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Detection of melanoma metastases with PET—Comparison of ^{18}F-5-FPN with ^{18}F–FDG☆

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ABSTRACT

Introduction: ^{18}F-5-fluoro-N-(2-(Diethylamino)ethyl)picolinamide (^{18}F-5-FPN) is a new positron-emission tomography (PET) radiopharmaceutical with potential for the detection of lymph node (LN) and pulmonary metastatic lesions of melanoma. We compared its performance with that of ^{18}F–deoxyglucose (^{18}F–FDG).
Methods: Cervical LN and lung melanoma metastasis models were established in C57BL/6 mice. Primary tumors were created by injection of melanoma cells into the pinna, and the resulting cervical LN metastases were evaluated. Lung metastases were created by intravenous injection of melanoma cells. The mice underwent ^{18}F–FDG and ^{18}F-5-FPN positron emission tomography (PET) imaging. A biodistribution study was conducted after imaging. Histopathologic evaluation of the tumors was also performed.
Results: LN metastases with a diameter < 1 cm were more visible on ^{18}F-5-FPN PET imaging than ^{18}F–FDG imaging. Quantitative analysis showed that the uptake of ^{18}F-5-FPN was significantly higher than that of ^{18}F–FDG, with values of 13.29 ± 3.80% ID/g and 7.24 ± 1.95% ID/g ($n = 5$, $P < 0.05$), respectively. LN-to-muscle ratios were 21.23 ± 6.02 and 4.50 ± 2.11 ($n = 5$, $P < 0.01$) for ^{18}F-5-FPN and ^{18}F–FDG, respectively. Biodistribution results were similar, with high uptake of ^{18}F-5-FPN in the LN. ^{18}F-5-FPN imaging manifested the pulmonary lesions clearly, while the ^{18}F–FDG imaging showed no uptake in lesions <2 mm. The related uptakes of ^{18}F-5-FPN and ^{18}F–FDG were 3.12 ± 1.17% ID/g and 1.48 ± 0.15% ID/g, respectively ($n = 5$, $P < 0.05$), with lung metastasis-to-muscle ratios of 8.16 ± 3.12 and 1.28 ± 0.18 ($n = 5$, $P < 0.01$), respectively. H&E and Prussian blue staining displayed pluri nucleated or mega nucleus cells and dark brown granules in the metastatic tissues, characteristic of melanoma.
Conclusions: ^{18}F-5-FPN targeted small metastatic lesions with a higher target-to-normal ratio of uptake than those of ^{18}F–FDG, which suggests its ability to detect metastatic lesions earlier than ^{18}F–FDG. Further studies with a wide range of melanoma cell lines should be needed to confirm the similar performance.

1. Introduction

Malignant melanoma is the most fatal common skin cancer, with increasing incidence, especially in the Caucasian population, in recent years. It has a high rate of metastasis, and has become a prominent public health problem in European countries, requiring close attention to diagnosis and treatment [1,2]. While immunotherapy, biological therapy and chemotherapy have been developed, and newer treatments are under development, melanoma treatment is not yet ideal [3]. According to the 2008 AJCC Melanoma Staging Database, the 10-year survival rate among 11,841 stage I patients with T_1 melanomas was 92%; one-year survival rates, however, among 7972 stage IV patients were 62% for M1a, 53% for M1b, and 33% for M1c, [4,5]. Effective treatment demands early diagnosis followed by early excision and close surveillance to reduce mortality [1,3].

Radiologic and molecular imaging in the diagnosis and staging of melanoma has been widely studied, and it is more likely to detect distant metastases or occult lesions. X-ray and computed tomography (CT) are inappropriate for early detection of metastases because of the extremely low sensitivity and high false-positive rates [6,7]. High-resolution ultrasound examination cannot reveal distant metastatic lesions [8]. Currently, molecular imaging has achieved prominence in the early diagnosis and accurate staging of metastatic melanoma [2,9]. The integration of scanning of single photon emission or positron-emission tomography (PET) and CT (SPECT/CT, PET/CT) has played a

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significant role in the management of malignant melanoma because its sensitivity shows an outstanding advantage over other traditional imaging technologies [9,10].

^{18}F–FDG, a glucose analog, capitalizes on high glucose consumption in tumors [11]. Although ^{18}F–FDG is commonly used in clinical imaging, reports suggested that the whole-body detection rate for the occult lesions in IB-II staging melanoma patients was considerably low [12]. ^{18}F–FDG also failed to validate metastatic lesions less than 1 cm in diameter located in the lung, liver or brain [10]. Therefore, it has limited capability to identify occult lesions or distant small metastases with low specificity and high false positive rate for melanoma, so that it is not suitable for early diagnosis of metastatic disease [12–14]. Several radiopharmaceuticals, including radiolabeled antibodies, α-melanocyte stimulating hormone (α-MSH) receptor [15–17], benzamide (BZA) [2,18,19] and BZA analogues [20–22], have been developed. Some of them, such as ^{18}F-6-fluoro-N-[2-(diethylamino)ethyl] pyridine-3-carboxamide (^{18}F–MEL050) [20,22] and picolinamide [21] (a BZA derivative), have shown promise for the diagnosis and prognostic evaluation of melanoma [9,21].

In our early research [9], a novel ^{18}F–labeled tracer, ^{18}F-5-fluoro-N-(2-(Diethylamino)ethyl)picolinamide (^{18}F-5-FPN), was prepared. ^{18}F-5-FPN had some advantages such as easy preparation, high stability, tight binding to melanin, and favorable pharmacokinetics, which suggested its potential value in the detection of malignant melanoma. As a BZA derivative, ^{18}F-5-FPN showed much higher binding to B16F10 cells (pigmented) than to A375m cells (nonpigmented) *in vitro* and *in vivo*. Static images acquired 1 h after ^{18}F-5-FPN injection displayed excellent tumor retention and very high tumor-to-background ratios in B16F10-bearing mice with 16.89 ± 6.08 for tumor-to-muscle ratio and 23.33 ± 10.34 ($n = 5$) for tumor-to-blood ratio [9]. The tumor-to-background ratio of ^{18}F-5-FPN in B16F10 tumor was ten times higher than that of ^{18}F–FDG (35.22 ± 7.02 *vs.* 3.29 ± 0.53) [9]. We speculated that ^{18}F-5-FPN may show better performance in the diagnosis of small lymph node and lung metastasis lesions of melanoma.

Therefore, in this study, further research was performed to verify the ability of ^{18}F-5-FPN PET to detect metastatic lesions, and compared it with ^{18}F–FDG. The aim was to demonstrate the potential clinical application of ^{18}F-5-FPN for the staging of malignant melanoma.

2. Materials and methods

2.1. Synthesis of ^{18}F-5-FPN and ^{18}F–FDG

We synthesized ^{18}F-5-FPN according to our preciously published protocol with a radiosynthesis module (GE TraceLab FX_{FN}; GE Healthcare, Milwaukee, WI) [9]. The radiochemical yields of 6.98% (n = 6) and the radiochemical purity of more than 95% were consistent with our previous results. Details of the procedures of the synthesis and chemical structure of the probe are available [9].

^{18}F–FDG was synthesized (GE-MX_{FDG}, General Electric™, Liege, Belgium) with radiochemical yields of 65% and radiochemical purity of >97%.

2.2. Cell culture

The B16F10 melanoma cell line was cultured in Dulbecco's modified Eagle's medium (Gibco™, Carlsbad California, USA) supplemented with 10% fetal bovine serum (Gibco) with 1% penicillin–streptomycin solution (100 U/mL; Beyotime™, Shanghai, China) maintained in a humidified incubator containing 5% CO_2 at 37 °C. Cells in the logarithmic growth phase were used in the subsequent protocols.

2.3. Preparation of animal metastasis models of melanoma

C57BL/6 specific pathogen-free mice (male, 4–6 weeks, HFK Bioscience Co., Ltd.™, Beijing, China) were maintained under specific pathogen-free conditions, within facilities approved by the Laboratory Animal Care of Huazhong University of Science and Technology, and in compliance with the regulations and standards of the Institutional Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science and Technology.

An aliquot of 2×10^5 B16F10 cells in 25 μL phosphate buffered saline (PBS) was injected subcutaneously into the right pinna of mice for the cervical lymph node (LN) metastasis preparation [23,24]. According to our preliminary experiments (unpublished data), to successfully prepare LN metastasis models, the primary tumor should be removed. At the 11th d after injection of B16F10 cells, the primary tumors on the right pinna were excised, and H&E and Prussian blue staining were performed. Then, mice with LN metastases were injected *via* tail vein with ^{18}F–FDG and ^{18}F-5-FPN on the 20th and 21st d after the injection of tumor cells, respectively, for PET scanning.

To prepare the pulmonary metastasis models, 2×10^5 B16F10 cells suspended in 100 μL PBS were injected *via* tail vein. ^{18}F–FDG and ^{18}F-5-FPN PET imaging were performed on the 13th and 14th d after the injection of tumor cells, respectively.

2.4. Small-animal PET scanning

All PET images were obtained with a small-animal PET scanner (BioCaliburn LH Raycan Technology Co., Ltd. ™, Suzhou, China). The intrinsic spatial resolution was 1.0 mm, the time resolution was 1.50 ns full-width at half-maximum, the energy resolution was 13.0% at 511 keV and the axial field of view was 5.3 cm. Mice were anesthetized with 2% isoflurane in 100% O_2 for maintenance during imaging. Mice were placed in the supine position, and static images for 10 min were acquired 1 h after injection of ^{18}F–FDG (3.7 MBq) after fasting for 6–8 h or ^{18}F-5-FPN (3.7 MBq) after routine feeding.

Open-source software (Amide.exe 1.0.4, SourceForge.net) was used for quantitative analysis of the image data. Regions of interest (ROIs) were manually drawn on the decay-corrected whole-body maximal intensity projection (MIP) images with surrounding healthy tissue as the background, followed by calculating the percentage injected dose per gram of tissue (% ID/g) and the ratio of tumor uptake to uptake by normal organs or tissues (tumor-to-normal ratio, TNR).

2.5. Biodistribution studies

Five mice with cervical LN metastases were sacrificed immediately after the ^{18}F-5-FPN PET scanning for biodistribution studies. LN metastases; normal tissues and organs, including, eye, blood, brain, heart, lung, liver, spleen, kidney and muscle were collected and weighed and their radioactivity measured with a gamma counter (2470 Automatic Gamma Counter WIZARD, PerkinElmer, Norwalk CT, USA). We calculated the % ID/g and the TNR values. The lesions were then stained for histopathology.

2.6. H&E and Prussian blue staining

The primary tumors and metastases were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4 μm slices for H&E and Prussian blue staining. For the Prussian blue staining, the slices were dewaxed, washed with distilled water, and then dyed dropwise with a mixture of 2% potassium ferrocyanide and 2% hydrochloric acid (1:1) for 20 min. The slices were washed with distilled water, counterstained with nuclear fast red for 10 min and dehydrated before sealing.

2.7. Statistical analysis

All quantitative data are presented as mean ± standard deviation (SD). Commercial software (GraphPad Prism 6.0 GraphPad Software™, La Jolla, CA, USA) was applied to analyze the data of the biodistribution study. Data were compared using the paired *t*-test with a P value <0.05 indicating significance.