Study on didanosine concentrations in cerebrospinal fluid

Implications for the treatment and prevention of AIDS dementia complex

• David M. Burger, Cees L. Kraayeveld, Pieter L. Meenhorst, Jan W. Mulder, Richard M.W. Hoetelmans, Cees H.W. Koks and Jos H. Beijnen

Introduction

Numerous studies have now demonstrated that the antiretroviral agent zidovudine modifies and prevents neurological disease associated with human immunodeficiency virus (HIV) infection, including the acquired immunodeficiency syndrome (AIDS) dementia complex [1-10]. With the introduction of zidovudine, the incidence of AIDS dementia complex has declined and dementia has become a rare disease in patients with AIDS on zidovudine [4 10]. It is generally believed that in order to inhibit HIV replication effectively in the central nervous system, zidovudine, or any other antiretroviral agent, must be able to cross the blood–brain barrier and achieve adequate concentrations in cerebrospinal fluid (CSF) [11 12].

Portegies *et al.* recently reported a relatively high incidence of AIDS dementia complex in patients participating in a randomized, double-blind trial comparing two doses of didanosine in patients with symptomatic HIV infection who were intolerant of zidovudine [13]. The incidence of AIDS dementia complex was 14% in 58 patients from Amsterdam and 11.1% in all 1,775 participants in the study. They suggested that this may be due to the low efficacy of didanosine on HIV-1 replication in the brain. As support for this hypothesis, they mentioned "...the fact that didanosine has not been detected in the cerebrospinal fluid" [13].

We present evidence that didanosine does cross the blood–CSF barrier and that detectable concentrations can be measured in CSF.

Methods

Four patients with AIDS who were on didanosine therapy were included in this study. All patients underwent a lumbar puncture for confirmation of a diagnosis of a neurological disorder and were using didanosine chronically. A corresponding blood sample was withdrawn within 5 min of the lumbar puncture. Samples were stored in glass (CSF) or polypropylene (plasma) tubes at –20°C. Each patient used the standard dose regimen, *i.e.* 250 mg of didanosine in sachets every 12 h, which is bioequivalent to 200 mg of didanosine in tablets every 12 h. Because the time after ingestion largely determines the plasma concentration, and therefore also the CSF/plasma ratio [14], all lumbar punctures were performed at a fixed time interval after the last dose, *i.e.* 4 h.

Concentrations of didanosine (provided by Bristol-Myers Squibb, Wallingford, USA) were determined in CSF and in plasma using a validated high-performance liquid chromatographic (HPLC) method developed at our laboratory [15]. Briefly, an aliquot of plasma was mixed with internal standard (inosine; Sigma, St. Louis, USA) and subjected to solid-phase extraction on preconditioned octadecyl extraction columns (Bakerbond SPE, J.T. Baker, Philipsburg,

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D.M. Burger, R.M.W. Hoetelmans, C.H.W. Koks and **J.H. Beijnen:** Department of Pharmacy, Slotervaart Hospital, Louwesweg 6, 1066 EC Amsterdam, the Netherlands.
Correspondence: D.M. Burger, Department of Clinical Pharmacy, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.
C.L. Kraayeveld: Department of Neurology, Slotervaart Hospital, Amsterdam, the Netherlands.
P.L. Meenhorst and **J.W. Mulder:** Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands.

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Abstract
It has been hypothesized that didanosine has a low efficacy in the prevention and treatment of patients with the dementia complex of acquired immunodeficiency syndrome (AIDS) because "...the drug has not been detected in the cerebrospinal fluid". We investigated didanosine concentrations in cerebrospinal fluid (CSF) and plasma of four patients with AIDS who were using didanosine chronically. Didanosine levels, 4 h after the last drug administration, averaged 0.16 (± 0.03) µmol/l in CSF and 0.70 (± 0.27) µmol/l in plasma. When compared with historical data from patients using zidovudine, didanosine concentrations in CSF appeared to be approximately half (on a molar base) those of zidovudine concentrations in the CSF. Whether this difference in CSF levels is the explanation for the presumed lower efficacy of didanosine in the prevention and treatment of AIDS dementia complex remains to be proven. However, it is clear from this study, in contrast with earlier suggestions, that didanosine is able to pass the blood–CSF barrier in human immunodeficiency virus-infected individuals.

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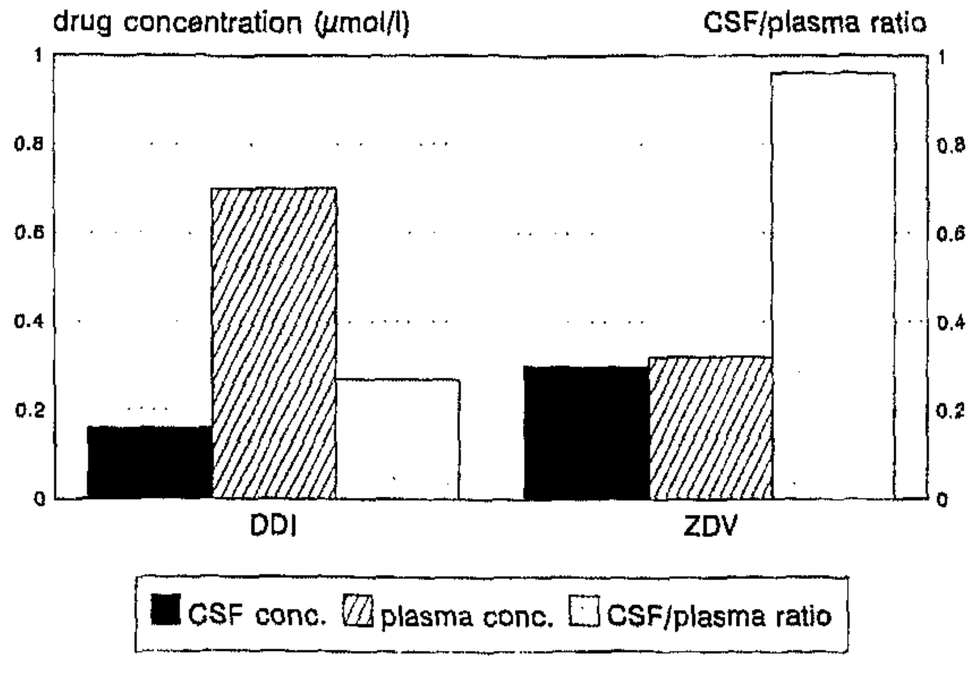
USA). After elution with methanol and evaporation of the solvent, the residue was redissolved in the mobile phase (960 ml of 0.005 mol/l phosphate buffer pH 6.8 + 40 ml methanol) and injected onto the HPLC column. Chromatography was carried out on an RP-8 analytical column (Merck, Darmstadt, Germany). The absorbance of the effluent was measured at 250 nm. The lower limit of quantitation was 0.04 µmol/l (0.01 mg/l). The standard curves for didanosine in plasma (0.04-40 µmol/l) were constructed by use of log-log regression analysis of didanosine/internal standard peak area ratios versus didanosine concentrations. Correlation coefficients of determinations were > 0.990 for all curves. Between-day and within-day variation of a quality control sample of 2 µmol/l didanosine were 12.8 and 10.0%, respectively.

In order to compare didanosine with zidovudine, data from five patients who used 200 mg of zidovudine every 8 h and who also underwent a lumbar puncture 4 h after the last dose were taken from a previous study [14]. Differences between zidovudine and didanosine concentrations (on a molar base) were tested by Student's t-test. A p value of 0.05 or less was considered to be significant.

Results

Individual and mean (± SD) concentrations of didanosine and zidovudine are listed in Table 1. Mean values are presented in Fig. 1. All patients on didanosine therapy had detectable concentrations of



▲ Figure 1
Comparison of cerebrospinal fluid and plasma concentrations of didanosine (DDI) and zidovudine (ZDV) 4 h after the last dose of didanosine (250 mg) or zidovudine (200 mg). Data are mean values of four (didanosine) or five (zidovudine) observations

Table 1 ***Cerebrospinal fluid and plasma concentrations of didanosine and zidovudine in patients with acquired immunodeficiency syndrome. Samples were taken 4 h after the last dose of didanosine (250 mg) or zidovudine (200 mg)***

Drug regimen	*Patient*	*CSF concentration (µmol/l)*	*Plasma concentration (µmol/l)*	*CSF/plasma ratio*
Didanosine 250 mg every 12 h	A	0.13	0.52	0.26
	B	0.14	0.91	0.16
	C	0.18	0.95	0.19
	D	0.20	0.42	0.47
	Mean	0.16	0.70	0.27
	SD	0.03	0.27	0.14
Zidovudine 200 mg every 8 h	E	0.60	0.63	0.95
	F	0.36	0.38	0.94
	G	0.09	0.08	1.10
	H	0.36	0.38	0.95
	I	0.11	0.12	0.88
	Mean	0.30	0.32	0.96
	SD	0.21	0.22	0.08
p Values		0.18	0.09	< 0.0001

didanosine in the CSF. The CSF and plasma concentrations of zidovudine were almost equal, whereas the CSF concentrations of didanosine were only 23% of those in plasma. As a result, the CSF/plasma ratio of didanosine concentrations was 3-4 times lower than the CSF/plasma ratio of zidovudine concentrations ($p < 0.0001$). However, this underestimates the CSF penetration of didanosine because absolute concentrations of didanosine were only approximately two times lower than zidovudine CSF concentrations ($p = 0.18$).

Remarkably, the between-subject variability in didanosine levels was lower than for zidovudine, both in plasma and in CSF samples. Two of the five patients receiving zidovudine had marginally lower CSF concentrations than any of the patients receiving didanosine.

Discussion

Our data are in agreement with other pharmacokinetic studies that reveal detectable didanosine concentrations in CSF, in humans [16-18] as well as in monkeys [19], dogs [20], and rats [21]. The observed low efficacy of didanosine in the prevention of HIV-1-related neurological complications leading to AIDS dementia complex in participants of the MRC-Alpha trial [13] thus cannot be explained by 'undetectable' CSF levels of the drug. The presumed lower efficacy of didanosine on HIV replication in the brain, compared to that of zidovudine, can also not be attributed to a lower CSF/plasma ratio of didanosine concentrations as suggested by some authors [12]. We have previously demonstrated that the CSF/plasma ratio, based on single plasma concentrations, is not an appropriate marker of drug penetration into CSF, because it is significantly influenced by the time since the last dose [14]. In addition, other reports are in conflict with the data from Portegies *et al.* [13] because improvement of HIV-related neurological disorder following the use of didanosine has been reported for both adults [16] and children [22]. Apparently, more clinical research is required to find out whether didanosine has an effect on the progression of AIDS dementia complex.

In order to compare the efficacy of antiretroviral drugs, drug levels in the CSF should be compared with parameters of antiretroviral drug activity, for example IC50 values. In this way, the two times lower absolute concentrations of didanosine in the CSF compared with those of zidovudine might be equalled by a greater sensitivity of HIV strains to didanosine. Further research in this area is needed. In addition, the uptake and subsequent phosphorylation of antiretroviral agents, including didanosine, by macrophages/monocytes of the brain or CSF has not been investigated yet.

Nevertheless, it is obvious that the penetration of didanosine into the CSF is far from optimal. This may be a problem for patients who do not tolerate zidovudine and who require high levels of didanosine in CSF to prevent or improve AIDS dementia complex. Two options that are currently in a preclinical stage are the development of prodrugs of didanosine with an enhanced uptake into the CSF [23] and a combination of didanosine with probenecid, which inhibits the efflux of didanosine from the CSF [24].

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