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Zidovudine pharmacokinetics in zidovudine-induced bone marrow toxicity

M. BARRY[1], J. L. HOWE[1], D. J. BACK[1], A. M. SWART[2], A. M. BRECKENRIDGE[1], I. V. D. WELLER[2], N. BEECHING[3] & F. NYE[3]
[1]Department of Pharmacology and Therapeutics, University of Liverpool, [2]Academic Department of Genitourinary Medicine, University College and Middlesex Hospital School of Medicine, London and [3]Regional Infectious Diseases Unit, Fazakerley Hospital, Liverpool

1 The major adverse effect of zidovudine (ZDV) is haematological toxicity which results in anaemia and granulocytopenia. The aim of the present study was to investigate if HIV-positive patients developing erythroid aplasia/hypoplasia are exposed to higher plasma concentrations of ZDV owing to impaired hepatic metabolism to the major metabolite, 3′-azido-3′-deoxy-5′-β-D-glucopyranuronosylthymidine (GZDV).

2 Twelve HIV-positive male patients were studied, six having developed bone marrow aplasia/hypoplasia within the first 6 months of ZDV therapy. Each of the patients exhibiting toxicity were matched for age, weight, risk factors for HIV infection and disease stage with patients who had no evidence of early bone marrow toxicity.

3 ZDV was administered orally in doses of 3–10 mg kg^{-1} and blood samples taken at intervals to 6 h. Urine was collected over the whole 6 h period. ZDV and GZDV were assayed by h.p.l.c.

4 There were no significant differences in the pharmacokinetic parameters between the two groups of patients. For patients with early bone marrow toxicity the elimination half-life of ZDV was 1.10 ± 0.16 h with an oral clearance of 2752 ± 1031 ml min^{-1} compared with values of 1.06 ± 0.18 h and 2843 ± 730 ml min^{-1} seen in the control group. Similarly there was no significant difference in the pharmacokinetics of GZDV or the urinary ratio of GZDV to ZDV.

5 Therefore, despite the fact that ZDV toxicity to haematopoietic progenitor cells has been previously shown to be dose related, there was no indication from this study that it is directly related to plasma concentrations of ZDV.

Keywords zidovudine pharmacokinetics bone marrow toxicity

Introduction

Zidovudine (3′-azido-3′-deoxythymidine; ZDV) is a thymidine analogue antiretroviral drug active against the human immunodeficiency virus (HIV) [1]. The efficacy of ZDV in the treatment of patients with the Acquired Immunodeficiency Syndrome (AIDS) and AIDS-related Complex (ARC) is established [2] although the clinical benefit to asymptomatic patients has recently been questioned [3]. Following oral administration approximately 65% of the drug is systemically available. The mean volume of distribution is 1.4 l kg^{-1} with plasma protein binding less than 25% [4]. The drug has a short elimination half-life of approximately 1 h, being metabolised extensively to an ether glucuronide, 3′-azido-3′-deoxy-5′-β-D-glucopyranuronosylthymidine (GZDV) [5] which is rapidly excreted in the urine [6]. The extent of glucuronidation may have profound effects on the disposition of ZDV. The major adverse effect of ZDV is haematological toxicity which results in anaemia and granulocytopenia. ZDV-induced myelosuppression is associated with more advanced disease, lower CD4 lymphocyte counts, decreased serum B_{12} or folic acid levels and baseline anaemia or neutropenia [7]. It is apparent that a small percentage of patients, <5%, develop erythroid aplasia/hypoplasia shortly after

Correspondence: Dr M. Barry, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

commencing treatment [8]. In children ZDV-induced haematological side-effects have been demonstrated to correlate with its steady state plasma concentration [9]. The aim of this study was to determine whether patients developing erythroid aplasia/hypoplasia are exposed to higher ZDV concentrations, possibly due to impaired glucuronidation of ZDV.

Methods

Subjects

ZDV pharmacokinetics were determined in six HIV-positive male patients aged 30–48 years who developed bone marrow erythroid aplasia/hypoplasia within the first 6 months of ZDV therapy, and in a control group of six HIV-positive male patients aged 32–47 years without evidence of early bone marrow toxicity. Patients were matched for age, weight, risk factors for HIV infection and disease stage according to the revised CDC definition for AIDS (Table 1). Two patients had asymptomatic HIV infection (CDC Group II) and ten patients had AIDS (CDC Group IV). All patients had normal liver function as assessed by the Pugh classification (Pugh score <5), which includes a combination of laboratory parameters of liver function and clinical features such as ascites and hepatic encephalopathy [10]. There was no evidence of renal dysfunction; urea and creatinine values were normal. Patients were not taking any medication that would be expected to interfere with ZDV glucuronidation [11, 12]. Approval for the study was granted by the local ethics committee and all patients provided written informed consent. On the study day patients attended following an overnight fast from 24.00 h. Patients were allowed fluids but did not eat for at least 4 h after commencing the study. An indwelling intravenous cannula was inserted in the cubital fossa to facilitate blood sampling. ZDV was administered orally in doses of 3–10 mg kg^{-1} (reflecting the dose variation in the clinical setting) at 09.00 h and blood samples were taken at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5 and 6 h after dosing. Urine samples were collected over the 6 h period. Plasma and and urine samples were exposed to a temperature of 58° C for at least 30 min in a waterbath. This treatment inactivates HIV [13], but does not affect ZDV or its metabolite.

Analytical methods

ZDV and its glucuronide metabolite (GZDV) were assayed in plasma using a modification of the method of Blum *et al.* [6]. Plasma samples were spiked with 5 μM of either A22U (Wellcome, Beckenham) or β-hydroxy-ethyltheophylline (Sigma) as internal standard and were ultrafiltered using an Amicon Centrifree Micro-partition System. The ultrafiltrates were subsequently analysed by h.p.l.c. Samples were eluted on an Ultratech 5ODS column (4.6 mm × 250 mm) using a mobile phase of 25 mM ammonium phosphate buffer, pH 7.2, with a linear gradient of 0–30% v/v acetonitrile over 35 min followed by a return to 100% buffer over 5 min and a 5 min reequilibration period with 100% buffer at a flow rate of 0.6 ml min^{-1}. Retention times of authentic standards (u.v. detection at 267 nm) were 19, 23, 26 and 28 min for GZDV (Wellcome, Beckenham), β-hydroxyethyltheophylline, A22U and ZDV (Wellcome, Beckenham), respectively. Peak height ratios of ZDV and GZDV to the internal standard were used to calculate concentrations from standard curves (range 0–20 μM and 0–40 μM for ZDV and GZDV respectively). Intraassay coefficients of variation were 8.1%, 1.7% and 2.1% for 0.25 μM, 2.5 μM and 20 μM ZDV respectively and 8.3%, 5.9% and 3.2% for 0.5 μM, 5.0 μM and 40 μM GZDV, respectively (n = 10). Interassay coefficients of variation were 5.7% and 7.9% for 2.5 μM ZDV and 5 μM GZDV, respectively (n = 5). Urine samples were diluted 1:100, spiked with 5 μM of internal standard (A22U) and analysed by h.p.l.c. as above. Intraassay coefficients of variation were 5.24% and 5.14% for 0.5 μM and 20 μM ZDV, respectively, and 8.73% and 9.59% for 1 μM and 40 μM GZDV, respectively (n = 10). Interassay coefficients of variation were 9.9% and 9.8% for 2.5 μM ZDV and 5 μM GZDV, respectively (n = 5). Limits of detection were 0.1 μM for ZDV and 0.2 μM for GZDV.

Pharmacokinetic calculations

C_{max} values (μmol l^{-1}) and t_{max} values (h) for ZDV and GZDV were noted directly from the data. The elimination rate constant (λ_z) was calculated by log linear

Table 1 Clinical details and drug treatment of patients participating in the study

Patient	*Age (years)*	*Sex*	*Weight (kg)*	*CDC classification*	*Bone marrow toxicity*	*Zidovudine dose (mg)*	*Other medications*
1	45	M	85	IV C_1	Yes	850	
2	47	M	75	IV C_1	No	800	Acyclovir 200 mg 4 hourly, Pentamidine (nebulised)
3	37	M	67	IV C_1	Yes	650	Paracetamol 500 mg prn., Fluconazole 50 mg b.d.
4	41	M	60	IV C_1	No	600	Ibuprofen 200 mg b.d.
5	39	M	63	IV C_2	Yes	650	
6	38	M	73	IV C_2	No	700	Cotrimoxazole 450 mg b.d., Volterol 75 mg prn.
7	30	M	60	IV C_2	Yes	600	
8	39	M	60	IV C_2	No	600	
9	40	M	64	IV C_1	Yes	250	Pentamidine (nebulised)
10	32	M	65	IV C_1	No	250	Cotrimoxazole 900 mg nocte
11	48	M	85	II	Yes	250	Pentamidine (nebulised)
12	46	M	90	II	No	250	Acyclovir 200 mg 4 hourly

Table 2 Pharmacokinetic parameters of ZDV and GZDV following an oral dose of ZDV to control patients and patients with bone marrow suppression. Data expressed as mean ± s.d. with the exception of t_{max}, median (range). 95% CI: 95% confidence intervals of the differences between the means for the two groups of patients

	ZDV			GZDV		
	Control patients (n = 6)	*Patients with bone marrow suppression (n = 6)*	*95% CI*	*Control patients (n = 6)*	*Patients with bone marrow suppression (n = 6)*	*95% CI*
t_{max} (h)	0.5 (0.5–0.75)	0.75 (0.5–1.25)	–	1.00 (0.75–1.25)	1.00 (0.75–1.5)	–
$t_{½}$ (h)	1.06 ± 0.18	1.10 ± 0.16	−0.26 to 0.16	1.11 ± 0.22	1.10 ± 0.19	−0.27 to 0.26
AUC normalised to 250 mg dose (μmol l^{-1} h)	5.8 ± 1.6	6.5 ± 2.7	−4.2 to 2.3	17.3 ± 4.5	17.8 ± 6.6	−7.7 to 7.2
Urinary ratio [GZDV]/[ZDV]	6.72 ± 1.80	8.17 ± 4.68	−8.05 to 3.24	–	–	–

There were no significant differences in either ZDV or GZDV pharmacokinetic parameters between the two groups of patients.

regression of the terminal portion of the plasma drug concentration-time curve using the method of least squares. The elimination half-life ($t_{½,z}$) was calculated from $\ln 2/\lambda_z$. The areas under the plasma ZDV and GZDV concentration-time curves (μmol l^{-1} h) were calculated using the linear trapezoidal rule. The urinary ratio of GZDV to ZDV was calculated from concentrations in total urine collected over the 6 h period. The pharmacokinetic parameters of ZDV and GZDV are expressed as mean values ± s.d. and were compared between the groups using the Mann-Whitney U-test. The power of the study was such that a difference of 50% in AUC could have been detected (as statistically significant; $P < 0.05$) with a probability of 75%.

Results

The pharmacokinetic parameters for ZDV and GZDV in the patients with early bone marrow toxicity and in the control group are shown in Table 2. The values were consistent with those reported by others [6, 14, 15]. The decline in plasma concentration was monoexponential. Variability in values of C_{max} and AUC partly reflects the fact that eight patients were given 10 mg kg^{-1} ZDV while four received a standard 250 mg dose. However, ZDV kinetics have been shown to be dose-independent up to a 10 mg kg^{-1} dose [6, 15]. Thus it is possible to normalise AUC values to a 250 mg dose (Table 2). There was no significant difference in the pharmacokinetic parameters between the two groups studied. AUC values for ZDV and GZDV, $t_{½,z}$ values of ZDV and urinary ratios of [GZDV] to [ZDV] are shown in Figure 1, each patient matched to the corresponding control. There was no evidence of an increased AUC of ZDV or a decrease in AUC of GZDV in patients with marrow toxicity when compared with the matched control patients.

Discussion

Toxicological data from the original Phase I study of ZDV in patients with AIDS indicated that neutropenia was more likely to occur at higher doses [16]. Subsequent studies of the efficacy and toxicity of low-dose ZDV found anaemia and neutropenia to be dose-related [17–19]. This is consistent with the findings of Balis *et al.* [9] who showed steady state plasma concentrations of ZDV to be higher in children with symptomatic HIV infection who developed severe neutropenia (neutrophils $<0.5 \times 10^9$ l^{-1}) compared with children who did not develop haematological toxicity. *In vitro* studies have supported this dose-dependence. ZDV inhibited Granulocyte-Macrophage Colony Forming Units (CFU-GM) and Erythroid Burst Forming Units (BFU-E) in a concentration-dependent fashion [20, 21], continuous exposure being more inhibitory than exposure for 1 h [20].

The aim of the present study was to investigate whether the haematological toxicity of ZDV was related to high concentrations of the drug in plasma, and if so, whether this increase was due to a reduced ability to conjugate ZDV in patients with toxicity. Apart from the hereditary hyperbilirubinaemias (Gilbert's Syndrome and Crigler-Najjar Syndrome) in which the uridine diphosphate glucuronosyl transferase (UGT) responsible for bilirubin conjugation is absent or has much reduced activity [22], there has been little evidence for polymorphism in glucuronidation activity. ZDV is glucuronidated in human liver microsomes from patients suffering Crigler-Najjar Syndrome [23] indicating that the UGT responsible for ZDV glucuronidation is not related to that responsible for bilirubin glucuronidation.

Polymorphism in the ester glucuronidation of fenofibric acid has recently been suggested [24], administration of the drug to patients yielding a bimodal distribution of urinary fenofibric acid: fenofibryl glucuronide. However ethinyloestradiol glucuronidation, whilst exhibiting great inter-individual variability in rate, was unimodally distributed in 110 patients [25]. Since ethinyloestradiol competitively inhibits the hepatic glucuronidation of ZDV *in vitro* [23, 26] this indicates the possible involvement of the same UGT isozyme in the metabolism of the two drugs and, therefore, it is unlikely that a marked polymorphism of ZDV glucuronidation exists. Indeed under conditions in which ZDV concentration approximated to the K_m for glucuronidation, polymorphism was not observed in 29 livers in which the glucuronidation of ZDV by microsomal fractions was studied [27], an observation supported by a normal distribution of the AUC(GZDV)/AUC(ZDV) ratio in 62 HIV-positive asymptomatic patients administered ZDV [14].

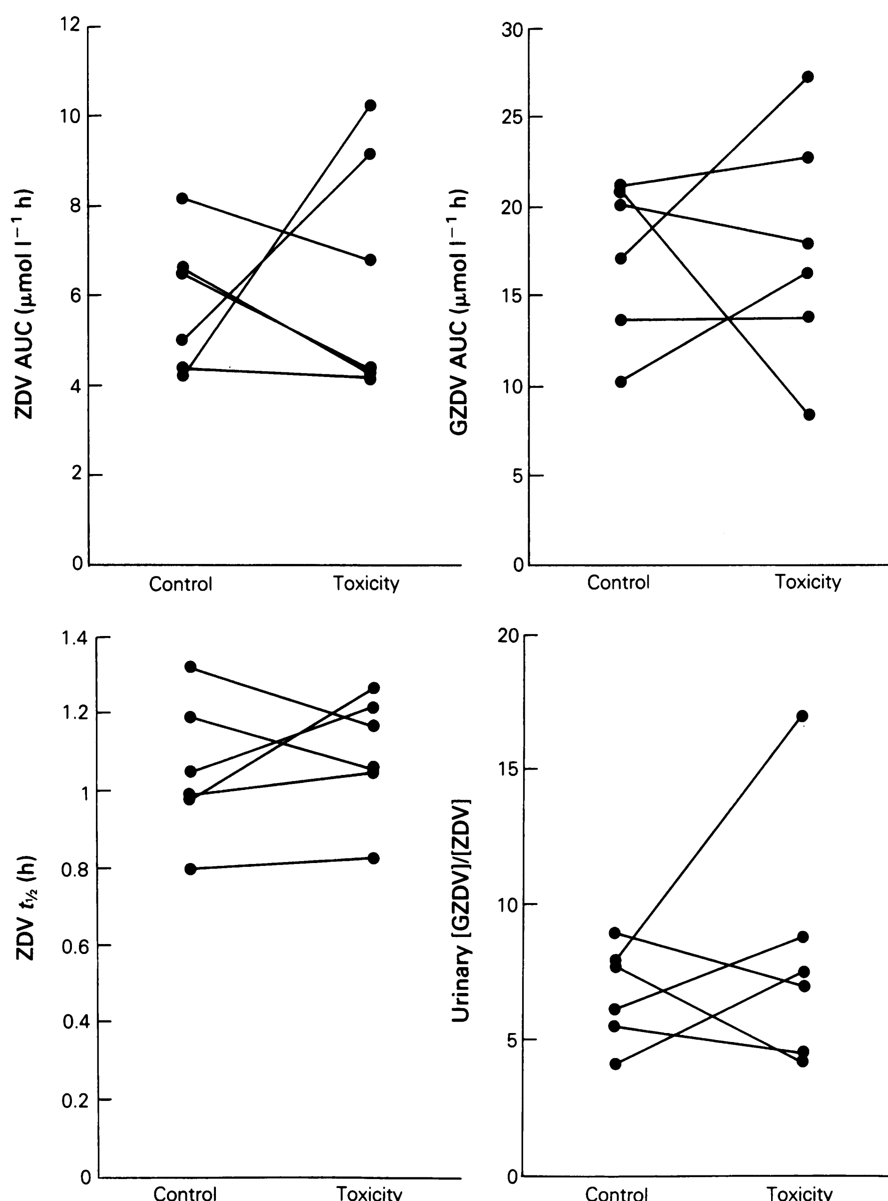

Figure 1 Kinetic parameters for ZDV and GZDV. Each patient with bone marrow toxicity is matched to the corresponding control patient. a) ZDV AUC normalised to a 250 mg dose, b) GZDV AUC normalised to a 250 mg dose, c) ZDV $t_{1/2}$, d) urinary [GZDV]/[ZDV].

The results of the present study would indicate that there is no difference in rate of glucuronidation of ZDV between control patients and those with bone marrow toxicity as ZDV pharmacokinetic parameters were similar in both groups. However, the relatively low power of the study reflects the small population of patients with zidovudine-induced bone marrow aplasia/hypoplasia and the significant intersubject variability in zidovudine pharmacokinetics. The monoexponential decline in plasma concentration observed in the patients studied agreed with previous studies [28–30] although some groups have reported biexponential declines [6, 9, 31] or even triexponential declines in some patients [32].

The mechanism of ZDV myelosuppression is unclear and several mechanisms have been proposed, for example the incorporation of ZDV triphosphate into the DNA of human bone marrow cells [33]. Haem synthesis, which occurs in the mitochondria of bone marrow cells may also be affected by ZDV through its inhibitory effects on mitochondrial DNA polymerase γ [34]. This inhibition occurs by competition with endogenous thymidine triphosphate rather than through incorporation into DNA [35]. The depletion of endogenous phosphorylated nucleotides may also contribute to ZDV-induced myelosuppression [36]. Decreased thymidine salvage resulting in decreased intracellular accumulation of thymidine nucleotides has been observed in thymidine-exposed H_9 cells when compared with unexposed cells [37]. Zidovudine-5′-monophosphate has also been shown to be inhibitory to thymidylate kinase with a resulting decrease in thymidine phosphate levels [38]. The active metabolite of ZDV against HIV, the 5′-triphosphate [38], is formed in human bone marrow CFU-GM [33]. Should the triphosphate be involved in toxicity, the kinetics of its formation would be important. The relationship of intracellular ZDV phosphate formation to plasma concentrations of the parent drug remains unclear, having been determined to be loosely correlated

with AUC in a small group of patients [39], but not to single measurements of plasma drug concentration [40].

Recent *in vitro* studies suggest that two previously unidentified ZDV catabolites, 3′-amino-3′deoxythymidine (AMT) and its glucuronide (GAMT) are formed in isolated hepatocytes and human liver microsomes [41, 42] and that AMT may play a significant role in ZDV-induced myelosuppression, being more toxic for CFU-GM and BFU-E than the parent compound, ZDV [41]. In addition both AMT and ZDV have been shown to decrease the rate of globin gene transcription in K-562 leukaemia cells with a corresponding decrease in haemoglobin synthesis [43]. Although AMT has been measured in patient plasma following the administration of radiolabelled ZDV (AUC approximately five times less than that of ZDV) [44] we were unable to measure AMT in the present study owing to chromatographic interference from endogenous compounds. The relationship of AMT to plasma concentrations of ZDV and to toxicity in patients remains to be established.

Several mechanisms appear to be involved in ZDV-induced myelosuppression. Despite the fact that ZDV toxicity to haematopoietic progenitor cells *in vitro* has been determined to be concentration-related [20], there was no indication from this study that it is directly related to plasma concentrations of ZDV.

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