NUCLEAR THEORY, Vol. 35 (2016)
eds. M. Gaidarov, N. Minkov, Heron Press, Sofia

Optimization of Production and Synthesis of the Radiopharmaceutical ^{18}F-FET in Kazakhstan for the Diagnosis of Brain Tumors

A.E. Ryskulov[1], **B.A. Prmantayeva**[1], **G.O. Ryskulova**[2]

[1]L.N. Gumilyov Eurasian National University, Astana, Kazakhstan

[2]Republican Diagnostic Center Branch of University Medical Center Corporate Fund, Astana, Kazakhstan

Abstract. This paper presents the results of research work of the authors for the production, synthesis and clinical application of a new radiopharmaceutical for Kazakhstan – ^{18}F-fluoroethyltyrosine (^{18}F-FET) with the use for diagnosis by the method of PET/CT. The article describes in detail the techniques for the production and preparation of the radiopharmaceutical. It has been pointed out in the article, that the production and synthesis of ^{18}F-FET, its high stability in the body, fast kinetics of accumulation in brain and tumor, its low accumulation in non-tumor tissue, as well as ease of synthesis, determine further assessment of ^{18}F-FET as amino acids indicator for cerebral and peripheral tumors.

1 Introduction

Discoveries in Nuclear Physics have a huge impact on almost all fields of human activity [1,2]. The successes of nuclear physics have also the exceptional value for medicine. The radionuclides are used in nuclear medicine as radiopharmaceuticals for early diagnosis and treatment of various pathologies.

Radiopharmaceutical (RPC) is a chemical compound containing in its molecule a certain radioactive nuclide allowed for administration to humans for diagnostic or therapeutic purposes. The distinctive feature of a diagnostic RPC in this case is the lack of pharmacological effect [3,4].

Nuclear medicine - section of clinical medicine that deals with the use of radionuclide pharmaceuticals for diagnosis and treatment [4]. Single-photon emission computed tomographic (SPECT) scanners and positron emission tomographic (PET) scanners are primarily used in diagnosis.

Until recently, the main diagnostic PET RPC for the diagnosis of malignant tumors, including brain tumors, remained ^{18}F-Fluorodeoxyglucose (^{18}F-FDG). However, drawbacks of ^{18}F-FDG, such as increased accumulation of the radiotracer in the cortex and the absence of hyper-fixation in many brain tumors, raise the necessity of use other tumor-seeking RPCs [5].

The aim of this work is to optimize the processes of production and synthesis of the ultra-short-lived positron-emitting radionuclide in the radioisotope

diagnosis using the method of positron emission tomography for the diagnosis of brain tumors in Kazakhstan.

2 Materials and Methods

The radionuclide fluorine-18 is obtained by irradiation of the nuclei of the isotope oxygen-18 by accelerated beam of protons with an energy of 15 MeV at the reaction:

$$^{18}\mathrm{O}\ (p, n)\ ^{18}\mathrm{F}\,.$$

The target substance is water, enriched in oxygen-18 isotope. The resulting fluorine-18 radionuclide is stabilized in chemical form of fluoride, fluorine-18 ($[^{18}\mathrm{F}]$, F^-).

2.1 Formation of radionuclides of Nitrogen-13 and Fluorine-17

With the indicated parameters of irradiation, oxygen-16 and oxygen-17, contained in the irradiated material as impurities, undergo nuclear reactions with the formation of radionuclides of nitrogen-13 (half-life – 9.96 min) and fluorine-17 (half-life – 70 s) respectively:

$$^{16}\mathrm{O}\ (p, \alpha)\ ^{13}\mathrm{N}\,,$$

$$^{17}\mathrm{O}\ (p, n)\ ^{17}\mathrm{F}\,.$$

Chemical forms of stabilization of nitrogen-13 is nitrogen gas $[^{13}\mathrm{N}]\mathrm{N}_2$. Radionuclide of fluorine-17 is stabilized in the chemical form of fluoride, fluorine-17.

The content of nitrogen-13 and fluorine-17 in preparation at the time of its manufacture cannot exceed 0.01% theoretically, which allows not identifying radionuclide impurities in the final product.

2.2 Production of Fluoride Tetrabutylammonium, Fluorine-18

$$\underbrace{(\mathrm{C_4H_9})_4\mathrm{N^+HCO_3^-}}_{\substack{\text{Tetrabutylammonium}\\ \text{hydrocarbonate}\\ \text{M.m 303}}} + \underbrace{^{18}F^-}_{\substack{\text{Fluoride,}\\ \text{fluorine-18}\\ \text{A.m 18}}} \xrightarrow[\mathrm{H_2O}]{} \underbrace{(\mathrm{C_4H_9})_4\mathrm{N^{+18}F^-}}_{\substack{\text{Tetrabutylammonium}\\ \text{fluoride, fluorine-18}\\ \text{M.m 260}}} + \underbrace{\mathrm{HCO_3^-}}_{\substack{\text{Hydrocarbonate}\\ \text{M.m 61}}}$$

Adverse reaction. Formation of chloride tetrabutylammonium:

$$\underbrace{(C_4H_9)_4N^+HCO_3^-}_{\text{Tetrabutylammonium hydrocarbonate M.m 303}} + \underbrace{Cl^-}_{\text{Chloride, A.m 35}} \xrightarrow[H_2O]{} \underbrace{(C_4H_9)_4N^+Cl^-}_{\text{Tetrabutylammonium chloride M.m 277}} + \underbrace{HCO_3^-}_{\text{Hydrocarbonate M.m 61}}$$

2.3 Preparation of O-(2-fluoroethyl)-L-Tyrosine

[^{18}F]Fluoride fixation and desorption

[^{18}F]fluoride is separated from ^{18}O-enriched water through an anion exchange resin (AG1 $\times$ 8, carbonate form) by a conventional method (Hamacher-etal., 1990). Elution of the activity is performed with 0.3 ml (35 mmol) of tetra-n-butyl ammonium hydrogen carbonate (pH $\approx$ 8) in water. Dehydration by azeotropic distillation is performed repeatedly with acetonitrile in a glass carbon (Sigradur®G) reaction vessel.

^{18}F-Fluorination

10 mg (14.8 micromoles) of O-(2-tosyloxyethyl)-N-trityl-L-tyrosine tert-butyl ester dissolved in 0.5 mL of dry acetonitrile (DNA quality), is added to the [^{18}F]fluoride without the addition of the carrier and is heated for 5 minutes at 85°C. Subsequently, the solution is evaporated to dryness in a stream of inert gas over 3 min, maintaining the temperature at 80-85°C.

Deprotection and solid phase extraction

At 30°C 1 ml of a mixture of trifluoroacetic acid in 1,2-dichloroethane (1:2, v/v) is added and stirred for 2 min, and then continued to stir for 7 min at 70°C. After cooling to room temperature, 5 ml of dichloromethane are added and the yellow-green solution for about 2 minutes passes through a cartridge of silica gel (LiChrolut®Si60; 0,2 g). The reaction vessel is filled with a mixture of diethyl ether/n-pentane (1:1) (5 mL) which is used to wash the cartridge. The washing step is repeated with 5 ml of the above-mentioned solvent mixture, and the (discolored) cartridge is purged with an inert gas (e.g. argon) while the reaction vessel is heated at 100°C for about 3 minutes.

Absorbed in silica gel ^{18}F-labeled amino acid is eluted with 2 ml of the heated solution of sodium glycinate (0.5 mol/l; pH 9.5), which leads to formation of FET-containing crude solution of the product (about 1.5 ml; pH 5-7) ready for use HPLC (High Performance Liquid Chromatography).

Synthesis of L-[^{18}F]FET is simple, effective and requires only commercially available chemicals and no complex equipment. The process of ^{18}F-fluoride

alkylation is simple and often used, starting with nucleophilic ^{18}F-fluoride. The whole synthesis terminates in less than 1 hour with a radiochemical yield of about 40% based on the ^{18}F-fluoride.

HPLC purification

Reversed phase HPLC purification of FET is performed using C18 phase (18 carbon atoms) with embedded polar groups (Prontosil® 120-5-C18-ace-EPS (250 × 10 mm) (Bischoff Analysentechnik GmbH, Leonberg, Germany)), eluent: ethanol/water (2/98, v/v), flow: 5 mL/min, k′= 9.1.

To provide high-quality cleaning and extend the column life, the system is treated (washed) immediately after the synthesis with approximately 200 ml mixture of ethanol/water (70/30, v/v). Storage in the presence of this solvent ensures aseptic conditions. Before starting the synthesis, column is pretreated with an ethanol/water mixture (2/98, v/v) for half an hour.

Formulation

FET, containing HPLC fraction (5-8 ml of a 2% ethanol), is ready for administration to humans after sterilizing filtration. In case of high activity concentration (>1.8 GBq/ml), the solution should be diluted with isotonic sodium chloride solution.

2.4 Quality Control

HPLC analysis

Radiochemical purity is analyzed using an analytical column Prontosil (250 × 4,6 mm) with a flow of 1 ml of ethanol/water (2/98). The retention time is comparable to the FET standard. Column capacity factor: k′=5.02.

Analysis by gas chromatography (GC)

GC was performed on: HP 5890 Series II Precolumn: FS-Phe-Sil desact. (8 m × 0,32 mm); column: OV 1701-DF-1.0 (50 m × 0,32 mm); helium flow: 12,5 ml/min, temperature program: with 5°C/min from 80° to 200°C; tR (ethanol): 4.16 min; tR (diethylether): 4.08 min.

Enantiomeric purity

The enantiomeric purity of the deprotected amino acid was analyzed using HPLC-system: Crownpak® CR (+) (150 × 4 mm) (Daicel Chemical Industries, Ltd.) with 20 mM perchloric acid; flow: 0.8 ml/min; capacity factor: k′(L-FET) = 12,5.

2.5 Data Collection

59-year-old man with recurrent astrocytoma was examined using the scanner ECATEXACT 951/R (CTI/Siemens, Knoxville, TN).

Doctor explains all the details of the study to the patient before the examination, patient gives written informed consent. The study is started after intravenous injection of 296 MBq of ^{18}F-FET on PET/CT scanner. After dynamic

emission tomography of the brain, full-body scanning is performed, covering the whole body from the base of the skull to the bladder (four overlapping positions of the couch, a 5-minute time of issue for each position).

Data were reconstructed by filtered back projection using a Hanning filter with a cutoff frequency of 0.8 Nyquist frequency. The number of pixels in the image were calibrated by activity concentration (Bq/g), and standardized uptake values (SUVs) were calculated using the formula: SUV = tissue concentration/injected dose/body weight.

2.6 Data Analysis

To determine the regions of interest (ROIs), frames of dynamic study were summed between 30 and 40 minutes.

Borders of the tumor were determined by 75% isocontours in successive sections. ROI of irregular shape was placed in the contralateral cortex. To define the input function, small annular ROIs were placed in eight consecutive slices in the area of right carotid artery, which is mounted in the first frame of the dynamic study. Time-activity curves (TACs) were calculated for tumor, normal brain and blood. Using these TACs, the influx of L-[^{18}F]FET into the tumor and normal brain was calculated by Goedde-Patlak analysis.

3 Results

3.1 Radiosynthesis

Depending on the concentration of tyrosine, ^{18}F-fluoroethylation resulted in saturation yields up to 75% $\pm$ 6.7% after 5 minutes. These results were observed using 45 mmol/L solution of the disodium salt of tyrosine in 300 ul of dimethyl sulfoxide.

3.2 Patient Study

Dynamic PET study showed a rapid and intensive consumption of L-[^{18}F]FET in brain tumor tissue. After 10 min post injection the tumor was clearly delineated from normal brain tissue (Figure 1). Concentration (given as SUV) of L-[^{18}F]FET, both in the tumor and normal brain cortex, steadily increased and reached 2.0 and 0.75, respectively, 40 minutes after injection (Figure 1). Due to the slow kinetics of accumulation in normal brain cortex, the tumor-to-cortex ratio continued to increase until the end of the study and reached a value of 2.7 after 40 min postinjection (Figure 2). Blood curve showed a biexponential diagram with a terminal half-life of 40 min (Figure 1).

This led to a strictly increasing tumor-to-blood ratio until the end of the study period (Figure 2).

Scanning of the entire body started in 40 min after injection and showed washout of radiotracer by the kidneys. Liver and pancreas did not show higher

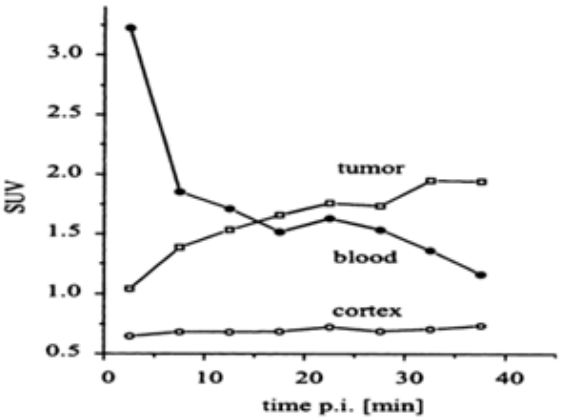

Figure 1. The change with time of activity localized in the blood pool (•), tumor (□) and the original tissue (cerebral cortex, ○) after intravenous administration of L-[^{18}F]FET to the patient with malignant brain tumor.

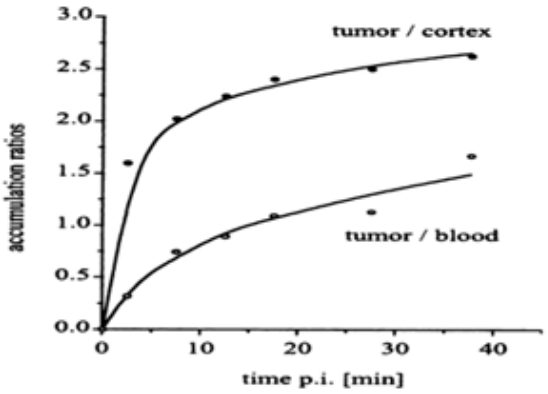

Figure 2. The change with time of L-[^{18}F]FET accumulation ratios: tumor-blood and tumor-cerebral cortex after intravenous administration of L-[^{18}F]FET to the patient with malignant brain tumor.

consumption of L-[^{18}F]FET, than the blood-pool activity. There was also no accumulation of the tracer in bones, bone marrow or intestine.

4 Discussion

Synthesis of [^{18}F]FET was performed in about 50 min with the overall radiochemical yield of 40%. Consumption of L-[^{18}F]FET in the brain reached $> 2\%$ ID/g between 30 and 60 min after injection. Consumption of D-isomer in brain was negligible, indicating penetration through the blood-brain barrier of spe-

cific amino acid transport system. L-[^{18}F]FET is not incorporated into proteins. HPLC examination of the brain, pancreas and tumor tissue at 10, 40 and 60 minutes post injection showed only unchanged L-[^{18}F]FET. Activity consumption in bones did not exceed 2% ID/g at 40 min after injection. In the first human study, L-[^{18}F]FET-PET allowed us to obtain a clear delineation of recurrent astrocytomas. At 35 min after injection, the tumor-to-cortex ratio was > 2.7. A tumor-to-blood ratio > 1.5 was reached at 30 minutes after injection and continued to increase. There was no significant accumulation of activity in peripheral organs in about 40 min after injection.

As the rate of protein synthesis in normal brain tissue several orders of magnitude lower than glucose uptake by the brain (approximately 0.5 nmol/g/min for leucine against 0.3 umol/100 g/min for glucose [6]), so the amino acid radiotracers have been proposed as alternative to FDG in describing the nature of the metabolism of brain tumors [7]. 2-[^{18}F]fluorotyrosine and p-[^{18}F]fluorophenylalanine did not find regular use due to their complex and low yield syntheses [8-10]. Synthesis of L-[^{18}F]FET is simple, effective and requires only commercially available chemicals and no complex equipment. The process of ^{18}F-fluoroalkylation is often used and is simple, starting with nucleophilic ^{18}F-fluoride [11,12]. The whole synthesis is terminated in less than 1 hour, with a radiochemical yield of about 40% based on the ^{18}F-fluoride.

Compared with all the other amino acids, studied until now, L-[^{18}F]FET shows the highest consumption by brain in last researches. Different from other synthetic amino acids, such as iodo-alpha-methyltyrosine (IMT), which is not retained in the brain, but shows the initial maximum consumption and is rapidly removed from the body [13], the kinetics of L-[^{18}F]FET in brain indicate a longer retention by an unknown mechanism [14, 15]. Slow washout was observed only after 1 hour.

Currently, for biopsy planning and differential diagnosis of recurrent tumors and postbeam changes, PET with labeled amino acids, such as fluoro-ethyltyrosine, is most widely used. This method is also widely used for radiotherapy planning. An important advantage of labeled amino acids in the diagnosis of brain tumors and relapses is the fact that a violation of the blood-brain barrier permeability does not affect their seizure in brain tumors. It was shown that ^{18}F-FET does not accumulate in areas of radiation necrosis.

In the Department of radioisotope diagnosis of the Republican Diagnostic Center in Astana the studies on PET/CT using ^{18}F-FDG RPC are currently carried out for the diagnosis of brain tumors. As it is known, in more than 90% of cases the PET/CT is performed with an analogue of glucose - ^{18}F-FDG.

During the period from 2013 by 2015, 21 studies of primary brain tumors were performed. All the patients were sent after surgery, radiation or chemotherapy. Among them were patients with medulloblastoma – 4 people (19.0%), astrocytoma - 4 people (19.0%), glioblastoma – 6 people (28.6%), oligodendroglioma – 4 people (19.0%), meningioma – 3 people (14.3%). After the study, 10 patients (47.6%) showed no tumor recurrence, 8 patients (38.0%) – correla-

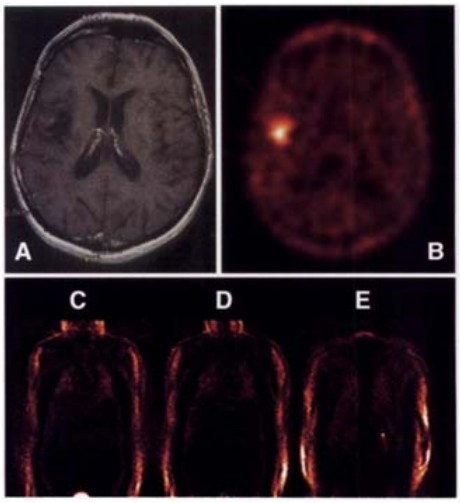

Figure 3. Comparative picture of L-[^{18}F]FET accumulation (B) and MR image after the administration of Gd-DTPA (A) to the patient with malignant brain tumor. Image shows drug activity between 30 and 45 min after injection of 296 MBq L-[^{18}F]FET. Tumor borders were defined by 75% isocontours. Lower row: regional body images of the same patient (from the ventral to dorsal: C, D, E).

tion with MRI is required, 3 patients (14.3%) – difficult to differentiate radiation pathomorphosis with continued tumor growth.

According to the results of our research and data in the literature, not all brain tumors may be diagnosed on PET/CT using ^{18}F-FDG.

Data are given in Table 1.

Table 1. Information value of PET with RPC for the diagnosis of primary brain tumors

Investigation method	Sensitivity, %	Specificity, %	Diagnostic accuracy, %
PET/CT with ^{18}F-FDG	80–86	80–93	—
PET/CT with ^{18}F-FET	93–95	96–99	97–98

We have examined other methods. One of these methods is the method of PET/CT with ^{18}F-FET. This is the method of brain tumors visualization by positron emission tomography using the radiopharmaceutical ^{18}F-fluoroethyl-tyrosine (tyrosine amino acid, labeled with isotope ^{18}F, ^{18}F-FET). It has a very high specificity to imaging brain tumors (gliomas), and is used in particular for their differentiation from healthy tissue (for example, for planning radiation therapy) and for the early detection of recurrence. We, together with scientists of Vivantes Clinic in Berlin, and the production base of nuclear medicine in Jlich (Germany) have conducted data processing of methodical experiments for the

production, quality control and clinical use of ultra-short-lived RPC ^{18}F-FET in neurooncology.

In most cases, ^{18}F-FET PET allows a clear distinction between tumors and inflammatory processes as well as between recurrence and radiation necrosis.

5 Conclusion

L-[^{18}F]FET is an ^{18}F-labeled amino acid for radionuclide diagnosis of brain tumors. High stability in the body, the rapid accumulation in the brain and tumors, low accumulation in non-tumor tissue, and ease of synthesis define further application of L-[^{18}F]FET as an amino acid tracer for tumors.

In contrast to [^{11}C]methionine, L-[^{18}F]FET did not accumulate in the bone marrow, kidneys or pancreas, and thus, may have utility in the detection of peripheral tumors. L-[^{18}F]FET can find widespread use, since it can be produced in high yield drug activity.

Hence, the production and synthesis of RPC L-[^{18}F]FET, its high stability in the body, fast kinetics of accumulation in the brain and tumor, low accumulation in non-tumor tissue and ease of synthesis strongly support the further evaluation of L-[^{18}F]FET as an amino acid tracer for tumor.

Well-known manufacturers of radiopharmaceuticals are the USA, Japan, Germany and other countries. In this direction, we have maintained close cooperation with scientists from Charité University and Vivantes Clinic (Berlin, Germany), production base of Nuclear Medicine (Jülich, Germany).

Optimization the processes of production and synthesis of modern radiopharmaceuticals in Kazakhstan, including ^{18}F-FET, will initiate the creation of a new class of drugs labeled with ultra-short-lived positron-emitting radionuclides.

The widespread adoption of PET/CT with the use of new RPCs in clinical practice in Kazakhstan to identify brain tumors, in addition to the impact on the diagnostic process, has an important economic component. This is because that by increasing the efficiency of diagnosis, detection of diseases at an earlier stage of their development and timely prescribing the adequate treatment, it is possible to significantly reduce the cost of treatment, decrease the duration of inpatient and outpatient treatment, improve prognosis, reduce disability and diminish mortality. Complete diagnostics, which combines computed and positron-emission tomography, significantly reduces the need for re-treatment.

References

[1] H.E. Johns, *The Physics of Radiology* (Atomizdat, Moscow, 1965) [in Russian].

[2] V. Chumakov, Postavit' diagnoz pomozhet atom (Atom will help to make a diagnosis) *V mire nauki: zhurnal* No 2 (2012) 3-9.

[3] V.Ju. Baranova (Ed.), *Isotopes: Properties, Production, Application* (Moscow, IzdAT, 2000) [in Russian].

[4] I.N. Bekman, *Lectures on Nuclear medicine* (Moscow, MGU, 2006) [in Russian].

[5] A.M. Granov, L.A. Tyutin (Eds.) *Positron Emission Tomography: A Guide for Physicians* (Foliant, St. Petersburg, 2008) 189-216.

[6] R.A. Hawkins, S.C. Huang, J.R. Barrio, R.E. Keen, D. Feng, J.C. Mazziotta, M.E. Phelps, *J. Cereb. Blood Flow Metab.* **9** (1989) 446-60.

[7] O. Schober, G.J. Meyer, C. Duden et al., *ROFO-Fortsch-Geb-Rontgenstr-Nuklearmed.* **147** (1987) 503-509.

[8] H.H. Coenen, K. Franken, P. Kling, G. Stöcklin, *Int. J. Radiat. Appl. Instrum.* **39** (1988) 1243-1250.

[9] H.H. Coenen, K. Franken, P. Kling, G. Stöcklin, *J. Nucl. Med.* **30** (1989) 1367-1372.

[10] C. Lemaire, M. Guillaume, L. Christiaens, A.J. Palmer, R. Cantineau, *Appl. Radiat. Isot.* **38** (1987) 1033-1038.

[11] W. Bodsch, H.H. Coenen, G. Stöcklin, K. Takahashi, K.A. Hossmann, *J. Neurochem.* **50** (1988) 979-983.

[12] H.H. Coenen, G. Stöcklin, *Radioisot. Klin. Forsch.* **18** (1988) 402-440.

[13] K.J. Langen, H.H. Coenen, N. Roosen, et al., *J. Nucl. Med.* **31** (1990) 281-286.

[14] H.J. Wester, C. Dittmar, M. Herz, K. Hamacher, M. Schwaiger, G. Stöcklin, *Nuklear-medizin*. **36** (1997) A75.

[15] H.J. Wester, C. Dittmar, M. Herz, R. Senekowitsch-Schmidtke, M. Schwaiger, G. Stöcklin, *J. Nucl. Med.* **38** (1997) 175.