Investigation of Transport Mechanism and Uptake Kinetics of O-(2-[^{18}F]Fluoroethyl)-L-Tyrosine In Vitro and In Vivo

Peter Heiss, Sabine Mayer, Michael Herz, Hans-Jürgen Wester, Markus Schwaiger and Reingard Senekowitsch-Schmidtke

Nuklearmedizinische Klinik and Poliklinik, Technische Universität München, Munich, Germany

The aim of the study was to investigate the transport mechanism and uptake kinetics of the new ^{18}F-labeled amino acid O-(2-[^{18}F]fluoroethyl)-L-tyrosine (L-[^{18}F]FET) and D-[^{18}F]FET in human SW 707 colon carcinoma cells and the in vivo biodistribution of this tracer in SW 707 tumor-bearing mice. **Methods:** SW 707 cells were incubated with L- and D-[^{18}F]FET under physiologic amino acid concentrations with and without the competitive transport inhibitors 2-amino-2 norbornane-carboxylic acid and α-(methylamino)isobutyric acid plus serine. For the investigation of the transport capacity, unlabeled L-FET was added to the samples. In addition, xenotransplanted mice were injected intravenously with L-[^{18}F]FET; killed 10, 30, 60 and 120 min after injection; and the radioactivity concentration in different organs was measured in a gamma counter. **Results:** The in vitro kinetic experiments showed a fast initial uptake of L-[^{18}F]FET into the cells up to 6 min, followed by a nearly constant tracer concentration. The accumulation factor, calculated as the ratio between intracellular and extracellular tracer concentration, ranged from 3.0 to 5.0. In comparison, D-[^{18}F]FET did not accumulate in the cells. Washing the cells in medium at 37°C, after a 30-min incubation with L-[F-18]FET, led to a rapid decrease of radioactivity, which demonstrates the bidirectional transport. In addition, experiments with increasing concentrations of unlabeled L-FET indicated a linear correlation between L-FET uptake rate and the extracellular concentration. Results of transport inhibition experiments with the specific competitive inhibitors demonstrated that the uptake of L-FET into SW 707 cells was caused mainly (>80%) by the transport system L. In the in vivo studies, the half-life ($t_{1/2\ \beta}$) of L-[^{18}F]FET in the plasma was determined to be 94 min and the uptake into the brain increased to 120 min with a brain-to-blood ratio of 0.86. The xenotransplanted tumor showed higher uptake of L-[^{18}F]FET (>6 %ID/g) at 30 and 60 min than all other organs, except the pancreas. The tumor-to-blood ratio reached about 2 between 30 and 120 min. **Conclusion:** L-[^{18}F]FET, which is transported by the specific amino acid transport system L, seems to be a potential amino acid tracer for tumor imaging and therapy monitoring with PET.

Key Words: PET; amino acids; O-(2-[^{18}F]fluoroethyl)tyrosine; tumor imaging

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The glucose analog (2-deoxy-2-[^{18}F]fluoro)-D-glucose (FDG) is routinely used in PET for tumor diagnosis. However, several limitations of this tracer, e.g., high uptake of FDG in brain and in nonmalignant, inflammatory cellular elements (*1*), demand the development of new imaging tracers.

In many studies, radiolabeled amino acids have proven to be useful for imaging tumors, especially for brain tumors (*2*) but also for peripheral tumors such as lymphoma (*3*), lung tumors (*4*) and breast cancer (*5*). Furthermore, it is under investigation whether amino acids are useful for tumor grading and for demonstrating therapeutic effects. In a previous study by Kubota et al. (*6*), the results showed that the accumulation of methionine was lower in macrophages compared with FDG. Consequently, one would expect fewer false-positive results with amino acids compared with FDG, specifically if inflammatory processes are coexisting.

Currently, the commonly used amino acid tracer for PET is [methyl-^{11}C]-L-methionine. However, because of the short half-life of ^{11}C ($t_{1/2}$ = 20 min), the availability of ^{11}C-labeled tracers is limited to PET centers with an in-house cyclotron, preventing the widespread use of these compounds. Therefore, an ^{18}F-labeled amino acid ($t_{1/2}$ = 110 min) would be more advantageous for routine PET application. Several ^{18}F-labeled amino acids have been evaluated, such as p-[^{18}F]fluorophenylalanine (*7*) and 2-[^{18}F]fluorotyrosine (*8*). The syntheses, however, were difficult and only low yields were obtained. In comparison, the synthesis of the new artificial ^{18}F-labeled amino acid tracer O-(2-[^{18}F]fluoroethyl)-L-tyrosine (L-[^{18}F]FET) is fast (less than 1 h), can be automated and the tracer was obtained in a high yield (*9,10*). In addition, first clinical results demonstrated the ability of this tracer for brain tumor imaging (*9*).

As previously shown, the accumulation of amino acids in tissue is due to the transport rather than protein synthesis (*2,11*). Therefore, the aim of this study was to characterize the transport mechanism responsible for the uptake of L-[^{18}F]FET into tumor cells and to investigate the uptake kinetics in human colon carcinoma cells SW 707 in vitro and in the corresponding xenotransplanted tumor in vivo.

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For correspondence and reprints contact: Reingard Senekowitsch-Schmidtke, MD, PhD, Nuklearmedizinische Klinik und Poliklinik, Technische Universität München, Ismaningerstr. 22, D-81675 Munich, Germany.

MATERIALS AND METHODS

Cell Culture and Xenotransplantation

All experiments were performed with the human colon carcinoma cell line SW 707, which was received from the Tumor Bank of the Cancer Research Center, Heidelberg, Germany. The cells were cultivated in RPMI 1640 medium with the physiologic glucose concentration (1 g/L) containing 5% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2. The medium was routinely renewed three times a week. Two days before the in vitro experiments, the cells were trypsinized and 2×10^5 cells per well were seeded in 24-well plates. For the in vivo studies, BALBc/-nu nu mice were subcutaneously injected with a 0.1-mL suspension containing 1×10^7 cells. The biodistribution experiments were performed 14–21 d later.

In Vitro Studies

Uptake Experiments. L-[^{18}F]FET and D-[^{18}F]FET were synthesized as published previously by Wester et al. (*9*). All experiments were performed in an RPMI 1640 medium with modified amino acid concentrations corresponding to the human plasma levels, including tyrosine. Before the incubation with L-[^{18}F]FET, the cells were preincubated for 30 min in 200 μL medium with and without the supplement of unlabeled L-FET (72 μmol/L) corresponding to the physiologic tyrosine concentration. Aliquots of 400 μL [^{18}F]FET (1.85 MBq/mL) were added and the cells were incubated at 37°C for 1, 2, 4, 6, 10, 20 and 30 min. After stopping the tracer uptake with 1 mL ice-cold phosphate-buffered saline (PBS), the cells were washed three times with PBS at 4°C and dissolved in 1.5 mL 0.1 N NaOH plus 2% Triton X. The radioactivity in the cells was measured with a gamma counter (Wallac, Turku, Finland). In addition, the number of cells per well and the mean volume of the cells were determined electromechanically (CASI 1; Schaerfe System GmbH, Germany) for the calculation of the intracellular tracer concentration. The results were expressed as cpm/10^6 cells or as accumulation factor, which is calculated as the ratio between the intracellular tracer concentration and the concentration in the incubation medium.

Release Experiments. After the incubation of the cells with L-[^{18}F]FET for 30 min, the medium was replaced by 600 μL tracer-free culture medium and the samples were incubated at 37°C for 2, 4, 6 and 20 min. After the incubation, the medium was removed and the cells were washed three times with ice-cold PBS. Dissolving and measuring of the activity in the cell pellets were performed as previously described.

Acid Precipitation. The samples were incubated with L-[^{18}F]FET for 20 and 30 min as previously described and the incubation medium was removed. The cells were detached with 0.5 mL ethylenediaminetetraacetic acid (1%), transferred into a cup and 0.5 mL 20% trichloroacetic acid was added. After 30 min on ice, the samples were centrifuged at 6000 rpm for 4 min. The supernatant was removed, and the pellet was washed three times in ice-cold PBS. Dissolving and measuring of the activity in the acid precipitable fraction were performed as previously described.

Competitive Inhibition Experiments. The inhibition experiments to characterize the transport system were performed with the specific competitive inhibitors for system L: 2-amino-2 norbornanecarboxylic acid (BCH); and for systems A and ASC: α-(methylamino)isobutyric acid (MeAIB) plus serine. The concentration of the inhibitors used was 15 mmol/L. Parallel experiments, which were performed with increasing concentrations of unlabeled L-FET, were performed to investigate the capacity of the transport system. The medium used for the incubation with L-[^{18}F]FET was the same as for the uptake experiments. After preincubation of the cells in 200 μL of this medium for 30 min, 200 μL [^{18}F]FET (1.85 MBq/mL) and 200 μL of one of the inhibitors or unlabeled L-FET (0–330 μmol/L) were added and the samples were incubated at 37°C for 2 min. After stopping the tracer uptake with 1 mL ice-cold PBS, the cells were washed three times, dissolved and the activity measured as previously described.

In Vivo Studies

Biodistribution. BALB c/-nu nu mice bearing human SW 707 colon carcinoma were injected into the tail vein with 1.85 MBq L-[^{18}F]FET in 0.1 mL saline. The animals were killed by cervical dislocation at 10, 30, 60 and 120 min after injection. Different organs were immediately dissected and weighed. The ^{18}F radioactivity was measured in a gamma counter, and the tissue uptake of the tracer was expressed as percentage of injected dose per gram of tissue (%ID/g).

Statistical Analysis

The nonparametric Mann-Whitney *U* test was used to analyze the data. A two-tailed *P* value of less than 0.05 was considered statistically significant.

RESULTS

Uptake and Release Studies

L-[^{18}F]FET showed a fast increasing initial uptake into SW 707 colon carcinoma cells for the first 6 min, followed by a nearly constant tracer concentration in the cells. The accumulation factor, calculated as the ratio between intracellular and extracellular tracer concentration, ranged from 3.0 to 5.0 (Fig. 1). The cell volume, which was used for the calculation of the intracellular tracer concentration, was measured electromechanically and determined as approximately $1.4 \times 10^{-6} \pm 0.09 \times 10^{-6}$ μL. Uptake experiments with D-[^{18}F]FET showed a low linear uptake over 20 min and the accumulation factor remained under 1 (Fig. 1). L-[^{18}F]FET uptake was also investigated after preincubation of the cells in culture medium supplemented with unlabeled L-FET (72 μmol/L). The concentration of 72 μmol/L corresponds to the L-tyrosine level reported for the human plasma (48–84 μmol/L) (*12*). No significant reduction of L-[^{18}F]FET uptake was found (Fig. 2), which is an indication that no saturation of the tracer accumulation occurred up to a concentration corresponding to the L-tyrosine level.

Samples, precipitated with trichloroacetic acid after an incubation with L-[^{18}F]FET for 20 and 30 min, demonstrated less than 1% of the radioactivity in the acid precipitable fraction. Therefore, no incorporation of L-[^{18}F]FET into proteins could be determined.

The release of L-[^{18}F]FET from the cells was demonstrated by washing the cells at 37°C in tracer-free medium (after an incubation with the tracers for 30 min). L-[^{18}F]FET was washed out very quickly (Fig. 3). After 6 min in the tracer-free medium, only 7% of the tracer concentration was measured in the cells.

In the competitive inhibition experiments, the specific transport inhibitors BCH and MeAIB plus serine were used

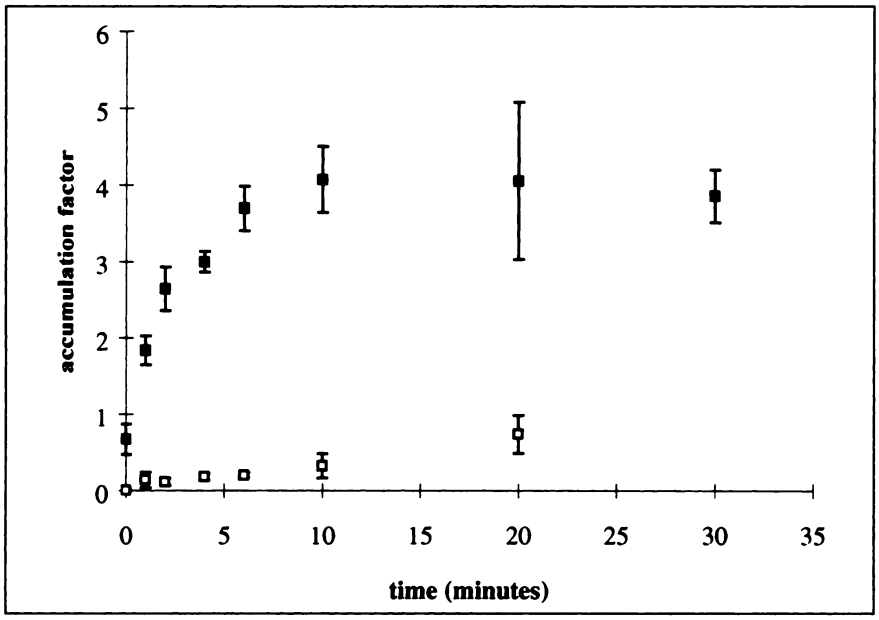

FIGURE 1. Uptake kinetics of L-[^{18}F]FET (■) and D-[^{18}F]FET (□) in SW 707 cells; calculated as accumulation factor between tracer concentration intracellular and in medium (mean ± SD).

to inhibit the three main transport systems of neutral amino acids. The addition of BCH, specific for system L, resulted in approximately 80% reduction of L-[^{18}F]FET uptake, whereas the addition of MeAIB plus serine, specific for systems A and ASC, caused no significant change ($P = 0.11$) in the tracer uptake (Fig. 4). The experiments with increasing unlabeled L-FET concentration (0–330 μmol/L) showed no significant change in the radioactivity concentration in the cells (Fig. 5). Therefore, no saturation of the transport capacity was found until a concentration was reached which was five times higher than the physiologic tyrosine level in the human plasma.

In Vivo Distribution

The tissue distribution data of L-[^{18}F]FET in athymic nude mice xenotransplanted with SW 707 colon carcinoma, expressed in %ID/g, are summarized in Table 1. The concentration in the blood decreased over time, and the plasma half-life time ($t_{1/2,\beta}$) was calculated at 94 min. In all organs, except the tumor and the brain, the highest activity concentration was measured at 10 min after injection. Tissue-to-blood ratio was only significantly higher than 1 for the pancreas and tumor. The maximum in the brain uptake for L-[^{18}F]FET was reached at 60 min after injection. The brain-to-blood ratio increased to 0.86 at 120 min for L-[^{18}F]FET (Fig. 6). The uptake of L-[^{18}F]FET into the tumor increased from 10 to 30 min and reached 6.4 ± 1.4 %ID/g. The tumor-to-blood ratio increased up to 30 min after injection and showed no significant change until 120 min with values around 2 (Fig. 6). The highest tissue uptake of L-[^{18}F]FET was found in the pancreas and a high

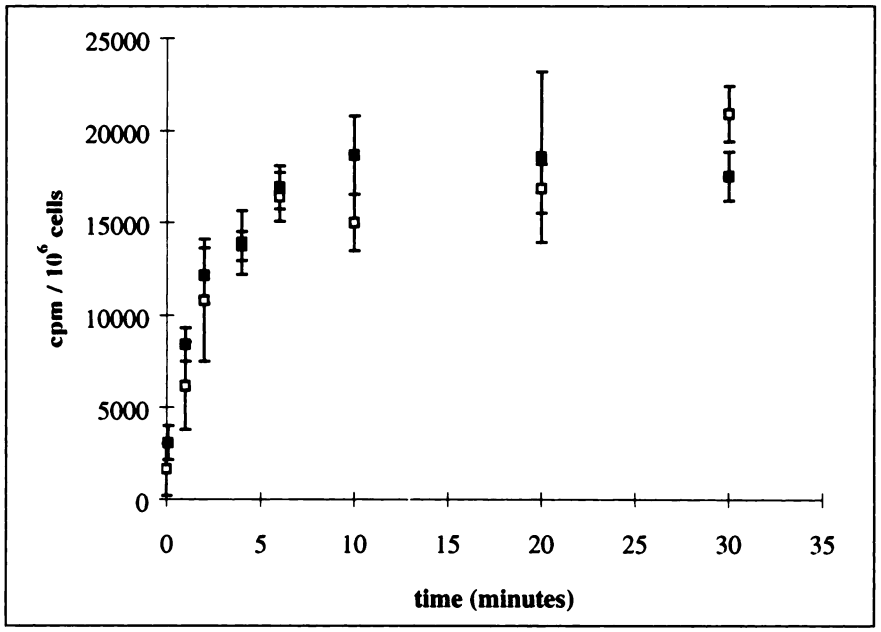

FIGURE 2. Uptake kinetics of L-[^{18}F]FET per 10^6 cells after preincubation with unlabeled L-FET (13 mg/L) for 30 min (□) and without adding unlabeled L-FET (■).

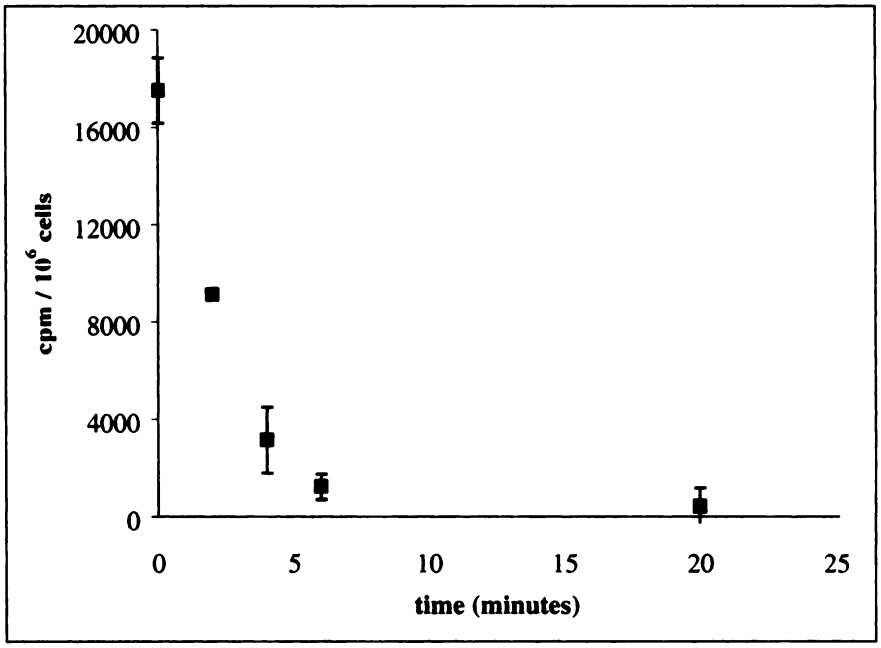

FIGURE 3. Release of L-[^{18}F]FET per 10^6 cells at 37°C after incubation for 30 min.

radioactivity concentration in the urine, but without accumulation in the kidney.

DISCUSSION

Radiolabeled, nonmetabolized amino acids, such as α-(N-[1-^{11}C]acetyl)-aminoisobutyric acid (*13*), L-3[^{18}F]fluoro-α-methyl tyrosine (*14–16*) and L-[^{18}F]FET (*9,10*), are promising agents for tumor imaging and eventually for therapy monitoring with PET. The fluorine label with a half-life of 110 min and the short, high-yielding radiosynthesis of L-[^{18}F]FET meet the requirements for routine application. Several studies have suggested that the amino acid transport is the dominating step for the increased tracer accumulation in the tumor tissue. Therefore, we investigated the transport system of L-[^{18}F]FET in tumor cells. The possible pathways for the transport of neutral amino acids are described as three major systems: A, ASC and L. Systems A and ASC serve mainly for the uptake of amino acids with short, polar or linear side chains. Branched and aromatic amino acids enter the cells mainly by system L (*17*). For L-tyrosine, the system L was determined as the main transport system in melanoma cells (*18,19*) and, as already shown in our laboratory, on SW 707 cells (Senekowitsch-Schmidtke et al., unpublished data). Analogous to these experiments, we investigated which system or combination of systems is involved in the uptake of L-[^{18}F]FET by competitive inhibition experiments with the transport inhibitors BCH and MeAIB plus serine. BCH is a specific competitive inhibitor for system L, whereas MeAIB inhibits specifically system A, and in combination with serine, also system ASC (*17*). In our experiments, we found a specific transport of L-[^{18}F]FET in SW 707 cells, which is mainly mediated by the system L; no substantial involvement of systems A and ASC could be demonstrated. Therefore, L-[^{18}F]FET enters the cells over the same pathway as L-tyrosine. System L has no requirement of sodium and energy sources and is unaffected by insulin in contrast to systems A and ASC (*20*). The activity of system L is sensitive to the presence of intracellular branched and aromatic amino acids because of the exchange properties of this system (*21*). Therefore, we performed our experiments under conditions in which all amino acids were existing in physiologic concentrations of the human plasma. The bidirectional transport of L-FET in SW 707 cells, which was expected because of the reversibility of system L in the amino acid transport (*21*), was demonstrated by our release experiments and the kinetic experiments after preincubation with unlabeled L-FET. Investigations of the transport capac-

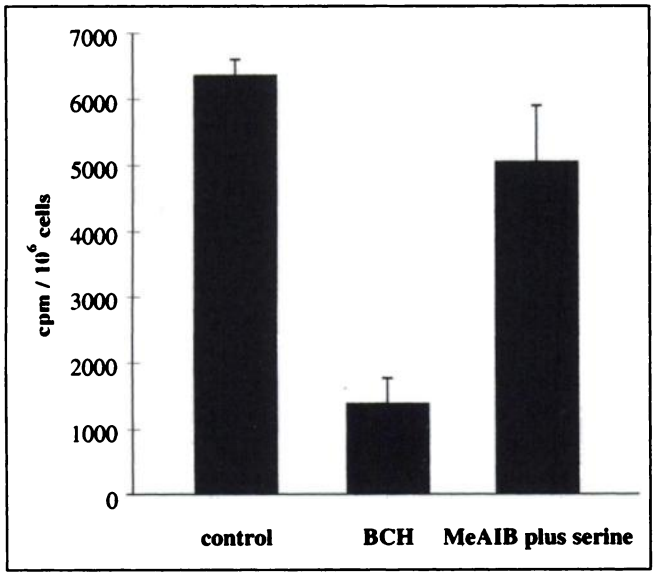

FIGURE 4. Uptake of L-[^{18}F]FET per 10^6 cells after coincubation with specific transport inhibitors (15 mmol/L) for 2 min; BCH as competitive inhibitor of system L, MeAIB plus serine as competitive inhibitor of systems A and ASC.

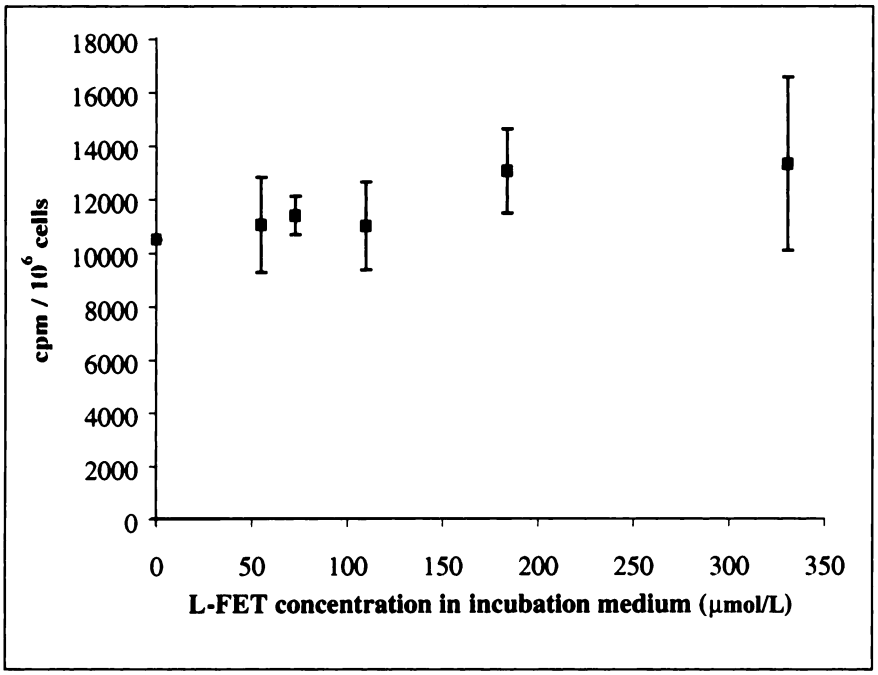

FIGURE 5. Uptake of L-[^{18}F]FET per 10^6 cells at different concentrations of L-FET in incubation medium.

ity for L-FET in SW 707 cells did not show a saturation up to the concentration five times higher (330 μmol/L) than the normal tyrosine concentration in the human plasma. The range of the normal L-tyrosine concentration is reported to be between 48–84 μmol/L (*12*). This result is in agreement with published data by Pankovich and Jimbow (*19*), who found in melanoma cells a saturation of the system L only at a tyrosine concentration higher than 200 μmol/L. Furthermore, the results are in accordance with the study by Tovar et al. (*20*), who showed under physiologic conditions no competition for transport through system L. In addition to the high capacity of the transport system, we found a dramatically slower transport rate for D-[^{18}F]FET. Therefore, we could demonstrate the stereospecificity of the FET uptake. This result is in agreement with the results found for the brain uptake in vivo by Wester et al. (*9*).

TABLE 1
Tissue Distribution of O-(2-[^{18}F]fluoroethyl)-L-tyrosine in Colon Carcinoma-Bearing Mice After Intravenous Injection

	%ID/g tissue			
Site*	10 min n = 12	30 min n = 12	60 min n = 12	120 min n = 6
Blood	4.2 ± 0.7	3.4 ± 0.3	2.9 ± 0.6	1.9 ± 0.3
Plasma	4.3 ± 0.7	3.6 ± 0.3	3.0 ± 0.5	1.9 ± 0.3
Brain	1.5 ± 0.3	2.0 ± 0.3	2.2 ± 0.6	1.6 ± 0.2
Pancreas	26.4 ± 5.2	18.5 ± 2.2	18.9 ± 5.3	15.0 ± 3.5
Heart	3.6 ± 0.5	3.0 ± 0.3	2.5 ± 0.4	1.6 ± 0.2
Liver	3.4 ± 0.5	2.9 ± 0.4	2.5 ± 0.4	1.7 ± 0.2
Kidney	4.1 ± 0.6	3.5 ± 0.4	3.0 ± 0.5	1.9 ± 0.2
Muscle	3.1 ± 0.7	2.8 ± 0.4	2.3 ± 0.4	1.3 ± 0.5
Colon	3.0 ± 0.7	3.2 ± 0.8	2.6 ± 0.6	1.4 ± 0.5
Tumor	4.0 ± 0.9	6.4 ± 1.4	6.3 ± 1.7	3.4 ± 1.6

*Only two values for urine were available, each at 10 min: 46.4 and 44.6 %ID/g.
%ID/g = percentage of injected dose per gram.

The kinetic experiments showed that after an initial increase of L-[^{18}F]FET lasting 6 min, an equilibrium of radioactivity in the cells was reached. Calculations of the tracer concentration resulted in a substantial accumulation of L-[^{18}F]FET in the cells. However, no incorporation of L-[^{18}F]FET into macromolecules could be demonstrated. Two different explanations for the accumulation in cells have been offered. Oxender et al. (*21*) suggested that amino acids such as methionine, with a high affinity to systems A and L, enter into the cells through system A and exit from the cells through system L as an exchange for other amino acids. Therefore, amino acids exclusively transported by system L can be highly concentrated as long as amino acids are present for the exchange mechanism. Alternatively, in regard to L-[^{18}F]FET, a second explanation is the reversible binding of this tracer to intracellular structures, which would also allow an accumulation. However, as recently published, no intracellular binding complex has yet been demonstrated by high-performance liquid chromatographic analysis of tumor tissue (*9*).

As shown in the in vitro experiments, the biodistribution experiments of L-[^{18}F]FET in xenotransplanted nude mice also showed an accumulation of L-[^{18}F]FET in the tumor with a continuous increase up to 30 min after injection and a tumor-to-blood ratio greater than 2 at 60 and 120 min. The blood clearance of L-[^{18}F]FET, with a half-life ($t_{1/2,\beta}$) of 96 min, was slower compared with L-3-[^{123}I]-α-methyl tyrosine (IMT), with a half-life of 57 min and other amino acids, such as L-tyrosine (*22*) and L-methionine (*23*). The time course

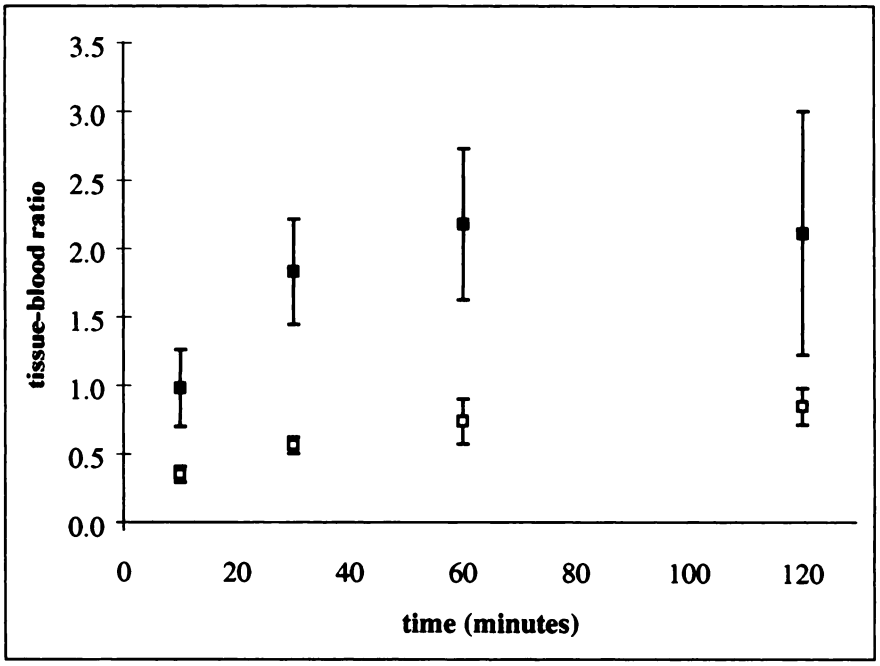

FIGURE 6. Time course of tumor-to-blood ratio (■) and brain-to-blood ratio (□) after intravenous injection of L-[^{18}F]FET in xenotransplanted mice (n = 12; mean ± SD).

of L-[^{18}F]FET in the blood is in accordance to nonmetabolized amino acid, such as IMT. No increase of radioactivity with time was observed compared with L-[3,5-H-3]tyrosine, which was measured in double labeling experiments. It has been previously demonstrated that L-[^{18}F]FET is not metabolized in vivo (*9*). Therefore, the kinetic model, which is necessary for quantitative PET studies, is less complex compared with the model used by [^{11}C]methionine. The retarded clearance of L-[^{18}F]FET led to an increase of tracer uptake in the brain up to 60 min with a continuously increasing ratio. A very high uptake of this tracer was found only for the pancreas. In comparison to published data for IMT (*19*) and L-3[^{18}F]fluoro-α-methyl tyrosine (*11*), the %ID/g of L-[^{18}F]FET was substantially lower in the kidney, but a high radioactivity concentration was found in the urine.

CONCLUSION

In our study, the in vitro experiments with SW 707 colon carcinoma cells demonstrated a rapid accumulation of the amino acid L-[^{18}F]FET. The stereospecific, high capacity uptake into the tumor cells occurred nearly exclusively through the system L. These properties would allow dynamic PET studies to provide information regarding both the capacity of the transport system of the tumor and changes in the transport system L because of the tumor therapy. Therefore, the results of this study indicate L-[^{18}F]FET, as a nonmetabolized L-tyrosine analog, and thus is a promising tracer for tumor imaging with PET.

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