [^{18}F]FEDAA1106 (10-20% of control) in the brain, suggesting high specific bindings of these tracers with PBR in the brain.



R = H: DAA1123
R = $^{11}CH_3$: [^{11}C]DAA1106
R = $^{18}FCH_2$: [^{18}F]FMDAA1106
R = $^{18}FCH_2CH_2$: [^{18}F]FEDAA1106

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DEVELOPMENT OF A ^{99m}Tc LABELLED ANTI-TNFa MONOCLONAL ANTIBODY FOR THE TARGETED IMAGING OF INFECTION

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Keywords: infection, TNF , infliximab

Tumour necrosis factor (TNF) is a potent proinflammatory cytokine which plays a key role in the acute phase response to inflammation and infection. TNF concentration is elevated at sites of infection and may provide a target for a radiopharmaceutical to image infection. Infliximab (Remicade, Centocor), a chimeric IgG1 monoclonal antibody against TNF is available for the immunotherapy of inflammatory diseases. Our aims were to radiolabel infliximab with ^{99m}Tc, to confirm binding to TNF and study its accumulation at sites of infection. An instant cold kit was prepared using 2-mercaptoethanol (ME) reduced infliximab and evaluated for radiolabelling with ^{99m}Tc. Radiochemical purity (RCP) of the resultant ^{99m}Tc-infliximab was determined following the addition of ^{99m}Tc-pertechnetate. The effect of ME modification on the TNF binding properties of the antibody was examined by a TNF binding assay (ELISA) to both human and rat TNF . As a prelude to human studies, biodistribution studies were performed in rats following induction of a S. aureus focal infection in the right thigh, comparing ^{99m}Tc-infliximab, ^{99m}Tc-HIG and ^{99m}Tc-DTPA.

RCP of ^{99m}Tc-infliximab was found to be 97.7 ± 0.4 % (n=5) for up to 4 hours post reconstitution. The binding assay showed that the modified antibody still retains 90% of its binding to human TNF while there is no binding to rat TNF . In rats, the infected to non-infected muscle ratios at 1 and 4 hours were 5.7 ± 0.8, 7.1 ± 1.2 for ^{99m}Tc-infliximab, 3.1 ± 1.1, 7.8 ± 1.2 for ^{99m}Tc-HIG and 3.5 ± 0.3, 1.9 ± 0.2 for ^{99m}Tc-DTPA respectively. ^{99m}Tc-DTPA as a marker of capillary permeability gave low ratios whereas both ^{99m}Tc-labelled IgG molecules showed substantially higher ratios, indicating that a non-TNF -specific binding mechanism is occurring in rats. In conclusion, infliximab can be efficiently labelled with ^{99m}Tc without a substantial reduction in its binding properties to human TNF . ^{99m}Tc-infliximab is expected to show potential in the targeting of infectious/inflammatory foci in humans given its specific binding to human TNF .

TRANSCELLULAR TRANSPORT OF 4-[I-125]-L-META-TYROSINE ACROSS MONOLAYERS OF KIDNEY EPITHELIAL CELL LINE LLC-PK1

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Keywords: 4-[^{125}I]iodo-L-*meta*-tyrosine, amino acid transport, LLC-PK1, proximal tubule, resorption

Aim: We investigated the carrier systems involved in transport of 4-[^{125}I]iodo-L-*meta*-tyrosine (^{125}I-*m*Tyr) (1)across a monolayer of the porcine kidney epithelial cell line LLC-PK1 (2). Like the brush border membrane of proximal cells, LLC-PK1 forms an oriented monolayer with microvilli and tight junctions (3). LLC-PK1 monolayers exhibit bidirectional transcellular transport (secretion and reabsorption) and accumulation of electrolytes and some nutrients (3). **Materials and Methods:** To create LLC-PK1 monolayers, cells were grown on a collagen-coated microporous (3 m) membrane (4.7 cm^2) in Transwells, in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, without antibiotics, in an atmosphere of 5% CO_2-95% air at 37 C. LLC-PK1 cells were seeded on the membrane at a density of 5×10^5 cells/cm^2 in 1.5 mL (upper side) or 2.0 mL (lower side) of the complete medium, and were used on the 4th or 5th day after inoculation. Transcellular transport (secretion and reabsorption) and accumulation were examined as follows. After removal of the culture medium, each side of the membrane was washed once with 2 mL of incubation medium for 10 min at 37 C. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM HEPES, pH 7.4. For the inhibition experiment, one of the following compounds was added at a final concentration of 1 mM: 2-amino-bicyclo-(2,2,1)-pentane-2-carboxylic acid (BCH), L-Arg, L-Tyr, 2-(methylamino)isobutyric acid (MeAIB), or probenecid. For the pH dependence experiment, the pH of the apical-side uptake solution was varied (from pH 5 to pH 8). The monolayers were incubated for 90 min at 37 C with 18.5 kBq ^{125}I-*m*Tyr. After incubation, transcellular transport was assessed by quantifying the radioactivity of the uptake solution (50 L) of each side using a well-type scintillation counter. For the accumulation experiment, the uptake solution was aspirated, and the monolayers of each side were rinsed rapidly twice with 2 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 1 N NaOH, and the radioactivity of each aliquot was quantified. **Results and Discussion:** Accumulation and transcellular transport of ^{125}I-*m*Tyr were significantly inhibited by BCH, L-Arg, and L-Tyr ($P<0.01$), suggesting that the amino acid transport systems L and $B^0/B^{0,+}$ mediate ^{125}I-*m*Tyr transport of each side of the monolayer. Probenecid did not significantly inhibit ^{125}I-*m*Tyr reabsorption, but it slightly inhibited secretion, suggesting that ^{125}I-*m*Tyr act as a substrate for an organic anion transport system to some extent. On the apical side, ^{125}I-*m*Tyr reabsorption was dependent on pH, suggesting that system A contributes to ^{125}I-*m*Tyr reabsorption.

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IN VITRO RESULTS OF CONJUGATION OF PEGULATED AVIDIN

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Introduction: The Avidin Biotin affinity system has been used to improve tumour targeting by radiolabelled agents. But Avidin has a short circulation time in-vivo hence low tumour uptake. Both theoretically can be increased by pegulation with concomitant reduction in immunogenicity.

Method, we prepared PEG-Avidin by conjugating 20 Kda PEG with Avidin at 400:1incubation ratio and reacted with HABA. To separate the un - reacted PEG the conjugate was passed down a 2-imminobiotin- Sepharose 4B column and the effluent collected, concentrated by dialysis and reacted with HABA to subjectively determine the amount of collected Avidin via a visible colour reaction.

Result, Pegulated Avidin obtained by this method and tested with the HABA reaction produced similar intensity colour reaction as unpurified pegulated Avidin.

Conclusion: Pegulated Avidin can be synthesized and can react with HABA after using 6 M Urea elution from a 2- imminobiotin- Sepharose 4B column suggesting retained Biotin affinity.

TRANSPORT SELECTIVITY OF 3-[I-125]IODO-L-TYROSINE IN HUMAN L-TYPE AMINO ACID TRANSPORTERS

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Keywords: 3-[^{125}I]iodo-L-tyrosine, L-tyrosine, human L-type amino acid transporter, *Xenopus laevis* oocyte, system L

Transport of large neutral amino acids with branched or aromatic side chains is mediated by system L, which is Na^+–independent (1). It is thought that system L is a major route of cellular uptake of branched or aromatic amino acids (1). The compound 3-[^{125}I]Iodo-L-tyrosine (^{125}I-Tyr), which is produced by 3-iodination of L-Tyr, acts as a biosynthetic substrate of triiodothyronine and thyroxine (thyroid hormone) (2). In the present study, we examined transport of ^{125}I-Tyr and L-[^{14}C(U)]-Tyr by the human L-type amino acid transporters hLAT1 and hLAT2, which are elements of system L. Uptake of these compounds and inhibition of uptake were examined using *Xenopus laevis* oocytes expressing hLAT1 or hLAT2 and the heavy chain of human 4F2 cell surface antigen (h4F2hc) (3,4). Functional expression of hLAT1 and hLAT2 requires formation of a hetero-dimer with h4F2hc (3,4). **Materials and Methods:** The animal experiments in this study were approved by the ethical committees of all universities involved. Defolliculated *Xenopus laevis* oocytes were injected with 15 ng of hLAT1 or hLAT2 cRNA and 10 ng of h4F2hc cRNA (mole ratio, 1:1). Two days after injection, the oocytes were incubated in ND96 solution (pH 7.4) containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2 2H_2O$, 1 mM $MgCl_2 6H_2O$, 5 mM HEPES and 18.5 kBq of ^{125}I-Tyr or L-[^{14}C(U)]-Tyr, at 25 C for 30 min, followed by assessment of uptake. In the inhibition experiment, the incubation solution also contained 3 mM of 1 of 21 naturally occurring L-amino acids. **Results:** Uptake of ^{125}I-Tyr by hLAT1-expressing *Xenopus laevis* oocytes was approximately 60-fold greater ($p<0.001$) than that of the control oocytes. Uptake of ^{125}I-Tyr decreased (<20%) in the presence of neutral amino acids with branched or aromatic side chains; i.e., Cys, Leu, Ileu, Phe, Met, Tyr, His, Trp and Val. Uptake of ^{125}I-Tyr via hLAT1 was approximately 3-fold greater than uptake via hLAT2 ($P<0.005$). There was no significant difference in L-[^{14}C(U)]-Tyr uptake between hLAT1-4F2hc- and hLAT2-4F2hc-expressing *Xenopus laevis* oocytes. Thus, ^{125}I-Tyr transport was isoform-selective, whereas L-[^{14}C(U)]-Tyr transport was not.

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IMPROVEMENT OF ^{99m}Tc-PERTECHNETATE THYROID ACCUMULATION BY MEANS OF DISPLACEMENT OF SERUM PROTEIN BINDING

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Keywords: ^{99m}Tc-pertechnetate, thyroid accumulation, serum protein binding, displacement

It is generally accepted that only free fraction of drugs in serum is concerned with pharmacological activity in therapeutic drugs. Concurrent administration of drugs with high protein binding affinity may produce competitive displacement at the specific binding site of serum protein, causing higher free drug concentration [1] and greater biological. Technetium-99m labeled mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$) is widely used for renal scintigraphy. At diagnostic concentrations, it has been shown that 90 % of ^{99m}Tc-MAG$_3$ were bound to serum protein [2]. In our previous study, we identified that bucolome was the prominent inhibitor of ^{99m}Tc-MAG$_3$ protein binding, and brought rapid kidney accumulation and rapid clearance of ^{99m}Tc-MAG$_3$, because bucolome treatment increased free fraction of ^{99m}Tc-MAG$_3$ in both *in-vitro* and *in-vivo* [3, 4].

Meanwhile, approximately 75% of ^{99m}Tc-pertechnetate (^{99m}Tc-TcO$_4^-$), a thyroid imaging agent, are also bound to serum protein. If displacers with high protein binding affinity inhibit ^{99m}Tc-TcO$_4^-$ serum protein binding, the thyroid accumulation of this tracer would be accelerated. As the results, it may produce better thyroid imaging with shorter waiting time and less radiation dose for patients. In this study, the displacement of ^{99m}Tc-TcO$_4^-$ serum protein binding has been also attempted to regulate its pharmacokinetics. For this purpose, the present study is undertaken to find the major displacers of ^{99m}Tc-TcO$_4^-$ binding to HSA for the improvement of its thyroid imaging.

The serum protein binding of ^{99m}Tc-TcO$_4^-$ was evaluated by ultrafiltration method (Tosoh; Ultracent-10). The inhibitory effects of several displacers of ^{99m}Tc-TcO$_4^-$ binding were examined in human and mouse serums. Mouse biodistribution studies of ^{99m}Tc-TcO$_4^-$ with or without bucolome, which has been identified as a displacer of binding site I on HSA, were also performed.

The free fraction rate of ^{99m}Tc-TcO$_4^-$ was increased 9.0% and 1.9% with a HSA site I displacer in *in-vitro* human and mouse serums, respectively. The effects of bucolome loading on ^{99m}Tc-TcO$_4^-$ distribution in mice were evaluated using 20 mg/kg of bucolome, a therapeutic dose for humans. The *in-vivo* free fraction of ^{99m}Tc-TcO$_4^-$ significantly increased in mouse. The peak of thyroid accumulation was shifted 30 min after the ^{99m}Tc-TcO$_4^-$ injection. The accumulation rate was extremely elevated more than 1.4 times compared with control condition, and then the radioactivity was rapidly disappeared. The results showed that bucolome treatment increased the free ^{99m}Tc-TcO$_4^-$ in *in-vivo* and the rapid and high thyroid accumulation was achieved. Since the binding affinity of ^{99m}Tc-TcO$_4^-$ and the displacement effect of bucolome in human serum were significantly greater than in mouse serum, in the human thyroid imaging, more effective displacement of bucolome was expected. Moreover, it suggested the possibility to control the pharmacokinetics of radiopharmaceuticals as well as more cost-effective diagnosis in clinical application.

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EVALUATION OF FACTORS AFFECTING THE RADIOLABELLING KINETICS OF A BIOACTIVE PEPTIDE

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Key words: Technetium-99m, radiolabelling conditions, bioactive peptide

In developing new ^{99m}Tc-labelled peptide-based radiopharmaceuticals for in vivo use, one of the important criteria is to radiolabel receptor-binding bioactive peptides with ^{99m}Tc under mild conditions. This is because harsh radiolabelling conditions may alter receptor-binding and biological characteristics of a peptide. In addition, optimization of the radiolabelling conditions of each specific peptide is crucial in order to maximize the radiolabelling yields and minimizes the formation of radiochemical impurities.

The purpose of this work is to investigate various radiolabelling parameters and to study their affect on the radiolabelling efficiency of a bombesin (BN) peptide coupled with a MAG_3 moiety at the N-terminus. We evaluated different parameters: concentration of the ligand (5 to 50 µg), amount of $SnCl_2$ (100 to 1200 µg), pH of the labelling mixture (pH 2 to 12) and reaction temperature (no heating and heating the mixture for 10-15 min at 95ºC).

Radiolabelling of MAG_3-BN(2-14) conjugate with ^{99m}Tc was accomplished via an exchange method in the presence of tartrate. It was observed that the amount of the ligand and the pH of the reaction mixture influence the radiolabelling yield significantly. A low amount of ligand (= 10 µg) results in low radiolabelling yield (up to 45%). The radiolabelling yield improved significantly (= 90%) when 20-40 µg of the peptide was used for radiolabelling. A maximum of 35% radiolabelling yield was obtained when radiolabelling was performed under acidic conditions (pH 5 or below) which improved considerably (> 90%) under alkaline (pH higher than 10) conditions. The amount of the reducing agent ($SnCl_2$) has no apparent affect on radiolabelling efficiency. Complexation with ^{99m}Tc without heating the labelling mixture was found to be very slow for MAG_3-BN(2-14) conjugate probably due to the slow labelling kinetics of MAG_3-containing peptides at ambient temperature. The pH of the labelling mixture and the reaction temperature are vital radiolabelling parameters for MAG_3-containing peptides. Alkaline pH combined with the elevated temperature facilitate removal of the thiol-protecting group and deprotonation of the amide-nitrogens in MAG_3 ligand, thus enhancing efficient complexation with ^{99m}Tc. The addition of ascorbic or citric acid was found to improve radiolabelling yield.

MAG_3-BN(2-14) conjugate, after radiolabelling with ^{99m}Tc under optimized conditions (25 µg, pH 12, 100 µL 5% ascorbic acid, 400 µg $SnCl_2$ and, heating for 15 min at 95ºC), was evaluated for its ability to bind in vitro with a human breast cancer cell line specific for BN-like peptides. In vitro stability was tested by incubation of ^{99m}Tc-MAG_3-BN(2-14) conjugate with plasma and by cysteine challenge. The results show that ^{99m}Tc-MAG_3-BN(2-14) conjugate retains its affinity for the desired receptors and also remains sufficiently stable in plasma and when challenged with excess amount of cysteine.

In conclusion, to retain maximum receptor affinity and preserve biological activity it is important that receptor-binding peptides be radiolabelled with ^{99m}Tc under suitable conditions, which must be optimized for each specific peptide according to type of chelator and nature of ^{99m}Tc-complex in order to achieve simple and efficient radiolabelling.

EFFECTS OF GELATIN ON PREPARATION OF ^{188}RE-TIN COLLOID FOR RADIOSYNOVECTOMY

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Keywords: ^{188}Re-tin colloid, Gelatin

Beta-emitting radiocolloids have been used for the treatment of rheumatoid arthritis. We have reported the preparation of ^{188}Re-tin colloid for radiosynovectomy [1, 2]. In this study, we evaluated the effects of gelatin on labeling efficiency, stabilization and particle size of ^{188}Re-tin colloid.

Lyophilized vials containing 10 mg $SnCl_2 \cdot 2H_2O$ and 1 g ascorbic acid were prepared. Radiolabeling was performed by boiling for 2 hr after addition of 0.1-N HCl and ^{188}Re-perrhenate freshly eluted with saline from ^{188}W/^{188}Re-generator (0.1 N HCl:^{188}Re-perrhenate = 1:4). The radiolabeled ^{188}Re-tin colloid was neutralized with adequate volume 0.2-M sodium phosphate buffer. Labeling efficiency was checked by chromatography (ITLC-SG/normal saline) and radioactivity was monitored by TLC scanner. The effect of gelatin on preparation of ^{188}Re-tin colloid was investigated. Gelatin was added to the vials containing $SnCl_2 \cdot 2H_2O$ before or after labelling (final concentration = 0.05%~1%). We evaluated the stability of ^{188}Re-tin colloid when gelatin (0.25~1%) was added to the reaction vials at room temperature for 24 hour. The particle size of each colloid preparation was determined by using filters with pore sizes of 10, 5, 1 and 0.22 m. One mL aliquot of each radiolabeled colloid was passed through the serially connected filters pore size between 0.22 ~10 m. After washing the filters with 4 mL of normal saline, the remained radioactivities of each filters and eluent were measured by dose calibrator.

The labelling efficiencies were maintained higher than 95% when the volume of ^{188}Re-perrhenate was < 2.4 mL. However, it decreased when the volume of ^{188}Re-perrhenate was over 2.4 mL. The ^{188}Re labelled colloid preparations were stable and was not affected by addition of gelatin before or after labelling. When gelatin was added to the reaction vials before labelling, the particle size of ^{188}Re-tin colloid was mainly between 1~10 m, and the portion of over 10 m and under 1 m were small (Table 1). However, when gelatin was added after labelling, the particle size of ^{188}Re-tin colloid was not changed compared to the preparation of ^{188}Re-tin colloid without gelatin.

Table 1. Particle Size Distribution of ^{188}Re-Tin Colloid in the Presence of Gelatine.

Particle size (?)	Distribution of particles (%)			
	Gelatin 0.05%	Gelatin 0.1%	Gelatin 0.15%	Gelatin 0.20%
> 10	3.3	14.8	10.8	2.6
5~10	34.6	37.5	10.3	3.1
1~5	56.2	40.9	63.6	6.8
1~0.2	1.4	2.5	9.4	65.7
< 0.2	4.5	4.2	6.0	21.8

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IN VIVO PHARMACOLOGICAL EVALUATION OF THE POTENT D1 RADIOLIGAND [^{11}C]A-69024 USING INTRACEREBRAL RADIOSENSITIVE b-MICROPROBES

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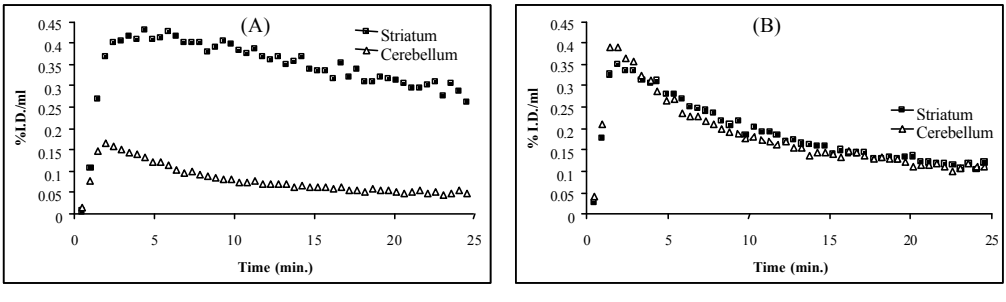
Keywords: Carbon-11, A-69024, D1 Receptor

The isoquinoline (+)-A-69024, [(+)-1-(2-bromo-4,5-dimethoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline] is a specific and enantioselective dopamine D1 receptor ligand (1). In the present study, the pharmacological profile of both carbon-11 radiolabelled enantiomers, (+)-[^{11}C]A-69024 and (-)-[^{11}C]A-69024, have been evaluated *in vivo* using intracerebral radiosensitive -microprobes in rodents. The enantiomers were prepared by *N*-alkylation of the racemic *N*-desmethyl precursor with [^{11}C]methyl triflate. Typically, 10 to 40 mCi of each enantiomer was obtained within 35 and 40 minutes of radiosynthesis following chiral semi-preparative HPLC purification with specific radioactivities ranging from 0.7 to 1.3 Ci/ mol.

Radiosensitive -microprobes were implanted simultaneously in the striatum and the cerebellum of isoflurane-anaesthetised rats. Following injection of (+)-[^{11}C]A-69024 (0.75 nmol ± 0.14: mean ± SD), the uptake was high in the striata at 5 min followed by a slow clearance. As a measure of specificity, the striatum to cerebellum ratio reached ~ 6 at 25 min (Fig. A). Pre-administration of the D1 antagonist SCH 23390 (1 mg/kg; *i.v.* 15 min before (+)-[^{11}C]A-69024) resulted in a reduction of the uptake in the striatum and was similar to that found in the cerebellum. A similar inhibition was observed when rats were pretreated using racemic A-69024 under the same experimental conditions (Fig. B).



The accumulation of (+)-[^{11}C]A-69024 in the striatum could also be displaced to the level of cerebellum using either SCH 23390 (1 mg/kg; *i.v.*), administered 20 min after (+)-[^{11}C]A-69024) or racemic A-69024, under the same conditions. The pharmacologically inactive enantiomer, (-)-[^{11}C]A-69024, displayed a striatum to cerebellum ratio of approximately 1 throughout the time course of the experiment. As a result, the uptake was determined to represent only non-specific binding.

These *in vivo* results suggest that the use of (+)-[^{11}C]A-69024 with radiosensitive -microprobes is feasible in pharmacological studies of dopamine D1 receptors. This simple technique will prove useful in associating and quantifying specific dopamine D1 receptor changes in animal models of neurodegeneration or neuroprotection.

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COMPUTER SIMULATION STUDY ON TRACER SELECTION PROBLEM OF REVERSIBLE AND IRREVERSIBLE RADIOLIGANDS FOR QUANTITATIVE MEASUREMENT OF ENZYMES OR RECEPTORS BY PET

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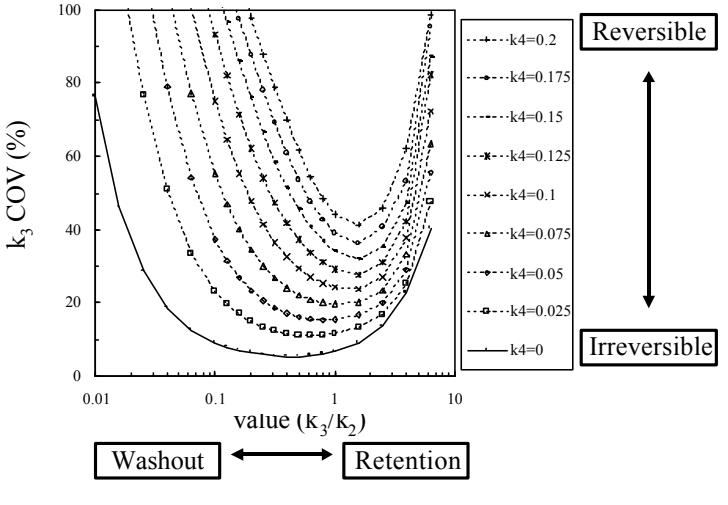
Keywords: Computer simulation, Tracer selection, Irreversible radiotracer, Reversible radiotracer, PET

Various AchE-specific radiotracers have been developed and clinically evaluated as tracers for early and differential diagnosis of Alzheimer disease and other demented disorders. They can be classified into two categories. One is irreversible tracers such as [^{11}C]MP4A and [^{11}C]PMP, which are lipophilic acetylcholine analogs, hydrolyzed into hydrophilic metabolites by AchE and thus trapped in the brain. The other is reversible tracers such as [^{11}C]physostigmine and [^{11}C]CP-126998, which are reversible inhibitor of AchE. In substrate-type tracers, k3 that represents hydrolysis rate of tracer with AchE is used as an AchE index, while in inhibitor-type tracers, either k3 or k3/k4 (BP; binding potential) is used. In this study, we have performed Monte Carlo simulation studies to examine the relationship between coefficient of variation (COV) of k3 and BP on tracer's properties.

In simulations, we have assumed that each compound has the same K1 (transport from blood to brain) and k2 (transport from brain to blood) and negligible non-specific binding. As shown in below, k3 COV (also BP COV, data not shown) was highly dependent on k3/k2 (Lassen's value; x-axis) and k4 (tracer's reversibility; each line). The k3 COV was lowest (i.e., k3 precision was highest) for k4=0 (dotted line) corresponding to irreversible tracers. As k4 increased, the precision of k3 became worse, suggesting that irreversible tracers can give more reliable k3 than reversible tracers. The k3 COV was quite dependent on value (k3/k2). As the value increased, k3 COV rapidly increased, suggesting that tracers with extremely high hydrolysis/binding rate may not be useful for accurate estimation of AchE activity/density in spite of high radioactivity uptake (flow-limitation effects). The optimal was about 0.5 in irreversible tracers and shifted to slightly higher values as k4 increased. These results suggest that in tracer selection we need to evaluate the value of tracers by dynamic PET study using monkeys.



CAN RECOMBINANT HUMAN ALBUMIN REPLACE HUMAN SERUM ALBUMIN IN DIAGNOSTIC RADIOPHARMACEUTICALS?

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Keywords: recombinant human albumin, diagnostic radiopharmaceuticals

Human serum albumin (HSA) is the raw material for three pivotal diagnostic radiopharmaceuticals: radioiodinated serum albumin (RISA), ^{99m}Tc-macroaggregated albumin (MAA) and ^{99m}Tc-albumin nanoparticles. However, concerns for the safety of human blood derived products due to potential contamination by infective agents (e.g., nvCJD) make alternative production methods necessary. Recombinant DNA technology is a promising method of albumin production avoiding problems associated with human-derived HSA. We have investigated the use of recombinant human albumin (rHA, Recombumin®, Delta Biotechnology Ltd., Nottingham, UK) as a direct replacement for HSA in diagnostic radiopharmaceuticals. This paper presents results from two studies comparing the biochemical and radiochemical properties of RISA and MAA prepared from rHA with both similar in-house products made from HSA and commercially-available RISA and MAA.

Soluble rHA and HSA structure was evaluated before and after iodination with stable NaI by gel electrophoresis, gel permeation chromatography, peptide mapping, electrospray mass spectrometry and free thiol assay. Radiochemical stability and biodistribution of ^{125}I or ^{131}I radiolabelled rHA was compared both with the similarly prepared radioiodinated HSA and a commercial RISA. ^{99m}Tc-MAA was prepared using previously published production methods by heating a mixture of albumin and stannous chloride in acetate buffer (pH 5.4) at 70 C for 20 minutes. A comparison of MAA produced from rHA (rMAA), with in-house produced HSA MAA (hMAA) and commercially-available MAA (cMAA) was undertaken. Parameters investigated include aggregate size, radiolabelling efficiency, radiochemical and aggregate stability at 4 C and *in vitro* (in whole blood) at 37 C and biodistribution and clearance rate of the aggregates in an animal model.

The results of the RISA study confirmed that there was no significant difference in the primary, secondary or tertiary molecular structure of the soluble rHA and HSA either prior to or after iodination. However, iodination did result in structural changes in both the HSA and rHA including loss of free thiol groups and protein degradation. Radiochemical stability and biodistribution of radioiodinated-rHA was compared both with a similarly prepared radioiodinated-HSA and a commercial product. The results showed that the rHA product had similar radiochemical characteristics to HSA products. The findings of the MAA study demonstrated that rMAA could be produced with similar morphology, labelling efficiency and stability to both hMAA and cMAA.

This study confirms the feasibility of using rHA as the base material for routine formulation and manufacture of both RISA and MAA. Our findings will be discussed with reference to the potential use of rHA as a direct replacement for HSA in commercially-available diagnostic radiopharmaceuticals.

SYNTHESIS OF 4-[^{18}F]FLUOROBENZOATE-(B^{1})-INSULIN: A POTENTIAL RADIOTRACER FOR IN VIVO PET STUDY OF INSULIN PHARMACOLOGY

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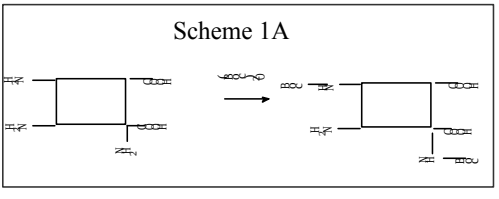
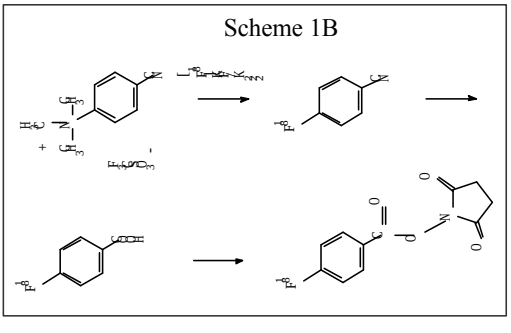
Insulin role as a vital hormone yields massive research on this compound. A possible role of insulin in essential brain functions, such as general learning processes, was also suggested and, to better investigate this issue, suitability of [^{124}I]iodoinsulin and positron emission tomography (PET) has been studied. However difficulties in the radiochemistry, poor imaging and high radiation exposure deriving from the use of ^{124}I led us to the decision to use fluorine-18.

We intended to label the hormone using N-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) . In order to achieve a regioselective labelling on B1 amino residue, which seems to be less essential for insulin biological activity, A1 and B29 amino residues were previously protected using $(BOC)_2O$ (Scheme 1, A).



Fluorine-18 was produced by proton bombardment of >95% ^{18}O-enriched water and the [^{18}F]KF/K_{222} complex was formed in the usual way using acetonotrile azeotropic distillation in a 5-ml V-shaped vial. The solvent was removed and 3.2 mg of 4-trimethylammoniumbenzonitrile trifluoromethansulfonate in 500 µl of anhydrous DMSO were added to the vial and heated (10 minutes, 90°C) to produce 4-[^{18}F]fluorobenzonitrile ([^{18}F]FBN). Crude [^{18}F]FBN was hydrolized with 5M NaOH (1 ml, 120°C) to 4-[^{18}F]fluorobenzoic acid. The product was purified by Sep-pack solid phase extraction (1x2 ml HCL dil, 1x3 ml H_2O) and eluted with 2.5 ml of acetonitrile into a new 5 ml V-shaped vial. The volume was reduced to approx. 500 µl and the vial was added with 300 µl of a 0.1 M N,N'-disuccinimidyl carbonate in acetonitrile and 300 µl of pyridine, closed and heated at 150 °C for 15 minutes (Scheme 1, B). The reaction mixture was then evaporated under nitrogen to small volume and removed from the heating bath and brought to dryness under nitrogen stream.



t-Boc-insulin in borate buffer was added to the crude [^{18}F]SFB and gently warmed (15 minutes, 50°C). The labeled hormone was separated from low molecular weight impurities by G-25 Sephadex SE-chromatography.

Product purity was assessed by HPLC using a Phenomenex C-18 reversed phase 250x3.9 mm (5µm, 250 Å) column and a two-eluent (solvent A= H_2O:ACN:TFA/95:5:0.1; solvent B= H_2O:ACN:TFA/50:50:0.1) linear gradient of 100%A to 100%B in 20 minutes. Cold products (non radioactive SFB and SFB-insulin) were used as reference standards.

Overall procedure was performed in approximatly one fluorine-18 half-life. End of synthesis radioactivity ranged 0.5 to 1 mCi (overall average decay-corrected yield = 9%, n=4). In vitro biological activity measurement and in vivo assessment of biodistribution in rats using a small animal PET scanner are in progress.

EVALUATION OF ^{99m}Tc-LAVELED HYNIC-NMA FOR SENTINEL LYMPH NODE DETECTION

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Keywords: sentinel lymph node (SLN), mannosyl-neoglycoalbumin (NMA), ^{99m}Tc, 6-hydrazinopyridine-3-carboxylate (HYNIC)

Both ^{99m}Tc colloids and ^{99m}Tc-labeled human serum albumin (HSA) are being used as radiopharmaceuticals for sentinel lymph node (SLN) detection. Although ^{99m}Tc-HSA eliminates faster from the injection sites than do ^{99m}Tc-colloids, ^{99m}Tc-HSA travels through the lymph node chain, which makes SLN identification difficult. This suggested that an addition of lymph node binding ability to HSA would provide ^{99m}Tc-radiopharmaceuticals useful for SLN detection. Prior studies demonstrated that mannosyl-neoglycoalbumin (NMA) showed receptor-mediated uptake in macrophages of the liver and spleen after intravenous injection (1). Since macrophages are present in lymph nodes, ^{99m}Tc-labeled NMA was synthesized and evaluated as a radiopharmaceutical for SLN detection.

^{99m}Tc-labeled NMA was synthesized by the reaction of ^{99m}Tc-tricine with HYNIC (6-hydrazinopyridine-3-carbocylate)-conjugated NMA (44 mannose and 0.8 HYNIC/HSA). Biodistribution of the radioactivity after subcutaneous injection of [^{99m}Tc](HYNIC-NMA)(tricine)$_2$ to mice foot pad was evaluated using [^{99m}Tc](HYNIC-HSA)(tricine)$_2$ and ^{99m}Tc colloidal rhenium sulphide (NANOCIS, CIS Bio International) as references.

The radioactivity levels in the popliteal (SLN in this model) increased by decreasing the amount of NMA in [^{99m}Tc](HYNIC-NMA)(tricine)$_2$ from 8 μg to less than 0.8 μg, suggesting that popliteal accumulation of the radiopharmaceutical was saturable. On the other hand, high radioactivity levels were observed in the blood, liver, spleen and lumber lymph node (secondary lymph node in this model) after injection of [^{99m}Tc](HYNIC-NMA)(tricine)$_2$ with 8 μg of NMA. The ability of [^{99m}Tc](HYNIC-NMA)(tricine)$_2$ to deliver high and selective radioactivity to the popliteal was clearly demonstrated in mice biodistribution studies, as shown in Figure 1. [^{99m}Tc](HYNIC-NMA)(tricine)$_2$ also demonstrated elimination from the injection site at a rate similar to and faster than that of [^{99m}Tc](HYNIC-HSA)(tricine)$_2$ and ^{99m}Tc colloidal rhenium sulphide, respectively. These findings indicated that [^{99m}Tc](HYNIC-NMA)(tricine)$_2$ would constitute a useful radiopharmaceutical for SLN detection.

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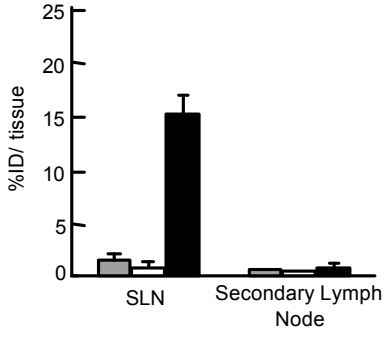

Figure 1. Radioactivity levels in the SLN and secondary lymph node at 1 h (A) post-subcutaneous injection of 0.08 μg [^{99m}Tc](HYNIC-NMA) (tricine)$_2$ (■), 0.08 μg [^{99m}Tc](HYNIC-HSA)(tricine)$_2$ (□) and [^{99m}Tc] colloidal rhenium sulphide (▩).

A FAST EQUILIBRIUM OF THE SELECTIVE SEROTONIN TRANSPORTER PET RADIOLIGAND [^{11}C]DADAM IS CONFIRMED IN HUMAN SUBJECTS

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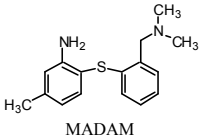
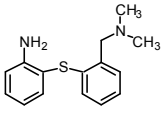
Key words: Human, PET, [^{11}C]DADAM, serotonin transporter, peak equilibrium

The role of the serotonin transporter in the treatment of depression is important. The selective serotonin transporter PET radioligands [^{11}C]MADAM (1) and [^{11}C]DASB (2), which belongs to the series of diphenyl sulfide derivatives suffer from too late specific binding peak equilibrium in brain stem being more than 70 min. The de-iodinated ADAM (DADAM, N,N-dimethyl-2-(2-aminophenylthio)benzylamine) has a Ki (nM) of 4, a logP of 2.70 and a logD 2.25 compared to MADAM of 1.65, 3.12 and 2.64. We have earlier demonstrated a fast peak equilibrium of [^{11}C]DADAM in monkeys (15-20 minutes) (3). The aim in this work was to confirm a fast peak equilibrium of [^{11}C]DADAM also in the human brain. Labeled metabolites were measured in human plasma by HPLC.



MADAM

Ki = 1.65 nM
logP = 3.12
logD = 2.64



DADAM

Ki = 4 nM
logP = 2.70
logD = 2.25

[^{11}C]DADAM was labeled using [^{11}C]methyl triflate in acetone (incorporation >75%). The total synthesis time including reversed-phase HPLC purification was about 30 minutes giving [^{11}C]DADAM with a radiochemical purity >99% and a SA of >37 GBq/ mol. The regional brain distribution [^{11}C]DADAM was examined in five healthy human subjects. A high accumulation of radioactivity was obtained in the brain after i.v. injection of [^{11}C]DADAM (3-5% at 15 min). There was a high uptake of radioactivity in the raphe, hippocampus and thalamus with a ratio to cerebellum of 1.6-3.2 obtained at 60 minutes. Specific [^{11}C]DADAM binding in raphe, hippocampus and thalamus reached equilibrium at 30 min which is much earlier than for [^{11}C]MADAM (70-90 min). The labeled metabolites in plasma were all polar with 20% unchanged radioligand at 40 min.

Conclusions: The main advantage with [^{11}C]DADAM compared to radioligands such as [^{11}C]MADAM and [^{11}C]DASB is the early specific binding peak equilibrium which should allow simplified clinical protocols. [^{11}C]DADAM demonstrate no significant difference to [^{11}C]MADAM with regards to metabolism measured in human plasma.

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SYNTHESIS AND IN VIVO EVALUATION OF [^{11}C]GLYBURIDE FOR IMAGING PANCREATIC b-CELLS

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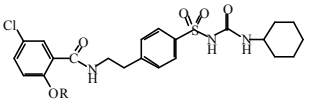
Keywords: Sulphonylurea receptors, [^{11}C]Glyburide, -cell imaging, carbon-11

Sulphonylurea is widely used to treat type II diabetes. It stimulates insulin release by binding to the pancreatic -cell membrane and blocking the ATP-sensitive potassium ion efflux [1]. Sulphonylurea binds to the -cell membrane with high affinity, and these binding sites have been named sulphonylurea receptors (SUR) [2]. We are interested in the development of a positron-labeled ligand for monitoring the SUR in vivo, which will provide a novel approach for probing the functions of -cells.

The lead compound for tracer development is glyburide (compound 1), which has a K_D of 0.3 nM for the SUR [3]. In vitro studies have shown that the uptake of [^{3}H]glyburide is proportional to the number of beta cells and the uptake is saturable [3]. These characteristics strongly suggested that positron-labeled glyburide might be a potential tracer for imaging -cells in vivo. Previously, radiolabeled glyburide analogs, such as [^{123}I]iodoglyburide [4] and [^{18}F]fluoroethoxyglyburide [5], have been evaluated. To our knowledge, there are no reports on the in vivo biodistribution of these tracers. Our aim is to evaluate the potential of [^{11}C]glyburide as a PET tracer for imaging pancreatic -cells.



R = Me, Glyburide, 1
R = H, Desmethylglyburide, 2

Glyburide was synthesized according to literature procedure [6] and its O-demethylated precursor 2 was prepared by O-demethylation of glyburide using BBr_3. All compounds were characterized by ^{1}HNMR and confirmed by high-resolution mass spectrum. [C-11]Glyburide was prepared by reacting precursor 2 (2 mg) with [C-11]methyltriflate in DMF (0.4 mL, plus 10 L of 5 N aq. NaOH) at 80 °C for 5 min. The crude product was purified by semi-preparative HPLC (Prodigy ODS-Prep, 10 , 10 x 250 mm; acetonitrile/0.1 M aq. HCO_2NH_4-CH_3CO_2H (0.5%) = 45/55, 10 mL/min, retention time 14 min). The synthesis time was 30 min and the radiochemical yield was 3% at end-of-synthesis (EOS) with a specific activity of 1.5 mCi/nmol at EOS.

Biodistribution study was performed using male Sprague Dawley rats (3 time points: 5, 15, and 45 min post tracer injection, and a blocking study with glyburide (1 mg/Kg) at 45 min, n = 3 per group). Each animal received 0.1 mCi of tracer via tail vein injection. At the specified time point, animals were sacrificed by decapitation and organs were removed, weighed, and counted. The uptake was expressed as percent injected dose per gram of tissue (%ID/g). At 45 min post-injection, the tissue uptakes for liver, kidney, lung, heart and pancreas were 14%, 0.14%, 0.1%, 0.08% and 0.03%, respectively. Co-injection of unlabeled glyburide had no effect on the tissue uptake, indicating the uptakes were mainly non-specific. In contrast to the promising in vitro data, our initial result suggested that [C-11]Glyburide is not a good tracer for imaging -cells.

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EFFECT OF STEROIDS ON THE *IN VITRO* BINDING OF THE SIGMA-1 RECEPTOR PET RADIOLIGAND [^{18}F]FPS IN THE RODENT HEART

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Introduction: Sigma-1 (? -1) receptors are expressed throughout the mammalian CNS and in organs of the immune and endocrine systems, including the heart. Numerous reports have shown that sigma receptor ligands modulate contractility, calcium influx, and beating rate in cultured cardiac myocytes[2]. However the effect of endogenous compounds, such as hormones, on the sigma receptor have not been evaluated in heart tissues. We have developed the high affinity (KD = 0.5 ± 0.2 nM) selective ? -1 PET radiotracer [^{18}F]FPS [1]. This study examines the effect of various steroids on [^{18}F]FPS binding *in vitro* in rodent heart membranes and autoradiographic slices.

Methods: *In vitro* inhibition constants (Ki) of selected steroids at [^{18}F]FPS labeled sigma receptors were determined (rat heart membranes, 3 mg/tube; 50 mM TRIS buffer, 1 µM $MgCl_2$, pH = 7.5, incubation time = 2 hr). The effect of co-administration of progesterone (1 µM), testosterone (1 µM), DHEA (1 µM), Estradiol (1 µM) and Estriol (1 µM) on *in vitro* [^{18}F]FPS binding was examined *via* membrane binding studies. Twenty micron frozen sections were prepared from normal rat hearts and the effect of selected hormones at 100 nM and 10µM was determined by co-incubation of heart sections with [F-18]FPS and hormones.

Results: Inhibition constants (Ki) determined for haloperidol and selected neuroactive steroids are shown in Table I. As expected, haloperidol, progesterone, testosterone and DHEA reduced [^{18}F]FPS binding, whereas estradiol and estriol did not. The affinity order of these compounds is the same as previously reported for ? -1 receptors. *In vivo* administration of progesterone, testosterone or DHEA significantly decreased [^{18}F]FPS uptake in peripheral organs that express ? receptors (heart, spleen, muscle, lung) was reduced (54-85%), while blood activity values were not significantly different. In membrane studies, progesterone, testosterone and estradiol reduced binding of [F-18]FPS to receptors in rat heart membranes. Testosterone and progesterone at 1 ? M were the most potent and reduced the specific binding of [F-18]FPS, as defined by unlabeled FPS at 1.0 ? M, by about 85 and 70%, respectively. The blocking of autoradiographic slices with selected hormones, followed the known binding affinity of hormones at the sigma receptor site.

Table 1

Test Ligand	Ki (nM)	n
FPS	0.5 ± 0.2	4
Haloperidol	14 ± 3	3
Estriol	> 1000	1
Estradiol	> 1000	1
Progesterone	36 ± 6	4
Testosterone	201 ± 11	3
DHEA	706 ± 89	3

Conclusion: [^{18}F]FPS binding in the rat heart is reduced *in vitro* by steroids with previously established ? -1 receptor affinity. Steroid concentration variation over the normal menstrual cycle and during menopause can have significant impact on heart function via the sigma receptor site.

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DETECTION OF DVT IN ANIMAL EXPERIMENTAL ANIMAL MODEL USING RADIOACTIVE LABEL TIROFIBAN – GPIIb/IIIa INHIBITOR

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Keywords: DVT, tirofiban, GPIIb/IIIa receptor inhibitor, experimental animal model

Detection of acute deep venous thrombosis (DVT) based on the bimolecular behaviour of components of the clotting process including the platelets and their specific expressed receptors have suggested like a new approach in nuclear medicine. The new venue has focused on the use of small peptide or peptidomimetic ligands with high specificity and incorporation of a convient nuclide for imaging purpose.

Tirofiban (N-(Butylsulfonyl)-4-O-(4-(4-piperidyl)-L-tyrosine is a non-peptide tyrosine derivate, highly selective, short acting inhibitor of fibrinogen binding to platelet glycoprotein IIb/IIIa.

The aim of our study was to introduce Tirofiban 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.

Iodine 125[I] – Tirofiban labeling was performed using Iodo-gen method and the quality control of the labelled product was checked, using TLC technique in 1mol/L HCl like solvent. The percentage of labelling was more than 95% without purification. Technetium 99m[Tc]-Tirofiban labeling 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. The percentage of the obtained complex was more than 95% and 99mTcO_4^- less than 5%. The labeled product was stable without changing the percent of labeling 2 hours at room temperature. The labelled Tirofiban has a fast blood clearance in the normal rat model (without induced thrombosis). More than 80% of injected dose (for both of used products) was eliminated from the circulation in the first hour after injection.

The biodistribution studies and visualisation of the labelled molecule was carried out using experimental model of thrombosis in male Wistar rat. Planar images were obtained 30 min, 2 and 24 hours after application of 2-6 x10^6cpm in 50-100 m 99m[Tc]-Tirofiban or 1.6-2.1 x10^6cpm in 50 m of iodine 125[I]-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 scarified animals was quantified thrombus/normal ratio and the obtain results were 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.

Our results 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.

OLFACTORY TRANSPORT OF INTRANASAL RUBIDIUM AND THALLIUM

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Keywords: ^{201}Tl-chloride, intranasal administration, olfactory transport, brain uptake, rubidium

Previously, we have examined a variety of trace-element delivery manner to the brain using a multitracer solution containing ^{7}Be, ^{46}Sc, ^{48}V, ^{51}Cr, ^{54}Mn, ^{59}Fe, ^{56}Co, ^{65}Zn, ^{74}As, ^{75}Se, ^{83}Rb, ^{85}Sr, ^{88}Y, ^{88}Zr, ^{95m}Tc and ^{103}Ru. The ^{83}Rb brain uptake by intranasal instillation was found approximately twice higher than those of other administration methods [1]. The result suggested that a large part of the intranasal Rb was delivered directly to the brain without passing the blood-brain barrier (BBB), and supposed that the Rb delivery was mainly due to olfactory transport [2]. The aim of this study is to demonstrate the existence of direct Rb delivery route to brain via the olfactory transport and to examine whether intranasal Tl behaves in the same manner as Rb. Rubidium-86 chloride and ^{201}Tl chloride physiological solutions were used as a tracer to examine the biobehavior of Rb and Tl, respectively.

Three, 6, 12, and 24 hours after ^{86}Rb and ^{201}Tl administration to two groups of mice such as intranasal (IN) and intravenous (IV) group, each mouse was sacrificed and the liver and head were dissected. In the IN-group, the ^{86}Rb and ^{201}Tl solution was administered only into the right nostril of mice to determine how Rb and Tl reached the brain, respectively. The heads were sagittally separated into the right and left sides, and then the following seven sectional-samples were collected from each side: non-olfactory epithelium including lateral turbinals (section A); olfactory epithelium including medial turbinals (section B); olfactory bulb (section C); brain section including olfactory tract (section D); brain section including striatum (section E); brain section including hippocampus (section F); brain section including cerebellum and medulla oblongata (section G). All samples were weighed immediately and subjected to -ray spectrometry. In addition, the autoradiographic images were examined to understand the brain regional distribution following IN administration.

As a result, throughout 24 hours after administration of ^{86}Rb and ^{201}Tl, although there were no significant differences between the blood uptake rates of the IN- and IV-groups, the brain uptakes of the IN-group were significantly higher than those of the IV-group, which only depend on the circulation. In the IN-group, high uptakes were observed in the section B and C of the right side from 3 to 12 hours. The uptakes in section A, B, and C of the right side were significantly higher than those of the left side at 3, 6, and 12 hours. The uptakes in section D and E of the right side were also higher than those of the left side. The similar tendency was found in the autoradiographic images, *i.e.* high ^{86}Rb and ^{201}Tl levels accumulated in the right side of head sections. By contrast, there were no significant differences between the uptakes of ^{86}Rb and ^{201}Tl in the right and left side head sections of the IV-group.

Intranasal ^{86}Rb and ^{201}Tl highly accumulated in the olfactory bulb along the axons of the olfactory neurons of the administered side and gradually passed along secondary and tertiary olfactory neurons into the telencephalon and the diencephalon. The unique behavior of intranasal ^{86}Rb and ^{201}Tl may be explained on their K-mimic properties *in vivo*. Anyway, it suggested the possibility to apply ^{201}TlCl to a variety of the rhinological studies as well as molecular neurobiological studies of olfactory nerve system.

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INFLUENCE OF THE CHELATOR ON THE *IN VITRO* AND *IN VIVO* BEHAVIOUR OF ^{99m}Tc(I) COMPLEXES WITH POLY(MERCAPTOIMIDAZOLYL)BORATES

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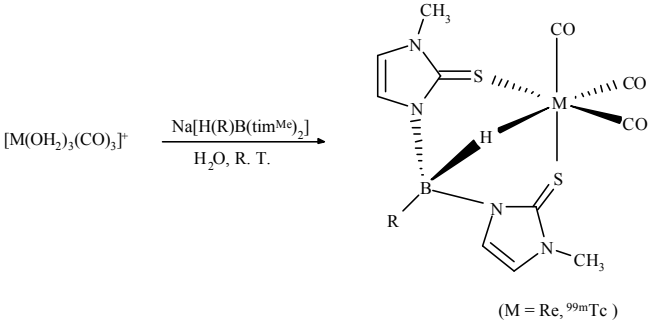
Keywords: Technetium, Rhenium, Tricarbonyl, Biological Studies, CNS

To extend the range of potential radiopharmaceuticals with the *fac*-$[M(CO)_3]^+$ moiety, we have studied a new class of sulfur donor chelators of the type $[H(R)B(tim^{Me})_2]^-$ and $[RB(tim^{Me})_3]^-$(tim^{Me}= 1-methyl-2-mercaptoimidazole, R = H, Me, Ph), which can be used as BFC agents for the labelling of selected biomolecules [1-3].

In this work we report on the synthesis, characterization and biological evaluation of rhenium and technetium complexes of the type: [M{ ^{3}-R(-H)B(tim^{Me})$_2$}(CO)$_3$] and [M{ ^{3}-RB(tim^{Me})$_3$}(CO)$_3$] (M = ^{99m}Tc, Re; R = H, Me, Ph) (Scheme 1).



Scheme 1

The novel compounds, which were characterized at the macroscopic and *non carrier* added level, are neutral and lipophilic and, in the case of ^{99m}Tc, were prepared in quantitative yield, at low ligand concentrations (10^{-6}-10^{-4} M). These features make these compounds particularly useful for the labelling of central nervous system (CNS) receptor ligands, and preliminary results on the derivatization of poly(mercaptoimidazolyl)borates with CNS receptor avid molecules will be also reported.

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LABELING OF VITAMIN C (ASCORBIC ACID) WITH ^{99m}Tc AND INVESTIGATING ITS BIODISTRIBUTION ON ALBINO WISTAR RATS

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Keywords: ascorbic acid, ^{99m}Tc, Biodistribution , ^{99m}Tc-ascorbic acid, albino Wistar rat

The aim of this study; is to label ascorbic acid with ^{99m}Tc and to investigate its biodistribution on rats.

In the study; ascorbic acid was labeled with ^{99m}Tc by using the stannuos chloride method. Quality control studies were carried out through RTLC, paper electrophoresis and RHPLC methods.

Labeling yield was found to be 93 5.0. ^{99m}Tc-ascorbic acid was stable through three hours at room temperature. The biodistribution studies related to ^{99m}Tc-ascorbic acid were performed on albino Wistar male rats. It was intravenously administered via the tail. Activities were counted by a Cd(Te) RAD 501single channel analyzer. The %ID/g (% injected dose-per gram of tissue weight) in organs and blood were determined at 15, 30, 60, 120, 180 minutes postinjection. Maximum uptake of ^{99m}Tc-ascorbic acid occured in liver, lung, heart, spleen, pancreas and prostate at 60. minutes after postenjection. The data shows a good uptake in prostate and kidneys of ^{99m}Tc-ascorbic acid.

SYNTHESIS OF NOVEL MULTI-LIGANDS TETRAAZAMACROCYCLES FOR RADIOLABELLING PROTEINS WITH ^{64}Cu FOR THERAPY.

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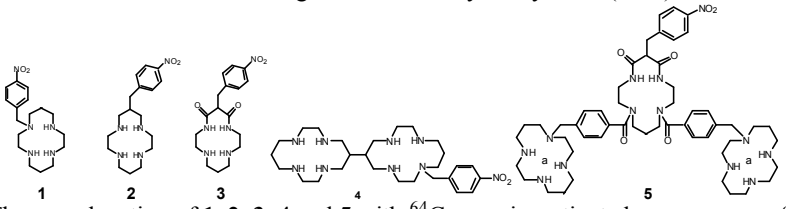
Keywords: ^{64}Cu, Tetraazamacrocycles, Bifunctional compound, Antibodies

To date, high specific activity of the radioimmunoconjugate is commonly obtained by increasing the available metal binding sites (chelating agents) on monoclonal antibodies (mAbs). However this approach is often not satisfactory as the immunoreactivity of the mAb is compromised.[1,2] The present study explores the potential of a number multi-ring tetraazamacrocyclic systems for radiolabelling mAbs to achieve high specific activities.

A series of mono and multi-ring tetraazamacrocyclic systems (1 - 5) have been synthesized.



The complexation of **1**, **2**, **3**, **4** and **5** with ^{64}Cu was investigated over a range of pH (4.0 – 8.0) and temperatures (4- 37 ºC) at micromolar concentration. Once the complexes were formed each was tested for stability in human sera at 37 ºC. Only 1, 2, 4 and 5 formed sufficiently stable ^{64}Cu complexes under appropriate conditions for use in radiolabelling mAbs.

The nitro group of **2** and **5** was reduced and then conjugated to B72.3 mAb using 1-etyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The effect of conditions such as pH, ligand, protein and EDC concentrations were investigated. The resultant radioimmunoconjugates displaying adequate immunoreactivity (>80%) were evaluated in nu/nu tumour bearing mice (see Table 1). The ^{64}Cu-**5a**-B72.3 contained almost two-fold higher (5.5 vs 3.5) ^{64}Cu molecules per mAb compared to ^{64}Cu-**2a**-B72.3.

Table. 1. Biodistribution of ^{64}Cu-2a-B72.3 and ^{64}Cu-5a-B72.3 in (nu/nu) mice bearing LS-174T tumours

Ligand	Tissue	Time post injection (hours)			
	(%ID/g)	1	16	24	48
2a (NH_2 form of **2**)	Tumour	4.7 0.6	12.5 3.9	23.7 4.3	35.3 11.2
	Blood	36.7 2.1	27.7 1.2	16.8 1.3	14.8 3.3
	Liver	11.3 0.5	13.04 0.9	12.1 0.7	10.9 2.7
5a (NH_2 form of **5**)	Tumour	4.1 0.2	10.1 0.8	20.6 2.1	29.6 1.1
	Blood	29.5 3.2	18.7 1.2	11.7 0.9	9.65 1.5
	Liver	28.7 1.9	32.4 2.4	28.7 7.5	30.5 0.6

The biodistribution data show higher uptake of ^{64}Cu-5a-B72.3 compared to ^{64}Cu-2a-B72.3 in the liver. However the total accumulated radioactivity in tumour of these radioimmunoconjugates was found to be higher compared to analogues systems in the literatures.[3,4,5]

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SYNTHESIS AND BIODISTRIBUTION STUDIES OF ^{99m}TC-EDTMP-Bz-FOLATE A NOVEL RECEPTOR SPECIFIC RADIOPHARMACEUTICAL

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Key words: Folate; BCA; Technetium-99m-tetraphosphonate; Skeletal agent

The objective of present study was to synthesize a novel bifunctional aminobenzyl ethylene diamine tetramethylene phosphonic acid (Ab-EDTMP) and to apply it to stable ^{99m}Tc-labeling of folate through its -carboxyl moiety and its biological evaluation. **Methods:** Aminobenzyl ethylene diamine tetramethylene phosphonic acid (Am-Bz-EDTMP) was prepared in 7 steps starting from an unsubstituted optically active amino acid L-phenylalanine. Conjugation of Am-Bz-EDTMP was carried out through the active ester of -carboxyl group of folic acid at pH 8.4 using triethylamine at ambient temperature. The Folate conjugated tetramethyl phosphonate (folate-Bz-EDTMP) was purified by reversed phase HPLC and characterized by mass spectroscopy. A stable complex with ^{99m}Tc was prepared using this multidentate phosphonate ligand. Lesion/normal bone ratios were determined from digitized images obtained from gamma camera in rabbits. **Results:** The Ab-EDTMP was prepared by a 7 steps synthesis starting from L-phenylalanine. The amide linked Bz-EDTMP-folate(g) conjugate was obtained by the reaction of active ester of carboxylate of folate with amino group of benzyl-EDTMP to serve as linker between the vitamin and the metal chelator, EDTMP. High resolution FAB mass spectroscopy analysis showed the folate-Bz-EDTMP $[M+H]^+$ parent peak ion peak at m/e = 965 (calculated 964) When folate conjugate was labeled with ^{99m}Tc the preparation has been found to be sufficiently stable in vitro as well as in vivo. Blood clearance of the tracer in rabbits was found to be more rapid (T1/2(F) = 27 1.4 minutes and T1/2(S) 3hr 48 8.4 minutes. Biodistribution in unanesthetized mice showed varying degrees of bone and soft - tissue uptake of this complex. It showed high bone uptake (4.93 0.22 % ID/g and bone/muscles 11.7 at 1 hour), low nonosseous uptake. Rabbit studies confirmed the ^{99m}Tc-Folate-Bz-EDTMPA results obtained in mice. Scintigraphic images were comparable to that obtained with ^{99m}Tc-MDP image. **Conclusion:** Above results suggests that ^{99m}Tc-Folate-Bz-EDTMP is a bone seeker agent in the normal subject. Based on these excellent biodistribution characteristics, [^{99m} Tc]-Folate-Bz-EDTMP supports the concept of its utility as radiopharmaceutical for imaging folate receptor positive tumor and palliative therapy of bone cancer using -emitting radionuclides coupled with folate derivative (anticancer drug).

AN IN VIVO/IN VITRO EVALUATION OF THE GINKGO BILOBA EXTRACT ON THE BIODISTRIBUTION OF THE SODIUM 99mTc-PERTECHNETATE AND ON THE MORPHOLOGY OF ORGANS ISOLATED FROM THE RATS

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Keywords: Ginkgo Biloba extract, 99mTc-pertechnetate, biodistribution

In nuclear medicine, radioactive tracers, called radiopharmaceuticals, are employed in the study of blood flow, metabolism, morphology of an organ and to evaluate the drug formulation and drug delivery systems. Many substances have been reported to affect the biodistribution of different radiopharmaceuticals. Ginkgo Biloba extract (EGb) is a phytoterapic used in the treatment of hypoxic conditions. This medicinal plant has several biological effects, specially, vasodilator, anti-inflammatory and anti-coagulant properties. It has antioxidant characteristics. We evaluated the influence of an EGb on the biodistribution of the 99mTc-pertechnetate (99mTcO4Na) and on the morphology of the organs from WISTAR rats. The animals were treated (6 days, intragastric via) with EGb (40 and 400 mg/mL). After that, 99mTcO4Na was injected and the animals were sacrificed (after 10 minutes). The organs were isolated and counted in a well counter. The percentages of radioactivity per organ (%ATI/organ) and radioactivity per gram (%ATI/gram) of each organ were calculated. Histological preparations were carried out with the pieces of organs withdrawn from the treated animals (400mg/mL EGb). The results showed that EGb altered (not significant, n=5, $p>0.05$) the biodistribution of the 99mTcO4Na in the kidneys and liver. A significant (n=5, $P<0.05$) decrease in the uptake of this radiopharmaceutical in the duoden after the treatment with EGb (40 and 400 mg/mL) was observed: %ATI/organ, 0.590,18 (control) to 0.380.06 (40mg/ml) and to 0.260.19 (400mg/ml) and %ATI/gram, 1.010.37 (control) to 0.550.09 (40mg/ml) and to 0.510.36 (400mg/ml). Morphological alterations on kidney and liver due to treatment (in vivo) were found.

We suggest that the action of this extract could generate metabolites capable to promote modifications in the organs and to alter the biodistribution of the 99mTcO4Na in the treated animals.

BIOBURDEN STUDIES: RECENT OBSERVATIONS ON A SIMPLE, RELIABLE AND GENERAL METHOD APPLIED TO P.E.T. RADIOPHARMACEUTICAL PRODUCTION

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Keywords: Bioburden studies, Microbial contamination, [^{18}F]FDG, [^{15}O]water

Over the years certain personnel from the FDA microbiology evaluation section have suggested that if appropriate in-process bioburden studies were conducted during radiopharmaceutical production, in conjunction with filter integrity testing, that routine sterility testing might be mitigated, or at least reduced in frequency. Our rationale for undertaking these studies was to determine the feasibility of conducting these in a simple manner during routine QC of clinical PET production. Furthermore, significant amounts of clinically useful product are being consumed to perform required sterility testing, and undue radiation exposure is associated with collecting the samples prior to release of product. Here we present our latest findings, which lead us to believe that these types of study can be easily incorporated into routine QC practices. More importantly, the results from these studies can provide valuable insights as to the sanitary cleanliness of synthesis modules, and offer a means of monitoring sources of contamination and confirmation of the effectiveness of remedial actions.

In our preliminary studies (*1*) we utilized a manually assembled Swinnex® filter which often presented problems with filter lock when incorporated in-line: this method has proved unacceptable. We have been able to conveniently overcome the latter problem by substituting a vented sterilizing filter unit, Millipore®, proximal to the final sterilizing filter (*2*). This simple ploy also avoided the time-consuming and manually intensive procedure involved in our earlier study. The distal filter is then used for subsequent bioburden analysis and the proximal filter for filter integrity. Moreover, this tandem arrangement of vented filters provides for double assurance of microbial trapping over a single filter. This procedure has been successfully applied to our [^{15}O]water QC sub-batch analysis.

During [^{18}F]FDG production we routinely dilute our sample with sterile water to reduce radiolytic decomposition in concentrated (>20mCi/mL) samples. The radiation exposure is also reduced to technologists who often experience difficulty in drawing small volume clinical doses. We achieve this by flushing the intermediate collection vessel in our synthesis unit. Furthermore, this procedure flushes precious [^{18}F]FDG out of hold-up volume of the sterile vented filter units into the final dose vial and simultaneously reduces the radioactivity in the filters to a level acceptable for timely determination of collected bioburden and filter integrity. However, we have found that some of our bioburden samples are contaminated with particulate materials. Pathological analysis of this material indicates that it is not of microbial origin. The material appeared to be alumina fines that were not flushed out during the purification column pre-wash. Our most recent findings indicate that the problem may be more subtle than at first suspected. We first attempted to overcome this problem of alumina fines by incorporating a pre-filter at the base of the purification unit. This 5µ unit, Pall®, was the largest porosity that we could find available in a sterile packaged form that would entrap particulates, yet permit the passage of microbes to the bioburden filter trap. This pre-filter provided for a much clarified product prior to final filtration and prevented potential clogging of narrow bore lines used in the transfer process. However, we have noted that on several occasions when back-flushing the bioburden filter that the eluate is still often cloudy. It appears at this time that not only alumina fines are being released by the purification unit but that some alumina is being leached in the FDG elution process which on final dilution comes out of solution. To overcome this latest problem, before aliquoting into the growth media, we filter the sample with yet another 5µ filter unit. Our preliminary findings using this latest methodology are proving to be encouraging.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF 9-[^{18}F]FLUORO-3,4-CYCLOPROPYLHEPTADECANOIC ACID FOR DETECTING HEART DISEASE BY PET

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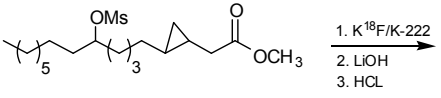
Keywords: heart, fatty acid, PET, imaging

Long-chain fatty acids are the major substrate for energy metabolism in the normally perfused, well oxygenated, myocardium. Nonesterified fatty acids are taken up by the normoxic myocardium with an extraction of 40-60% and either esterified to a triglyceride or metabolized by beta-oxidation. In contrast, under conditions of reduced oxygen delivery to heart tissue, such as ischemia and hypoxia, there is a dramatic decrease in fatty acid metabolism. Thus, an alteration of fatty acid uptake is considered to be a sensitive marker of ischemia and myocardial damage.

Radiolabeled fatty acids that initially display myocardial blood flow and eventually show adequate myocardial retention associated with metabolic activity are attractive candidates for clinical evaluation of regional discrepancies in heart blood flow and fatty acid metabolism which occur in ischemic heart disease and cardiomyopathies (1, 2).

We have prepared 9-[^{18}F]fluoro-3,4-cyclopropylheptadecanoic acid ([^{18}F]FCPHA) as part of a new series of modified fatty acids. This fatty acid analog was designed to enter the myocardium by the same long chain fatty acid carrier protein mechanism as natural fatty acids, however, the cyclopropyl moiety in the , -position blocks beta-oxidation and thus the tracer is trapped in the myocardium. [^{18}F]FCPHA was synthesized by nucleophilic substitution of the corresponding mesylate fatty acid ester with dry K^{18}F/Kryptofix-222 in acetonitrile at 120 C for 10 min, which gave 90% F-18 incorporation. The ^{18}F-labeled ester was concentrated (nitrogen stream), dissolved in hexane/ethyl acetate (85:15), and purified using a silica Sep-Pak. After solvent removal, the ester was dissolved in methanol (1 mL) and treated with 0.5 mL of 1M LiOH at 80 C for 10 min. The reaction mixture was concentrated to <1 mL, acidified with 10% HCL (2 mL) and extracted with ether. Solvent evaporation provided [^{18}F]FCPHA in 40% radiochemical yield. The radiotracer was formulated in saline/ethanol (90:10) for animal studies.



Scheme 1. Synthesis of [^{18}F]FCPHA

Biodistribution of [^{18}F]FCPHA was performed in Sprague Dawley rats (male, 300-350 grams). The non-anesthetized animals were injected with 40 Ci in 200 l via the tail vein and sacrificed at 5 and 60 min post injection. The organs were excised and counted in a gamma counter. Heart accumulation (%DPG) for [^{18}F]FCPHA at 5 and 60 minutes were 1.55±0.66 and 1.43±0.14, respectively. Lung activity (%DPG) was 0.47±0.12 at 5 min and 0.33±0.16 for 60 min, while liver uptake (%DPG) was 0.87±0.15 at 60 min. The heart-to-blood ratios at 5 and 60 min for [^{18}F]FCPHA, an indicator of image resolution, were 25.8 and 20.4, respectively. Heart-to-lung ratios at 5 and 60 min were 3.3 and 4.6, respectively. Bone accumulation, an indication of defluorination, was 0.16% DPG at 5 min and increased to 0.70% DPG after 60 min. These results indicate the potential of [^{18}F]FCPHA for evaluating myocardial fatty acid metabolism by PET.

References

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SYNTHESIS AND EVALUATION OF BISPHOSPHONATE DERIVATIVE LABELED WITH RHENIUM-186 USING MAG3 AS A CHELATING GROUP

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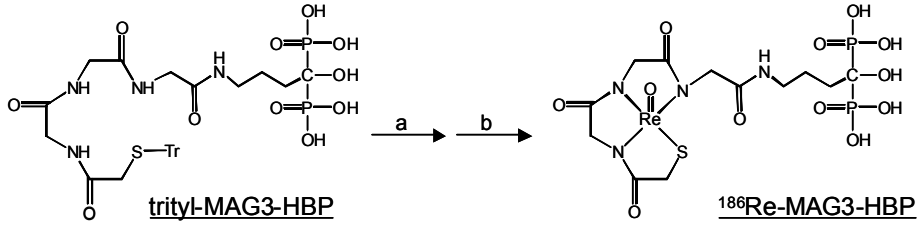
The development of radiopharmaceuticals for relieving the pain of osteoblastic bone metastases has attracted great interest in the nuclear medical field. To date, some compounds including ^{186}Re-HEDP have been synthesized. However, ^{186}Re-HEDP showed slow blood clearance and high gastric uptake of radioactivity since it is a polynuclear complex and unstable *in vivo*, leading to generation of $^{186}ReO_4^-$. In this study, based on the idea of bifunctional radiopharmaceuticals, we designed a ^{186}Re-labeled bisphosphonate complex with high *in vivo* stability. MAG3 ligand was chosen as ^{186}Re-chelating group based on the stability and hydrophilicity of ^{186}Re-MAG3 complex, and this ligand was introduced to the bisphosphonate structure containing a hydroxyl group at its central carbon (MAG3-HBP).

A precursor, trityl-MAG3-HBP was synthesized by coupling trityl-MAG3 and 4-amino-1-hydroxybutylidene-1,1-bisphosphonate. After deprotection of the trityl group of this precursor, ^{186}Re-labeling was performed by reaction with $^{186}ReO_4^-$ and $SnCl_2$ in citrate buffer (Scheme 1). After purification by HPLC, ^{186}Re-MAG3-HBP was obtained with high radiochemical purity (over 95%). The stability of ^{186}Re-MAG3-HBP was comparatively studied with that of ^{186}Re-HEDP by incubating in oxygen-saturated phosphate buffer. There was no measurable decomposition of ^{186}Re-MAG3-HBP over a 24-hour incubation period, while ^{186}Re-HEDP remained only approximately 25% intact 24 hour post-incubation. In a biodistribution study with normal mice, ^{186}Re-MAG3-HBP exhibited more rapid and higher bone uptake and faster blood clearance compared with ^{186}Re-HEDP. In addition, stomach accumulation of radioactivity after administration of ^{186}Re-MAG3-HBP was lower than that of ^{186}Re-HEDP. These results indicated that the newly developed ^{186}Re-MAG3-HBP has a high affinity for bone and high *in vivo* stability.

In conclusion, we developed a highly stable ^{186}Re-labeled bisphosphonate, ^{186}Re-MAG3-HBP. This agent showed a high bone accumulation and rapid blood clearance, leading to high bone-to-blood ratios of the radioactivity. Gathered data indicated that ^{186}Re-MAG3-HBP holds great potential for palliation of metastatic bone.



Scheme 1 Radiosynthesis of ^{186}Re-MAG3-HBP.
Reagents: a, trifluoroacetic acid, triethylsilane; b, $^{186}ReO_4^-$, $SnCl_2$.

COMPARATIVE BIODISTRIBUTION OF [111IN] DTPA-HEGF AND [111IN] BZ-DTPA-HEGF IN NORMAL MICE

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Keywords: [^{111}In] DTPA-hEGF, [^{111}In] Bz-DTPA-hEGF, biodistribution, normal mice

EGF-receptors (EGFR) are overexpressed in gliomas, as well as in tumours of breast, lung and urinary bladder. For this reason, EGFR may be an attractive target for both visualisation and therapy of malignant tumours with the use of radioactive nuclides. Natural ligand of EGFR, epidermal growth factor (EGF) is a small 53-amino-acid protein with a molecular weight of about 6 kDa. Low molecular weight of EGF enables better intratumoral penetration in comparison with larger targeting proteins, such as antibodies. [^{111}In] DTPA-EGF was proposed for targeting of glioblastoma and breast cancer, and its tumour-targeting properties were confirmed in animal studies. However, indium label on dianhydride DTPA derived conjugate might be challenged *in vivo* by transferrin, with subsequent transfer to bone marrow. It might be of value to use backbone-modified benzyl-DTPA as bi-functional chelating agent. Experience with labelled somatostatin analogues indicated that the use of different chelating agents could change pharmacokinetics of targeting peptide. The goal of this study was to evaluate how substitution of heptadentate DTPA for octadentate Bz-DTPA affects biodistribution of indium labelled recombinant human EGF (hEGF) in normal mice.

[^{111}In] DTPA-hEGF and [^{111}In] Bz-DTPA-hEGF were prepared in our laboratory as it was described earlier (Liljegren Sundberg Å., Orlova A., Bruskin A., Gedda L., Carlsson J. and Tolmachev V. [^{111}In] Bz-DTPA-hEGF: preparation and *in vitro* characterisation of potential anti-glioblastoma targeting peptide. *Cancer Biotherapy and Radiopharmaceuticals, in press*). Both compounds have similar affinity for the EGFR, about 2 nM. Compounds were injected in tail vein of NMRI mice. At 0.5, 1, 4 and 24 h post injection, animals were sacrificed by heart puncture in groups of three. Such organs and tissues as heart, urinary bladder, pancreas, liver, kidney, spleen, lungs, large and small intestine, stomach, brain, submaxillary gland, thyroid, muscle, skin as well as samples of blood, urine and faeces were collected, and their radioactivity was measure using automatic gamma-spectrometer. One group of animals was pre-injected with 100 g of non-labelled hEGF in order to estimate receptor specificity of the uptake in different organs.

Results of the study demonstrated that uptake of both compounds in liver, spleen, pancreas, intestines, thyroid and submaxillary gland was most likely receptor-mediated since it could be blocked with large amount of non-labelled EGF. Blood clearance of both compounds was fast (less than 0.5 % ID/g at 1 h p.i.), mainly through receptor-mediated uptake in liver and glomerular filtration. Uptake in a majority of organs was similar, and difference in biodistribution was within errors. However, indium uptake in the case of [^{111}In] DTPA-hEGF was significantly higher in kidneys and bones. Elevated uptake in bone might be explained by release of free indium from DTPA. “Negative patches” in proximal tubulae mediate re-uptake of excreted peptides in kidneys. Additional negative charge provided by Bz-DTPA might be reason of reduced reabsorbtion of [^{111}In] Bz-DTPA-hEGF.

In conclusion, [^{111}In] Bz-DTPA-hEGF seems to have more favourable *in vivo* distribution in comparison with [^{111}In] DTPA-hEGF.