Nuclear Science and Techniques **20** (2009) 163–169

Radiosynthesis and biodistribution of [^{18}F]-tetracosactide using a semi-automated [^{18}F]SFB production module

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Abstract In order to prepare a specific melanocortin type 2 receptor (MC2R) ligand, β$^{1\text{-}24}$-corticotrophin was prepared in one-step reaction with [^{18}F] SFB and β-1-24-corticotrophin pharmaceutical solution (1 mg/mL, pH=6.5). [^{18}F]SFB was prepared in a semi-automated module in two steps with an overall radiochemical yield of 47% to EOB (not-decay corrected) in 90 min. The ^{18}F-labeled intermediates and ^{18}F-labeled peptide was checked by RTLC and HPLC. The results show that the radiochemical purity is >95% and the yield to EOB (not-decay corrected) is 29% for final ^{18}F-labeled peptide at optimized conditions. Preliminary *in vivo* studies in normal mice were performed to determine biodistribution of the ^{18}F-labeled peptide for 150 min. The results show that the major tracer uptake is consistent with the natural distribution of MC2R receptors in mammals. Testes/blood and testes/muscle ratios for ^{18}F-labeled peptide at 150 min were 184 and 1.56, respectively, and adipocyte/blood and adipocyte/muscle ratios at 120 min were 221 and 142, respectively. The data support the specific receptor binding of the radiolabeled peptide as reported for MC2R receptor accumulation in adipocytes and testes and demonstrates the retention of biological activity of the peptide. This tracer can be used in detection of MC2R distribution in malignancies and sex organ diseases.

Key words Mc2r, β-1-24-corticotrophin, Fluorine-18, Radiolabeling, Biodistribution

1 Introduction

β-1-24-corticotrophin consists of the first 24 amino acids occurring in the natural adrenocorticotrophic hormone (ACTH). Fig. 1 shows the chemical structure of corticotrophin. Like ACTH, it stimulates adrenocortical production of glucocorticoids and mineralocorticoids, and to lesser extent androgens, which explains its therapeutic effect in conditions responsive to glucocorticoid treatment. The ACTH receptor belongs to a subfamily of the G protein- coupled receptor superfamily, the melanocortin receptor family, which consists of the ACTH receptor (or MCR2), MSH-R (or MCR1) and three other receptors (MCR-3 to MCR-5)[1, 2]. ACTH is crucial for the development of the adrenal cortex and may play an essential part in adaptational processes like adrenal hypertrophy[3]. The ACTH receptor is mainly expressed in the adrenal cortex, but has been identified in human skin[4], in an ovarian steroid cell tumor[5] and in rodent adipocytes[6] β-1-24-corticotrophin, through the Melanocortin-2 receptor (MC2R), is of the most potent trophic stimulus of the adrenal cortex, stimulating both steroidogenesis and protein synthesis[7,8]. By stimulating aldosterone and glucocorticoid synthesis and secretion (corticosterone in rodents and cortisol in humans and bovine), ACTH agonists binding to MC2R plays a pivotal role in homeostasis, metabolism and stress response[9].

Since early 1990's, N-succinimidyl-4-[^{18}F] fluorobenoate ([^{18}F]SFB) have been used as a bifunctional agent for ^{18}F-labeling of peptides and antibody fragments[10, 11], and there have been recent developments in the production of [^{18}F]SFB in order to minimize the manual procedure and enhance the amount of radioactivity[12,13].

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Received date: 2009-03-06

In this report, a ^{18}F-radiolabeling strategy was employed using [^{18}F]SFB and ^{18}F-labeled $\beta^{1\text{-}24}$-corticotrophin for PET imaging of Mc2r receptors. Radiochemical and chemical purity of the radiotracer was controlled using HPLC/RTLC. Biodistribution study of the tracer was performed to demonstrate the accumulation of the tracer in the vital organs.

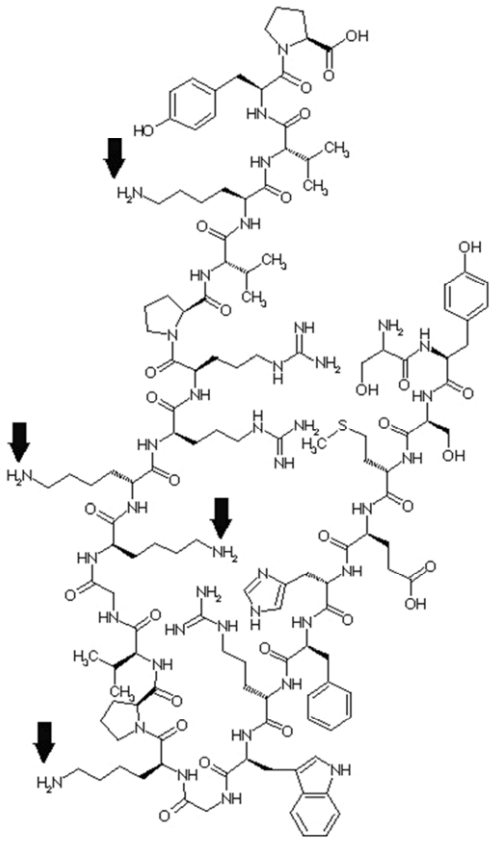

Fig. 1 Chemical structure of corticotrophin and possible sites of conjugation with [^{18}F]SFB.

2 Experimental

The chemicals were purchased from Aldrich Chemical Company Inc., UK. Thin-layer chromatography (TLC) of non-radioactive products was run on silica gel polymer-backed (F 1500/LS 254, 20 cm × 20 cm, TLC Ready Foils Schleicher & Schuell®) or glass plates (25 cm × 35 cm, E-Merck). Acetonitrile used for labeling experiments was of `Sure-Seal™` grade (Aldrich). The specific activity of radiolabeled peptide was calculated using a standard curve from cold peptide. Radiochromatography was performed on Bioscan AR-2000 (France), using polymer-backed silica gel papers. Analytical HPLC to determine the specific activity was performed on a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-vis (Shimadzu) using Partisphere C-18 column 250 mm × 4.6 mm, (Whatman, USA). Eluents: A, CH_3CN (0.1% TFA); B, water (0.1% TFA) 20% A up to 50% A in 20 min. The eluent was with a flow rate of 1.3 mL/min.

The identity of the intermediate products was confirmed by comparing radiochromatograms of ^{18}F-labeled compounds with the UV-chromatograms of non-labelled reference materials. Analytical HPLC was also used to determine the specific radioactivity of ^{18}F-labeled peptide. A standard curve was generated to calculate the mass of the final solution. The production of the labeled compound was performed in an automated fluorination module designed and manufactured locally. The purification of radiolabeled peptide was performed by C_{18} Sep-Pak™ short columns, which were purchased from Waters.

Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd Ed. (approved by Iranian Ministry of Health and Medical Education).

2.1 Preparation of potassium [^{18}F]fluoride

[^{18}F]fluoride was produced via ^{18}O(p, n)^{18}F reaction by bombarding an isotopically enriched ^{18}O-H_2O target with 18 MeV protons on an IBA Cyclone 30 cyclotron in a cylindrical Au-plated silver target. At EOB aqueous solution of [^{18}F] fluoride (2.405×10^4 MBq) in ^{18}O-enriched water was captured on a cromafix PS-HCO_3 (Macherey-Nagel, Germany) Sep-Pak cartridge and then washed with deionized water (3 mL). [^{18}F]fluoride ions on the cartridge was eluted using 500 μL of K_2CO_3 (0.166 mol/L) solution. The eluted mixture was transferred to a 10-mL conical vial containing 800 μL dry actonitrile and kryptofix (25 mg, 66.4 μmol), and dried under a gentle stream of N_2 gas at 85˚C. The azeotropic drying step was repeated at least two times with 1 mL portions of acetonitrile.

2.2 Preparation of 4-[^{18}F]fluorobenzoic acid ([^{18}F]FBA) using an automated module

A mixture of ethyl 4-(trimethylammonium) benzoate trifluormethane-sulfonate (30mg, 84μmol) in anhydrous dimethylsulfoxide (1 mL) was added to the residue ob-

tained above, and heated to 110°C for 15 min while stirring under N_2 atmosphere. The reactor was cooled by 18°C water follow by the addition of diethyl ether (9 mL) to the residue. After mixing for 1 min, the solution mixture was passed through a silica Sep-Pak column to remove possible amounts of fluoride anion and/or K222 while transferred to the second reactor. The ethereal mixture was evaporated using a N_2 flow at 60°C for 5 min. For hydrolysis process, 1 mol / L NaOH (1 mL) was added to the residue and the mixture was heated at 100°C for 7 min. After cooling for 1 min, the mixture was acidified using 1 mol/L HCl (1.6 mL) and diluted by the addition of DD H_2O (8 mL). The final mixture was passed through a C_{18} sep-Pak column. The column was washed with 0.01 mol / L HCl (1 mL) followed by N_2 flushing in order to remove any liquids from the column bed for 4 min. An anhydrous $MgSO_4$ packed column (5 mm × 15 mm) was connected to the bottom of the C_{18} column and the C_{18} column was eluted using anhydrous acetonitrile while the whole organic phase was passed through $MgSO_4$ column[14]. About 1.332×10^4~1.369×10^4 MBq of [^{18}F]FBA was produced in this procedure and whole process took about 65 min with a radiochemical yield of 55% to EOB (not-decay corrected). Fig. 2 shows schematic diagram to the semi-automated module used to produce [^{18}F]FBA in this study.

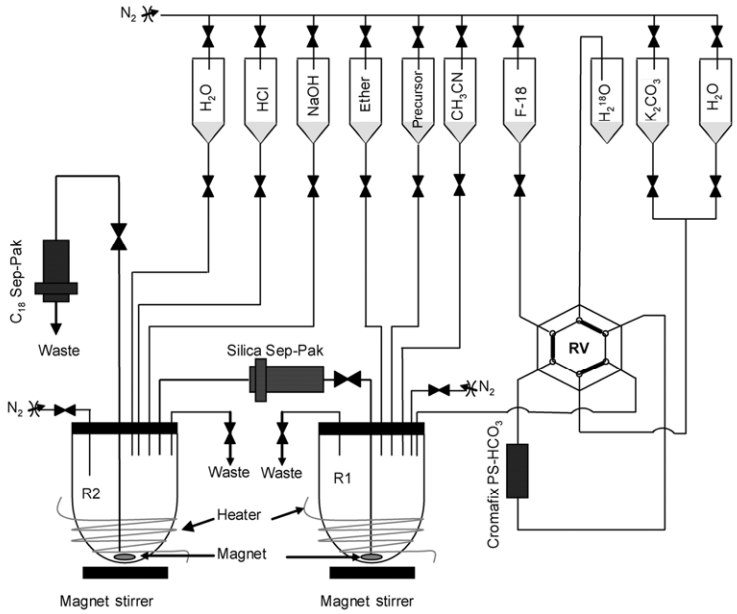

Fig. 2 Schematic diagram to the semi-automated module. Reactor 2 (R2) is equipped with a cold finger cooling system.

2.3 Preparation of N-succinimidyl-4- [^{18}F]SFB and ^{18}F-labeled $\beta^{1\text{-}24}$-corticotrophin

The final solution was evaporated to 1 mL using a flow of inert gas. Dicyclohexyl carbodiimide (DCC) (12 mg, 58 μmol) and N-hydroxusuccinimide (NHS) (6.6 mg, 58 μmol) were added to the residue and the mixture was stirred at room temperature for 15 min. Water (10 mL) was added to terminate the esterifiaction reaction and decomposed DCC to urea compound. The mixture was passed through a pre-conditioned C_{18} Sep-Pak column followed by elution of the column by anhydrous acetonitrile (3 mL). The mixture was evaporated using a flow of N_2 gas and slight heating while being mildly stirred. Then an aqueous solution of $\beta^{1\text{-}24}$-corticotrophin (1 mg/mL) was added to the residue, stirred at room temperature for 30 min. The mixture was passed through an activated C_{18} Sep-Pak followed by passing DDH_2O (1 mL). The column was eluted using one-mL fractions of 0.1 mol/L citrate buffer (pH 5) and each fraction was checked by RTLC. The final formulation was passed through a 0.22 micrometer filter.

2.4 Quality control of intermediates and final radiolabeled peptide

2.4.1 Radio thin layer chromatography

The active solutions were checked for radiochemical purity by TLC on a polymer-backed silica gel layer

eluted with solvent systems of (A) Hex: EtOAc = 6: 1; (B) CH_2Cl_2: EtOAc = 4 : 1 and (C) n-butanol: water: HOAc = 4 : 1 : 1. The R_f values of intermediates were calculated using authentic samples prepared by cold fluorinations. The RTLC tests were confirmed by co-spotting of ^{19}F-compound and intermediate active solutions. The results showed that they migrated at the same R_f. Table 1 demonstrates the RTLC data for radiolabeled intermediates and peptide.

2.4.2 High performance liquid chromatography

The purity of all intermediates and the final product was checked using analytical reverse phase HPLC (Partisphere-5 C-18, Whatman, 250 mm × 4.6 mm). A gradient system was used with solvents of (D) CH_3CN (with 0.1% TFA) or (E) H_2O (with 0.1% TFA, at 1.3 mL/min), starting with a mixture of 20% D: 80% E (t = 0.0 min) to 50% D and 50% E (t = 20 min). The retention time for [^{18}F]FBA and [^{18}F]SFB was 9.30 and 12.04, respectively. Radiolabeled peptides show multiple peaks at 4~9 min region (Fig. 3).

Table 1 RTLC chromatogram data for labeled compounds on silica paper using three solvent systems

Chemical species	[^{18}F]Fluoride	[^{18}F]FEB	[^{18}F]FBA	[^{18}F]SFB	^{18}F-labeled peptide
Solvent A	0.0	0.75	0.0	0.2	0.0
Solvent B	0.0	0.9	0.26	0.8	0.0
Solvent C	0.0	≈1	0.62	0.85	0.18 and 0.32

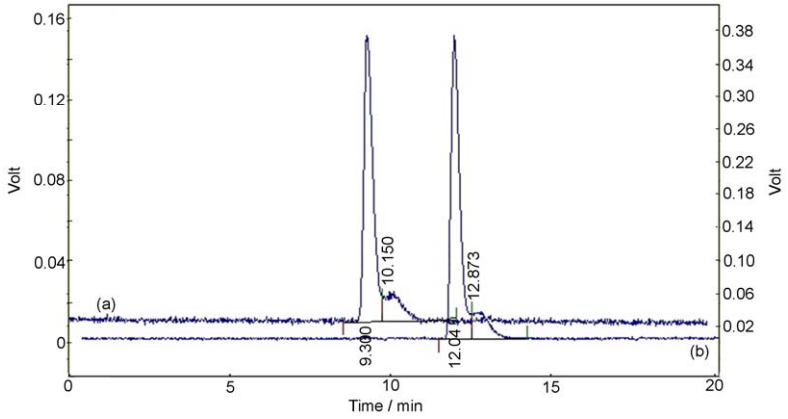

Fig. 3 HPLC radio-chromatograms of (a) [^{18}F]FBA and (b)[^{18}F]SFB on a reversed phase column using gradient system.

2.5 Stability testing of the radiolabeled peptide

A sample of the purified ^{18}F-labeled peptide (74 MBq) was kept at room temperature for 5 h and its radiochemical purity was checked by RTLC (Solvent C) every 30 min to determine the stability of the ^{18}F-labeled peptide.

To 74 MBq of ^{18}F-labeled peptide, 500 mL of freshly prepared human serum was added and the resulting mixture was incubated at 37°C for 3 h. Aliquots (5 μL) were analyzed by RTLC (Solvent C) after 0, 15, 30, 60, 120 and 180 min of incubation to determine the serum stability of the ^{18}F-labeled peptide.

2.6 Biodistribution of $\beta^{1\text{-}24}$-corticotrophin in normal mice

^{18}F-labeled $\beta^{1\text{-}24}$-corticotrophin was administered to normal mice. A volume (50~100 μL) of final solution containing (2.96±0.10) MBq radioactivity was injected intravenously to mice through the tail vein. The animals (n=3) were sacrificed at 15, 90, 120 and 150 min. The specific activity of different organs was calculated as percentage of tissue count per gram using an HPGe detector.

3 Results and discussion

3.1 Radiolabeling and quality control

Radiolabeld peptides are potent targeting probes in nuclear medicine due to their known structure and better pharmacokinetic compared to antibodies. The simple structure and lack of 3rd and 4th sterrical structures compared to proteins and antibodies increase their stability in wider pH ranges and chemical conditions as well as *in vivo* conditions. These advantages

enable radiochemists to apply chemical reactions more confidently on peptides.

Based on our works on receptor-based peptide radioligands of buserelin, gonadorelin[15] and corticotrophin[16], we were interested in labeling the latter peptide with ^{18}F using famous bifunctional agent[17], [^{18}F] SFB. The biological activity of radiolabeled peptide was interesting, because many disease conditions are related to ACTH receptor.

The bifunctional agent of [^{18}F] SFB was prepared using a semi-automated module preliminary used for routine [^{18}F] FDG production. Starting from ethyl 4-(trimethylammonium) benzoate trifluormethane- sulfonate according to reported reaction[18], the labeling was performed to obtain [^{18}F] FBA in 55% yield to EOB (not-decay corrected) and radiochemical purity of >98.5%. After esterifiaction of [^{18}F] FBA using NHS and DCC as the dehydrating agent, [^{18}F] SFB was obtained in 47% yield to EOB (not-decay corrected) and radiochemical purity of >99.5%. Also the polarity of the labeled compound was changed and in reverse phase HPLC a time shift from 9.3 min ([^{18}F] FBA) to 12.04 ([^{18}F] SFB) was observed.

Lys-NH_2 groups are the most susceptible sites of conjugation and all these sites can be conjugated with [^{18}F] SFB in aqueous media at mild alkaline conditions. RTLC in various solvents can easily demonstrate the peptide radioconjugation as shown in Table 1. The presence of some side products such as dicyclohexylurea, as well as retention of some small molecule precursors can be eliminated using solid phase extraction by C-18 column. The radiochemical purity of the final mixture was enhanced as shown in Fig. 4.

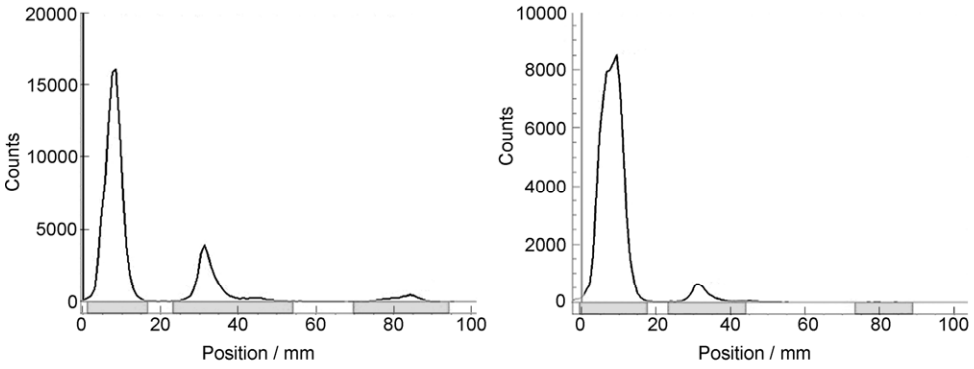

Fig. 4 The radiochemical purity of $\beta^{1\text{-}24}$-corticotrophin before (left) and after (right) solid phase purification. TLC radiochromatograms were taken in solvent B.

Fig. 5 shows the HPLC radio-chromatograms of the purified final radiolabeled mixture. In HPLC control, the ^{18}F-radiolabeled peptide mixtures are eluted faster than [^{18}F]SFB and other possible intermediates, the peaks at 4~9 min are the radiolabeled peptides (Fig. 1). There are at least 4 possible conjugation sites on the peptide resulting in 4 radiolabeled species. The peak at 9.5 min is possibly related to [^{18}F]FBA since during conjugation, hydrolysis of the succinimidyl ester is possible, too. The peak at 11.88 min is possibly the remaining unreacted [^{18}F]SFB. Over 98% of radiochemical species are related to radiolabeled peptides that looked suitable for animal experiments. The radiochemical yield for peptide conjugation reaction was calculated based on RTLC data (76%, decay corrected to [^{18}F]SFB). The overall yield for $\beta^{1\text{-}24}$-corticotrophin was 29% to EOB (not decay corrected). ^{18}F-labeled $\beta^{1\text{-}24}$-corticotrophin was stable in citrate buffer solutions for at least 5 h as detected by RTLC. The radiochemical purity of ^{18}F-labeled peptide decreased in human serum media (from 96.0% at 0 min to 91.2% at 180 min).

3.2 Biodistribution studies

The distribution of $\beta^{1\text{-}24}$-corticotrophin among tissues was determined for male mice. A volume (0.1 mL) of final $\beta^{1\text{-}24}$-corticotrophin solution of 2.96 MBq radioactivity was injected into the dorsal tail vein. The radioactivity injected into each mouse was measured by counting the 1-mL syringe before and after injection in a curiemeter with a fixed geometry.

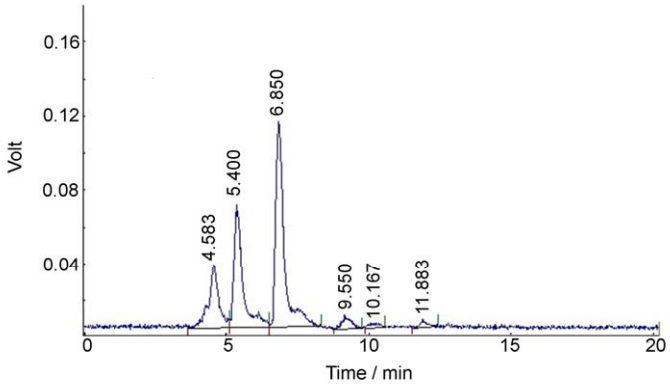

Fig. 5 HPLC radio-chromatogram of final $\beta^{1\text{-}24}$-corticotrophin on a reversed phase column using gradient system.

The animals were sacrificed by ether asyxphycation at 15, 90, 120 and 150 min after injection, the tissues (blood, heart, spleen, kidneys, liver, intestine, stomach, lung, brain, muscle, sternum, fat and testes) and feces were weighed and the specific activities were determined with a γ-ray scintillation as a percentage of count per gram of tissue (Fig. 6).

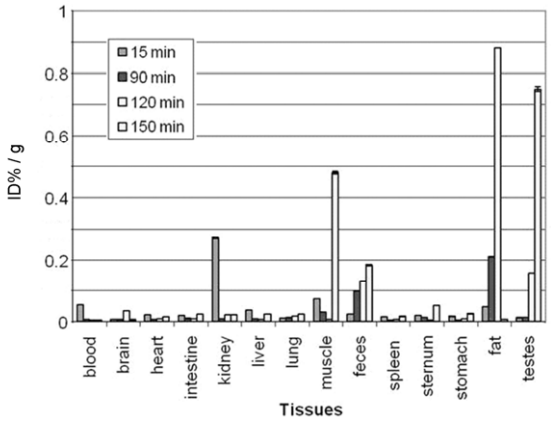

Fig. 6 Bio-distribution of $\beta^{1\text{-}24}$-corticotrophin in normal mice 15~150 min post-injection via their tail vein.

In serum, β-1-24-corticotrophin degraded rapidly by enzymatic hydrolysis, while most of activity was washed out by kidneys first to inactive oligopeptides, then to free amino acids[19].

At 120 min, most of radioactivity accumulated in the fat tissues. Surveying the literature, a great portion of Mc2r is present in adipocytes[20, 21]. This is strongly confirmed by this study (Fig. 6), where the tracer was mostly accumulated in testes, the predominent organs of accumulation throughout the studied time period. This was in accordance with the reported literature[22].

4 Conclusion

Production of [^{18}F]SFB was performed using an in-house made semi-automated machine in 90 min with a yield of 55% to EOB (not-decey corrected). Total labeling of $\beta^{1\text{-}24}$-corticotrophin took about 120 min, with a yield of 29% to EOB (not-decay corrected). After SPE purification, no significant amount of free ^{18}F as well as ^{18}F-labeled intermediates were observed in final preparation (<5% in all cases). The final preparation was administered to normal mice and bio-distribution of the radiotracer was checked up to 150 min later. Testes/blood, testes/muscle ratios for ^{18}F-labeled peptide at 150 min were 184 and 1.56, respectively, and adipocyte/blood and adipocyte/ muscle ratios at 120 min were 221 and 142, respectively. A concise and thorough work on RTLC and HPLC of [^{18}F]SFB production and application in the radiolabeling of peptides was presented in this work. $\beta^{1\text{-}24}$-corticotrophin can be a good probe for imaging of ACTH receptors with potential use in adrenal and sex organ malignancies using PET.

Acknowledgement

Authors wish to thank Mr S. Daneshvari for the animal studies, and cyclotron operating team for their helps.

References

1 Cone R D, Mountjoy K G, Trends Endocrin Met, 1993, **4**(7): 242-247.

2 Mountjoy K G, Robbins L S, Mortrud M T, *et al.* Science, 1992, **257** (5074): 1248-1251.

3 Baxter J D, Tyrrel J D, the Adrenal Cortex. In: Felig P, Baxter J D, Broadus A E, *et al.* eds. Endocrinology and

Metabolism. 2nd Ed. New York: McGraw-Hill, 1986: 511-692.

4 Slominski A, Ermak G, Mihm M. J Clin Endocrinol Metabol, 1996, **81** (7): 2746-2749.

5 Lin C J, Alexander A A J, Latronico A C, *et al.* J Clin Endocrinol Metabol, 2000, **85** (3) : 1211-1214.

6 Boston B A, Cone R D. Endocrinology, 1996, **137**(5): 2043-2050.

7 Thomas M, Keramidas M, Monchaux E, *et al.* Microsc Res Techniq, 2003, **61**(3): 247-251.

8 Sewer M B, Waterman M R, Microsc Res Techniq, 2003, **61**(3): 300-307.

9 Dallman M F, la-Fleur S E, Pecoraro N C, *et al.* Endocrinology, 2004, **145**(6): 2633-2638.

10 Vaidyanathan G, Zalutsky M R. Nucl Med Biol, 1992, **19**(3): 275-281.

11 Vaidyanathan G, Zalutsky M R. Bioconjugate Chem, 1994, **5**(4): 352-356.

12 Mading P, Fuchtner F, Wust F. Appl Radiat Isot, 2005, **63**(3): 329-332.

13 Wust F, Hultsch C, Bergmann R, *et al.* Appl Radiat Isot, 2003, **59**(1): 43-48.

14 Cheng D, Yin D, Zhang L, *et al.* J Fluorine Chem, 2007, **128**(3): 196-201.

15 Jalilian A R, Shanehsazzadeh S, Akhlaghi M, *et al.* J Radioanal Nucl Ch, 2003, **278**(1): 123-129.

16 Jalilian A R, Shanehsazzadeh S, Akhlaghi M, *et al.* Radiochim Acta, 2008, **96**: 435-439.

17 Toretsky J, Levenson A, Weinberg I N, *et al.* Nucl Med Biol, 2004, **31**(6): 747-752.

18 Haka M S, Kilboum M R, Watkins G L, *et al.* J Labelled Cmpd Radiopharm, 1989, **27**(7): 823-833.

19 SYNACTHEN DEPOT.® Data Sheet, Novartis New Zealand Limited Private Bag 47909 Ponsonby 6-8 Mackelvie Street, Grey Lynn AUCKLAND, 15 Feb., 2006

20 Harmer S C, Pepper D J, Cooke K, *et al.* J Endocrinol, 2008, **196**(1): 149-158.

21 Noon L A, Bakmanidis A, Clark A J L, *et al.* J Mol Endocrinol, 2006, **37**(3): 415-420.

22 O'shaughnessy P J, Fleming L M, Jackson G, *et al.* Endocrinology, 2003, **144**(8): 3279-3284.