Studies on the Metabolism of the Novel Antitumor Agent \([N\text{-methyl}-^{11}\text{C}]N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide}\) in Rats and Humans prior to Phase I Clinical Trials


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ABSTRACT

This study reports on the biodistribution and metabolism of the \({}^{11}\text{C}\)-labeled novel antitumor agent \(N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide}\) (DACA) (also known as NSC 601316) in rats (plasma and tissues) and humans (plasma). Information on plasma metabolites was uniquely obtained in humans prior to Phase I clinical trial following i.v. injection of \([{}^{11}\text{C}]\text{DACA}\) at tracer dose.

DACA was labeled in the \(-\text{methyl}\) position using no-carrier-added \({}^{11}\text{C}\)iodomethane. Rapid high-performance liquid chromatography methods were developed for metabolite analysis of \([{}^{11}\text{C}]\text{DACA}\). The metabolism of \([{}^{11}\text{C}]\text{DACA}\) was investigated in patients by plasma sampling. The biodistribution and metabolism of \([{}^{11}\text{C}]\text{DACA}\) was investigated in rats by plasma sampling, sacrifice experiments with tissue samples, and imaging using positron emission tomography scanning.

Analysis of human plasma demonstrated rapid and extensive metabolism of \([{}^{11}\text{C}]\text{DACA}\). The levels of \([{}^{11}\text{C}]\text{DACA}\) in plasma decreased from 77 ± 8% (SD) at 5 min to 25 ± 5% at 45 min postinjection. Seven radioactive metabolites were observed in human plasma, and one was identified as \([{}^{11}\text{C}]\text{DACA}-N\text{-oxide}\).

Rapid clearance of \({}^{11}\text{C}\) radioactivity from rat blood, plasma, and major organs was observed. The half-life of \({}^{11}\text{C}\) radioactivity clearance in rat blood between 15 and 90 min was calculated to be 3.2 h; the levels of \([{}^{11}\text{C}]\text{DACA}\) in rat plasma decreased from 69 ± 3% (SD) at 2 min to 29 ± 1.5% at 25 min. The number of radioactive metabolites in rat plasma was the same as in human plasma except that the parent compound was present in the metabolite fraction. A new metabolite was identified as the \([{}^{11}\text{C}]\text{DACA}-N\text{-oxide}\). Analysis of rat tissues showed rapid and extensive metabolism in tissues, particularly liver and kidney; however, \([{}^{11}\text{C}]\text{DACA}\) (i.e., the parent compound) was the major radioactive component in the lung, heart, and brain at 40 min. Positron emission tomography scanning using \([{}^{11}\text{C}]\text{DACA}\) in the rat showed little retention of \({}^{11}\text{C}\) radioactivity in major organs with rapid excretion via gut and kidney. The rat data were consistent with animal (mouse and rat) preclinical data obtained with previously used techniques with longer-lived isotopes.

Labeling of potential anticancer drugs with positron-emitting radionuclides and performing in vivo preclinical evaluation at tracer doses in animals and humans prior to Phase I clinical trials provides unique information that could speed up the assessment of the drug and could potentially assist drug development programs. In this example, there was no unexpected interspecies difference in metabolism of DACA that would have alerted us to make a change in the planned Phase I study.

INTRODUCTION

Standard preclinical evaluation of anticancer compounds uses radiolabeled compounds, usually tritium or \(^{13}\text{C}\), with sacrifice experiments and HPLC analysis, to determine biodistribution and metabolism in animals. By using compounds labeled with positron-emitting radionuclides, e.g., \(^{11}\text{C}\) \((t_{1/2} = 20.4 \text{ min})\), it is possible to obtain that information, as well as in vivo pharmacokinetic data, by animal tissue and plasma sampling in combination with PET scanning. By using compounds labeled at the “no-carrier-added” level (i.e., associated with only micrograms of stable compound) it would be possible to safely obtain biodistribution and pharmacokinetics in humans using PET scanning. Information on metabolism can be obtained by the analysis of serial plasma samples from patients undergoing PET studies. We investigated this approach by radiolabeling a promising anticancer agent, DACA \((N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide})\), also known as NSC 601316, with \(^{11}\text{C}\) and using it to obtain biodistribution and metabolism data in rats and plasma metabolites in humans. The results of human PET studies with \([{}^{11}\text{C}]\text{DACA}\) will be reported elsewhere.

DACA (I) (Fig. 1) is an acridine derivative developed as a potential anticancer drug (1, 2). DACA has shown a 90% cure rate against advanced Lewis lung carcinoma and causes little myelosuppression in mice (2). Other acridine derivatives, amsacrine (3) and CI-921 (4, 5), have only marginal activity toward this type of tumor. DACA has also shown sensitivity to wild-type, multidrug-resistant sublines of the P388 murine and Jurkat human leukemia cell lines (2). DACA exhibits its cytotoxic activity by inhibiting both topoisomerase I and II and demonstrates reduced susceptibility to P-glycoprotein and topoisomerase II-mediated multidrug resistance mechanisms (6, 7). It was selected for clinical evaluation by the United Kingdom Cancer Research Campaign.

MATERIALS AND METHODS

Male Sprague Dawley rats were obtained from Harlan Olac Ltd. (Bicester, United Kingdom). All chemicals were of analytical or HPLC grade. Ammonium formate, heptane sulfuric acid, triethylamine, and phosphoric acid were purchased from BDH Ltd. HPLC-grade methanol and acetonitrile were purchased from Fisons. HPLC column \(\mu\text{-Bondapak C}_{18}\) (30 × 0.78 cm i.d.) was purchased from AnaChem Ltd. Millipore filters (0.2 μm) were purchased from Gelman Sciences. The following standards were prepared by reported methods (1, 8): DACA (I) \((N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide})\); NSC 601316; acridone (III) \((N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide})\); DACA-N-oxide (IV) \((N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide-N-oxide})\); N-monomethyl-DACA (V) \((N\text{-}[2\text{-}(\text{methylamino})\text{ethyl}]\text{acridine-4-carboxamide})\); 7-hydroxycridone (VI) \((N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]7\text{-hydroxycridone-4-carboxamide})\); acridone-N-oxide (VII) \((N\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{acridine-4-carboxamide-N-oxide})\); N-monomethyl-acridone (VIII) \((N\text{-}[2\text{-}(\text{methylamino})\text{ethyl}]\text{acridone-4-carboxamide})\). Radioactivity was counted using a gamma counter (Compugamma 1282, Pharmacia LKB Biotechnology AB) with automatic decay correction.

Radiochemical Synthesis. \([{}^{11}\text{C}]\text{DACA}\) (II) was produced in 50 min by an \(N\text{-methylene reaction of N-desmethyl precursor, N-monomethyl-DACA (V)}\)
using \(^{14}C\)iodomethane (9). The product was purified using HPLC and formulated as the hydrochloride in isotonic saline, pH 5.5. The average yield of \(^{14}C\)DACA was 2.88 GBq (range, 1.74—4.44 GBq) with a specific activity of 2.7 GBq/\mu l at end of synthesis, corresponding to 10 \mu g (34 nmol) of stable DACA. The radiochemical and chemical purity was greater than 99%.

HPLC Separation of DACA from Its Putative Metabolites. A standard solution containing DACA (I) and metabolites (II)–(VIII) (20 \mu g each) was injected onto a reverse-phase HPLC column (μ-Bondapak C\(_{18}\), 30 × 0.78 cm i.d.; pore size, 10 \mu m). The column was eluted at a flow rate of 3 ml/min with a mixture of acetonitrile and water (32:68, v/v) containing triethylammonium phosphate (10 mM, pH 3.5) and heptane sulfonic acid (5 mM). The eluent was monitored for UV absorbance at 254 nm.

Biodistribution of \(^{14}C\)DACA in Rats. The work was carried out by licensed investigators in accordance with the Home Office’s Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (10). Adult male rats (n = 3) weighing 250—300 g were anesthetized using isoflurane mixed with nitrous oxide and oxygen. The ventral tail artery, for sampling blood, and one lateral tail vein, for injecting \(^{14}C\)DACA, of each rat were catheterized using a polythene catheter (0.58 id., 0.96 outer diameter). The rats were allowed to recover from the anesthesia in Bollman restraining cages for at least 1 h and were heparinized prior to injection of \[^{14}C\]DACA.

The rats were given an injection via the tail vein of a bolus of formulated \(^{14}C\)DACA (74—185 MBq, 6.82—17.04 nmol) and killed at selected time intervals (1.5, 5, 15, 25, and 40 min). Samples of brain, lung, heart, kidney, and liver were removed, blotted, cut into small pieces, and added to ice-cold methanol (20 ml) containing DACA (20 \mu g). The tissues were homogenized using a Polytron homogenizer set at one-half of the maximum for 1—2 min, and the homogenates were centrifuged at 2000 \times g for 5 min. The remaining pellets and duplicate aliquots (200 \mu l) of methanol supernatant were taken for counting. The remaining methanol supernatant containing the extracted \(^{14}C\) radioactivity was rotary evaporated at 80°C and the resulting residue taken up in 0.1 M ammonium formate (3 ml) and filtered. Duplicate aliquots (100 \mu l) were taken for counting. The processed sample (1 ml) was then analyzed using the semiautomated HPLC system. The HPLC conditions and the acquisition and processing of the data were as described above.

Determination of AUCs. The AUCs for rat plasma and tissue concentration versus time were calculated by summing the total \(^{14}C\) radioactivity or \[^{14}C\]DACA found in plasma and tissues over a period of 40 min. The tissue concentration was corrected for blood volume, and the results are expressed as MBq/g. The AUCs and the ratio of total \(^{14}C\) radioactivity in tissue to total \(^{14}C\) radioactivity in plasma (AUC\(_{\text{plasma}}/\text{AUC}\)) and the ratio of \[^{14}C\]DACA in tissue to \[^{14}C\]DACA in plasma (AUC\(_{\text{DACA}}/\text{AUC}\)) were determined.

Rat PET Study. An anesthetized rat, after an i.v. injection of \[^{14}C\]DACA (≈10 MBq), was scanned dynamically for 45 min using the ECAT-953B PET camera (CTI PET Systems; Ref. 12). The images were visualized using Analyze software (13) and rescaled so that the count range of each could be represented by the same arbitrary 8-bit color scale.

Human Plasma Analysis. \[^{14}C\]DACA at tracer dose (10 MBq) was measured for 45 min using the ECAT-953B PET camera (CTI PET Systems; Ref. 12). The images were visualized using Analyze software (13) and rescaled so that the count range of each could be represented by the same arbitrary 8-bit color scale.

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Table 1. Retention times of DACA (I) and some of its putative metabolites on a reverse-phase HPLC column.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
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</thead>
<tbody>
<tr>
<td>7-Hydroxy acridone (VI)</td>
<td>5.6—6.5</td>
</tr>
<tr>
<td>Acridone (III)</td>
<td>7.9—8.9</td>
</tr>
<tr>
<td>N-Monomethyl-acridone (VIII)</td>
<td>7.9—8.9</td>
</tr>
<tr>
<td>Acridone-N-oxide (VII)</td>
<td>8.5—10.1</td>
</tr>
<tr>
<td>N-Monomethyl-DACA (V)</td>
<td>13.1—15.0</td>
</tr>
<tr>
<td>DACA (I)</td>
<td>15.0—16.5</td>
</tr>
<tr>
<td>DACA-N-oxide (IV)</td>
<td>16.5—18.5</td>
</tr>
</tbody>
</table>

Further eluted using the same solution at 5 ml/min for 2 min. The AQF was collected for counting. The SPE column was then eluted at a flow rate of 3.0 ml/min in the reverse direction using a mixture of acetonitrile and water (32:68, v/v) containing 10 mM triethylammonium phosphate, pH 3.5, and 5 mM heptane sulfonic acid. This operation transferred the retained \[^{14}C\]DACA and any remaining metabolites on the SPE column onto the HPLC column (μ-Bondapak C\(_{18}\), 30 × 0.78 cm i.d.; 10 \mu particle size). The HPLC eluent was monitored sequentially for UV absorbance at 254 nm and for radioactivity. Duplicate aliquots (5 ml) of AQF from the SPE column and the HPLC eluent were taken for counting. Both detectors were linked to a personal computer-based integrator, which recorded the chromatogram and allowed the correction of the data for background radioactivity and physical decay, integration of peaks and thus calculation of the percentage of each radioactive component in each analyte. The amount of unchanged \[^{14}C\]DACA at a given time point was calculated using the percentage of each radioactive component in each analyte and the radioactivity present in the AQF.

Rat Tissue Analysis. Rats (n = 3), catheterized as for the biodistribution studies, were given an injection via the tail vein of a bolus of formulated \[^{14}C\]DACA (74—185 MBq, 6.82—17.04 nmol) and killed at selected time intervals (1.5, 5, 15, 25, and 40 min). Samples of brain, lung, heart, kidney, and liver were removed, blotted, cut into small pieces, and added to ice-cold methanol (20 ml) containing DACA (20 \mu g). The tissues were homogenized using a Polytron homogenizer set at one-half of the maximum for 1—2 min, and the homogenates were centrifuged at 2000 \times g for 5 min. The remaining pellets and duplicate aliquots (200 \mu l) of methanol supernatant were taken for counting. The processed sample (1 ml) was then analyzed using the semiautomated HPLC system. The HPLC conditions and the acquisition and processing of the data were as described above.

Pretreatment and Analysis of Radioactivity. Radioactivity was determined by scintillation counting and the AUCs for rat plasma and tissue concentration versus time were calculated by summing the total \(^{14}C\) radioactivity or \[^{14}C\]DACA found in plasma and tissues over a period of 40 min. The tissue concentration was corrected for blood volume, and the results are expressed as MBq/g. The AUCs and the ratio of total \(^{14}C\) radioactivity in tissue to total \(^{14}C\) radioactivity in plasma (AUC\(_{\text{plasma}}/\text{AUC}\)) and the ratio of \[^{14}C\]DACA in tissue to \[^{14}C\]DACA in plasma (AUC\(_{\text{DACA}}/\text{AUC}\)) were determined.

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Figure 1. Structure of DACA (I).

Fig. 1. Structure of DACA (I).

Fig. 2. Time course of \(^{14}C\) radioactivity in rat blood and plasma after i.v. administration of \[^{14}C\]DACA at tracer dose.

![Graph](image-url)
from the Hammersmith Hospital Research Ethics Committee, and authorization for the use of the radionuclide was given by the United Kingdom Administration of Radioactive Substances Advisory Committee. The average amount of activity used was 464.5 MBq (range, 175–667 MBq), associated with 12.6 μg (range, 4.6–18.5 μg) of stable DACA. Blood samples (5 ml) for analysis were taken via an arterial line (arterial samples were used because these patients were also undergoing PET scanning and already had an arterial line inserted) into heparinized syringes at 5, 15, 25, 45, and 60 min after i.v. administration of [11C]DACA (II). DACA (I) and compounds (IV) and (V) were added as references, and CFP was prepared and analyzed using the semiautomated metabolite system as described above.

RESULTS

HPLC Separation of DACA from Its Putative Metabolites. The HPLC retention times of DACA (I) and some of its putative metabolites (II)–(VIII) are given in Table 1. Under the isocratic conditions used, near-baseline separation of DACA (II) from its metabolites (II)–(VIII) was achieved. Metabolites (II) and (VIII) coeluted, metabolites (VII) and (IX) coeluted, and partial separation between metabolites (II) and (VIII) was obtained.

Clearance of [11C]DACA from Rat Blood and Plasma. Clearance of [11C]radioactivity from blood and plasma after a bolus injection of [11C]DACA is shown in Fig. 2. After an initial peak, the radioactivity cleared rapidly from both blood and plasma, so that by 90 min after injection, the radioactivity content per gram of plasma was 0.05% of that injected. The blood:plasma ratios were ~1 and did not change during the course of the study.

In Vivo Biodistribution of [11C]DACA in Rat. The distribution of [11C]radioactivity expressed as radioactivity content (MBq/g of tissue) from 1–70 min after injection of [11C]DACA for each tissue sampled is shown in Fig. 3. In general, the tissues examined showed only slight retention of radioactivity. The initial distribution (1–2 min) of radioactivity per gram of tissue was maximal in lung (5.4%), followed by kidney (4%) and heart (2%). By 70 min, the levels had decreased to 0.8% for lung, 0.7% for kidney, and 0.1% for heart. In the case of lung and kidney, these values were still significantly higher than that in the blood, suggesting retention of [11C]DACA or metabolites. In the case of spleen and duodenum, accumulation of radioactivity (1–2%) was observed between 5 and 15 min, followed by slow clearance. Again, the radioactivity levels still remained substantially higher than those in the blood. The radioactive content in colon, liver, cerebrum, and cerebellum was <1% of the injected dose at early time points and decreased to slightly higher than that in blood at 70 min. In the case of fat, testis, and skeletal muscle, radioactivity content was very low (<0.2%), and no significant change was observed over 70 min. In urine, significant accumulation of radioactivity was observed compared to blood and tissues and reached 4.8% of injected dose at 70 min.
Table 2 Retention times of [14C]DACA (II) and its radioactive metabolites in rat plasma (Met1–Met7) on a reverse-phase HPLC column

<table>
<thead>
<tr>
<th>Radioactive metabolite</th>
<th>Retention time (min)</th>
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<tbody>
<tr>
<td>Met1</td>
<td>3.2–4.4</td>
</tr>
<tr>
<td>Met2</td>
<td>4.4–5.3</td>
</tr>
<tr>
<td>Met3</td>
<td>5.3–8.0</td>
</tr>
<tr>
<td>Met4</td>
<td>7.1–10.1</td>
</tr>
<tr>
<td>Met5</td>
<td>11.6–14.5</td>
</tr>
<tr>
<td>[14C]DACA (II)</td>
<td>14.5–16.5</td>
</tr>
<tr>
<td>Met6</td>
<td>16.5–18.5</td>
</tr>
<tr>
<td>Met7</td>
<td>25–30</td>
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</table>

Fig. 5. Percentage of [14C]DACA and its radioactive metabolites in serial plasma samples from a rat as a function of time after i.v. injection of [14C]DACA.

Analysis of Rat Plasma. Serial plasma samples were analyzed using the semiautomated metabolite analysis system. Plasma proteins were removed using the SPE column. In control experiments, in which plasma samples were spiked with DACA (I) (20 μg) and [14C]DACA (II), >98% of the activity was retained by the SPE column, and >98% of the retained activity was transferred from the SPE column onto the HPLC column. A typical HPLC trace of [14C]DACA (I) and its radioactive metabolites in rat plasma 15 min postinjection of [14C]DACA is shown in Fig. 4. The plasma sample had been spiked with reference DACA (I) and reference metabolites (III)–(VIII) prior to analysis. The radioactive peaks are referred to as Met1–Met7 (Fig. 4). The retention times of [14C]DACA and Met1–Met7 are given in Table 2.

Met1–Met5 eluted before [14C]DACA, whereas Met6 and Met7 eluted after [14C]DACA. A typical metabolic profile of the percentage of [14C]DACA (II) and Met1–Met7 in rat plasma, together with the radioactivity in AQF as a function of time after injection of [14C]DACA, is shown in Fig. 5. For simplicity, the percentages of radioactivity in Met1–Met7 were summed for an individual time point and are referred to as [14C]-labeled metabolites, and the results from three rat studies are given in Table 3 along with the percentages of radioactivity as [14C]DACA and in AQF. In general, [14C]DACA decreased from 69 ± 3% (SD) at 2.5 min to 29 ± 1.5% at 25 min postinjection, whereas the radioactivity in AQF increased from 8 ± 3% at 2.5 min to 30 ± 2 at 25 min, and [14C]-labeled metabolites increased from 23 ± 6% to 42 ± 3% for the same time period. However, it was noted that the percentage of radioactivity in each metabolite component was <10%.

PET Imaging of a Rat. The PET image of a rat scanned dynamically is shown in Fig. 6. The scan illustrates the redistribution in time of the [14C] radioactivity injected, from blood to intestinal contents and urine (Fig. 6, a–e) within the 45 min of the scan duration. The lower panels are cross sections through the regions shown in the upper panels indicated by the horizontal yellow lines. Sequentially, the maximal signal moved from atrium and lung (Fig. 6, a and b) through kidney and liver (Fig. 6, c and d) to small intestine and then to bladder and large intestine (Fig. 6e). In rats, therefore, excretion was mainly via the kidney and gut.

Analysis of Rat Tissues. The HPLC profile of [14C]DACA and its radiolabeled metabolites in various rat tissues at 15 min postinjection of [14C]DACA is shown in Fig. 7. The results are expressed as [14C] radioactivity (MBq/g of tissue) normalized to an injectate of 100 MBq and have been corrected for blood volume. The blood volumes in brain, heart, lung, kidney, and liver were taken to be 3, 20, 50, 1.6, and 27%, respectively (14). In general, all of the tissues examined contained [14C]DACA and metabolites Met1–Met7 in varying amounts, which changed with time. The amount of [14C]DACA itself in the lung, kidney, heart, brain, liver, and plasma over 1.5–40 min is shown in Fig. 8. For comparison, the amount of total radioactivity (normalized to a injectate of 100 MBq and expressed as MBq/g of tissue) present in these tissues is also shown. Expressed as a tissue concentration, the amount of [14C]DACA in rat lung was always higher than that in any of the other tissues examined. In the lung, heart, kidney, brain, heart, and plasma, [14C]DACA paralleled the clearance of [14C] radioactivity. The lowest concentration of [14C]DACA was observed in the liver (<0.1 MBq/g during 1.5–40 min), although there was a slight indication of accumulation of [14C]DACA between 1.5 and 15 min postinjection.

The tissue concentrations of [14C] radioactivity and [14C]DACA expressed as AUC together with the ratios of [14C] radioactivity or [14C]DACA in tissue to plasma are given in Table 4. The accumulation of [14C]DACA in the lung, kidney, heart, brain, and liver was 90, 24, 13, 8, and 1.7 times higher than that in plasma, respectively.

Table 3 Percentage of [14C] radioactivity as [14C]DACA, [14C]AQF, and other [14C]-labeled metabolites in rat (n = 3) and human (n = 22) plasma

<table>
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<tr>
<td>2.5</td>
<td>68.90 ± 3.46</td>
<td>7.85 ± 2.58</td>
<td>23.25 ± 5.90</td>
<td>77.50 ± 8.42</td>
<td>4.24 ± 2.4</td>
<td>18.26 ± 9.70</td>
</tr>
<tr>
<td>5</td>
<td>54.83 ± 9.27</td>
<td>18.70 ± 0.03</td>
<td>26.47 ± 6.41</td>
<td>68.56 ± 11.61</td>
<td>9.04 ± 5.47</td>
<td>22.40 ± 11.96</td>
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<tr>
<td>10</td>
<td>43.53 ± 3.33</td>
<td>25.78 ± 3.77</td>
<td>30.69 ± 3.60</td>
<td>52.39 ± 12.34</td>
<td>21.11 ± 8.27</td>
<td>26.50 ± 10.97</td>
</tr>
<tr>
<td>15</td>
<td>33.33 ± 1.48</td>
<td>27.79 ± 4.17</td>
<td>38.88 ± 2.10</td>
<td>47.43 ± 10.95</td>
<td>23.63 ± 5.42</td>
<td>28.94 ± 6.07</td>
</tr>
<tr>
<td>20–25</td>
<td>28.87 ± 1.45</td>
<td>29.56 ± 2.07</td>
<td>41.57 ± 3.0</td>
<td>34.56 ± 8.01</td>
<td>30.07 ± 7.49</td>
<td>35.37 ± 10.10</td>
</tr>
<tr>
<td>40–45</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>23.22 ± 6.13</td>
<td>32.48 ± 7.49</td>
<td>44.30 ± 10.10</td>
</tr>
<tr>
<td>60</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>20.40 ± 5.38</td>
<td>33.64 ± 16.13</td>
<td>45.96 ± 4.74</td>
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</tbody>
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Fig. 6. Images of an anesthetized rat, scanned using an ECAT-953B PET camera, following i.v. injection of 10 MBq of $^{11}$C-DACA. Top, horizontal slices through the image volume with the rat orientated nose-down, acquired at 0–5 s (a), 5–10 s (b), 1–2 min (c), 5–10 min (d), and 40–45 min (e) after injection. Bottom, coronal slices of the same volumes taken at the levels indicated by the lines on the horizontal slices. The images, visualized using Analyze software (13), have been rescaled so that the count range of each could be represented by the same arbitrary 8-bit color scale.

After an initial peak, the radioactivity cleared rapidly from both blood and plasma, such that by 60 min after injection, the radioactivity decreased to 0.001% of that injected activity per ml. The blood to plasma ratios were ~0.8 and did not change during the course of the study.

Analysis of Human Plasma. Five serial plasma samples from each patient were analyzed. The HPLC profile (Fig. 10) was similar to that observed for rat plasma (Fig. 4) except that the proportions of $^{11}$C-labeled metabolites differed. $^{11}$C-labeled metabolites Met1–Met5 eluted before and Met6 and Met7 eluted after the $^{11}$C-DACA peak. The percentage of $^{11}$C-DACA and Met1–Met7, together with the radioactivity in the AQF over 45 min, is shown in Fig. 11. Again, for simplicity, the radioactivity in Met1–Met7 was summed, and the results are given in Table 3, along with $^{11}$C-DACA and AQF. $^{11}$C-DACA decreased from 77 ± 8% (SD) at 2.5 min to 20 ± 5% at 60 min, whereas the radioactivity in AQF increased from 4 ± 2% at 2.5 min to 34 ± 16% at 60 min. The $^{11}$C-metabolites increased from 18 ± 10% at 2.5 min to 45 ± 5% at 60 min.

DISCUSSION

This study describes the preclinical ex vivo and in vivo evaluation in the rat of the anticancer agent DACA labeled with the positron-emitting radionuclide $^{11}$C. This involved sacrifice experiments and rat imaging using PET scanning. The work also describes how information on plasma metabolites was uniquely obtained in humans prior to Phase I clinical trial following i.v. injection of $^{11}$C-DACA at tracer dose.

After i.v. administration of $^{11}$C-DACA at tracer dose in rat and patients, $^{11}$C radioactivity cleared rapidly from blood and plasma (Figs. 2 and 9). Both curves were optimally represented by a two-exponent fit. Due to the short half-life of $^{11}$C and the consequently short duration of the study (90 min), it is not possible to determine t$_{1/2}$ accurately. However, the half-life of $^{11}$C radioactivity in rat blood between 15 and 90 min was calculated to be 3.2 h. This figure compares well with published data obtained in the mouse (15) and rats (16) using $^{3}$H-DACA at different dose ranges. In the mouse, over the dose range of 9–121 µmol/kg, DACA displayed linear kinetics, and the plasma concentration-time profile was fitted to a two-compartment model with t$_{1/2}$ = 1.6 ± 0.6 h. In the rat, after i.v. administration of 18, 55, and 81 µmol/kg $^{3}$H-DACA the plasma concentration-time profile exhibited biphasic elimination kinetics over 8 h postadministration; t$_{1/2}$ = 2.1 ± 0.7 h. Over this dose range, the kinetics of DACA were found to be first-order. The blood:plasma ratio for $^{11}$C-DACA in rat was ~1 and did not change during the course of the study, indicating that there was no accumulation of $^{11}$C radioactivity in RBC. A similar blood:plasma concentration ratio of 1 has been reported in mice after i.v. injection of $^{3}$H-DACA (15).

The metabolism of $^{11}$C-DACA in rat plasma was extensive and rapid (Figs. 4 and 5). A number of distinct radioactive peaks were observed. Due to the constraints of the short half-life of $^{11}$C and the need to analyze serial plasma samples rapidly, HPLC analyses were carried out under isocratic conditions. Consequently, some of the radioactive metabolites coeluted especially near the solvent front, and the separation achieved was not adequate to assign each radioactive peak as a single component; they were referred to as Met1–Met7 (Figs. 4 and 5). Met1–Met5 were less lipophilic than $^{11}$C-DACA, and...
Metabolites observed in the in vitro studies were identified in mouse urine, bile, and feces using $^1$H nuclear magnetic resonance and fast atom bombardment mass spectroscopy analysis (8). HPLC analysis of plasma samples from mice after i.v. administration of 30 µmol/kg $[^3]$H-DACA also showed the presence of at least five radioactive metabolites with persistently long half-life; however, they were not identified (15).

In rat plasma, at 2 min, $[^{11}]$C-DACA represented ~69% of the total $^{11}$C radioactivity; it decreased to ~29% at 25 min (Table 3). Paxton et al. (16) also found that at 2 min, 70–80% of the $^3$H radioactivity in rat plasma was due to $[^3]$H-DACA. Although a number of $^{11}$C-labeled metabolites were observed in rat plasma, the AQF contained Met6 and Met7 were more lipophilic. Met6 coeluted with reference compound (IV) (Table 1) and by inference was predicted to be the metabolite DACA-N-oxide. A broad span of radioactivity was observed eluting prior to the $[^{11}]$C-DACA peak, and this was referred to as Met5. Because $[^{11}]$C-DACA was labeled in the N-methyl position and only one of the methyl groups was labeled with $^1$C, only 50% of the predicted demethylation pathway would be detected. On this basis, it was difficult to say to what degree demethylation was taking place. No attempt was made to assign the other $^{11}$C radioactive peaks observed because adequate separation was not achieved. The in vivo metabolism of DACA has also been investigated in mice after both i.p. (17) and i.v. (15) administration of $[^3]$H-DACA.
METABOLISM OF $[^{11}]$C-DACA IN RATS AND HUMANS

Fig. 8. The distribution of total $[^{11}]$C radioactivity (■) and $[^{11}]$C-DACA (□) in rat tissues compared with plasma over a period of 1.5–40 min after i.v. injection of $[^{11}]$C-DACA.

~30% of radioactivity at 25 min. This was probably due to the presence of polar, conjugated compounds (e.g., glucuronides). The levels of other radioactive metabolites increased to 42% during the course of the study. The metabolite corrected plasma curve was satisfactorily fitted to a single exponential plus constant.

A PET scan of a rat (Fig. 6) showed redistribution of $[^{11}]$C radioactivity, suggesting little tissue retention and rapid excretion. This was confirmed by sacrifice experiments. $[^{11}]$C-DACA biodistribution studies in the rat showed initial accumulation of $[^{11}]$C radioactivity followed by rapid washout. The results reported are in broad agreement with the tissue distribution in mice following i.v. (15) and i.p. (17) administration of $[^{3}]$H-DACA. Although the numbers of radioactive metabolites observed in tissues were similar, the relative amounts of $[^{11}]$C-DACA and Met1–Met7 differed for each tissue and plasma. The clearance of $[^{11}]$C-DACA paralleled that of total $[^{11}]$C radioactivity in the case of lung, kidney, heart, and brain. $[^{11}]$C-DACA was the major component in the lung and heart. In the case of brain, $[^{11}]$C-DACA and the most lipophilic radioactive metabolite, Met7, were the only two major components. $[^{3}]$H-DACA has been reported to cross the blood-brain barrier in mice and to be retained after intracarotid administra-
Table 4  Tissue concentrations of $^{11}$C radioactivity and $[^{11}C]DACA$ expressed as AUC (MBq/g/min) and ratios of $^{11}$C radioactivity and $[^{11}C]DACA$ in rat tissue to plasma

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AUC (MBq/g/min)</th>
<th>AUC/AUC&lt;sub&gt;p&lt;/sub&gt; ratio</th>
<th>AUC (MBq/g/min)</th>
<th>AUC/AUC&lt;sub&gt;p&lt;/sub&gt; ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>3.84</td>
<td>1</td>
<td>1.68</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>73.55</td>
<td>19.15</td>
<td>24.44</td>
<td>14.57</td>
</tr>
<tr>
<td>Kidney</td>
<td>77.89</td>
<td>7.03</td>
<td>1.71</td>
<td>1.02</td>
</tr>
<tr>
<td>Liver</td>
<td>26.98</td>
<td>7.03</td>
<td>1.71</td>
<td>1.02</td>
</tr>
<tr>
<td>Heart</td>
<td>19.41</td>
<td>5.06</td>
<td>12.69</td>
<td>7.56</td>
</tr>
<tr>
<td>Brain</td>
<td>16.45</td>
<td>4.28</td>
<td>8.32</td>
<td>4.96</td>
</tr>
</tbody>
</table>

The concentration of $^{11}$C radioactivity and $[^{11}C]DACA$ in plasma and tissues was expressed as AUC, and the ratio of AUC in tissues to AUC in plasma was determined (Table 4). The latter ratio was high for lung, kidney, heart, and brain compared to the level in plasma over 40 min after i.v. administration of $[^{11}C]DACA$. In mice, the ratio of $^3$H radioactivity after an i.p. administration of $[^3H]DACA$ (410 μmol/kg) has been reported (17). Although the values differed, the findings cannot be directly compared because the difference could be due to a number of factors, such as species difference, route of administration, effect of dose, and duration of study.

Analysis of serial plasma samples from patients following i.v. administration of $[^{11}C]DACA$ showed that the metabolism of $[^{11}C]DACA$ was extensive and rapid (Figs. 10 and 11). Several radioactive metabolites were observed, and the profile was similar to that seen in the rat plasma except that the proportions differed. By inference with reference compound DACA-N-oxide (IV), it can be concluded that N-oxidation was occurring and producing Met6. No significant radioactive peak (Met5) was observed coeluting with N-monomethyl-DACA (V). It appears that in human plasma, polar or conjugated species were also being produced.

In conclusion, the methodology developed has been successfully used to establish the fate of $[^{11}C]DACA$ at tracer doses. Studies with $[^{11}C]DACA$ in animals gave biodistribution and metabolism results that were broadly similar to those obtained in the conventional way using tritiated DACA. In this particular case, we also determined the levels of unchanged $^{11}$C]DACA and its radioactive metabolites in serial tissue samples from rats. The methodology developed has been successfully applied to establish the fate of $[^{11}C]DACA$ at tracer doses in human plasma for the first time in studies carried out prior to Phase I clinical trials of DACA. This approach of labeling compounds with short-lived positron-emitting radionuclides with preclinical evaluation in animals and humans has the potential to provide unique pre-Phase I information.
REFERENCES


Studies on the Metabolism of the Novel Antitumor Agent \[N\text{-}methyl-^{11}\text{C}]N\text{-}2\text{-}(\text{dimethylamino})\text{ethyl}\text{]acridine-4-carboxamide in Rats and Humans prior to Phase I Clinical Trials}

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