Comparative Biodistribution and Metabolism of Carbon-11-labeled N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating Analogues

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ABSTRACT

The tricyclic carboxamide N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) is a DNA-intercalating agent capable of inhibiting both topoisomerase I and II and is currently in Phase II clinical trial. Many related analogues have been developed, but despite their potential in vitro cytotoxicities, they exhibit poor extravascular distribution. As part of an ongoing drug development program to obtain related “minimal intercalators” with lower DNA association constants, we have compared the biodistribution and metabolism profiles of the prototype compound, DACA, with three analogues to aid rational drug selection. All of these compounds share a common structural feature, N-dimethyl side chain, which was radio labeled with the positron-emitting radioisotope, carbon-11. This strategy was selected because it allows promising candidates emerging from preclinical studies in animals to be evaluated rapidly in humans using positron emission tomography (PET).

The acridine DACA, the phenazine SN 23490, the pyridoxamine SN 23719, and the dibenzodioxin SN 23935 were found to be cytotoxic in in vitro assays with an IC50 of 1.4–1.8 μM, 0.4–0.6 μM, 1.3–1.6 μM, and 24–36 μM, respectively. In HT29, U87MG, and A375M cell lines, Ex vivo biodistribution studies with carbon-11 radio labeled compounds in mice bearing human tumor xenografts showed rapid clearance of 11C-radioactivity (parent drug and metabolites) from blood and the major organs. Rapid hepatobiliary clearance and renal excretion were also observed. There was low (<5% of injected dose/gram (%ID/g)) and variable uptake of 11C-radioactivity in three tumor types for all of the compounds. Tumor (U87MG) to blood 11C-radioactivity for [11C]DACA, [11C]phenazine-1-carboxamide (SN 23490), [11C]pyridoxamine-8-carboxamide (SN 23719), and [11C]dibenzo[1,4]dioxin-1-carboxamide (SN 23935) at 30 min were 2.9 ± 1.1, 2.3 ± 0.6, 2.6 ± 0.6, and 0.7 ± 0.2, respectively. For SN 23719, the distribution of 11C-radioactivity in normal tissues and tumors determined ex vivo was in broad agreement with that determined in vivo by whole body PET scanning. [11C]DACA was rapidly and extensively metabolized to several plasma metabolites and a major tumor metabolite. In contrast, [11C]SN 23935, [11C]SN 23490, and [11C]SN 23719 showed less extensive metabolism. In the tumor samples, the parent [11C]DACA and [11C]SN 23719 represented between 0.3 and 1.5%ID/g, whereas [11C]SN 23490 and [11C]SN 23719 represented between 1.5 and 2.8%ID/g.

In conclusion, by using a strategy with 11C-labeling, we have determined the tissue distribution and metabolic stability of novel tricyclic carboxamides with the view of selecting analogues with potentially better in vivo activity against solid tumors. SN 23490 and SN 23719 had more favorable distribution and metabolic stability compared with DACA and SN 23935 and may warrant further development. The radiolabeling strategy used allows ex vivo and in vivo evaluation of promising anticancer agents in animals and offers the potential of rapid translation to studies in humans using PET.

INTRODUCTION

DACA3 (NSC 601316, SN 22995; Fig. 1 I) is a tricyclic DNA-intercalating agent with broad spectrum in vivo activity against both leukemia and solid tumors (1). DACA interacts with both topoisomerase I and topoisomerase II-DNA complexes (2, 3). Cytotoxicity is thought to be attributable to this dual action, as well as its ability to overcome both classic (p-glycoprotein) and atypical (topoisomerase II-dependent) multidrug resistance (4, 5). On the basis of its novel mechanism of action and antitumor activity, DACA was selected for clinical development. Two Phase I clinical studies have been completed (6, 7), and the drug is now undergoing Phase II clinical trials.

Despite their cytotoxic properties, a common limitation of tricyclic carboxamides is their poor extravascular distributive properties (8, 9). This is particularly true where a cationic charge is located on the DNA-binding pharmacophore. Thus, in vitro cytotoxicity generally translates into in vivo activity against leukemias but not necessarily to activity against remotely sited solid tumors. Structure-activity studies led to the notion that, whereas intercalative binding was essential, pharmacophores with lower DNA association constants possessed the additional property of exhibiting higher equilibrium unbound drug levels and, hence, better distribution into solid tumors (9–12). As part of a drug development program to produce tricyclic carboxamides with a minimum aromatic region to ensure intercalative binding, “minimal intercalators,” and with better distributive properties, three structural types have emerged as potential candidates. These include the phenazine carboxamides (e.g., SN 23490; Fig. 1 II; Ref. 13), arylquinoline carboxamides (e.g., SN 23719; Fig. 1 III; Ref. 14), and dibenzodioxin carboxamides (e.g., SN 23935; Fig. 1 IV; Ref. 9). Although initial studies in tumor cell lines and in mouse s.c. tumors showed interesting activity profiles (9, 13), comparative pharmacokinetic studies have not been performed. Another important consideration for selection of tricyclic carboxamides is metabolic stability. Experience with DACA showed that the drug was extensively metabolized in vivo (15–18). The production of several metabolites that could be active or inactive reduced the ability to predict tissue drug profiles from plasma levels. Studies with PET also suggested that more tumor and normal tissue information could have been gained if DACA was less extensively metabolized.

3 The abbreviations used are: DACA, N-[2-(dimethylamino)ethyl]acridine-4-carboxamide; PET, positron emission tomography; SN 23490, (9-methoxyphenazine-1-carboxamide; SN 23719, 2-(4-pyridyl)quinoline-8-carboxamide; SN 23935, dibenzo[1,4]dioxin-1-carboxamide; %ID/g, percentage of injected dose/million; HPLC, high performance liquid chromatography; SRB, sulforhodamine B; SN 25699, N-[2-(methylamino)ethyl]-dibenzo[1,4]dioxin-1-carboxamide; mp, melting point; 6, chemical shift in parts/million downfield from the standard; J, coupling constant in Hz; s, singlet; t, triplet; q, quartet; br, broadened; SN 25696, N-[2-(methylamino)ethyl]-9-methoxyphenazine-1-carboxamide; SN 25700, N-[2-(methylamino)ethyl]-2-(4-pyridyl)quinoline-8-carboxamide; TCA, trichloroacetic acid.

MATERIALS AND METHODS

All of the chemicals were of analytical or HPLC grade. Heptane sulfonic acid, triethylamine, and phosphoric acid were purchased from BDH Chemicals (Poole, United Kingdom). SRB, trichloroacetic acid, and acetic acid were purchased from Sigma Chemical Co. (Poole, United Kingdom). HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific United Kingdom. SRB, trichloroacetic acid, and acetic acid were purchased from Sigma Chemical Co. (Poole, United Kingdom). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific United Kingdom. HPLC column "Bondapak C18 (30 × 0.78 cm inside diameter; 10-µm size) was purchased from Phase Separations Ltd. (Flintshire, United Kingdom). Millipore filters (0.2 µm) were purchased from Gelman Sciences (Northampton, United Kingdom). The following standards were prepared by reported methods (1): DACA (Fig. 1 I); [11C]DACA (Ia); SN 24390 (IIa); SN 23719 (IIIa); [11C]SN 23719 (IIa); SN 23935 (IVa), and SN 23935 (Iva).

Synthesis of N-Desmethyl Precursors of SN 23490, SN 23719, and SN 23935. SN 25699 (a precursor to SN 23490) was prepared as follows. A solution of dibenzo(1,4)dioxin-1-carboxylic acid (1.0 g; 4.38 mmol) was added. The mixture was stirred at 50 – 60°C for 30 min, then cooled to −10°C, and treated with N-methylethylenediamine (1.5 ml; 17.52 mmol). After an additional 3 h at −10°C, excess dimethylformamide and amine were removed under reduced pressure, and the solution was diluted with aqueous sodium carbonate. The resulting precipitate was collected, dried, and dissolved in a minimum volume of methanol. This solution was treated with dry hydrochloric gas, then diluted with ethylacetate to give SN 25699. Yield, 0.75 g (52%), mp (methanol-ethylacetate), 251–254°C. 

\[ \text{SN 25699} \]

\[ \text{N-Desmethyl Precursors of SN 23490, SN 23719, and SN 23935.} \]

SN 23719 (III), R = CH₃

\[ [1\text{C}]\text{SN 23719 (IIla), R = } ^{13}\text{CH}_3 \]

SN 23935 (IV), R = CH₃

\[ [1\text{C}]\text{SN 23935 (Iva), R = } ^{13}\text{CH}_3 \]

Fig. 1. Structures of DACA (I), [11C]DACA (Ia); SN 24390 (IIa); [11C]SN 23719 (IIla); SN 23719 (IIIb); [11C]SN 23719 (IIla); SN 23935 (IVa), and SN 23935 (Iva).

To support rational selection of tricyclic carboxamides with better distributive and metabolic profiles, we have radiolabeled lead compounds representing the three aforementioned structural types with carbon-11 and studied their biodistribution and metabolism in mice bearing human tumor xenografts (U87 MG glioma, A375M melanoma, and HT29 colon adenocarcinoma). This was possible because all of the structural types possessed a common side chain that could be radiolabeled with carbon-11. Carbon-11 radiolabeling is an alternative to carbon-14 and has the added advantage that such studies can be extended to humans using PET. As a prelude to this, biodistribution of the most promising candidate was also established in tumor-bearing mice using the small animal PET scanner.

BOD DISTRIBUTION AND METABOLISM OF RADIOLABELLED DACA ANALOGUES

To support rational selection of tricyclic carboxamides with better distributive and metabolic profiles, we have radiolabeled lead compounds representing the three aforementioned structural types with carbon-11 and studied their biodistribution and metabolism in mice bearing human tumor xenografts (U87 MG glioma, A375M melanoma, and HT29 colon adenocarcinoma). This was possible because all of the structural types possessed a common side chain that could be radiolabeled with carbon-11. Carbon-11 radiolabeling is an alternative to carbon-14 and has the added advantage that such studies can be extended to humans using PET. As a prelude to this, biodistribution of the most promising candidate was also established in tumor-bearing mice using the small animal PET scanner.
Radiochemical Synthesis. $[^{11}C]$DACA (Fig. 1 Ia) was produced by N-methylation of the N-desmethyl precursor (N-monomethyl-DACA) using $[^{11}C]$(iodomethane as described previously (16). The three analogues, $[^{11}C]$SN 23490 (Fig. 1 Ib), $[^{11}C]$SN 23719 (Fig. 1 Iia), and $[^{11}C]$SN 23935 (Fig. 1 Iva), were prepared using the same reaction conditions except the N-desmethyl precursor (SN 25699, SN 25696, or SN 25700) was varied (19). The radiolabeled analogues were then purified using normal-phase HPLC (μ; Porasil; 300 × 7.8 mm; 10-μm size) eluted with a mixture chloroform and ethanol [95:5, v/v] at a flow rate of 3 ml/min. The formulated products were analyzed using analytical HPLC (Bondclone; 250 × 3.9 mm; Phenomenex). The column was eluted with a mixture of ammonium formate (0.1 M) and methanol [50:50, v/v] at a flow rate of 1.5 ml/min.

In Vitro Cytotoxicity Assay. The sensitivity of U87MG, A375M, and HT29 human tumor cell lines to DACA and analogues was evaluated by the SRB assay (20). Cells were seeded in 96-well plates at a concentration of 2.5 × 10^3 cells/well in a final volume of 100 μl of RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml); Life Technologies, Ltd., Paisley, United Kingdom). Twenty four h later, DACA, SN 23490, SN 23719, and SN 23935 were added at concentrations ranging between 0.2 and 200 μM, and the cells were incubated at 37°C for 72 h. One column of eight wells on each plate was left untreated as controls. Cells were fixed by gently adding cold TCA to give a final concentration of 10%. The cells were incubated at 4°C for 1 h and washed five times with water to remove growth medium, TCA, and other contaminants. Plates were air-dried and stored before staining. TCA-fixed cells were stained with SRB (0.4% w/v dissolved in 1% acetic acid) for 30 min. Stained cells were washed four times with 1% acetic acid to remove unbound dye. Tris base (15 mEq; pH 10.5) was added to the wells, and the plates were shaken gently for 10 min to dissolve the dye. Absorbance in each well was measured in an automatic microplate spectrophotometer (Spectramax; Molecular Devices, Sunnyvale, CA) at 564 nm, and results were expressed as treated/control cell survival. From these, the IC_{50} was determined.

Ex Vivo Biodistribution. The work was carried out by licensed investigators in accordance with the United Kingdom Home Office’s “Guidance on the Operation of the Animals (Scientific Procedures) Act 1986” (Her Majesty Stationary Office, Feb., 1990). Female ICRF strain athymic nude (nu/nu) mice (obtained from Harlan United Kingdom Ltd., Bicester, United Kingdom) 3–4 months of age at the time of experiment (body weight, 19–21 g) were used. Tumor xenografts of the human glioma U87MG, the nonmelanotic melanoma A375M, and the colonic adenocarcinoma HT29 were established bilaterally in the flanks of mice by s.c. injection of 5 × 10^6 cells in 100 μl of tissue culture medium. Experiments were performed approximately 6 weeks later when the tumors were 5–8 mm in diameter. A bolus injection of $[^{11}C]$-radiolabeled compound (11 MBq) was given via a lateral tail vein. At selected times after injection from 5 to 90 min, mice were sacrificed by exsanguination via cardiac puncture under general anesthesia (isoflurane inhalation). Blood, normal tissues (brain, heart, lung, liver, kidney, spleen, stomach, small intestine, skeletal muscle, colon, and gallbladder), and tumor samples were rapidly dissected. The tissue samples were weighed, and the radioactivity was measured using an automatic gamma counter with automatic decay correction (Compugamma 1282; Pharmacia LKB Biotechnology). The results were expressed as %ID/g of tissue.

Analysis of Plasma Samples. Blood samples were taken via cardiac puncture at 5, 15, and 30 min after i.v. injection of $[^{11}C]$DACA, $[^{11}C]$SN 23490, $[^{11}C]$SN 23719, and $[^{11}C]$SN 23935. An aliquot of blood was taken for measurement of radioactivity, and the remaining sample was dispensed into a heparinized tube and centrifuged (2000 g for 2 min). Plasma samples were then equilibrated with ice-cold methanol and centrifuged (2000 g for 3 min). The resulting pellets and duplicate aliquots (100 μl) of methanol supernatant were taken for measurement of radioactivity. The remaining methanol supernatant was analyzed using analytical HPLC (Bondclone; 250 × 3.9 mm; Phenomenex). The column was eluted with a mixture of ammonium formate (0.1 M) and methanol [50:50, v/v] at a flow rate of 1.5 ml/min.
containing the extracted $^{11}$C-radioactivity was rotary evaporated at 60°C, and the resulting residue was dissolved in HPLC mobile phase (3 ml) and filtered. Duplicate aliquots (100 μl) were taken for counting. The processed sample (1 ml) was spiked with the appropriate unlabeled reference compound and then injected onto an HPLC column ("μ" Bondapak C18). The column was eluted with a mixture of triethylammonium phosphate (10 mM; pH 3.5) and acetonitrile (65:25 v/v) at a flow rate of 3 ml/min. The HPLC eluent was monitored sequentially for radioactivity and UV absorbance at 254 nm. Both detectors were linked to a computer-based integrator that recorded the chromatogram and allowed the correction of the data for physical decay, background radioactivity, and integration of peaks and, thus, calculation of the amount of radioactive component in each analyte.

Analysis of Tumor Samples. Tumor samples were rapidly cut into small pieces to which ice-cold methanol (20 ml) was added. The samples were homogenized using a Ultra-Turrax Homogenizer (Janke & Kunkel, KG, IKA) for 2 min, and the homogenate was centrifuged (2000 g for 5 min). The resulting pellets and duplicate aliquots (100 μl) of the methanol supernatant containing the $^{11}$C-radioactivity were taken for measurement of radioactivity. The remaining methanol supernatant was evaporated at 60°C under reduced pressure. The resulting residue was dissolved in HPLC mobile phase (3 ml) and filtered. Duplicate aliquots (100 μl) were taken for measurement of radioactivity. The processed sample (1 ml) was then analyzed by HPLC as described above for plasma samples.

During sample preparation, the extraction of radioactivity from plasma and tumors was >95%, and the recovery of radioactivity after concentration of sample by rotary evaporation ranged between 90 and 96% with 4–10% of the radioactivity sticking to the glass vessel.

PET Imaging. As a prelude to clinical studies with [$^{11}$C]SN 23719, we performed PET imaging with this radiotracer and compared the images with the ex vivo biodistribution data. The tail vein of a nude mouse bearing the HT29 tumor was catheterized after induction of anesthesia (isofluorane/N₂O/O₂) and placed in a thermostatically regulated holder. The mouse was given a bolus injection of [$^{11}$C]SN 23719 (4.4 MBq) via the catheterized tail vein. The anesthetized mouse was scanned for 1 h in a HIDA positron emission tomograph (Oxford Positron Systems, Oxford, United Kingdom; Ref. 21). The acquired list mode data were sorted into 0.5-mm sinogram bins for image reconstruction. The images were visualized using Analyze software (22).

RESULTS

Radiochemistry. [$^{11}$C]DACA (Fig. 1 Ia), [$^{11}$C]SN 23490 (Fig. 1 IIa), [$^{11}$C]SN 23719 (Fig. 1 IIIa), and [$^{11}$C]SN 23935 (Fig. 1 IVa) were produced in good radiochemical yield, in the range of 2–4 GBq at end of synthesis with specific radioactivity, in the range of 20–70 GBq/mmol, and corresponding to 16–33 μg of stable compound. When the products were formulated in saline alone, radiolysis was observed. This was overcome by the addition of either hydrochloric acid/ethanol or L-ascorbic acid before formulation with NaCl solution (0.9%). The chemical and radiochemical purity of the products was >95%.

In Vitro Cytotoxicity Assay. The effect of different concentrations of DACA, SN 23490, SN 23719, and SN 23935 on the growth of the three tumor-derived cell lines (U87MG, A375M, and HT29) are illustrated in Fig. 2. All of the drugs were active on the cell lines with IC₅₀ of 1.8, 1.4, and 1.4 μM, respectively, for DACA; 0.5, 0.6, and 0.4 μM, respectively, for SN 23490; 1.6, 1.3, and 1.3 μM, respectively, for SN 23719; and 36.3, 23.7, and 27.1 μM, respectively, for SN 23935. Hence, SN 23490 was the most cytotoxic, DACA and SN 23719 were next, and SN 23935 was the least cytotoxic.

Fig. 3. Tumor:blood ratio of $^{11}$C-radioactivity after i.v. injection of (a) [$^{11}$C]DACA, (b) [$^{11}$C]SN 23490, (c) [$^{11}$C]SN 23719, and (d) [$^{11}$C]SN 23935 in mice bearing human tumor xenografts U87MG, A375M, and HT29 at 5, 15, and 30 min.
exhibited similar cytotoxicities, and SN 23935 was the least cytotoxic. It is of importance that DACA, SN 23490, and SN 23719 also showed biphasic cytotoxicity in U87MG.

**Tissue Biodistribution.** The biodistribution of $^{11}$C-radioactivity in the three tumor types and normal tissues after injection of $[^{11}\text{C}]{\text{DACA}}$, $[^{11}\text{C}]{\text{SN}}$ 23490, $[^{11}\text{C}]{\text{SN}}$ 23719, and $[^{11}\text{C}]{\text{SN}}$ 23935 is summarized in Table 1. The values are comprised of both parent and metabolite radioactivities. The uptake of $^{11}$C-radioactivity varied for each tumor type and was low ($<5\%\text{ID/g}$). For all of the compounds, kidney, liver, and lungs showed the highest uptake of $^{11}$C-radioactivity at 5 min. $^{11}$C-radioactivity decreased at 15 min and 30 min. In small intestine, $^{11}$C-radioactivity increased at 15 min and decreased at 30 min. The high uptake of radioactivity in small intestine at 5 and 15 min was thought to reflect hepatobiliary clearance, because gall bladder $^{11}$C-radioactivity was high at these time points (data not shown). $^{11}$C-radioactivity levels were lower for all of the other tissues. Overall, there were no obvious trends in normal tissue distribution for the different compounds.

Fig. 4. A typical radiochromatographic profile of $[^{11}\text{C}]{\text{DACA}}$, $[^{11}\text{C}]{\text{SN}}$ 23490, $[^{11}\text{C}]{\text{SN}}$ 23719, and $[^{11}\text{C}]{\text{SN}}$ 23935 and their radioactive metabolites in plasma (Fig. 4, a–d) and HT29 tumor (Fig. 4, e–h) samples at 5 min after injection.
Comparative Tumor:Blood Ratios of Radioactivity. To obtain an index of extravascular distribution, we expressed $^{11}$C-radioactivity in tumors as a ratio of the blood $^{11}$C-radioactivity (Fig. 3). Overall, there was a trend toward increasing tumor:blood ratios, with time reflecting retention in the tumors. For all of the tumor types, $[^{11}]$C$SN\ 23935$ showed the lowest tumor:blood ratio (1.5). For A375M and HT29, the analogue $[^{11}]$C$SN\ 23719$ had the most favorable tumor: blood ratios, 3.6 ± 1.5 and 3.2 ± 1.1, respectively, at 30 min. For U87 MG, $[^{11}]$C$DACA\,\ [^{11}]$C$SN\ 23490$, and $[^{11}]$C$SN\ 23719$ showed relatively high ratios of 3.6 ± 1.5, 2.3 ± 0.6, and 2.6 ± 0.6, respectively, at 30 min.

In Vivo Metabolite Profile. Typical radiochromatograms of mouse plasma and U87MG tumor samples 5 min after i.v. injection of $[^{11}]C$DACA, $[^{11}]$C$SN\ 23490$, $[^{11}]$C$SN\ 23719$, and $[^{11}]$C$SN\ 23935$ are shown in Fig. 4, a–h. In plasma $[^{11}]C$DACA (retention time, 17 min), several unidentified radioactive polar metabolites and one less polar radioactive metabolite were observed (Fig. 4a). Similarly, for the $^{11}$C-labeled analogues, a number of unidentified polar metabolites and one less polar radioactive metabolite were found in plasma (Fig. 4b–d). Analysis of U87MG tumor samples after a 5-min injection of $[^{11}]$C$DACA$ showed the parent compound and several unidentified polar radioactive metabolites (Fig. 4e). One major radioactive metabolite (retention time, 9 min) was predominant. In contrast, with the $^{11}$C-analogues, the most predominant radioactive component was the parent compound with low levels of other more polar radioactive metabolites (Fig. 4, f–h). The analysis of A375M and HT29 tumor samples at 5 min showed similar profiles for $[^{11}]$C$DACA$ and its three analogues to that observed for the U87 MG tumor (data not shown).

Analysis of tumor samples at other time points (15 and 30 min) showed similar radiochromatographic profiles, but the amounts of individual radioactive components differed with time.

The amount of parent $[^{11}]$C$DACA$, $[^{11}]$C$SN\ 23490$, $[^{11}]$C$SN\ 23719$, and $[^{11}]$C$SN\ 23935$ expressed as percentage of the total radioactivity in plasma and in the three tumor types over 30 min is shown in Fig. 5. In plasma, $[^{11}]$C$DACA$ represented 8% of the total radioactivity at 30 min after injection. The corresponding values for $[^{11}]$C$SN\ 23490$, $[^{11}]$C$SN\ 23719$, and $[^{11}]$C$SN\ 23935$ were 11, 23, and 14%, respectively. In all of the three tumors, $[^{11}]$C$DACA$ represented <30% of the total $^{11}$C-radioactivity by 30 min, whereas the analogues represented between 43 and 87% of the total $^{11}$C-radioactivity at the same time point. Of the analogues, $[^{11}]$C$SN\ 23719$ represented >78% of the total $^{11}$C-radioactivity in all of the tumor types examined.

The relationship between total $^{11}$C-radioactivity compared with the radioactivity represented by the parent $[^{11}]$C$DACA$, $[^{11}]$C$SN\ 23490$, $[^{11}]$C$SN\ 23719$, and $[^{11}]$C$SN\ 23935$ in tumors is shown in Fig. 6. For $[^{11}]$C$DACA$, rapid and extensive metabolism was observed in all of the tumor types. In contrast, $[^{11}]$C$SN\ 23490$, $[^{11}]$C$SN\ 23719$, and $[^{11}]$C$SN\ 23935$ were less extensively metabolized. The parent drug represented <1.5%ID/g for $[^{11}]$C$DACA$ and $[^{11}]$C$SN\ 23935$, whereas $[^{11}]$C$SN\ 23490$ and $[^{11}]$C$SN\ 23719$ represented >1.5%ID/g.

PET Imaging. Fig. 7 a–d illustrates the distribution of $[^{11}]$C$SN\ 23719$ in a HT29 tumor-bearing mouse. There was a high distribution of $^{11}$C-radioactivity in liver, kidneys, small intestine, and urine. The tumor was not visualized because of the low levels of $^{11}$C-radioactivity in the tumor compared with the above normal tissues. As with
ex vivo biodistribution studies, the distribution of [11 C]SN 23719 in brain and muscle was also low.

DISCUSSION

Our previous work with [11 C]DACA in rats and in patients has provided information on metabolism, plasma kinetics, normal tissue biodistribution, and tumor uptake (15, 16). Tumor uptake was low and variable.4 Additionally, it was found that [11 C]DACA was rapidly and extensively metabolized in vivo (15, 16).

Enhanced tumor uptake, reduced metabolism, and longer half-lives could improve the therapeutic efficacy of this class of compounds. Earlier identification of lead compounds that possess all or some of these properties could make an important contribution to the selection of potential candidates for further development. As a contribution to the ongoing drug development program on this series of DNA-intercalators, we have compared the biodistribution and metabolite profiles of [11 C]DACA with three analogues exhibiting anticancer activity.

DACA and the three structurally distinct analogues, SN 23490, SN 23719, and SN 23935, share a common structural feature; they all have the same side chain containing a terminal N,N-dimethyl group. This allowed the three analogues to be radiolabeled with carbon-11 in the same position as [11 C]DACA using a similar radiochemistry strategy. The 11 C-analogues were produced with high specific radioactivity and in good radiochemical yield with high radiochemical and chemical purity.

As part of the preclinical evaluation, the cytotoxicities of SN 23490, SN 23719, and SN 23935 were compared with DACA in the same human tumor cell lines that were subsequently implanted in mice. The cytotoxicity of the compounds in these cell lines had not been determined previously. It was found that the cytotoxicity of DACA (IC50, 1.4 μM) in two of the tumor types was in good agreement with the range (0.09–3.4 μM) reported for mouse and some human cell lines and in a panel of primary human melanoma cultures derived from fresh surgical melanoma specimens (23). In U87 MG cells, both SN 23490 and SN 23719 showed similar biphasic cytotoxicities (Fig. 2) as reported previously (24) for DACA. This effect was not observed with SN 23935, which was considerably less cytotoxic (IC50, 24–36 μM) compared with the other analogues. In the present study, SN 23490 was found to be the most cytotoxic (IC50, 0.4–0.6 μM) and SN 23719 to be as cytotoxic as DACA (IC50, 1.3–1.6 μM). Although the cytotoxicity of SN 23719 has not been studied previously in human tumor cell lines, in vitro studies with 935.1 mouse fibrosarcoma cells showed it had an IC50 of 148 nM (14).

The tissue distribution of [11 C]DACA observed in human tumor xenografts in nude mice were in agreement with our previously published data in rats (15) and in nontumor and syngeneic tumor-bearing mice after i.v. and i.p. administration of [3 H]DACA (25–27). The tissue distribution of 11 C-analogues were in broad agreement with [11 C]DACA (Table 1). The tumor uptake of [11 C]DACA and 11 C-analogues varied in the three tumor types but was generally low (<5%ID/g). The modest uptake observed for [11 C]DACA and the 11 C-analogues is not uncommon for anticancer agents; e.g., Shani et al. (28) have shown that the uptake of radiolabeled 5-fluorouracil in 15 s.c. implanted tumors in mice and rats was low (~2%ID/g in mice and ~0.8%ID/g in rats). They concluded that the therapeutic efficacy observed with 5-fluorouracil is not attributable to enhanced uptake in the tumor compared with normal tissues, but rather that the tumor cells may be more sensitive to the active metabolites than are normal cells.

For all of the radiotracers, the tumor:blood ratios generally increased with time, suggesting the retention of radioactivity in tumors. The lowest tumor:blood ratio was observed for [11 C]SN 23935 (<1.5), and the highest was observed for [11 C]SN 23719 in the three

![Fig. 6. The striped panels represent the total [11 C]-radioactivity (%ID/g), and the shaded panels represent the unchanged parent, [11 C]DACA, [11 C]SN 23490, [11 C]SN 23719, and [11 C]SN 23935 (%ID/g) in U87MG, A375M, and HT29 tumor samples at 5, 15, and 30 min after injection of the radiotracer.](image)
tumor types, indicating the most favorable extravascular uptake of \(^{11}\text{C}\)-radioactivity into tumors. We could not determine the half-lives because of a limitation of our methodology (i.e., the short half-life of carbon-11) where a limited number of time points and associated data could be collected.

The metabolism of DACA has been well established both \textit{in vitro}, using subcellular fractions of rat liver (29, 30) and rat isolated hepatocytes (31), and \textit{in vivo} (mouse, rat, and human; Refs. (32, 15, 16, 18). These studies have shown that DACA is extensively metabolized. In mouse (32) and in humans (18), the major biotransformations of DACA involve \(N\)-oxidation of the tertiary amine side chain, and 9(10\(H\))acridone formation followed hydroxylation at C-7 with subsequent glucuronidation. \(N\)-demethylation of the tertiary amine side chain of DACA has been observed but as a minor pathway. In mouse urine, \(\sim 1\%\) of the administered dose was present as \(N\)-monomethyl-DACA (32), whereas in human urine, \(<5\%\) of the administered dose was present as \(N\)-monomethyl-DACA (18).

Before this study, the metabolism of the three analogues examined here had not been investigated. The studies with \(^{11}\text{C}\)DACA and its analogues were carried out at the no-carrier-added level; \textit{i.e.}, the amount of radiolabeled compound was associated with only \(\mu\)g of stable drug. Identification of radiolabeled metabolites was difficult under such conditions because of the low level of compound and will be the subject of another study carried out on pharmacological doses of DACA and its analogues.

However, by analogy with the metabolism of DACA, the tertiary amine side chains of the three analogues might be expected to undergo \(N\)-oxidation and, to a minor extent, \(N\)-demethylation. Additionally, the pyridine ring nitrogen of SN 23719 could undergo \(N\)-oxidation. In the case of SN 23490, the 9-methoxy group would be expected to demethylate, followed by hydroxylation. Also, it is possible that SN 23935 might undergo chromophore hydroxylation probably at the C-6/7 positions. In the present study, analysis of plasma samples after i.v. injection of \(^{11}\text{C}\)DACA, \(^{11}\text{C}\)SN 23490, \(^{11}\text{C}\)SN 23719, and \(^{11}\text{C}\)SN 23935 showed in all of the cases the presence of the parent radiotracer, several polar, and one less polar radioactive metabolites. For \(^{11}\text{C}\)DACA, the less polar radioactive metabolite in plasma was tentatively identified as \(^{11}\text{C}\)DACA-\(N\)-oxide (15). By analogy with \(^{11}\text{C}\)DACA, the less polar radioactive metabolite observed from the three \(^{11}\text{C}\)-analogues could be products of \(N\)-oxidation of the tertiary

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**Fig. 7.** Whole body coronal projection image of an unanesthetized HT29 tumor-bearing mouse after i.v. injection of \(^{11}\text{C}\)SN 23719 (4.4 MBq). PET images were acquired with the mouse positioned prone using a HIDA-PET Scanner. The panels show selected 0.5-mm thick slices through the image volume (C-25, C-33, C-41) acquired at (a) 0–5 min, (b) 5–20 min, (c) 20–40 min, and (d) 40–60 min after injection. Images were visualized using Analyze software (22). Image intensities in the different panels have been scaled to the maximum signal intensity for each panel. Arrows represent position of the tumor.
amine side chain to give the corresponding N-oxide(s). In our studies, substantial N-demethylation of [11C]DACA or its analogues was not observed because >95% of radioactivity was recovered during sample preparation. If volatile [11C]methanol, the by-product of N-demethylation, had been present, it would have been lost during sample processing.

Analysis of tumor samples after i.v. injection of [11C]DACA showed significant contribution from a single radioactive metabolite that was more polar than [11C]DACA. In contrast, for the three [11C]-analogues, the majority of the radioactivity was present as the parent radiotracer with low levels of other very polar radioactive metabolites. Similar profiles were observed for the other two tumor types, A375M and HT29. Although there were slight variations overall, the rate of metabolism was in the order [11C]DACA > [11C]SN 23935 > [11C]SN 23490 > [11C]SN 23719 in all of the three tumor types. Our initial hypothesis was that lipophilicity may influence the rate of metabolism; i.e., more lipophilic compounds will reach metabolic sites readily and hence be metabolized more rapidly. The four compounds have widely varying lipophilicities with SN 23935 being the most lipophilic, as shown by their relative lipophilicity (R_{lip}) values measured by liquid-liquid TLC: SN 23935, 0.01; DACA, 0.1; SN 23490, 0.42; SN 23719, 0.87 (33). Our findings from this limited series of compounds indicated that the least lipophilic compounds are the least metabolized. These results highlight the complexity of the process of uptake and retention of drugs and the variability between different tumor types. However, other factors such as vascularity, protein binding, and DNA binding could all contribute to the uptake and retention of anticancer agents in tumors.

Because SN 23719 showed similar cytotoxicity to DACA and exhibited better extravascular distribution and metabolic stability in tumors, the in vivo biodistribution of this analogue was investigated in a HT29 tumor-bearing mouse using PET. In the PET images, liver, kidneys, and the bladder could be clearly observed because uptake of [11C]-radioactivity was high (in the range of 6–22%ID/g). The images also showed rapid hepatobiliary clearance with high signal intensity in the gut. During the PET study, the mouse was anesthetized, which resulted in decreased gut motility, and thus increased signal intensity was observed. However, the ex vivo biodistribution results showed a decrease of [11C]-radioactivity in the gut at 30 min after injection. During biodistribution studies, the animals were conscious and able to excrete the [11C]-radioactivity from the gut.

The uptake of [11C]SN 23719 in the HT29 tumor was not sufficiently high (in the range of 1.8–2.4%ID/g) to be visualized in the presence of higher [11C]-radioactivity in the surrounding tissues. However, this does not preclude analysis of regions of interest defined for the tumor or normal tissues to obtain pharmacokinetic data in vivo. Visualization of the tumor was not the prime goal of this strategy. What was more important was to determine whether or not the active drug was present in the tumor. As with the ex vivo studies, this observation does not necessarily mean that SN 23719 will not be an effective therapeutic agent. Although DACA is extensively metabolized and is rapidly cleared, it still has potential as an anticancer agent and is currently being evaluated in Phase II clinical trials. Analogues that exhibit comparable or more favorable cytotoxicity, pharmacokinetics, and metabolism could offer better therapeutic efficacy.

In conclusion, this study has demonstrated for the first time the in vivo biodistribution and tumor metabolic profile of [11C]DACA and its analogues, [11C]SN 23490, [11C]SN 23719, and [11C]SN 23935, at a tracer dose in mice bearing human tumor xenografts. We have shown that PET images acquired for one of the analogues, [11C]SN 23719, were in general agreement with the ex vivo biodistribution data with the exception of gut where a difference was observed because of the effect of the anesthesia. We have demonstrated that the uptake and retention of the radiolabeled analogues were variable in the three tumor types. Although the absolute uptake of the [11C]-analogues by the tumors was not high, both [11C]SN 23490 and [11C]SN 23719 had more favorable pharmacokinetics and metabolic profiles in tumors compared with [11C]DACA and [11C]SN 23935. Therefore, these analogues warrant further development. The radiolabeling strategy used allows ex vivo and in vivo evaluation of promising anticancer agents and offers the potential of rapid transition to studies in humans using PET.

REFERENCES


Comparative Biodistribution and Metabolism of Carbon-11-labeled N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating Analogues

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