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The effect of tumor size on F-18-labeled fluorodeoxyglucose and fluoroerythronitroimidazole uptake in a murine sarcoma model

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The purpose of this study was to evaluate the effect of tumor size on the uptake of ^{18}F-fluorodeoxyglucose (FDG) and fluoroerythronitroimidazole (FETNIM) in a murine sarcoma model. ICR mice were xenografted with sarcoma 180 cell line and tumors were allowed to grow to a weight of 0.26–5.82 grams. ^{18}F-FDG and ^{18}F-FETNIM were injected intravenously in separate groups of mice, and after 1 hr, the tumors were excised and radiotracer uptake was measured. In another group of mice tumors were autoradiographically analyzed and subjected to H & E staining. In both the FDG and FETNIM group, per-gram radiotracer uptake by a tumor was inversely proportional to tumor weight. ^{18}F-FETNIM correlated more ($r = -0.593$, $p < 0.05$) than ^{18}F-FDG ($r = -0.447$, $p < 0.05$). Autoradiographic studies revealed that FDG accumulated in viable tumor areas, whereas FETNIM accumulated in both viable and partially necrotic areas. In the case of ^{18}F-FETNIM, a direct correlation between tumor weight and the no-uptake-area to total-tumor-area was demonstrated. We concluded that increased tumor size is associated with decreased uptake of ^{18}F-FDG and FETNIM, though this depends on the type of radiotracers and distribution of necrosis.

Key words: F-18-FDG, F-18-fluoroerythronitroimidazole, sarcoma, tumor size, autoradiography

INTRODUCTION

POSITRON EMISSION TOMOGRAPHY (PET) has been widely used in the evaluation of cancer patients. The current application of PET in oncology is in differentiating and characterizing indeterminate lesions, differentiating recurrent disease from treatment effects, staging and evaluating the extent of disease, and monitoring the success or failure of therapy.[1] ^{18}F-fluorodeoxyglucose (FDG), an analogue of glucose, is most commonly used. In addition, ^{11}C-methionine, an amino acid, and ^{18}F-nitroimidazole, a hypoxic agent, have been tried for the assessment of cancer.[2,3] Yang et al.[4] recently synthesized a new nitroimidazole analogue, ^{18}F-fluoroerythronitroimidazole (FETNIM), and reported that in mammary-tumor-bearing rats, the uptake of this was higher than that of ^{18}F-fluoromisonidazole.

To allow all objective assessment of the amount of tracer uptake in clinical PET studies, several quantitative methods have been applied. The standardized uptake value (SUV) is a semiquantitative method and frequently used. Tracer kinetic modelings such as compartment analysis and Patlak analysis are more accurate, but require several blood samples,[1,2] but the quantitation of radiotracer uptake involves problems such as the recovery coefficient, the partial volume effect, an incorrect lumped constant and tissue heterogeneity[5,6]; the last-named is thought to originate in the heterogeneity of blood flow, and tumor metabolism and necrosis.[6]

Variation of tumor size enhances tissue heterogeneity. A number of reports have noted that the uptake of radiotracers by a tumor varies as a function of tumor size[7–10]; uptake of radiolabeled phospholipid, liposome and monoclonal antibodies varied according to tumor size. There has, however, been no report describing the uptake of positron-emitting radiotracers, and we evaluated the

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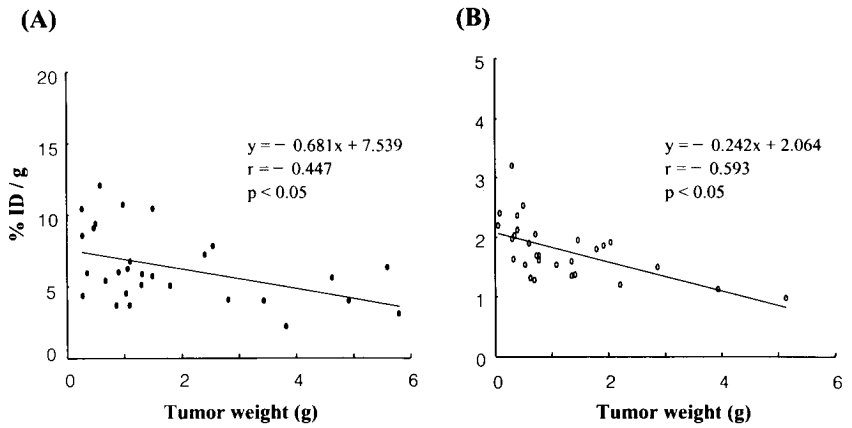

Fig. 1 Relationship between sarcoma 180 tumor weight and tumor uptake of percentage injected dose per gram tumor of ^{18}F-FDG (A) and ^{18}F-FETNIM (B).

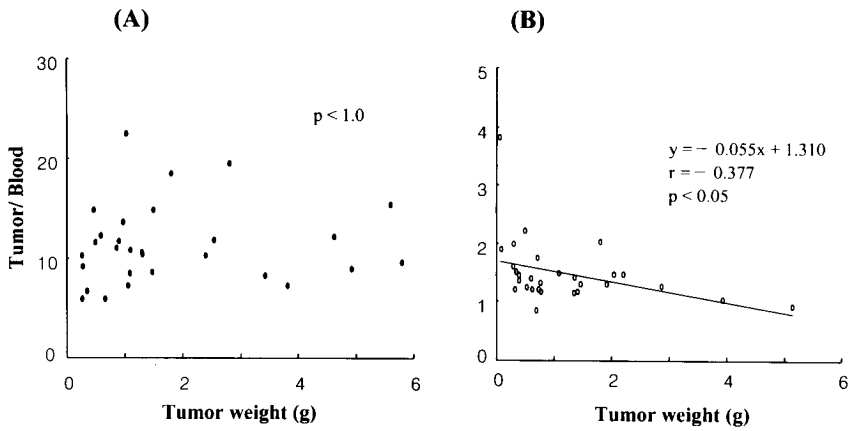

Fig. 2 Relationship between sarcoma 180 tumor weight and tumor-to-blood ratio of ^{18}F-FDG (A) and ^{18}F-FETNIM (B).

relationship between tumor size and tumor uptake of ^{18}F-FDG and ^{18}F-FETNIM.

MATERIALS AND METHODS

Radiopharmaceuticals

^{18}F-FDG and ^{18}F-FETNIM were synthesized by the modification of reported methods.[4,11] ^{18}F was produced by bombardment with 13 MeV proton to ^{18}O enriched (> 95%) water in a TR13 cyclotron (EBCO Technologies); it was captured in a AG1-X8 microcolumn and eluted with 1 m*l* of tetrabutylammonium bicarbonate (TBAB) solution in MeCN. The eluted ^{18}F-fluoride was dried by purging nitrogen gas at 80°C. After drying the azeotrophic mixture, 2 m*l* of MeCN was added, prior to further drying. To the dried tetrabutylammonium ^{18}F-fluoride, 30 mg of tetra-acetyltrifluoromannopyranoside in 3 m*l* MeCN (for ^{18}F-FDG) or 10 mg of tosylerythronitroimidazole in 3 m*l* of MeCN (for ^{18}F-FETNIM) was added and heated for 20 min at 80°C. After evaporation of the MeCN by nitrogen purging at 80°C, 4 m*l* of 1.5 N HCl was added and reacted for 20 min at 110°C. The reaction mixture was passed through an ion retardation column (AG11A8) to effect neutralization a C18 Sep-Pak to effect decolorization and a neutral alumina Sep-Pak to remove fluoride. After washing with 10 m*l* of distilled water, all the eluants were passed through a 0.22-μm syringe filter. Labeling efficiency and radiochemical purity of both products were about 60% and > 97%, respectively, when determined by silica gel TLC (1 × 6 cm), with 95% MeCN as a solvent.

Tumor model

Male ICR mice were xenografted with sarcoma 180 cell line[12] by implanting 2×10^6 cells s.c. in their left flank. The tumors were allowed to grow for 1–4 weeks until they reached the desired size; they were usually grew to 0.5–1 g within about 10 days of injection. At the time they were sacrificed, the average weight of the mice was 34.8 ± 3.6 g. All data from this animal experiment were normalized for the overall mouse weight (30 g).

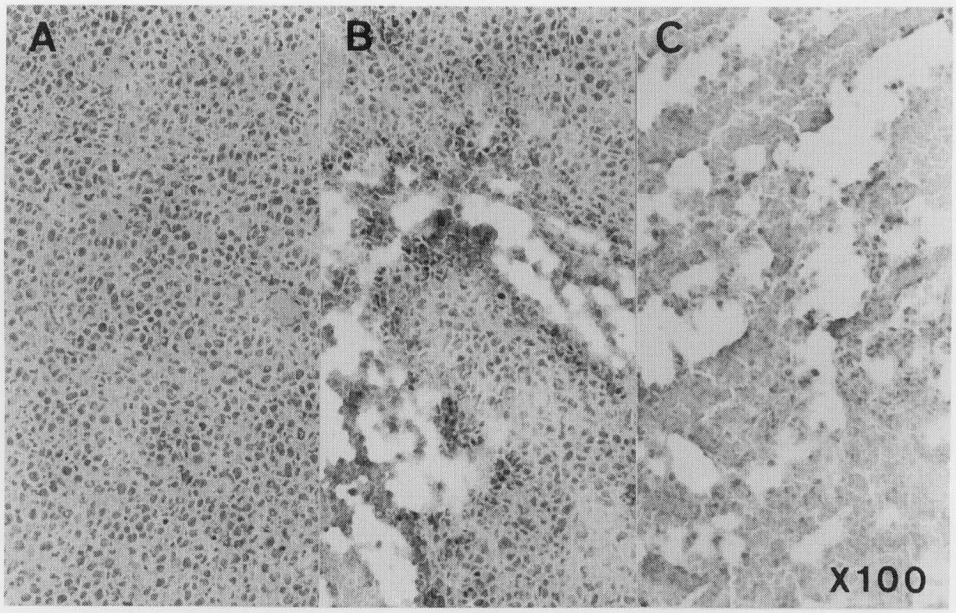

Fig. 3 Histopathologic images of sarcoma 180 tumor sections. A: Viable tumor cell area; B: Partially necrotic area; C: Totally necrotic area

Biodistribution

ICR mice bearing sarcoma 180 xenografts were intravenously injected with ^{18}F-FDG (185 kBq, n = 31) or ^{18}F-FETNIM (185 kBq, n = 29) and the tumors used in the present study weighed 0.26–5.82 g.

The mice were killed 1 hr after the administration of the radiopharmaceuticals and the tissues were weighed and counted. Biodistribution data were represented as a percentage of the injected dose per gram of tumor.

Autoradiography

ICR mice bearing sarcoma 180 xenografts were intravenously injected with ^{18}F-FDG (18.5–37 MBq, n = 12) or ^{18}F-FETNIM (18.5–37 MBq, n = 12). They were killed 1 hr postinjection and the tumors were excised and then frozen; they were cut into 10-μm or 20-μm sections on a cryostat immediately or after brief storage at −70°C. For autoradiographic studies, 20-μm sections were exposed to the image plate of a Bio-imaging analyzer (BAS2500, Fuji Photo Film, Tokyo, Japan). The histologic structures in each set of sections were identified from the adjacent 10 μm section stained with hematoxylin and eosin (H & E).

By means of volumetry, we measured the ratio of the no-uptake-area to the total-tumor-area, as seen in autoradiographic images.

Statistical analysis

Values are expressed as the mean ± s.d. Radiotracer uptake in the tumor and tumor weight were compared by linear regression analysis, and the ratio of the no-uptake-area to the total-tumor-area and tumor weight were also compared, by simple regression analysis. Differences in mean values were assessed by Student's t-test. A p value of less than 0.05 was considered significant.

RESULTS

Effect of tumor mass on tumor uptake

Tumor uptake expressed as a percentage of the injected dose per gram tumor of ^{18}F-FDG (6.27 ± 2.53%ID/g) was significantly higher than that of ^{18}F-FETNIM (1.77 ± 0.48%ID/g). The tumor-to-blood ratio of FDG was also higher than that of FETNIM. In ICR mice injected with ^{18}F-FDG and ^{18}F-FETNIM, tumor uptake was inversely proportional to tumor weight, as shown in Figure 1. ^{18}F-FETNIM showed a closer correlation ($r = -0.593$, $p < 0.05$) than ^{18}F-FDG ($r = -0.447$, $p < 0.05$). As shown in Figure 2, the tumor-to-blood ratio of ^{18}F-FETNIM also decreased as the tumor enlarged ($r = -0.377$, $p < 0.05$) but tumor-to-blood ratio of ^{18}F-FDG was independent of tumor size.

In normal organs, there was no statistically significant correlation between tumor weight and uptake of FDG or FETNIM.

Autoradiography and H & E staining

One pathologist (J.J.J.) reviewed the H & E sections of tumors and classified the lesions as viable tumor areas, or partially or totally necrotic areas (Fig. 3).

In tumors, the distribution of ^{18}F-FDG and ^{18}F-FETNIM was not the same. In ^{18}F-FDG autoradiograms, the pattern of uptake coincided with the distribution of viable tumor cells. High FDG uptake corresponded to the area of highest viable tumor cell density (Fig. 4A); areas of partial necrosis and total necrosis were associated with low levels of FDG uptake. In ^{18}F-FETNIM autoradiograms,

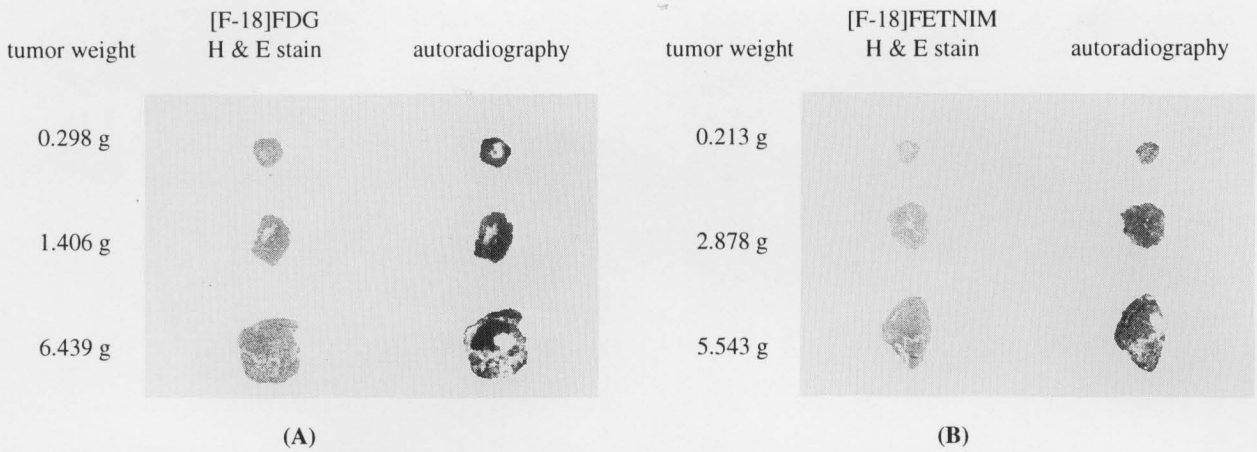

Fig. 4 H & E stained and autoradiographic images in cases of ^{18}F-FDG (A) and ^{18}F-FETNIM (B). In ^{18}F-FDG, autoradiograms show decreased uptake areas of FDG even in small tumors. In ^{18}F-FETNIM, however, autoradiograms show relatively homogeneous uptake of FETNIM in small tumors, and decreased uptake areas in larger tumors.

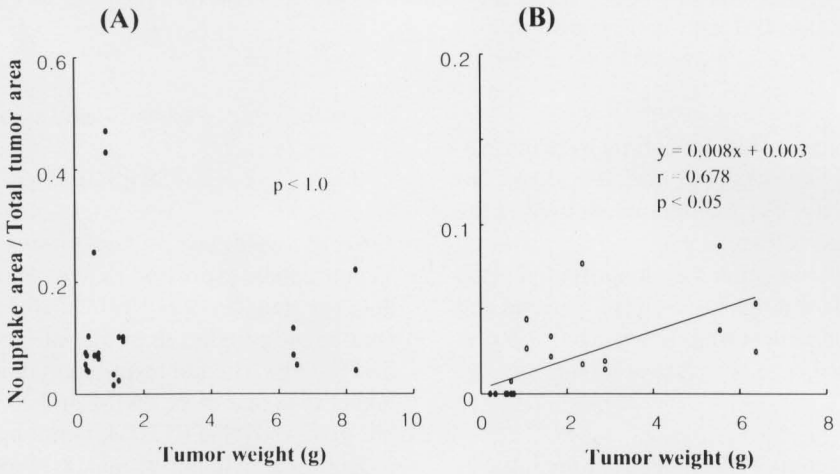

Fig. 5 Relationship between sarcoma 180 tumor weight and no-uptake-area to total-tumor-area in cases of ^{18}F-FDG (A) and ^{18}F-FETNIM (B).

the pattern of uptake coincided with the distribution of viable tumor areas and partially necrotic areas (Fig. 4B). High FETNIM uptake corresponded to areas of viable tumor and partial necrosis; areas of total necrosis were associated with low levels of FETNIM uptake.

By means of volumetry, we measured the ratio of the no-uptake-area to the total-tumor-area, as seen in autoradiographic images. In the case of ^{18}F-FDG, there was no relationship between this ratio and tumor weight (Fig. 5A) but in the case of ^{18}F-FETNIM, a direct correlation between the ratio and tumor weight was demonstrated ($r = 0.678$, $p < 0.05$) (Fig. 5B).

DISCUSSION

The principal object of this study was to examine the effect of tumor mass and necrosis on the uptake of ^{18}F-FDG and ^{18}F-FETNIM by tumors. Because the resulting tumor enlarged rapidly and quickly became necrotic, sarcoma 180 cell line was selected for transplantation in ICR mice; the effect of the tumor mass and necrotic area could thus be easily determined.

FDG is the most popular radiotracer in clinical PET studies and FETNIM is a new nitroimidazole analogue; we evaluated the uptake of these two radiotracers. Yang et al.[4] found that both ^{18}F-FETNIM and ^{18}F-fluoromisonidazole selected hypoxic cells, but FETNIM was more hydrophilic and the tumor-to-blood ratio was higher than in the case of fluoromisonidazole. They also demonstrated that in a tumor, FETNIM could help differentiate the hypoxic and necrotic regions.

In many animal studies, it has been reported that the uptake of several radiotracers varied directly with tumor size. Patel et al.,[7] with ^{111}In-phospholipid vesicles, dem-

onstrated decreased uptake as the tumor mass increased. Ogihara et al.[8] showed that the uptake of radiolabeled liposome decreased, and several investigators have demonstrated decreased uptake of radiolabeled antibodies as tumor mass increased.[9,10] Epenetos et al.[13] reported an inverse relationship between nonspecific antibody uptake and tumor size, and we noted a similar phenomenon in the case of ^{18}F-FDG and ^{18}F-FETNIM.

An inverse correlation between tumor mass and uptake was found in ICR mice injected with ^{18}F-FETNIM. The effect of tumor mass was also observed with ^{18}F-FDG, but was less obvious than with ^{18}F-FETNIM. The uptake pattern in partially and totally necrotic areas of the tumor mass could explain areas of different effects of tumor mass. ^{18}F-FDG accumulated in areas of viable tumor cells, but not in partially or totally necrotic areas. ^{18}F-FETNIM accumulated in viable and partially necrotic (hypoxic) areas, but not in those that were totally necrotic. The percentage of totally necrotic areas in a tumor mass increased according to tumor size ($r = 0.678$, $p < 0.05$). The larger the totally necrotic area, the less radiotracer uptake per gram was noted. This phenomenon is seen in Figure 4. This is why the effect of tumor size was more obvious in the case of ^{18}F-FETNIM.

Several factors such as the extent of necrosis, the degree of fibrosis, blood flow and infiltration of inflammatory cells may modify FDG uptake. Using macroautoradiography, Brown et al.[6] found that in a breast cancer model, 80% of ^{18}F-FDG accumulated in viable cancer cells. Average FDG uptake in areas of either inflammatory infiltrating cells or necrotic tissue was lower than in areas of cancer cells, but Kubota et al. reported that FDG accumulated not only in tumor cells but also in the inflammatory cell elements which appeared in association with tumor growth or necrosis.[14] Neither experiment demonstrated uptake of ^{18}F-FDG in totally necrotic areas of a tumor.

In this study, we found that the extent of necrosis was related to tumor size, which in turn might be related to blood flow. The geometry of this flow is a dominant constraint on radiotracer uptake[10]; the internal volume of a tumor is often found to be more poorly perfused as its size increases,[15] whereas it would be expected that a relatively large necrotic zone would be inaccessible to any blood-borne agent.[16]

It is well known that a small tumor responds to radiotherapy better than a large tumor,[17] because a large tumor may have many radioresistant hypoxic cells. It might be expected that hypoxia tracer FETNIM uptake by a large tumor could be higher than that by a small tumor, but result of this study was the opposite. Although we could not measure the amount of hypoxic tissue in tumors, we speculated that the hypoxic fraction did not increase with the increase in tumor size in this animal model, as Shimamoto et al. reported.[18] Sarcoma 180 tumors enlarged rapidly and quickly became necrotic with a relatively small hypoxic area.

This study indicates that tumor size can change the result of PET; smaller lesions may acquire a greater amount of positron-emitting radiotracer. Many investigators have tried to correlate radiotracer uptake of a tumor to its pathologic grade, proliferative activity or growth rate.[19–24] To separate benign from malignant lesions in marginal cases, quantitation such as SUV is vitally important.[5] In addition, the SUV of an individual tumor nodule can be measured before and after therapy and any change used as an index of therapeutic response. If SUV or quantitative data for the evaluation of tumor metabolism is used without considering tumor size, significant misunderstanding might occur.

In clinical PET, however, the SUV of a small tumor is frequently found to be lower than that of a larger tumor in the same patient, a finding contrary to the findings of this experiment. Low SUV in a small tumor may very well be due mainly to recovery coefficient errors and partial volume effects. Keyes reported that for these reasons, the apparent SUV of a tumor less than 2 cm in diameter might be lower than the true value.[5] When these effects were accurately corrected, greater uptake of radiotracer might be apparent in a small tumor. In addition, total *in vivo* uptake increases linearly with tumor size; a larger tumor tends to be better visualized, even if radiotracer accumulates more densely in one that is smaller.

Finally, analysis of this single tumor model is not sufficient in order to make a general prediction as to FDG and FETNIM uptake in human tumors. The relationship between tumor size and the proportion of necrosis might vary according to the histology of the tumor, but in the case of the same histology, such as the metastasis from a tumor, the size may affect the uptake of FDG and FETNIM. Several tumor lines of different origin need to be examined in future.

CONCLUSION

This study demonstrated that increased tumor size is associated with decreased uptake of ^{18}F-FDG and FETNIM. The effect of tumor size on the accumulation of radiopharmaceuticals depends on the extent of necrosis and the type of radiopharmaceuticals involved. Quantitation of tumor uptake may be limited by these factors, and tumor size should be considered.

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