Chapter 5

Feasibility of tumor imaging using L-3-[\textsuperscript{123}I]-iodo-alpha-methyl-tyrosine in extra-cranial tumors.

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5.1 SUMMARY

L-3-[\textsuperscript{123}I]-iodo-alpha-methyl-tyrosine (IMT) is a modified amino acid. It is reported to be avidly taken up in brain tumors reflecting amino acid transport and is suitable for SPECT.

Methods: To determine whether tumors outside the brain can accumulate this tracer, we injected 300-450 MBq IMT in 20 patients with different tumors (5 breast cancer, 4 lung tumors of which 1 benign, 2 carcinoid liver metastases, 4 soft tissue tumors of which 1 benign, 3 malignant lymphoma and 2 primary brain tumors). Tumor size ranged from 1 - 12 cm. Imaging was repeated after radiotherapy in 2 patients with breast cancer. Histology was available in all cases. Dynamic scans, whole body imaging and SPECT were
performed in the first hour and repeated 3 hr after injection. Plasma samples were analyzed for IMT, free $^{123}$Iodide and other metabolites.

**Results**: All primary tumors were visualized. Tumor to background (T/B) ratios ranged from 1.1 - 3.8 on planar and from 1.3 - 6.2 on SPECT images. Tumor uptake peaked in the first hour. Two carcinoid lesions in the liver tumors exhibited no IMT uptake above liver background. T/B ratios in a benign bone inflammatory process and a focal pulmonary vasculitis were less than 1.2 (planar) and 1.9 (SPECT) and could be differentiated from uptake in all malignant non brain tumors. IMT was rapidly cleared from the plasma (3.6%±0.6(s.d.) %I.D (injected dose)/L at 10 min p.i.). Minor in-vivo deiodination was present (<1% of I.D. 1 hr p.i.). No other metabolites were found. Normal distribution consists of some uptake in brain, liver, spleen, muscles, pancreatic region, intestinal structures and massive uptake and excretion in kidneys and bladder. **Conclusion**: IMT has potential as a metabolic tracer also in tumors outside the brain.

### 5.2 INTRODUCTION

Considerable interest has been shown in metabolic imaging of tumors using positron emission tomography (PET) with $^{18}$F-fluoro-deoxyglucose (FDG) or radiolabeled amino acids such as L-$^{[11]}$C-methyl]-methionine or L-$^{[1-^{11}]}$C]-tyrosine (1-3). Uptake of these tracers in tumors is based on the increased metabolic demand of tumor tissue compared to normal tissue and is hypothesized to represent tumor vitality (4-6). Potential use in oncology includes characterization of anatomic lesions, tumor staging and evaluation of therapy. FDG is avidly taken up by almost all kinds of tumors, representing increased glucose consumption, but it also accumulates in inflammatory tissues (7,8). Several studies have suggested that imaging with radiolabeled amino acids visualizes protein synthesis and amino acid transport phenomena (9-11). These processes are generally accelerated in tumors (12). Since amino acids play a minor role in the metabolism of inflammatory cells, these tracers might be more tumor specific than FDG (13,14).

Because of the limited availability and the cost of PET there is a demand for similar compounds for use in a conventional nuclear medicine department. SPECT using FDG is an option but, aside from the limited specificity, clinical oncological application is hampered by detection difficulties such as a limited resolution, low sensitivity for small lesions and septal penetration in ultra high energy collimators (15).

The $^{123}$I-labeled amino acid L-3-$^{[123]}$I-iodo-alpha-methyl-tyrosine has been introduced for imaging of brain tumors. It was demonstrated that uptake in brain tumors represents amino acid transport and thus a step in tumor metabolism (16-20). IMT is not incorporated into protein. Increased transmembrane transport is thought to be the main determinant of the uptake.
process (16). Sensitivity and specificity both between 80% and 100% are reported (18,19,21). Interestingly, the uptake increases with higher grading of gliomas and differentiation between high and low grade gliomas appears to be feasible (21). IMT uptake changes may predict the response to chemo- and radiotherapy and detect recurrent brain tumors (22-24).

Thus far, no data exist on the use of IMT in human tumors outside the brain. It was applied as a melanoma seeking agent and used for scintigraphy of the adrenals and pancreas, both mainly in animal studies some 20 years ago (25,26). In experimental rat tumors IMT uptake was found to be associated with amino acid transport, tumor perfusion and diffusion (27). Because of the good uptake of 11C-tyrosine demonstrated with PET in various tumors outside the brain (28-30) and the initial common step (amino acid transport from plasma into the cell) in the proposed uptake mechanism, we undertook a study to determine whether IMT is taken up in human tumors outside the brain, and if so, to what extent and at what time. In addition, normal uptake patterns were qualitatively studied.

5.3 METHODS

Patients
Twenty patients (10 male, 10 female, mean age 61 yr, range 23 - 81 yr), randomly recruited from various clinical oncological departments, with known or suspected malignancies were studied. In all patients histopathological material was obtained by biopsy or operation after the IMT study. The selection criterion was the availability of a histological diagnosis and location outside the vicinity of the kidneys and the urinary tract (because IMT is excreted by the kidneys). All patients were studied 1-3 weeks before therapy was started. Eighteen of the 20 patients had a malignant tumor, in two cases benign inflammatory processes were confirmed by histology. Two patients with breast cancer were studied twice: before and 6 weeks after the termination of external radiotherapy. Table 1 summarizes patient characteristics.

Written informed consent was obtained from all patients. The study was approved by the Medical Ethics Committee of the Groningen University Hospital.

Synthesis and quality control
Synthesis of IMT was carried out as described by Krummeich et al. (31). Briefly, Iodo-gen™ iodination with high quality Na123I (specific activity > 5000 Ci/mmol, obtained from Amersham Cygne, Eindhoven, the Netherlands) of the precursor L-alpha-methyl-tyrosine was performed in a borate buffer. IMT was purified by elution with saline containing 5% ethanol over a C-18 SepPak® cartridge (Waters, Milford, Mass. USA) preconditioned with methanol followed by saline containing 5% ethanol. After filtration through a sterile 0.22 µm Millex
GV filter (Millipore®, Sa, Molsheim, France) a colorless ready-to-inject solution was obtained. Samples were demonstrated to be sterile and pyrogen-free. Quality control was performed by HPLC on a RP-18 column (Multosorb 100 4.6) using H₂O/Ethanol/Acetic-acid 87.5/10/2.5 v/v/v, containing 2.5 g/l ammoniumacetate as eluent. Radiochemical purity was over 99% in all cases. The overall synthesis time, including purification and quality control was less than 1 hr. Radiochemical yield was 50 - 65%.

Imaging
After an overnight fast, imaging was started immediately after the intravenous injection of 300-450 MBq IMT. Fifteen min prior to injection 10 drops of Lugol's solution were given orally to prevent possible thyroid uptake of free ¹²³Iodine. A large-field-of-view double headed gamma camera (MULTISPECT 2, Siemens Inc, Hoffman Estates, Illinois, USA) was used with a medium energy all purpose collimator and a 15% window centered on the 159 keV photopeak of ¹²³Iodine. System resolution was 12 mm FWHM at 10 cm distance.

A 30 min dynamic scan (60 frames of 30 seconds each) of the tumor area was acquired in a 128 x 128 matrix, followed by whole body scanning or SPECT of the tumor area. After SPECT additional spot views were recorded to obtain whole body information in all cases. SPECT included 64 views (2 x 32; 5.6°/step) of 40 seconds duration each in a 128 x 128 matrix format with a zoom factor of 1.45. This corresponds to a pixel dimension of 3.3 mm. Whole body scans were carried out with a scan speed of 15 cm/min. The initial scan procedures took 60-80 min. After these initial scans patients were allowed to eat. SPECT with additional spot views or total body scanning was repeated 3 hr p.i. and in the first 3 patients also at 24 hr p.i.

Transaxial tomograms were reconstructed without prefiltering using filtered back-projection with a Butterworth filter of 6th order and a cutoff frequency of 0.275 Nyquist. No attenuation correction was performed except for brain SPECT studies where first order correction using Chang's method was applied with an attenuation coefficient of 0.11 cm⁻¹. An estimation of tumor washout during SPECT acquisition was obtained by analyzing tumor ROI's on the first and last SPECT image.

The reported radiation burden of IMT is 0.007 mSv/MBq, yielding an effective dose equivalent of 2.5 - 3.5 mSv (32).

Analysis of metabolism
At 0, 10, 20, 30 min, 1, 3 and 24 hr p.i. heparinized blood samples were collected in the first 10 patients. After centrifugation (3,000 G, 10 min) plasma samples were analyzed for total and tri-chloro-acetic-acid (TCA) precipitable radioactivity using a gamma counter (Compugamma, LKB Wallac, Finland) together with a defined 1% aliquot of the injected material as reference. The measured radioactivity was expressed as percent of the injected dose per liter plasma [%ID/l]. In addition, relative fractions of parent compound IMT, ¹²³Iodide
and possible other metabolites were determined by elution of plasma samples
over a C18 Sep-Pak cartridge, preconditioned with methanol followed by
saline containing 5% ethanol. In two patients 3 and 24 hr urine portions were
collected and analyzed for total radioactivity and metabolites in the same way
as the plasma samples.

Table 1. Patient characteristics and IMT scintigraphic findings

<table>
<thead>
<tr>
<th>Nr</th>
<th>Age</th>
<th>Sex</th>
<th>Tumor histology</th>
<th>Size (cm)</th>
<th>---Visual score*---</th>
<th>----T/B ratio*---</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>F</td>
<td>Carcinoid liver metastasis</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>-Small lesion, no SPECT</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>M</td>
<td>Carcinoid liver metastasis</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>-No SPECT</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>M</td>
<td>Lung cancer (non-small-cell)</td>
<td>4</td>
<td>+</td>
<td>++</td>
<td>-No other lesions present</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>F</td>
<td>Lung cancer (non-small-cell)</td>
<td>4</td>
<td>+</td>
<td>++</td>
<td>-Mediastinal involvement (CT) detected</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>M</td>
<td>Lung cancer (small-cell)</td>
<td>3</td>
<td>++</td>
<td>+++</td>
<td>-All lymph node metastases detected</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>M</td>
<td>Lung vasculitis lesion (benign)</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>-Benign very active inflammatory process</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>F</td>
<td>Breast cancer (ductal)</td>
<td>4</td>
<td>++</td>
<td>+++</td>
<td>-Axillary lesion 3 cm detected, T/B ratio 6.2</td>
</tr>
<tr>
<td>7A</td>
<td></td>
<td></td>
<td>--after radiotherapy</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>-No tumor palpable</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>F</td>
<td>Breast cancer (ductal)</td>
<td>4</td>
<td>++</td>
<td>3.6</td>
<td>-2 small bone metastases not detected</td>
</tr>
<tr>
<td>8A</td>
<td></td>
<td></td>
<td>--after radiotherapy</td>
<td>1</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>F</td>
<td>Breast cancer (ductal)</td>
<td>3</td>
<td>+</td>
<td>++</td>
<td>-Axillary micrometastasis not detected</td>
</tr>
<tr>
<td>10</td>
<td>81</td>
<td>F</td>
<td>Breast cancer (ductal)</td>
<td>3</td>
<td>+</td>
<td>++</td>
<td>-1 possible bone metastasis not detected</td>
</tr>
<tr>
<td>11</td>
<td>45</td>
<td>F</td>
<td>Breast cancer (ductal)</td>
<td>3</td>
<td>+</td>
<td>+++</td>
<td>-No other lesions</td>
</tr>
<tr>
<td>12</td>
<td>82</td>
<td>M</td>
<td>Malignous fibrous histiocytoma</td>
<td>8</td>
<td>++</td>
<td>+</td>
<td>-No other lesions</td>
</tr>
<tr>
<td>13</td>
<td>57</td>
<td>F</td>
<td>Bone tumor femur (benign)</td>
<td>12</td>
<td>–</td>
<td>+</td>
<td>-MRT: osteoblastoma, biopsy: inflammation</td>
</tr>
<tr>
<td>14</td>
<td>29</td>
<td>M</td>
<td>Chondrosarcoma elbow</td>
<td>2</td>
<td>+</td>
<td>1.7</td>
<td>-Axillary micrometastasis not detected</td>
</tr>
<tr>
<td>15</td>
<td>66</td>
<td>M</td>
<td>High grade sarcoma (knee)</td>
<td>8</td>
<td>++</td>
<td>+++</td>
<td>-No other lesions</td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td>M</td>
<td>Mixed oligo-astrocytoma</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>-Low grade process</td>
</tr>
<tr>
<td>17</td>
<td>76</td>
<td>F</td>
<td>Glioblastoma multiforme</td>
<td>3</td>
<td>–</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>69</td>
<td>M</td>
<td>Non Hodgkin's lymphoma arm</td>
<td>5</td>
<td>++</td>
<td></td>
<td>-T-cell lymphoma skin</td>
</tr>
<tr>
<td>19</td>
<td>48</td>
<td>M</td>
<td>Hodgkin's lymphoma chest</td>
<td>4</td>
<td>++</td>
<td>+++</td>
<td>-No other lesions</td>
</tr>
<tr>
<td>20</td>
<td>55</td>
<td>F</td>
<td>Hodgkin's lymphoma chest</td>
<td>5</td>
<td>++</td>
<td>+++</td>
<td>-No other lesions</td>
</tr>
</tbody>
</table>

T/B ratio = tumor to background ratio, planar 20 min p.i., SPECT 60 min p.i.
F=female. M=male.
* — = not visible, + = just visible, ++ = visible, +++ = clearly visible
† empty table entry: no SPECT performed
CHAPTER 5

Data analysis
Without knowledge of the clinical and histopathological data all images were visually analyzed for tumor uptake and abnormal extra-tumoral uptake. Normal uptake patterns were visually assessed from whole body scans and from spot views. Regions of interest (ROIs) were placed manually over areas of abnormal uptake on 10 min summed initial dynamic images, on the SPECT slices with maximal lesion visibility and/or on spot views. ROIs were drawn at 80% of the maximal pixel value around the lesion under study (33). A representative, usually contralateral background region was defined and ROI-size normalized tumor-to-background ratios were calculated. To relate tumor uptake to normal organ uptake, tumor-to-bloodpool ratios and tumor-to-liver ratios were calculated using a ROI method. All IMT scintigraphic findings were finally compared to standard conventional images (CT, MRI, mammography, ultrasound) and histology.

5.4 RESULTS

Metabolism
IMT was rapidly cleared from the plasma: 10 min after injection only 3.6% (± 0.6% s.d.) of total radioactivity was present per liter plasma. Therefore approximately 90% of the tracer has left the plasma compartment within the first 10 min (figure 1). The shape of the plasma disappearance curve was bi-phasic. Minor deiodination took place starting from 7.5% (of plasma radioactivity, 10 min after injection) and rising to 24.5% free 123Iodide 3 hr after injection (table 2). This amounts to mean plasma iodide levels at 10 min, 1 hr and 3 hr of 0.8% ID (range 0.4 - 1%), 0.7% ID (range 0.4 - 0.9%) and 0.6% ID (range 0.4 - 0.7%) of the injected dose respectively.

Renal excretion amounted to 40-50% within the first three hr and to 65-85% of the injected dose in 24 hr. In the urine excreted in the first 3 hr 95% or total radioactivity consisted of intact IMT and the remaining 5% of free iodide. In the 24 hr-urine 10-15% free iodide was found, the remainder was intact IMT. Since intact IMT and free iodide together accounted for ~100% of the radioactivity both in plasma and in urine, it was concluded that no other labeled metabolites are formed.

A slowly decreasing fraction of plasma activity was TCA precipitable, but IMT added to plasma in vitro also gave a TCA precipitable fraction of 24% (table 2). These data indicate co-precipitation of IMT with plasma proteins.

No immediate nor delayed side effects were observed after the administration of the radiopharmaceutical.
**Figure 1.** Plasma clearance of L-3-[\(^{123}\)I]-lodo-alpha-methyl-tyrosine. Total radioactivity (%ID/l plasma) with standard deviation (sd) vs time (hr).

**Table 2.** Analysis of plasma radioactivity after administration of IMT (±SD).

<table>
<thead>
<tr>
<th>Time p.i</th>
<th>%Intact IMT</th>
<th>%Iodide-123</th>
<th>%Rest*</th>
<th>%TCA precipitable†</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>89.8 ± 3.3</td>
<td>7.5 ± 2.8</td>
<td>2.3 ± 1.1</td>
<td>23.4 ± 4.5</td>
</tr>
<tr>
<td>20 min</td>
<td>86.3 ± 4.6</td>
<td>10.8 ± 4.7</td>
<td>2.2 ± 1.2</td>
<td>23.5 ± 4.6</td>
</tr>
<tr>
<td>30 min</td>
<td>83.5 ± 7.5</td>
<td>12.8 ± 4.9</td>
<td>3.2 ± 3.3</td>
<td>22.0 ± 4.4</td>
</tr>
<tr>
<td>1 hr</td>
<td>78.9 ± 8.0</td>
<td>18.0 ± 6.5</td>
<td>2.6 ± 2.2</td>
<td>20.5 ± 3.8</td>
</tr>
<tr>
<td>3 hr</td>
<td>73.0 ± 8.5</td>
<td>24.5 ± 7.9</td>
<td>2.1 ± 2.1</td>
<td>19.6 ± 5.0</td>
</tr>
<tr>
<td>24 hr</td>
<td>32.5 ± 3.1</td>
<td>61.6 ± 8.0</td>
<td>3.6 ± 1.4</td>
<td>15.8 ± 4.4</td>
</tr>
</tbody>
</table>

* Activity remaining on the C18 SepPak column
† Control experiment: (after in vitro addition of IMT to plasma) 23.7±5.8% TCA precipitable
TCA=tri-chloro-acetic acid
Figure 2. Whole body scans in normal person acquired 0.5, 3 and 24 hr after injection of 350 MBq L-3-[^113]I)-lodo-alpha-methyl-tyrosine.

Normal scintigraphic appearance
The images indicate a high concentration in the kidneys and urinary tract. In the brain diffuse uptake was noted in the first hour, which had disappeared after 3 hr. Some uptake in the naso/oropharyngeal area, salivary glands, thyroid and stomach was seen on the early scans, slightly increasing after 3 hr. No uptake was observed in the thoracic region, except for minor bloodpool and myocardial activity (n=2) during the first 3 hr. Modestly intense uptake was present in the liver without the typical pattern of hepatobiliary clearance (no gallbladder or bile duct visualization). Some splenic uptake was observed. Accumulation in the pancreatic region and intestinal structures was noted in the first hour after injection but was quite variable between patients and between the first and third hour in individual patients. Possible pancreatic uptake was present in 7 of the 20 patients (35%) during the first hour only, but was hard to separate from neighboring structures. In general, the 3-hr images showed slowly increasing bowel activity. In obese patients it was clearly noted that uptake in muscles was higher than in subcutaneous fat. The scintigrams obtained 24 hr after administration showed uptake in the thyroid, naso/oropharynx and salivary glands and rather high uptake in stomach and small-intestinal structures. Most radioactivity (including tumor uptake) had disappeared by this time and therefore these 24-hr scintigrams were omitted after the first 3 patients. In figure 2 an example is shown.
Tumor uptake

All malignant primary tumors were visualized. Examples are presented in figures 3 and 4. Only the two metastatic carcinoid tumors in the liver were not visualized, one of which was very small. Tumor to background ratios (table 1) peaked within the first hour after administration and had decreased significantly in nearly all cases 3 hr p.i. (Figure 5). During the ~30 min SPECT acquisition tumor washout was estimated between 10 and 20% of the initial uptake, in the same range as neighboring normal tissues. After 24 hr most radioactivity had disappeared and no tumor uptake was observed anymore. When SPECT was performed (n=16) this greatly improved the tumor to background ratio and lesion detectability.

In patient 5 with small cell lung cancer, pathological IMT uptake was observed in mediastinal and supraclavicular metastases (Figure 4). In 2 cases of breast cancer, axillary metastases were present: in patient 7 intense IMT uptake was noted in an axillary lymph node metastasis while a microscopic metastasis was missed on the IMT SPECT scan of patient 9. Another
microscopic axillary lymph node metastasis could not be distinguished on planar IMT images of patient 14. In two cases of bone metastases, as shown on a bone scintigram, no IMT uptake could be assessed on planar images (patients 8,10).

Patient 1 was imaged two weeks after ileocecal resection of a large carcinoid tumor. During operation a 1 cm liver metastasis was suspected after liver palpation, but biopsy showed only normal liver tissue. No abnormal IMT uptake was noted in the liver postoperatively. Patient 2 had a 5 cm carcinoid liver metastasis that showed no IMT uptake above the liver background. No SPECT was performed in this patient.

Compared to the non brain tumors, the IMT uptake in the 2 brain tumors (one low grade mixed oligo-astrocytoma and one high grade glioma) was fairly low, with tumor uptake only slightly above the brain background.

In two patients with breast cancer imaging was repeated 6 weeks after the termination of radiotherapy. In both cases a marked decrease in uptake was present, in accordance with the clinical assessment (in patient 4 complete disappearance and in patient 8 partial disappearance of palpable lesions) (Figure 6).

*Figure 4. (A) Anterior planar image and (B) coronal SPECT slice of the chest in patient 5 showing intense uptake in the primary tumor near the right pulmonary hilus, lower and upper mediastinal lymph nodes and bilateral supraclavicular metastases. Image obtained 20 min (planar) and 60 min (SPECT) after administration of 450 MBq L-3-[\textsuperscript{123}]Iodo-alpha-methyl-tyrosine.*
Uptake in benign processes

In patient 13 very faint uptake, just above background and only detectable by SPECT, was observed in a large bony process dorsolateral to the right upper femur. MRI suggested this to be a osteoblastoma, on incisional biopsy, however, a non-specific cortical thickening was found with some inflammatory cells. In patient 6 a 2 cm coin lesion in the right lung showed very faint IMT uptake (exclusively on SPECT) and proved to be a very active focal vasculitis after pathological examination.

In patient 1 minor IMT uptake was observed in the operation field in the lower right abdomen 2 weeks after ileocecal resection of the large carcinoid gut tumor. In patient 10 multiple pulmonary granulomata exhibited no IMT uptake. Also another benign lesion, a fibrous nodular hyperplastic lesion in the liver of patient 11, showed no IMT uptake.

**Figure 5.** Tumor uptake, represented by the tumor/background ratio (T/B ratio) in time, for all patients. (A) Data obtained from dynamic planar images and spot views. (B) Data obtained from early and delayed SPECT images (n=16). (C) Average values (sd) for T/B ratios, tumor-to-bloodpool and tumor-to-liver ratios vs time.
5.5 DISCUSSION

While considerable experience exists in the imaging of brain tumors with the artificial amino acid IMT, no data are available regarding the use in other tumors. This study shows the feasibility of IMT and SPECT for the detection and therapy evaluation of extra cranial tumors: we found good tumor uptake in breast tumors, lung tumors, malignant lymphomas and soft tissue sarcomas. Tumor to background ratios in these non-brain tumors were even the same or higher than those reported in primary brain tumors (17,18,21,34). Tumor uptake appeared to peak rapidly after administration, within the first 15-30 min, as was also observed in brain tumors (18.). All tumors remained visible during the first hour, but after 3 hr T/B ratios had considerably decreased in nearly all cases.

The uptake mechanism of IMT in brain tumors was studied by Langen et al. They showed that IMT is not incorporated in protein, is not further metabolized and after rapid uptake, slowly washes out of the brain. In addition, they demonstrated that brain and primary brain tumor uptake can be diminished by infusion of amino acids and therefore concluded that the uptake is mediated by amino acid transport, presumably by the large neutral amino acid carrier system in the blood-brain barrier (16,17). This competitive effect of amino acid loading on IMT uptake was much lower in 1 metastasis and 2 meningeomas. This finding suggests a different way of uptake in non brain tumors. However, the presence of the blood-brain barrier and the potential differences in the Michaelis-Menten constant for amino acid transport in brain tissue versus non-brain tissue, make the translation of observations in brain tumor to tumors elsewhere in the body very difficult (35).

The uptake mechanism of IMT in these tumors outside the brain is not known. However, some clues can be derived from our data and from the literature. The first clue is that IMT tumor-to-background ratios peaks around 30 minutes, while at that time total plasma-activity has already decreased to ~6% of the injected dose (Figure 1). Minor bloodpool activity can be caused by uptake into erythrocytes, a known phenomenon of amino acids. The second clue: tumor to bloodpool ratios rise during the first hour and then remain stable (Figure 5C). Although non-specific uptake by passive diffusion related to tumor bloodflow could surely be present, these rising ratios may suggest (partial) specific uptake. The third clue: during the 30-40 minutes SPECT acquisition normal tissue washout was not different from tumor washout (both 10-20% of the initial value). The fourth clue is that the tumor to background ratios of many patients may be too high for just non-specific uptake. Although we did not separately assess tumor bloodflow, a limitation of this study, it is unlikely that tumor perfusion is e.g. 4 - 6 times higher than perfusion in neighboring normal tissues.

Together these clues may lead to the hypothesis that cellular tumor uptake is rapid and higher in malignant tissue than in normal tissue, while
Figure 6. SPECT images (volume rendered projections) of patient 7 showing intense uptake in a 3 cm left-sided breast carcinoma and in a 3 cm axillary metastasis. Minor uptake in the cardiac bloodpool is observed. (A) Before radiotherapy. (B) 6 weeks after the termination of radiotherapy a marked reduction in tumor uptake is noted. Images obtained 1 hr after administration of 340 MBq L-3-[^123]I-iodo-alpha-methyl-tyrosine.

Cellular washout of this artificial amino acid is the same for tumors and normal tissues. However, Deehan et al. suggested IMT, but also ^1^C-labeled amino acids uptake in artificial rat tumors to be related to bloodflow and diffusion and to a lesser extent amino acid transport phenomena. On the other hand they observed good tracer penetration in poorly vascularized areas (27). In contrast with their findings, other studies have demonstrated that (at least for ^1^C-labeled amino acids) specific tumor uptake is present (10). In addition, all amino acids can enter cells by passive diffusion (36). Therefore, the exact uptake mechanism for IMT and the possible fraction of non-specific uptake remain unclear. Although some influence of tumor perfusion is evident (and even necessary) for any tumor tracer, like ^20^Tl, ^99m^Tc-Sestamibi or ^18^F-FDG, this may not be clinically relevant as long as specific tumor uptake is also present. It is evident that more research is warranted in this area.

In addition to the tumor uptake analysis, normal whole body patterns of IMT uptake were qualitatively established. IMT is rapidly cleared from the plasma (fig 1), but minor bloodpool activity but also myocardial uptake is noted on the images of a minority of our patients (also on the images provided by Schmidt et al). This may be caused by amino acid transporter activity of erythrocytes and myocardial cells (36). Uptake in the brain is present during
the first hour but has almost completely disappeared at 3 hr. Although IMT is a stable tracer (deiodination in plasma of 0.6% of the injected dose, range 0.4 - 0.7%, 60 min p.i.), uptake in the thyroid, salivary glands, stomach and intestine was seen at 1 hr and 3 hr and more at 24 hr. Langen et al. found lower rates of deiodination in 3 patients (18). Uptake in liver and spleen was present at 1 and 3 hr p.i. Although biodistribution studies in mice have shown high uptake in the pancreas (26) we only observed possible pancreatic uptake in 35% of our patients, which was low and hard to distinguish from neighboring tissue uptake. In all patients uptake in intestinal structures was present on the early images. The pattern of this was variable between patients and changing over time in individual patients. Intestinal uptake cannot be attributed to hepatobiliary clearance, since gallbladder or bile duct visualization was absent in all dynamic studies were the liver was in the field of view. These intestinal uptake patterns are also noted on PET amino acid studies and may be caused by amino acid uptake in metabolically active intestinal tissue. All patients were imaged after an overnight fast, but possibly intestinal and pancreatic uptake depends on dietary conditions of the preceding day. Our findings regarding the normal uptake and metabolic behavior of IMT are in close agreement with a recent publication by Schmidt et al., who quantitatively described whole body kinetics in six brain tumor patients (32).

The combination of intense renal and bladder activity, liver uptake and variable uptake in intestinal structures, stomach and pancreas makes abdominal pathology hard to investigate. Especially on SPECT filtered backprojection reconstructions bladder/kidney artefacts are to be expected and therefore, in this study, we excluded patients with tumors in these areas. More experience is required, but organ uptake may seriously limit clinical application in the abdominal area.

While good uptake in various primary tumors and their metastases was present, microscopic disease in axilla and bone was below the detection limit as this is the case for virtually every non-invasive and even invasive procedure.. The iso-intense uptake in the patient with a 5 cm carcinoid liver metastasis may be attributed to a very low metabolic rate in this tumor, that had been present for many years, but probably also the omission of SPECT in this case. Although minor uptake in some inflammatory processes was observed, this could be distinguished from the more intense uptake in all other malignant tumors outside the brain (SPECT T/B ratio > 1.9 and planar T/B ratio > 1.2 in all cases), but not from the two brain tumors. Kuwert also found minor uptake in some non tumorous brain processes limiting the differentiation between benign and low grade malignant brain processes (21).

Some studies have suggested IMT SPECT to be of value in determining the response to radiotherapy of brain tumors, information that is hard to obtain by means of CT or MRI investigations (6,24). The correspondence between the IMT uptake and the clinical response after radiotherapy in the two patients with breast cancer in our study may lead to the speculation that IMT SPECT
can provide useful clinical information on treatment evaluation in tumors outside the brain as well. Further work is warranted in this field.

Although our patient group is rather small and only contains a few tumor types, general tumor uptake of IMT appears to be of the same magnitude as uptake of $^{201}$TI, $^{99m}$Tc-MIBI or $^{67}$Ga. The extent of uptake appears not different from uptake in brain tumors. Also, uptake kinetics in peripheral tumors are similar to brain tumor kinetics. Since uptake in brain tumors reflects transport of amino acids, it is tempting to suggest that some part of the uptake in extracranial tumors is associated with amino acid transport. Addition of a general amino acid SPECT tracer might be a valuable addition to diagnosis and therapy evaluation although interfering normal abdominal uptake and not completely neglectable free iodide production may limit whole body analysis. Furthermore it remains to be demonstrated whether IMT uptake is indeed a measure for amino acid transport activity in vivo.

Like in brain tumors, the optimal scan protocol requires imaging within the first ~60-90 min after administration because of the highest tumor to background ratios. SPECT is recommended. The procedure proved to be a simple, patient friendly, one-day investigation with a reported low radiation burden. Further work in carefully selected patient groups is necessary to validate this method for tumor detection, staging and restaging as well as further elucidating the uptake mechanism in non brain tumors.

5.6 CONCLUSION

In this study we have shown that tumors outside the brain can be visualized using the labeled amino acid L-3-$^{[123]}$I-Iodo-alpha-methyl-tyrosine.
CHAPTER 5

5.7 REFERENCES


