1-(2-DEOXY-2-FLUORO-β-D-ARABINOFURANOSYL)-5-[^{36}Cl]-CHLOROURACIL; RADIOSYNTHESIS AND PRELIMINARY BIODISTRIBUTION STUDIES IN AN EXPERIMENTAL MURINE TUMOR MODEL

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his outstanding contributions to the area of nucleic acid chemistry.

The nucleoside to nucleic acids pathways are attractive targets for molecular imaging of cell proliferation, for radionuclide-based radiotherapy, for following molecular processes and for drug design, development and delivery. This paper describes the preparation of several radiolabelled nucleosides for comparitive study as markers of tumour cell proliferation. 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-[^{36}Cl]chlorouracil (F[^{36}Cl]ClAU)[1] was synthesized by reaction of 1-(2-deoxy-2-fluoro-arabinofuranosyl)uracil (FAU) with Na^{36}Cl in the presence of 2 M HNO_3 (32% radiochemical yield; 13.5 MBq/mmol). 1-(2-Deoxy-2-fluoro-arabinofuranosyl)-5-fluoro/bromo/iodouracils radiolabelled with carbon-14 ([2-^{14}C]FFAU; 1.5 GBq/mmol), bromine-82 (F[^{82}Br]BrAU; 167 MBq/mmol) and iodine-125 (F[^{125}I]IAU; 10.4 GBq/mmol) were synthesized according to literature methods. These radiolabelled halopyrimidine nucleosides were administered by i.v. injection into BDF_1 mice bearing transplanted Lewis Lung tumors. Uptake of test compounds by target (tumor) tissue was low, and not dissimilar to the reported uptake of their high-specific-activity 5-halopyrimidine nucleoside counterparts. Selected biodistribution data are presented. In general, clearance from blood was rapid, with less that one percent of the injected dose remaining in the blood within 1 h of injection. Tumor uptake was approximately 2% of the injected dose per g of tumor for F[^{36}Cl]ClAU and F[^{125}I]IAU, with [2-^{14}C]FFAU showing less that 2% per g and F[^{82}Br]BrAU with over 3% per g at this time after injection. Data are compared to those for the respective high-specific-activity counterparts.

Keywords: Radiosynthesis; Radioactive labelling; F[^{36}Cl]ClAU; Antitumor drugs; Nucleosides; 5-Halopyrimidines; Biodistribution.

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5-Halopyrimidine nucleosides that have undergone substitutional and/or configurational changes at one or more carbon atoms in the sugar moiety, particularly the 1-(2′-deoxy-2′-fluoroarabinofuranosyl)uracils[1] have been of particular interest, when radiolabelled, for diagnostic imaging of cell proliferation and gene therapy. These compounds are generally bioisosteric substitutes for their 5-halo-2′-deoxyuridine analogues, but offer greater resistance to catabolism by pyrimidine phosphorylases[2]. Several nucleosides have made their way to the market as antivirals, but of the many of antitumor pyrimidines synthesized since 5-fluorouracil (5FU)[3], 1-(β-D-arabinofuranosyl)cytosine (araC) and 9-(β-D-arabinofuranosyl)-2-fluoroadenine 5′-monophosphate (fludarabine) are among the few arabinose nucleosides in common clinical use as antitumor drugs[4]. Radiohalogenated nucleosides have substantial radiodiagnostic imaging and/or radiotherapeutic potential. Examples include 1-(2,3-dideoxy-3-[^{18}F]fluoro-β-D-ribofuranosyl)-5-methyluracil (fluorothymidine; FLT)[5], 2′-deoxy-5-[$^{123/125}$I]-iodouridine[6], 2′-deoxy-5-[^{76}Br]bromouridine[7] and 2′-deoxy-5-[^{211}At]astatouridine[8].

The chlorine radioisotopes are inappropriate for imaging or therapy because of their decay characteristics (high energy beta/gamma or positron emission; half-lives generally <1 h or very long). Consequently, there has been little interest within the nuclear medicine community to develop either chlorinated or radiochlorinated nucleosides, with the exception of 2′-[^{34m}Cl]chloro-2′-deoxyuridine[9] and the ^{18}F-labelled chloronucleoside, 1-(2-deoxy-2-[^{18}F]fluoro-β-D-arabinofuranosyl)-5-chlorouracil[10].

Radionucleoside imaging to monitor the thymidine kinase – ganciclovir gene therapy paradigm[11,12] was based on the pioneering work of Elion[13] and followed early attempts to image herpes encephalitis by exploiting herpes thymidine kinase (HSV-TK) in infected tissue[14]. Arabinofuranosyl nucleosides such as 1-(β-D-arabinofuranosyl)-5-iodouracil (FIAU)[15] as well as in the ribo analogues such as 1-(β-D-ribofuranosyl)-5-iodouracil (FIRU)[16,17] have subsequently been developed for nuclear imaging.

The radiosynthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-[^{36}C])chlorouracil is now reported. High specific activity radiotracers are known to be required for receptor-based imaging, but there is no general agreement on the advantages of high specific activity for monitoring enzymatic processes such as phosphorylation *in vivo*. Preliminary biodistribution data for this series of low specific activity radiolabelled 1-(2-deoxy-2-fluoroarabinofuranosyl)-5-halouracil nucleosides are compared to literature data for their high-specific-activity counterparts (Fig. 1).

EXPERIMENTAL

Materials and Methods

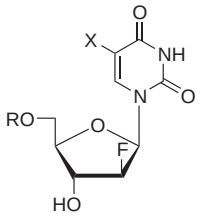
Chemicals and reagents were reagent grade. The major synthon, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FAU) was synthesized according to a literature procedure[18].

High performance reverse-phase chromatography. HPLC separations of products in reaction mixtures were performed on a preparative Whatman Magnum 9 Partisil 10 C_{18}-ODS 25 cm reverse phase column using a Waters HPLC system consisting of Model 510 solvent pumps, Model 860 gradient controller, Model U6K injector and a Hewlett Packard Model 1040A diode array ultraviolet detector, with methanol/water (20:80 v/v; 1.8 ml/min) as eluting solvent. Eluent fractions corresponding to all UV-active peaks were collected and aliquots were assayed for Cl-36 radioactivity using Soluene-350 (Packard Instrument Co.) fluor and Beckman 9000 liquid scintillation counter.

Radioactive precursors. Sodium [^{36}Cl]chloride (Na^{36}Cl; ~13 MBq/mmol) was purchased from Amersham International. Ammonium [^{82}Br]bromide (specific activity ~170 MBq/mmol) was prepared by irradiation of NH_4Br (97.8% ^{81}Br, ORNL, USA) at a neutron flux of 1×10^{12} n/cm^2/s in the University of Alberta SLOWPOKE nuclear reactor. Sodium [^{125}I]iodide (Nordion International, Inc.) was purchased through the Edmonton Radiopharmaceutical Centre, as a no-carrier-added solution in 0.1 M NaOH; carrier NaI was added before use to lower the specific activity to ~10 GBq/mmol. [2-^{14}C]Uracil (l.5 GBq/mmol) was prepared by acid hydrolysis of [2-^{14}C]2,2′-anhydrouridine, which was an intermediate product in the total synthesis of [2-^{14}C]2′-deoxyuridine from Ba[^{14}C]CO_3[19].

Nucleosides

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-chlorouracil (F[^{36}Cl]ClAU) (13.5 MBq/mmol) was synthesized by heating (135 °C, 1 h) an aqueous solution of Na^{36}Cl (16.5 MBq; 10.3 mg, 0.177 mmol in 0.5 ml H_2O) with a solution of FAU (21 mg, 0.085 mmol) in 2 mM HNO_3 (3 ml). After cooling to room temperature, this solution was carefully neutralized (1 M NaOH) to pH 6.5 and subsequently passed through a Sep-Pak® C_{18} cartridge (Waters Associates). The crude product was recovered from the cartridge by elution with methanol/water (80:20 v/v; 5 ml). The eluent was concentrated to 2–3 ml under a gentle stream of dry nitrogen gas, then purified by preparative reverse-phase HPLC. The desired product, F[^{36}Cl]ClAU, eluted with a retention time (18.5 min) identical to that for authentic FClAU[20], was recovered in



X	Abbreviation
F	FFAU
Cl	FClAU
Br	FBrAU
I	FIAU

FIG. 1
1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-halouracils

46% chemical yield from FAU, and 32% radiochemical yield from $Na^{36}Cl$. The radiochemical purity was determined by radio-HPLC to be greater than 99%. The specific activity, determined by liquid scintillation counting and HPLC mass quantification, was 13.5 MBq/mmol.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-fluoro-[2-^{14}C]uracil ([^{14}C]FFAU) (1.5 GBq/mmol) was prepared by coupling 3,5-*O*-diacetyl-2-fluoroarabinofuranosyl bromide to [2-^{14}C]uracil as reported previously[20].

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-[^{82}Br]bromouracil (F[^{82}Br]BrAU) (167 MBq/mmol) and *1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-[^{125}I]iodouracil* (F[^{125}I]IAU) (10.4 GBq/mmol) were prepared by halogenation of FAU using $NH_4{}^{82}Br$ and $Na1^{25}I$, respectively, as reported[21].

Tissue distribution studies were made in male BDF_1 mice (18–22 g; University of Alberta Health Sciences Animal Facility) bearing transplanted, subcutaneous Lewis lung tumors, following procedures described elsewhere[22]. Mice were dosed by intravenous injection of 50–150 μg of nucleoside via the tail vein. Animals were exsanguinated by cardiac puncture following asphyxiation with CO_2. Tissues collected upon necropsy were lightly blotted to remove superficial blood, then weighed wet. For F[^{82}Br]BrAU- and F[1^{25}I]IAU-dosed mice, tissues and the remaining carcass were transferred to plastic vials for γ-counting in a Beckman 8000 well-type counter. For [^{14}C]FFAU and F[^{36}Cl]ClAU, tissue samples were taken for analysis by liquid scintillation (LS) counting. ^{36}Cl measurements were made directly following solubilization of the tissue in Soluene-350 (Packard Instrument Co.), bleaching with 30% H_2O_2 and mixing with scintillation fluor, whereas ^{14}C was measured after combustion of samples using a Harvey OX300 Biological Oxidizer and mixing with fluor. All LS measurements were made with a Beckman 9000 counter.

RESULTS

Radiochlorination of FAU with $Na^{36}Cl$ under electrophilic conditions proceeded smoothly to afford semi-preparative HPLC-purified F[^{36}Cl]AU in 32% radiochemical yield, without apparent reduction in the specific activity; the peak elution volume was just over 1 min, between 18 and 19 min after injection. The remaining radiochromatographic peaks were tentatively identified as ^{36}Cl anion (not quantified) and 28% of 5-[^{36}Cl]chlorouracil. HPLC showed six UV-active components in the crude reaction mixture, three of which were radioactive: the major component (32%) was confirmed by co-chromatography and UV spectral analysis to be F[^{36}Cl]ClAU. Radiochemical yields were determined by liquid scintillation counting of aliquots of eluent collected periodically to coincide with elution of each peak region; these data were plotted as a histogram of activity as a function of elution time and compared to the UV elution profile. On analytical HPLC, reference standards co-eluted with four of the six peaks detected in the chlorine-36 synthesis mixture: uracil (7.6 min), 5-chlorouracil (9.7 min), unreacted FAU (10.6 min) and the desired product, FClAU (16.6 min); two UV peaks at 6 and 9 min were not identified. On-line UV spectral analysis provided additional confirmation of the identity of each eluted fraction.

The four radionucleosides studied all showed biodistribution properties characterized by rapid clearance from blood ($T_{1/2}$ < 30 min) and most other tissues, indicating that there was little metabolic trapping in the tissues examined. At all time intervals, radioactivity concentrations (% of injected dose per g) were highest for F[^{82}Br]AU. The low levels of incorporation by liver and tumor, which for all compounds tested essentially followed the blood clearance time course, was interpreted as representing perfusion and transient effects rather than metabolic trapping. The tumor to blood ratios 1 h after injection reached values of about 1.6 for F[^{36}Cl]ClAU and F[^{82}Br]BrAU, and for F[^{82}Br]BrAU, continued to increase gradually over a 3-h period to a maximum of 4. Selected biodistribution data are presented. In general, clearance from blood was rapid, with less than 1% of the injected dose remaining in the blood within 1 h of injection. Tumor uptake was approximately 2% of the injected dose per g of tumor for F[^{36}Cl]ClAU and F[^{125}I]IAU, with [2-^{14}C]FFAU showing less that 1% per g and F[^{82}Br]BrAU with over 3% per g at this time after injections. Summary data, together with literature data for their high-specific activity counterparts, are presented in Table I.

TABLE I
Uptake of radiolabelled 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-halouracil *in vivo* in tumor-bearing mice

Nucleoside	Percent of injected dose per g tissue, 2 h[a]			Spleen: Blood	Tumor: Blood	Specific activity (MBq/mmol)	Reference
	blood	spleen	tumor				
F[^{36}Cl]ClAU	1.3 ± 0.1	1.2 ± 0.5	2.0 ± 0.3	0.9	1.5	13	this work
[^{18}F]FClAU	1.9 ± 1.0	2.4 ± 0.9	2.4 ± 0.8	1.3	1.3	>74 × 10^6	10
[^{14}C]FFAU	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.5	1.0	1.5 × 10^3	this work
[^{3}H]FFAU	4.5 ± 0.6	4.2 ± 0.7	nd[b]	0.9	nd[b]	444 × 10^3	5
F[^{82}Br]BrAU	1.5 ± 0.6	1.7 ± 1.1	3.3 ± 1.8	1.1	2.2	167	this work
[^{18}F]FBrAU	1.4 ± 0.6	3.2 ± 1.4	1.7 ± 0.5	2.3	1.2	>74 × 10^6	10
F[^{125}I]IAU	1.7 ± 0.9	1.0 ± 0.4	2.0 ± 0.6	0.6	1.2	10.4 × 10^3	this work
F[^{125}I]IAU	0.4 ± 0.1	1.7 ± 0.8	0.7 ± 0.2	4.2	1.8	213 × 10^6	28

[a] Derived from linear extrapolation between 1 and 3 h data for F[^{82}Br]BrAU and F[^{125}I]IAU. [b] nd, not determined.

DISCUSSION

Radionuclides of several biologically-important elements, especially carbon-14, tritium (hydrogen-3), phosphorous-32, sulfur-35 and iodine-131, have been used to study biochemical processes, drug metabolism and drug distribution since these nuclear reactor products became routinely available in the late 1940's. Unfortunately, many reactor-produced radionuclides decay with beta and beta-gamma emissions which, although often suitable for radiotherapy, are not suited or poorly suited for medical imaging[23]. Cyclotron-produced, neutron-deficient, positron-emitting radionuclides such as carbon-11 were first produced by Lawrence in the late 1930's [24], but mainstream production of these radionuclides only became a reality with the establishment of the Hammersmith medical cyclotron[25]. Numerous medical cyclotrons are now in operation globally, producing fluorine-18 ($T_{1/2}$ 109 min), carbon-11 ($T_{1/2}$ 20 min), iodine-124 ($T_{1/2}$ 4 days) and other positron emitters, as well as many other non-positron emitting radionuclides. Although the positron emitters are ideally suited for imaging by positron emission tomography (PET), the short half-lives of the commonly used PET tracers can be limiting in studies of basic biochemistry and in drug development work.

Effects of halosubstitution on pyrimidine nucleoside metabolism, transport and toxicity have been the subject of intensive study[2,26–31] and extensive review[4,32]. Despite the biochemical potential of the chlorinated antimetabolite nucleosides, their development as radiotracers is severely constrained by the decay properties of the chlorine radioisotopes: in general, the radioisotopes have hard particulate emissions (>4 MeV) or in the case of chlorine-36, a moderate-energy β (0.71 MeV) but with virtually no γ-ray component and an extremely long half-life (>300 000 y) with a correspondingly very low specitic activity. Thus, although synthesis with chlorine radioisotopes is relatively facile using either nucleophilic substitution, as e.g. for 2′-deoxy-2′-[^{34m}Cl]chlorouridine[9], or electrophilic radiochlorination as for F[^{36}Cl]ClAU in this report, radiochlorinated nucleosides have no real future in diagnostic or therapeutic clinical nuclear medicine.

The future of chlorinated nucleosides, especially as radiotracers for positron emission tomography (PET), may lie in the use of dihalonucleosides using the 'other' halogen as the radiolabel, thereby retaining the desirable biochemical properties and gaining the advantage of a superior tracer radionuclide. In the case of FClAU, for example, either F or Cl can be used as the radiolabel, to obtain the otherwise identical compound, 1-(2-deoxy-2-[^{18}F]fluoro-β-D-arabinofuranosyl)-5-chlorouracil ([^{18}F]FClAU)[10] or F[^{36}Cl]ClAU as now reported. The chemistry of chlorine-36 radiolabelling

at C-5 has considerable advantage over radiofluorination at the C-2 arabinose position, despite the necessity of using oxidizing conditions which necessitate the implementation of strict radiological containment measures. Nucleophilic radiofluorination at the arabino C-2 position is not possible because of participation of the C-2 carbonyl oxygen, thereby necessitating fluorination of the sugar prior to coupling and final deprotection[10,33]. These additional steps require substantial chemical expertise; they are also time consuming, leading to lower recovered radiochemical yields at each additional step, as well as through radioisotope decay losses which are substantial for this 109 min half-life of fluorine-18.

The introduction of chlorine at the C-5 position of uracil and uracil-based nucleosides proceeded smoothly. The radiochlorination reaction provided virtually identical results to preliminary reactions conducted under identical 'cold' conditions. In three modelling reactions, the chemical yields of FClAU were 31, 38 and 56%, compared to 46% chemical yield based on FAU for the radioactive synthesis. The radiochemical yield of F[^{36}Cl]ClAU was 32% based on chlorine-36, indicative of radiotracer dilution by ubiquitous chlorine in the system. Hydrolysis of the substrate, FAU, to uracil occurred as anticipated under these conditions. Chlorouracil was formed, either by hydrolysis of the expected product or by chlorination of uracil. In the radioactive synthesis, this reaction leads to reduced radiochemical yields. No attempts were made to further optimize reaction conditions.

The biodistributions of four 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-halouracils, including F[^{36}Cl]ClAU, were compared in BDF_1 mice bearing Lewis lung tumors. Data are presented in Table II. As with their 2-fluororibose analogues[34,35], the 2-fluoroarabinose nucleosides were rapidly cleared from the blood, and there was little accumulation of radioactivity in any organ or tissue, including the liver. A notable exception, however, was the apparent lack of tumour uptake of [^{14}C]FFAU, which approached only 0.1% of injected dose within 1 h, compared to almost 1% reported by Mercer for FFRU[34] under similar experimental conditions. F[^{82}Br]BrAU, on the other hand, reached about 0.5% of the injected dose compared to approximately 0.1% for F^{82}BrRU[32]. Literature data for tumour:blood ratios were close to unity for [^{14}C]FFAU, F[^{36}Cl]ClAU and F[1^{25}I]IAU 1 h after injection, but the ratios for F[^{82}Br]BrAU rose steadily over the 3-h period of study. Persistently high levels of radioactivity in blood and kidneys of animals receiving F^{82}BrRU are thought to reflect the presence of free radiobromide, paralleling the rapid *in vivo* debromination of 5-[^{82}Br]bromo-2′-deoxyuridine (BUdR) reported by Kriss et al.[36], but this phenomenon was not investigated fur-

TABLE II

Uptake of radioactivity in selected tissues and tumors after i.v. injection of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-halouracils into BDF_1 mice bearing Lewis Lung tumors. Data are expressed as percent of injected dose per organ tissue (mean) ± standard deviation (n = 5)

Organ/Tissue	Time (min) after i.v. injection					
	5	10	15	30	60	180
			[2-^{14}C]FFAU			
Blood[a]	5.8 ± 5.9	4.0 ± 0.8	4.9 ± 0.9	1.6 ± 0.2	0.5 ± 0.1	<0.1
Muscle	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 100	0.1 ± 0.0	<0.1	<0.1
Liver	5.3 ± 0.9	3.6 ± 0.5	2.3 ± 0.6	1.5 ± 0.3	0.3 ± 0.40	<0.1
Kidney	4.3 ± 0.1	3.6 ± 0.4	3.4 ± 0.6	1.8 ± 0.2	0.3 ± 0.0	<0.1
Tumor	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	<0.1
			F[^{36}Cl]ClAU			
Blood	nd	nd	nd	3.5 ± 0.3	2.4 ± 0.6	1.2 ± 0.1[b]
Muscle	nd	nd	nd	3.0 ± 0.6	1.7 ± 0.7	0.8 ± 0.3[b]
Liver	nd	nd	nd	3.5 ± 0.8	1.8 ± 0.9	1.2 ± 0.5[b]
Kidney	nd	nd	nd	7.9 ± 1.6	2.3 ± 1.1	1.1 ± 0.3[b]
Tumor	nd	nd	nd	3.7 ± 0.4	1.9 ± 0.4	2.0 ± 1.0[b]
			F[^{82}Br]BrAU			
Blood	29.5 ± 2.5	14.7 ± 5.8	16.9 ± 6.5	12.8 ± 12.3	2.7 ± 0.7	1.6 ± 0.4
Muscle	3.2 ± 0.6	2.0 ± 0.9	1.9 ± 1.0	1.2 ± 1.1	0.3 ± 0.1	0.1 ± 0.0
Liver	23.1 ± 2.5	10.7 ± 4.7	14.0 ± 5.7	8.2 ± 6.7	1.7 ± 0.4	0.8 ± 0.2
Kidney	29.7 ± 5.0	18.9 ± 6.4	20.0 ± 7.4	15.4 ± 10.1	3.8 ± 1.2	1.1 ± 0.3
Tumor	1.5 ± 1.2	1.0 ± 0.2	1.7 ± 1.5	1.7 ± 2.8	0.5 ± 0.2	0.7 ± 0.4
			F[^{125}I]IAU			
Blood	4.60 ± 0.3	3.2 ± 0.8	4.1 ± 2.2	2.7 ± 0.9	2.5 ± 0.8	1.0 ± 0.5
Muscle	1.8 ± 0.1	1.2 ± 0.3	1.1 ± 0.5	0.9 ± 0.4	0.8 ± 0.4	0.2 ± 0.1
Liver	4.0 ± 0.3	2.7 ± 0.7	2.6 ± 1.2	1.8 ± 0.9	1.7 ± 0.9	0.4 ± 0.2
Kidney	13.5 ± 2.0	11.1 ± 3.4	11.2 ± 5.7	6.4 ± 4.1	6.3 ± 5.2	0.8 ± 0.3
Tumor	1.7 ± 0.3	1.7 ± 0.6	2.0 ± 0.4	2.3 ± 0.7	2.3 ± 0.7	1.6 ± 0.4

[a] Blood calculated as 6% of the total body weight. [b] Measurements at 120 min after injection. nd, not determined.

ther. The presence of a fluorine atom at either the C-2-ribose (down) or C-2-arabinose (up) position in the respective pyrimidine nucleoside series imparts stability to the glycosidic bond[37], making these nucleosides much less susceptible to phosphorolytic cleavage[2]. The introduction of F in either the ribose (C-2′ 'down') or arabinose (C-2′ 'up') configuration does affect the partition coefficient, and therefore it is unlikely that diffusion into cells creates differences in their uptake. Furthermore, fluorine configuration at C-2 appears to exert no appreciable influence on their respective interaction with a nucleoside transmembrane transporter[29]. It would therefore be reasonable to conclude that low uptake of these arabinose nucleosides is due to their inability to interact with molecular targets (e.g. enzymes) within cells rather than to their inability to enter cells.

Direct comparisons of F[^{36}Cl]ClAU uptake to reported data for [^{18}F]FClAU (Table II) are difficult because of both animal model and specific activity differences. The specific activity of the fluorine-18 labelled counterpart (>74 TBq/mmol) is more that one million times greater that the unavoidably low specific activity (13 MBq/mmol) of F[^{36}Cl]ClAU, resulting in dose differences of a similar order of magnitude. Furthermore, the chlorine-36 nucleoside was studied in a murine model, whereas the fluorine-18 compound was injected into nude nice bearing human HT-29 tumours. It is surprising, then, that the biodistributions have strikingly similar tumour:blood ratios (Table I), and is somewhat contradictory to the established dogma that specific activity limits sensitivity. Although the 'high specific activity' undoubtedly applies to receptor-binding radioligands, enzyme and transport substrate capacity, at least with nucleosides, override specific activity, at least within the limits of these fluoroarabinofuranosyl nucleoside studies.

Although qualitative and quantitative studies of metabolism and excretion were not undertaken as part of this study, the data reported here for [^{14}C]FFAU, F[^{36}Cl]ClAU, F[^{82}Br]BrAU and F[^{125}I]IAU indicate that, in terms of dose, they are not efficiently utilized in the Lewis lung tumor/BDF_1 murine model. They appear to offer little encouragement for use as radiopharmaceuticals for tumour imaging in diagnostic oncology, in comparison to their ribose analogues. Similarly, they do not appear to be useful for biochemical investigation of *in vivo* mammalian nucleoside and nucleic acid metabolism by virtue of their low utilization rate and the relative difficulty in synthesis compared to the corresponding 2′-deoxyuridines. Of course, in the case of tissue engineered to express TK trans-genes that readily phosphorylate the fluoroarabinofuranosyl nucleosides relative to mammalian TK, they have been demonstrated to be superior diagnostics for

imaging the proliferation of these cells[37–39]. The long half-life and chemical stability of the chlorine-36 radiolabel do confer substantial advantage, however, for metabolic and biochemical studies, offering the opportunity for chemical characterization as well as radioassay, with no half-life time constraint.

ABBREVIATIONS

FAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil;
FBrAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-bromouracil;
FClAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-chlorouracil;
FFAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-fluorouracil;
FIAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil;
FIAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil;
FFRU, 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-5-fluorouracil;
FIRU, 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-5-iodouracil;
FLT, 1-(2,3-dideoxy-3-[^{18}F]fluoro-β-D-ribofuranosyl)-5-methyluracil, fluorothymidine;
FU, 5-fluorouracil;
FUDR, 2′-deoxy-5-fluorouridine;
TK, thymidine kinase;
HSV-TK, herpesvirus type-1 thymidine kinase.

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REFERENCES

1. Watanabe K. A., Reichman U., Hirota K., Lopez C., Fox J. J.: *J. Med. Chem.* **1979**, *22*, 21.
2. Gati W. P., Kugyelka J. G., Knaus E. E., Wiebe L. I. in: *Current Applications in Radiopharmacology* (M. W. Billinghurst, Ed.), pp. 316–322. Pergamon Press, Toronto 1986.
3. Heidelberger C., Chaudhuri N. K., Danneberg P., Mooren M., Griesbach L., Duschinsky R., Schnitzer R. J., Pleven E., Scheiner J.: *Nature* **1957**, *179*, 663.
4. Matsuda A., Sasaki T.: *Cancer Sci.* **2004**, *95*, 105.
5. Shields A. F., Grierson J. R., Koxawa S. M., Zheng M.: *Nucl. Med. Biol.* **1996**, *23*, 17.
6. Semnani E. S., Wang K., Adelstein S. J., Kassis A. I.: *J. Nucl. Med.* **2005**, *46*, 800.
7. Boni R., Blauenstein P., Dummer R., von Schulthess G. K., Schubiger P. A., Steinert H. C.: *Melanoma Res.* **1999**, *9*, 569.
8. Larsen R. H., Vaidyanathan G., Zalutsky M. R.: *Int. J. Radiat. Biol.* **1997**, *72*, 79.
9. Abrams D. N., Knaus E. E., Wiebe L. I., Helus F., Maier-Borst W.: *Int. J. Appl. Radiat. Isot.* **1984**, *35*, 1045.

10. Alauddin M. M., Shahinian A., Park R., Tohme M., Fissekis J. D., Conti P. S.: *Nucl. Med. Biol.* **2004**, *31*, 399.
11. Knaus E. E., Wiebe L. I., Morin K. W.: Canadian 2,202,891, 1994.
12. Morin K. W., Wiebe L. I., Knaus E. E., Balzarini J., De Clercq E.: *Eur. J. Nucl. Med.* **1994**, *21*, S-125.
13. Elion G. B., Furman P. A., Fyfe J. A., de Miranda P., Beauchamp L., Schaeffer H. J.: *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716.
14. Samuel J., Gill M. J., Iwashina T., Tovell D. R., Tyrrell D. L., Knaus E. E., Wiebe L. I.: *Antimicrob. Agents Chemother.* **1986**, *29*, 320.
15. Tjuvajev J. G., Stockhammer G., Desai R.: *Cancer Res.* **1995**, *55*, 6126.
16. Morin K. W., Knaus E. E., Wiebe L. I., Xia H., McEwan A. J. B.: *Nucl. Med. Commun.* **2000**, *21*, 129.
17. Nanda D., de Jong M., Vogels R., Havenga M., Driesse M., Bakker W., Bijster M., Avezaat C., Cox P., Morin K., Naimi E., Knaus E., Wiebe L., Smitt P. S.: *Eur. J. Nucl. Med. Mol. Imag.* **2002**, *29*, 939.
18. Howell H. G., Brodfuehrer P. R., Brundilige S. P., Benigni D. A. Sapino C.: *J. Org. Chem.* **1988**, *53*, 85.
19. Giziewicz J., Gati L. J., Knaus E. E., Mercer J. R., Flanagan R. J., Wiebe L. I.: *Appl. Radiat. Isot.* **1985**, *36*, 227.
20. Misra H. K., Wiebe L. I., Knaus E. E.: *J. Lab. Comp. Radiopharm.* **1987**, *24*, 1107.
21. Misra H. K., Knaus E. E., Wiebe L. I.., Tyrrell D. L.: *Appl. Radiat. Isot.* **1986**, *37*, 901.
22. Lee Y. W., Abrams D. N., Wiebe L. I., Knaus E. E.: *Int. J. Nucl. Med. Biol.* **1984**, *11*, 262.
23. Cherry S. R., Sorenson J. A., Phelps M. E.: *Physics in Nuclear Medicine*, 3rd ed. Saunders, Philadelphia 2003.
24. a) Lawrence E. O., Cooksey D.: *Phys. Rev.* **1936**, *50*, 1131; b) Lawrence E. O.: *Science* **1939**, *90*, 407.
25. http://www.mrc.ac.uk/index/public-interest/public-news_centre/public-news_list/public-news_2005/public-news_3_june_2005.htm
26. Grant A. J., Feinberg A., Chou T.-C., Watanabe K. A., Fox J. J., Philips F. S.: *Biochem. Pharmacol.* **1982**, *31*, 1103.
27. Coderre J. A., Santi D. V., Matsuda A., Watanabe K. A., Fox J. J.: *J. Med. Chem.* **1983**, *26*, 1149.
28. Schinazi R. F., Fox J. J., Watanabe K. A., Nahmias A. J.: *Antimicrob. Agents Chemother.* **1986**; *29*, 77.
29. Xu L., Gati W. P., Knaus E. E., Wiebe L. I.: *Advances in Radiopharmacology*. Proc. 6th Int. Symp. Radiopharmacol., p. 283. University Wollongong Press, Wollongong, Australia 1990.
30. Klecker R. W., Katki A. G., Collins J. M.: *Mol. Pharmacol.* **1994**, *46*, 1204.
31. Brust P., Haubner R., Friedrich A., Scheunemann M., Anton M., Koufaki O. N., Hauses M., Noll S., Noll B., Haberkorn U., Schackert G., Schackert H. K., Avril N., Johannsen B.: *Eur. J. Nucl. Med.* **2001**, *28*, 721.
32. Cabrita M. A., Baldwin S. A., Young J. D., Cass C. E.: *Biochem. Cell Biol.* **2002**, *80*, 623.
33. Mangner T. J., Klecker R. W., Anderson L., Shields A. F.: *Nucl. Med. Biol.* **2003**, *30*, 215.
34. Mercer J. R., Knaus E. E., Wiebe L. I.: *J. Med. Chem.* **1987**, *30*, 670.
35. Mercer J. R., Xu L., Knaus E. E., Wiebe L. I.: *J. Med. Chem.* **1989**, *32*, 1289.
36. Kriss J. P., Maruyama Y., Tung L. A., Bond S. B., Revesz L.: *Cancer Res.* **1963**, *23*, 260.

37. Cho S. Y., Ravasi L., Szajek L. P., Seidel J., Green M. V., Fine H. A., Eckelman W. C.: *J. Nucl. Med*. **2005**, *46*, 1923.
38. Bettegowda C., Foss C. A., Cheong I., Wang Y., Diaz L., Agrawal N., Fox J., Dick J., Dang L. H., Zhou S., Kinzler K. W., Vogelstein B., Pomper M. G.: *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1145.
39. Soghomonyan S. A., Doubrovin M., Pike J., Luo X., Ittensohn M., Runyan J. D., Balatoni J., Finn R., Tjuvajev J. G., Blasberg R., Bermudes D.: *Cancer Gene Ther.* **2005**, *12*, 101.