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ORIGINAL ARTICLE

Plasma pharmacokinetic evaluation of cytotoxic agents radiolabelled with positron emitting radioisotopes

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Abstract
Purpose This study aimed to evaluate the utility of plasma pharmacokinetic analyses of anti-cancer agents from data obtained during positron emission tomography (PET) oncology studies of radiolabelled anti-cancer agents.
Patients and methods Thirteen patients were administered fluorine-18 radiolabelled 5-FU ([^{18}F]5-FU) admixed with 5-FU, corresponding to a total 5-FU dose of 380–407 mg/m^2 (eight patients) and 1 mg/m^2 (five patients). Nine patients received 2.2–19.2 μg/m^2 of carbon-11 radiolabelled *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide ([^{11}C]DACA) at 1/1,000th of phase I dose, as part of phase 0 microdosing study. Radioactivity of parent drug obtained from arterial blood samples, the injected activity of the radiolabelled drug, and the total dose of injected drug were used to obtain plasma drug concentrations. Plasma pharmacokinetic parameters were estimated using model-dependent and model-independent methods.
Results 5-FU plasma concentrations at therapeutic doses were above the Km and a single compartment kinetic model was best used to fit the kinetics, with a mean half-life of 8.6 min. Clearance and volumes of distribution (V_d) obtained using both model-dependent and model-independent methods were similar. Mean (SE) clearance was 1,421(144), ml min^{-1} and 1,319 (119) ml min^{-1} and the mean (SE) V_d was 17.3 (1.8) l and 16.3 (1.9) l by the model-independent method and model-dependent methods, respectively. In contrast, with 1 mg/m^2, plasma concentrations of 5-FU were less than the Km and a two-compartment model was used to best fit the kinetics, with the mean 5-FU half-life of 6.5 min. The mean (SE) clearances obtained by the model-independent method and model-dependent methods were 3,089 (314) ml min^{-1} and 2,225 (200) ml min^{-1}, respectively and the mean (SE) V_d were 27.9 (7.0) l and 2.3 (0.4) l, by the model independent and dependent methods, respectively. Extrapolation of $AUC_{0–Clast}$ to $AUC_{0–\infty}$ was less than 3% in both these cohort of patients. A two-compartment model with a mean half-life of 42.1 min was used to best fit the kinetics of DACA; considerable extrapolation (mean 26%) was required to obtain $AUC_{0–\infty}$ from $AUC_{0–Clast}$. Mean (SE) clearance of DACA was 1,920 (269) ml min^{-1}, with the model-independent method and 1,627 (287) ml min^{-1} with the model-dependent method. Similarly, V_d [mean (SE)] of DACA with the model-independent and model-dependent methods were 118 (22) l and 50 (15) l, respectively.
Conclusions Pharmacokinetic parameters can be estimated with confidence from PET studies for agents given at therapeutic doses, whose half-lives are significantly less than the total sampling time during the scan. Tracer studies performed alone, wherein plasma levels below the Km are expected, may also provide valuable information on drug clearance for the entire range of linear kinetics. However,

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drugs with half-lives longer than the sampling duration are inappropriate for PET plasma pharmacokinetic evaluation.

Keywords PET · Pharmacokinetics · Tracer studies · 5-Fluorouracil · *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide · Microdosing

Introduction

Pharmacokinetic evaluation has proved invaluable in the pharmacological development of therapy and greatly aided the formulation of an administration strategy such as schedule, dose and route of administration. However, the inability to select concentration–time values that discriminate anti-tumour activity from normal tissue toxicity has limited the utility of plasma pharmacokinetics in anti-cancer drug development. This limitation is mainly due to an inadequate understanding of basic patho-physiological processes of cancer and of the differences between normal and tumour tissues. Moreover, the heterogeneous nature of tumours and inter-organ differences in drug handling make extrapolation of plasma data to normal tissue and tumours difficult. Despite this, pharmacokinetic parameters obtained from plasma data have generally been used to explain tissue pharmacokinetic behaviour and served to optimise therapy with anti-cancer agents such as methotrexate [1] and carboplatin [2]. The need to further understand tissue drug pharmacokinetics together with the quest to widen the narrow therapeutic index of cytotoxic therapy has led to utilisation of non-invasive methods such as magnetic resonance spectroscopy (MRS) [3] and positron emission tomography (PET) [4], which allow additional tissue pharmacology to be evaluated.

Positron emission tomography is a quantitative and highly sensitive dynamic imaging modality that can provide in vivo tissue and tumour data with high temporal resolution, not available by other means. This allows the quantification of picomolar quantities of administered compounds and makes it an ideal tool in the evaluation of tissue pharmacology of radio labelled drugs, administered at tracer (microgram) quantities in man. These advantages, aided by recent developments in radiochemical synthesis of organic compounds, allowing a substantial number of new drug candidates to be radio labelled with positron-emitters has generated an increased interest in the "microdosing" study concept, wherein PET studies are performed after tracer amounts of radiolabelled drugs are administered [5]. This will potentially aid anti-cancer drug development (lead selection and optimisation), as currently three of four cases of new chemical entities under development fail as drug candidates in clinical trials. It is also likely to reduce the associated costs and demands to perform pre-clinical animal experiments [5]. In general, PET can provide information on distribution of drugs to tumour and normal tissue, drug clearance, target modulation and also help to predict response to therapy. Since blood sampling is usually performed during PET pharmacokinetic studies to generate tissue pharmacokinetic parameters, an envisaged increase in "phase 0" microdosing studies prior to the conventional phase I clinical trials is likely to provide a unique opportunity in obtaining early plasma pharmacokinetic data in man, prior to that classically obtained in phase I and II clinical trials.

We therefore evaluated the utility of plasma pharmacokinetic analyses of anti-cancer agents performed during PET studies of radiolabelled anti-cancer agents. These studies were performed as part of our anti-cancer drug development translational research programme and includes plasma pharmacokinetic data obtained from the world's first microdosing study of an anti-cancer agent, performed prior to a conventional phase I clinical trial in man [6]. Additionally, plasma pharmacokinetics of another well-established anti-cancer agent, 5-fluorouracil (5-FU) was also evaluated. In contrast to conventional plasma pharmacokinetic studies, where plasma drug versus time concentrations are obtained from venous blood samples usually up to several hours after drug administration and used in the analyses, results reported in this paper are based on limited arterial blood sampling data. Arterial blood sampling was limited by the short scan duration (due to the short half-life of the radioisotopes used and patient comfort) and was performed to obtain an arterial input function, used for tissue pharmacokinetic modelling. In addition, plasma drug concentrations were non-classically obtained from measurements of plasma radioactivity, the contribution by the parent and metabolites to the total radioactivity and the total dose of drug injected.

Materials and methods

Plasma pharmacokinetic analyses in patients taking part in PET studies to evaluate the normal tissue and tumour pharmacokinetics were performed for two anti-cancer agents, 5-fluorouracil (5-FU) and *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA). Fluorine-18 and carbon-11 were used to radiolabel 5-FU [7, 8] and DACA [9], respectively. Three groups of patients with cancer were included in this study in whom discrete arterial blood was sampled at pre-determined time points for evaluation of metabolites and for calibration of the continuous online samples. We have used data obtained from discrete arterial blood samples to obtain plasma pharmacokinetic parameters in this paper.

The first group consisted of patients taking part in a 5-FU biomodulation PET study, where [^{18}F]5-FU-PET was performed after 5-FU was administered either on its own or in combination with biomodulators [10]. Eight patients who underwent [^{18}F]5-FU-PET studies in the absence of biomodulator co-administration were included in this group. All patients received intravenous [^{18}F]5-FU admixed with unlabeled 5-FU. The dose of unlabelled 5-FU co-administered with [^{18}F]5-FU was 375–400 mg/m^2, corresponding to a total 5-FU (labelled and unlabelled) dose of 380–407 mg/m^2. Plasma drug concentrations were obtained from discrete arterial samples typically at 5, 10, 20, 30, 40 and 60 min in this cohort of patients and were used in the analysis. The second group included those taking part in another biomodulation trial of 5-FU with eniluracil, an inactivator of dihydropyrimidine dehydrogenase (DPD), the primary rate-limiting pyrimidine metabolising enzyme [11]. Five patients who underwent [^{18}F]5-FU-PET studies, about 1 week before being started on oral eniluracil/5-FU were included in this group. The total dose of 5-FU (labelled and unlabelled) administered was 1 mg/m^2 and discrete arterial samples typically obtained every half minute from 1.5 min until 5 min and thereafter at 10, 30 and 60 min were used in the analysis. The third group of nine patients were those taking part in a novel microdosing study performed prior to a conventional phase I trial of DACA, as part Cancer Research, UK's novel drug development programme [6]. The dose of DACA administered (2.2–19.2 μg/m^2) was 1/1,000th of the phase I starting dose of 9 mg/m^2. Plasma drug concentrations calculated from discrete arterial samples typically obtained at 2.5, 5, 10, 20, 40 and 60 min after the injection of tracer amount of [^{11}C]DACA in this cohort of patients, were used in the analysis in this group of patients.

Discrete arterial blood samples at the pre-determined time points corrected for radioactive decay were collected and analysed for plasma radioactivity and the contribution of the parent drug and metabolites to total radioactivity, as described previously [6, 10, 11]. Plasma concentrations of the injected drug were calculated from the injected activity of the radiolabelled drug (MBq), the total dose of injected drug (labelled and unlabelled 5-FU or DACA in mg; derived from specific radioactivity) and plasma radioactivity of parent drug (5-[^{18}F]FU or [^{11}F]DACA; MBq/ml) at discrete time points. Plasma pharmacokinetic parameters were estimated using model-dependent (ADAPT® II, Release 4, D. D'Argenio and A. Shumitizsky, Los Angeles, CA, USA) [12] and model-independent methods. The robustness of fits was assessed using the Akaike information criterion. The trapezoidal method was used to calculate the AUC with the model-independent method. Non-parametric tests were used to make statistical comparisons in all the studies with P values ≤ 0.05 being considered to be statistically significant.

Results

Figure 1a–c illustrate the mean time–activity curves (TACs), corrected for radioactive decay. These TACs are not normalised for the injected activity (or do not account for differences in injected activity between patients) for the total radiotracer and parent compound. The lesser magnitude of peak plasma activity observed with full doses of 5-FU is mainly a result of the timing of blood samples. In contrast to the first sample being obtained at 5 min with full doses of 5-FU, blood samples obtained from 1.5 min and 2.5 min onwards, with tracer doses of 5-FU and DACA, respectively. This enabled samples to be obtained when peak plasma activity was reached, in patients receiving tracer doses of 5-FU and DACA. Mean TACs for full and tracer doses [^{18}F]5-FU and [^{11}C]DACA corrected for radioactive decay and not normalised for the injected activity are plotted in Fig. 1d for comparison.

Full dose 5-FU pharmacokinetics

The mean (range) radioactivity of injected [^{18}F]5-FU was 168 (93–210) MBq/m^2 and the total dose of 5-FU (labelled and unlabelled) administered was 380–407 mg/m^2 in the first group of patients. Arterial samples were taken at the pre-determined time-points except in two patients (patients nos. 1 and 8), where the 40 and 60 min samples were replaced by a single 45 min sample. In one patient (patient no. 1), the 45 min sample was venous. The mean (SE) contribution of [^{18}F]5-FU to total radioactivity at 5 and 10 min was 91% (1.5) and 81% (2.3), respectively, which decreased to 26% (4.3) at 30 min and 4% (0.8) at 60 min. Figure 1a illustrates total ^{18}F and [^{18}F]5-FU TACs, corrected for radioactive decay. [^{18}F]α-fluoro-β-alanine was the primary radiolabelled metabolite identified.

Plasma concentration versus time curves of 5-FU at full doses for the eight patients are illustrated in Fig. 2a. Maximal plasma 5-FU concentrations [mean (SE)] of 37,833 (2,758) ng/ml [corresponding to 291 μM (average); range 219–407 μM] were reached at earliest sampled time point after injection (5 min). 5-FU kinetics appeared to follow a single compartment kinetic model with a mean (SE) half-life of 8.6 min (Table 1). The mean clearance and volume of distribution obtained using both model-dependent and model-independent methods were similar indicating the validity of the model used (Table 4). The mean (SE) clearances obtained by the model-independent method and

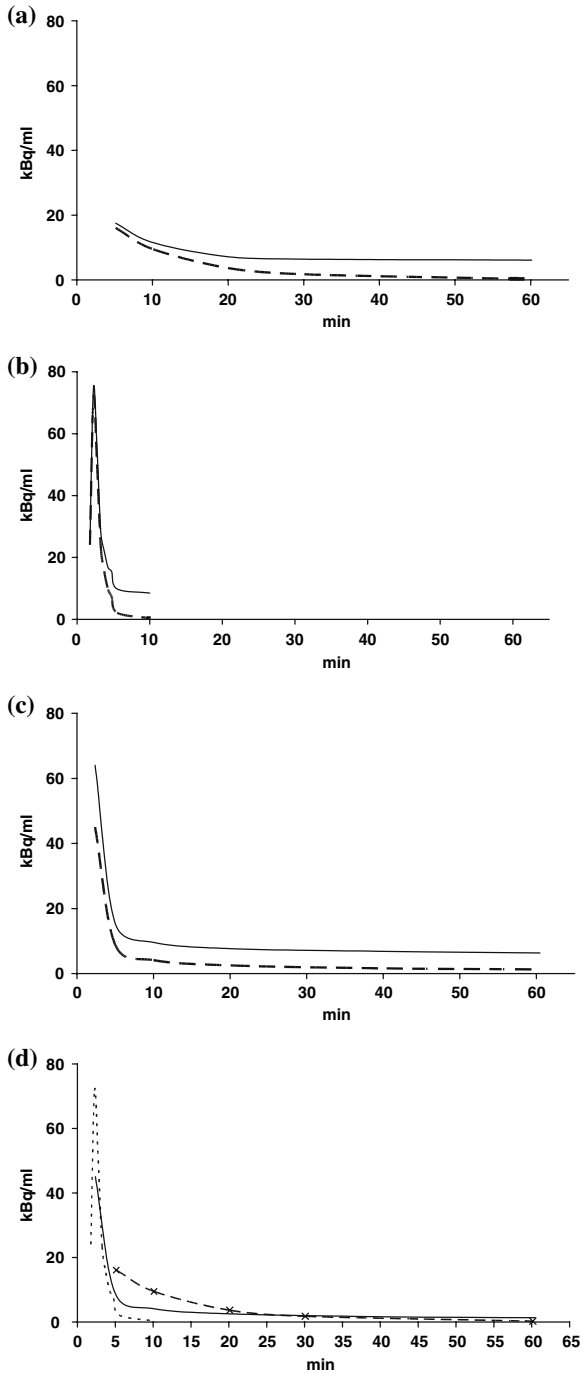

Fig. 1 (**a**–**c**) Mean time–activity curves (TACs) corrected for radioactive decay for total radiotracer (*unbroken line* ^{18}F or ^{11}C) and parent compound (*broken line* 5-[^{18}F]FU or [^{11}C]DACA. Mean TACs with full dose 5-FU (**a**), tracer doses of 5-FU (**b**), and tracer doses of DACA (**c**) are illustrated separately for comparison. The mean (range) radioactivity of [^{18}F]5-FU injected with full dose studies was 168 (93–210) MBq/m^2 (**a**), and 202 (180–218) MBq/m^2 with tracer doses of 5-FU (**b**). Mean (range) of [^{11}C]DACA injected with tracer doses was 301 (194–395) MBq/m^2 (**c**). Mean TACs for full dose 5-FU (*broken line with crosses*), tracer dose of 5-FU (*broken line*) and DACA (*unbroken line*) are also shown for comparison in (**d**). All TACs illustrated in this figure have been corrected for radioactive decay but not been normalised to account for differences in injected activity between patients

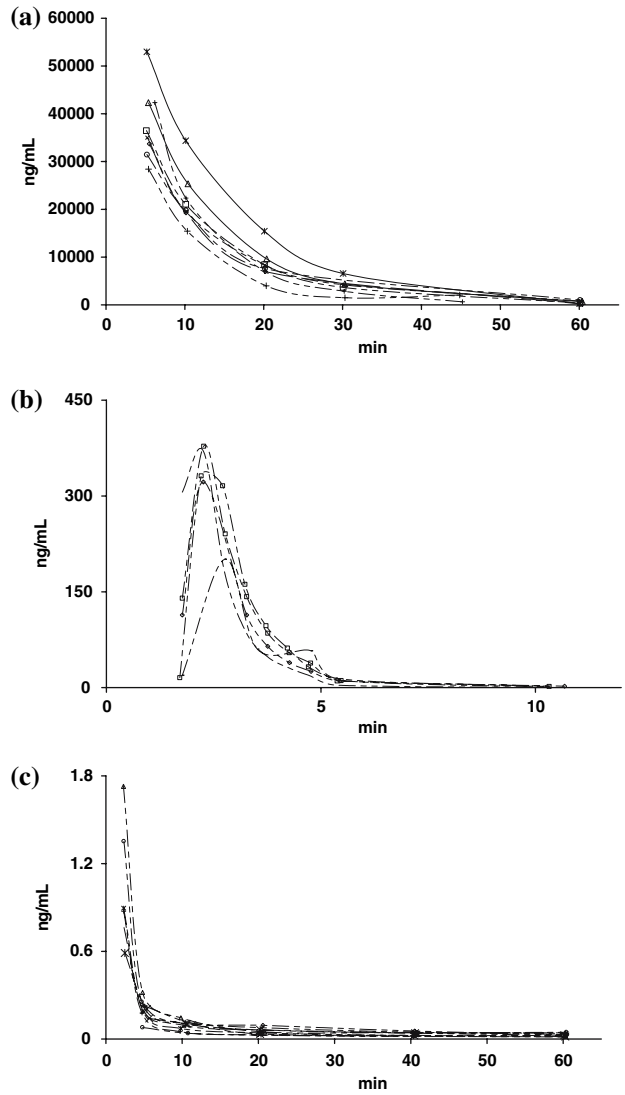

Fig. 2 Plasma time versus concentration curves for (**a**) 5-FU at full dose (380–407 mg/m^2) (**b**) 5-FU at tracer doses (1 mg/m^2) and (**c**) DACA at tracer doses (4.1–9.9 μg/m^2). Please note that the *X* and *Y* axes for the three figures are different

model-dependent methods were 1,421(144) ml min^{-1} and 1,319 (119) ml min^{-1}, respectively. Similarly, the mean (SE) volume of distribution values were 17.3 (1.8) l and 16.3 (1.9) l, by the model-independent and model-dependent methods, respectively. The mean percentage extrapolation from C_{last} to obtain $AUC_{0-\infty}$ with the model-independent method was less than 2%. Individual plasma clearance and volume of distribution values obtained using model-independent methods are shown in Table 1.

Tracer dose 5-FU pharmacokinetics

The mean (range) radioactivity of injected [^{18}F]5-FU was 202 (180–218) MBq/m^2 and the total dose of 5-FU

Table 1 Pharmacokinetic parameters of 5-FU at full doses

Patient no.	$t_{1/2}$ (min)	$AUC_{0-\infty}$ (ng ml^{-1} min)	Extrap(%)	Clearance (ml min^{-1})	V_d (l)
1	6.9	481,529	1.34	1,348	13.3
2	11.8	533,088	3.28	1,320	22.5
3	7.7	489,600	0.47	1,316	14.6
4	8.6	505,730	0.89	1,563	19.3
5	9.4	609,244	1.47	1,150	15.6
6	10.6	509,874	2.41	1,591	24.4
7	7.5	815,265	0.39	839	9.0
8	6.1	317,628	4.14	2,243	19.6
Mean (SE)	8.6 (0.7)	532,744 (49,592)	1.8 (0.5)	1,421[a] (144)	17.3 (1.8)

The half-life was calculated by fitting a two-compartmental model and the percentage extrapolated to obtain $AUC_{0-\infty}$ using the model-dependent method is given in column 4. $AUC_{0-\infty}$, clearance and V_d in the table were calculated by the model-independent method

[a] Corresponds to clearance of 798 (70) ml min^{-1} m^{-2}

Table 2 Pharmacokinetic parameters of 5-FU at tracer doses

Study no.	$t_{1/2}$ (min)	$AUC_{0-\infty}$ (ng ml^{-1} min)	Extrap(%)	Clearance (ml min^{-1})	V_d (l)
9	9.5	612	8.6	3,055	41.9
10	9.4	607	0.4	2,506	34.3
11	8.8	525	1.1	3,264	41.3
12	1.9	412	1.4	4,175	11.7
13	3.0	687	0.2	2,444	10.4
Mean (SE)	6.52 (1.7)	569 (47)	2.3 (1.6)	3,089[a] (314)	27.9 (7.0)

The half-life was calculated by fitting a two-compartmental model and the percentage extrapolated to obtain $AUC_{0-\infty}$ using the model-dependent method is given in column 4. $AUC_{0-\infty}$, clearance and Vd in the table were calculated by the model-independent method

[a] Corresponds to clearance of 1,764 (167) ml min^{-1} m^{-2}

(labelled and unlabelled) administered was 1 mg/m^2. Arterial samples were taken at the pre-determined time points. Unlike patients who received full doses of 5-FU, rapid metabolism of [^{18}F]5-FU was observed in patients who received tracer doses of 5-FU (Fig 1b, d). The mean (SE) contribution of [^{18}F]5-FU to total radioactivity at 2 min was 95% (1.1), which decreased to 25% (2.1) at 5 min and 4% (0.8) at 10 min. The contribution of [^{18}F]5-FU to total radioactivity was <1% at 30 min. Figure 1b illustrates total ^{18}F and [^{18}F]5-FU TACs, corrected for radioactive decay, which demonstrates rapid metabolism of [^{18}F]5-FU at tracer doses compared with full doses (Fig. 1a). [^{18}F]α-fluoro-β-alanine was the primary radioactive metabolite identified.

Plasma concentration versus time curves of 5-FU at tracer doses for the five patients are illustrated in Fig. 2b. Maximal plasma 5-FU concentrations [Avg (SE)] of 321 (32) ng/ml [corresponding to 2.5 μM (average); range 1.6–2.9 μM] were reached at 2 min post-injection. A two-compartment model was used to best fit the kinetics of 5-FU given at a dose of 1 mg/m^2. Despite the rapid metabolism of 5-FU, there was little difference in the concentration of 5-FU between two successive time points in one patient (Fig. 2b) resulting in a poor fit of the model used. The mean half-life of 5-FU was 6.5 min (range 1.9–9.5 min; Table 2). The mean (SE) clearances obtained by the model-independent method and model-dependent methods were 3,089 (314) ml min^{-1} and 2,225 (200) ml min^{-1}, respectively, and the mean (SE) volume of distribution values were 27.9 (7.0) l and 2.3 (0.4) l, by the model independent and dependent methods, respectively (Table 4). The mean percentage extrapolation from C_{last} to obtain $AUC_{0-\infty}$ with the model-independent method was 2.3%. Individual plasma clearance and volume of distribution values obtained using model-independent methods are given in Table 2.

Pharmacokinetics of DACA at 1/1,000 phase I starting dose

The mean (range) radioactivity of injected [^{11}C]DACA was 301 (194–395) MBq/m^2 and the mean total dose of DACA administered in this group of nine patients was 6.9 μg/m^2 (range 4.1–9.9 μg/m^2). Arterial samples were taken at the pre-determined time points except in three patients (patient nos. 20, 21 and 22), where 2.5 min

Table 3 Pharmacokinetic parameters of DACA at tracer doses

Patient no.	$t_{1/2}$ (min)	$AUC_{0-\infty}$ (ng ml^{-1} min)	Extrap(%)	Clearance (ml min^{-1})	V_d (l)
14	36.36	8.28	26.99	1,122	59
15	35.07	6.09	20.86	1,280	65
16	39.24	4.98	18.54	1,950	110
17	51	10.59	32.15	1,699	125
18	53.13	11.53	25.87	937	72
19	43.06	2.56	34.93	3,277	204
20	57.55	4.11	34.36	3,016	250
21	27.5	6.09	19.74	2,215	88
22	35.69	6.51	20.54	1,783	92
Mean (SE)	42.07 (3.3)	6.75 (0.98)	25.99 (2.2)	1,920 (269)	118 (22)

The half-life was calculated by fitting a two-compartmental model and the percentage extrapolated to obtain $AUC_{0-\infty}$ using the model-dependent method is given in column 4. $AUC_{0-\infty}$, clearance and V_d in the table were calculated by the model-independent method

[a] Corresponds to clearance of 1,139 (131) ml min^{-1} m^{-2}

Table 4 Comparison of pharmacokinetic parameters derived by model-independent and model-dependent methods

5-FU	Clearance (ml min^{-1})		V_d (l)		$AUC_{0-\infty}$ (ng ml^{-1} min)	
	Model independent	Model dependent	Model independent	Model dependent	Model independent	Model dependent
5-FU 380–407 mg/m^2	1,421 (144)	1,319 (119)	17.3 (1.8)	16.3 (1.9)	532,744 (49,592)	570,002 (53,028)
5-FU 1 mg/m^2	3,089 (314)	2,225 (200)	27.9 (7.0)	2.3 (0.4)	569 (47)	782 (51)
DACA 4.1–9.9 μg/m^2	1,920 (269)	1,627 (287)	118 (22)	50 (15)	6.75 (0.98)	8.47 (1.36)

samples were not done. The mean (SE) contribution of [^{11}C]DACA to total radioactivity at 2.5 min and 5 min was 72% (2.8) and 58% (2.1), respectively. This decreased to 23% (2.3) at 40 min and 20% (1.8) at 60 min. Figure 1c illustrates total ^{11}C and [^{11}C]DACA TACs curves, corrected for radioactive decay. In contrast to tracer doses of 5-FU, where tracer doses were similarly administered as with DACA, the absence of rapid elimination of DACA allowed plasma levels to be estimated to the very end of the sampling period in this cohort of patients. One of the seven radioactive metabolites observed was identified as [^{11}C]DACA-N-oxide [13].

Plasma concentration versus time curves of DACA for the nine patients are illustrated in Fig. 2c. A two-compartment model was used to best fit the kinetics of DACA given at tracer doses of 1/1,000 of the phase I starting dose. The mean half-life of DACA was 42.1 min (range 27.5–57.6 min; Table 3). Mean (SE) clearance of DACA was 1,920 (269) ml min^{-1}, with the model-independent method and 1,627 (287) ml min^{-1} with the model-dependent method. Similarly, V_d [mean (SE)] of DACA with the model-independent and model-dependent methods was 118 (22) l and 50 (15) l, respectively (Table 4). Overall, considerable extrapolation (mean 26%; range 18.5%–34.9%) was required to obtain $AUC_{0-\infty}$ from $AUC_{0-Clast}$ with the model-independent method. Individual plasma clearance and volume of distribution values obtained using model-independent methods are given in Table 3.

A comparison of model-dependent and model-independent pharmacokinetic parameters for all the three cohort of patients is given in Table 4.

Discussion

Traditionally, pharmacokinetic analyses are performed by serial evaluation of venous plasma samples up to a few days after injection of the drug. With increasing utilisation of PET to evaluate normal tissue and tumour pharmacokinetics of drugs radiolabelled with short half-lived positron-emitters, there is an opportunity to evaluate the plasma pharmacokinetics of these agents, at the same time. This is the first of such reported analyses where conventional plasma pharmacokinetic methodology has been used to derive plasma pharmacokinetic parameters, from arterial samples obtained during the PET scanning procedure. In this analyses, plasma pharmacokinetics of two anti-neoplastic agents, 5-FU [14], a fluorinated pyrimidine analogue in clinical use for more than 40 years and DACA, a topoisomerase I and II inhibitor were evaluated by our group. Studies with carbon-11 radiolabelled DACA were performed as part of a novel drug development strategy, where

DACA microdosing studies were initiated prior to phase I clinical studies [6].

A number of plasma pharmacokinetics studies performed with 5-FU have demonstrated that peak plasma 5-FU concentrations of 100–1,000 μM are reached after intravenous bolus injections of 400–600 mg/m^2, followed by rapid metabolism with a primary half-life of 6 to 20 min that is known to vary significantly between patients. Plasma pharmacokinetic data have been fitted to a single-compartmental [15, 16], a two-compartmental model [17] and a three-compartment model [18]. 5-FU demonstrates a non-linear decrease in systemic clearance [17, 18], with increasing doses, with an apparent Km of 15 μM (1,950 ng/ml) [17]. Clearance values of 5-FU have been reported to be in the range of 0.46–0.75 l/min/m^2 for intravenous bolus 5-FU doses of 330–500 mg/m^2 [18–20]. With the continuous infusional schedules, where steady state 5-FU plasma concentrations (Css) below the Km are observed, 5-FU demonstrates linear kinetics. Css range between 0.005 and 0.011 μM (for 300–500 mg/m^2) [21] to 3.4–7.5 (for 1,250–2,250 mg/m^2) [22]. Clearance values with continuous infusional schedules are also much higher compared with intravenous bolus injectional schedules and range between 1.1 and 4 l/min/m^2 [17, 22–24]. These clearance values, which are significantly higher than the hepatic perfusion (1.5 l/min) are most likely due to the significant plasma metabolism of 5-FU by DPD, the primary rate-limiting pyrimidine metabolising enzyme in lymphocytes [23].

In our cohort of patients, plasma pharmacokinetics of 5-FU were evaluated at full therapeutic doses (380–407 mg/m^2) and tracer doses (1 mg/m^2) of 5-FU. A single compartmental model was used to fit the pharmacokinetic data of 5-FU at full dose as also used by others [15, 16]. Overall, plasma pharmacokinetic parameters of 5-FU at full therapeutic doses including plasma levels, half-life, clearance and V_d were consistent, with previously published data [18–20]. There was also concordance in plasma pharmacokinetic parameters derived using the model-dependent and model-independent methods with full doses of 5-FU underlining the utility of PET derived PK parameters.

As expected, plasma concentrations below the Km (15 μM; 1,950 ng/ml) were observed in our cohort of patients who received tracer doses of 5-FU (1 mg/m^2). In contrast to full doses of 5-FU, there was rapid clearance of 5-FU with tracer doses of 5-FU. 5-FU clearance in this cohort of patients, who received tracer doses of 5-FU was similar to values obtained with prolonged infusional schedules of 5-FU at doses, wherein plasma levels 5-FU are also below Km [17, 22–24]. However, a 12-fold variation in the V_d values obtained with the model-dependent and model-independent methods, despite minimal (2%) extrapolation of $AUC_{0-tlast}$ to $AUC_{0-\infty}$, suggests that the two-compartmental model used may be incorrect, despite good fits being obtained in four of the five patients. These discrepancies in parametric values also highlight the limited accuracy in the determination of terminal half-life, especially with rapidly metabolising agent such as 5-[^{18}F]FU administered at tracer doses, as small changes in terminal half-life are likely to have a large impact on the parametric values. It is therefore imperative that frequent blood sampling is performed when accurate data are required as in tissue kinetic modelling studies using PET. For this reason, continuous on-line arterial sampling is carried out in PET pharmacokinetic studies, from which a continuous plasma time–activity curve is derived by fitting the plasma metabolite and radioactivity calibration data obtained from discrete arterial blood samples to the online continuous sampling data. However, it is heartening to note from our studies that despite the caveats, clearance values obtained with tracer doses (1.8 l/min/m^2) are broadly in keeping with the clearance of 5-FU given as a prolonged continuous infusions (1.1–4 l/min/m^2), wherein plasma concentrations reached are also less than the Km (15 μM; 1,950 ng/ml). In contrast, where the mass of the drug associated with radioactivity is large, as with full dose 5-FU and the half-life of the drug is less than the sampling/scanning time it is possible to determine pharmacokinetic parametric values with confidence and good congruence with both the model- dependent and independent methods.

DACA, a topoisomerase I and II inhibitor exhibits linear pharmacokinetics when given either as a 3 or a 120 h infusion schedules. Classical pharmacokinetic studies performed with these schedules during early drug development demonstrated that DACA has a mean terminal elimination half-life of 2 h [25–27]. However, there was considerable variation in parametric values for volume of distribution at steady state (V_{ss}) and clearance with both the 3 h and 120 h infusions. V_{ss} values with the 3 h infusion were 0.5 to 4 l/kg (mean 0.7 l/kg) [25] and 84 to 207 l/m^2 (mean 138.1 l/m^2) with the 120 h infusion schedule [26]. Clearance values of 1 (±0.4) l/h/kg [25] and 84 l/h [27] were obtained with the 3 h infusion and with the 120 h infusion, clearance values of 69 (±30) l/h were attained, representing inter-patient variability of 44% [26].

In contrast to the rapid metabolism of 5-FU at tracer doses, which prevented estimation of plasma drug levels at latter time points, despite being radiolabelled with a longer half-lifed radioisotope (half-life of ^{18}F 110 min), we were able to estimate plasma levels of DACA until the end of the planned sampling duration. This was due to longer elimination half-life of DACA (2 h from clinical studies) [25–27], which allowed the estimation of plasma DACA levels, despite being radiolabelled with carbon-11 (half-life 20 min), which has a shorter half-life compared to fluorine-18 (half-life 110 min), thus underlying the importance of both the biological and radioactive half-life in such studies.

In our cohort of patients, who underwent PET studies with tracer quantities of radiolabelled DACA, a mean terminal elimination half-life of 42 min was observed. As expected from a half-life that is two-thirds of the total sampling time, a 26% extrapolation was required to generate $AUC_{0-\infty}$ and is likely to introduce considerable uncertainty in the parametric values especially in those obtained using the model-independent method. Consequently, considerable differences were observed between parameters obtained by the model dependent and independent methods, which are also likely to result from uncertainties in the determination of terminal half-life. Clearance values for DACA obtained in our study using the model-dependent method (1,920 ml/min corresponding to 115 l/h) were higher than obtained from clinical studies and reflect the limited duration of sampling, especially for an agent whose half-life is known to be longer than the scan duration. Although it is possible that sampling of blood for a longer duration may have provided an accurate drug pharmacokinetic data, limited half-life of positron emitters makes it difficult to sample data for prolonged durations. The limited data obtained precludes us from making any prediction

In this paper, we have explored the utility of plasma pharmacokinetic parameters derived from arterial blood sampling performed during PET tracer studies of two anticancer agents, 5-FU and DACA. These studies provide valuable information and guidance on the utility of PET plasma pharmacokinetic data and the associated limitations. Although there is significant first pass extraction of 5-FU from portal venous blood by the liver [17], there is little evidence to suggest that drug concentrations in peripheral arterial blood are significantly different from that in venous blood, as 5-FU or DACA are not preferentially extracted by other tissues. Moreover, there is unlikely to be differential in metabolism of 5-FU by plasma lymphocytes in the arterial and venous blood. Therefore, both arterial and venous blood sampling are entirely appropriate in obtaining plasma pharmacokinetic parameters for 5-FU and DACA.

We found full concordance in the pharmacokinetic parameters for 5-FU given at therapeutic bolus doses (380–407 mg/m^2). Rapid equilibration of radiolabelled and unlabelled 5-FU ensured that the sampled radioactivity data at latter time points was less noisy and allowed quantification until final sampling times. Moreover, the limited half-life of 5-FU (<10 min) compared with the total sampling time (60 min) in our study allowed for minimal extrapolation and hence confidence in reliability of the models used. Despite the supportive arguments of a short half live and a longer sampling time with tracer doses of 5-FU alone in our second cohort of patients, the rapid clearance of 5-FU at plasma levels below Km introduces uncertainties in the estimation of the elimination rate constant and therefore also on parameters such as V_d, using the model dependent method. On the other hand, the minimal extrapolation of the AUC required, allows the estimation of 5-FU clearance using the model-independent method less dependent on the elimination rate constant. 5-FU clearance values obtained in our study with tracer doses (1.8 l/min/m^2) were similar to that for prolonged venous infusions of 5-FU (1.1 to 4 l/min/m^2), where plasma concentrations are also below Km (15 μM; 1,950 ng/ml). Although, clearance values were similar to that obtained in the linear kinetic range of 5-FU, we advice caution in the extrapolation of all PET pharmacokinetic parameters to explain continuous venous infusions, as parameters such as V_d are normally performed when steady state levels are reached with continuous infusions and AUC for the total duration of infusion is used in the calculation of pharmacokinetic parameters.

Finally, with DACA, the long half-life compared to the limited sampling time led to more than 25% extrapolation, leading to inaccuracies with the parameter estimates. Pharmacokinetic data for such a compound, which is known to have a terminal elimination half-life longer than the scan duration is certain to be associated with errors, as was seen in our cohort of patients. Although there was significant inter-patient variability in clearance of DACA with the early clinical studies [25–27], PET pharmacokinetic clearance values were much higher compared to the clinical studies. Although PET pharmacokinetic studies can contribute significant tissue pharmacokinetic data with such agents [28, 29], it is only possible to obtain the approximate clearance of such agents from the blood data alone.

Our conclusion from this evaluation is that pharmacokinetic parameters can be estimated with confidence, especially for agents whose half-lives are significantly less than the total sampling time. Tracer studies in combination with full therapeutic doses for such agents can provide pharmacokinetic data similar to that obtained with conventional early clinical studies. Tracer studies performed alone, wherein plasma levels below the Km are attained, may also provide information of clearance values for the entire range of linear kinetics. Finally, a drug with half-life which is longer than the sampling duration is inappropriate for PET plasma pharmacokinetic evaluation and there is little added value in such assessment, other than for exploratory purposes. Although it is difficult to be precise, on sampling duration vis a vis terminal elimination half-life of the drug, first principles and supportive evidence from our studies suggest that meaningful data can be obtained from PET tracer studies of anti cancer agents, where sampling times are at least four times the terminal elimination half-life of the agent.

In conclusion, we have demonstrated that PET tracer kinetic studies can provide meaningful plasma pharmacokinetic data for selected anti-cancer agents radiolabelled with short half-life positron emitters. Overall, we have underscored

the value of performing plasma pharmacokinetics, when PET tissue pharmacokinetic studies are being performed.

References

1. Stoller RG et al (1977) Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 297(12):630–634
2. Calvert AH et al (1989) Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 7(11):1748–1756
3. Wolf W, Waluch V, Presant CA (1998) Non-invasive 19F-NMRS of 5-fluorouracil in pharmacokinetics and pharmacodynamic studies. NMR Biomed 11(7):380–387
4. Saleem A, Aboagye EO, Price PM (2000) In vivo monitoring of drugs using radiotracer techniques. Adv Drug Deliv Rev 41(1):21–39
5. Bergstrom M, Grahnen A, Langstrom B (2003) Positron emission tomography microdosing: a new concept with application in tracer and early clinical drug development. Eur J Clin Pharmacol 59(5, 6):357–366
6. Saleem A et al (2001) Pharmacokinetic evaluation of N-[2-(dimethylamino)ethyl]acridine-4-carboxamide in patients by positron emission tomography. J Clin Oncol 19(5):1421–1429
7. Brown GD (1999) Radiolabelling of anticancer drugs with short-lived positron emitters for PET studies. University of London, London, p 256
8. Brown GD et al (1993) A practical synthesis of 5-[18F]Fluorouracil using HPLC and a study of its metabolic profile in rats. J Label Compd Radiopharm 32:521–522
9. Brown GD et al (1994) Carbon-11 labelling of the antitumour agent, [N-^{11}C-methyl] NSC 601316 for pre-clinical evaluation in man by PET. J Label Compd Radiopharm 35:558
10. Harte RJ et al (1999) Tumor, normal tissue, and plasma pharmacokinetic studies of fluorouracil biomodulation with N-phosphonacetyl-L-aspartate, folinic acid, and interferon alfa. J Clin Oncol 17(5):1580–1588
11. Saleem A et al (2000) Modulation of fluorouracil tissue pharmacokinetics by eniluracil: in-vivo imaging of drug action. Lancet 355(9221):2125–2131
12. D'Argenio DZ, Schumitzky A (1997) A program package for simulation and parameter estimation in pharmacokinetic systems. Comput Programs Biomed 9(2):115–134
13. Osman S et al (1997) Studies on the metabolism of the novel antitumor agent [N-methyl-11C]N- [2-(dimethylamino)ethyl]acridine-4-carboxamide in rats and humans prior to phase I clinical trials. Cancer Res 57(11):2172–2180
14. Heidelberger C et al (1957) Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. Nature 179(4561):663–666
15. Finan PJ et al (1987) The relationship between plasma pharmacokinetics and tissue metabolites of 5-fluorouracil (5-FU) in patients with colorectal cancer. Eur J Surg Oncol 13(4):349–353
16. Fraile RJ et al (1980) Pharmacokinetics of 5-fluorouracil administered orally, by rapid intravenous and by slow infusion. Cancer Res 40(7):2223–2228
17. Collins JM et al (1980) Nonlinear pharmacokinetic models for 5-fluorouracil in man: intravenous and intraperitoneal routes. Clin Pharmacol Ther 28(2):235–246
18. van Groeningen CJ et al (1988) Pharmacokinetics of 5-fluorouracil assessed with a sensitive mass spectrometric method in patients on a dose escalation schedule. Cancer Res 48(23):6956–6961
19. Heggie GD et al (1987) Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res 47(8):2203–2206
20. MacMillan WE, Wolberg WH, Welling PG (1978) Pharmacokinetics of fluorouracil in humans. Cancer Res 38(10):3479–3482
21. Spicer DV et al (1988) Reevaluation of the maximum tolerated dose of continuous venous infusion of 5-fluorouracil with pharmacokinetics. Cancer Res 48(2):459–461
22. Erlichman C, Fine S, Elhakim T (1986) Plasma pharmacokinetics of 5-FU given by continuous infusion with allopurinol. Cancer Treat Rep 70(7):903–904
23. Fleming RA et al (1992) Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. Cancer Res 52(10):2899–2902
24. Thyss A et al (1986) Clinical pharmacokinetic study of 5-FU in continuous 5-day infusions for head and neck cancer. Cancer Chemother Pharmacol 16(1):64–66
25. Kestell P et al (1999) Plasma pharmacokinetics of N-[2-(dimethylamino)ethyl]acridine-4-carboxamide in a phase I trial. Cancer Chemother Pharmacol 44(1):45–50
26. Propper DJ et al (2003) Use of positron emission tomography in pharmacokinetic studies to investigate therapeutic advantage in a phase I study of 120-hour intravenous infusion XR5000. J Clin Oncol 21(2):203–210
27. Twelves CJ et al (1999) Phase I and pharmacokinetic study of DACA (XR5000): a novel inhibitor of topoisomerase I and II. CRC Phase I/II Committee. Br J Cancer 80(11):1786–1791
28. Aboagye EO et al (2001) Extraction of 5-fluorouracil by tumor and liver: a noninvasive positron emission tomography study of patients with gastrointestinal cancer. Cancer Res 61(13):4937–4941
29. Meikle SR et al (1998) Pharmacokinetic assessment of novel anticancer drugs using spectral analysis and positron emission tomography: a feasibility study. Cancer Chemother Pharmacol 42(3):183–193