Imaging proliferation in human leukemia-tumor bearing mice with ^{18}F-FLT: Comparison with ^{18}F-FDG PET

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Abstract

Leukemia threatens human life due to its uncontrolled proliferative malignancy. 3'-deoxy-3'-^{18}F-fluorothymidine (^{18}F-FLT) has been suggested as a new positron emission tomography (PET) tracer for imaging tumor proliferation. *The aim of the study* was to investigate the usefulness of ^{18}F-FLT PET for imaging human leukemia-tumor bearing mice, compared with fluorine-18-fluorodesoxyglucose (^{18}F-FDG PET). *In vitro the experiments* of ^{18}F-FLT and ^{18}F-FDG uptake were performed in K562 cell lines at various time points and radioactive tracer uptake was measured in a gamma counter. *^{18}F-FLT and ^{18}F-FDG* PET imaging were performed both in the same mouse when eight tumor-bearing mice models of human chronic myeloid leukemia were established successfully by injecting K562 cells. Regions of interest were drawn over the tumor, the crossed normal tissue was regarded as background and the ratio of tumor to non-tumor counts (T/NT) in tissues was calculated. *A higher uptake of ^{18}F-FLT* (15min, 5.73±0.05%; 30min, 5.90±0.06%; 60min, 6.16±0.19%; 120min, 6.32±0.08%) than that of ^{18}F-FDG (15min, 1.05±0.10%; 30min, 1.11±0.14%; 60min, 1.14±0.37%; 120 min, 1.36±0.25%) was observed in K562 cells in the tracer uptake experiment. Ratios of T/NT of ^{18}F-FLT PET (0.5h, 5.39±0.42; 1h, 4.88±0.43; 2h, 3.81±0.38) were higher than those of ^{18}F-FDG PET/CT (0.5h, 0.34±0.12; 1h, 0.21±0.06; 2h, 0.13±0.05) after injection. Both uptake and T/NT differences of ^{18}F-FLT versus ^{18}F-FDG were significant (P>0.05). *In conclusion*, ^{18}F-FLT and ^{18}F-FDG quantitative and semi-quantitative uptake measurements resulting from cell lines and PET imaging respectively suggested a promising potential of ^{18}F-FLT for metabolic imaging of human chronic myeloid leukemia.

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Introduction

In clinical oncology, most investigators are using the glucose analogue ^{18}F-fluorodeoxyglucose (^{18}F-FDG) as PET radiotracer for characterizing malignant tumors. Although this radiotracer generally has high uptake in cancerous lesions and low uptake in benign lesions, it is not tumor specific, and worse, it may not provide direct information about cellular proliferation which is vital for clinical management of cancer patients. The other radiotracer ^{18}F-fluorothymidine (^{18}F-FLT), a pyrimidine analogue, can be used to define the salvage pathway of DNA synthesis to reveal cell proliferation in vivo [1]. Several studies have indicated that ^{18}F-FLT can be useful for non-invasive assessment of the proliferation rate of several types of cancer, such as colorectal, esophageal, breast and laryngeal cancer and acute leukemia [2-5].

Chronic myeloid leukemia is a clonal disease of hematopoietic progenitor cells which partially or completely loose the ability to differentiate and to respond to normal regulations of cellular proliferation but maintain an abnormally increased proliferation capacity. We wondered whether measurement and imaging of proliferation with ^{18}F-FLT PET could be a better noninvasive tool for imaging proliferation in human chromic myeloid leukemia, compared with ^{18}F-FDG PET. For this purpose, uptake assay of ^{18}F-FDG and ^{18}F-FLT in K562 cells and tumor uptake comparison between ^{18}F-FDG and ^{18}F-FLT in K562-bearing mice with PET were performed in this study.

Materials and methods

Radiopharmaceuticals

Synthesis of ^{18}F-FLT was performed according to the method of Machulla et al (2000) [6, 7]. The ^{18}F-FLT was produced by ^{18}F-fluorination of the 4,4'-dimethoxytrityl-protected anhydrothymidine, followed by a deprotection step. After purification by reversed-phase high-performance liquid chromatography, the product was made isotonic and passed

through a 0.22-μm filter. Fluorine-18-FLT was produced with a radiochemical purity of >95% and a specific activity of <10TBq/mmol. The radiochemical yield was 7.5±5.1% (at end of bombardment). Fluorine-18-FDG was acquired from daily supplies for routine clinical diagnoses (Huashan PET centre, Shanghai, China).

Chemicals

Medium RPMI (Roswell Park Memorial Institute) 1640, fetal bovine serum (FBS) and penicillin/streptomycin (10,000IU/mL) were obtained from GIBCO Company (USA). All chemicals were of reagent grade.

Cell lines

Cell lines K562 derived from human chronic myeloid leukemia were purchased from the Institute of Cell Biology, Chinese Academy of Science (Shanghai, China). K562 cell lines were cultured in RPMI 1640 medium supplemental with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (10,000IU/mL). The cells were incubated at 37°C in humidified air with 5% CO_2/95% air atmospheric. The medium was replaced thrice weekly.

Radiotracer uptake experiments

Cells lines K562 (1X10^{5}cells per well) were seeded in 24-well plates (Costar, Corning, USA). Cells were grown overnight preceding the uptake experiment. The radiotracer (37KBq ^{18}F-FDG, 37KBq ^{18}F-FLT) was added to each well using a volume of 5μL normal saline (NS). Cells were incubated at 37 °C for 15, 30, 60 and 120min, then centrifuged at 10,000R/min for 5min. The supernatant was removed and cells were washed twice with RPIM 1640 medium. Cells were finally incubated in 1mL RPMI 1640 medium and then radioactive tracer uptake was measured in a gamma counter. Standard pipe with only 1mL RPMI 1640 medium and 37KBq radiotracer was included in every experiment as a reference to be able to compare the tracer uptake among the cells (uptake in cancer cells/ uptake in standard pipe). Experiments were performed twice with triplicate wells.

Animals and tumor models

Fourteen five-week-old male Balb/C nude mice (13g) were obtained from the Animal Laboratory of the Chinese Academy of Sciences (Shanghai, China, SCXK 2007-0005). Subcutaneous tumors were established in the right shoulder of Balb/C nude mice by inoculating 2.4X10^7 cells in 100μL of RPMI 1640 medium. Mice were bred and maintained in a specific pathogen-free animal facility with a standard light/dark cycle, at 22±1°C and a relative humidity of 40%-70%. Food and water were supplied ad libitum. And in this study, cages and water bottles were changed once a week. Tumor size in the shoulder was determined by caliper measurement at least twice a week by the formula V=1/2(lXwXh) (l, length; w, width; h, height of the tumor). When the tumors reached a volume of >500mm^3, PET with ^{18}F-FLT and ^{18}F-FDG experiment was performed 15-17 days after implantation. The experimental protocol was conducted with sterile techniques in accordance with the Guide for the Care and Use of Laboratory [8].

^{18}F-FLT and ^{18}F-FDG PET imaging

Firstly, mice were fasted overnight before each ^{18}F-FDG injection but allowed free access to water. Secondly, mice were injected with ^{18}F-FLT or ^{18}F-FDG, 555MBq/kg, via lateral tail vein. Intravenous pentobarbital, 30mg/kg, was given before PET scanning. ^{18}F-FLT or ^{18}F-FDG PET images were acquired during 20min at a single-bed position with the tumor in the center of the field, on a Discovery LS PET system (GE Healthcare Bio-Science Corp.). The acquisition took place at the time point of 0.5h, 1h and 2h after each tracer injection, respectively.

The region of interest (ROI) was drawn on the PET images manually by qualitative assessment over the tumor and the crossed normal tissue as background. The ratio of tumor to non-tumor (T/NT) in tissues was also calculated.

Statistical analysis

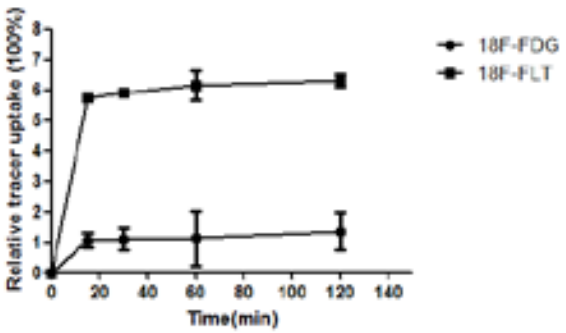
Calculations were made in SPSS 17.0. All data were expressed as mean±SD and differences between the various groups (^{18}F-FLT and ^{18}F-FDG) were tested for statistical significance using 2-sided Student's *t* test for independent samples. A value of P less than 0.05 was considered statistically significant.

Results

Cell uptake assay of ^{18}F-FDG and ^{18}F-FLT

The uptakes of ^{18}F-FLT and ^{18}F-FDG in K562 cells were both evaluated and the results are showed in Graph 1. During the first 15min incubation, rapid cell accumulation of ^{18}F-FLT and ^{18}F-FDG was noted. Later on time, the cellular uptakes of ^{18}F-FLT and ^{18}F-FDG became pretty stable.

The K562 cell uptake values of ^{18}F-FLT after 15, 30, 60, 120min of tracer incubation at 37°C were 5.73±0.05%, 5.90±0.06%, 6.16±0.19% and 6.32±0.08%, respectively. In comparison, cell uptake values of ^{18}F-FDG after 15, 30, 60, 120min of tracer incubation at 37°C were 1.05±0.10%, 1.11±0.14%, 1.14±0.37% and 1.36±0.25%, respectively. A significantly higher uptake of ^{18}F-FLT than that of ^{18}F-FDG was observed. The ratio of ^{18}F-FLT uptake was almost 5 times higher than that of ^{18}F-FDG at each time point in Graph 1.



Graph 1. Relative ^{18}F-FDG and ^{18}F-FLT uptake in the K562 cell lines after 15, 30, 60 and 120min. Tracer uptake in the cancer cells was normalized to the uptake in standard pipe. Error bars indicate standard errors (n=4).

Different uptake patterns between ^{18}F-FDG and ^{18}F-FLT in K562- bearing mice

In this study, tumor-bearing human chronic myeloid leukemia models were successfully established in eight mice. When the volume of tumors reached >500mm^3, PET with ^{18}F-FLT and ^{18}F-FDG was performed. One typical image of ^{18}F-FLT and ^{18}F-FDG PET on a tumor-bearing mouse is shown in Figures 1 and 2.

As seen in Figure 1, there was hardly any significant ^{18}F-FDG uptake in the right shoulder which was seeded with tumor-bearing human chronic myeloid leukemia, at 0.5h (Fig. 1A), 1h (Fig. 1B) and 2h (Fig. 1C) after radiotracer injection. However, obvious ^{18}F-FLT uptake was clearly seen in the tumor foci at 0.5h (Fig. 2A), 1h (Fig. 2B) and 2h (Fig. 2C) after radiotracer injection. Moreover, the radioactivities of ^{18}F-FLT PET decreased gradually 0.5h after the radiotracer injection.

Tumor/NT ratios of ^{18}F-FLT PET after 0.5, 1 and 2h of tracer injection were 5.39±0.42, 4.88±0.43 and 3.81±0.38, respectively. And T/NT ratios of ^{18}F-FDG PET after 0.5, 1 and 2h of tracer injection were 0.34±0.12, 0.21±0.06 and 0.13±0.05, respectively.

A significantly higher T/NT ratio of ^{18}F-FLT PET than that of ^{18}F-FDG PET was viewed and this difference was significant (P>0.05).

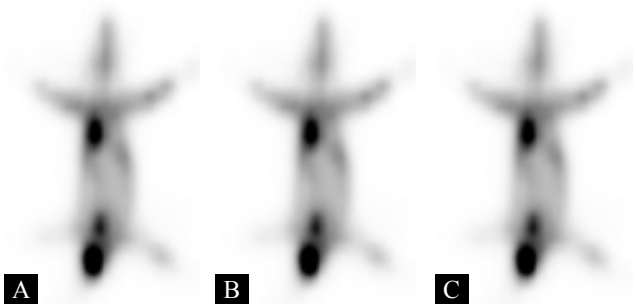


Figure 1. Coronal PET images of ^{18}F-FDG show nearly no uptake of ^{18}F-FDG in the right shoulder at 0.5h (A), 1h (B) and 2h (C) after injection.

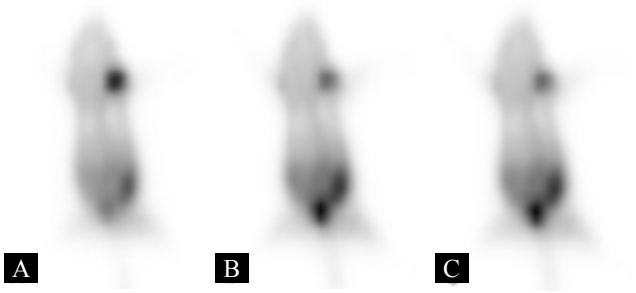


Figure 2. Coronal PET images with ^{18}F-FLT demonstrate increased uptake of ^{18}F-FLT in the right shoulder at 0.5h (A), 1h (B), 2h (C) after injection of the radiopharmaceutical in the same mouse.

Discussion

Much as a very useful radiotracer for cancer diagnosis, ^{18}F-FDG may be found to have limited value for those cancer cells with normal or low glucose metabolism, as in our study K562 cell lines derived from human chronic myeloid leukemia. On the other hand, ^{18}F-FLT is a substrate for cytoplasmic thymidine kinase 1 (TK1) [9]. The rationale for using ^{18}F-FLT PET in measuring the proliferative activity of tumors is based on the S-phase-specific induction of TK1 at the transcriptional and posttranscriptional level [10]. So ^{18}F-FLT regarded as a cell proliferation tracer, may be more cancer-specific than ^{18}F-FDG in those cancers with low glucose metabolism but high proliferation rates [11-13].

Chronic myeloid leukemia is a clonal stem cell disorder characterized by excessive proliferation of cells of the myeloid series. The usefulness of ^{18}F-FDG PET for the diagnosis and monitoring of chronic myeloid leukemia after treatment has been reported. [14] Several studies have reported that ^{18}F-FLT PET was able to visualize extramedullary manifestation sites of acute myeloid leukemia [2]. This is the first study investigating ^{18}F-FLT PET as a potential tool for imaging tumor-bearing human chronic myeloid leukemia in mice compared to ^{18}F-FDG PET.

Our first thought could be that both ^{18}F-FLT and ^{18}F-FDG are taken up by malignant cells but that the demand for glucose is larger than that for thymidine. However, in our study, in contrast to ^{18}F-FDG, an elevated ^{18}F-FLT uptake was observed in all K562 cells with significant difference (Graph 1). And in the PET study, we found a higher relative uptake of ^{18}F-FLT than ^{18}F-FDG in K562 tumor (Fig. 1 and 2).

Other studies [15,16] have reported that the glucose analogue showed a biodistribution that was expected on the basis of clinical ^{18}F-FDG PET scans, that is, high and physiologic uptake in brain, heart and inflamed muscle, besides renal excretion and accumulation in urine. In contrast to ^{18}F-FDG, ^{18}F-FLT accumulated more in bone, bone marrow, and healthy muscle. Therefore, ^{18}F-FLT could reflect cellular proliferation of bone marrow in patients with chronic myeloid leukemia. Other researchers [14] confirmed that ^{18}F-FDG could also image chronic myeloid leukemia, but there was nearly no uptake of ^{18}F-FDG in the tumor bearing K562 cell lines. This can illustrate the reason why one could have thought that ^{18}F-FDG could not image chronic myeloid leukemia in mice.

Furthermore, after the first 15min, the cellular uptakes of ^{18}F-FLT and ^{18}F-FDG became pretty stable (Graph 1), and the radioactivity of ^{18}F-FLT PET dropped gradually 0.5h after the radiotracer injection (Fig. 2), so the optimal time for acquisition should be after 15min and not after 1h.

Our findings suggest that ^{18}F-FLT is a better indicator of cell proliferation than ^{18}F-FDG when working with cultured cells and animal tumors in agreement with other studies [17, 18]. According to our knowledge, this is the first study of comparing these two radiopharmaceuticals in the same mice. The limitations of our study are the small sample, and that further studies should investigate whether ^{18}F-FLT is also better in humans.

In conclusion, compared with ^{18}F-FDG PET, ^{18}F-FLT PET was better for imaging chronic myeloid leukemia in mice.

The authors declare that they have no conflicts of interest.

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