Comparison of [18F]FHPG and [124/125I]FIAU for imaging herpes simplex virus type 1 thymidine kinase gene expression

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Abstract. Various radiotracers based on uracil nucleosides (e.g. [124I]2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil, [124I]FIAU) and acycloguanosine derivatives (e.g. [18F]9-[(3-fluoro-1-hydroxy-2-propoxy) methyl] guanine, [18F]FHPG) have been proposed for the non-invasive imaging of herpes simplex virus type 1 thymidine kinase (*HSV1-tk*) reporter gene expression. However, these radiotracers have been evaluated in different in vitro and in vivo models, precluding a direct comparison. Therefore, we directly compared [18F]FHPG and radioiodinated FIAU to assess their potential for PET imaging of transgene expression. The uptake of [125I]FIAU, [18F]FHPG and [3H]acyclovir was determined in vitro using four different *HSV1-tk* expressing cell lines and their respective negative controls. The in vitro tracer uptake was generally low in non-transduced parental cell lines. In *HSV1-tk* expressing cells, [3H]acyclovir showed approximately a twofold higher tracer accumulation, the [18F]FHPG uptake increased by about sixfold and the [125I]FIAU accumulation increased by about 28-fold after 120-min incubation of T1115 human glioblastoma cells. Similar results were found in the other cell lines. In addition, biodistribution and positron emission tomography (PET) studies with [18F]FHPG and [124/125I]FIAU were carried out in tumour-bearing BALB/c mice. Significantly higher specific accumulation of radioactivity was found for [125I]FIAU compared with [18F]FHPG. The ratio of specific tracer accumulation between [125I]FIAU and [18F]FHPG increased from 21 (30 min p.i.) to 119 (4 h p.i.). PET imaging, using [124I]FIAU, clearly visualised and delineated *HSV1-tk* expressing tumours, whereas only a negligible uptake of [18F]FHPG was observed. This study demonstrated that in vitro and in vivo, the radioiodinated uracil nucleoside FIAU has a significantly higher specific accumulation than the acycloguanosine derivative [18F]FHPG. This suggests that [124I]FIAU should be the preferred reporter probe for PET imaging of *HSV1-tk* gene expression. Thus, further attempts to develop suitable PET tracers for the assessment of *HSV1-tk* gene expression should also focus on 18F-labelled uracil derivatives.

Keywords: Gene therapy – FHPG – FIAU – HSV-1 – Thymidine kinase – Positron emission tomography

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Introduction

Gene therapy offers promising new treatment modalities in oncology and other diseases [1]. The suicide gene, herpes simplex virus type 1 thymidine kinase (*HSV1-tk*), has already been successfully employed in a variety of tumour models, both in vitro and in vivo [2, 3]. A prodrug with low toxicity (e.g. ganciclovir) becomes toxic

only in cells where the *HSV1-tk* gene is expressed, thus resulting in the selective killing of transfected cells. Gene therapy is moving from experimental settings to clinical applications. Phase I/II clinical trials for brain, breast, colon and other tumours are now being performed [4].

To apply gene therapy successfully in vivo to humans, it is important to develop methods that determine the location, the level and the duration of gene expression over time. Common reporter genes (e.g. β-galactosidase) allow monitoring of transgene expression only ex vivo. However, scintigraphic imaging methods can be used to determine non-invasively reporter gene expression in vivo [5]. One such approach uses the *HSV1-tk* gene as the reporter gene [6]. HSV1-TK is less substrate specific than human thymidine kinases and phosphorylates not only thymidine but a wide range of nucleoside analogues. Phosphorylation of these substrates results in negatively charged metabolites remaining trapped inside the transduced cells. Thus a specific signal, derived from the trapped radiolabelled reporter probe, permits assessment of transgene expression.

The principle of in vivo imaging of herpes simplex virus thymidine kinase expression was first demonstrated visualising herpes encephalitis with [^{14}C]2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{14}C]FMAU) [7]. Later this strategy was successfully used for the assessment of gene transfer and expression. In 1995, Tjuvajev et al. employed the radiolabelled uracil nucleoside derivative [$^{131/124}$I]2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil ([$^{131/124}$I]FIAU) as the reporter probe for *HSV1-tk* reporter gene expression and successfully imaged RG2TK brain tumours in vivo [8]. Subsequently, they demonstrated non-invasive imaging of *HSV1-tk* gene transfer and expression in animal models with clinical gamma camera systems and positron emission tomography (PET) [9, 10]. Radiolabelled FIAU was also used to successfully visualise adenoviral gene transfer and expression of *HSV1-tk* in vivo [11]. Meanwhile various tracers, based on acycloguanosine derivatives, have been developed, including [^{18}F]9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG), [^{18}F]9-(4-fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG), [^{18}F]8-fluoroacyclovir ([^{18}F]FACV), [^{18}F]8-fluoroganciclovir ([^{18}F]FGCV) and [^{18}F]8-fluoropenciclovir ([^{18}F]FPCV) [12, 13, 14, 15]. However, these tracers were evaluated using different in vitro and in vivo models, precluding direct comparison.

Therefore, we studied radiolabelled FHPG and FIAU in vitro and in vivo, to compare a uracil nucleoside and an acycloguanosine derivative in the same experimental setting regarding their potential for PET imaging of transgene expression.

Materials and methods

Synthesis of [^{18}F]FHPG. The precursor was synthesised by a modified procedure [12]. The sodium salt of 9-[(1, 3-dihydroxy-2-propoxy)methyl]guanine was neutralised with 0.1 *N* hydrochloric acid. The free nucleobase 9-[(1, 3-dihydroxy-2-propoxy)methyl] guanine was recrystallised from water and combined with *p*-anisylchlorodiphenyl-methane to yield *N*-(*p*-anisyldiphenylmethyl)-9-[1-(*p*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl] guanine (35%). Tosylation produced the *N*^{2}-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-(p-toluolsulfonyl-oxy)-2 propoxy]methyl]guanine (75%), which was fluorinated with potassium fluoride and Kryptofix 2.2.2. in acetonitrile to yield *N*-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethyl)-3-fluoro-2-propoxy]methyl]-guanine. Reflux with acetic acid produced the reference compound, 9-[(1-hydroxy-3-fluoro-2-propoxy)methyl] guanine (85%). Characterisation of the product was carried out using elemental analysis and ^{1}H-NMR spectrometry. HPLC analyses demonstrated 95% purity.

[^{18}F]FHPG was synthesised as reported previously [12]. Briefly, an aqueous solution of 5.7–9.8 GBq [^{18}F]fluoride was delivered to a solution of Kryptofix 2.2.2 and K_2CO_3 (15 mg Kryptofix and 2.77 mg K_2CO_3 in 86% aqueous acetonitrile). The water was removed by repeated azeotropic evaporation, acetonitrile (400 µl) containing *N*-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-(p-toluolsulfonyl-oxy)-2-propoxy]methyl]guanine (2.5 mg) was added and the solution was heated for 20 min at 120°–130°C. The intermediate was treated with 1 *N* acetic acid (180 µl) at 120°C for 3 min. After neutralisation with 1 *N* sodium hydroxide solution (80 µl), the reaction mixture was passed through a silica Sep-Pak cartridge (Merck, Silicagel). Purification was achieved using preparative HPLC on an RP18 column (RP18, Lichrosphere, 5% acetonitrile in water, flow rate 5 ml/min). The resulting solution was passed through a polystyrene cartridge (Merck, LiChrolut EN). The product was eluted with ethanol (2 ml) and characterised by co-injection of the unlabelled reference compound (RP18, Lichrosphere, 5% acetonitrile in water, t_R=17.8 min, flow rate 0.5 ml/min). Ethanol was removed under reduced pressure and the residue was dissolved in phosphate-buffered saline (PBS) pH 7.4. The overall radiochemical yield was generally 9% based on [^{18}F]F$^-$ resolubilised in acetonitrile. The radiochemical purity was ≥99% with a specific activity of 14.8 TBq/mmol.

Synthesis of radioiodinated FIAU. 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FAU) was labelled following a procedure described by Vaidyanathan and Zalutsky [16]. Briefly, 5-trimethylstannyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) uracil (FTAU, 50 µg) was dissolved in chloroform (200 µl). Then 10 µl acetic acid/hydrogen peroxide (3:1) and 50–150 MBq no-carrier-added (n.c.a.) [^{125}I]sodium iodide (specific activity >0.6 TBq/mg iodide, Amersham Pharmacia Biotech, Buckinghamshire, UK) or 37 MBq [^{124}I]sodium iodide in 100 µl 0.004 *M* sodium hydroxide solution (Radiopharmacy, University Hospital Tübingen, Germany) was added. After 30 s ultrasound, the product was isolated using RP-HPLC (10%–50% MeCN/H_2O/0.1% trifluoroacetic acid, 30 min, 1 ml/min, column: YMC-J'sphere ODS-H80, 4.5× 150 mm, 4 µm). The solvent was evaporated off under reduced pressure, dissolved in water (1 ml), passed through an Alltech maxi-clean C-18 cartridge (Alltech Assoc. Inc. Deerfield, Ill., USA), washed with water (4 ml) and eluted with methanol (4 ml). Radiochemical purity, determined by HPLC, was generally >98%. The methanol was removed in vacuo and the residue dissolved

with PBS pH 7.4. The overall radiochemical yield, after RP-HPLC, was approximately 70%.

Cell lines and culture conditions. The murine fibrosarcoma cell line, CMS-5, and the *HSV1-tk* expressing CMS-STK cell line were obtained from the Institute of Experimental Oncology, Technische Universität München. Rat Morris hepatoma cells (MH3924A), containing the *HSV1-tk* gene (LXSNtk8) or an empty vector (LXSN), wild type human mammary carcinoma cells (MCF7) and their respective *HSV1-tk* expressing cell line (LNCtk11) and human glioblastoma cells (T1115) were obtained from the Deutsches Krebsforschungszentrum, Heidelberg. CMS cells were cultured in Dulbecco modified Eagle's minimum essential medium (DMEM) plus 10% fetal calf serum (FCS), MH3924A cells in Roswell Park Memorial Institute medium (RPMI) plus 20% FCS, MCF7 cells in RPMI plus 10% FCS and T1115 cells in Basal Medium Eagle (BME) medium completed with 200 m*M* L-glutamine, 10 m*M* hydroxyethyl-piperazine-ethansulfonic acid (HEPES) and 10% FCS. The cells were seeded, at a density of 10^3–10^4 cells/cm^2, in 24-well culture plates and incubated in humidified 5% CO_2/95% air at 37°C. The experiments were conducted after 3–4 days in culture, with cell densities of between 2 and 4 10^5 cells per well (equivalent to about 100–150 µg protein).

Vector construction and transfection of tumour cells. Transfection of the *HSV1-tk* gene and characterisation of the CMS-STK fibrosarcoma cell line, the MCF7 mammary adenocarcinoma cells and rat liver Morris Hepatoma (MH3924A) cells have been reported elsewhere [17, 18, 19]. The 1.13-kb *Eco*RI *Bam*HI fragment, containing the *HSV1-tk* gene, was excised from pGEX2T-tk-f+ (provided by G. Folkers, Department of Pharmacy, Eidgenössische Technische Hochschule Zürich) and cloned between the cytomegalovirus (CMV) immediate-early promoter and the SV40 splice and polyA site of the expression vector pUHD10–1, resulting in pUHD-tk [20]. For transfection, T1115 cells were seeded at a density of 1.5×10^4 cells per cm^2 and transfected with a mixture of 5 µg expression vector pUHD-tk, 5 µg selection plasmid pSV-2(neo) and 70 µl Lipofectin (Gibco), in a total volume of 10 ml serum-free medium (Optimem, Gibco). After 6-h incubation with intermittent shaking, the transfection mixture was removed and cells were grown for 36 h in complete culture medium. Cells were then replated, at a density of 5×10^3 cells per cm^2, and kept under selection pressure (200 µg/ml G418) for 10 days. A number of stable transfected clones were obtained. Clone T1115/pUHD-TK/pSV-2(neo)#2 was selected as the most sensitive cell line to ganciclovir for further experiments.

Cell culture uptake studies. All in vitro experiments were performed in triplicate and repeated at least once. For uptake studies, the culture medium was replaced by 0.5 ml (per well) of the incubation medium (culture medium containing 20 m*M* HEPES, pH 7.4, 37°C). The media contained 30–40 kBq/well [side-chain-2-^{3}H]acyclovir (specific activity 1.295 TBq/mmol; Sigma, St. Louis, USA), 0.5–0.7 MBq/well [^{18}F]FHPG and 30–50 kBq/well [^{125}I]FIAU. After 5-, 10-, 15-, 30-, 60- and 120-min incubation periods, the well plates were cooled to 4°C. The medium was removed and the cells rinsed four times with 1 ml of ice-cold phosphate-buffered solution (pH 7.4) containing Mg^{2+} (0.5 mmol/l) and Ca^{2+} (0.9 mmol/l). The cells were solubilised by shaking the plates for 30 min with modified 500 µl Lowry reagent. Aliquots were taken for the protein determination assay (Sigma, Deisenhofen, Germany) and the radioactivity measurements. For ^{3}H measurement, the vials contained 4 ml scintillation cocktail. The count vials were assayed for gamma radiation (^{18}F, ^{125}I), in a multichannel well-type sodium iodine gamma counter (COBRA II, Packard Instrument Company, Meriden, USA). The ^{3}H count vials were assayed in a multichannel well-type beta counter (TRICARB, Packard Instrument Company, Meriden, USA). Corrections for background activity and efficiency were performed based on calibrated standards and ^{3}H counts were quench corrected.

Biodistribution studies. Biodistribution studies were carried out using tumour-bearing BALB/c mice following the principles of laboratory animal care (NIH publication no. 86-23, revised 1985). Handling of animals was approved by the Committee of Veterinarian Medicine of the State of Bavaria. Tumours were generated by subcutaneous injection of a suspension (10^6 cells in 100 µl) of CMS-STK cells in the left flank and of non-transduced parental CMS-5 cells in the right flank. The animals were grouped in sets of five for each assay time point. A mixture of approximately 250 kBq [^{125}I]FIAU and 700 kBq [^{18}F]FHPG was injected into a tail vein. The mice were sacrificed and dissected at 30, 60, 120 and 240 min after tracer injection. *HSV1-tk* expressing and non-transduced parental tumours, blood, plasma, muscle, liver, kidney, heart, brain, lung, spleen, intestine and femur were sampled and weighed, and the radioactivity in each tissue was measured using a gamma counter (1480Wizard3, Wallac, Turku, Finland). Results were expressed as the percentage of the injected dose per gram of tissue. Each value represents the mean±SEM of five animals.

PET studies. In BALB/c mice bearing CMS-STK tumours at the left flank and non-transduced parental CMS5 tumours at the right flank, 3–4 MBq [^{124}I]FIAU or [^{18}F]FHPG was injected into a tail vein. Emission scans over 30 min were obtained 2 h after tracer injection using a whole-body PET scanner (ECAT EXACT, Siemens

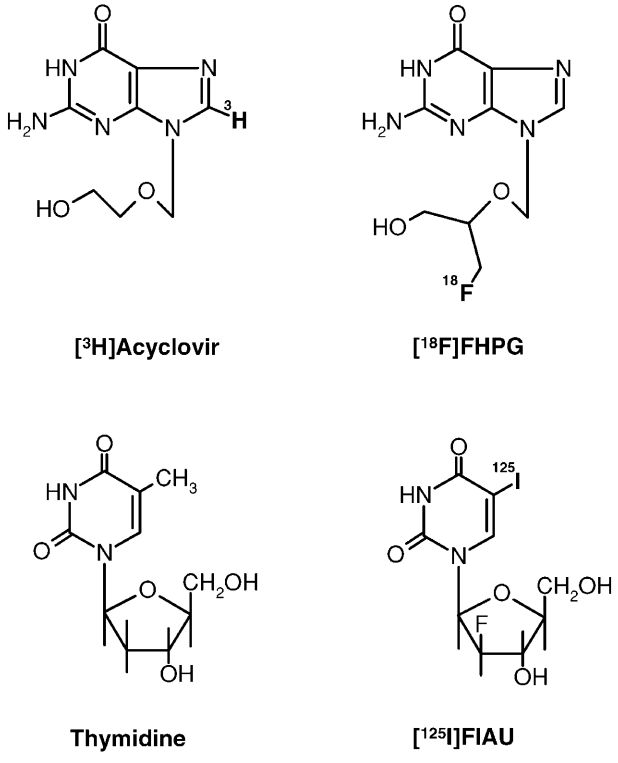

Fig. 1. Structure of [^{3}H]acyclovir, [^{18}F]FHPG, thymidine and [^{125}I]FIAU

CTI, Knoxville, Tenn., USA). This scanner provides an axial field of view of 15.8 cm, resulting in 47 transverse slices with a slice separation of 3.4 mm. Consecutive transmission scans were performed using germanium-68 rod sources. Emission data, corrected for random events, dead time and attenuation, were reconstructed using an iterative algorithm (OSEM with four subsets and eight iterations). The resulting in-plane image resolution of axial images was approximately 5–6 mm full-width at half-maximum (FWHM).

Results

Cell uptake studies

The uptake of [^{3}H]acyclovir, [^{18}F]FHPG, and [^{125}I]FIAU (for structures, see Fig. 1) was studied in four *HSV1-tk* expressing cell lines and their negative controls, namely the human glioblastoma T1115, the human mammary adenocarcinoma MCF7, the murine fibrosarcoma CMS-5 and the rat liver Morris Hepatoma cell line MH3924A. Generally low uptake values were observed for these tracers in non-transduced parental cells (Fig. 2). The highest uptake values were observed for [^{125}I]FIAU in all non-transduced parental cell lines, indicating a lower selectivity of the uracil derivative, resulting in higher unspecific uptake.

Only small differences in tracer uptake between *HSV1-tk* expressing and non-transduced parental cells were found for [^{3}H]acyclovir (Figs. 2, 3). As shown in Fig. 3, the cell uptake ratio in vitro (*HSV1-tk* expressing

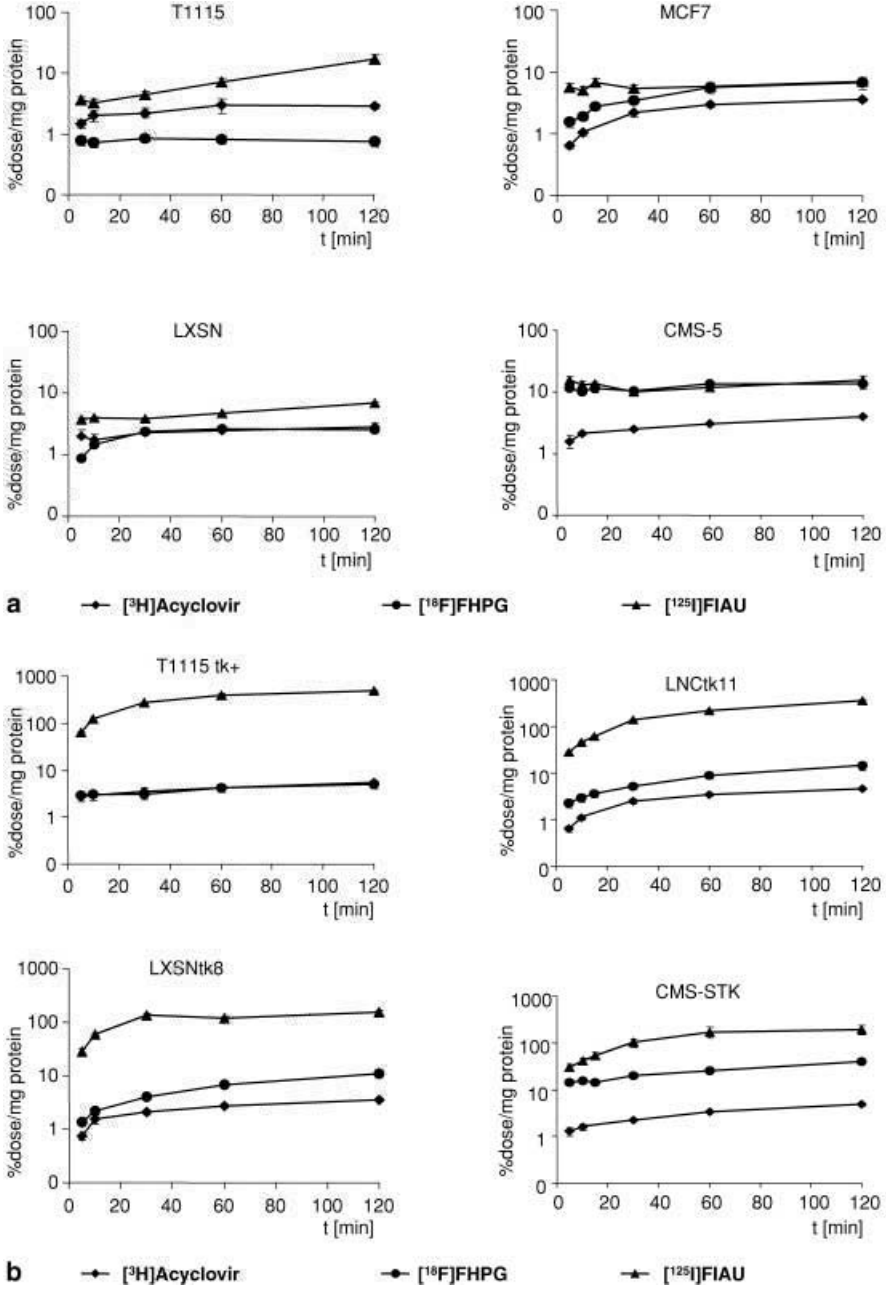

Fig. 2. Results of the in vitro uptake studies of four different parental non-transduced (**a**) and the respective *HSV1-tk* expressing cell lines (**b**). The cell lines were incubated for up to 120 min with [^{3}H]acyclovir, [^{18}F]FHPG and [^{125}I]FIAU and the uptake expressed as % dose/mg protein (mean±SEM). There was a statistically significant difference between the uptake of [^{18}F]FHPG and that of [^{125}I]FIAU in all cell lines (P<0.01). For some data points, error bars are not visible because SEM was smaller than the size of the symbol

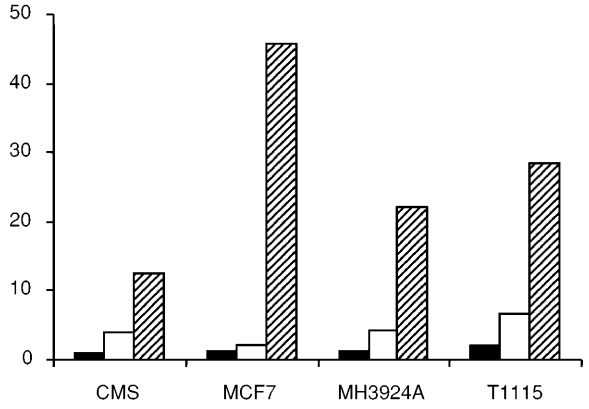

Fig. 3. Cell uptake ratios in vitro (*HSV1-tk* expressing vs non-transduced parental cells) after 120-min incubation for [^{3}H]acyclovir (*closed bar*), [^{18}F]FHPG (*open bar*) and [^{125}I]FIAU (*striped bar*). There was a statistically significant difference between the uptake of [^{18}F]FHPG and that of [^{125}I]FIAU in all cell lines (P<0.001)

Table 1. Biodistribution data of [^{125}I]FIAU in tumour-bearing BALB/c mice at 30, 60, 120 and 240 min p.i.

	Time post injection			
	30 min	60 min	120 min	240 min
Blood	4.99±0.11	3.54±0.31	2.19±0.41	0.43±0.13
Serum	5.92±0.14	4.17±0.37	2.50±0.42	0.46±0.12
Brain	0.46±0.04	0.35±0.04	0.22±0.04	0.04±0.01
Heart	4.43±0.14	2.75±0.31	1.33±0.23	0.19±0.05
Lung	3.95±0.14	2.55±0.33	1.62±0.29	0.30±0.08
Liver	3.90±0.15	2.34±0.22	1.24±0.23	0.18±0.04
Spleen	2.66±0.08	2.15±0.26	1.97±0.41	1.70±0.75
Kidney	16.5±0.6	9.5±1.04	3.02±0.57	0.51±0.11
Intestine	3.65±1.17	3.70±0.41	2.57±0.61	1.04±0.36
Muscle	2.97±0.09	1.79±0.30	0.98±0.22	0.50±0.33
Femur	2.05±0.35	1.61±0.20	1.29±0.27	0.49±0.15
CMS-5	2.44±0.25	2.31±0.32	1.85±0.22	0.69±0.18
CMS-STK	8.56±1.1	12.7±2.4	12.7±3.8	10.5±3.9

Values are given as % ID/g and represent the means±SEM (n=5)

vs non-transduced parental cells) after 120-min incubation ranged between 1.2 (CMS-5) and 2.1 (T1115). Using [^{18}F]FHPG, higher uptake values were observed in all *HSV1-tk* expressing cell lines compared with non-transduced parental cells (Figs. 2, 3); the cell uptake ratio ranged between 2.1 (MCF7) and 6.6 (T1115) (Fig. 3). However, significantly higher uptake values were found for [^{125}I]FIAU in *HSV1-tk* expressing cells, with cell uptake ratios ranging between 12.4 (CMS-5) and 45.9 (MCF7). The uptake of [^{125}I]FIAU in *HSV1-tk* expressing cells was between 5- and 100-fold higher than that of [^{18}F]FHPG and between 40- and 100-fold higher than that of [^{3}H]acyclovir. Data were tested for significance using the two-tailed t test with Bonferroni correction for multiple comparisons. Statistically significant differences were found between the uptake of [^{18}F]FHPG and [^{125}I]FIAU in all *HSV1-tk* expressing cell lines (P<0.01) as well as the cell uptake ratios (Fig. 3) in vitro (P<0.001).

Table 2. Biodistribution data of [^{18}F]FHPG in tumour-bearing BALB/c mice at 30, 60, 120 and 240 min p.i.

	Time post injection			
	30 min	60 min	120 min[a]	240 min[a]
Blood	0.98±0.07	1.01±0.16	0.05±0.01	0.01±0.002
Serum	0.94±0.07	0.94±0.14	0.16±0.06	0.01±0.001
Brain	0.10±0.03	0.14±0.01	0.02±0.004	0.004±0.001
Heart	0.97±0.07	0.92±0.14	0.04±0.01	0.02±0.01
Lung	1.06±0.06	1.05±0.18	0.05±0.01	0.02±0.003
Liver	1.30±0.10	1.32±0.18	0.07±0.01	0.01±0.001
Spleen	1.02±0.08	1.01±0.15	0.05±0.01	0.01±0.001
Kidney	3.29±0.20	3.12±0.75	0.11±0.02	0.02±0.001
Intestine	3.70±1.00	12.9±2.4	2.10±1.17	1.10±0.73
Muscle	1.38±0.07	2.18±0.54	0.15±0.03	0.01±0.003
Femur	0.83±0.08	1.36±0.24	0.57±0.46	0.10±0.01
CMS-5	1.02±0.09	1.73±0.26	0.33±0.18	0.07±0.02
CMS-STK	1.29±0.11	2.23±0.18	0.40±0.26	0.15±0.03

Values are given as %ID/g and represent the means±SEM (n=5)
[a] n=4

Biodistribution studies

The biodistribution data for [^{125}I]FIAU and [^{18}F]FHPG are summarised in Tables 1 and 2. The initial tracer uptake (30 min p.i.) in *HSV1-tk* expressing tumours was 8.6%±1.1%ID/g for [^{125}I]FIAU and 1.3%±0.1%ID/g for [^{18}F]FHPG. There was a statistically significant difference between the accumulation of [^{125}I]FIAU and [^{18}F]FHPG (P<0.05). The ratio between *HSV1-tk*-positive and non-transduced parental tumours was then 3.5 for [^{125}I]FIAU and 1.3 for [^{18}F]FHPG, increasing to 15.2 and 2.1 respectively 240 min after tracer injection. [^{125}I]FIAU accumulation in *HSV1-tk* expressing tumours reached a maximum at 1 h p.i (12.7%±2.4%ID/g) and remained close to this level over the entire observation period (10.5%±3.9%ID/g at 4 h p.i.). The highest uptake values for [^{18}F]FHPG were also found 1 h p.i (2.2%±0.2%ID/g). However, in contrast to FIAU, the tracer accumulation rapidly decreased to 0.2%± 0.03% ID/g 4 h p.i. The specific tracer accumulation of *HSV1-tk* expressing tumours was low (Table 3), with the maximum at 1 h p.i. (0.5%±0.1%ID/g). The specific tracer accumulation of [^{125}I]FIAU was significantly higher, ranging between 6.1±1.3 and 10.8±3.7. The ratio of specific tracer accumulation between [^{125}I]FIAU and [^{18}F]FHPG increased from 21 at 30 min p.i. (P<0.01) to 119 at 4 h

Table 3. Specific accumulation of radioactivity for [18F]FHPG and [125I]FIAU in vivo (*HSV1-tk* expressing fibrosarcoma tumours compared with non-transduced tumours)[a]

Time p.i.	FHPG	FIAU	FIAU/ FHPG	*P*
30 min	0.3±0.1	6.1±1.3	21	<0.01
60 min	0.5±0.1	10.4±2.3	21	<0.001
120 min	0.1±0.09[b]	10.8±3.7	103	<0.05
240 min	0.08±0.02[b]	9.8±3.7	119	<0.05

Values are given as %ID/g and represent the means±SEM (n=5)
[a] Specific accumulation of radioactivity in *HSV1-tk* expressing tumours is obtained by subtracting the non-specific accumulation of radioactivity in non-transduced tumours from the total accumulation of radioactivity found in *HSV1-tk* expressing tumours
[b] n=4

p.i. (P<0.05). We observed rapid elimination from the body for both tracers. However, at 30 min p.i., [18F]FHPG derived radioactivity in the blood was fivefold lower than that of [125I]FIAU and at 240 min p.i. approximately 40-fold lower. [125I]FIAU showed an exponential elimination with a half-life of approximately 1 h. In contrast, a plateau phase was observed for [18F]FHPG in the blood between 30 min and 60 min p.i., followed by an exponential elimination ($t_{1/2}$~30 min).

PET studies

PET imaging of tumour-bearing BALB/c mice 2 h after tracer injection demonstrated a low uptake of [18F]FHPG in CMS-STK tumours but a clearly visible accumulation of [124I]FIAU (Fig. 4). There was no specific uptake visible in the non-transduced parental tumours. However, significant radioactivity accumulation was observed in the unblocked thyroid gland.

Discussion

Efforts to develop radiolabelled reporter probes for the non-invasive imaging and quantification of herpes viral thymidine kinase (*HSV1-tk*) gene expression have focussed on uracil nucleoside (e.g. FIAU) and acycloguanosine derivatives (e.g. FHPG). For overview see [5, 21]. In this study we compared [18F]FHPG and radioiodinated FIAU for PET imaging of *HSV1-tk* gene expression and found a significantly higher specific accumulation of radiolabelled FIAU in vitro and in vivo. The uptake of [125I]FIAU by *HSV1-tk* expressing cells in vitro was between 5- and 100-fold higher than that of [18F]FHPG. More importantly, in vivo the specific tracer accumulation of [125I]FIAU in *HSV1-tk* expressing tumours was between 21- and 119-fold higher than that of [18F]FHPG.

Using four different cell lines, namely the human glioblastoma T1115, the human mammary adenocarcinoma MCF7, the murine fibrosarcoma CMS-5 and the rat liver Morris Hepatoma cell line MH3924A, we found [18F]FHPG in vitro uptake ratios (*HSV1-tk* expressing vs non-transduced parental cells) of between 2.1 and 6.6 (Fig. 3) after 2-h incubation. Our in vitro data regarding [18F]FHPG are comparable to previously reported results. Alauddin et al. studied the uptake of [18F]FHPG in HT-29 human colon cancer cells at various time points and found a fourfold (at 1 h), eightfold (at 3 h) and 12-fold (at 5 h) higher accumulation in *HSV1-tk* expressing cells compared with negative controls [22]. Other groups have reported large differences in [18F]FHPG accumulation in *HSV1-tk* expressing cells in vitro. Hospers et al., using C6 rat glioma cells, found after 2 h approximately a 35 times higher [18F]FHPG uptake in *HSV1-tk* expressing cells as compared with negative controls [23]. De Vries et al. compared the uracil nucleoside derivative [11C]-2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil

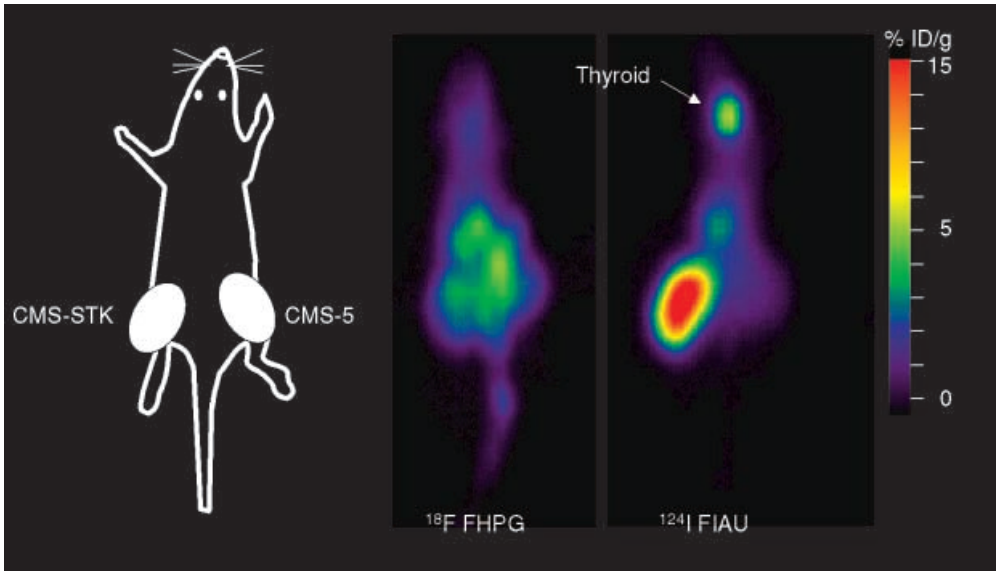

Fig. 4. PET imaging shows summed coronal slices of tumour-bearing BALB/c mice 2 h after tracer injection into a tail vein. There is low uptake of [18F]FHPG in CMS-STK tumours but clearly visible accumulation of [124I]FIAU. No specific uptake is visible in the non-transduced parental tumours but significant radioactivity accumulation is observed in the unblocked thyroid gland in the [124I]FIAU image

([^{11}C] FMAU) with [^{18}F]FHPG and reported a specific uptake of [^{11}C]FMAU in the *HSV1-tk* expressing PA-317 packaging as well as the C6 glioma cell line but [^{18}F]FHPG accumulation in *HSV1-tk* expressing C6 cells only [24]. Differences in [^{18}F]FHPG accumulation between various *HSV1-tk* expressing cell lines could be related to variations in specific transport systems as well as the level of *HSV1-tk* gene expression in the cell lines used in these studies. Cell type-specific differences in uptake rates of nucleosides have been reported for facilitated transport and non-mediated permeation [25]. Haberkorn et al. found that ganciclovir is transported into MH3924A and MCF7 cells predominantly via the nucleoside carrier [26]. However, inhibition and competition experiments demonstrated a relatively slow transport of ganciclovir across the cell membrane. Therefore, differences between [^{18}F]FHPG and [^{125}I]FIAU tracer accumulation may be at least partly due to differences in tracer transport through the cell membrane.

In our study, the uptake of [^{3}H]acyclovir, [^{18}F]FHPG and [^{125}I]FIAU in vitro was generally low in non-transduced parental cell lines (Fig. 2). However, the uptake of the uracil analogue [^{125}I]FIAU was slightly higher in all four non-transduced parental cell lines, indicating a lower selectivity of the uracil derivative. In contrast, we found significant differences in the accumulation of [^{3}H]acyclovir, [^{18}F]FHPG and [^{125}I]FIAU in *HSV1-tk* expressing cells. Using the T1115 human glioblastoma cells, [^{3}H]acyclovir showed approximately a twofold higher tracer accumulation in *HSV1-tk* expressing compared with non-transduced parental cells, whereas the [^{18}F] FHPG uptake increased approximately sixfold and the [^{125}I]FIAU accumulation 28-fold after 120-min incubation. Similar results were found for the other cell lines, demonstrating significantly higher phosphorylation of radiolabelled FIAU by the herpes viral thymidine kinase.

These in vitro findings were confirmed by our in vivo studies. We observed a significantly higher specific tracer accumulation of [^{125}I]FIAU compared with [^{18}F] FHPG in tumour-bearing BALB/c mice (Table 3). Four hours after tracer injection, [^{18}F]FHPG showed only a 2.1-fold higher accumulation in *HSV1-tk* expressing fibrosarcoma tumours (CMS-STK) compared with non-transduced tumours (CMS-5). This is in contrast to the findings of Hospers et al., who reported approximately 15-fold higher tracer accumulation in *HSV1-tk* expressing tumours in nude rats at 2 h post injection [23]. However, in vivo studies by Alauddin et al. offered comparable findings to ours [22]. Using tumour-bearing nude mice, they revealed a threefold higher accumulation of [^{18}F]FHPG in *HSV1-tk* expressing tumours 2 h after tracer injection, and the uptake ratio between transduced and non-transduced tumours had increased approximately sixfold 5 h after tracer injection [22].

The contrast between transduced and non-transduced tumours is an important factor when comparing different tracers for scintigraphic imaging of transgene expression. We found ratios of the specific tracer accumulation between [^{125}I]FIAU and [^{18}F]FHPG ranging from 21 (at 30 min p.i.) to 119 (at 4 h p.i.), demonstrating the advantage of radiolabelled FIAU. PET imaging, using [^{124}I]FIAU, clearly visualised and delineated *HSV1-tk* expressing tumours, whereas only a negligible uptake of [^{18}F]FHPG was observed (Fig. 4). The closer similarity of FIAU with the structure of thymidine may be the most likely explanation for the higher accumulation in *HSV1-tk* expressing cells (Fig. 1). Of note, there is also some phosphorylation by the mammalian thymidine kinase, resulting in a lower selectivity of uracil nucleosides. However, our PET and biodistribution studies revealed a significantly higher specific tracer accumulation of radioiodine-labelled FIAU compared with [^{18}F]FHPG.

Both tracers have shown a high stability in vivo without significant metabolites in the blood [13, 19]. Using [^{123}I]FIAU and gamma camera imaging some radioiodine uptake has been observed in the thyroid gland [19]. However in humans, the thyroid can be easily blocked with cold iodine or sodium perchlorate. In this study, we found no significant activity derived from [^{18}F]FHPG in the bone. Both tracers showed low activity concentrations in the brain, indicating low permeation through the blood-brain barrier. [^{18}F]FHPG and radiolabelled FIAU are eliminated predominantly via the renal excretion pathway. However, after 1 h, significant tracer amounts of [^{18}F]FHPG were found in the intestine, suggesting some hepatobiliary excretion (Table 2). In contrast to previous reports [9, 10], which suggested a wash-out period of unbound radiolabelled FIAU of at least 24 h, Haubner et al. have recently shown that visualisation of *HSV1-tk* gene expression is possible by conventional gamma camera imaging and [^{123}I]FIAU as early as 0.5 h after tracer injection [19].

Regarding the clinical application of radiotracers for PET imaging: there is a clear advantage of ^{18}F labelling owing to the availability and length of its physical half-life. Nucleophilic fluorination of the FHPG precursor and subsequent HPLC purification led to [^{18}F]FHPG of high radiochemical purity with a specific activity of approximately 14 TBq/mmol. Nevertheless, the radiochemical yield of this labelling method was poor (approximately 10%). In contrast, labelling of FAU using the radioiodination procedure recently introduced by Vaidyanathan and Zalutsky resulted in high radiochemical yields ranging between 70% and 80%, as well as high radiochemical purity and specific activities [16]. However, the use of [^{124}I]iodine has certain disadvantages, mainly due to the low positron yield (24%) and the limited availability. So far, there is no synthesis described for the ^{18}F labelling of FIAU and, therefore, the main advantage of using acycloguanosine derivatives is the availability of ^{18}F-labelled compounds. Gambhir et al. compared [^{14}C]ganciclovir and [^{18}F]ganciclovir in vivo following adenoviral directed transfer of the *HSV1-tk* reporter gene to the liver [14, 27]. After 1 h, the ratio be-

tween infected and control animals in the liver for the two compounds was 31 and 6, respectively, indicating a lower phosphorylation of [^{18}F]ganciclovir by the herpes viral thymidine kinase. Comparing various tracers in cell culture, [^{3}H]penciclovir showed a twofold higher accumulation than ganciclovir [15]. Subsequently, ^{18}F-labelled penciclovir derivatives ([^{18}F]FPCV and [^{18}F]FHBG) were developed [13, 28]. These showed a twofold higher accumulation than the corresponding ganciclovir derivatives [^{18}F]FGCV and [^{18}F]FHPG.

Recently, MacLaren et al. reviewed preliminary data comparing varying tracers in *HSV1-tk* expressing C6 cells and found FIAU and FHBG to be the better reporter probes [21]. However, detailed data concerning a direct comparison of radioiodinated FIAU and [^{18}F]FHBG have not yet been reported. Gambhir et al. recently described a mutant herpes simplex virus thymidine kinase enzyme that utilises acycloguanosine derivatives more effectively and thymidine less effectively than wild-type *HSV1-tk* [29]. This interesting approach resulted in an enhanced sensitivity (by a factor of ~2) for imaging reporter gene expression. However, it is important to note that receptor-based reporter gene imaging systems have been developed (e.g. D_2-receptor/3-(2′-[^{18}F]fluoroethyl) spiperone [30], and the somatostatin receptor subtype 2/[^{99m}Tc]-somatostatin-avid peptide [31]). MacLaren et al. estimated a comparable sensitivity of the mutant herpes simplex virus thymidine kinase enzyme and [^{18}F]FHBG. In addition, there have been great improvements in imaging gene expression in small animals that are transparent to visible light by expression of fluorescent proteins and luciferases [32]. By systemic application of an enzyme substrate to the animals (e.g. luciferin), gene expression can be quantitatively measured with sensitive optical camera systems. However, this method will be of limited value in human applications.

In conclusion, the radioiodine labelled uracil nucleoside, FIAU, exhibited a significantly higher specific accumulation in *HSV1-tk* expressing cells compared with the acycloguanosine derivative [^{18}F]FHPG, both in vivo and in vitro. Therefore, it is suggested that further attempts to develop suitable PET tracers for the assessment of *HSV1-tk* gene expression should also focus on ^{18}F-labelled uracil derivatives, since in clinical applications, non-invasive imaging of reporter gene expression with ^{18}F-labelled reporter probes would facilitate repetitive measurements.

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