Brain and Brain Tumor Uptake of L-3-[^{123}I]Iodo-α-Methyl Tyrosine: Competition with Natural L-Amino Acids

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SPECT studies with L-3-[^{123}I]iodo-α-methyl tyrosine (IMT) were carried out in 10 patients with different types of brain tumors—first under fasting conditions (basal) and a week later during intravenous infusion of a mixture of naturally-occurring L-amino acids (AA load). An uptake index (UI) was calculated by dividing tissue count rates by the integral of plasma count rates. The UI decreased by 45.6% ± 15.4% ($n = 10$, $p < 0.001$) for normal brain and by 53.2% ± 14.1% for gliomas ($n = 5$, $p < 0.01$) during AA load compared to basal conditions, while two meningiomas and a metastasis showed only a minor decrease (23.9 ± 5.7%, n.s.). Two pituitary adenomas could not be delineated on the SPECT scans. These data indicate that IMT competes with naturally-occurring L-amino acids for transport into normal brain and gliomas. Transport characteristics of IMT into tumors of non-glial origin appear to be different from those of gliomas. For both types of tumors, it is advisable to perform IMT-SPECT under fasting conditions.

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Position emission tomography (PET) studies using carbon-11-labeled amino acids have proven to be useful for diagnosis of brain tumors and therapy control (*1–6*). The synthetic amino acid L-3-[^{123}I]iodo-α-methyl tyrosine (IMT) has been shown to be a promising tracer for conventional nuclear medicine (*7–11*). Recently, we investigated the intracerebral kinetics of IMT in patients with brain tumors (*12*). In that study, it was shown that the stability of tracer concentration in the brain is appropriate for single-photon emission computed tomography (SPECT) studies with rotating gamma camera systems. The accumulation of IMT in normal brain tissue and in tumors without disruption of the blood-brain barrier (BBB) suggested a facilitated transport via a carrier system, although IMT is not incorporated into protein (*12*).

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It remains questionable, however, whether the accumulation of IMT is nonspecific, especially in tumors with disruption of the BBB. Animal experiments have shown that noniodinated α-methyl tyrosine competes with large neutral amino acids (phenylalanine, leucine, methionine, valine) for a single transport site in the BBB (*13*). The present study examines whether the transport of IMT into the brain and into brain tumors can be affected by competition with naturally-occurring L-amino acids in order to prove that IMT utilizes one of the amino acid carrier systems.

MATERIALS AND METHODS

SPECT studies with IMT were carried out in 10 patients with brain tumors. Information concerning this group of patients is given in Table 1. Two patients had Grade II astrocytomas, three patients malignant gliomas, two patients meningiomas, one patient a metastasis from a bronchial carcinoma, and two patients pituitary adenomas (GH-secreting adenomas). All patients who were treated surgically had recurrent or residual tumors of at least 2 cm in diameter. Histopathologic data were obtained by surgery or stereotactic biopsy. All patients had SPECT studies after 12 hr fasting (basal study) and within 1 wk thereafter during infusion of amino acids (AA load study). No therapeutic interventions were carried out between these two studies. In order to achieve a maximal competitive effect, a mixture of naturally-occurring L-amino acids usually used for parenteral nutrition (Aminoplasmal PO 10%, B. Braun Melsungen) was infused via a central venous catheter at the highest possible rate recommended by the manufacturer (120 ml/hr).

A liter of the amino acid solution had the following composition: 4.8 g L-isoleucine, 8.4 g L-leucine, 10.4 g L-lysine, 2 g L-methionine, 4.2 g L-phenylalanine, 4.8 g L-threonine, 2 g L-tryptophane, 6.4 g L-valine, 8.6 g L-arginine, 5.4 g L-histidine, 7 g glycine, 12.4 g L-alanine, 7 g L-proline, 0.9 g L-aspartic acid, 0.9 g L-asparagine, 0.59 g L-cysteine, 9 g L-glutamic acid, 1.8 g L-ornithine, 3.2 g L-serine, and 2 g L-tyrosine. The infusion was started 90 min before tracer injection and continued throughout the SPECT study.

All SPECT studies were started 15 min after intravenous injection of 370 MBq IMT. IMT was prepared as previously described with a specific activity of >25 GBq/mmol (0.6 Ci/mmol) (*7,8*). Sixty-four images of 35 sec each were acquired using

TABLE 1
Patient Data

Patient no.	Age (yr)	Sex	Tumor type and grade	Pre-treatment Surg.	Rad.	Chemo.
Gliomas						
1	41	M	Glioma III	y	y	n
2	31	M	Astrocytoma II	n	n	n
3	44	M	Astrocytoma II	n	n	n
4	64	F	Glioblastoma IV	y	y	n
5	39	M	Astrocytoma II–III	n	n	n
Others						
6	65	M	Meningioma	n	n	n
7	57	M	Meningioma	n	n	n
8	50	M	Metastasis	n	n	n
9	56	M	Pituitary adenoma	y	n	n
10	36	M	Pituitary adenoma	y	n	n

a Philips Diagnost Tomo-Gamma Camera (Einthoven, The Netherlands) with a 30° slant-hole collimator (360° rotation). The 30° slant-hole collimator and a special headholder were used to allow a minimum radius of rotation so that a resolution of about 14 mm FWHM was achieved (*14*). The radius of rotation was kept constant during the basal and the AA load study in each patient, and the patients were repositioned by checking the orbito-meatal line using two ^{57}Co point sources. Orbitomeatal parallel slices were reconstructed by ramp-filtered backprojection after uniform attenuation correction. Eight samples of venous blood were taken at 1, 3, 6, 10, 15, 20, 35, and 60 min postinjection, and the plasma activity of each was measured in a scintillation well counter cross-calibrated with the gamma-camera. For cross-calibration, a syringe with 18.5 MBq (0.5 mCi) of IMT was measured as a standard at a 15-cm distance from the collimator surface, which was the radius of rotation during brain SPECT. The standard was diluted in 1000 ml of water and an aliquot of 1 ml was measured in the well counter to determine the calibration factor. This procedure was performed in each case. The plasma input function was determined and corrected for the non-IMT radioactivity after HPLC analysis as described previously (*15*). Within 1 wk before or after the SPECT studies, the patients were studied with transmission computed tomography (CT) or magnetic resonance imaging (MRI).

The basal SPECT studies were evaluated by regions of interest (ROIs) placed over the tumor, i.e., the area with increased uptake, and over normal brain tissue. The ROIs of normal brain included areas of gray and white matter that were not involved by tumor tissue or edema according to the CT or MRI scan. These ROIs were transferred to the corresponding SPECT scans in the AA load study. In the two cases of pituitary adenomas, tumor uptake was similar to that of the surrounding tissue so that tumors could not be delineated on the SPECT scans and no quantitative data are available for the tumors in these two patients. An uptake index (UI) was calculated by dividing the count rates in tissue (counts ml^{-1} min^{-1}) by the integral of plasma count rates (counts ml^{-1}). The integral of plasma activity was taken from injection time up to the mean time of the SPECT study (i.e. 35 min postinjection). These data were used to quantify the percent change of tumor and brain uptake during the AA load study versus the study under fasting conditions (basal study). Furthermore, the tumor uptake was quantified in terms of the tumor/brain ratio using the average counts per pixel in each region. In order to measure the washout of tracer during SPECT acquisition, the first and last projection images of each SPECT study were evaluated by a ROI over the brain. The relative changes of all data between the basal and the AA load study were statistically evaluated by the paired t-test.

RESULTS

All tumors with the exception of the two pituitary adenomas showed increased IMT uptake compared to normal brain tissue (see Table 3). UI decreased by 45.6% ± 15.4% ($n = 10$, $p < 0.001$) for normal brain and by 53.2% ± 14.1% ($n = 5$, $p < 0.01$) for gliomas during AA load compared to basal conditions, while the two meningiomas and the metastasis showed only a minor decrease ($n = 3$, 23.9% ± 5.7%, ns, Tables 2 and 3 and Fig. 1). CT, MRI, and SPECT scans of Patient 3 (astrocytoma Grade II) are shown in Figure 2. The tumor is not visible on the

TABLE 2
IMT Uptake Index (UI) for Normal Brain Tissue and IMT Washout During SPECT Acquisition Under Fasting Conditions (Basal) and During Infusion of Amino Acids (AA Load)

Patient no.	UI (brain tissue) Basal	AA load	% decrease	(%) washout during SPECT (15–50 min p.i.) Basal	AA load
1	0.032	0.012	62.5	34.7	39.0
2	0.025	0.011	56.0	40.0	42.0
3	0.027	0.012	55.6	42.0	44.0
4	0.023	0.008	65.2	23.5	29.0
5	0.015	0.010	33.3	37.6	41.0
6	0.017	0.009	47.1	24.4	34.0
7	0.016	0.008	50.0	34.4	38.9
8	0.013	0.007	46.2	34.8	42.2
9	0.012	0.008	33.3	38.0	41.0
10	0.046	0.032	30.4	36.0	40.6
mean	0.021 ± 0.011	0.011 ± 0.007	45.6 ± 15.4 ($p < 0.001$)	34.5 ± 6.1	39.2 ± 4.5

TABLE 3
IMT Uptake Index (UI) for Tumor Tissue and Tumor/Brain Ratios Under Fasting Conditions (Basal) and During Infusion of Amino Acids (AA Load)

Patient no.	UI (tumor tissue)		% decrease	Ratios Tumor/Brain Tissue	
	Basal	AA load		Basal	AA load
Gliomas					
1	0.053	0.024	54.7	1.39	1.61
2	0.031	0.014	54.8	1.43	1.40
3	0.037	0.014	62.2	1.55	1.59
4	0.040	0.014	65.0	1.74	1.83
5	0.034	0.024	29.4	2.12	2.25
mean	0.039 ± 0.009	0.018 ± 0.005	53.2 ± 14.1 ($p < 0.01$)	1.65 ± 0.30	1.74 ± 0.33
Others					
6	0.033	0.026	21.2	1.79	2.74
7	0.023	0.016	30.4	1.23	1.60
8	0.015	0.012	20.0	1.31	2.27
mean	0.024 ± 0.009	0.018 ± 0.007	23.9 ± 5.7	1.44 ± 0.30	2.20 ± 0.57

contrast-enhanced CT scan, indicating an intact BBB. CT and SPECT scans of the patient with a brain metastasis are shown in Figure 3. The tumor/brain ratios for gliomas remained nearly constant during AA load compared to basal conditions (n = 5, 1.65 ± 0.30 versus 1.74 ± 0.33), while the two meningiomas and the brain metastasis showed considerable increases of the tumor/brain ratios (1.44 ± 0.30 versus 2.20 ± 0.57, Table 3). The mean washout of IMT from the whole brain increased from 34.5 ± 6.1% during the basal study to 39.2% ± 4.5% during AA load (n = 10, ns, Table 2). There was no correlation between the increase of IMT washout and the decrease of the IMT uptake index.

DISCUSSION

It is well known that the transport of a specific amino acid into the brain can be inhibited by an infusion of large amounts of other amino acids that utilize the same carrier system (*13*). It has also been shown for gliomas that the transport of ^{11}C-L-methionine can be inhibited by infusion of branched-chain amino acids (*6*). The significant reduction of IMT uptake by normal brain tissue and gliomas during infusion of a mixture of naturally-occurring L-amino acids proves that IMT utilizes one of the carrier systems for amino acids. Since we used a mixture of L-amino acids in order to obtain a maximal competitive effect, it remains to be shown which of the transport

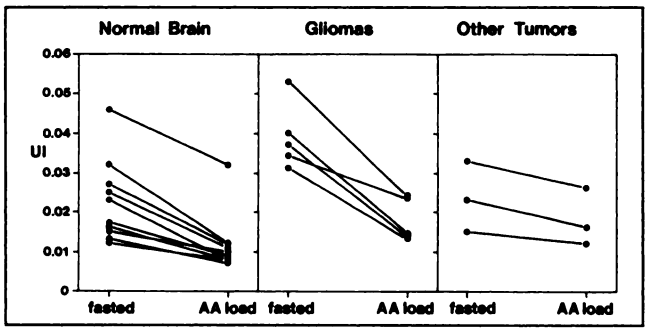

FIGURE 1. Changes of the IMT uptake index (UI) in normal brain tissue, gliomas, and other brain tumors (two meningiomas, one metastasis) under fasting conditions and during infusion of amino acids. Normal brain and gliomas exhibit significant decreases of UI while other tumors showed only a minor decrease.

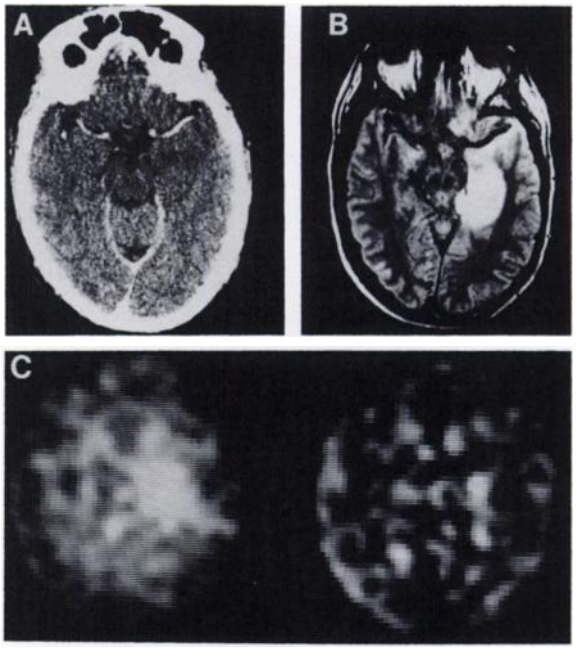

FIGURE 2. Astrocytoma Grade II: CT (A), MRI (B), and IMT-SPECT scans (C) under fasting conditions (left) and during infusion of amino acids (right). SPECT scans are normalized to the same integral of plasma activity. The tumor is not visible on the CT scan. There is a significant decrease of IMT uptake in both normal brain and tumor tissue during amino acid infusion.

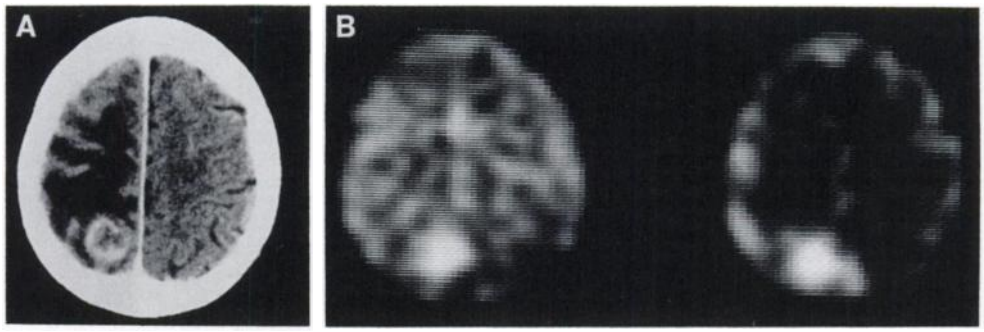

FIGURE 3. Metastasis: CT (A) and IMT-SPECT scan (B) under fasting conditions (left) and during infusion of amino acids (right). Images are normalized to the same integral of plasma activity. There is a significant decrease of tracer uptake in normal brain, but there is no change of IMT uptake in tumor tissue during amino acid infusion.

systems of amino acids is involved here. The carrier system for large neutral amino acids that also transports the non-iodinated α-methyl tyrosine is likely to be involved (*13*).

An UI calculated by dividing the tissue count rates by the integral of plasma count rates was the method of quantification in our study. The integral of plasma count rates, corrected for non-IMT radioactivity, provides a reliable measure of what amount of tracer is available for transport into the brain and brain tumors. The calculated UI presents only a relative value for each patient, since quantification of tracer concentrations with SPECT is difficult because of lack of adequate methods for attenuation correction. For the measurement of intraindividual changes, however, the problems of quantification inherent with the SPECT method are of minor importance because the procedure for measurement was identical in both the basal and AA load scan. A simpler approach to quantification of tracer uptake such as a differential absorption ratio (tracer uptake in relation to injected dose and body weight) was, in our opinion, not acceptable because tracer distribution in the body could be influenced by the amino acid infusion and would therefore have produced unreliable results.

It is possible that there is small increase of the IMT washout rate from the brain from 34.5 ± 6.1 during the basal study to 39.2 ± 4.5 during amino acid load. This small increase might have influenced the UI slightly, but it cannot, in our opinion, explain the decrease of the UI in the range of 50% as observed in normal brain tissue and gliomas. No correlation between the increase of IMT washout and the decrease of the UI could be detected in the ten patients.

The decreases of IMT uptake under AA load yielded variable results for different tumor types. For tumors of glial origin, such as glioblastomas and astrocytomas, the effects were similar to normal brain tissue. This indicates that accumulation of IMT in these brain tumors is not a simple, passive diffusion due to disruption of the BBB. Similar results for gliomas have been reported using ^{11}C-L-methionine and PET (*6*). In the meningiomas and the brain metastasis, however, only minor decreases of IMT uptake during AA load could be observed. It cannot be excluded that this minor decrease would be statistically significant if a larger number of patients with meningiomas and metastases would have been investigated. In addition, due to the small number of patients, there is also no statistically significant difference between gliomas and meningiomas or the metastasis. Nevertheless, the minor decrease suggests that IMT uptake in meningiomas and brain metastases may be mediated only by a disruption of the BBB, i.e., simple, passive diffusion. This, however, is unlikely because the uptake of IMT is very high, and by way of explanation the following hypothesis is offered which may also explain the lack of competitive effects in meningiomas and metastases. It is well known that the transport of neutral amino acids into the brain differs significantly from transport into other tissues of the body (*16,17*). The Michalis-Menten constant for neutral amino acid transport in tissues other than brain is far above physiologic levels, i.e., tissues other than the brain are independent of competitive effects *in vivo*. Since meningiomas and brain metastases belong to extracerebral tissues, no competitive effect is expected at physiologic levels, which were not exceeded by the amino acid infusion in our study.

Therefore it cannot be excluded that the uptake of IMT in meningiomas and the brain metastasis is also due to a specific amino acid transport.

Since the competitive effects in gliomas and normal brain tissue are similar, the tumor/brain ratio is independent of the nutritional status (Table 2). This makes quantification of therapeutic effects in gliomas simple because the dietary status need not be the same in the pre- and post-treatment studies. Nevertheless it is advisable to study patients under fasting conditions because the brain and brain tumor uptake is higher and gives better count rates in the SPECT study and thus provides better delineation of gliomas. Moreover, the washout of activity from the brain is slower under fasting conditions, which is better for the long-lasting SPECT acquisition with rotating gamma camera systems (Table 1).

In brain metastases and meningiomas, however, there is a considerable influence of the dietary status on the tumor/brain ratio, which may result in increases from 1.31 to 2.27 (70%) as shown in Patient 8 with a brain metastasis. Therefore, in the case of tumors originating from tissues external to the brain, the quantification of therapeutic effects requires a constant dietary status or more complex methods of quantification.

In summary, the significant reduction of IMT uptake in normal brain tissue and in gliomas during AA load proves that IMT utilizes one of the amino acid carrier systems. Therefore, IMT can be used as a tracer of amino acid transport in SPECT studies. The spatial resolution is limited, but it may be improved with new generations of SPECT systems. Thus, SPECT studies with IMT may offer clinical potentials similar to that of PET studies using ^{11}C-labeled amino acids.

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EDITORIAL

Saturation of Amino Acid Uptake by Human Brain Tumor Demonstrated by SPECT

Saturation of human blood-brain barrier (BBB) transport of positron-labeled amino acids has been demonstrated by PET. In this issue, Langen et al. examines saturation of amino acid BBB transport in several types of tumors using SPECT to image a single-photon analog of tyrosine. It is alpha-methyltyrosine labeled in the 3 position of the ring with ^{123}I and is referred to as IMT. The importance of this report is that it represents, to my knowledge, the first SPECT examination of human BBB amino acid uptake saturation in several types of brain tumors.

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An intravenous preload of a commercial parenteral nutritional supplement containing the 20 common amino acids was given before, during, and after the labeled IMT was given intravenously.

Ten patients were studied: five with gliomas; two with meningiomas; two with pituitary adenomas; and one with metastases. The two pituitary adenomas could not be seen by SPECT, so no tumor data are presented in these two cases, but normal brain was measured. The patients were fasted for 12 hr, scanned, and tumor and brain uptake of IMT was measured. One week later they were scanned with the amino acid preload, the infusion being given intravenously prior to and during the scan.

The results of this paper suggest that amino acid competition can be demonstrated in non-neoplastic brain and in gliomas. These findings are compatible with the hypothesis suggested by Davson and Oldendorf that glial cells, through some humoral mechanism, cause brain capillaries to become structurally, functionally, and chemically altered to create the BBB (*1*). Recent studies in tissue culture support this hypothesis (*2*).

Since almost any major disruption of brain cellular integrity results in loss of the BBB, it may well be shown that loss of BBB represents very abnormal or dead astrocytes in the region. One might speculate that in metastatic tumors and meningiomas there are no astrocytes present. Capillaries in these lesions are permeable to all small molecules,