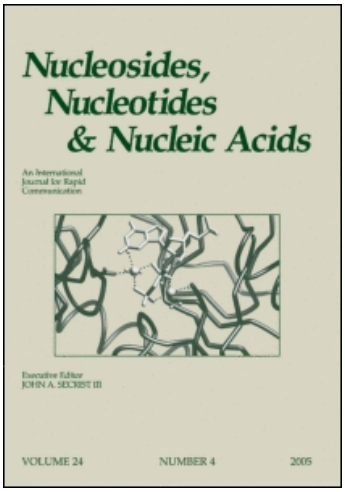

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In Vivo Evaluation of the Uptake of [123I]FIAU, [123I]IVFRU and [123I]IVFAU by Normal Mouse Brain: Potential For Noninvasive Assessment of HSV-1 Thymidine Kinase Gene Expression in Gliomas

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IN VIVO EVALUATION OF THE UPTAKE OF [^{123}I]FIAU, [^{123}I]IVFRU, AND [^{123}I]IVFAU BY NORMAL MOUSE BRAIN: POTENTIAL FOR NONINVASIVE ASSESSMENT OF HSV-1 THYMIDINE KINASE GENE EXPRESSION IN GLIOMAS

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□ *Radioiodinated 5-iodo-1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)uracil (F*IAU) is most commonly used for noninvasive assessment of herpes simplex virus type 1 thymidine kinase (HSV-1*-tk*) gene expression. However, it does not permeate the intact blood–brain barrier (BBB) because of its moderate lipophilicity. In this work, three iodo-nucleosides, FIAU, IVFRU, and IVFAU, were radiolabeled with iodine-123 and tested for permeation of the BBB in mice and for potential measurement of HSV-1*-tk *gene expression in gliomas. The results demonstrate that brain uptake and retention of these nucleosides is not directly related to their lipophilicity. The low brain uptake of IVFAU, in conjunction with its higher and constant brain/blood ratio, may reflect greater stability against hydrolysis of the* N*-glycosidic bond. In vivo PET evaluations of [^{124}I]IVFRU and [^{124}I]IVFAU in tumor-bearing mice are warranted.*

Keywords Blood–brain barrier (BBB); gene expression, brain permeability; herpes simplex virus type 1 thymidine kinase (HSV-1-TK)

Noninvasive imaging technologies to monitor gene expression in animals and humans are being investigated to provide useful information on the molecular state and alteration in transcriptional activity, as well as for therapy planning and efficiency readout. The widely used nuclear imaging

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techniques, single photon emission computed tomography (SPECT) and positron emission tomography (PET), provide the possibility to determine spatial distribution of exogenous enzymes such as the herpes simplex virus type 1 thymidine kinase (HSV-1-TK) by using radiolabeled enzyme-specific substrates at low, subpharmacological concentrations. The most widely used HSV-1-TK substrates are [I*]FIAU,[1,2] a nucleoside analogue for SPECT and PET, and the fluorine-18 labeled acycloguanosine derivative [^{18}F]-FHBG (9-(3-[^{18}F]-fluoro-3-hydroxy-methylbutyl)guanine).[3] Various other radiolabeled nucleoside substrates have been synthesized and investigated for imaging vector-mediated gene expression.[4–6]

Recently, the expression of nucleoside transporters, which belong to two gene families, has been investigated: equilibrative nucleoside transporters (Slc29 consists of four genes ENT1-4)[7] and concentrative nucleoside transporters (Slc28, consists of three members CNT1-3).[8] In those studies it has been shown that expression of the nitrobenzylthioinosine (NBMPR)-sensitive equilibrative nucleoside transporter in the BBB (ENT2), is greater than expression of ENT1 and CNT2 in neurons, and that expression of both ENT2 and ENT1 is greater in neurons than in astrocytes or C6 glioma cells.[9] Uridine crosses the rat BBB via the CNT2 proteins, the high-affinity transporters for purines like adenosine as well as for uridine, and is taken up by the rat brain more efficiently than cytidine under physiological conditions.[10] Since little was known about nucleoside transporters in brain, nucleoside design was oriented toward lipophilic species like the moderately lipophilic FIAU (log P −0.14[2]), which does not permeate the intact blood–brain barrier (BBB).[11]

To increase brain permeation, more lipophilic nucleoside analogues, for example, IVDU (5-(*E*)-(2-iodovinyl)-2′-deoxyuridine),[12,13] 3-MeIVDU (5-(*E*)-(2-iodovinyl)-N^3-methyl-1-(2-deoxy-β-D-ribofuranosyl)uracil),[14] IVFRU (5-(*E*)-(2-[^{125}I]iodovinyl)-2′-fluoro-2′-deoxyuridine),[15,16] and IVFAU (5-(*E*)-(2-[^{125}I]iodovinyl)-2′-fluoro-2′-deoxyarabino-uridine)[15] have been developed. The aim of the current work is to compare biodistribution and brain uptake of *IVFRU and *IVFAU with F*IAU (see structures in Figure 1) in mice, to assess their potential for imaging and measurement of HSV-1-*tk* gene expression in gliomas.

MATERIALS AND METHODS

FIAU was purchased from ABX Pharma, and its precursor Mandau [5-trimethylstannyl-1-(2-deoxy-2-fluoro-ß-D-arabinofuranosyl)uracil] was synthesized in our laboratory. IVFRU, IVFAU, and their (*E*)-5-(2-(trimethylsilyl) vinyl) precursors (TMSiVFRU and TMSiVFAU) were synthesized in the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Canada (see structures in Figure 2).[15] No-carrier-added (n.c.a.) sodium

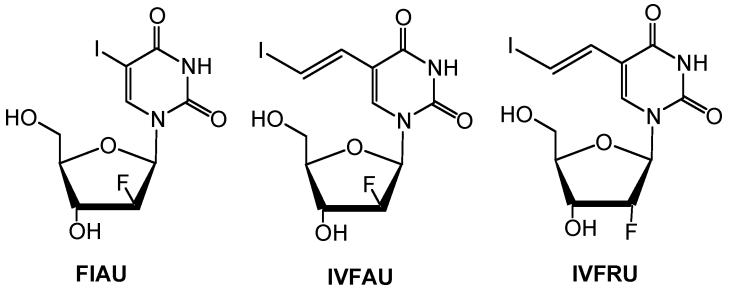
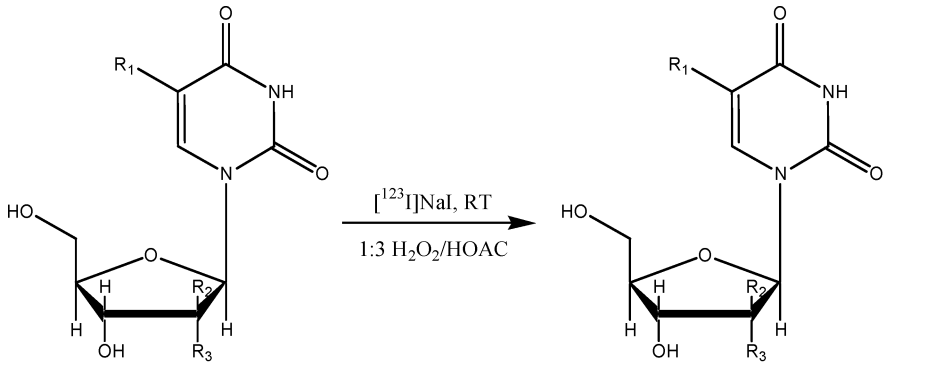


FIGURE 1 Chemical structures of nucleoside analogues.

[^{123}I]iodide (Na[^{123}I]I) was obtained from Amersham-Cygne, The Netherlands (specific activity: 3700 TBq/mg (100 Ci/mg)). High-performance liquid chromatography (HPLC) was conducted using the following system: Knauer solvent pump K1001 (Berlin, Germany) combined with an ultraviolet detector (λ, 254 nm) and a Geiger-Müller detector (Berthold, Wildbad, Germany).

Radiolabeling

All three tracers were labeled according to reported electrophilic iodination reaction procedures.[17] Briefly, [^{123}I]FIAU was prepared by electrophilic iodination–destannylation of Mandau (0.3 mg; 0.73 μmol), which was dissolved in ethanol/acetic acid (v/v; 50/50; 100 μL) and vortex mixed. Aqueous NaOH (100 μL; 0.1 N) was added to Na[^{123}I]I (6 μL; 74 MBq) and mixed with the Mandau solution. H_2O_2/acetic acid (v/v; 1:3; 100 μL) was added to the mixture in a 2.0-mL conical reaction vial (borosilicate with Teflon rubber septum) and then the reaction was



TMSnFAU: R_1= $SnMe_3$, R_2=F, R_3=H
TMSiVFRU: R_1= CH=CH$SiMe_3$, R_2=H, R_3=F
TMSiVFAU: R_1= CH=CH$SiMe_3$, R_2=F, R_3=H

FIAU: R_1=I, R_2=F, R_3=H
IVFRU: R_1= CH=CHI, R_2=H, R_3=F
IVFAU: R_1= CH=CHI, R_2=F, R_3=H

FIGURE 2 Radioiodination of nucleoside analogues.

sonicated (3 minutes) at room temperature in an ultrasonic bath (Sonorex Super RK 52 H, Bandelin). After adding aqueous NaOH (100 μL; 5 N) for neutralization, the mixture was purified by HPLC. The reaction solution was loaded on a preparative column (Adsorbosil RP C-18, 10 μm, 250 $\times$10-mm column) (Alltech, Unterhaching, Germany) with a 0.5-mL HPLC glass syringe and eluted using an isocratic solvent system of 10% ethanol in 50 mmol/L NaH_2PO_4; flow 5.0 mL/minutes). The [^{123}I]FIAU fraction was collected. For injection into mice, the [^{123}I]FIAU solution was diluted with sterile physiologic saline to reduce the ethanol concentration to 5% and passed through a 0.22-μm filter. The radiochemical purity was determined by analytical HPLC using an isocratic solvent of 10% methanol in 50 mM/L K_2HPO_4 at pH 4.3 (Econosil RP C18, 5-μm column [Alltech]; 250 $\times$ 4.6 mm; flow 1 mL/minutes). An iodination–desilylation method was used for IVFRU and IVFAU labeling. The respective trimethylsilyl- precursor (TMSiVFRU or TMSiVFAU; 500 μg, 1.53 μmol) was dissolved in 20% acetic acid in ethanol (10 μL), with workup as for FIAU labeling. The mobile phase for purification was NaH_2PO_4/EtOH = 70:30. Quality control of the n.c.a. products was performed on the same analytical column using the same eluent as for purification, with a flow 1.0 mL/minutes. The 30% ethanolic solutions of IVFRU and IVFAU were diluted to 10% ethanol with sterile saline, for murine biodistribution studies. The radiosynthesis of [^{123}I]I-nucleoside analogs is shown schematically in Figure 2. The specific activity of [^{123}I]IVFRU was measured by HPLC with UV detection compared to known concentrations of authentic standard compound.

Lipophilicity Measurement

The octanol/water partition coefficient (log P) was measured by mixing 0.04 MBq of [^{123}I]IVFRU or [^{123}I]IVFAU with 1-octanol (1 mL) and buffer (1 mL; pH 7.4, 0.05 M phosphate) in a plastic or a glass test tube. The test tube was vortex mixed for 2 minutes at room temperature and then centrifuged for 5 minutes. Aliquots (0.2 mL) from 1-octanol and buffer layers were transferred to a plastic or glass test tube and counted in an automatic well-type γ-counter (Berthold, MAG312 Model 43). Adherence of radioactivity to the walls of the test tubes was measured and also the efficiency of radiometric counting in octanol versus water was considered. The log P value was determined by calculating the logarithm of the ratio of counts per minute of 1-octanol to that of buffer.

Biodistribution in Mice

The experimental procedures used were in accordance with the guidelines on the use of living animals in scientific investigations and the German

law on the protection of animals, and followed the principles of laboratory animal care. Groups of 3–6 male nude mice (20–30 g) were injected with [^{123}I]FIAU or [^{123}I]IVFRU or [^{123}I]IVFAU (10–20 μL, 1.5–1.7 MBq) into the femoral vein under anesthesia (0.9% NaCl solution of ketanest and rompun), which was given 30 minutes before tracer injection. The mice were sacrificed by cardiac excision at 2, 15, 60, and 120 minutes postinjection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic well-type γ-counter. The percentage of the injected dose per gram (%ID/g) organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material.

RESULTS

All three nucleoside analogues were radiosynthesized in a short time at room temperature, with radiochemical yields ranging from 70 to 85%. These n.c.a. products, isolated using RP-HPLC, reached radiochemical purities of greater than 95%. The retention times for radioiodide [^{123}I$^-$], [^{123}I]FIAU, [^{123}I]IVFRU, and [^{123}I]IVFAU were 4.26, 9.1, 13, and 16.5 minutes, respectively. The three tracers were stable at room temperature, and the radiochemical purity of 95–97% did not change within 24 hours after elution from the HPLC column. The UV detection limit was 0.001 μg/mL for IVFRU and 0.008 μg/mL for IVFAU. The n.c.a. and the authentic iodinated samples exhibited identical HPLC retention times. The overall radiosynthesis required circa 45 minutes. Reproducibility was high, which would make these tracers suitable for routine clinical production.

The partition coefficients (log P) of these three nucleoside analogues were -0.13 (FIAU), 1.22 (IVFRU), and 1.24 (IVFAU), respectively. These data, which parallel the reverse-phase HPLC retention times indicated above, are similar to the published lipophilicity measurements of these compounds, where the log P values are -0.14,[2] 1.21, and 1.20, respectively.[17] Adherence of radioactivity to the walls of the test tubes during the measurements of the lipophilicity did not measurably influence the log P value and could therefore be neglected (<0.1%). Similarly, the differences in counting efficiency for the gamma energy of 159 keV in water and octanol lay within the experimental error (about 10%). The density of the octanol is 0.8 and its Z/A is 0.5; the Z/A for water is 0.44.

Radioactivity accumulations in organs are given in percentage of injected dose per gram (%ID/g). Biodistribution of FIAU, IVFRU, and IVFAU in mice are shown in Tables 1–3. Only the uptake in thyroid is calculated for the whole organ and shown as %ID/thyroid. The blood/brain ratios of these three tracers are given in Table 4.

TABLE 1 Biodistribution of [^{123}I]FIAU in mice ($n = 6$); % injected dose/g organ

Organ	2 minutes	15 minutes	60 minutes	120 minutes
Blood	5.24 ± 1.34	5.22 ± 0.85	5.32 ± 1.20	3.37 ± 1.94
Heart	3.88 ± 1.59	3.21 ± 1.03	3.03 ± 0.83	1.28 ± 0.25
Lungs	4.49 ± 2.04	4.59 ± 2.29	3.27 ± 0.68	1.57 ± 0.11
Liver	4.80 ± 2.21	4.66 ± 1.24	4.17 ± 0.66	1.88 ± 0.33
Kidneys	14.08 ± 8.26	10.34 ± 2.27	9.17 ± 0.72	2.90 ± 0.46
Spleen	2.40 ± 1.24	1.88 ± 1.57	2.82 ± 0.81	1.13 ± 0.10
Muscle	0.98 ± 0.60	3.12 ± 1.61	4.38 ± 3.18	1.05 ± 0.15
S. intestine	1.32 ± 0.90	3.11 ± 2.73	4.10 ± 2.64	3.74 ± 2.94
Stomach	2.18 ± 1.15	3.11 ± 3.32	2.71 ± 1.66	1.19 ± 0.34
Brain	0.17 ± 0.07	0.25 ± 0.07	0.28 ± 0.06	0.25 ± 0.09
Thyroid	0.14 ± 0.15	0.14 ± 0.09	0.18 ± 0.08	0.04 ± 0.03

DISCUSSION

The lipophilicity of FIAU is significantly lower than that of IVFRU and IVFAU, which reflects the influence of the substitution group at the C-5 position of the uracil ring. FIAU has iodine in position 5 and fluorine in the 2′-up (arabino) position. Comparing the more lipophilic tracers IVFRU and IVFAU with FIAU, the vinyl group at C-5 clearly adds to their higher lipophilicity. The unique structural difference between IVFRU and IVFAU is the fluorine in the C-2′-position. The fluorine substituent in the upper (arabino) orientation for IVFAU, and down (ribo) for IVFRU could potentially affect solubilities in two ways: through changes in the nucleobase-sugar (N-1–C-1′) torsion angle (χ), or sugar ring pucker (C-3′–C-4′ torsion; δ). Fluoride size approaches that of hydrogen, and it can participate in hydrogen bonding, and since F electronegativity is similar to that of hydroxyl, insertion of fluorine at C-2′ would not be expected to greatly influence solubility. In other arabino–ribo "pairs," like FIAU (log P −0.13) and FIRU (log P −0.26), but differences are observed experimentally even

TABLE 2 Biodistribution of [^{123}I]IVFRU in mice ($n = 6$); % injected dose/g organ

Organ	2 minutes	15 minutes	60 minutes	120 minutes
Blood	21.53 ± 1.16	12.67 ± 1.56	11.03 ± 0.42	8.70 ± 2.30
Heart	8.53 ± 3.30	5.36 ± 1.96	3.50 ± 0.25	2.72 ± 0.55
Lungs	8.08 ± 1.23	6.31 ± 1.31	5.21 ± 0.55	4.09 ± 0.82
Liver	6.75 ± 0.40	4.28 ± 0.62	3.61 ± 0.36	2.75 ± 0.54
Kidneys	7.68 ± 0.34	5.06 ± 2.92	3.85 ± 0.46	3.48 ± 0.20
Spleen	3.64 ± 0.20	3.06 ± 1.05	2.26 ± 0.24	1.82 ± 0.36
Muscle	1.49 ± 0.18	2.38 ± 0.90	1.92 ± 0.22	1.63 ± 0.37
S. intestine	4.35 ± 0.80	4.88 ± 1.59	2.93 ± 0.54	2.42 ± 0.66
Stomach	4.00 ± 1.13	2.85 ± 1.19	2.50 ± 0.29	2.74 ± 0.65
Brain	0.51 ± 0.05	0.52 ± 0.20	0.32 ± 0.02	0.25 ± 0.09
Thyroid	0.52 ± 0.30	0.57 ± 0.15	0.47 ± 0.03	0.56 ± 0.20

TABLE 3 Biodistribution of [^{123}I]IVFAU in mice ($n = 6$); % injected dose/g organ

Organ	2 minutes	15 minutes	60 minutes	120 minutes
Blood	4.60 ± 0.23	2.60 ± 0.24	2.37 ± 0.46	2.58 ± 0.73
Heart	6.20 ± 0.25	3.25 ± 0.29	1.97 ± 0.16	1.91 ± 0.33
Lungs	4.46 ± 0.10	2.98 ± 0.53	2.03 ± 0.19	2.27 ± 0.35
Liver	10.84 ± 0.38	5.97 ± 0.71	4.08 ± 0.58	4.01 ± 0.83
Kidneys	10.45 ± 1.17	4.83 ± 0.29	3.26 ± 0.40	3.38 ± 0.65
Spleen	3.60 ± 0.30	2.16 ± 0.23	1.47 ± 0.09	1.50 ± 0.40
muscle	1.45 ± 0.49	1.47 ± 0.66	1.27 ± 0.42	1.14 ± 0.11
S. intestine	8.90 ± 3.18	10.61 ± 3.34	7.14 ± 3.31	8.06 ± 0.05
Stomach	2.72 ± 0.81	2.07 ± 0.39	2.48 ± 0.17	0.27 ± 0.06
Brain	0.19 ± 0.02	0.17 ± 0.04	0.16 ± 0.03	0.17 ± 0.04
Thyroid	0.36 ± 0.30	0.32 ± 0.08	0.42 ± 0.03	0.50 ± 0.06

when simple additivity-based log P calculations [19] indicate that they have identical partition coefficients (−0.13).[16,20]

Biodistribution data in mice show significantly different features among the three tracers (Tables 1–3), especially the comparison IVFRU with FIAU and IVFAU. In spite of the big lipophilicity difference between FIAU and IVFAU, these two compounds exhibit similar in vivo behavior. The brain uptake of FIAU (0.17%ID/g at 2 minutes postinjection (p.i.)) increases slightly to 0.25%ID/g, which doesn't change at 15 minutes p.i. through 120 minutes p.i. IVFAU has a similar brain uptake (0.19%ID/g) at 2 minutes p.i., but it is steady at this level during the next 2 hours. However, IVFAU shows much more rapid blood clearance, ($T_{1/2}$ 15 minutes) compared with FIAU ($T_{1/2} > 2$ hours). Thus [^{123}I]IVFAU has a much higher brain/blood ratio than [^{123}I]FIAU (Table 4). The highest ratios are reached at 120 minutes for FIAU and 15 minutes for IVFAU; the IVFAU ratio remains stable for the full 120 minutes p.i. injection period. In heart, spleen, and stomach, [^{123}I]FIAU and [^{123}I]IVFAU behave similarly, except for the higher uptake of IVFAU at 2 minutes after injection (Tables 1, 3). [^{123}I]IVFAU exhibits a much higher uptake in the liver (11%ID/g at 2 minutes p.i.) and slower washout (4%ID/g at 120 minutes) as compared to [^{123}I]FIAU (4.8%ID/g at 2 minutes and 1.9%/ID/g at 120 minutes, respectively), which may reflect

TABLE 4 Brain uptake and brain/blood ratio of [^{123}I]I-labeled FIAU, IVFRU, and IVFAU

Time	Brain uptake (%ID/g)			Brain/blood ratio (×10)		
(minutes)	FIAU	IVFRU	IVFAU	FIAU	IVFRU	IVFAU
2	0.17	0.51	0.19	0.03	0.24	0.40
15	0.25	0.52	0.17	0.02	0.40	0.65
60	0.28	0.32	0.16	0.05	0.29	0.68
120	0.25	0.25	0.17	0.07	0.29	0.66

the higher lipophilicity of [^{123}I]IVFAU. However, more [^{123}I]IVFAU is found in small intestine. In kidney, [^{123}I]FIAU shows not only a higher initial uptake than [^{123}I]IVFAU (14 and 10%ID/g at 2 minutes p.i., respectively) but also a slower clearance (9 and 3%ID/g at 60 minutes p.i., respectively). Biodistribution of the ribose derivative [^{123}I]IVFRU differs from the other two arabinose derivatives mainly with respect to blood radioactivity, which reaches 22%ID/g at 2 minutes after injection and clears half of the radioactivity after 60 minutes; [^{123}I]FIAU and [^{123}I]IVFAU show 5.2 and 4.6%ID/g at 2 minutes, respectively. The relatively high blood levels would explain the higher lung and heart concentrations of [^{123}I]IVFRU (8.1 and 8.5%ID/g at 2 minutes p.i.) in comparison with the other two tracers which are 4.5 and 3.9%ID/g for [^{123}I]FIAU and 4.5 and 6.2%ID/g for [^{123}I]IVFAU at 2 minutes after injection. Furthermore, [^{123}I]IVFRU, with higher brain uptake (0.5%ID/g at 2 minutes p.i.), differs from [^{123}I]FIAU and [^{123}I]IVFRU, both with approximately 0.2%ID/g at 2 minutes p.i. The brain uptake for both IVFRU and FIAU are approximately 0.3%ID/g 60 minutes after injection, in contrast with IVFAU with only 0.2%ID/g.

Although IVFRU has the highest brain uptake and IVFAU the lowest, their brain/blood ratios give another order, IVFAU > IVFRU > FIAU, owing to the blood radioactivity. The higher and constant brain/blood ratio of IVFAU may reflect greater stability against hydrolysis of the N-glycosidic bond or deiodination, differences in mediated transport or differences in rates of phosphorylation (trapping). These effects could be attributable to the steric effects of the C-2′-F substituent. In vivo PET evaluations of [^{124}I]IVFRU and [^{124}I]IVFAU in tumor-bearing mice are warranted to determine effects of the pathologically compromised integrity of the BBB. The virtually constant levels of radioactivity in stomach, and the low and decreasing amount of radioactivity in thyroid indicate that all three nucleoside analogues are not significantly deiodinated in vivo. Both IVFRU[21] and FIAU[1] have been shown to be very good substrates for HSV-1 TK.

The blood–brain barrier (BBB) consists of endothelial cells of the brain capillaries, which possess tight interendothelial cell junctions, show few pinocytotic vesicles, and lack fenestrae.[22] Therefore, the cerebral endothelium acts as a lipophilic physical barrier that restricts the passive passage of hydrophilic compounds into the brain. BBB permeation improves with increasing lipophilicity of the permeant,[23,24] but decreases again after reaching a maximum lipophilicity (log P 2–3).[25,26] However, some lipid-soluble substances such as vinblastine (log P 1.7) and vincristine (log P 2.1) can hardly enter the brain.[27] In this study, IVFRU and IVFAU, with the comparable lipophilicities (log P 1.2), show the same poor BBB permeation. The usual relationship between BBB permeability and drug lipophilicity obviously does not apply to these compounds. However, complex active and passive transport mechanisms[22,23] and other factors (like the permeability of the BBB during infection) are known to influence

drug movement from blood into the central nervous system (CNS). Thus, the investigation of brain uptake in tumor-bearing mice has been planed, to find out whether expression of HSV-1 *tk* can give some influence on increasing brain permeability comparing within the normal mice in this work. Since all three nucleosides are effectively transported by the equilibrative, NBMPR-sensitive nucleoside transporter,[20] the low uptake in brain suggests that nucleoside-specific transport is not modulating uptake into brain. However, it is possible that although mediated transport "in" is fast, lack of phosphorylation by nucleoside kinases results in equally rapid egress, since this transporter is equilibrative. Thus, efforts to develop more lipophilic nucleoside analogues will have to be supplemented by determination and subsequent incorporation of other factors involved in moving nucleosides across the BBB, and keeping them there. This approach will advance the application of radiotracers for noninvasive imaging of HSV-1 thymidine kinase gene expression in gliomas.

CONCLUSION

Facile, rapid radioiodination of the uracil nucleoside analogues FIAU, IVFRU, and IVFAU using iodine radioisotopes useful for both SPECT (^{123}I) and PET (^{124}I), has been demonstrated. The biodistribution data for [^{123}I]FIAU, [^{123}I]IVFRU, and [^{123}I]IVFAU in mice show that their uptake by brain is not in direct proportion to their (relative) lipophilicities. Although all three tracers investigated in this study show poor accumulation of BBB in normal mice brain, it remains necessary to determine their biodistributions in tumor-bearing mice, especially because of the high blood/brain ratio of IVFAU. PET studies of HSV-1 *tk* gene expression with ^{124}I-labeled nucleosides in tumor-bearing cats are proposed to find out which of these substrates is more suitable for PET assessment of HSV-1 *tk* gene expression in gliomas.

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