value of octreotide scintigraphy in patients with lung cancer. *Eur J Nucl Med* 1994;21:1106–1113.
12. Bong SB, VanderLaan JG, Louwes H, Schuurman JJ. Clinical experience with somatostatin receptor imaging in lymphoma. *Semin Oncol* 1994;21(suppl):46–50.
13. Van Den Anker-Lugtenburg PJ, Krenning EP, Oei HY, et al. Somatostatin receptor scintigraphy in the initial staging of Hodgkin's disease. *Br J Haematol* 1996;93:96–103.
14. Weckbecker G, Raulf F, Stolz B, Bruns C. Somatostatin analogs for diagnosis and treatment of cancer. *Pharmacol Ther* 1993;60:245–265.
15. Weckbecker G, Tolcsvai L, Liu R, Bruns C. Preclinical studies on the anticancer activity of the somatostatin analogs octreotide. *Digestion* 1993;54:98–103.
16. Zlock DW, Greenspan FS, Clark OH, Higgins CB. Octreotide therapy in advanced thyroid cancer. *Thyroid* 1994;4:427–431.
17. Hipkin RW, Friedman J, Clark RB, Eppler CM, Schonbrunn A. Agonist induced desensitization, internalization, and phosphorylation of the sst 2A somatostatin receptor. *J Biol Chem* 1997;272:13869–13876.

Glucose Transporter Protein-Independent Tumor Cell Accumulation of Fluorine-18-AFDG, a Lipophilic Fluorine-18-FDG Analog

Atsuo Waki, Yasuhisa Fujibayashi, Yasuhiro Magata, Akira Yokoyama, Norihiro Sadato, Tatsuro Tsuchida, Yasushi Ishii and Yoshiharu Yonekura

Biomedical Imaging Research Center, Fukui Medical School, Fukui, Japan

Fluorine-18-fluorodeoxyglucose (FDG) is used clinically for tumor diagnosis, but its mechanism of accumulation in tumor cells is complicated because two factors, glucose transporter protein (GLUT) and hexokinase, govern [^{18}F]FDG uptake directly. We selected a lipophilic [^{18}F]FDG analog, 1,3,4,6-tetra-acetyl-2-[^{18}F]-2-deoxy-D-glucose ([^{18}F]AFDG), to regulate the effects of hexokinase and evaluated its characteristics in an in vitro cell culture system. **Methods:** Fluorine-18-AFDG was synthesized by the method used to produce [^{18}F]FDG, as an intermediate of [^{18}F]FDG. Fluorine-18-AFDG uptake study was performed with LS180 tumor cells, and its metabolites were also investigated by thin-layer chromatography. To evaluate the relationship between [^{18}F]AFDG and GLUT, we also examined [^{18}F]AFDG uptake in the presence of cytochalasin B or with increased medium glucose concentration. The effects of lowered temperature (4°C) on [^{18}F]AFDG uptake were also investigated. **Results:** Fluorine-18-AFDG (lipophilicity: octanol/water = 3.5) uptake was 3.3-fold higher than that of [^{18}F]FDG. Metabolic analysis showed that [^{18}F]AFDG was extremely stable in the incubation medium but was quickly hydrolyzed and metabolized to 2-fluoro-[^{18}F]-2-deoxy-D-glucose-6-phosphate ([^{18}F]FDG-6P) in tumor cells. Fluorine-18-FDG-6P accounted for approximately 45% of the total radioactivity after a 60-min incubation of [^{18}F]AFDG. Incubation with 50 μM cytochalasin B did not affect [^{18}F]AFDG uptake. In medium with double the control glucose level, [^{18}F]FDG uptake was decreased by about 50%, but [^{18}F]AFDG uptake was not affected. Fluorine-18-AFDG uptake and [^{18}F]FDG-6P production did not show saturation and increased linearly with addition of a 10-fold higher concentration of [^{18}F]AFDG. Lowered incubation temperature caused decreased [^{18}F]AFDG uptake due to reduced [^{18}F]FDG-6P production. **Conclusion:** Fluorine-18-AFDG rapidly penetrated the cell membrane as a result of its high lipophilicity and was metabolized to [^{18}F]FDG-6P within cells. Fluorine-18-AFDG was thus characterized as "GLUT-independent [^{18}F]FDG."

Key Words: glucose metabolism; deoxyglucose; cultured tumor cells; glucose analogs; hexokinase

J Nucl Med 1998; 39:245–250

Tumors generally have high glycolytic activity (*1–4*). Based on this characteristics, PET with ^{18}F-fluorodeoxyglucose (FDG) has become a clinically useful tool for tumor imaging because of its high sensitivity and high specificity (*5,6*). The entrance of [^{18}F]FDG is mediated by glucose transporter proteins (GLUTs), and [^{18}F]FDG is then metabolized to [^{18}F]FDG-6-phosphate) by hexokinase. In general, [^{18}F]FDG-6P cannot be further metabolized, so that ^{18}F radioactivity is retained as [^{18}F]FDG-6P in the cells. In the brain, glucose metabolism is believed to be governed by hexokinase. Based on this consideration, quantification of the glucose metabolic rate has been performed with [^{18}F]FDG (*7,8*).

On the other hand, the contribution of hexokinase to the accumulation of [^{18}F]FDG in tumors is not similar to that in the brain. High hexokinase activity in tumors has been reported (*9–11*), but decreased phosphatase activity (*12*) and increased GLUT expression (*13–16*) are also considered to be the cause of [^{18}F]FDG accumulation in tumors. In addition, hypoxic conditions, such as low oxygen supply due to poor perfusion, are thought to enhance glycolytic metabolism (*17,18*), and high [^{18}F]FDG accumulation in "hypoxic tumor cells" has been reported in accordance with increased GLUT expression (*19*). Thus, it is difficult to use [^{18}F]FDG accumulation to monitor glucose metabolic rate of tumors, based on hexokinase activity.

Monitoring accumulation of a membrane-permeable [^{18}F]FDG analog with considerable affinity to hexokinase might be a better parameter for the evaluation of glucose metabolism in tumors. We selected 1,3,4,6-tetra-acetyl-2-[^{18}F]-2-deoxy-D-glucose ([^{18}F]AFDG) as a candidate for such a molecule (its structure is shown in Fig. 1). In this study, an in vitro cell culture system was used to determine whether [^{18}F]AFDG instantly penetrates the cell membrane due to its high lipophilicity, whether [^{18}F]AFDG is hydrolyzed to [^{18}F]FDG after penetration and whether [^{18}F]FDG produced from [^{18}F]AFDG is phosphorylated by hexokinase and retained in the cells. Based on these considerations, the possibility of use of [^{18}F]AFDG as a tracer for monitoring tumor glucose metabolism will be discussed.

MATERIALS AND METHODS

Fluorine-18-FDG and Fluorine-18-AFDG Synthesis

Fluorine-18-FDG and its intermediate [^{18}F]AFDG were produced by the method of Hamacher et al. (*20*) with an automated [^{18}F]FDG synthesis system (NKK Co. Ltd., Tokyo, Japan). Briefly, ^{18}F anion, synthesized from [^{18}O]H_2O in the reaction ^{18}O(p,n)^{18}F,

Received Sep. 9, 1996; revision accepted Apr. 15, 1997.
For correspondence or reprints contact: Atsuo Waki, PhD, Biomedical Imaging Research Center, Fukui Medical School, 23-3, Shimoaizuki, Matsuoka-cho, Yoshida-gun, Fukui 910-11, Japan.

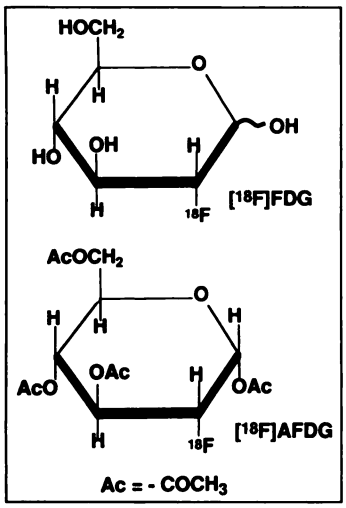

FIGURE 1. Structures of [^{18}F]FDG and [^{18}F]AFDG.

and 26 mg of Kriptfix 2.2.2 in 1 ml of dry acetonitrile were mixed in the reaction vessel. The mixture was evaporated to dryness with helium at 80°C. Then, 20 mg of 1,3,4,6-tetraacetyl-2-O-trifluoromethanesulfunyl-β-D-mannopyranose (Triflate) in 1 ml of dry acetonitrile were added, and the substitution reaction was performed for 5 min. After the addition of water to the reaction mixture, the product was purified by passing through a Sep-Pak C18 column. The acetonitrile extract was concentrated twice by evaporation and resolved in 2 ml of dimethylsulfoxide (in the case of [^{18}F]AFDG production) or 3 ml of 2 *N* HCl (hydrolyzed to [^{18}F]FDG). For [^{18}F]FDG synthesis, the hydrolyzation reaction was performed at 130°C for 5 min, the reaction mixture was then passed through ion retardation resin and the solvent was replaced with distilled water. There was no difference in specific activity between [^{18}F]FDG and [^{18}F]AFDG (~1 Ci/μmol) because the same synthesis method was used.

Analysis of Fluorine-18-FDG and Fluorine-18-AFDG

Analyses of [^{18}F]FDG and [^{18}F]AFDG were performed by radio high-performance liquid chromatography on a 3.9 × 300 mm carbohydrate analysis column [eluent, CH_3CN/H_2O (95:5); flow, 2 ml/min]. The eluted volumes of [^{18}F]FDG and [^{18}F]AFDG were 4.1 ml and 2.5 ml, respectively.

Cell Culture

The human colon adenocarcinoma cell LS180 was a kind gift from Dr. Sakahara (Kyoto University Hospital, Kyoto, Japan). LS180 cells were maintained under a 5% CO_2-humidified atmosphere at 37°C. Cells were grown and maintained in RPMI 1640 medium (Gibco/BRL) supplemented with 10% fetal bovine serum and 50 μg/ml streptomycin sulfate throughout the experiment. At confluency, cells were dissociated with 0.05% trypsin/0.2% EDTA. Tumor cell viability was assessed by the trypan blue dye exclusion test using an Olympus inverted microscope (Tokyo, Japan).

Fluorine-18-FDG and Fluorine-18-AFDG Uptake Experiment: Time Course Studies

LS180 cells were trypsinized and seeded at a concentration of 20 × 10^4 in 24-well plates, and the accumulation experiment (Experiment A) was performed after 24 hr. Briefly, 5 μCi of [^{18}F]FDG or [^{18}F]AFDG were added to each well and incubated for 2, 5, 10, 30 or 60 min. After incubation, the medium was removed, and cells were washed three times with ice-cold phosphate-buffered saline (PBS). Lysis was performed with 500 μl of 0.2 *N* NaOH, and then the radioactivity of the whole lysate was counted with an autowell gamma counter (model ARC-2000; Aloka). Protein content of the whole lysate was then measured with bicinchoninic acid protein assay reagent and converted to cell number, and the scales of graphs were represented. The remaining radioactivity in wells of the plates was <5% of total activity.

For the investigation of temperature-dependent accumulation of [^{18}F]AFDG, another accumulation experiment (Experiment B) was performed because the cells became detached from the plates in the cold. LS180 was trypsinized and suspended at a concentration of 1 × 10^6 cells/ml in 1 ml of culture medium. The suspension was preincubated at 15 min at 4°C or 37°C, and then [^{18}F]AFDG was incubated for 2, 5, 10, 30 or 60 min. At each time point, the cells were collected by centrifugation at 2000 rpm in a microcentrifuge and washed twice with ice-cold PBS, and then the cells was pelleted and lysed with 0.2 *N* NaOH. Radioactivity of the whole lysate was counted with an autowell gamma counter. Protein content was assayed and converted to cell number, as described above.

Fluorine-18-FDG and Fluorine-18-AFDG Metabolite Analysis

Fluorine-18-FDG and [^{18}F]AFDG metabolite analysis was performed by thin-layer chromatography (TLC) on silica gel 60 with a solvent system of acetonitrile/water (95:5). The cells, incubated with [^{18}F]FDG or [^{18}F]AFDG, as in Experiment A, were scraped with 100 μl of 50% ethanol and centrifuged at 15,000 rpm for 10 min in a microcentrifuge, and the supernatant was used for metabolite analysis. The cell number was assessed and corrected with those of other wells in the same plate. The radioactivity of the supernatant was usually over 95% of that in the whole lysate. The metabolites in 25 μl of the supernatant were separated by TLC, and the plates were cut into 5-mm-wide strips and then counted with a gamma counter. The total radioactivity of each metabolite was calculated with the proportion of metabolites analyzed by TLC. R_f values of synthesized and purified [^{18}F]FDG and [^{18}F]AFDG were obtained, and that of [^{18}F]FDG-6P was obtained by metabolite analysis of [^{18}F]FDG in cells. R_f values of [^{18}F]AFDG, [^{18}F]FDG and [^{18}F]FDG-6P were 0.88, 0.61 and 0.11, respectively.

Effects of Glucose Transporter Protein Inhibitor on Accumulation of Fluorine-18-AFDG

Cytochalasin B (CB) is known to inhibit glucose transport activity of GLUT. Cytochalasin B was reported to inhibit [^{3}H]3-O-methylglucose ([^{3}H]3-OMG) transport at concentrations of 50 μ*M* or less (*21,22*). We investigated [^{3}H]3-OMG, [^{18}F]FDG and [^{18}F]AFDG transport in the presence or absence of 50 μ*M* CB. Incubation was performed for short periods (1 min) only, to exclude the effects of CB on energy status of cells. Other experimental conditions were similar to those described above except with addition of tracers (10 μ*M*).

Effects of Glucose Concentration on Accumulation and Metabolism of Fluorine-18-FDG and Fluorine-18-AFDG

The media used in this experiment were of three different glucose concentrations: 200 mg/dl glucose as normal culture, 400 mg/dl (2× glucose medium) and 2000 mg/dl (10× glucose medium). The three kinds of medium were based on glucose-free RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 μg/ml streptomycin sulfate and glucose at the indicated concentrations. The cells were incubated with [^{18}F]FDG or [^{18}F]AFDG for 60 min in normal 2× or 10× glucose concentration medium and washed three times with ice-cold PBS, and then the cells were scraped with 50% ethanol and centrifuged at 15,000 rpm. Metabolite analysis of supernatants and correction of cell number were performed as described above.

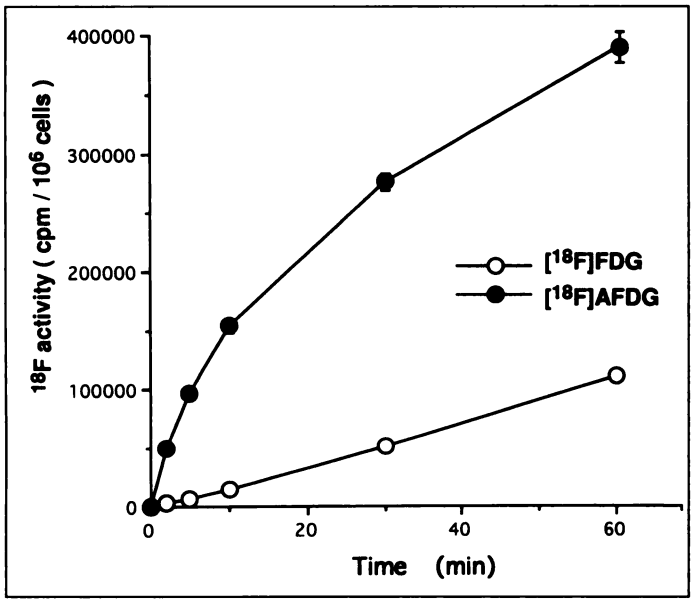

FIGURE 2. Fluorine-18-AFDG and [^{18}F]FDG uptake in LS180: a time course study. Fluorine-18-AFDG differed from [^{18}F]FDG in uptake manner and showed a 3.3-fold higher uptake than did [^{18}F]FDG. Data represent mean ^{18}F activities per 10^6 cells (n = 4).

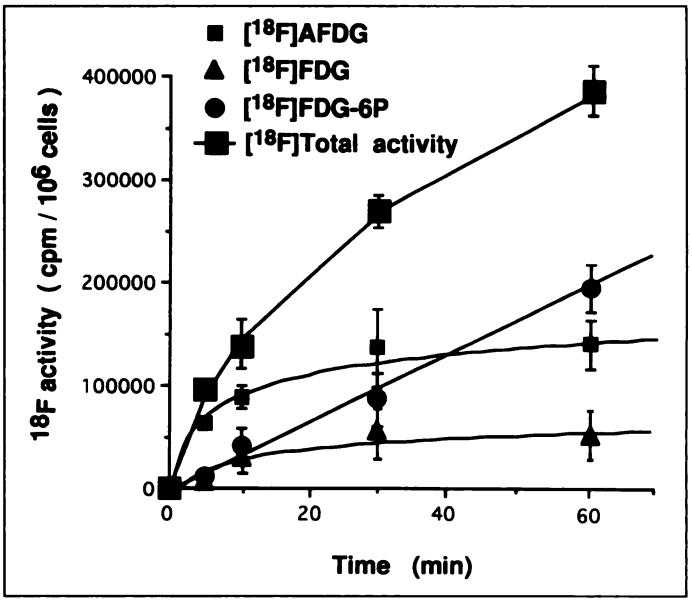

FIGURE 3. Metabolite analysis of [^{18}F]AFDG in tumor cells. This graph was plotted from the results of TLC analysis of [^{18}F]AFDG metabolites by LS180 cells. Fluorine-18-AFDG was shown to be metabolized to [^{18}F]FDG-6P, and among the metabolites, only [^{18}F]FDG-6P level increased linearly until 60 min. Data represent mean ^{18}F activities per 10^6 cells (n = 4). Experiments were repeated several times, and the same results were obtained in each experiment.

RESULTS

Fluorine-18-AFDG Accumulation in Tumor Cells

Figure 2 shows accumulation curves of [^{18}F]FDG and [^{18}F]AFDG into cells up to 60 min. Fluorine-18-FDG accumulation increased linearly, and radioactivity in the cells was about 1.1×10^5 cpm/10^6 cells after 60 min. Fluorine-18-AFDG accumulation increased nonlinearly until 60 min. At 10 min, radioactivity in the cells (1.0×10^5 cpm/10^6 cells) had already reached the value of [^{18}F]FDG at 60 min. During the experimental period, no degradation of [^{18}F]AFDG in the incubation medium was detected (TLC analysis; data not shown).

Analysis of Metabolites of Fluorine-18-AFDG in Tumor Cells

We also investigated the metabolism of [^{18}F]AFDG in tumor cells by TLC analysis (Fig. 3). Three peaks were found and confirmed to be [^{18}F]AFDG, [^{18}F]FDG and [^{18}F]FDG-6P from their R_f values. At 5 min after the addition of [^{18}F]AFDG, most of the radioactivity was found as nonmetabolized [^{18}F]AFDG, with only a small amount as [^{18}F]FDG and [^{18}F]FDG-6P. The radioactivity levels of [^{18}F]AFDG and [^{18}F]FDG in the cells had almost plateaued at 10 min. However, [^{18}F]FDG-6P increased linearly at a constant rate during the experimental period, and at 60 min, 40%–50% of the radioactivity in the cells was found to be [^{18}F]FDG-6P.

Effect of Fluorine-18-AFDG Concentration on Fluorine-18-AFDG Accumulation and Fluorine-18-FDG-6P Production

To investigate whether the hydrolyzation process regulated [^{18}F]AFDG accumulation, we assessed the effects of amount of [^{18}F]AFDG on the accumulation of [^{18}F]AFDG (Fig. 4). The accumulation of [^{18}F]AFDG increased linearly with the amount of [^{18}F]AFDG added. No saturation of [^{18}F]AFDG accumulation was seen with addition of a 10-fold amount of [^{18}F]AFDG compared to controls. Fluorine-18-FDG-6P production also increased linearly.

Effects of Incubation Temperature on Fluorine-18-AFDG Accumulation and Fluorine-18-FDG-6P Production

Time-activity curves of [^{18}F]AFDG at 37°C and 4°C are shown in Figure 5A. The accumulation of radioactivity at 4°C was about 30% of that at 37°C. Figure 5B shows metabolites of [^{18}F]AFDG at 60 min of incubation. After 60 min of incubation at 4°C, ~80% of the radioactivity was nonmetabolized [^{18}F]AFDG, and the remainder was [^{18}F]FDG-6P.

Relationship Between Fluorine-18-AFDG Accumulation and Glucose Transporters

The transport of [^{3}H]3-OMG and [^{18}F]FDG in the presence of CB was decreased by ~50%. On the other hand, the transport

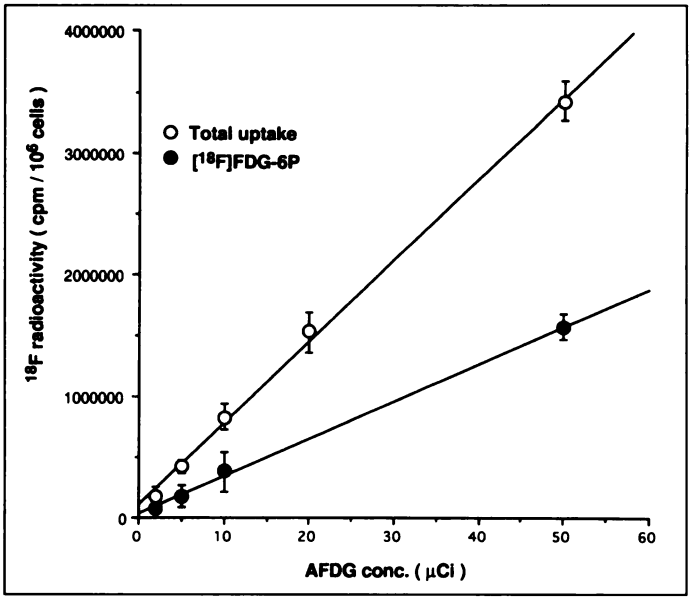

FIGURE 4. Effects of [^{18}F]AFDG concentration on [^{18}F]AFDG accumulation and [^{18}F]FDG-6P production. Fluorine-18-AFDG uptake and [^{18}F]FDG-6P production increased linearly with the amount of [^{18}F]AFDG added. Data represent mean ^{18}F activities per 10^6 cells (n = 4).

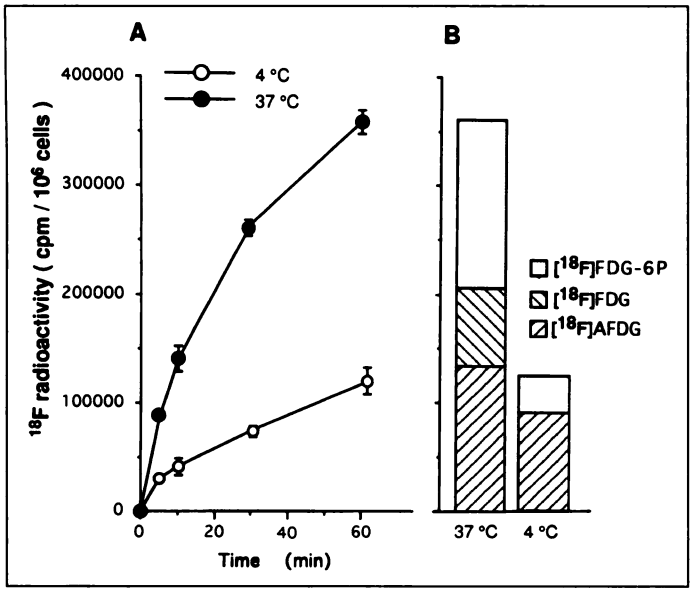

FIGURE 5. Effects of incubation temperature on [^{18}F]AFDG uptake and [^{18}F]FDG-6P production. (A) Time-activity curves of [^{18}F]AFDG at 37°C and 4°C. (B) Metabolites of [^{18}F]AFDG are shown after a 60-min incubation. Fluorine-18-FDG-6P production was markedly decreased at 4°C, but [^{18}F]AFDG accumulation was ~80% of control at 37°C after a 60-min incubation. Data represent mean ^{18}F activities per 10^6 cells (n = 4).

of [^{18}F]AFDG in the presence of CB was not different from that in controls (Fig. 6).

The effects of medium glucose concentration on [^{18}F]FDG and [^{18}F]AFDG accumulation are shown in Figure 7. In the [^{18}F]FDG experiment, both total radioactivity and [^{18}F]FDG-6P production were decreased when glucose concentrations of the incubation medium were increased to 2-fold and 10-fold,

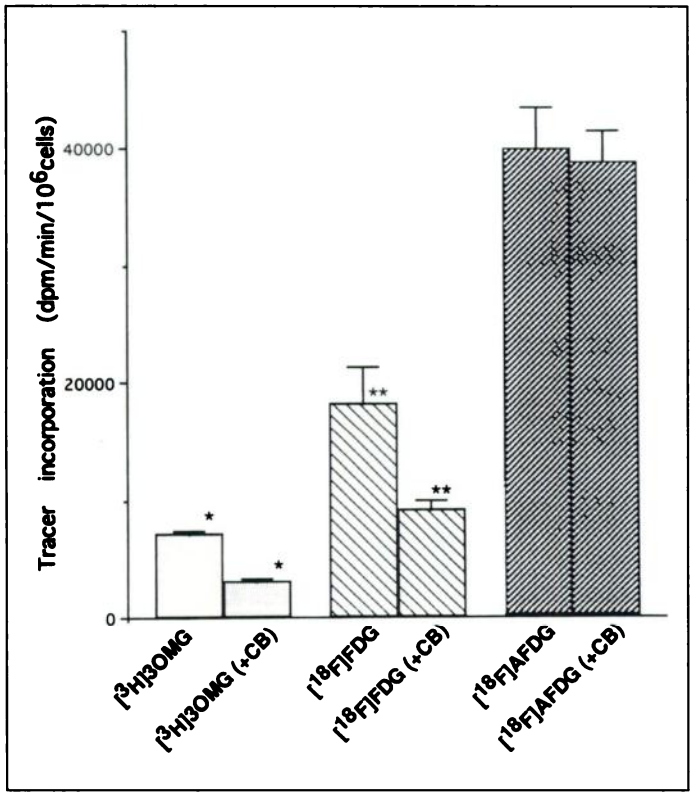

FIGURE 6. Effects of CB on [^{3}H]3-OMG, [^{18}F]FDG and [^{18}F]AFDG uptake. Cytochalasin B, a GLUT inhibitor, markedly affected [^{3}H]3-OMG and [^{18}F]FDG uptake. On the other hand, [^{18}F]AFDG were not influenced by CB. Data represent mean radioactivities per 10^6 cells (n = 4).

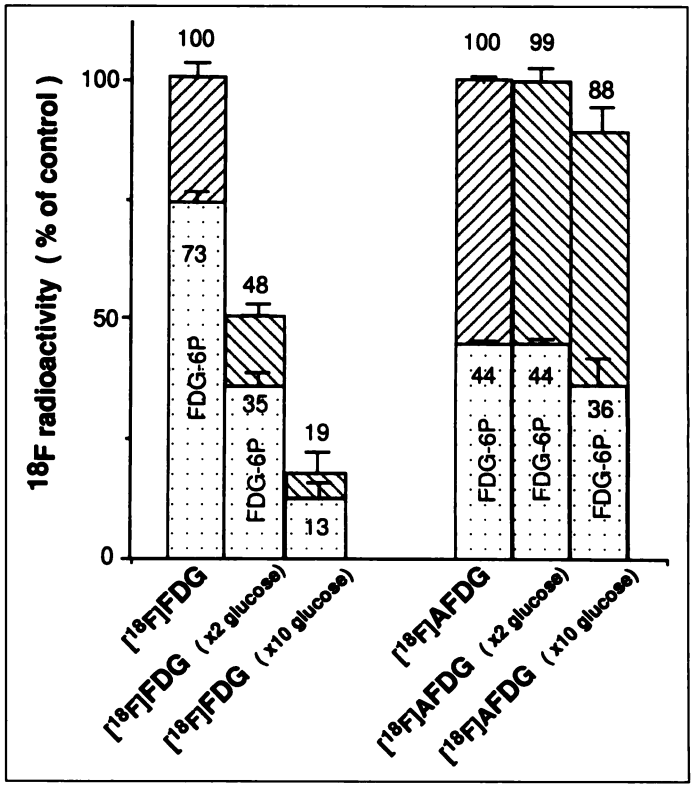

FIGURE 7. Effects of medium glucose concentration on [^{18}F]FDG, [^{18}F]AFDG uptake and [^{18}F]FDG-6P production. The numbers above the columns show percentages of ^{18}F activity compared to respective total uptake in controls. Fluorine-18-AFDG uptake and [^{18}F]FDG-6P production on incubation with [^{18}F]AFDG were not influenced by medium glucose concentration. Both [^{18}F]FDG uptake and [^{18}F]FDG-6P production at [^{18}F]FDG incubation were decreased to ~50% when medium glucose concentration was increased to 2-fold compared to controls. Data represent mean percentages of control of ^{18}F activity per 10^6 cells (n = 4).

respectively, compared to controls. On the other hand, in the [^{18}F]AFDG experiment, neither of these parameters showed any significant changes.

DISCUSSION

The most unique feature of [^{18}F]AFDG is its high lipophilicity (octanol/water = 3.5 ± 0.3; 37°C). In addition, this compound is an intermediate of [^{18}F]FDG synthesis, so that it can be easily obtained in most PET centers routinely producing [^{18}F]FDG. Some glucose analogs with side chain modifications have been reported previously, but these studies focused mainly on their affinity to GLUT (*23,24*), with no consideration to the membrane penetrability of these compounds based on lipophilicity. The higher accumulation of [^{18}F]AFDG relative to [^{18}F]FDG observed in this study suggested that [^{18}F]AFDG is accumulated by a mechanism different from that responsible for uptake of [^{18}F]FDG. Thus, we investigated the contribution of GLUT to the uptake of [^{18}F]AFDG. If the membrane transport of [^{18}F]AFDG was GLUT-dependent, it would be suppressed by addition of carrier glucose or CB, a GLUT inhibitor. Indeed, [^{18}F]FDG uptake showed clear suppression by these two treatments. However, no suppression of [^{18}F]AFDG uptake was observed, indicating that [^{18}F]AFDG is taken up by simple penetration, based on its high lipophilicity. It has been reported that bulky side chain substitution of glucose clearly diminished its affinity to GLUT (*24*). This also supports our observations concerning the mechanism of [^{18}F]AFDG uptake.

Analysis of metabolites indicated that [^{18}F]AFDG was fully metabolized through [^{18}F]AFDG to [^{18}F]FDG-6P, as expected. In the [^{18}F]AFDG accumulation experiment, [^{18}F]FDG-6P

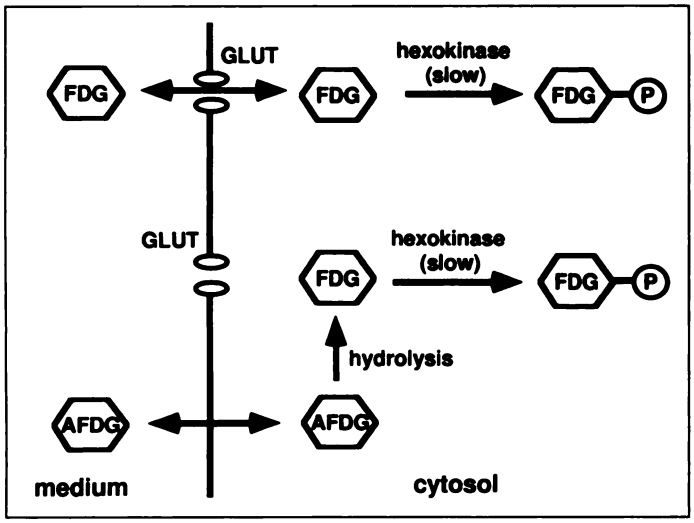

FIGURE 8. Schematic representation of the [^{18}F]AFDG uptake mechanism.

content increased at a constant rate. On the other hand, [^{18}F]AFDG and [^{18}F]FDG contents were almost constant after a 10-min incubation of [^{18}F]AFDG. This radioactivity of [^{18}F]AFDG was thought to be nonmetabolized [^{18}F]AFDG in both cytosolic and plasma membrane fractions, and both were thought to be almost saturated after a 10-min incubation. Thus, the increase in total radioactivity into cells during the incubation period from 30 to 60 min was thought to indicate the production of [^{18}F]FDG-6P.

Hydrolysis is the next step necessary for [^{18}F]AFDG metabolism. Therefore, we investigated the effects of [^{18}F]AFDG concentration on radioactivity accumulation and [^{18}F]FDG-6P production. Total radioactivity and [^{18}F]FDG-6P production were thought to be dependent on the amount of [^{18}F]AFDG added, and [^{18}F]FDG-6P production did not show saturation kinetics. Thus, at least in this study, the results indicated that hydrolyzation was not the rate-limiting step within the range of [^{18}F]AFDG added. However, the level of hydrolyzation ability in cells was thought to be important, and the hydrolyzation step may vary between different tumor cell lines. Even in tumor cells, in this study, the presence of a large fraction of AFDG in the cold suggests that the hydrolyzation process becomes rate-limiting under these conditions.

From these results, the mechanism of [^{18}F]AFDG uptake was expected to be as follows (Fig. 8):

1. Fluorine-18-AFDG is taken up by simple diffusion (i.e., not through GLUT).
2. Four acetylated sites of [^{18}F]AFDG are hydrolyzed quickly to [^{18}F]FDG intracellularly.
3. Fluorine-18-FDG derived from [^{18}F]AFDG is phosphorylated to [^{18}F]FDG-6P.

We then focused our attention on the relationship between cellular activity and [^{18}F]AFDG accumulation levels. Cellular activity is generally reduced by lowering the temperature, along with decreased rates of chemical and enzymatic reactions. In this study, accumulated radioactivity and [^{18}F]FDG-6P production in the cells were decreased when incubated at 4°C. More interestingly, the decrease in [^{18}F]FDG-6P production was greater (reached 15% of the 37°C control) than that of accumulated radioactivity (reached to 30% of the 37°C control). The amount of nonmetabolized [^{18}F]AFDG was not markedly reduced. However, [^{18}F]FDG, which was seen in controls, was not observed. On the other hand, the amount of nonmetabolized [^{18}F]AFDG was not markedly reduced. Thus, the hydrolyzation step was thought to be rate-limiting at low temperature or cellular activity was thought to be generally reduced.

The level of [^{18}F]FDG-6P production from [^{18}F]AFDG in the tumor cells was a 2.1-fold higher than that from [^{18}F]FDG at 60 min. This was thought to be because of the differences in supplement systems, i.e., the participation of GLUT. In many types of cells, GLUT is thought to regulate glucose metabolism. For example, hypoxia has been reported to increase GLUT expression in tumor cells (*19*). Even in some normal cells, insulin or mitochondrial inhibitors are known to increase glucose accumulation based on increased GLUT expression (*25–28*). Fluorine-18-FDG and other glucose analogs are known to enter cells via GLUT, and therefore, the uptake of those tracers is thought to mainly reflect the levels of GLUT expression. On the other hand, the uptake of [^{18}F]AFDG may show glucose metabolism from a different viewpoint from other glucose analogs, such as [^{18}F]FDG.

Fluorine-18-FDG accumulation was shown to be affected by glucose level both in vitro (*29*) and in vivo (*30*). In this study, accumulated radioactivity and [^{18}F]FDG-6P production were paradoxically reduced in medium with increased glucose concentration. In the case of [^{18}F]AFDG, on the other hand, no effect of medium glucose level was observed in 2× glucose medium, and even in 10× glucose medium, only a small decrease of [^{18}F]AFDG uptake was observed. The difference in the metabolism of [^{18}F]AFDG and [^{18}F]FDG was thought to be due to their different mechanisms of uptake, i.e., lipophilic penetration and carrier-mediated transport, respectively. Therefore, it may be said that the effect of glucose concentration on [^{18}F]FDG accumulation is the result of competition between [^{18}F]FDG and glucose at GLUT. Thus, although [^{18}F]AFDG is similar to [^{18}F]FDG with regard to phosphorylation by hexokinase, the mechanism by which [^{18}F]AFDG is taken up by cells differs markedly from that of [^{18}F]FDG.

Hexokinase activity has been reported to be correlated with parameters of malignancy such as growth rate and differentiation level (*31*). A tracer that can measure hexokinase activity selectively could provide useful information about malignancy of tumors. On the other hand, overexpression of GLUT, especially GLUT1, in tumor cells has been reported by many researchers (*13–16*). Some kinds of oncogenes have been reported to be closely correlated with overexpression of GLUT (*32,33*). The level of GLUT expression might be a useful additional parameter for tumor characterization. In hypoxic tissue, glycolysis has been reported to be enhanced along with increased expression of GLUT (*19*), and GLUT expression could also be considered as a marker of hypoxic tumors. From the results of this study, [^{18}F]AFDG was thought to be GLUT-independent [^{18}F]FDG. Its detail of character is still unknown, but further basic as well as clinical trials using such well-characterized tracers will provide new perspectives in the field of oncology.

CONCLUSION

Fluorine-18-AFDG rapidly penetrated the cell membrane as a result of its high lipophilicity and was metabolized to [^{18}F]FDG-6P within cells. Fluorine-18-AFDG was thus characterized as "GLUT-independent [^{18}F]FDG." Further studies using both [^{18}F]AFDG and [^{18}F]FDG could resolve glucose metabolism in tumor cells, especially the relationship between GLUT and hexokinase.

REFERENCES

1. Warburg O. The metabolism of the carcinoma cell. In: *The metabolism of tumors*. New York: Richard R. Smith Inc.; 1931:129–169.
2. Warburg O. On the origins of cancer cells. *Science* 1956;123:309–314.

3. Weinhouse S. Glycolysis, respiration and anomalous gene expression in experimental hepatomas. Clows memorial lecture. *Cancer Res* 1972;32:2007–2016.
4. Hatanaka M. Transport of sugars in tumor cell membranes. *Biochim Biophys Acta* 1974;355:77–104.
5. Braams AW, Pruim J, Nikkels GJ. Nodal spread of squamous cell carcinoma of the oral cavity detected with PET-tyrosine, MRI and CT. *J Nucl Med* 1996;37:897–901.
6. Wahl RL, Cody R, Hutchins G, et al. Positron-emission tomographic scanning of primary and metastatic breast carcinoma with the radiolabeled glucose analog 2-deoxy-2-[^{18}F]-fluoro-D-glucose [Letter]. *N Engl J Med* 1991;324:200.
7. Sokoloff L, Reivich M, Kennedy C, et al. The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem* 1977;28:897–916.
8. Kuhl DE, Metter EJ, Riege WH. Patterns of local cerebral glucose utilization determined in Parkinson's disease by the [^{18}F]fluorodeoxyglucose method. *Ann Neurol* 1984;15:419–424.
9. Kester MV, Phillips TL, Gracy RW. Changes in glycolytic enzyme levels and isoenzyme expression in human lymphocytes during blast transformation. *Arch Biochem Biophys* 1977;183:700–708.
10. Kraaijenhagen RJ, Rijksen G, Staal EJ. Hexokinase isoenzyme distribution and regulatory properties in lymphoid cells. *Biochim Biophys Acta* 1980;631:402–410.
11. Rempel A, Bannasch P, Mayer D. Differences in expression and intracellular distribution of hexokinase isoenzymes in rat liver cells of different transformation stages. *Biochim Biophys Acta* 1994;1219:660–668.
12. Gallagher BM, Fowler JS, Gutterson NI, et al. Metabolic trapping as a principle of radiopharmaceutical design: some factors responsible for the biodistribution of [^{18}F]2-deoxy-2-fluoro-D-glucose. *J Nucl Med* 1978;19:1154–1161.
13. Nishioka T, Oda Y, Seino Y, et al. Distribution of the glucose transporters in human brain tumors. *Cancer Res* 1992;52:3972–3979.
14. Brown RS, Wahl RL. Overexpression of GLUT-1 glucose transporter in human breast cancer: an immnohistochemical study. *Cancer* 1993;72:2979–2985.
15. Yamamoto T, Seino Y, Fukumoto A, et al. Overexpression of facilitated glucose transporter genes in human cancer. *Biochem Biophys Res Commun* 1990;170:223–230.
16. Nishioka T, Oda Y, Seino Y, et al. Distribution of the glucose transporters in human brain tumors. *Cancer Res* 1992;52:3972–3979.
17. Mueller-Klieser W, Vaupel P, Manz R, Schmidseder R. Intracapillary oxyhemoglobin saturation of malignant tumors in humans. *Int J Radiat Oncol Biol Phys* 1981;7:1397–1404.
18. Brown JM, Giaccia AJ. Tumor hypoxia: the picture has changed in the 1990s. *Int J Radiat Biol* 1994:65:95–102.
19. Clavo AC, Brown RS, Wahl RL. Fluorodeoxyglucose uptake in human cancer cell lines is increased by hypoxia. *J Nucl Med* 1995;36:1625–1632.
20. Hamacher K, Coenen HH, Stocklin G. Efficient stereospecific synthesis of no-carrier-added 2-[F-18]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *J Nucl Med* 1986;27:235–238.
21. Shetty M, Loeb JN, Ismail-Beigi F. Enhancement of glucose transport in response to inhibition of oxidative metabolism: pre- and posttranslational mechanisms. *Am J Physiol* 1992;262:C527–C532.
22. Klip A, Logan WJ, Li G. Hexose transport in L6 muscle cells. Kinetic properties and the number of [^{3}H]cytochalasin B binding sites. *Biochim Biophys Acta* 1982;687:265–280.
23. Polt, R, Porreca F, Szabo L, et al. Glycopeptide enkephalin analogues produce analgesia in mice: evidence for penetration of the blood-brain barrier. *Proc Natl Acad Sci* 1994;91:7114–7118.
24. Gateley SJ. Iodine-123-labeled glucose analogs: prospects for a single-photon-emitting analog of fluorine-18-labeled deoxyglucose. *Nucl Med Biol* 1995;22:829–835.
25. Cartee GD, Douen AG, Ramlal A, et al. Stimulation of glucose transport in skeletal muscle by hypoxia. *J Appl Physiol* 1991;70:1593–1600.
26. Koivisto UM, Martinez-Valdez H, Bilan PJ, et al. Differential regulation of the GLUT-1 and GLUT-4 glucose transport systems by glucose and insulin in L6 muscle cells in culture. *J Biol Chem* 1991;266:2615–2621.
27. Bashan N, Burdett E, Guma A, et al. Mechanisms of adaptation of glucose transporters to changes in the oxidative chain of muscle and fat cells. *Am J Physiol* 1993;264: C430–C440.
28. Mercado CL, Loeb JN, Ismail-Beigi F. Enhanced glucose transport in response to inhibition of respiration in clone 9 cells. *Am J Physiol* 1989;257:C19–C28.
29. Lindholm P, Minn H, Leskine-Kallio S, et al. Influence of the blood glucose concentration on FDG uptake in cancer: a PET study. *J Nucl Med* 1992;34:1–6.
30. Wahl RL, Henry CA, Ethier SP. Serum glucose effects on tumor and normal tissue accumulation of [^{18}F]fluoro-2-deoxy-D-glucose (FDG) in rodents with mammary carcinoma. *Radiology* 1992;183:643–647.
31. Knox WE, Jamdar SC, Davis PA. Hexokinase, differentiation and growth rates of transplanted rat tumors. *Cancer Res* 1970;30:2240–2244.
32. Flier JS, Mueckler MM, Usher P, et al. Elevated levels of glucose transport and transporter messenger RNA are induced by *ras* or *src* oncogenes. *Science* 1987;235: 1492–1495.
33. Birnbaum MJ, Haespel HC, Rosen OM. Transformation of fibroblasts by FSV rapidly increases glucose transporter gene transcription. *Science* 1987;235:1485–1498.

Tumor Metabolic Rates in Sarcoma Using FDG PET

Janet F. Eary and David A. Mankoff
Division of Nuclear Medicine, University of Washington, Seattle, Washington

In a busy clinical environment, the arterial blood sampling and long imaging time used for the determination of tumor metabolic rates are not always feasible. In this study, the relationship of tumor standard uptake value (SUV) and metabolic rate of FDG (MRFDG) was investigated in a group of patients with sarcoma. To further investigate the implications of reducing blood sampling requirements for determining tumor metabolic rate, the relationship between FDG blood clearance, obtained from serial venous blood sampling and from a hybrid method of early cardiac blood pool imaging, and late venous blood sampling was analyzed. **Methods:** Comparisons of the sarcoma SUV and MRFDG obtained using graphical analysis, dynamic FDG imaging and venous blood sampling were made. Also, venous and hybrid blood time-activity curves were analyzed for similarity and for their effect on the estimated tumor metabolic rate. **Results:** For this group of patients with sarcoma (n = 42), the tumor SUV and MRFDG had a consistent relationship, with an overall correlation coefficient of 0.94. The MRFDG, determined by venous blood sampling, had a 6% average overestimate, compared to the same value obtained by the hybrid method of early blood pool imaging and late venous sampling. **Conclusion:** Both the correlation of SUV and MRFDG and the hybrid blood pool/tumor imaging protocol provide clinically feasible methods for obtaining tumor metabolic rate information in a busy clinical PET service.

Key Words: FDG; sarcoma; PET; metabolic rate

J Nucl Med 1998; 39:250–254

A goal of our institution is to provide information on pretreatment tumor grade in sarcoma and to assess response to therapy. Sarcomas present clinically as large masses that are often heterogeneous, and the biological behavior of these tumors is thought to be driven by the highest-grade region of the tumor. The characteristics of this tumor region are the data upon which treatment decision and planning are based. A practical problem that exists in treatment is that biopsy of a small portion of tumor does not yield the overall character of the tumor and may miss those clinically significant high-grade areas.

We proposed that, in our sarcoma tumor population, FDG imaging would provide objective information on the entire tumor and that generation of the local metabolic rate of FDG (MRFDG) of the tumor from dynamic quantitative imaging would improve performance of FDG static imaging and semiquantitative analysis for assessing tumor grade (*1–3*). However, because of the practical problems associated with clinical

Received Sep. 6, 1996; revision accepted Mar. 27, 1997.
For correspondence or reprints contact: Janet F. Eary, MD, University of Washington Medical Center, Division of Nuclear Medicine, Box 356113, Seattle, WA 98195-6113.