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Original Research Article

Evaluation of Therapeutic Effects of Radiotherapy during Treatment of Lung Adenocarcinoma in Mice with Positron Emission Tomography Imaging of ^{18}F-FLT and ^{18}F-FDG

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Abstract

Purpose: *To assess the role of 3'-deoxy-3'-^{18}F-fluoro-thymidine (^{18}F-FLT) and 2'-deoxy-2'-^{18}F-fluoro-deoxy-glucose (^{18}F-FDG) positron emission tomography (PET) imaging in the evaluation of the therapeutic effects of radiotherapy during treatment of lung adenocarcinoma.*

Methods: *Eighteen mice with lung adenocarcinoma were randomly divided into 2 groups; each group was randomly paired and evenly divided into three smaller groups, namely, A, B and C. Group A served as the control group without any treatment; mice in group B were received radiotherapy in sites of tumor. A single dose of 2000 cGy, 6MV x-ray was used in this experiment; mice in group C also received radiotherapy at sites of tumors two days before the experiment using the same procedure and dose as group B. Micro PET imaging was taken after intravenous injection of ^{18}F-FLT and ^{18}F-FDG through the mice's tail.*

Results: *The intake ratio of ^{18}F-FLT and ^{18}F-FDG was much higher at the tumor sites. After radiotherapy, ^{18}F-FLT uptake was significantly lower than that of the control group ($p < 0.05$), while there was no obvious change of ^{18}F-FDG uptake. There was a significant decrease in T/NT value of FLT PET imaging group 24 and 48 h after radiotherapy; a significant difference could be seen, compared with that before radiotherapy ($p < 0.05$).*

Conclusion: *Change in ^{18}F-FLT uptake induced by radiotherapy is more sensitive than that of ^{18}F-FDG. Intake of ^{18}F-FLT is lowered more significantly after radiotherapy than that of ^{18}F-FDG, and this can serve as evidence that ^{18}F-FLT is an effective tracer to monitor the therapeutic effect of radiotherapy on malignant tumors.*

Keywords: *3'-deoxy-3'-^{18}F-fluoro-thymidine, 2'-deoxy-2'-^{18}F-fluoro-deoxy-glucose, Radiotherapy, Positron Emission Tomography imaging, Cell proliferation, Lung adenocarcinoma*

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INTRODUCTION

Positron emission tomography (PET) is a rapidly developing imaging technology in recent years, which has significantly benefited the diagnosis, staging and especially the therapeutic effect evaluation of radiotherapy for malignant tumors [1-4]. ^{18}F-FDG is the most commonly-used tracer in PET-CT clinical examination, but there may be some false positives since ^{18}F-FDG is not a tumor-specific tracer and can be ingested by inflammatory cells, granulation tissue, etc. ^{18}F-FLT, the derivative of thymidine, has drawn a lot of attention because it can reflect the value-added features of tumors [5,6].

The purpose of this study was to assess the bio-distribution regularity of ^{18}F-FLT in mice and the feasibility of PET imaging by constructing the lung adenocarcinoma model of tumor-bearing mice; mice with lung adenocarcinoma were treated with radiotherapy. The role of ^{18}F-FDG and ^{18}F-FLT in monitoring the therapeutic effects of radiotherapy treated lung adenocarcinoma was evaluated by comparing the changes of PET imaging of tumor ingesting ^{18}F-FDG and ^{18}F-FLT before and after the treatment.

EXPERIMENTAL

Material

Six-week old Balb/c-nu mice, weighing 18 to 20 g, half male and female, provided by Experimental Animal Center of PLA General Hospital, were reared in the laminar flow racks in the Experimental Animal Center of PLA General Hospital according to the Specific Pathogen Free (SPF) standard. Experimental animal use certificate issued is no. 2006E00699.

Tracers: 3-deoxy -3-^{18}F-fluoro-thymidine (^{18}F-FLT) and ^{18}F-fluoro-deoxy-glucose (^{18}F-FDG) provided by the Nuclear Medicine PET Laboratory of People's Liberation Army General Hospital. FT603 well-type detector was made by Beijing 261 Factory. PET imaging system: eXplore VISTA-CT MicroPET/CT, GE, made in USA. Linear accelerator: Swedish Elekta Precise linear accelerator. Tumor lines: A549 human lung adenocarcinoma cell line, provided by the Cancer Institute of Chinese Academy of Medical Sciences Test solution: DMEM medium (Gibco, USA), RPMI-1640 medium (Gibco, USA), fetal bovine serum (Huamei biological preparation company) and trypsin (Sigma, USA).

Establishment of tumor-bearing mice model

Single cell suspension (2×10^5/mL) was conventionally prepared using the normal sub-cultured A-549 tumor cell line of lung adenocarcinoma, 0.2 mL of which was taken and subcutaneously inoculated in the right armpit of the mice. The subcutaneous inoculations of the mice resulted in tumors whose diameters ranged from 1.0 to 1.2 cm. The experiment was performed in 6 or 7 days after inoculation.

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

The tumor-bearing mice were randomly and evenly divided into the FDG group and FLT group of 3. The two groups were injected with 0.2 mL ^{18}F-FLT (3.7 MBq) and 0.2 mL ^{18}F-FDG (3.7 MBq) respectively via the tail veins. PET imaging and determination of bio-distribution measure were carried out in 60 min after the injection.

After being anaesthetized with 2 mL of 10 % chloral hydrate, the mice were laid on the micro PET/CT beds, with limbs fixed with tape, and were scanned for 10min. The 3D mode was used to capture images. The scanning results were analyzed by dispersed and random counting and reconstruction of the cross-sectional images of the coronal plane, transverse section and sagittal plane.

Image data analysis

The software package of MicroPET/CT acquisition system was used to analyze the image data. A region of interest (ROI) was outlined in the best level of tumor selected in the image. A ROI of the same size was outlined in the contralateral tumor-free area as a control. The average count value and standard deviations were recorded within the ROI with the unit of value/pix. The T/NT value was calculated.

Evaluation of the therapeutic effect of radiotherapy

Eighteen mice with lung adenocarcinoma were randomly divided into ^{18}F-FLT group and ^{18}F-FDG goups and then each group was randomly paired and evenly divided into three smaller groups, namely, group A, B and C. Group A served as the control group without any treatment; group B were anaesthetized with isoflurane one day before the experiment, and then received radiotherapy in sites of tumors while being placed under fixed linear accelerator.

A single dose of 2000 cGy, 6 MV energy and x-ray were used in this experiment; mice in group C also received radiotherapy two days before the experiment in sites of tumors with the same methods and radiotherapy dose as group B. Methods of microPET/CT imaging of the tumor-bearing mice and image data analysis were same as those of group B.

Statistical analysis

For statistical analysis, SPSS 12.0 statistical software was used to carry out t-test and correlation analysis of the mean of two groups. $P < 0.05$ was considered statistically significant.

RESULTS

In vivo biodistribution and PET imaging

Biodistribution of two tracers in tumor-bearing mice

The tumor-bearing mice both in the FLT group and the FDG group were killed 60 min after injection, and their organs and tissues were separated to detect radioactive distribution (Table 1). The radioactive uptake of the FLT group was significantly lower than that of the FDG group. Tissues or organs with the highest radioactive uptake were, in order of the heart, tumor and kidney; except for heart and kidney, the T/NT values of tumors to other normal tissues were all greater than 2. Organs or tissues of the FDG group with the highest radioactivie uptake were, in order of the kidney, tumors, spleen and liver; except for the first three, the T/NT values of tumors to other normal tissues were all greater than 2. There was a significant difference ($p < 0.05$) between the two groups' T/NT values of tumors to heart, liver and kidney.

After comparing the biodistribution of the two tracers in tumor-bearing mice, it could be seen that in the FDG group, except for heart and kidney, the T/NT values of tumors to other normal tissues were all greater than 2; in the FDG group; except for kidney, spleen and liver, the T/NT values of tumors to other normal tissues were all greater than 2. There was a significant difference between the two groups' T/NT values of tumors to heart, liver and kidney ($p < 0.05$).

Moreover, PET imaging was made in the FLT group and FDG group, 60 min after injection. After being processed by the software package of microPET/CT acquisition system, it could be seen that the tumor uptake was significantly higher than before. A region of interest (ROI) was outlined in the best level of tumor in the image and another ROI of the same size was outlined in the contralateral tumor-free area as a control. The average count value and standard deviations were recorded within the ROI with the unit of value/pix. There were significant differences between the T/NT values of FLT group and FDG in 60 min after the injection (FLT 2.4 ± 0.2, FDG 4.3 ± 0.3, $p < 0.05$).

MicroPET imaging (coronal plane) of ^{18}F-FLT in mice with lung adenocarcinoma 60 min after injection showed that tumors inoculated in the left forelimbs of mice were evenly assimilated. MicroPET imaging (coronal plane) of ^{18}F-FDG in mice with lung adenocarcinoma 60 min after injection showed that tumors inoculated in the left forelimbs of mice had significant uptake. Tumor necrosis could be seen in intratumoral areas with low intake.

Therapeutic effect of radiotherapy

Tumor % ID/g of the two tracers in tumor-bearing mice of the two groups are shown in Table 2. In the radiation experiments, six groups of mice with two tracers were killed 60 min after injection. The well-type detector was used to detect the radioactivity distribution (% ID/g) of tumors. The tumor tissue % ID/g of group B and group C, which were treated with radiotherapy in FDG group, decreased a little bit more than that of the control group (A), but there was no significant statistical difference ($p > 0.05$), while the tumor tissue % ID/g of group B and group C in the FLT group, decreased much more significantly when compared with group A. The difference between them was statistically significant ($p < 0.05$).

The tumor tissue % ID/g of group B and group C in the FDG group, decreased a little bit more than that of the control group A after radiotherapy, but it was of no significant statistical difference ($p > 0.05$).

Table 1: Comparison of the biodistribution of the two tracers in lung adenocarcinoma models (mean±SD)

Organ	%ID/g		T/NT	
	FDG group	FLT group	FDG group	FLT group
Heart	26.77±5.45	0.49±0.12	0.38±0.11	3.21±1.15*
Liver	2.17±0.48	0.69±0.11	4.65±1.39	2.27±0.56*
Spleen	4.43±0.63	0.87±0.12	2.25±0.75	1.80±0.42
Lung	4.29±0.71	0.61±0.15	2.35±0.63	2.58±0.71
Kidney	5.27±1.15	1.55±0.21	1.92±0.47	1.03±0.21*
Muscle	3.09±0.49	0.51±0.17	3.27±0.89	3.11±0.85
Tumor	10.11±2.11	1.57±0.43		

**There is statistical difference between the two groups ($p < 0.05$)*

Table 2: Tumor (% ID/g) of tumor-bearing mice in each group (mean±SD)

	Group A	Group B	Group C
FDG group	10.24±1.73	8.73±1.71	7.97±1.15
FLT group	1.41±0.38	0.71±0.08*	0.33±0.07*

** Statistical difference could be seen when compared with group A (P < 0.05)*

Tumor tissue % ID/g of group B and group C in the FLT group decreased much more significantly than that of group A after radiotherapy. The difference between them was statistically significant ($p < 0.05$).

From PET imaging of tumor-bearing mice in the FLT and FDG groups in 24 h and 48 h after radiotherapy and the PET imaging of those in control groups, it can be seen that FLT and FDG uptake of tumors were both high before radiotherapy and theT/NT values of the control group and radiotherapy group were not statistically different in 0 h. The T/NT value of the FDG group was higher than that of the FLT group. The T/NT values of the FLT group decreased significantly 24 h and 48 h after radiotherapy and there was a significant difference when compared with that before radiotherapy (3.3 ± 0.5, 1.7 ± 0.3, 1.2 ± 0.2, $p < 0.05$), while no significant differences of the T/NT values could be seen in the control group ($p > 0.05$). There was a significant difference between the T/NT values of the control group and the radiotherapy group 24 h and 48 h of radiotherapy ($p < 0.05$), while it was of no significant difference in the FDG group.

DISCUSSION

Early evaluation of anti-tumor therapy is of great importance for the assessment of the therapeutic scheduled screening and prognosis of diseases. Presently, the clinical evaluation of anti-tumor efficacy is mainly achieved by radiographic techniques, which can determine the effects of treatment by early observation of the changes of tumor size before and after the treatment. However, a series of biological development process is involved in the changes of tumor size. The changes of tumor size can not only be caused by changes in tumor cell proliferation, but can also be induced by other factors, such as tissue fibrosis, inflammatory cell infiltration and changes in tissue fluid.

In addition, some new anticancer drugs which can inhibit the development of tumors instead of killing tumor cells, cannot change the size of tumor. Therefore, general radiological imaging techniques can only indirectly reflect the functional status of the tumor tissue and have some flaws in the evaluation of anti-tumor therapy efficacy.

Positron emission tomography (PET), which has been widely used, can noninvasively detect the positron radionuclide distribution in the body and reflect the physiological, pathological, biochemical, and metabolic changes in human tissues at the molecular level. PET imaging, which can sensitively and accurately reflect the abnormal conditions of tumor perfusion, metabolism, protein synthesis, DNA replication and cell proliferation, is an effective tool of tumor diagnosis and anti-tumor efficacy evaluation [5,6].

The radioactive tracers used in PET imaging are mainly drugs labeled by "organic" positron emitters, including ^{11}C, ^{13}N, ^{15}O, and ^{18}F, which can be divided into categories of metabotropic tracer, bound tracer and blood perfusion tracer etc., according to their biochemical function. The development of PET, to some extent, depends on the development and application of positron tracer. Moreover, ^{18}F-fluoro-deoxy-glucose (^{18}F-FDG) is the most widely used tracer up to now, whose biological behavior is similar to that of glucose. It is taken up and phosphorylated by cells via the same pathway as glucose and is stranded in mitochondria due to the failure of further metabolism. Influenced by local hypoxia and changes of tumor biological behavior, a significantly increased protein expression of glucose transporter and highly active glycolysis can be seen in malignant tumor cells. Therefore, the uptake and retention of ^{18}F-FDG in tumor tissue is several or even dozens of times higher than that in normal tissues.

However, ^{18}F-FDG is not a tumor-specific tracer. It can result false positives [7], since it can also be ingested by inflammatory cells, and granular tissues. In recent years, nucleoside metabolic PET tracers have made significant progress. Currently, 3'-deoxy-3'-^{18}F-fluoro-thymidine, ^{18}F-FLT is the most promising nucleoside metabolic tracer, which can indirectly reflect the state of tumor cell proliferation by interacting with the key enzyme, thymidine kinase -1 in the DNA synthesis and salvage pathway, in order to specifically diagnose tumors [8]. The biodistribution and PETimaging of ^{18}F-FLT and ^{18}F-FDG in models of mice with lung

adenocarcinoma were compared and studied in this experiment.

It was observed that although % ID/g of the FLT group was significantly lower than that of the FDG group, the radioactivity uptake of tumors in both groups were relatively high; T/NT values of tumors to heart, lungs and muscles in FLT group were greater than 2; a significantly radioactive hot zone of the tumor issues could be seen in the PET imaging, which was quite clear and consistent with the detection results of % ID/g obtained by the well-type detector. It can be concluded that ^{18}F-FLT, which can clearly distinguish tumor tissues from other normal tissues, can be applied in the PET imaging of lung malignant tumors. Its low background in heart enables it to make up the inadequacies of ^{18}F-FDG as a tracer. It can better distinguish breast tumor from mediastinal metastases to further improve the efficiency of clinical diagnosis and staging.

The impacts of radiotherapy on ^{18}F-FDG uptake is complex [9], since the repair mechanism activation of cell damages induced by radiation, causes stenosis and occlusion of tumor vessels occurring after radiotherapy and the hypoxia caused by swelling and edema of tumor tissues, can all lead to the increase of ^{18}F-FDG uptake by starting pathways of anaerobic glycolysis and aerobic oxidation, but the proliferation of tumor cells was inhibited, so it is difficult for ^{18}F-FDG to reflect the therapeutic effects accurately.

Although there are a large number of reports on application of ^{18}F-FDG in evaluation of antitumor efficacy [10], ^{18}F-FDG can mainly reflect the *in vivo* glucose utilization, instead of detecting tumor reactions specifically, while ^{18}F-FLT can better reflect the therapeutic effects of tumors when applied to measure changes of tumor proliferation activity.

In this study, radiotherapy intervention was made on mice with lung adenocarcinoma. The uptake and PET imaging of the two tracers in tumors before and after radiotherapy were compared and analyzed. A rapid decrease of tumor tissue % ID/g could be found in the FLT group in 24 h and 48 h after radiotherapy, which was of significant statistical difference compared with the control group; while there was no significant difference between the % ID/g values of the FDG radiotherapy group and control group. PET imaging of tumor tissues of tumor-bearing mice in FLT control group was clear. The T/NT values of the FLT group decreased significantly in 24 h and 48 h after radiotherapy and there was a significant difference when compared with the one before radiotherapy while no significant differences of the T/NT values could be seen in the control group. There was a significant difference between the T/NT values of the control group and the radiotherapy group in 24 h and 48 h of radiotherapy, while it was of no significant difference in the FDG group. It can be seen that ^{18}F-FLT is also able to monitor early the effects of radiotherapy in treating tumors noninvasively, which enables the implementation of a much more appropriate treatment protocol.

CONCLUSION

Both ^{18}F-FLT and ^{18}F-FDG, can serve as suitable lung cancer PET tracers. Compared with ^{18}F-FDG, ^{18}F-FLT is more sensitive in capturing the early reactions of tumors during radiotherapy. It significantly correlates with tumor proliferation activity and can provide accurate biological information for the assessment of therapeutic efficacy and determination of best treatment protocols.

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