Pharmacokinetics of 2-fluorodideoxycytidine (2FddC) in patients infected with human immunodeficiency virus

P. E. O. WILLIAMS¹, G. J. MUIRHEAD¹, D. SERENI², F. S. ROUSSEAU², K. EDELMAN^{3*} & M. HOOKER³

¹Pharmacokinetics and Metabolism Department, Roche Products Ltd, Hertfordshire, ²Hôpital Cochin, Paris, France and

³St Stephen's Hospital, London, England

- 1 The aim of this study was to estimate an oral dosage regimen of 2FddC giving peak plasma drug concentrations close to the antiretroviral IC₅₀ of 150 ng ml⁻¹.
- 2 A total of 55 doses (40 intravenous infusions and 15 oral solutions) were given to 21 patients. One group ($n = 6-11$) received single doses of 0.01 mg kg⁻¹ intravenously (i.v.), 0.1 mg kg⁻¹ i.v. and 0.1 mg kg⁻¹ orally (p.o.) in that order. The other group ($n = 8-10$) received single doses of 0.03 mg kg⁻¹ i.v., 0.3 mg kg⁻¹ i.v. and 0.3 mg kg⁻¹ p.o. in that order. Blood and urine samples were collected up to 24 h after each dose for drug assay by h.p.l.c. — u.v.
- 3 The peak plasma concentrations of 2FddC were proportional to dosage across the range 0.03 to 0.3 mg kg⁻¹. After intravenous dosing, the mean (%CV) volume of distribution was 60 (28) l and the mean (CV%) plasma clearance was 23 (23) l h⁻¹. On average, 71% of the dose was recovered unchanged in urine and renal clearance exceeded concurrent creatinine clearance.
- 4 Plasma concentrations reached mean peaks of 37 and 96 ng ml⁻¹ after oral doses of 0.1 and 0.3 mg kg⁻¹, respectively. The mean absolute bioavailability was 50% within a 95% confidence interval of 20 to 80%.
- 5 The adverse events were usually mild or moderate in severity and were generally attributed to the disease rather than the drug.
- 6 It is predicted that an oral dosage of 30 mg 8-hourly would result in little or no drug accumulation and that plasma drug concentrations would be close to 150 ng ml⁻¹ at peak.

Keywords pharmacokinetics 2-fluorodideoxycytidine HIV-1

Introduction

The reverse transcriptase enzyme of human immunodeficiency virus (HIV-1) has been a target for drug action since the discovery that analogues of naturally occurring nucleosides could inhibit the enzyme and prevent the incorporation of viral DNA into the host genome. Clinical use of nucleoside analogues such as zidovudine, dideoxyinosine and dideoxycytidine (ddC) in AIDS patients has been accompanied by improvements in surrogate markers of disease activity (Butler *et al.*, 1991). However, dose-limiting side-effects have occurred with each of these drugs e.g. peripheral neuropathy after dideoxycytidine. A possible reason for this neuropathy is the interaction of ddC with mammalian gamma-

polymerase. 2FddC (1-(2,3-dideoxy-2-fluoro- β -D-threo-pentofuranosyl)-cytosine) is a nucleoside analogue which inhibits the growth of HIV-1 in C8166 T-cells (IC₅₀ = 150 ng ml⁻¹). The drug is metabolised intracellularly to 2FddC triphosphate, which inhibits reverse transcriptase ($K_i = 67$ ng ml⁻¹) to a much greater extent than it inhibits mammalian gamma polymerase ($K_i = 6000$ ng ml⁻¹) (Wong-Kai-In *et al.*, 1991). The present study was designed to determine the pharmacokinetics of 2FddC in patients with AIDS. From this information, an estimate could be made of the oral dosage regimen which should give peak plasma concentrations close to 150 ng ml⁻¹.

*Current address: St Mary Abbots Hospital, London, England

Correspondence: Dr P. E. O. Williams, Pharmacokinetics and Metabolism Department, Roche Products Ltd, Hertfordshire

Methods

Patient selection

The protocol was approved by the Ethics Committees of Westminster Hospital, London and of Groupes Hospitaliers Cochin, St Vincent de Paul, Ste Anne, Paris. Each patient gave written informed consent to participation.

Patients attending St Stephen's Clinic or Hôpital Cochin, were considered for inclusion in the study if they were males between 18 and 45 years of age and were seropositive for HIV antibody.

Patients were excluded if they had a current history of intravenous drug abuse, hepatitis B, received anti-retroviral agents within the 4 weeks before the first dose, had any opportunistic infections within the previous month, had evidence of peripheral neuropathy, or a leucopenia, or serum creatinine concentration above $120 \mu\text{mol l}^{-1}$.

Each patient underwent a complete medical examination shortly before entering the study, and samples were collected for haematology, serum biochemistry and urinalysis.

Study procedures

The study involved two parallel staggered groups of patients. The first group received intravenous infusions over 1 h of 0.01 and 0.1 mg 2FddC per kg bodyweight and an oral dose of 0.1 mg kg^{-1} , in that order. The second group received infusions of 0.03 and 0.3 mg kg^{-1} followed by an oral dose of 0.3 mg kg^{-1} . These single doses were separated from each other by at least 2 weeks.

On each dosing day, the patients attended the clinic by 09.00 h, having fasted from 22.00 h on the previous evening. Each patient received a light snack 2 h after dosing and a standard lunch 5 h after dosing.

2FddC was dissolved in sterile isotonic saline for intravenous infusion at $0.5\text{--}0.8 \text{ ml kg}^{-1}$ by a syringe pump (Perfusor Secura F.T.) through an indwelling catheter into a forearm vein.

2FddC was dissolved in water for drinking from a plastic beaker which was rinsed with 100 ml of water, and the rinsings were also drunk.

Blood pressures and heart rates were recorded manually immediately before and 1, 2, 4, 8 and 24 h after each dose. Blood and urine samples were collected for laboratory safety tests immediately before and 5 days after each dose. Adverse events were graded by the investigators on a three-point scale (mild, moderate or severe) and the relationship to dosing was assessed as not related, remote, possible or probable.

Blood samples for drug assay were collected into heparinized plastic tubes immediately before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 24 h after each dose. Plasma was separated from each sample by centrifugation within 4 h of sample collection and stored at 4°C overnight before heat inactivation (see below).

A pre-dose urine sample was provided by each patient and then total collections of all urine were made over 0–4 h, 4–8 h and 8–24 h after dosing. The volume of each collection was measured and an aliquot (10 ml) was

stored at 4°C until heat inactivation.

Samples were heated in a water bath for 2 h at 56°C at the investigational centres to inactivate HIV. They were then transported on solid carbon dioxide to the analytical laboratory where the heat inactivation was repeated. The stability of 2FddC under these conditions was confirmed.

Assays

Concentrations of 2FddC in plasma and urine were measured by h.p.l.c. with detection by ultraviolet absorbance (u.v.).

For the plasma assays, aliquots (1 ml) were mixed with the internal standard, dideoxycytidine and transferred onto primed propylsulphonate Bond-Elut[®] columns (Waters) for solid-phase extraction of the drugs. The columns were washed with buffer (0.5 M acetic acid adjusted to pH 3 with 0.1 M ammonium acetate) and with an acetonitrile:buffer mixture (90:10). The drugs were eluted from the columns with methanol:ammonia (90:10) and the eluents were evaporated to dryness under nitrogen. The residue was reconstituted in 0.01 M ammonium acetate and injected into the h.p.l.c. system which comprised a Hewlett Packard 1050 autosampler, a Hypersil ODS 5μ column, 4.6 mm i.d. $\times$ 250 mm long, and a guard column with the same packing. The eluent was 0.01 M ammonium acetate:acetonitrile (98.5:1.5) at a flow rate of 2 ml min^{-1} . The drug and internal standard had retention times of approximately 15 and 11 min, respectively, and were detected by u.v. at 274 nm.

For urine assays, a similar analytical method was employed. Aliquots (0.2 ml) were mixed with 0.02 ml 1 M hydrochloric acid, 0.2 ml buffer (0.5 M acetic acid adjusted to pH 3 with 1 M ammonium acetate) and 1.6 ml water. No internal standard was added because of chromatographic interference from endogenous compounds. The h.p.l.c. system was the same as above except that the analytical column was a Waters Novapak C₁₈ 4μ cartridge in a radial compression module (8 mm i.d. by 100 mm long), with a μ Bondapak C₁₈ guard column. The mobile phase was 0.01 M ammonium acetate:acetonitrile (96.7:3.3) delivered at a rate of 1.8 ml min^{-1} .

Spiked standards and quality control samples were processed along with each batch of unknowns. Calibration curves were constructed by weighted linear regression of the peak height ratios (2FddC:ddC) from plasma or peak height from urine against standard concentrations. The minimum quantifiable levels (MQL) were set by the concentrations in the lowest standards, namely 2 ng ml^{-1} in plasma and 250 ng ml^{-1} in urine. The assays were linear across the ranges $2\text{--}1019 \text{ ng ml}^{-1}$ in plasma and $250\text{--}50,000 \text{ ng ml}^{-1}$ in urine. Inter-assay precisions for the quality control samples during the study were $\pm 5\%$ and $\pm 6\%$ in plasma (20 and 200 ng ml^{-1}) and urine (1100 and 22000 ng ml^{-1}), respectively. The precision at the MQL was $\pm 4\%$ in plasma and $\pm 10\%$ in urine.

Pharmacokinetic parameters

Individual plasma drug concentration-time profiles were subjected to standard 'model-independent' analysis

(Gibaldi & Perrier, 1982). The absolute bioavailability, F , after oral dosing was calculated from the ratio of the clearance after i.v. dosing to the apparent clearance after oral dosing. In addition, an open two-compartment pharmacokinetic model was fitted to the data after intravenous dosing of 0.03, 0.1 and 0.3 mg kg⁻¹. The MKMODEL program was used for curve-fitting by nonlinear regression (Holford, 1982).

Results

A total of 21 patients were enrolled for the study and tolerability was evaluated in all. Patients from London were numbered 2–10 and those from Paris were numbered 21–26 and 31–36. Their mean (%CV) age was 33 (26) years and their mean (%CV) weight was 67 (19) kg. They received a total of 55 doses (40 i.v. and 15 p.o.) from a potential total of 63 doses (42 i.v. and 21 p.o.) if all patients had completed the study. The patients who withdrew from the study did so because they were unwilling to wait until sufficient patients had received the lower dosages to permit safe escalation. No patient withdrew because of adverse events.

Tolerability

A total of 99 adverse events were recorded in 20 of the 21 patients during the study. The majority of these events were mild in intensity. Fifty of the events were considered to be unrelated to dosing. Of the remaining 49 adverse events, 22 were considered to be remotely related, 19 possibly and 8 probably related to dosing with 2FddC (Table 1). The most common adverse events were nausea and vomiting which were reported most frequently after 2FddC at a dose of 0.3 mg kg⁻¹ i.v. The most serious adverse events, all unrelated to 2FddC, were a lymphoma in patient number 3, angina in patient 26 and a recurrence of cerebral toxoplasmosis in patient 34.

Although there were some changes in the results of laboratory safety tests, they were not apparently related to dosing with 2FddC. There were a few diastolic blood pressures below the 'lower limit of normal' (60 mm Hg)

but these abnormalities were not related to the dosage of 2FddC and were not regarded as clinically significant. Abnormalities of systolic blood pressure or heart rate were not seen.

Pharmacokinetics

Two sets of samples could not be analysed; from patient 6 after 0.01 mg kg⁻¹ because of plasma coagulation and from patient 22 after 0.1 mg kg⁻¹ p.o. because of chromatographic interference. Hence only 53 of the 55 profiles were available for pharmacokinetic evaluation. Furthermore, the drug concentrations after the 0.01 mg kg⁻¹ i.v. dosage were too low for pharmacokinetic analysis and the absolute bioavailability was assessable in only 14 of the 21 patients because of withdrawals.

Plots of the mean plasma 2FddC concentrations are shown in Figure 1 after i.v. dosing and Figure 2 after oral dosing. A summary of the pharmacokinetic parameter

Figure 1 Mean plasma concentrations of 2FddC after intravenous infusion [bars are standard errors] □ 0.3 mg kg⁻¹, $n = 10$; ○ 0.1 mg kg⁻¹, $n = 9$; △ 0.03 mg kg⁻¹, $n = 10$; ▽ 0.01 mg kg⁻¹, $n = 10$. ----- MQL.

Table 1 Number of adverse events possibly or probably related to dosing with 2FddC grouped by most recent dose

Adverse event	2FddC dose (number of patients)			
	0.03 mg kg ⁻¹ i.v. ($n = 10$)	0.3 mg kg ⁻¹ i.v. ($n = 10$)	0.1 mg kg ⁻¹ p.o. ($n = 7$)	0.3 mg kg ⁻¹ p.o. ($n = 8$)
Malaise		1		
Abdominal ache	1			
Nausea		5		2
Vomiting	1	3		2
Drowsiness		2		
Headache		2		
Blurred vision during infusion		1		
Euphoria		1		
Lightheadedness		1		
Raised γ GT		1	2	
Raised AST			1	
Raised bilirubin			1	

Key: γ GT = gamma-glutamyl transpeptidase, AST = aspartate transaminase.

Table 2 Mean (%CV) pharmacokinetic parameters of 2FddC after intravenous dosing

Dose (number of patients)	Total clearance (l h ⁻¹)	Renal clearance (l h ⁻¹)	Elimination ⁺ half-life (h)	V (l)	C _{max} (ng ml ⁻¹)	t _{max} (h)	F (%)
0.03 mg kg ⁻¹ i.v. (n = 10) [IQR]	25.2 (15) [22.1–28.3]	* (50)	1.97 (37) [1.47–2.16]	70.2 (3) [57.5–78.4]	41.5 (19) [37.3–40.3]	nc	*
0.10 mg kg ⁻¹ i.v. (n = 9) [IQR]	22.8 (15) [21.0–24.6]	15.2 (50) [10.7–18.8]	1.78 (23) [1.50–2.10]	55.9 (20) [48.3–61.8]	144 (15) [125–151]	nc	*
0.3 mg kg ⁻¹ i.v. (n = 9) [IQR]	21.2 (29) [17.7–26.0]	13.2 (32) [9.79–15.5]	1.83 (12) [1.61–2.02]	54.9 (26) [42.9–66.9]	460 (19) [394–502]	nc	*
<i>Median [IQR] of the pharmacokinetic parameters after oral dosing</i>							
0.1 mg kg ⁻¹ p.o. (n = 6)	54.4 [33.3–63.4]	12.3 [8.95–15.8]	* (12)	* (26)	40.4 [12.0–59.1]	1.25 [1.0–3.38]	50.9 [39.9–75.5]
0.3 mg kg ⁻¹ p.o. (n = 8)	60.6 [26.9–84.6]	16.5 [10.9–18.1]	* (12)	* (26)	64.1 [51.4–166]	1.25 [0.875–1.88]	34.0 [25.8–62.4]

Key: *Implies not calculable, nc implies not calculated.

⁺Elimination half-life refers to the terminal phase.

[IQR] implies interquartile range.

Figure 2 Mean plasma concentrations of 2FddC after single oral solution doses [bars are standard errors] ◆ 0.3 mg kg⁻¹, n = 8; ■ 0.1 mg kg⁻¹, n = 6; ----- MQL.

values is given in Table 2. The results from the patients in London did not differ substantially from those in Paris. The patients who did not complete the study gave results similar to those who completed the study.

Intravenous dosing

After the end of the intravenous infusion, which lasted 1 h, plasma concentrations of 2FddC declined rapidly. This decline was more rapid during the first sampling interval from 1 to 1.5 h than during subsequent intervals. Thereafter the decline in drug concentration was log-linear with a half-life of approximately 2 h. 2FddC was rarely detectable 24 h after dosing although concentrations near 2 ng ml⁻¹ in some of the patients given 0.3 mg kg⁻¹

doses suggested that a slower terminal phase may have been present.

The mean plasma clearance was 23 l h⁻¹ and the distribution volume was 60 l. The mean plasma clearances at 0.03, 0.1 and 0.3 mg kg⁻¹ were not significantly different from each other ($P > 0.05$) and not significantly correlated with bodyweight ($P > 0.05$). An unpaired Student's *t*-test showed no significant difference between the mean volume of distribution at 0.03 and 0.1 mg kg⁻¹ ($P > 0.05$). However a paired *t*-test indicated that the mean volume was significantly higher after the 0.03 than after the 0.3 mg kg⁻¹ dose ($P < 0.01$). After the 19 intravenous doses of 0.1 or 0.3 mg kg⁻¹, the volume of distribution was significantly correlated with patient's bodyweight ($P < 0.01$) but this explained only 44% of the total variance.

A two-compartment open model with elimination from the central compartment provided adequate fits to almost all the data-sets after 0.03, 0.1 and 0.3 mg kg⁻¹ i.v. Sparse or irregular data prevented fitting of the data from four patients after 0.03 mg kg⁻¹ and one patient after 0.3 mg kg⁻¹. The mean (%CV) estimate over all the fits were 24 (35%) l for the central compartment volume, 2.2 (75%) h⁻¹ for the rate constant in the rapid phase and 0.28 (44%) h⁻¹ for the rate constant in the terminal phase. These rate constants corresponded to half-lives of 0.32 h and 2.4 h respectively. The mean volume of distribution at steady state was found to be 58 l. The results from the 'model-independent' analyses and the model-fitting corresponded closely.

Urine samples were not assayed after 0.01 and 0.03 mg kg⁻¹ dosage. The rate of appearance of 2FddC in urine appeared to follow the decline in plasma concentrations closely. After intravenous doses of 0.1 or 0.3 mg kg⁻¹, 73% of the total urinary recovery appeared in the 0–4 h collection, which spanned about two elimination half-lives. The total recovery of 2FddC after both dosages

was 67% of the administered dose. Therefore the renal clearance averaged two-thirds of the total plasma clearance at 14 l h^{-1} . This value exceeded the average concurrent creatinine clearance in these patients of 6.71 h^{-1} , calculated by the method of Cockcroft & Gault (1976). 2FddC is not bound to plasma proteins in man and so the renal clearance represented that of free drug.

Oral dosing

The plasma concentrations of 2FddC were more variable after oral than after intravenous dosing as shown by the coefficients of variation for most of the pharmacokinetic parameters of around 50% and 25% respectively. Peak plasma drug concentrations between 7.6 and 67 ng ml^{-1} occurred 1 to 5 h after $0.1 \text{ mg kg}^{-1} \text{ p.o.}$

The corresponding figures after the $0.3 \text{ mg kg}^{-1} \text{ p.o.}$ dosage were 34 to 178 ng ml^{-1} between 0.5 and 3 h after dosing. After the peaks, the plasma drug concentrations in individual patients declined rapidly but half-lives could not be calculated because of sparse or irregular data in the decline phase. The elimination half-life from the corresponding intravenous dose was used to extrapolate from $\text{AUC}(t)$ to infinite time in each profile. The mean oral clearance was 58 l h^{-1} leading to an estimate of 51% for the absolute bioavailability with a 95% confidence interval from 20 to 80%.

The results from the urinary assay of 2FddC after oral dosing were consistent with those after intravenous dosing in that the renal clearance values were around 14 l h^{-1} . They confirmed that a similar estimate of absolute bioavailability (43%) could be derived from the ratio of urinary recoveries.

Discussion

2FddC was reasonably well-tolerated, in view of the clinical condition of the study population; the majority of the adverse events were attributed to the disease rather than to the drug, and there was no evidence of drug-related change in the laboratory parameters.

The decline in plasma 2FddC concentrations after the end of the intravenous infusions indicated rapid distribution. Model-fitting estimated the half-life of this phase to be 0.32 h on average. The volume of distribution was around 60 l, slightly greater than total body water. The dose-relationship in the volume of distribution seemed to arise from the paucity of data in the terminal phase after the 0.03 mg kg^{-1} doses. The peak plasma

concentrations and AUCs of 2FddC were proportional to dosage.

The predominant phase of decline in 2FddC plasma concentrations was matched by the appearance of the drug in urine. Tubular secretion contributed to this excretion which occurred at a rate about double that of glomerular filtration.

After oral dosing of 2FddC solutions, plasma drug concentrations rose more slowly and to lower levels than after intravenous infusion. After the peaks, the decline in plasma drug concentrations was somewhat slower than after the intravenous doses and may have been influenced by a prolonged absorption phase. The bioavailability of 2FddC is incomplete in some patients, as shown by the mean absolute bioavailability close to 50%. The determination of F from plasma data was probably more reliable than that from urinary recoveries because of the large potential influence of incomplete urine collections. The incomplete absorption of the drug may relate to its high water solubility ($6.7 \text{ g } 100 \text{ ml}^{-1}$ at 20° C) and low lipid solubility (octanol-water partition coefficient of 0.08 at pH 7.4 and <0.01 at pH 1.2).

The results of this study are in keeping with limited data reported after the same dosage of the nucleoside analogue, dideoxycytidine (Klecker *et al.*, 1988). The oral bioavailability of dideoxycytidine seems to be higher at 80% than that of its fluorinated analogue, 2FddC (50%). Both of these figures exceed the bioavailability of dideoxyinosine which averaged 21% in 22 children with symptomatic HIV infection (Butler *et al.*, 1991). The pharmacokinetics of zidovudine are different since glucuronide conjugation is the primary elimination mechanism rather than renal excretion of unchanged drug (Collins & Unadkat, 1989).

The pharmacokinetics of 2FddC appeared to be governed by simple linear processes. Hence it could be predicted that an oral dosage regimen of 30 mg (approximately 0.5 mg kg^{-1}) every 8 h should result in little drug accumulation in plasma and average peak concentrations of 150 ng ml^{-1} . However, preclinical testing suggested significant toxicity at total daily dosages in excess of $1 \text{ mg kg}^{-1} \text{ day}^{-1}$. Therefore, a maximum oral dosage of 20 mg every 8 h was recommended for the first study of the efficacy of 2FddC in AIDS patients. This dosage should provide peak plasma drug concentrations close to 100 ng ml^{-1} and an average concentration of 60 ng ml^{-1} at steady state.

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