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Nuclear Medicine and Biology 34 (2007) 717–725
NUCLEAR MEDICINE AND BIOLOGY
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Preparation and biological evaluation of 2-amino-6-[^{18}F]fluoro-9-(4-hydroxy-3-hydroxy-methylbutyl) purine (6-[^{18}F]FPCV) as a novel PET probe for imaging HSV1-tk reporter gene expression

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Received 5 January 2007; received in revised form 22 May 2007; accepted 11 June 2007

Abstract

Introduction: 2-Amino-6-[^{18}F]fluoro-9-(4-hydroxy-3-hydroxy-methylbutyl) purine (6-[^{18}F]FPCV) was prepared via a one-step nucleophilic substitution and evaluated as a novel probe for imaging the expression of herpes simplex virus type 1 thymidine kinase (HSV1-tk) reporter gene.
Methods: Log *P* of 6-[^{18}F]FPCV was calculated in octanol/phosphate-buffered saline (PBS). Stability studies were performed in PBS and bovine serum albumin (BSA). Cell uptake was performed at various time points in wild-type cells and transduced cells. For in vivo studies, tumors were grown in nude mice by inoculation with C6 cells, wild type and tk positive. The radiotracer was intravenously injected to animals, and micro-PET imaging was performed. Biodistribution of 6-[^{18}F]FPCV was performed on another group of animals at different time points.
Results: Log *P* of 6-[^{18}F]FPCV was −0.517. 6-[^{18}F]FPCV was fairly stable in PBS and BSA at 6 h. The tracer uptake in C6-tk cells was 5.5–18.8 times higher than that in wild-type cells. The plasma half-life of 6-[^{18}F]FPCV was as follows: α $t_{1/2}$=1.2 min and β $t_{1/2}$=73.7 min. The average ratio of tumor uptake between the transduced tumor and the wild-type tumor was 1.69 at 15 min.
Conclusion: Biological evaluation showed that 6-[^{18}F]FPCV is a potential probe for imaging HSV1-tk gene expression. However, its in vivo defluorination may limit its application in PET imaging of gene expression.

Keywords: 6-[^{18}F]FPCV; HSV1-tk; Gene expression; PET

1. Introduction

Recently, several groups have investigated the herpes simplex virus type 1 thymidine kinase (HSV1-tk) as a reporter and/or suicide gene, which was applied in biomedical science or gene therapy of cancer [1,2]. Small-animal positron emission tomography (PET) is ideally suited for preclinical imaging of cancer biology. Small-animal PET imaging of reporter gene HSV1-tk in vivo using corresponding reporter probes could provide valuable information for monitoring gene therapy of cancer [3,4]. A lot of radiolabeled nucleoside derivatives have been under investigation as probes for PET imaging of reporter gene HSV1-tk [5–13]. However, the short half-life of ^{11}C and the potential for deiodination of ^{124}I are less than optimal for imaging purposes, and ^{18}F-fluoride production is relatively straightforward and readily available [5]. Accordingly, the ^{18}F-

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doi:10.1016/j.nucmedbio.2007.06.005

labeled nucleoside derivatives should be more advantageous. The ^{18}F-labeled compounds, which include mainly pyrimidine nucleoside derivatives and acylguanosine derivatives, are currently investigated as probes of PET imaging reporter gene HSV1-tk [6–13]. The chemical structures of probes are shown in Fig. 1 [5–15]. Comparing the probes, we found that each probe has its advantages and disadvantages. Although ^{18}F-labeled pyrimidine nucleoside derivatives were better than ^{18}F-labeled acylguanosine derivatives in sensitivity, the routine radiosynthesis of pyrimidine nucleosides were multistep and time-consuming. As to acylguanosine derivatives, fluorinated penciclovir analogues (including 8-[^{18}F]FPCV and [^{18}F]FHBG) have many advantageous characteristics over fluorinated ganciclovir (including 8-[^{18}F]FGCV and [^{18}F]FHPG). The improved pharmaceutical characteristics of penciclovir led to the

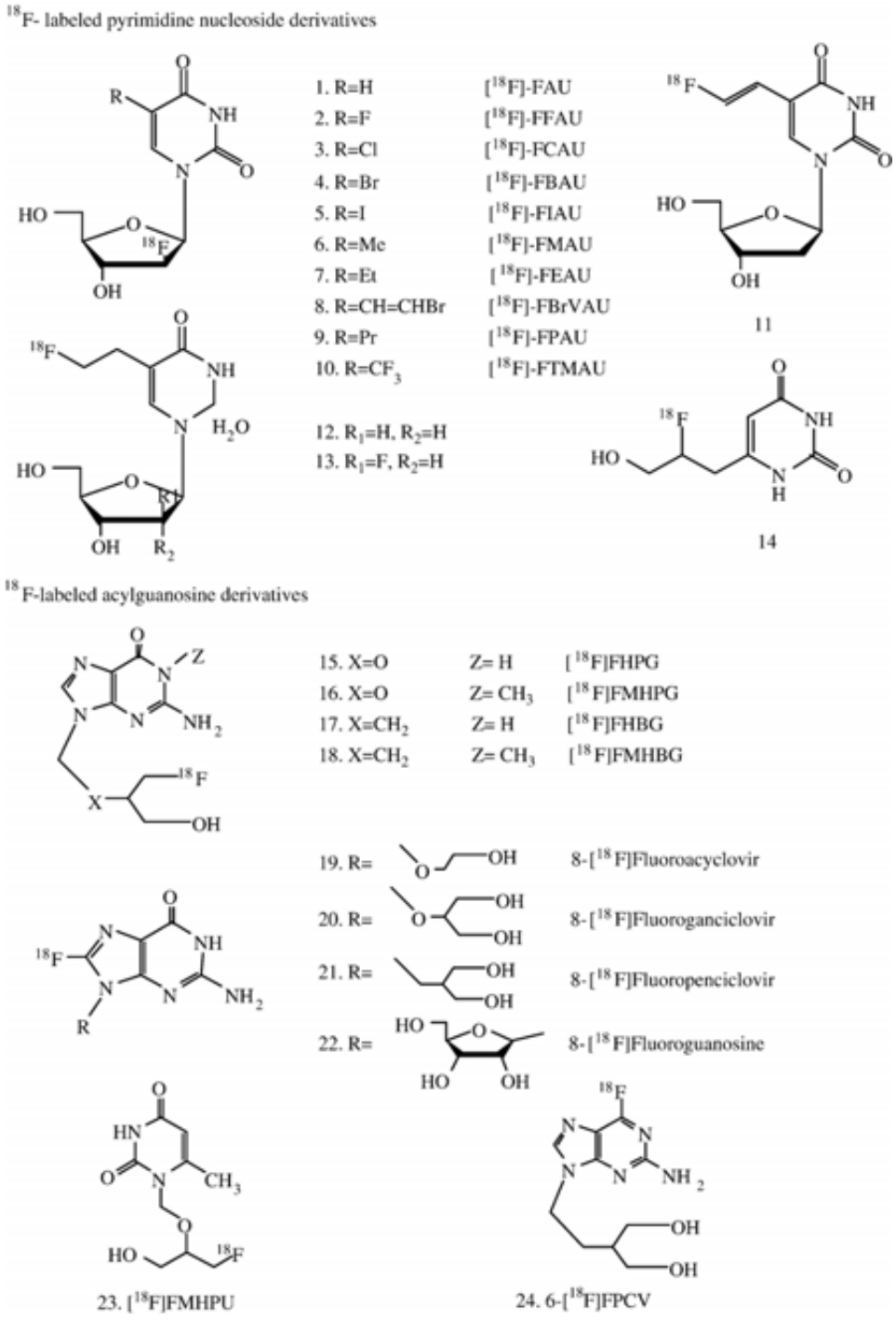

Fig. 1. ^{18}F-labeled PET imaging probes for HSV1-tk reporter gene expression.

investigation of its further potential use as a probe for PET imaging of the reporter gene HSV1-tk [14]. We originally developed a one-step facile nucleophilic substitution method for the preparation of fluorinated penciclovir analogue 2-amino-6-[^{18}F]fluoro-9-(4-hydroxy-3-hydroxy-methylbutyl) purine (6-[^{18}F]FPCV) as a new probe for PET imaging HSV1-tk reporter gene expression [15]. In this work, we report the biological evaluation of 6-[^{18}F]FPCV as a new PET probe for imaging HSV1-tk reporter gene expression in vitro and in vivo.

2. Materials and methods

2.1. Chemicals

2-Amino-9-(4-hydroxy-3-hydroxy-methylbutyl)-*N*,*N*,*N*-trimethyl-9H-purine-6-ammonium chloride (**25**), used as the precursor, and the authentic 2-amino-6-fluoro-9-(4-hydroxy-3-hydroxy-methylbutyl) purine (6-FPCV) were synthesized based on a method developed in our laboratory [16]. No-carrier-added [^{18}F]F$^-$ was supplied by Amersham Kexing Pharmaceuticals Co., Ltd. (Shanghai, China) and was produced via ^{18}O(p, n) ^{18}F reaction using enriched [^{18}O] H_2O on a cyclotron-30 (IBA, Belgium). The other solvents and reagents were commercially available and used without further purification unless stated.

Dulbecco's minimum essential medium (DMEM) and trypsin [2.5% (w/v)] were purchased from Gibco BRL; geneticine (G418), fetal calf serum (FCS) and bovine serum albumin (BSA) were purchased from BioDev Technologies, Inc. (Beijing, China). Goat polyclonal anti-HSV1 thymidine kinase was bought from Santa Cruz; rabbit antigoat HRP/IgG, cell lysis buffer for Western and IP, Prestained Protein Molecular Weight Marker, SDS-PAGE Sample Loading Buffer (5×) and phenylmethanesulfonyl fluoride were purchased from Beyotime Institute of Biotechnology. Phosphate-buffered saline (PBS; final concentrations: 137 mM sodium chloride, 10 mM phosphate, 2.7 mM potassium chloride, pH 7.31) was prepared in our lab.

2.2. Cell lines

C6 rat glioma cells (used as a control cell line) and C6 rat glioma cells transfected with HSV1-tk (C6-tk; gifts from Prof. Jianren Gu, State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Shanghai, China) were cultured as monolayers in DMEM supplemented with 10% FCS and 1% penicillin–streptomycin. Cells were grown in a humidified atmosphere with 5% CO_2 at 37°C. Stable resistance in the C6-tk cells was assured by culturing this line in the presence of 0.1 mg/ml of G418.

2.3. Animal

Chinese Kunming mice (weight, 18–22 g; 6–8 weeks old) and Balb/C nude mice (weight, about 20 g; 6–8 weeks old) were purchased from the Shanghai Experimental Animal Center, Chinese Academy of Sciences. In vivo studies were conducted on Chinese Kunming mice and model animals (tumor-bearing nude mice) at 10 to 180 min after injection. Tumors were grown in Balb/C nude mice by inoculation of 1 million C6 cells (wild type) under the skin on the left flank and 1 million C6-tk cells (HSV1-tk positive) on the right flank. All of the experiments were performed according to China's laws on animal experimentation and the Guide to the Care and Use of Laboratory Animals.

2.4. Western blot analysis

Cell lysate protein was separated by electrophoresis on a 10% polyacrylamide SDS-containing gel at 200 V for 45 min and transferred to a polyvinylidene difluoride membrane at 250 mA for 60 min. The membranes were incubated with anti-HSV1-tk polyclonal antibody [1:5000 dilution in TBS-Tween (TBS with 0.05% Tween)] at 4°C overnight. After washing with TBS-Tween, the membranes were incubated with rabbit antigoat HRP/IgG (1:5000 dilution in TBS-Tween) at 4°C for 1 h. Then, chemiluminescence was detected using the Lumi-Light Plus Western blotting kit (Roche Diagnostics, Mannheim, Germany) after they were washed with TBS-Tween. Membranes were exposed to KODAK Biomax MR films. Films were scanned, and band intensities were determined by densitometry using Diversity One PDI software.

2.5. Immunohistochemistry for HSV1-tk expression

The HSV1-tk expression in cell lines and tumor tissues was tested immunohistochemically with the HSV1-tk-directed antibody. After toasting in a baker at 60°C for 1 h, deparaffinization and three washes with PBS, sections were incubated in 3% H_2O_2 in PBS for 10 min and washed again. The sections, after antigen reparation with EDTA reparation buffer and antigen blocking with rabbit antiserum, and cell-crawling slice were reacted for 2 h at 37°C with goat polyclonal anti-HSV1 thymidine kinase. The sections were reacted with rabbit antigoat biotinylated IgG (1:300 dilution; DAKO, Denmark) and streptavidin (1:300; DAKO) each for 15 min at 37°C. 3-Amino-9-ethyl-carborole was used as the chromogen, which provided a red stain color in areas of HSV1-tk expression. Sections were counterstained with hematoxylin. After differentiation, dewatering with gradient alcohol and mounting with neutral resin, the sections were then tested under a microscope.

2.6. Preparation of 6-[^{18}F]FPCV

The radiosynthesis of 6-[^{18}F]FPCV was carried out with minor modifications to the procedure previously reported, which was synthesized following a method developed in our laboratory as shown in the synthetic scheme (Fig. 2). Briefly, the precursor compound (Compound **25**) was reacted by conventional nucleophilic substitution with K^{18}F/Kryptofix 2.2.2 in DMF at 60°C for 15 min to afford 6-[^{18}F]FPCV. The crude product was purified by a Silica Sep-Pak cartridge using a 50% MeOH/CH_2Cl_2 eluent (4 ml). The pure product was treated with PBS after removal of the solvent.

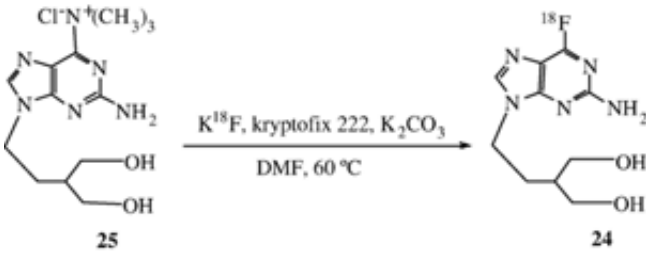

Fig. 2. Radiosynthesis scheme of 6-[^{18}F]FPCV.

2.7. Quality control of 6-[^{18}F]FPCV

PET probe 6-[^{18}F]FPCV in PBS was visually inspected for clarity, absence of color and particulates. An aliquot of the probe solution was removed for the determination of pH using standard pH paper. Chemical and radiochemical purities were also assessed on this aliquot by analytical thin-layer chromatography (TLC) on a silica gel plate 60 F_{254} visualized with a Bioscan AR-2000 radioanalyzer or high-performance liquid chromatography (HPLC) analysis performed on a Dionex system equipped with a P680 pump, PDA-100 photodiode array detector and a NaI(Tl) scintillation detector. The radioactive product was identified by μ-Bondapak C_{18} analytical column (3.9×300 mm) and elution with CH_3CN:H_2O (9:1, v/v) at a constant flow rate of 1.0 ml/min, using 6-FPCV as reference compound (t_R=6.22 min). The moles in an aliquot of final product solution with known amount of radioactivity were determined using HPLC method. The specific radioactivity was calculated by dividing the radioactivity with the number of moles of 6-[^{18}F]FPCV [6].

2.8. Partition coefficient study

Usually, the final partition coefficient value was expressed as log *P*. Log *P* of 6-[^{18}F]FPCV was determined by measuring the distribution of radioactivity in 1-octanol and PBS. A 5-μl sample of 6-[^{18}F]FPCV in PBS was added to a vial containing 1 ml each of 1-octanol and PBS. After vortexing for 5 min, the vial was centrifuged for 5 min to ensure complete separation of layers. Then, 5 μl of each layer was pipetted into other test tubes, and ^{18}F counts were measured using a gamma counter (Shanghai Rihuan Instruments, China). The measurement was repeated three times. Counts of the sample were calculated, and log *P* values were calculated using the following formula [10]:

$$\text{Log } P = \text{Log}\left(\frac{\text{counts in octanol}}{\text{counts in water}}\right). \quad (1)$$

2.9. In vitro stability

The stability of 6-[^{18}F]FPCV was assayed by measuring the radiochemical purity (RCP) at different times after preparation at room temperature. For in vitro stability in PBS or BSA, 100 μl of 6-[^{18}F]FPCV was pipetted into 2 ml of PBS or BSA and incubated in PBS at room temperature or BSA at 37°C. Radio-TLC was carried out to assess the RCP after incubating for different time intervals [17].

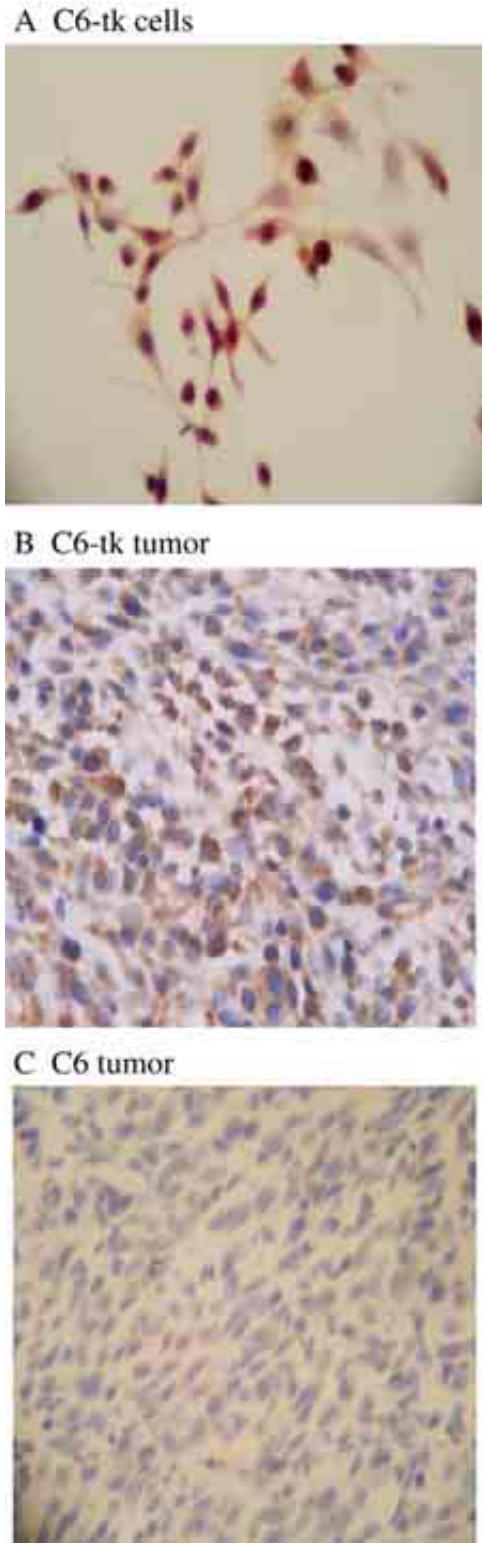

Fig. 3. Immunohistochemical staining of C6-tk cells and the slices of C6-tk and C6 tumors using an anti-HSV1-tk antibody.

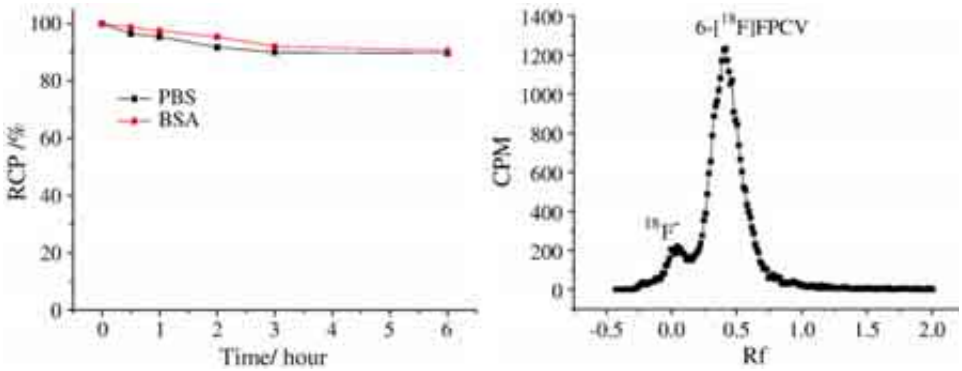

Fig. 4. In vitro stability of 6-[^{18}F]FPCV in PBS and BSA.

2.10. Accumulation of 6-[^{18}F]FPCV in tumor cells

Half million C6 cells and C6-tk cells were seeded in 6-well culture plates with 2 ml of DMEM supplemented with 10% FCS (the culture medium of C6-tk cells in the presence of 0.1 mg/ml of G418). Monolayers were grown, and 5 μCi of 6-[^{18}F]FPCV was added to the medium after 24-h incubation at 37°C. The culture medium was removed after 30–180 min of incubation, and the monolayers were washed three times with 2 ml of cold PBS. The cells were harvested from the culture plates by treatment with 0.3 ml of 0.25% trypsin for 3 min. The cells were resuspended in 2 ml of culture medium to neutralize the trypsin. A 50-μl sample was taken to assess cell viability with trypan blue and count the number of viable cells under a microscope. The radioactivity in the cell suspensions was measured in a gamma counter and normalized to the number of viable cells in the cell suspensions. 6-[^{18}F]FPCV accumulation was expressed as the percentage of the tracer dose accumulated per million cells (%dose/10^6 cells) [18].

2.11. Assay for blood clearance of 6-[^{18}F]FPCV

For blood clearance analysis, five normal mice were intravenously injected with 20 μCi 6-[^{18}F]FPCV. Blood samples were drawn through lateral tail vein at several time points from 1 to 180 min. The blood samples were weighed, and their radioactivity was counted and corrected with a standard 6-[^{18}F]FPCV solution prepared at the time of injection. Counts per minute per milligram of blood (cpm/mg) were plotted as a function of time [19].

2.12. Biodistribution of 6-[^{18}F]FPCV in normal mice and model animals

The in vivo biodistribution of 6-[^{18}F]FPCV in healthy mice was assessed by injecting Chinese Kunming mice with 20 μCi of activity in 100 μl of PBS. Animals were then sacrificed at various time points (10, 60, 90 and 180 min) after injection, and their organs were harvested, weighed and counted to determine the percentage of activity incorporated into the tissues. Each time (data) point was carried out in quintuples. This was expressed as percentage of injected dose per gram of tissue (%ID/g±S.D.).

Biodistribution studies on tumor-bearing nude mice were performed at the 15-min time point. Three model animals

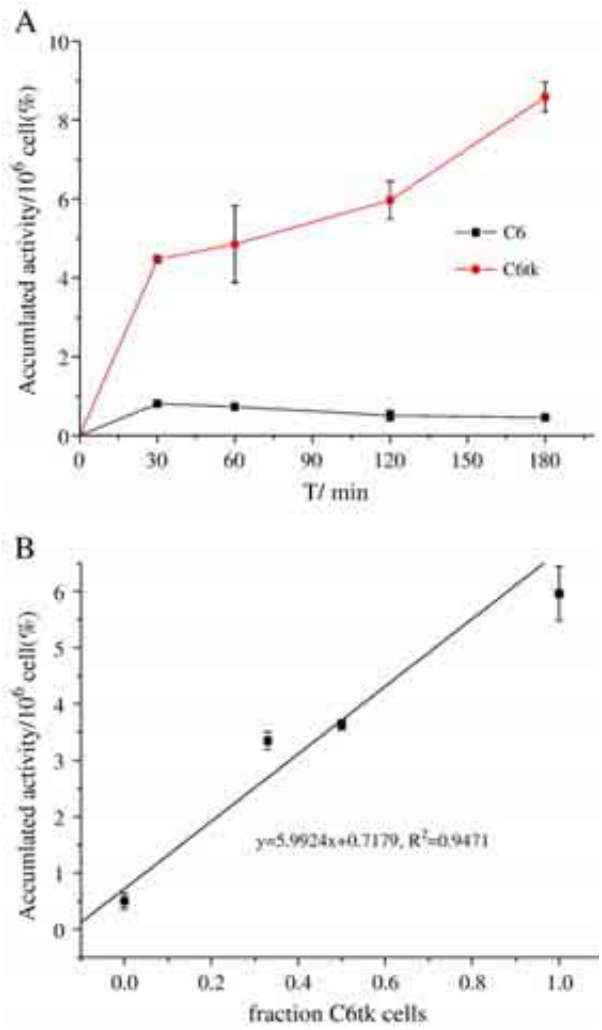

Fig. 5. In vitro 6-[^{18}F]FPCV uptake (%dose/10^6 cells). (A) The uptake of 6-[^{18}F]FPCV versus incubation time in C6-tk and C6 cells. (B) The uptake of 6-[^{18}F]FPCV is proportional to increasing C6-tk cell concentration.

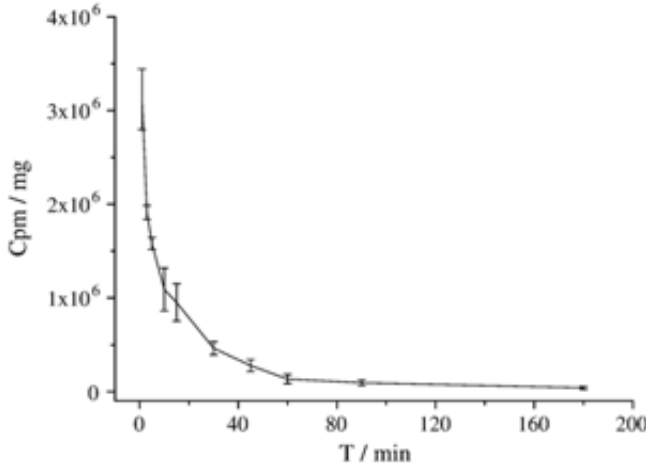

Fig. 6. The time–activity curves (n=5) of 6-[^{18}F]FPCV for blood of normal mouse.

were sacrificed 15 min after injection, and then, tumors were removed, weighed and counted to determine the percentage of activity incorporated into the tissues. This was expressed as %ID/g±S.D.

2.13. Small-animal PET studies

For small-animal PET, animals (n=2) were injected with 6-[^{18}F]FPCV (1.85 MBq, 50 μCi) or [^{18}F]FDG (7.4 MBq, 200 μCi) through the tail vein, and imaging was performed using a micro-PET scanner (Concorde Microsystems, Inc.; spatial resolution of 1.2 mm). Static scans were performed for 30 min with 10-min acquisitions. Initial 40-min dynamic scans of 6-[^{18}F]FPCV were performed to determine the kinetics for tumor uptake of 6-[^{18}F]FPCV (3.7 MBq, 100 μCi) using the following five consecutive frames: 6×10 s, 6×30 s, 6×60 s, 4×300 s, 1×600 s. Images were reconstructed using an ordered-subset expectation maximization algorithm. Region of interest was drawn, and measurements of radioactivity in the C6 and C6-tk were obtained from the sequential images.

3. Results

3.1. Cell line and tumor characterization

The C6-tk cell line showed an intense immunoreactive band for the HSV1-tk protein located at 45 kDa by Western blot experiment. HSV1-tk-directed antibody immunohistochemistry displayed a heterogeneous HSV1-tk expression in the C6-tk cell line (Fig. 3A). The control C6 cells were negative. Scoring for C6-tk revealed strong HSV1-tk staining in approximately 60% of the C6-tk cells. Immunohistochemical staining of the slices of C6-tk and C6 tumors is shown in Fig. 3B and C. The HSV1-tk-directed antibody clearly stained the cells in C6-tk tumors, whereas the control C6 tumors had negative results.

3.2. Radiosynthesis and quality control analysis of 6-[^{18}F]FPCV

The radiosynthesis time of 6-[^{18}F]FPCV was 35–45 min in one step from end of bombardment. 6-[^{18}F]FPCV in PBS solution was colorless at pH 7.0–8.0. 6-[^{18}F]FPCV was characterized by radio-TLC and HPLC, which showed an R_f of 0.63 and a retention time of 6.2 min. The radiochemical yield of 6-[^{18}F]FPCV was 45–55% with more than 99% RCP. The average specific activity of 6-[^{18}F]FPCV was 200 mCi/μmol in five runs.

3.3. Octanol/Water partition coefficient studies

Log P of 6-[^{18}F]FPCV is −0.517, which is in accordance with the calculation of ChemDraw software (log P=−0.52). The corresponding value for [^{18}F]FHBG is −1.19, which is calculated using ChemDraw software.

3.4. In vitro stability

In vitro stability of 6-[^{18}F]FPCV was determined in PBS or BSA using silica gel TLC, in which [^{18}F]fluoride stays at the origin and 6-[^{18}F]FPCV stays at the front (R_f=0.63). An aliquot of the preparation was removed for the determination by radio-TLC at the following time points after incubation: 0.5, 1, 2, 3 and 6 h. After 6 h, the RCP of 6-[^{18}F]FPCV in PBS or BSA is more than 90%. Good in vitro stability in PBS and serum solutions at physiological temperatures was observed, and the results are shown in Fig. 4.

3.5. In vitro uptake studies of 6-[^{18}F]FPCV

C6 rat glioma cells transfected with HSV1-tk (C6-tk) and control C6 cells were incubated with 6-[^{18}F]FPCV for 30–180 min. The tracer uptake in HSV1-tk containing C6-tk cells was 5.5 to 18.8 times higher than that in control cells at 30–180 min, respectively, and reached up to 8.6±0.38% dose/10^6 cells after 3 h of incubation, whereas very low uptake was found in C6 control cells (0.45±0.03%dose/10^6 cells; Fig. 5A). In vitro cellular 6-[^{18}F]FPCV accumulation

Table 1
Biodistribution data of 6-[^{18}F]FPCV in normal mice over time

Tissue	%ID/g			
	10 min	60 min	90 min	180 min
Blood	2.06±0.58	0.27±0.02	0.12±0.03	0.07±0.01
Muscle	1.60±0.41	0.37±0.06	0.17±0.07	0.11±0.05
Bone	3.58±0.30	7.60±1.06	9.05±0.77	10.04±0.23
Spleen	1.58±0.19	0.17±0.01	0.07±0.01	0.05±0.01
Kidney	4.05±0.63	0.41±0.20	0.15±0.05	0.08±0.00
Pancreas	1.13±0.40	0.21±0.05	0.05±0.02	0.04±0.03
Stomach	0.90±0.20	0.21±0.03	0.10±0.03	0.07±0.02
Intestine	3.05±0.76	0.79±0.46	0.13±0.09	0.02±0.00
Liver	4.47±1.26	0.30±0.02	0.16±0.02	0.07±0.02
Heart	2.47±0.09	0.31±0.07	0.08±0.01	0.04±0.01
Lung	1.96±0.40	0.21±0.11	0.08±0.01	0.05±0.01
Brain	0.15±0.03	0.06±0.01	0.05±0.01	0.02±0.00
Skin	1.51±0.11	0.20±0.06	0.09±0.05	0.04±0.01

Data are the mean±standard deviation of five animals.

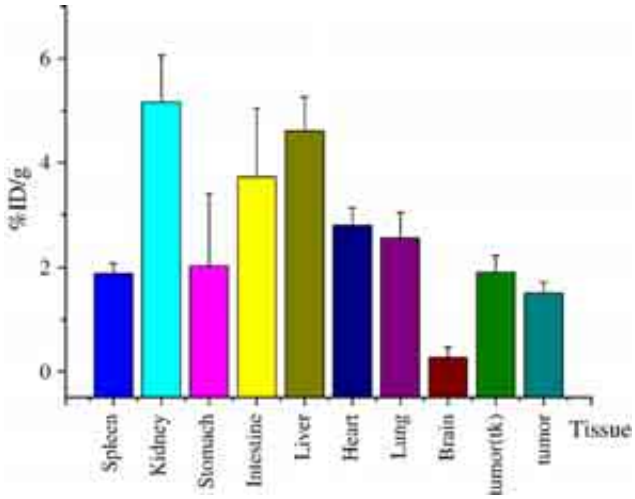

Fig. 7. Biodistribution of 6-[^{18}F]FPCV in tumor-bearing nude mice at the 15-min time point.

linearly increased with increasing portions of C6-tk cells expressing HSV1-tk. An excellent linear correlation was found between the cell uptake of 6-[^{18}F]FPCV with the fraction of C6-tk cells (Fig. 5B).

3.6. Blood clearance of 6-[^{18}F]FPCV

The blood clearance curve for 6-[^{18}F]FPCV in normal mice (n=5) is shown in Fig. 6. Error bars represent the standard deviation. The mean time–activity curve was fitted as a pharmacokinetic two-compartment model and expressed as shown in the equation below. A rapid decrease was observed, followed by a very slow clearance after 30 min. The plasma half-life ($t_{1/2}$) of the tracer in the blood of normal mice was as follows: α $t_{1/2}$=1.2 min and β $t_{1/2}$=73.7 min, which were calculated using Drug and Statistics software DAS 2.0 (Wuhu Gauss Data Analysis Ltd.).

$$C = 59552.06e^{-0.6t} + 368.6405e^{-0.0094t}, \quad (2)$$

where C is the amount of radioactivity per milligram (cpm/mg) in blood of normal mouse and t denotes the time when the sample was taken.

3.7. In vivo biodistribution studies of 6-[^{18}F]FPCV in normal mice and model animals

An in vivo biodistribution study using normal mice was undertaken. The mice were injected with 6-[^{18}F]FPCV through the tail vein and sacrificed at various time points after intravenous injection so that an understanding of the fate of 6-[^{18}F]FPCV in vivo over time could be determined. The results of this study are depicted in Table. 1. A disproportionate uptake of activity into bone, as compared to other tissue, was observed and then increased over time. It was ascertained that this was a result of defluorination, with uptake of fluoride into bone. In vivo studies indicated that 6-[^{18}F]FPCV was undergoing visible defluorination after 1 h. These studies showed that 6-[^{18}F]FPCV was unsuitable as a potential tracer to image reporter gene HSV1-tk expression for long periods.

In an effort to quantify the tracer uptake in C6-tk and C6 tumor, a final biodistribution study involving tumor-bearing nude mice was undertaken. This study was sampled at 15-min time points according to the biodistribution study of normal mice; 50 μCi 6-[^{18}F]FPCV was injected in the tail

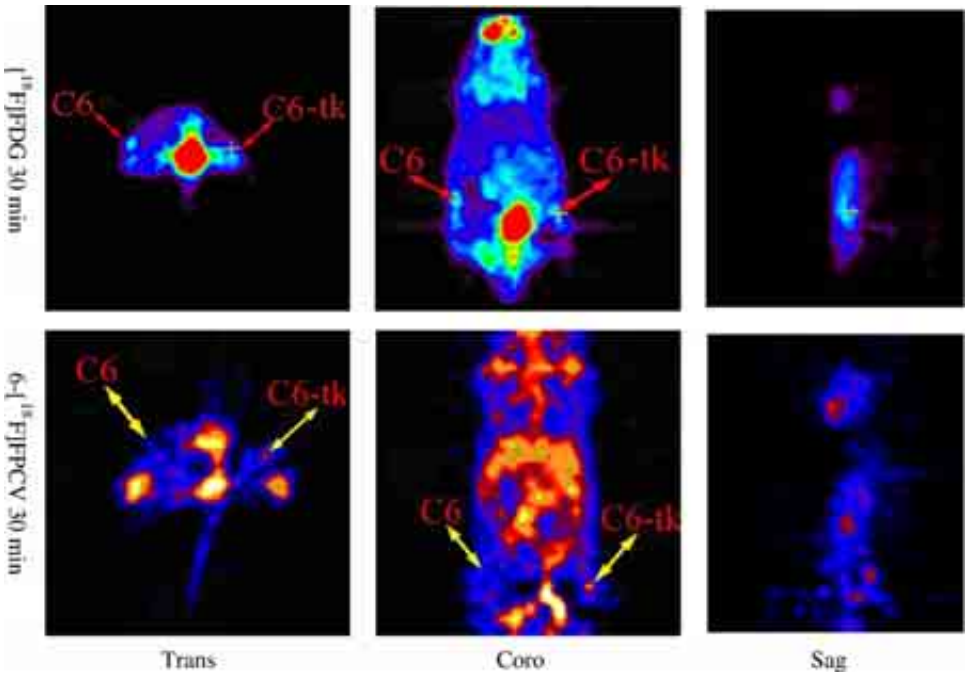

Fig. 8. Micro-PET imaging by 6-[^{18}F]FPCV and [^{18}F]FDG in tumor-bearing nude mice.

vein of the model animal. After 15 min, the mice were sacrificed, and then tumors and some organs were dissected and measured with a gamma counter. Biodistribution data for 6-[^{18}F]FPCV in tumor-bearing nude mice are shown in Fig. 7. The percentage of activity trapped in the tumor of C6-tk (1.91±0.30) was higher than that in the tumor of C6 (1.51±0.20). The average ratio of tracer accumulation between the tumors was 1.69 at 15 min postinjection. The other organs' uptake of tumor-bearing nude mice was similar with biodistribution of 6-[^{18}F]FPCV in normal mice.

3.8. Micro-PET studies of 6-[^{18}F]FPCV in model animals

Micro-PET images on tumor-bearing mice using 6-[^{18}F]FPCV and [^{18}F]FDG are shown in Fig. 8 (top row: 30 min of [^{18}F]FDG; bottom row: 30 min of 6-[^{18}F]FPCV). Tumors were grown with C6 cells, with wild-type cells on the left flank and transduced cells on the right flank. As the images show, the micro-PET images of [^{18}F]FDG show significant uptake both in wild-type C6 tumor and C6-tk tumor at 30 min; some activity remained in the organs and in background circulation. However, 6-[^{18}F]FPCV accumulates visibly in C6-tk tumors grown on the right flank; the left flank, containing C6 tumor, does not show any significant uptake except circulating background activity. 6-[^{18}F]FPCV uptake in the kidney, liver, heart, lung, spleen and intestine is consistent with the biodistribution results of normal mice.

6-[^{18}F]FPCV time–activity curves of C6-tk and C6 tumors in initial 40-min dynamic scans are plotted as Fig. 9. The time–activity curves showed an initial rapid uptake of radioactivity in all tumors, followed by a washout of radioactivity in C6 tumor that was inversely related to C6-tk tumor.

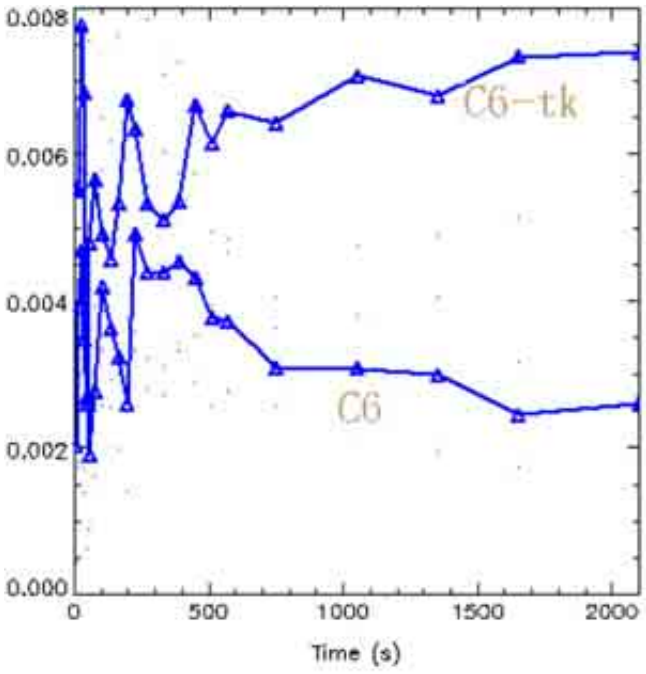

Fig. 9. 6-[^{18}F]FPCV time–activity curves of C6-tk and C6 tumors in initial 40-min dynamic scans.

4. Discussion

Radionuclide reporter gene imaging (SPECT or PET) involves a complementary "reporter gene" and "reporter probe." PET reporter gene imaging method can assess the reporter gene HSV1-tk enzyme in vivo, measure gene expression as an indicator of gene delivery and vector targeting and make noninvasive, repeated and quantitative monitoring of the level of enzyme in gene therapy [20–22]. The novel fluorinated penciclovir analogue 6-[^{18}F]FPCV was developed as a new agent for PET imaging of reporter gene HSV1-tk, which aimed at attaining higher lipophilicity than [^{18}F]FHBG to facilitate passive diffusion across cell membranes while retaining or increasing selective phosphorylation by HSV1-tk. 6-[^{18}F]FPCV was synthesized in good yields. The log P value of 6-[^{18}F]FPCV (log P=−0.517) indicates that this probe is significantly more lipophilic compared to [^{18}F]FHBG (log P=−1.19).

The amount of radioactivity of 6-[^{18}F]FPCV was rapidly decreased in normal mouse blood and followed by a very slow clearance after 30 min. It is beneficial to micro-PET imaging of the probe for decreasing imaging background. 6-[^{18}F]FPCV demonstrates rapid equilibration across cell membranes and selective accumulation in HSV1-tk-transfected C6 cells compared to wild-type C6 cells from in vitro 6-[^{18}F]FPCV accumulation, which was higher or comparable to that obtained with [^{18}F]FHBG and [^{18}F]FHPG in the same cell lines [23]. The uptake ratio (C6-tk to C6 cells) of 6-[^{18}F]FPCV is likely to be higher (more comparable to that of [^{18}F]FHBG) because of the very high uptake in C6-tk cells [23].

In vivo studies, including blood clearance and biodistribution, were performed to understand the pharmacokinetics of 6-[^{18}F]FPCV. Micro-PET imaging was performed for noninvasive measurement of the tracer distribution that reflects gene expression by comparing 6-[^{18}F]FPCV and [^{18}F]FDG PET imaging. Micro-PET images are consistent with the biodistribution data reflecting relative uptake of 6-[^{18}F]FPCV in C6-tk tumors. The results of the biological evaluation presented here suggest that 6-[^{18}F]FPCV could be applied as an alternative PET imaging agent for early imaging reporter gene HSV1-tk expression but is unsuitable to image HSV1-tk expression for long periods due to in vivo defluorination of 6-[^{18}F]FPCV. Further work is required to explore the reasons of in vivo defluorination of 6-[^{18}F]FPCV and to clear them or redesign pyrimidine nucleoside derivatives with pharmaceutical and physiochemical characteristics closer to the natural nucleoside so that incorporation of the compound into the target tissues should be possible.

5. Conclusion

Preparation of 6-[^{18}F]FPCV had been accomplished in yields and purity sufficient for PET imaging studies. Initial in vitro uptake studies were promising, and the in vivo

evaluation showed the potential of 6-[^{18}F]FPCV as a novel probe for HSV1-tk reporter gene early imaging with micro-PET. Unfortunately, 6-[^{18}F]FPCV is unsuitable as a potential tracer to image reporter gene HSV1-tk expression for long periods due to in vivo defluorination of 6-[^{18}F]FPCV.

Acknowledgments

The authors wish to thank Amersham Kexing Pharmaceuticals Co., Ltd., for supplying No-carrier-added [^{18}F]F^{-} solution. We also thank Dr. Zonghai Li of Shanghai Cancer Institute and Jifei Ma of Shanghai Institute of Applied Physics for their help with the cell culture. This project was supported by the Natural Science Foundation of Shanghai (No. 02ZB14061), the Knowledge of Innovation Project of the Chinese Academy of Sciences (No. KJCXI-SW-08) and the National Natural Science Foundation of China (No. 20601028).

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