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¹²³I/¹²⁵I-labelled 2-iodo-L-phenylalanine and 2-iodo-D-phenylalanine: comparative uptake in various tumour types and biodistribution in mice

Veerle Kersemans¹, Bart Cornelissen¹, Ken Kersemans², Matthias Bauwens², Rudi A. Dierckx³, Bart De Spiegeleer¹, John Mertens², Guido Slegers¹

¹ Laboratory for Radiopharmacy, Universiteit Gent, Harelbekestraat 72, B-9000 Gent, Belgium

² Laboratory for Medical Imaging and Physics, Vrije Universiteit Brussel, Brussel, Belgium

³ Division of Nuclear Medicine, Gent University Hospital, Gent, Belgium

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Abstract. *Purpose:* In vitro in the R1M cell model and in vivo in the R1M tumour-bearing athymic model, both [¹²³I]-2-iodo-L-phenylalanine and [¹²³I]-2-iodo-D-phenylalanine have shown promising results as tumour diagnostic agents for SPECT. In order to compare these two amino acid analogues and to examine whether the observed characteristics could be generalised, both isomers were evaluated in various tumour models.

Methods: Transport type characterisation in vitro in A549, A2058, C6, C32, Capan2, EF43fgf4, HT29 and R1M cells with [¹²³I]-2-iodo-L-phenylalanine was performed using the method described by Shotwell et al. Subsequently, [¹²³I]-2-iodo-L-phenylalanine and [¹²³I]-2-iodo-D-phenylalanine tumour uptake and biodistribution were evaluated using dynamic planar imaging and/or dissection in A549, A2058, C6, C32, Capan2, EF43fgf4, HT29 and R1M inoculated athymic mice. Two-compartment blood modelling of the imaging results was performed.

Results: In vitro testing demonstrated that [¹²³I]-2-iodo-L-phenylalanine was transported in all tumour cell lines by LAT1. In all tumour models, the two amino acid analogues showed the same general biodistribution characteristics: high and specific tumour uptake and renal tracer clearance. Two-compartment modelling revealed that the D-isomer showed a faster blood clearance together with a faster distribution to the peripheral compartment in comparison with [¹²³I]-2-iodo-L-phenylalanine.

Conclusion: [¹²³I]-2-iodo-L-phenylalanine and its D-isomer are promising tumour diagnostic agents for dynamic

planar imaging. They showed a high and similar uptake in all tested tumours. [¹²³I]-2-iodo-D-phenylalanine showed better tracer characteristics concerning radiation dose to other organs.

Keywords: [¹²³I]-2-iodo-phenylalanine – Radiolabelled amino acids – Tumour imaging – D-amino acid – Tracer evaluation

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Introduction

¹⁸F-labelled fluorodeoxyglucose (¹⁸F-FDG) is by far the most common radiopharmaceutical in clinical use. However, there are several well-known limitations to the use of ¹⁸F-FDG in oncology: not all tumours show an elevated glucose metabolism, brain uptake is high and the tracer lacks tumour specificity. The last-mentioned property is of utmost importance for the study of residual and recurrent tumour lesions after primary therapy [1–3].

Another interesting target for metabolic tumour imaging is the increased protein metabolism and amino acid uptake in the generally rapidly proliferating cancer cells. Over the past decade, many research groups have reported the overexpression of both amino acid transporter systems A and LAT1. The role of LAT1 is to provide cells with essential amino acids for cell growth and tissue development, which is reflected in its high upregulation in fetal tissues and tumours. The latter observation led to the development of various radiolabelled amino acids for tumour imaging [2, 4].

In contrast to ¹⁸F-FDG, amino acids play a marginal role in the metabolism of inflammatory cells. Radiolabelled amino acids may thus be more suitable than ¹⁸F-FDG for tumour imaging [3, 5]. Further studies with unnatural

Veerle Kersemans (✉)
Laboratory for Radiopharmacy,
Universiteit Gent,
Harelbekestraat 72,
B-9000 Gent, Belgium
e-mail: veerle.kersemans@utoronto.ca
Tel.: +32-9-2648065, Fax: +32-9-2648071

amino acids, which could not be incorporated into cell proteins, showed significant correlation between tracer uptake, and thus amino acid transport, and proliferation. Amino acid transport tracers are accordingly valuable in reflecting the tumour malignancy. Moreover, these experiments showed that incorporation into the cell proteins of radiolabelled amino acids was not necessary in order to image cancer [2].

During the 1980s, it was shown that some D-amino acids, in comparison to their L-analogues, were taken up preferentially in tumours [6]. More recently, it was demonstrated that D-amino acids were transported by the same amino acid transporter systems: LAT1 showed a high affinity for radiolabelled D-amino acids [7]. These features, together with the highly stereospecific translation machinery and the presence of D-amino acid oxidase in kidneys and liver to metabolise exogenously D-amino acids, could make D-amino acids promising tumour diagnostic tools [8].

In addition to [^{123}I]-2-iodo-L-phenylalanine, our research group has developed [^{123}I]-2-iodo-D-phenylalanine. The diagnostic value of both new tracers was demonstrated in vivo in an R1M (rhabdomyosarcoma) inoculated athymic mouse model [9, 10]. The aim of this study was to investigate whether these new tracers could diagnose a variety of tumours in inoculated athymic mice.

Materials and methods

All the conventional products mentioned were at least of analytical or clinical grade and were obtained from Sigma-Aldrich. The solvents were of high-performance liquid chromatography (HPLC) quality (Chemlab, Belgium).

Synthesis of precursor 2-iodo-L-phenylalanine and 2-iodo-D-phenylalanine

2-Iodo-L-phenylalanine and 2-iodo-D-phenylalanine were prepared from 2-bromo-L-phenylalanine and 2-bromo-D-phenylalanine (Pep-Tech Corp, USA), respectively, as described previously, using the Cu^{1+} assisted nucleophilic exchange under acidic and reducing conditions. The mean reaction yield obtained for 2-iodo-L-phenylalanine and 2-iodo-D-phenylalanine was 74% and 65%, respectively, and no contamination of the other isomer was detected by chiral chromatography for each reaction [9, 10].

Radiochemistry

[$^{123/125}\text{I}$]-2-iodo-L-phenylalanine and [$^{123/125}\text{I}$]-2-iodo-D-phenylalanine Radioiodination with $^{123}\text{I}^-$ (222 MBq; 10–20 μl) or $^{125}\text{I}^-$ (37 MBq; 10 μl) (Nordion Europe, Belgium) on 1.0 mg 2-iodo-L-phenylalanine or 2-iodo-D-phenylalanine was performed by Cu^{1+} assisted isotopic exchange under acidic and reducing conditions (0.2 mg CuSO_4 , 2.5 mg citric acid, 0.5 mg SnSO_4 , 1.3 mg gentisic acid in 565 μl ; 60 min at 100°C) [9, 10]. The reaction mixture was drawn up in a syringe containing the appropriate amount of “make-up solution” (tri-sodium citrate dihydrate, 71 mM) to render the solution isotonic and to adjust the pH to at least 4. The reaction mixture was sent through a 0.22- μm Ag filter (Millipore, Belgium)

to remove the free $^{123/125}\text{I}^-$ and through a sterile 0.22- μm filter (Millipore, Belgium) into a sterile vacuum vial. Quality control was achieved by HPLC and Sep-Pak C18 (Waters, Belgium). Radio-labelling of both tracers with $^{123}\text{I}^-$ or $^{125}\text{I}^-$ resulted in a radiochemical purity of >99% and a specific activity of 65 GBq/mmol (^{123}I labelling) and 11 GBq/mmol (^{125}I labelling). Chiral chromatography showed no transformation to the analogous isomer.

[^{123}I]-iodo-human serum albumin Radioiodination of human serum albumin (HSA) with $^{123}\text{I}^-$ was performed by electrophilic substitution using the Iodogen (1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycylcouracil) technique: 50 μg HSA together with 111 MBq $^{123}\text{I}^-$ (10 μl) and 140 μl 0.1 M KH_2PO_4 buffer at pH 8.5 was added to an Iodogen (Pierce, Belgium)-coated vial. After 15 min at room temperature the mixture was removed from the Iodogen-coated vial to stop the reaction and was sent through a 0.22- μm Ag filter to remove the free $^{123}\text{I}^-$ from [^{123}I]-iodo-HSA and through a sterile 0.22- μm filter to sterilise into a sterile vacuum vial. The specific activity was 4.10⁴ GBq/mmol. Quality control was achieved using a standard prepacked PD-10 SEC column (size exclusion chromatography, Amersham Pharmacia Biotech, Sweden) using PBS/HSA as the eluting agent.

In vitro experiments

To check the presence of the L-type amino acid transporter LAT1, we performed a short in vitro evaluation of [^{125}I]-2-iodo-L-phenylalanine uptake in all cell lines used. The method we used has been described by Shotwell et al. and is frequently used to determine the relative contribution of the individual neutral transporter systems involved [11]. Using R1M cells and 4F2hc-LAT1 transfected oocytes, respectively, Mertens et al. and Yanagida et al. described in earlier work that [^{125}I]-2-iodo-D-phenylalanine is taken up by the same transporter system with the same affinity for LAT1 as compared with its L-isomer [7, 12].

Cell model A549 human non-small cellular lung cell carcinoma, C32 human amelanotic melanoma, Capan2 human pancreatic carcinoma and HT29 human colon adenocarcinoma cells were purchased from Janssen Pharmaceutica (Beerse, Belgium). A2058 human melanoma and EF43fgf4 mouse breast carcinoma cells were obtained from the Laboratoire de Biologie des Tumeurs et du Développement, ULg (Liège, Belgium). C6 rat glioma and R1M rhabdomyosarcoma cells were purchased from BEFY-VUB (Brussels, Belgium). Cell cultures were maintained and treated according to ATCC recommendations. A549, A2058, C6, C32, Capan2, EF43fgf4, HT29 and R1M cells were cultured in complete medium (Ham's F12 for C6, McCoy's 5A for Capan2 and HT29 and DMEM for A549, A2058, C32, EF43fgf4 and R1M), enriched with 10% of fetal bovine serum (FBS) and supplemented with 1% penicillin-streptomycin and 1% L-glutamine. Weekly split ratios were 1:20, 1:10, 1:5, 1:4, 1:20, 1:10 and 1:20 for A2058, A549, C32, C6, Capan2, EF43fgf4, HT29 and R1M, respectively. All cell lines were grown in an anchorage-dependent manner in tissue culture flasks and Falcon six-well tissue culture plates.

All in vitro experiments were carried out in six-well plates (Novolab, Belgium), using three wells for each data point. Influx of [^{125}I]-2-iodo-L-phenylalanine radioactivity was studied both in an Na^+ -containing buffer (“HEPES⁺”: pH 7.4; 100 mM NaCl, 2 mM

KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM HEPES, 5 mM Tris, 1 g/l glucose and 1 g/l bovine serum albumin) and in an Na⁺-free buffer ("HEPES": pH 7.4; 100 mM choline-Cl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM HEPES, 5 mM Tris, 1 g/l glucose and 1 g/l bovine serum albumin). The influx was terminated by physical withdrawal of the buffer and by washing three times with ice-cold phosphate-buffered saline (PBS). Subsequently, the cells were lysed from the well with 1 ml of 0.1 M NaOH. The radioactivity of the samples was counted using the auto gamma-counting system (Cobra II Series, Canberra Packard, Meriden, CT, USA).

Time dependency Cells were incubated for different times (0.5, 1, 2.5, 5, 7.5, 10, 15 or 30 min) in 1 ml of HEPES⁺ or HEPES⁻ containing 37 kBq [¹²⁵I]-2-iodo-L-phenylalanine.

Inhibition of [¹²⁵I]-2-iodo-L-phenylalanine influx In inhibition experiments the cells were incubated with 37 kBq [¹²⁵I]-2-iodo-L-phenylalanine for 10 min in HEPES⁺ buffer supplemented with 1 mM L-phenylalanine (the natural amino acid transported by LAT), 1 mM 2-iodo-L-phenylalanine (the "cold" precursor), 1 mM BCH (2-amino-2-norbornane-carboxylic acid, a LAT transport system specific inhibitor) or 1 mM MeAIB (methyl amino isobutyric acid, an A/ASC transport system specific inhibitor).

In vivo experiments

Laboratory animals The study protocol was approved by the local ethical committee for animal studies and was within the rules of Belgian legislation. Guidelines of the National Institute of Health principles of laboratory animal care were followed.

Water and food was freely available during the experimental period.

Swiss *nu/nu* mice (*n*=15 per cell line, except R1M: *n*=30) (Bioservices, The Netherlands) were injected subcutaneously (SC) in the right flank (armpit region) with 5·10⁶ A549, A2058, C6, C32, Capan2, HT29 or R1M cells. Female NMRI mice (*n*=15) were injected SC in the right armpit region with 5·10⁶ EF43fgf4 cells. Normal tumour growth curves were obtained using sliding caliper measurement and the estimated volume formula $V = 0.4a^2 \cdot b$, *a* and *b* being the short and the long axis of the tumour respectively [9, 13]. The imaging experiments started when the tumour reached a volume of 0.5 ml. Imaging experiments with [¹²³I]-2-iodo-L-phenylalanine, [¹²³I]-2-iodo-D-phenylalanine and [¹²³I]-iodo-HSA were carried out on the same mouse population (per cell line). An interval of 6 days was left between experiments to allow for radioactive decay. Dissection experiments with [¹²⁵I]-2-iodo-L-phenylalanine were performed on each cell line and in the same mouse population as used for imaging experiments. Additionally, dissection experiments with [¹²⁵I]-2-iodo-D-phenylalanine were carried out in the R1M athymic mouse model to confirm the conclusions obtained by imaging for this isomer.

During all imaging experiments, the animals were anaesthetised by intraperitoneal injection of a dilution (1:3) of pentobarbital (1.5 mg in 75 µl of Nembutal 60 mg/ml from Ceva Santé Animale, Belgium). For the biodistribution experiments by dissection, the animals were killed by cervical dislocation without sedation and the organs of interest were dissected.

All tracers ([^{123/125}I]-2-iodo-L-phenylalanine, [^{123/125}I]-2-iodo-D-phenylalanine and [¹²³I]-iodo-HSA) were injected intravenously (IV) in the lateral tail vein.

Dynamic planar imaging Imaging was performed using a gamma camera (Toshiba GCA-9300 A/hg) in planar mode equipped with a high-resolution parallel-hole collimator. After the IV bolus injection of the [¹²³I]-labelled product in the lateral tail vein, the data were recorded on a 128×128 matrix (field of view 23.5×12.5 cm) and with a photopeak window set at 15% around 159 keV. Dynamic planar imaging (DPI) to determine the steady state of tumour tracer uptake consisted of 90 frames (48×10 s, 28×20 s and 14×60 s) and the DPI for the displacement study contained 90 frames of 30 s.

Time-activity curves were obtained by region of interest (ROI) analysis using a magnetic resonance imaging (MRI) maximum intensity projection as described previously [14]. The tracer uptake was recorded as the percentage injected activity (IA) per pixel, taking into account a dose calibration and background correction following the guidance of Thie et al. [15]. The differential uptake ratio (DUR) was calculated as: $[(\text{counts}_{\text{tissue}} \times \text{pixels}_{\text{total body}}) / (\text{pixels}_{\text{tissue}} \times \text{counts}_{\text{total body}})]$.

First, a [¹²³I]-iodo-HSA study was performed to measure the relative blood pool distribution to correct the uptake of [¹²³I]-2-iodo-D-phenylalanine and [¹²³I]-2-iodo-L-phenylalanine in the tumours for blood pool activity. Ten tumour-bearing mice of each mouse population were injected with 7.4 MBq [¹²³I]-iodo-HSA. Ten dynamic planar images of 1 min were acquired starting 10 min p.i. The tumour to contralateral background ratio (RTB) was calculated and the overall mean for all animals determined. Statistical analysis (ANOVA $\alpha=0.05$; SPSS 13.0 software package) was performed to compare the [¹²³I]-iodo-HSA uptake in the tumour tissue with that in the contralateral background tissue.

Immediately after injection of 18.5 MBq [¹²³I]-2-iodo-L-phenylalanine or [¹²³I]-2-iodo-D-phenylalanine, a dynamic acquisition was performed to determine the steady state of tracer uptake in the tumour. Second, at steady state a displacement study with L-phenylalanine (200 µl IV of a 145 mM solution) was performed. ROIs were drawn using an MRI maximum intensity projection as described previously [9]. Tumour [¹²³I]-2-iodo-L-phenylalanine or [¹²³I]-2-iodo-D-phenylalanine uptake was compared with the uptake in the contralateral background area and the RTB calculated. The significance of displacement of [¹²³I]-2-iodo-L-phenylalanine or [¹²³I]-2-iodo-D-phenylalanine activity by L-phenylalanine was calculated at a 95% confidential interval.

The data obtained by DPI for the blood curve for [¹²³I]-2-iodo-L-phenylalanine and [¹²³I]-2-iodo-D-phenylalanine were fitted to a two-compartment model with IV bolus injection, without a lag time and with first-order elimination, using WinNonlin 4.0.1. This kinetic model was chosen by analogy with [¹²³I]-3-iodo- α -methyltyrosine [16]. The primary parameters V_1 , $k_{1,0}$, $k_{1,2}$ and $k_{2,1}$ were determined for both tracers in all cell lines.

Statistical analysis was performed using a two-factor ANOVA (tumour type and [¹²³I]-2-iodo-L-phenylalanine or [¹²³I]-2-iodo-D-phenylalanine) on the DPI results to investigate whether tumour uptake was different amongst the tumour types and both tracers.

Dissection: biodistribution of [¹²⁵I]-2-iodo-L-phenylalanine or [¹²⁵I]-2-iodo-D-phenylalanine The IA was calculated by weighing

the syringes before and after injection of the tracer and by the use of a dilution series of the injected tracer solution, which was also weighed and counted for radioactivity using the auto gamma-counting system (Cobra II Series, Canberra Packard, Meriden, CT, USA).

Fifteen A549, A2058, C6, C32, Capan2, EF43fgf4, HT29 or R1M tumour-bearing mice were injected with 7.4 kBq [125 I]-2-iodo-L-phenylalanine and 15 R1M tumour-bearing mice were injected with 7.4 kBq [125 I]-2-iodo-D-phenylalanine, 6 days after the last imaging experiment with 123 I tracer was performed. At various time points (2, 30, 60, 120 and 180 min) post injection, three animals per time point were sacrificed. The organs and tissues were removed, washed and weighed. The blood was collected and weighed. The radioactivity of the samples was counted by use of an auto gamma-counting system (Cobra II Series, Canberra Packard, Meriden, CT, USA). The amount of radioactivity in the organs and tissues was calculated as % injected dose per gram (%ID/g): $[(\text{activity}_{\text{tissue}})/(\text{weight}_{\text{tissue}} \times \text{activity}_{\text{injected}})]$.

Results

In vitro experiments

The results of the *in vitro* experiments are summarised in Table 1. All tested cell lines showed comparable [125 I]-2-iodo-L-phenylalanine uptake in HEPES⁺ and HEPES⁻. In all cell lines, tracer uptake in HEPES⁺ was blocked to a large extent by L-phenylalanine, 2-iodo-L-phenylalanine and BCH but to only a minor extent by MeAIB. All cell lines demonstrated similar uptake values, with the exception of HT29 and EF43fgf4. The latter cell lines showed significantly lower [125 I]-2-iodo-L-phenylalanine uptake values.

Fig. 1. DPI images showing uptake of [123 I]-2-iodo-D-phenylalanine in the R1M tumour model as a function of time for a tumour volume of 0.50 cm³. Each image is the result of the summation of 11 consecutive frames. Relative scaling was used (see coloured tool). B bladder, P pancreatic zone, K kidney, T tumour, C calibration syringe

In vivo experiments

Planar imaging

DPI with [123 I]-iodo-HSA showed no significant difference (all $p > 0.05$) between the blood flow in the tumour and the contralateral reference leg for A549, A2058, C32, Capan2, HT29 and R1M tumours ($\text{RTB}_{[^{123}\text{I}]\text{-iodo-HSA}} = 1.2 \pm 0.1$; 1.0 ± 0.1 , 1.1 ± 0.1 , 1.2 ± 0.1 , 1.1 ± 0.1 and 1.1 ± 0.2 , respectively). The rat glioma tumour (C6) showed a slightly elevated $\text{RTB}_{[^{123}\text{I}]\text{-iodo-HSA}}$ of 1.4 ± 0.2 ($p = 0.03$) and the EF43fgf4 inoculated mice showed an $\text{RTB}_{[^{123}\text{I}]\text{-iodo-HSA}}$ of 3.2 ± 1.1 ($p < 0.001$).

Representative DPI images of [123 I]-2-iodo-D-phenylalanine uptake in the R1M tumour model are displayed in Fig. 1. DPI images of [123 I]-2-iodo-L-phenylalanine uptake have been presented elsewhere [9]. Net [123 I]-2-iodo-L-phenylalanine and [123 I]-2-iodo-D-phenylalanine tumour uptake at steady state (plateau phase) was high for all

Table 1. *In vitro* uptake of [125 I]-2-iodo-L-phenylalanine at equilibrium ($t = 15$ min)

Cell line	% Uptake/million cells		% Inhibition			
	HEPES ⁻	HEPES ⁺	L-Phe	2-I-L-Phe	BCH	MeAIB
A549	12.16 $\pm$ 3.61	10.37 $\pm$ 2.25	71	77	79	0
A2058	10.33 $\pm$ 0.93	10.06 $\pm$ 1.29	72	81	83	18
C6	12.34 $\pm$ 2.28	12.51 $\pm$ 1.68	79	85	87	0
C32	12.94 $\pm$ 2.39	15.79 $\pm$ 1.65	88	90	85	0
Capan2	13.25 $\pm$ 2.22	15.31 $\pm$ 1.62	89	90	90	0
EF43fgf4	2.62 $\pm$ 0.44*	3.83 $\pm$ 0.45*	79	82	75	9
HT29	5.27 $\pm$ 0.79*	7.47 $\pm$ 1.76*	80	83	78	2
R1M	14.12 $\pm$ 1.13	14.33 $\pm$ 0.92	90	89	93	6

L-Phe L-phenylalanine, 2-I-L-Phe 2-iodo-L-phenylalanine, BCH 2-amino-2-norbornane-carboxylic acid, MeAIB methyl amino isobutyric acid

*Significantly different at $\alpha = 0.05$

Table 2. In vivo [123 I]-iodo-L-phenylalanine and [123 I]-iodo-D-phenylalanine tumour and contralateral background uptake on DPI for the tested tumour types at steady state ($t=30$ min p.i.; $n=15$)

Cell line	L or D ^a	Tumour Mean $\pm$ SD (DUR)	Contralateral background Mean $\pm$ SD (DUR)	RTB Mean $\pm$ SD	Bladder ^b Mean $\pm$ SD (DUR)
A549	L	0.84 $\pm$ 0.08	0.49 $\pm$ 0.07	1.72 $\pm$ 0.23	4.0 $\pm$ 0.8
	D	0.88 $\pm$ 0.11	0.40 $\pm$ 0.03	2.17 $\pm$ 0.28	4.8 $\pm$ 0.9
A2058	L	0.80 $\pm$ 0.09	0.53 $\pm$ 0.03	1.50 $\pm$ 0.22	3.6 $\pm$ 0.9
	D	0.91 $\pm$ 0.09	0.49 $\pm$ 0.11	1.85 $\pm$ 0.21	6.3 $\pm$ 1.1
C6	L	1.12 $\pm$ 0.07	0.65 $\pm$ 0.15	1.73 $\pm$ 0.28	2.9 $\pm$ 0.4
	D	0.70 $\pm$ 0.06	0.30 $\pm$ 0.08	2.31 $\pm$ 0.21	10.5 $\pm$ 0.6
C32	L	0.99 $\pm$ 0.09	0.53 $\pm$ 0.05	1.88 $\pm$ 0.18	2.6 $\pm$ 0.5
	D	1.06 $\pm$ 0.11	0.55 $\pm$ 0.09	1.92 $\pm$ 0.22	6.2 $\pm$ 0.9
Capan2	L	0.87 $\pm$ 0.11	0.48 $\pm$ 0.07	1.83 $\pm$ 0.25	2.7 $\pm$ 0.7
	D	0.89 $\pm$ 0.09	0.39 $\pm$ 0.05	2.29 $\pm$ 0.21	3.3 $\pm$ 0.5
EF43fgf4	L	0.92 $\pm$ 0.07	0.46 $\pm$ 0.09	1.99 $\pm$ 0.19	4.5 $\pm$ 0.7
	D	1.13 $\pm$ 0.16	0.55 $\pm$ 0.09	2.04 $\pm$ 0.15	7.6 $\pm$ 0.9
HT29	L	0.90 $\pm$ 0.08	0.42 $\pm$ 0.06	2.12 $\pm$ 0.26	2.5 $\pm$ 0.5
	D	0.80 $\pm$ 0.06	0.36 $\pm$ 0.08	2.20 $\pm$ 0.41	6.8 $\pm$ 0.8
R1M	L	1.19 $\pm$ 0.09	0.63 $\pm$ 0.10	1.89 $\pm$ 0.16	3.2 $\pm$ 0.7
	D	0.99 $\pm$ 0.08	0.59 $\pm$ 0.07	1.68 $\pm$ 0.18	7.7 $\pm$ 0.9

RTB tumour to contralateral background ratio

^a[123 I]-iodo-L-phenylalanine or [123 I]-iodo-D-phenylalanine

^bTracer accumulation in the bladder was determined 30 min p.i.

tumours and is summarised in Table 2. No significant differences ($\alpha=0.05$) were observed between the two amino acid analogues or amongst the various tumour types. The mean RTB for [123 I]-2-iodo-L-phenylalanine and [123 I]-2-iodo-D-phenylalanine tumour uptake differed significantly ($p<0.05$) from the [123 I]-iodo-HSA study with the exception of the EF43fgf4 tumour. The tracer uptake in the tumour was displaced rapidly to a small but significant

extent ($p<0.05$) (Table 3) after IV administration of L-phenylalanine for all tumour types tested.

Blood elimination of both tracers showed renal clearance to the bladder in all tumour models. With the exception of the A549 and Capan2 inoculated athymic mice, in which both tracers showed the same elimination pattern, 2-iodo-D-phenylalanine accumulated in higher amounts in the bladder at 30 min p.i. in comparison with its L-analogue in all tested tumour models (Table 2). The latter was illustrated by the DPI experiments as well as by the applied two-compartment modelling. The results obtained using the pharmacokinetic model are presented in Table 4. Besides the difference in blood elimination velocity of the two tracers, the model showed that [123 I]-2-iodo-D-phenylalanine was taken up faster in the peripheral compartment. However, its elimination velocity from the latter compartment was comparable to that of [123 I]-2-iodo-L-phenylalanine in all tumour models. The observed tumour uptake of both 2-iodo-L-phenylalanine and 2-iodo-D-phenylalanine fitted the calculated curve for the proposed kinetic model in all tumour types (all $R^2>95\%$).

Table 3. Displacement of [123 I]-iodo-L-phenylalanine and [123 I]-iodo-D-phenylalanine activity in the tumour by L-phenylalanine using DPI ($t=45$ min p.i.)

Cell line	[123 I]-2-iodo-L-phenylalanine		[123 I]-2-iodo-D-phenylalanine	
	%Pa	%D ^b	%Pa	%D ^b
A549	60	32.6 $\pm$ 19.0*	85	22.3 $\pm$ 17.4*
A2058	57	13.2 $\pm$ 9.9*	53	13.9 $\pm$ 9.2*
C6	57	10.8 $\pm$ 1.8*	63	10.7 $\pm$ 4.1*
C32	50	7.1 $\pm$ 2.3*	55	8.3 $\pm$ 1.9*
Capan2	61	21.4 $\pm$ 11.3*	59	18.1 $\pm$ 3.7*
EF43fgf4	30	6.0 $\pm$ 3.1*	39	9.2 $\pm$ 2.5*
HT29	40	11.3 $\pm$ 4.8*	48	10.8 $\pm$ 3.3*
R1M	72	12.3 $\pm$ 4.2*	50	9.6 $\pm$ 2.3*

^aPercentage of all animals in which a significant displacement of radioactivity was observed ($n=15$)

^bPercentage reduction in tracer uptake upon administration of L-phenylalanine (mean $\pm$ SD)

*Significant at $\alpha=0.05$

Biodistribution by dissection

The biodistribution data of [125 I]-2-iodo-L-phenylalanine in all tumour-bearing athymic mice are shown in Tables 5 and 6. Those of [125 I]-2-iodo-D-phenylalanine in R1M are shown in Table 7.

The net [125 I]-2-iodo-L-phenylalanine tumour uptake reached equilibrium by 60 min for all tumour types. Mean %ID/g values at equilibrium of 2.93 $\pm$ 0.22, 7.33 $\pm$ 0.25,

Table 4. Kinetic fitting of the observed blood curve for [123 I]-iodo-L-phenylalanine (2-I-L-Phe) and [123 I]-iodo-D-phenylalanine (2-I-D-Phe) uptake with the calculated blood curve using a two-compartment model

Cell line	Two-compartment model fit (R^2)		V_1 (activity total body/ (DUR)		$k_{1,0}$ (1/min)		$k_{1,2}$ (1/min)		$k_{2,1}$ (1/min)	
	2-I-L-Phe	2-I-D-Phe	2-I-L-Phe	2-I-D-Phe	2-I-L-Phe	2-I-D-Phe	2-I-L-Phe	2-I-D-Phe	2-I-L-Phe	2-I-D-Phe
A549	0.9809	0.9937	9.57±0.21	11.52±0.15	0.027±0.001	0.030±0.002	0.36±0.02	0.48±0.02*	0.24±0.04	0.33±0.06
A2058	0.9894	0.9843	7.53±0.14	6.86±0.18	0.026±0.004	0.072±0.006*	0.70±0.05	0.59±0.06	0.32±0.03	0.42±0.07
C32	0.9893	0.9901	9.96±0.16	9.11±0.21	0.020±0.003	0.046±0.006	0.44±0.02	0.65±0.05*	0.25±0.01	0.32±0.05
C6	0.9904	0.9794	9.46±0.16	13.45±0.57*	0.033±0.002	0.076±0.005	0.64±0.03	1.86±0.17*	0.43±0.02	0.95±0.05*
Capan2	0.9936	0.9921	9.84±0.12	10.08±0.17	0.020±0.003	0.029±0.007*	0.57±0.02	0.69±0.08	0.41±0.01	0.40±0.04
EF43fgf4	0.9902	0.9897	8.98±0.16	11.36±0.12*	0.043±0.004	0.059±0.005	0.66±0.03	0.85±0.07*	0.30±0.01	0.33±0.06
HT29	0.9835	0.9580	12.28±0.24	12.52±0.47	0.020±0.002	0.037±0.003	0.57±0.04	0.48±0.07	0.50±0.03	0.56±0.07
R1M	0.9868	0.9964	9.65±0.20	7.10±0.12*	0.035±0.003	0.075±0.003	0.80±0.04	1.09±0.04*	0.42±0.02	0.41±0.02

The primary parameters V_1 , $k_{1,0}$, $k_{1,2}$ and $k_{2,1}$ were determined for both tracers in all cell lines

*Values for the two isomers significantly different at $\alpha=0.05$

4.43±0.23, 4.76±0.18, 9.19±0.34, 3.10±0.23, 2.65±0.17 and 6.68±0.28 were reached for A549, A2058, C6, C32, Capan2, EF43fgf4, HT29 and R1M tumours respectively. At the same time point, [125 I]-2-iodo-L-phenylalanine activity in the blood reached a %ID/g of 1.80±0.34, 2.08±0.49, 2.05±0.75, 2.73±0.37, 1.47±0.49, 3.38±1.06, 3.83±0.89 and 1.69±0.52 and in the contralateral leg, a %ID/g of 1.23±0.19, 1.76±0.19, 1.83±0.19, 2.01±0.25, 2.71±0.21,

1.50±0.16, 1.21±0.21 and 0.97±0.17 for A549, A2058, C6, C32, Capan2, EF43fgf4, HT29 and R1M respectively.

The net [125 I]-2-iodo-D-phenylalanine tumour uptake reached equilibrium from 60 min, with a mean %ID/g value of 4.02±0.26. At the same time point, [125 I]-2-iodo-D-phenylalanine activity in the blood and in the contralateral leg tissue reached a %ID/g of 1.90±0.30 and 1.18±0.10 respectively.

Table 5. Biodistribution by dissection of [123 I]-iodo-L-phenylalanine in A549, A2058, C6 and C32 inoculated athymic mice ($n=3$), expressed as %ID/g

Time (min)	A549 (%ID/g ±SD)					A2058 (%ID/g ±SD)				
	2	30	60	120	180	2	30	60	120	180
Blood	6.81±2.21	2.62±0.31	1.80±0.34	1.61±0.28	1.41±0.19	5.94±1.98	4.15±0.51	2.08±0.49	1.51±0.38	1.49±0.31
Brain	1.15±0.66	1.31±0.26	0.91±0.45	1.06±0.23	0.77±0.17	1.57±0.52	1.68±0.21	1.74±0.29	1.67±0.25	0.96±0.17
Stomach	1.55±0.85	2.58±0.59	1.77±0.47	1.79±0.65	1.19±0.53	2.22±0.89	3.95±1.16	4.74±0.89	5.48±1.03	2.75±0.56
Liver	4.39±1.03	1.37±0.34	1.36±0.21	1.43±0.26	1.13±0.31	4.19±0.76	2.32±0.33	1.97±0.21	1.52±0.27	1.42±0.19
Kidneys	23.03±8.21	3.83±0.84	3.00±0.79	2.10±0.53	1.99±0.49	14.80±5.24	3.76±0.73	3.46±0.58	3.57±0.47	2.71±0.50
Pancreas	18.47±6.89	12.33±3.61	10.14±4.11	16.13±2.89	8.43±3.35	21.98±7.29	19.84±4.12	19.11±5.31	19.21±4.24	11.81±3.85
Contr. bckgr.	2.70±0.56	1.23±0.46	1.23±0.19	1.07±0.24	1.00±0.15	3.74±0.43	1.85±0.22	1.76±0.19	1.72±0.17	1.07±0.23
Tumour	1.70±0.42	3.13±0.29	2.93±0.22	2.88±0.27	2.55±0.21	1.56±0.45	7.18±0.27	7.33±0.25	9.09±0.31	7.07±0.29
RTB	0.63±0.67	2.56±0.52	2.38±0.48	2.68±0.39	2.56±0.37	0.41±0.51	3.96±0.36	4.16±0.41	5.25±0.39	6.59±0.54
Time (min)	C6 (%ID/g ±SD)					C32 (%ID/g ±SD)				
	2	30	60	120	180	2	30	60	120	180
Blood	5.90±1.58	4.28±1.11	2.05±0.75	1.93±0.51	1.50±0.39	5.15±2.01	2.93±0.61	2.73±0.37	1.88±0.21	1.85±0.27
Brain	1.26±0.54	1.60±0.17	1.45±0.28	1.51±0.30	0.92±0.16	1.31±0.38	1.40±0.20	1.25±0.27	1.50±0.24	1.02±0.19
Stomach	1.95±1.21	4.23±1.35	4.46±1.09	3.81±0.74	2.31±0.83	1.31±0.61	1.37±0.55	1.66±0.47	0.96±0.50	1.65±0.46
Liver	3.36±0.37	1.77±0.29	1.13±0.31	1.27±0.21	1.14±0.28	4.94±0.39	2.81±0.26	1.85±0.23	1.39±0.27	1.27±0.13
Kidneys	10.91±2.58	5.41±1.04	2.61±0.61	2.79±0.67	2.53±0.51	6.55±1.18	4.15±0.97	3.29±0.71	2.99±0.52	3.33±0.54
Pancreas	9.42±5.19	11.77±3.27	12.51±3.80	12.65±2.36	14.47±4.09	23.60±7.66	18.56±5.21	22.28±6.84	8.97±4.84	12.30±4.64
Contr. bckgr.	2.94±0.22	1.74±0.30	1.83±0.19	1.27±0.17	1.07±0.19	4.16±0.31	2.22±0.27	2.01±0.25	1.41±0.31	1.43±0.15
Tumour	1.92±0.39	3.44±0.24	4.43±0.23	2.08±0.18	1.23±0.17	2.28±0.22	4.97±0.39	4.76±0.18	3.28±0.23	3.25±0.12
RTB	0.65±0.51	1.98±0.47	2.42±0.57	1.64±0.28	1.15±0.43	0.55±0.37	2.24±0.42	2.37±0.39	2.32±0.29	2.27±0.21

Contr. bckgr. contralateral background, RTB tumour to contralateral background ratio

Table 6. Biodistribution by dissection of [^{123}I]-iodo-L-phenylalanine in Capan2, EF43fgf4, HT29 and R1M inoculated athymic mice ($n=3$), expressed as %ID/g

Time (min)	Capan2 (%ID/g $\pm$ SD)					EF43fgf4 (%ID/g $\pm$ SD)				
	2	30	60	120	180	2	30	60	120	180
Blood	4.76 $\pm$ 1.02	2.49 $\pm$ 0.67	1.47 $\pm$ 0.49	1.91 $\pm$ 0.23	1.93 $\pm$ 0.31	6.56 $\pm$ 0.99	4.56 $\pm$ 1.13	3.38 $\pm$ 1.06	2.95 $\pm$ 0.79	3.02 $\pm$ 0.83
Brain	0.68 $\pm$ 0.49	2.41 $\pm$ 0.51	1.88 $\pm$ 0.47	1.77 $\pm$ 0.41	1.45 $\pm$ 0.48	1.58 $\pm$ 0.12	2.44 $\pm$ 0.23	2.45 $\pm$ 0.28	2.11 $\pm$ 0.21	1.61 $\pm$ 0.19
Stomach	2.52 $\pm$ 1.16	2.45 $\pm$ 0.60	4.59 $\pm$ 0.58	4.57 $\pm$ 0.51	4.52 $\pm$ 0.47	2.82 $\pm$ 0.84	5.60 $\pm$ 0.56	3.39 $\pm$ 0.38	3.31 $\pm$ 0.35	2.75 $\pm$ 0.47
Liver	3.41 $\pm$ 0.39	1.95 $\pm$ 0.24	1.42 $\pm$ 0.29	2.60 $\pm$ 0.37	1.77 $\pm$ 0.31	3.95 $\pm$ 0.41	2.12 $\pm$ 0.27	1.26 $\pm$ 0.35	1.65 $\pm$ 0.30	1.51 $\pm$ 0.21
Kidneys	10.84 $\pm$ 2.64	3.80 $\pm$ 1.11	3.41 $\pm$ 0.51	3.40 $\pm$ 0.57	2.71 $\pm$ 0.43	10.32 $\pm$ 2.49	4.67 $\pm$ 0.87	3.70 $\pm$ 0.71	2.54 $\pm$ 0.61	3.34 $\pm$ 0.73
Pancreas	13.21 $\pm$ 6.52	13.17 $\pm$ 4.10	5.98 $\pm$ 3.87	16.96 $\pm$ 3.54	18.56 $\pm$ 2.57	17.73 $\pm$ 5.68	15.17 $\pm$ 3.54	10.77 $\pm$ 3.88	12.71 $\pm$ 4.08	12.03 $\pm$ 2.61
Contr. bckgr.	3.53 $\pm$ 0.33	2.86 $\pm$ 0.28	2.71 $\pm$ 0.21	2.42 $\pm$ 0.39	2.34 $\pm$ 0.32	2.16 $\pm$ 0.18	2.17 $\pm$ 0.21	1.50 $\pm$ 0.16	1.59 $\pm$ 0.27	1.47 $\pm$ 0.25
Tumour	4.05 $\pm$ 0.29	8.62 $\pm$ 0.47	9.19 $\pm$ 0.34	8.91 $\pm$ 0.31	8.86 $\pm$ 0.20	3.02 $\pm$ 0.28	3.10 $\pm$ 0.23	2.04 $\pm$ 0.19	1.90 $\pm$ 0.22	1.71 $\pm$ 0.17
RTB	1.15 $\pm$ 0.46	3.01 $\pm$ 0.51	3.38 $\pm$ 0.43	3.68 $\pm$ 0.47	3.78 $\pm$ 0.39	1.39 $\pm$ 0.31	1.43 $\pm$ 0.27	1.36 $\pm$ 0.23	1.19 $\pm$ 0.30	1.16 $\pm$ 0.28
Time (min)	HT29 (%ID/g $\pm$ SD)					R1M (%ID/g $\pm$ SD)				
	2	30	60	120	180	2	30	60	120	180
Blood	8.91 $\pm$ 3.64	6.88 $\pm$ 2.14	3.83 $\pm$ 0.89	3.88 $\pm$ 1.06	3.81 $\pm$ 0.69	3.89 $\pm$ 1.13	2.00 $\pm$ 0.71	1.69 $\pm$ 0.52	1.66 $\pm$ 0.39	1.70 $\pm$ 0.32
Brain	4.12 $\pm$ 0.58	3.29 $\pm$ 0.62	2.61 $\pm$ 0.38	1.71 $\pm$ 0.47	1.25 $\pm$ 0.51	0.73 $\pm$ 0.21	0.72 $\pm$ 0.28	1.06 $\pm$ 0.17	0.86 $\pm$ 0.23	0.67 $\pm$ 0.21
Stomach	2.56 $\pm$ 1.39	8.09 $\pm$ 2.41	6.01 $\pm$ 1.76	3.36 $\pm$ 1.36	3.47 $\pm$ 1.24	1.84 $\pm$ 0.49	2.64 $\pm$ 0.43	5.50 $\pm$ 0.34	3.68 $\pm$ 0.45	3.45 $\pm$ 0.40
Liver	5.29 $\pm$ 0.49	3.01 $\pm$ 0.34	2.97 $\pm$ 0.52	3.29 $\pm$ 0.23	3.04 $\pm$ 0.54	1.91 $\pm$ 0.38	0.70 $\pm$ 0.23	0.88 $\pm$ 0.28	0.60 $\pm$ 0.23	0.80 $\pm$ 0.31
Kidneys	14.08 $\pm$ 5.04	14.68 $\pm$ 4.66	8.35 $\pm$ 2.54	5.71 $\pm$ 3.19	5.81 $\pm$ 1.82	10.19 $\pm$ 2.16	2.37 $\pm$ 0.45	2.34 $\pm$ 0.37	1.57 $\pm$ 0.29	1.45 $\pm$ 0.26
Pancreas	30.16 $\pm$ 8.79	15.06 $\pm$ 5.13	9.88 $\pm$ 3.68	9.66 $\pm$ 4.87	7.84 $\pm$ 4.03	11.28 $\pm$ 3.16	6.89 $\pm$ 2.58	13.47 $\pm$ 1.99	13.89 $\pm$ 4.27	13.00 $\pm$ 1.56
Contr. bckgr.	1.21 $\pm$ 0.19	1.63 $\pm$ 0.11	1.21 $\pm$ 0.21	1.26 $\pm$ 0.27	1.50 $\pm$ 0.15	1.66 $\pm$ 0.25	0.53 $\pm$ 0.31	0.97 $\pm$ 0.17	0.80 $\pm$ 0.26	0.72 $\pm$ 0.16
Tumour	2.72 $\pm$ 0.25	1.60 $\pm$ 0.27	1.63 $\pm$ 0.12	2.65 $\pm$ 0.17	2.17 $\pm$ 0.19	2.40 $\pm$ 0.15	3.82 $\pm$ 0.11	6.50 $\pm$ 0.21	5.91 $\pm$ 0.19	4.73 $\pm$ 0.19
RTB	2.25 $\pm$ 0.31	0.98 $\pm$ 0.29	1.35 $\pm$ 0.23	2.10 $\pm$ 0.37	1.45 $\pm$ 0.31	1.44 $\pm$ 0.31	7.22 $\pm$ 0.33	6.68 $\pm$ 0.28	7.42 $\pm$ 0.41	6.53 $\pm$ 0.36

Contr. bckgr: contralateral background, RTB tumour to contralateral background ratio

For all tumour types tested, [^{125}I]-2-iodo-L-phenylalanine was cleared very rapidly through the kidneys. Significant uptake of radioactivity in the pancreas was observed. No significant accumulation of radioactivity was observed in other abdominal organs such as liver, lungs, small intestine and large intestine, or in the brain.

[^{125}I]-2-iodo-D-phenylalanine showed the same pattern of clearance as its L-isomer. Significant uptake of radioactivity in the pancreas was observed but it was lower in

comparison with the L-analogue. No significant accumulation of radioactivity was observed in other abdominal organs such as liver, lungs, small intestine and large intestine, or in the brain.

Table 7. Biodistribution by dissection of [^{123}I]-iodo-D-phenylalanine in R1M inoculated athymic mice ($n=3$), expressed as %ID/g

Time (min)	%ID/g $\pm$ SD			
	2 min	30 min	60 min	120 min
Blood	8.59 $\pm$ 1.30	3.73 $\pm$ 1.47	1.90 $\pm$ 0.30	1.24 $\pm$ 0.44
Brain	1.96 $\pm$ 0.40	1.09 $\pm$ 0.42	0.74 $\pm$ 0.11	0.42 $\pm$ 0.08
Heart	6.72 $\pm$ 0.53	3.06 $\pm$ 1.24	1.41 $\pm$ 0.11	0.85 $\pm$ 0.29
Lung	5.97 $\pm$ 0.75	2.75 $\pm$ 1.21	1.32 $\pm$ 0.15	0.82 $\pm$ 0.24
Stomach	4.14 $\pm$ 1.72	5.96 $\pm$ 1.79	3.25 $\pm$ 0.51	3.38 $\pm$ 1.36
Spleen	3.17 $\pm$ 1.36	2.91 $\pm$ 0.72	1.42 $\pm$ 0.37	1.10 $\pm$ 0.39
Liver	4.32 $\pm$ 1.80	2.65 $\pm$ 0.59	1.78 $\pm$ 0.28	1.31 $\pm$ 0.78
Kidneys	9.43 $\pm$ 2.30	3.52 $\pm$ 0.30	2.19 $\pm$ 0.15	1.43 $\pm$ 0.32
Small intestines	3.06 $\pm$ 0.37	2.92 $\pm$ 0.43	2.10 $\pm$ 0.10	2.41 $\pm$ 0.57
Large intestines	3.89 $\pm$ 0.99	1.21 $\pm$ 0.32	0.87 $\pm$ 0.10	2.00 $\pm$ 0.60
Fatty tissue	1.40 $\pm$ 0.30	1.14 $\pm$ 0.28	0.34 $\pm$ 0.04	0.36 $\pm$ 0.09
Contr. bckgr.	6.75 $\pm$ 0.37	3.08 $\pm$ 0.24	1.18 $\pm$ 0.10	0.78 $\pm$ 0.28
Pancreas	19.75 $\pm$ 9.01	11.75 $\pm$ 6.40	12.11 $\pm$ 3.14	7.47 $\pm$ 3.26
Tumour	12.18 $\pm$ 0.31	11.01 $\pm$ 0.25	4.02 $\pm$ 0.26	2.52 $\pm$ 0.44
RTB	1.80 $\pm$ 0.46	3.57 $\pm$ 0.28	3.41 $\pm$ 0.32	3.21 $\pm$ 0.48

Contr. bckgr: contralateral background, RTB tumour to contralateral background ratio

Discussion

Our research group developed both [^{123}I]-2-iodo-*L*-phenylalanine and [^{123}I]-2-iodo-*D*-phenylalanine for SPECT imaging. Tracer uptake of both amino acid analogues was characterised and evaluated in vitro as well as in vivo in an R1M rhabdomyosarcoma athymic mouse model, respectively [9, 12]. Both tracers showed several major advantages over the routinely used tumour tracers ^{18}F -FDG and [^{123}I]-3-iodo- α -methyltyrosine: a much high tumour specificity, minor accumulation in inflammatory regions and no accumulation in the kidneys and thus the possibility of imaging peripheral cancers besides brain tumours. Moreover, owing to the unnatural character of the *D*-isomer, this tracer might have future potential as a systemic radiotherapy tool. These promising characteristics led to the evaluation of both tracers in tumours showing a high prevalence in human. This paper evaluated whether the observed characteristics could be generalised and thus whether both tracers could be used as general tumour-detecting agents in conjunction with DPI.

The in vitro results of all cell lines tested strongly suggested the presence of LAT1: the [^{125}I]-2-iodo-*L*-phenylalanine uptake was Na^+ independent. Moreover, the tracer uptake could be blocked by >70% by the LAT-specific inhibitor BCH, by “cold” 2-iodo-*L*-phenylalanine and by the natural amino acid *L*-phenylalanine whereas the A/ASC transport system-specific inhibitor MeAIB blocked only a minor proportion of the tracer uptake (<20%) (Table 1). The latter hypothesis was confirmed in vivo by the displacement study: significant displacement of the radioactivity in the tumour could be detected for all tumour types (all $p < 0.05$; Table 3). This shows that the transporter system involved is of the “obligatory exchanger” type such as LAT1 and that the tumour uptake is specific.

Concerning the biodistribution results obtained by DPI and dissection, both amino acid analogues showed the same distribution pattern as in the R1M rhabdomyosarcoma athymic mouse model. [^{125}I]-2-iodo-*L*-phenylalanine and [^{125}I]-2-iodo-*D*-phenylalanine both accumulated quickly to reach high amounts in all tumours. However, the dissection results showed that the EF43fgf4 and the HT29 tumours showed a lower RTB as compared with the other cell lines. This observation was confirmed by the in vitro study: although the uptake was specific, tracer uptake was lower. These results could indicate that EF43fgf4 and HT29 overexpressed the LAT1 transporter in lower concentrations as compared with the other cell lines, which illustrated similar tracer uptake values.

Because few membrane transporter proteins have been identified, investigators have to rely on kinetic and competitive inhibition experiments to determine and characterise individual transporter systems. As a result, displacement studies using *L*-phenylalanine were performed in vivo by analogy with the in vitro experiments. These studies revealed that the tracer uptake in vivo was specific. The latter observations, together with the fast renal clearance of [^{125}I]-2-iodo-*L*-phenylalanine and [^{125}I]-2-iodo-*D*-phenylalanine from the blood and their non-

significant uptake in the abdominal organs and brain in all tumour models, make both tracers suitable as powerful and general tumour-detecting agents.

Just like [^{123}I]-2-iodo-*L*-tyrosine, [^{123}I]-4-iodo-*L*-phenylalanine and [^{123}I]-3-iodo- α -methyltyrosine uptake in rodents [3, 17–19], [^{125}I]-2-iodo-*L*-phenylalanine showed high uptake in the pancreas. This is typical for labelled amino acid analogues in rodents. This feature might be due to the high protein turnover in the pancreas. In patients, no significant pancreatic uptake was observed for 2-iodo-*L*-tyrosine or [^{123}I]-3-iodo- α -methyltyrosine [16, 20].

By analogy with [^{123}I]-3-iodo- α -methyltyrosine, two-compartment modelling was applied in order to form an idea of the pharmacokinetics of both tracers in different tumour-bearing athymic mouse models. The two tracers followed almost the same trend in the different tumours as in the R1M model. The modelling revealed that in comparison to its *L*-analogue, [^{123}I]-2-iodo-*D*-phenylalanine was cleared more rapidly from the blood in all tumour models, with the exception of A549 and Capan2. This faster blood clearance will lead to lower tumour to blood ratios and less radiation burden for the animal. These results were confirmed by DPI: for the same tumour models the *D*-isomer reached higher tracer accumulation in the bladder at the same time point. Additionally, the faster uptake of the *D*-isomer in the peripheral compartment but its equal elimination velocity from this compartment in comparison to [^{123}I]-2-iodo-*L*-phenylalanine in all tumour models, except HT29, Capan2, C6 and A2058, should result in better oncological imaging characteristics.

Although two-factor ANOVA pointed out that both tracers did not reveal significant differences in tumour uptake, [^{123}I]-2-iodo-*D*-phenylalanine demonstrated better oncological imaging characteristics concerning the radiation burden as mentioned above.

The EF43fgf4 tumour on the one hand showed high [$^{123/125}\text{I}$]-2-iodo-*L*-phenylalanine and [$^{123/125}\text{I}$]-2-iodo-*D*-phenylalanine tumour uptake but on the other hand was the only tumour type with a high RTB for [^{123}I]-HSA. This could imply that other tracers should be used to detect this tumour type. However, the displacement study showed that the tracer uptake in the tumour was specific and the RT-PCR results illustrated a functional LAT1 transporter. Both findings could lead to an additional effect: tumours with high vascularity could be better visualised because of the specific tracer uptake and the high tracer availability due to the vascularisation.

Besides the [^{123}I]-2-iodo-phenylalanine isomers, other radiolabelled amino acids have been developed and evaluated for diagnostic use in oncology. [^{123}I]-4-iodo-*L*-phenylalanine was developed but owing to its high blood pool activity and high uptake in inflammatory tissue, this compound remains inferior to the isomers described in this report [19]. Other radiolabelled phenylalanine analogues for SPECT tumour imaging include [^{123}I]-4-amino-3-iodo-phenylalanine and [^{123}I]-*L*-iodo-1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid but too few data are available to compare their tumour diagnostic value with that of existing tracers. Concerning the tyrosine analogues,

[^{123}I]-2-iodo-L-tyrosine (^{123}I -2IT) has gained interest over the past few years. This tracer could overcome the limitations of [^{123}I]-L-3-iodo- α -methyltyrosine (rapid renal elimination and accumulation in the urinary tract) and ^{18}F -FDG (high and fast tumour uptake and rather low uptake in grey matter and inflammatory lesions). However, recent clinical evaluation of ^{123}I -2IT resulted in one false positive result, and Kersemans et al. reported better imaging characteristics for [^{123}I]-2-iodo-L-phenylalanine [9, 20].

Conclusion

[^{123}I]-2-iodo-L-phenylalanine and its D-isomer are promising tumour diagnostic agents for dynamic planar imaging. Both showed high uptake in those tumours with a high prevalence in humans. Moreover, 2-iodo-D-phenylalanine showed better tracer characteristics concerning radiation dose to other organs.

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