# Transport Mechanisms of 3-[$^{123}$I]Iodo-α-Methyl-L-Tyrosine in a Human Glioma Cell Line: Comparison with [$^{3}$H-methyl]-L-Methionine

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The amino acid analog 3-[$^{123}$I]iodo-α-methyl-L-tyrosine (IMT) is under clinical evaluation as a SPECT tracer of amino acid transport in brain tumors. This study investigated the carrier systems involved in IMT transport in human glioma cells in comparison with [$^{3}$H-methyl]-L-methionine ($^{3}$H-MET). **Methods:** Human glioma cells, type 86HG-39, were cultured and incubated for 1 min at 37°C with IMT and $^{3}$H-MET in the lag phase (1.2 d after seeding), exponential growth phase (3 d after seeding), and plateau phase (8 d after seeding). Experiments were performed in the presence and absence of $Na^+$, during inhibition of system L amino acid transport by 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH), and during inhibition of system A amino acid transport by 2-(methylamino)-isobutyric acid (MeAIB). **Results:** IMT and $^{3}$H-MET uptake decreased by 55%–73% when the cells entered from the exponential growth phase into the plateau phase ($P < 0.05$; n = 3–11). Inhibition by BCH reduced uptake of IMT in the lag phase, exponential growth phase, and plateau phase by 90%–98% ($P < 0.001$; n = 3–6) and the uptake of $^{3}$H-MET by 73%–83% ($P < 0.001$; n = 3–11). In a $Na^+$-free medium $^{3}$H-MET uptake was reduced by 23%–33% ($P < 0.05$; n = 3–11), whereas IMT uptake was not significantly different. MeAIB showed no significant effect on IMT or $^{3}$H-MET uptake in either phase. **Conclusion:** Transport of both IMT and $^{3}$H-MET depends on the proliferation rate of human glioma cells in vitro and is dominated by BCH-sensitive transport. These data indicate that system L is induced in rapidly proliferating glioma cells and is the main contributor to the uptake of both tracers. $^{3}$H-MET transport showed a minor $Na^+$ dependency that was not attributable to system A. The similarity of transport mechanisms of both tracers emphasizes the clinical equivalence of IMT SPECT and $^{11}$C-MET PET for the diagnostic evaluation of gliomas.

**Key Words:** amino acid transport; 3-[$^{123}$I]iodo-α-methyl-L-tyrosine; [$^{3}$H-methyl]-L-methionine; glioma cells

**J Nucl Med 2000; 41:1250–1255**

The use of radiolabeled amino acids in combination with PET has been shown to improve the diagnostic evaluation of brain tumors (*1–3*). Introduction of the amino acid analog 3-[$^{123}$I]iodo-α-methyl-L-tyrosine (IMT) as a tracer for SPECT offers a widespread application of this methodology (*4,5*). Although IMT is not incorporated into cerebral proteins (*5,6*), this tracer shows results that are comparable with those of the standard tracer [$^{11}$C-methyl]-L-methionine ($^{11}$C-MET) and PET (*7,8*). This is explained by the fact that transport phenomena play an important role in the increased accumulation of amino acids in cerebral gliomas (*9,10*). Initial clinical investigations emphasize the potential of SPECT with IMT for tumor grading and diagnosis of recurrence (*11–13*).

In vitro and in vivo studies revealed a similar transport of IMT across the blood–brain barrier compared with its parent L-tyrosine—i.e., saturable, carrier and temperature dependent, ouabain inhibitable, and cross-inhibitable by L-tyrosine (*6*). A clinical SPECT study showed that IMT uptake into the brain and cerebral gliomas is inhibited by coinfusion of L-amino acids (*14*).

However, it remains to be shown which specific carrier systems are involved in IMT transport in tumor cells and whether expression of the different carrier systems is influenced by proliferative activity of these cells. In this study we addressed these questions using a human glioma cell line. For comparison, [$^{3}$H-methyl]-L-MET ($^{3}$H-MET) was chosen because most clinical experience with gliomas was gained with PET and $^{11}$C-MET. Direct comparison of the transport mechanisms of both tracers in human glioma cells appears valuable because clinical results with $^{11}$C-MET and PET are used as a reference for SPECT with IMT.

## MATERIALS AND METHODS

### Cell Culture

The human glioblastoma cell line 86HG-39 was obtained from the Institute of Neuropathology, Heinrich-Heine-University, Düsseldorf, Germany. The immunohistochemical and morphologic criteria of this cell line have been described in detail (*15*). The cells were grown as a monolayer in Iscove's modified Dulbecco's medium (IMDM, pH 7.4; GIBCO, Karlsruhe, Germany) containing

Received Jun. 1, 1999; revision accepted Oct. 27, 1999.
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a 1% mixture of penicillin and streptomycin (10,000 IU/10 mg/mL) and 10% (volume/volume) fetal calf serum (Seromed, Berlin, Germany) at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air. An initial inoculum of $0.5 \times 10^6$ viable cells was added to 25-$cm^2$ vials (Greiner, Lemgo, Germany) containing 10 mL medium. The growth curve of the cell line is shown in Figure 1. The glioma cells progressed through a characteristic growth cycle of lag phase (days 0–2), exponential growth phase (days 3–6), and plateau phase (day 6 onward). The lag phase was the time after subculture and replating, during which time no increase in cell number was evident. The exponential growth phase corresponded to the period of exponential increase in cell number after lag phase. Toward the end of the exponential growth phase, the culture became confluent and entered the plateau phase.

### Transport Assay

Transport assays with the glioma cells were performed at different times after seeding. The method of measuring transport has been reported (*16,17*). The IMDM was discarded, and the cells were washed twice with Hank's balanced salt solution (HBSS: 136.6 mmol/L NaCl, 5.4 mmol/L KCl, 4.2 mmol/L $NaHCO_3$, 2.7 mmol/L $Na_2HPO_4$, 1 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, 0.44 mmol/L $KH_2PO_4$, and 0.41 mmol/L $MgSO_4$, pH 7.8) and depleted in 5 mL of the same buffer for 30 min at 37°C. The medium was again discarded, and the transport assay was started by adding 18.5 kBq $^3$H-MET (Amersham Buchler, Braunschweig, Germany) in 5 mL HBSS containing 50 nmol L-methionine (specific radioactivity, 370 MBq/mmol) or 18.5 kBq IMT in 5 mL HBSS containing 50 nmol iodo-α-methyl-L-tyrosine (specific radioactivity, 370 MBq/mmol), respectively. IMT was prepared as described (*18*) with a radiochemical yield of 80% ± 5% and a radiochemical purity of >99%.

The incubation was terminated after 60 s by pouring off the medium and rapidly rinsing the monolayer twice with 7 mL ice-cold phosphate-buffered saline solution (Seromed). After washing, the cells were treated with a mixture of 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid and resuspended in HBSS.

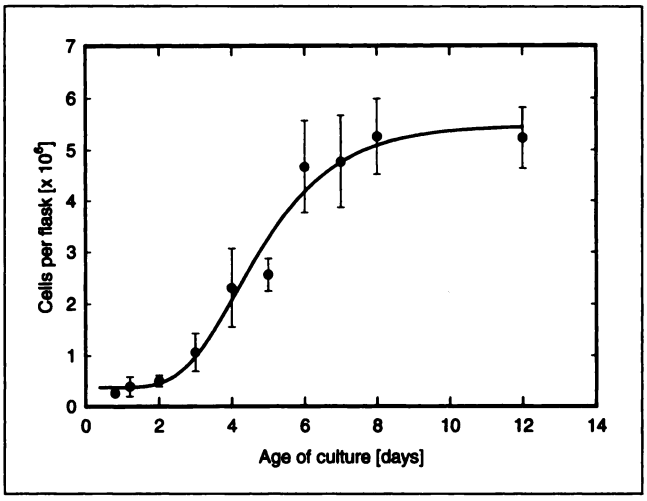


**FIGURE 1.** Cell growth curve of 86HG-39 human glioma cells. After seeding into vials as monolayers, cells progressed through characteristic growth cycle of lag phase (days 0–2), exponential growth phase (days 3–6), and plateau phase (day 6 onward). Transport experiments were performed 1.2, 3, and 8 d after seeding.

The number of cells/mL was determined by a Coulter counter (model ZM; Coulter, Krefeld, Germany). Uptake of IMT was measured with a γ counter (Berthold, Bad Wildbad, Germany), and uptake of $^3$H-MET was measured in a liquid scintillation counter (Packard Instrument, Dreieich, Germany) after dissolving the cells in perchloric acid and adding liquid scintillation cocktail (Ultima gold XR; Canberra Packard, Dreieich, Germany). The accumulation of IMT and $^3$H-MET was expressed in nmol/$10^6$ cells/min.

Experiments were repeated in the presence of either 10 mmol/L 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH; Sigma Chemical Co., Deisenhoven, Germany) to block amino acid transport through system L or 20 mmol/L 2-(methylamino)-isobutyric acid (MeAIB; Sigma) to block system A transport. All experiments were performed in the presence and absence of $Na^+$. In $Na^+$-free experiments, NaCl was replaced by choline chloride and sodium phosphate was replaced by potassium phosphate.

Each experiment was done in triplicate, averaged, and repeated 3–11 times on different days.

### Data Analysis

Statistical analysis was performed using a statistical software package (SAS mixed procedure; Statistical Analysis Systems, Cary, NC). Because the variation in uptake rates was considerably different for experiments with different cell numbers but was proportional to the corresponding mean values, a logarithmic transformation was used for variance stabilization. This approach leads to a multiplicative model for the original data that is more appropriate for evaluating the relevant relative changes rather than the absolute changes. This leads to a commonly used linear model after transformation. A multivariate analysis for repeated measurements was performed, and results were corrected for multiple testing according to Tukey–Kramer (*19*). $P < 0.05$ was considered to be significant.

## RESULTS

Uptake rates of IMT and $^3$H-MET in the lag phase, exponential growth phase, and plateau phase with different compositions of the transport medium are depicted in the bar graphs in Figures 2A and B, respectively. IMT and $^3$H-MET uptake decreased by 55%–73% when the cells progressed from the exponential growth phase into the plateau phase ($P < 0.05$; n = 3–11), indicating that transport of both tracers is dependent on the proliferative activity of the cells. Inhibition by BCH reduced the uptake of IMT in the lag phase, exponential growth phase, and plateau phase by 90%–98% ($P < 0.001$; n = 3–6) and the uptake of $^3$H-MET by 73%–83% ($P < 0.001$; n = 3–11). These data reflect the dominating role of system L-like transport for both tracers. The absence of $Na^+$ did not influence IMT uptake, but $^3$H-MET uptake was reduced by 23%–33% ($P < 0.05$; n = 3–11). MeAIB had no significant effect on uptake of IMT and $^3$H-MET in any phase, which excludes a significant role of system A-like transport in this cell line.

The dependency of tracer uptake and proliferative activity of the cells was evaluated further by plotting the tracer uptake rates against the cell number. This approach was useful because the cell number on different days after seeding varied considerably between the different experimental settings. Double logarithmic plots of IMT uptake with

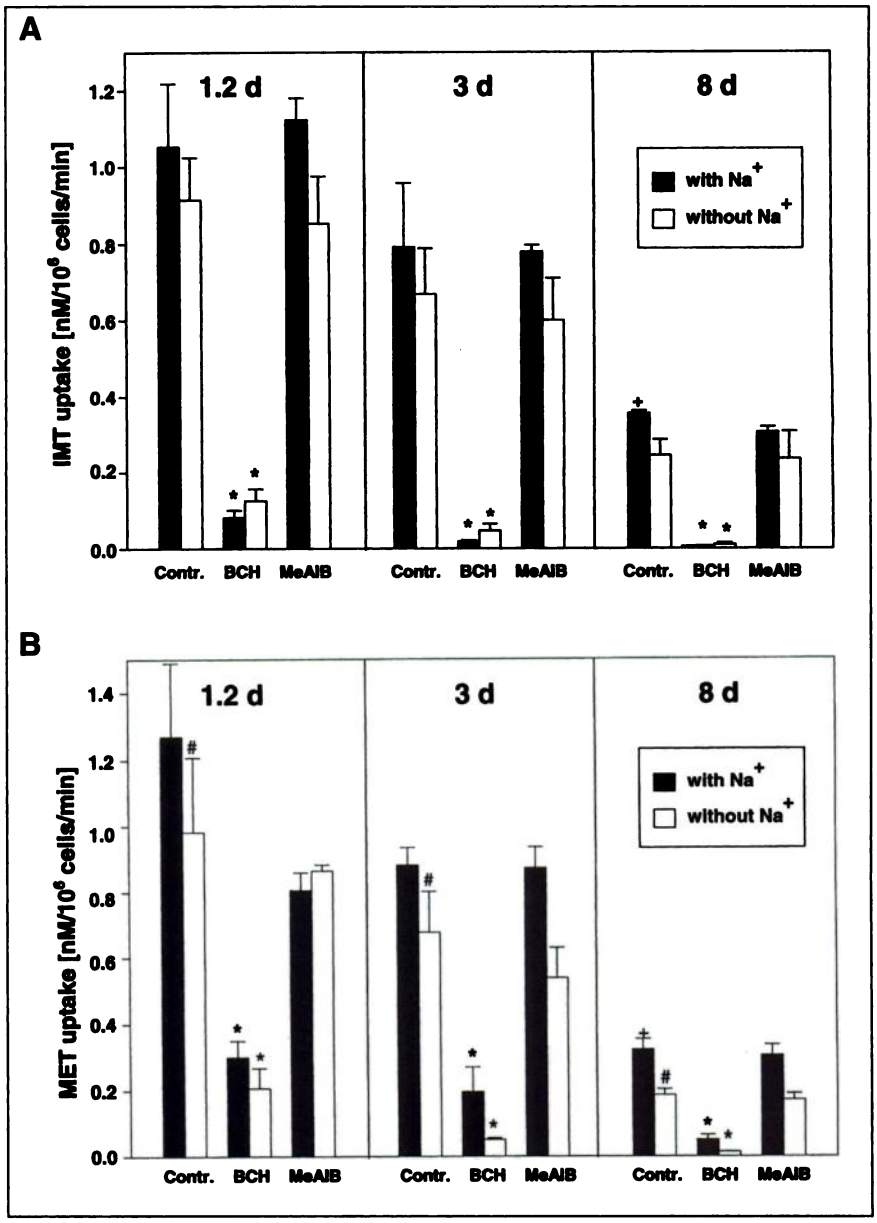


**FIGURE 2.** IMT uptake (A) and $^3$H-MET uptake (B) in 86HG-39 human glioma cells in lag phase (left, 1.2 d after seeding), exponential growth phase (middle, 3 d after seeding), and plateau phase (right, 8 d after seeding). Values are mean ± SEM. Uptake is lower in plateau phase than in lag or exponential growth phase (IMT, $^+P < 0.05$, n = 3–6; $^3$H-MET, $^+P < 0.01$, n = 3–11), indicating that IMT and MET uptake is dependent on proliferative activity of cells. Inhibition by BCH reduced uptake of IMT in all phases by 90%–98% ($^*P < 0.001$ versus control [Contr.]; n = 3–6) and of $^3$H-MET by 73%–83% ($^*P < 0.001$ versus control; n = 3–6), indicating dominant role of system L amino acid transport. In contrast with IMT, depletion of $Na^+$ led to minor reduction of $^3$H-MET uptake ($^\#P < 0.05$). System A inhibitor MeAIB shows no significant effect on IMT or $^3$H-MET uptake in either phase.

and without BCH inhibition versus cell number are shown in Figure 3A (medium with $Na^+$). The regression coefficient, b, for both plots is significantly different from zero (control, b = −0.328; BCH, b = −0.309; $P < 0.0001$), indicating that IMT uptake is dependent on the proliferative activity of the cells. The influence of BCH is independent of cell number, which confirms the dominant role of system L for IMT transport in all phases of cell growth. Figure 3B shows the same plots for $^3$H-MET (medium with $Na^+$). For both plots, b is significantly different from zero (control, b = −0.485; BCH, b = −0.435; $P < 0.0001$). Again, the influence of BCH is independent of cell number, which indicates a similar role of system L for $^3$H-MET transport in all phases of cell growth compared with IMT. The inhibitory effect of BCH appears less pronounced than that for IMT but is not significantly different from IMT data.

## DISCUSSION

The investigation of brain tumors with radiolabeled amino acids (especially $^{11}$C-MET and PET) has provided additional diagnostic information compared with CT and MRI. The clinical impact of this method has been reported in initial studies of large series of patients (*20,21*).

Although an increased rate of protein synthesis was initially assumed to be the driving force for increased uptake of amino acids in gliomas, it is now more obvious that transport phenomena play a major role in the uptake process. Thus, an inhibition of protein synthesis in mice did not influence the uptake of MET in the brain and transplanted tumors (*9*). A PET study in glioma patients with the amino acid analog L-[2-$^{18}$F]fluorotyrosine, which is incorporated into proteins (*22*), showed that the difference in uptake

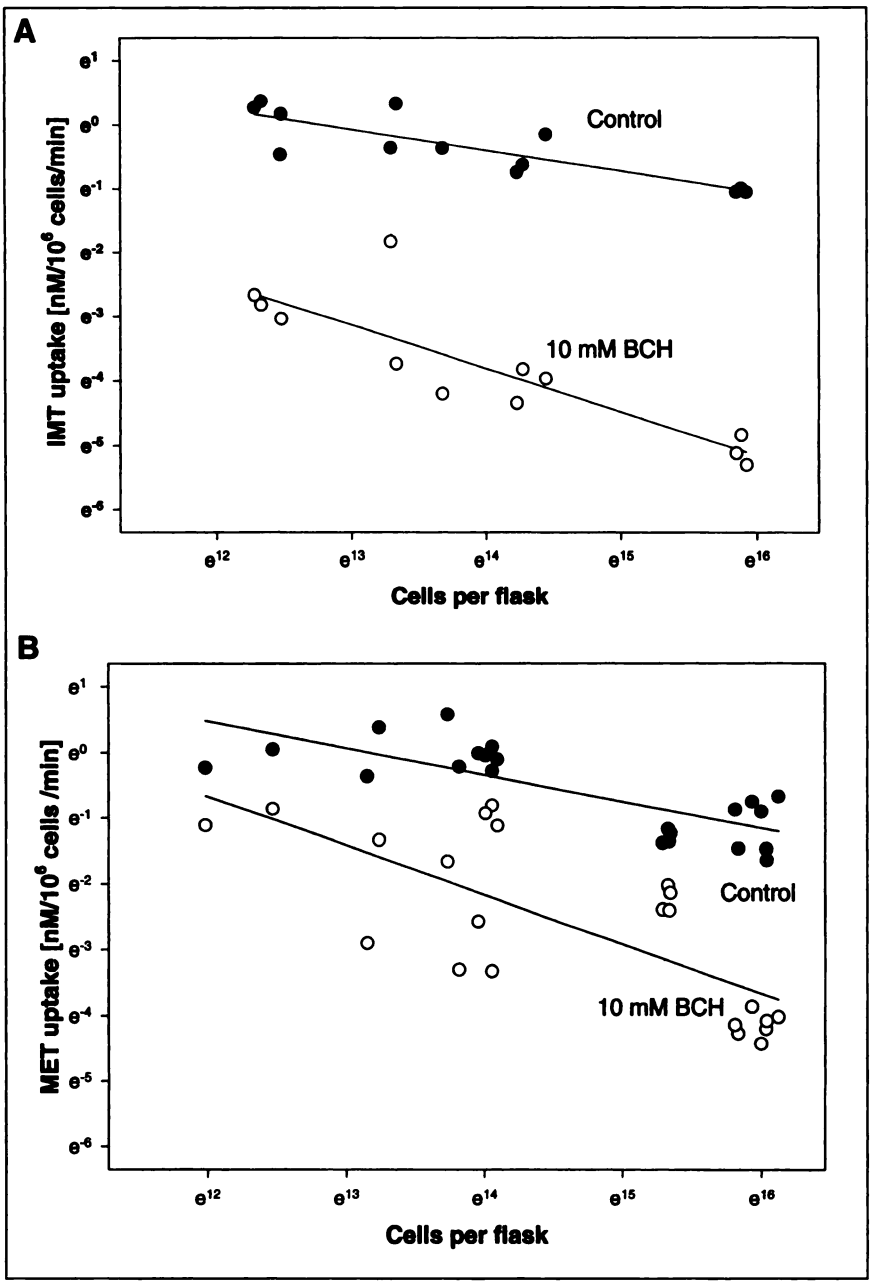


**FIGURE 3.** IMT and $^3$H-MET uptake in 86HG-39 human glioma cells as function of cell number with and without BCH inhibition (double logarithmic scale, $Na^+$-containing medium). Each data point represents 3 determinations. Regression coefficient, b, for all plots is significantly different from zero (IMT control, b = −0.328; BCH, b = −0.309; $^3$H-MET control, b = −0.485; $^3$H-MET control, b = −0.485; BCH, b = −0.435; $P < 0.0001$), indicating that IMT and $^3$H-MET uptake is dependent on proliferative activity of cells. Influence of BCH is independent of cell number, confirming dominant role of system L for IMT and $^3$H-MET transport in all phases of cell growth. BCH effect is less pronounced for $^3$H-MET, but results are not significantly different from IMT data.

between gliomas and normal brain was associated with an increase of the rate constant of tracer transport, $K_1$. The rate constant $k_3$, which describes the binding to the metabolic compartment, was not altered or even decreased in gliomas (*10*).

Futhermore, the uptake of the amino acid analog IMT into normal brain tissue and brain tumors is comparable with that of other large neutral amino acids, although this tracer is not incorporated into cerebral proteins (*5,6*). Brain tumor imaging with IMT and SPECT has been shown to be of similar diagnostic value as studies with $^{11}$C-MET and PET (*7,8*).

In this study we have investigated the mechanisms of IMT and $^3$H-MET transport into human glioma cells in vitro. IMT and $^3$H-MET transport is increased significantly in the lag phase and exponential growth phase compared with the plateau phase, and uptake is dominated by BCH-sensitive transport—i.e., transport through system L (Fig. 2).

It is interesting to note that transport is already upregulated in the lag phase and precedes rapid cell segmentation. The biologic meaning of this observation is unclear. IMT and $^3$H-MET uptake plotted as a function of cell number with and without BCH inhibition (Fig. 3) confirmed that the influence of BCH is independent of cell number—i.e., system L-like transport of IMT and $^3$H-MET is similar in all phases of cell growth.

This finding indicates that system L is induced in rapidly proliferating glioma cells and is the main contributor to the uptake of both tracers. There was a minor $Na^+$ dependency of $^3$H-MET uptake, which indicates a partial energy-dependent transport of this tracer. However, inhibition by

MeAIB was insignificant for both tracers in the exponential growth phase and in the plateau phase, which rules out a relevant transport through system A. Thus, uptake of IMT and $^{3}$H-MET in this human glioma cell line is dominated by system L-like transport, which supports the observation that clinical results for IMT and $^{11}$C-MET are comparable in patients with brain tumors (*7*).

These results agree with the results of many experimental studies. The natural parent of IMT, L-tyrosine, is transported predominantly by system L, whereas system A plays a negligible role (*23,24*). For L-methionine, uptake through system L is also the major route of entry into most human cells (*25*), although a contribution of system A to L-methionine uptake has been identified in some cell lines (*26*).

The expression of amino acid transport systems during proliferation and malignant transformation is a domain of major interest. System A is 1 of the few identified transport systems that is expressed strongly in transformed and malignant cells and appears to be a target of proto-oncogene and oncogene action (*27*). Fewer data are available concerning the importance of system L in this context (*28*).

However, recent progress in the structural analysis of system L emphasizes its involvement in malignant transformation and proliferation. System L-like transport was shown to be linked to the expression of the 4F2 surface antigen, which is known as a marker of normal and neoplastic cell growth (*29*). The 4F2 antigen is a membrane glycoprotein that consists of a heavy chain and a light chain. The heavy chain was identified as a modulatory unit of system L-like transport, whereas the function of the light chain remains unclear. Recently, complementary DNAs were cloned (E16 protein, LAT1), which exhibited transport patterns corresponding to system L when coexpressed with 4F2 heavy chain, and thus are identified as the light chain of 4F2 and assumed to represent the system L transporter molecule (*30,31*). A strong expression of LAT1 was reported in some tumor cell lines, and partial or incomplete sequences of LAT1 were shown to be upregulated on the mitogenic stimulation of lymphocytes (*30*).

Determination of the relationship between $^{11}$C-MET or IMT uptake in gliomas and proliferation, biologic aggressiveness, or histologic grading of these tumors is under clinical investigation. Recent studies showed significantly longer survival times in glioma patients with low $^{11}$C-MET uptake than in patients with high $^{11}$C-MET uptake (*20*) and a dependence of $^{11}$C-MET uptake on the presence of anaplasia in histologic tumor samples (*32*). Furthermore, a significant correlation between IMT uptake in gliomas and expression of the proliferation marker Ki-67 has been reported (*33*). The results of this in vitro study support the hypothesis that IMT and $^{11}$C-MET uptake are dependent on cell proliferation.

It must be recognized that in vitro results may not be transferred directly to the in vivo situation, and the results may be different for other glioma cell lines. The lack of a blood–brain barrier or blood–tumor barrier, respectively, limits the transferability of the results.

## CONCLUSION

The transport of IMT and $^{3}$H-MET is dependent on the proliferation rate of human glioma cells in vitro and is dominated by BCH-sensitive transport. This finding indicates that system L is induced in rapidly proliferating glioma cells and is the main contributor to the uptake of both tracers. The similarity of transport mechanisms of both tracers emphasizes the clinical equivalence of IMT SPECT and $^{11}$C-MET PET in the diagnostic evaluation of gliomas.

## ACKNOWLEDGMENTS

The authors thank Margit Meurer, Sabine Wilms, and Markus Cremer for technical assistance in cell culture experiments; Bettina Palm, Erika Wabbals, and Silke Bode for technical assistance in radiosynthesis of 3-[$^{123}$I]iodo-α-methyl-L-tyrosine; and Doris Beaujean for secretarial assistance.

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