

Acyclovir Levels in Serum and Cerebrospinal Fluid after Oral Administration of Valacyclovir

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The possible involvement of herpesviruses in the pathogenesis of multiple sclerosis (MS) was recently investigated in a clinical trial of valacyclovir in patients with MS. As an important part of that survey we performed an independent pharmacokinetic study in order to determine the concentration of acyclovir in cerebrospinal fluid (CSF). The concentrations of acyclovir in serum and CSF were measured at steady state after 6 days of oral treatment with 1,000 mg of valacyclovir three times a day. Samples were obtained from 10 patients with MS. All patients had normal renal function, and none had signs of a damaged blood-CSF barrier. The maximum concentration of acyclovir in serum was reached after 1 to 3 h (mean \pm standard deviation [SD], $27.1 \pm 5.6 \mu\text{M}$), and the minimum concentration in serum was $3.1 \pm 1.1 \mu\text{M}$ (mean \pm SD). The acyclovir concentrations in CSF at 2 and 8 h were essentially stable, with the mean \pm SD levels being 2.5 ± 0.9 and $2.3 \pm 0.7 \mu\text{M}$, respectively. Similar levels were recorded in serum and CSF samples from five other MS patients after 6 months of oral treatment with valacyclovir at identical dosages. The area under the concentration-time curve (AUC) for acyclovir in CSF to the AUC for acyclovir in serum (CSF/serum AUC ratio) was approximately 20%. We conclude that the improved bioavailability previously reported for valacyclovir in plasma results in higher concentrations in CSF, while the CSF/serum AUC ratio remains constant.

Acyclovir is a purine nucleoside analogue with effects against human herpesvirus infections. The bioavailability of orally administered acyclovir is limited to 15 to 30% (23), and the level of passage across the blood-brain barrier has been assumed to be low, probably due to the relatively low lipophilicity of acyclovir (8). It was previously found (14) that upon oral administration the acyclovir concentration in cerebrospinal fluid (CSF) is only 13 to 52% of that found in plasma. When sustained and high concentrations of acyclovir in plasma are desirable, improved bioavailability can be achieved with valacyclovir, the hydrochloride salt of the L-valyl ester of acyclovir. After oral administration, valacyclovir is rapidly and almost completely converted to acyclovir on the first pass through the liver and gives rise to concentrations in plasma three to five times higher than those after administration of the corresponding doses of oral acyclovir (4). It is not known whether similar increases also involve the central nervous system (CNS) and CSF compartments. High acyclovir levels are required for the treatment of severe CNS infections in order to increase the antiviral efficacy and broaden the antiviral spectrum. CNS and CSF acyclovir levels are also of interest when patients are evaluated for symptoms or signs of suspected neurotoxicity. The pharmacokinetic study described here was performed as a part of a larger survey studying the possible involvement of herpesviruses in the pathogenesis of multiple sclerosis (MS) (3). The main purpose of the present study was to establish whether oral administration of valacyclovir at 1,000 mg three times a day (t.i.d.) would yield concentrations in CSF

in the range required for the treatment of herpesvirus infections.

MATERIALS AND METHODS

Study population. Ten patients (two men and eight women) with clinically definite MS (17) with a relapsing-remitting course were enrolled in a 6-day open-label pharmacokinetic study of oral valacyclovir at 1,000 mg t.i.d. Demographic data for the patients are presented in Table 1. All were included during a clinically stable period; and the median disability score, evaluated with the expanded disability status scale (EDSS) (12), was 2.0 (range, 1.0 to 5.0). Three patients were receiving no other medication, four patients were receiving oral contraceptives only, two patients were being treated for allergic diseases (one with inhaled corticosteroids and a beta-2-agonist and the other with an oral antihistamine), and the last patient was being treated orally with oxybutynin and iron tablets. None of the patients had received steroid treatment within the 4 weeks prior to entry into the study, and none had previously been treated with any other immunomodulatory drug. All patients had normal renal function, i.e., creatinine clearance (CL) ≥ 35 ml/min or serum creatinine level $\leq 120 \mu\text{mol/liter}$. The study medication was provided by GlaxoWellcome (Mölnådal, Sweden), and the study was approved by the National Board of Health and the Ethics Committee of Göteborg University, Sweden. All patients gave informed consent prior to inclusion in the study.

The patients noted the time that they took each dose, and for all patients, the last dose on day 6 was taken 8 h before administration of the final dose on day 7. The administration of the last dose was controlled by the study nurse. No special measures were taken to control food intake. Blood samples were obtained before treatment and on day 7 for safety analysis. Blood and CSF samples for pharmacokinetic analysis were obtained on day 7. Sampling of serum samples was done immediately before and at 1, 1[1/2], 2, 2[1/2], 3, and 8 h after administration of the last dose. The CSF samples were obtained by two separate lumbar punctures, the first at 2 h after administration of the last dose and the second at 8 h after administration of the last dose. It was performed with a needle 0.7 by 75 mm and the patient in a supine position. All samples were stored at -80°C before analysis. One serum sample and one CSF sample could not be obtained and were missing for analysis.

Five pairs of CSF and serum samples were obtained from five other MS patients, three women 21, 31, and 38 years of age and two men 24 and 53 years of age, who had been treated for 6 months with the same valacyclovir dosage

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TABLE 1. Demographic and clinical characteristics of the patients

Characteristic	Mean	SD	Range
Age (yr)	31	8	20–46
No. of men/no. of women	2/8		
Disease duration (yr)	7	6	1–18
EDSS	2.5	1.2	1–5
Albumin ratio	4.9	1.4	2.8–8.5
Wt (kg)	69	13	53–90
Creatinine CL (ml/min)	110	20	65–130

regimen described above. These patients participated in a randomized, double-blind, placebo-controlled trial to test for a possible effect of valacyclovir in patients with MS (3). Sampling was performed at the termination of the trial. The length of time from the time of administration of the last dose to the time of sampling was not recorded.

Laboratory analysis. Acyclovir concentrations were measured by high-pressure liquid chromatography with fluorescence detection after solid-phase extraction (24). The limit of quantification was 0.5 μM , and the interassay variation was less than 10%. The albumin concentrations in serum and CSF were quantified by electroimmunoassay as described by Laurell (13).

Statistics and pharmacokinetics. Data are presented as means \pm standard deviations (SDs). Pharmacokinetic parameters were assessed by noncompartmental methods, and calculation of the area under the concentration-time curve (AUC) was based on the linear trapezoidal method (20). Linear regression on the log concentration versus time was used to calculate the terminal elimination rate constant (k_{el}). The apparent CL and the volume of distribution (V) were calculated as $CL = \text{dose}/AUC_{0-8}$ (where AUC_{0-8} is the AUC from 0 to 8 h) and $V = CL/k_{el}$, respectively, and the half-life was equal to $\ln(2)/k_{el}$.

Nonlinear mixed-effect modeling with a first-order absorption, first-order elimination model was also used. Covariate equations were selected on the basis of the objective function value provided by the NONMEM program (version 4). Five models were compared: CL with and without proportional dependence on creatinine CL, V with and without proportional dependence on body weight, and V with or without interindividual variance. CL was assigned an interindividual variance in all models. An exponentially distributed error model was chosen. The first-order method was used to estimate parameters.

The paired t test was used to compare the maximum concentration in serum (C_{max}) and the minimum (trough) concentration in serum (C_{min}) for acyclovir.

The albumin ratio ([CSF albumin concentration/serum albumin concentration] $\times 1,000$) was calculated and was used to evaluate the function of the blood-CSF barrier (10). The value was compared with the CSF acyclovir concentration/serum acyclovir concentration ratio. Regression analysis was used to investigate a possible influence of the blood-CSF barrier on the CSF acyclovir concentration.

RESULTS

The average \pm SD peak level in serum 2 h after administration of the last dose was $23.5 \pm 5.7 \mu\text{M}$ (range, 18.1 to 36.0 μM) (Fig. 1). The C_{min} 6 h later was $3.1 \pm 1.1 \mu\text{M}$ (range, 2.4 to 5.9 μM). Individual peak levels in serum were reached between 1 and 3 h after drug administration, with a mean \pm SD C_{max} of $27.1 \pm 5.6 \mu\text{M}$ (range, 18.4 to 36.0 μM) during this interval. The apparent CL was 35 ± 6 liters/h, V was 110 ± 20 liters, and the terminal half-life was 2 h 10 min \pm 22 min.

Analysis of the pharmacokinetic data for the population gave very similar mean values for CL and V . The model that best fit the data had an absorption rate constant of 1.41 liters/h, a CL of 34.6 liters/h, and a V of $1.75 \cdot$ body weight liters. The interindividual coefficient of variation for clearance was 22%, and the intraindividual residual coefficient of variation was 39%. The interindividual variance for V and the dependence of CL on renal function were both nonsignificant. The improvement of fit to the data was highly significant between the base model and the final model ($\chi^2 = 42$; degrees of freedom = 2; $P < 0.001$).

The CSF acyclovir concentrations at 2 and 8 h were 2.5 ± 0.9 and $2.3 \pm 0.7 \mu\text{M}$ (mean \pm SD), respectively. No statistically significant difference between these levels in CSF was found, while the difference between C_{max} and C_{min} in serum was highly significant ($P < 0.0001$).

The CSF acyclovir concentration/serum acyclovir concentration ratio was calculated to determine the blood-CSF passage

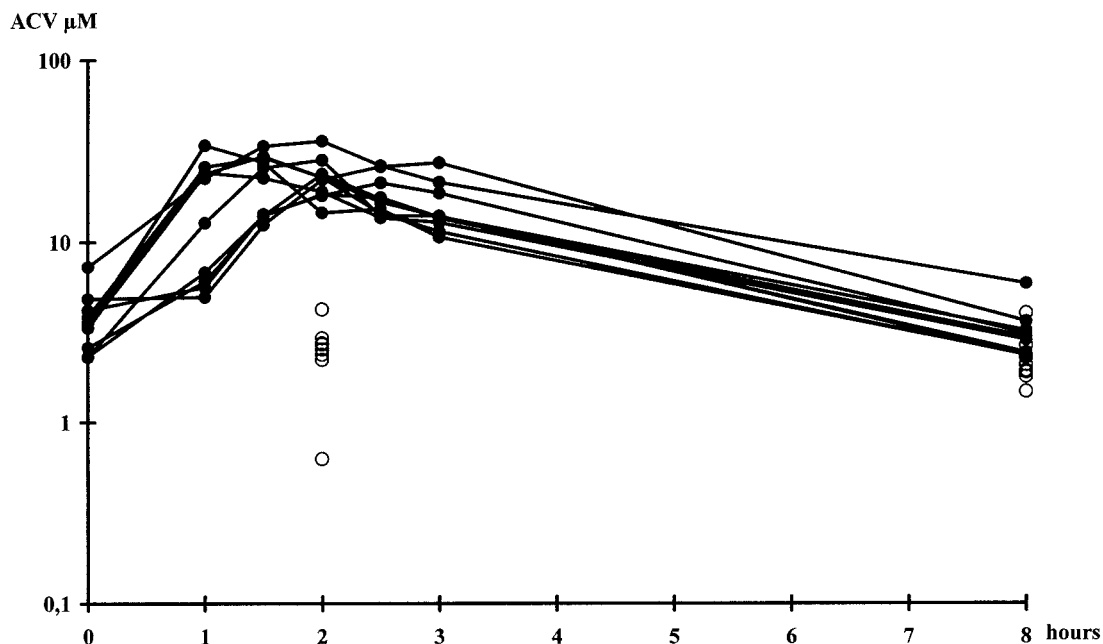


FIG. 1. Acyclovir (ACV) concentrations in serum versus time for each patient. Sampling was performed at steady state after administration of the last oral dose of valacyclovir at 1,000 mg t.i.d. The unconnected open circles are CSF acyclovir concentrations.

of acyclovir. The ratio at 2 h was $11\% \pm 4\%$ (mean \pm SD; range, 3 to 14%), and that at 8 h was $74\% \pm 9\%$ (range, 60 to 85%). Thus, the ratio was essentially dependent on the concentration of acyclovir in serum. The ratio of the AUC for acyclovir in CSF at 2 h plus the AUC for acyclovir in CSF at 8 h/AUC for acyclovir in serum was $19\% \pm 3\%$ (mean \pm SD).

The influence of the blood-CSF barrier function on the penetration of acyclovir into CSF was investigated. None of the patients demonstrated a damaged blood-CSF barrier; i.e., none had an elevated CSF albumin concentration/serum albumin concentration ratio (2). No relationship was found between the CSF acyclovir concentration/serum acyclovir concentration ratio and the CSF albumin concentration/serum albumin concentration ratio.

The acyclovir concentrations in serum and CSF were also compared with those obtained for five MS patients after long-term valacyclovir treatment. They had been treated for 6 months in a trial (3) that used the same valacyclovir dosage and administration regimen used in the present pharmacokinetic study. No sign of accumulation of acyclovir in the CSF was recorded, and serum and CSF acyclovir levels were essentially the same as those in the present pharmacokinetic study. The mean acyclovir concentration in serum was $16.7 \mu\text{M}$ (range, 1.21 to $27.9 \mu\text{M}$), and the mean acyclovir concentration in CSF was $3.4 \mu\text{M}$ (range, 2.0 to $4.6 \mu\text{M}$).

No serious adverse events were reported, and no drug-related abnormalities were detected from the results of hematology and biochemistry tests during the study. After the lumbar puncture, one patient had a mild headache, four patients had low back pain, and one patient had both a headache and low back pain.

DISCUSSION

Biotransformation of valacyclovir is virtually complete after oral administration on the first pass through the liver, with each gram of valacyclovir yielding approximately 700 mg of acyclovir and 300 mg of the amino acid valine. We have previously shown (14) that CSF acyclovir levels are slightly below $1 \mu\text{M}$ in MS patients after acyclovir is administered orally at 800 mg t.i.d. The CSF acyclovir levels found in the present study were three times higher, which is consistent with the three to five times higher bioavailability reported for valacyclovir (4). This finding implies that acyclovir transport to the CSF is not saturated at the concentrations attained in this study.

Although the serum acyclovir concentrations after oral administration of valacyclovir at 1,000 mg t.i.d. varied considerably during the 8 h over which they were observed, it would remain in excess of the *in vitro* 50% inhibitory concentration (IC_{50}) for most clinical isolates of herpes simplex virus type 1 (HSV-1) and HSV-2 (0.1 to $3.9 \mu\text{M}$) and was also at the IC_{50} for most clinical isolates of varicella-zoster virus (1.3 to $6.7 \mu\text{M}$). The acyclovir C_{max} reaches the IC_{50} for Epstein-Barr virus (4.4 to $13.3 \mu\text{M}$) but only partially reaches the IC_{50} s for cytomegalovirus (CMV; 10 to $>200 \mu\text{M}$) and human herpes virus type 6 (17.7 to $57.7 \mu\text{M}$) (1, 5, 21). The CSF acyclovir concentration was at the inhibitory level for HSV-1 and HSV-2 and was partially at the inhibitory level for varicella-zoster virus. However, although several studies have reported that

acyclovir has reduced antiviral activity for the treatment of established diseases caused by Epstein-Barr virus, CMV, and human herpesvirus 6, it appears to prevent reactivation of these viruses during prophylactic or combination treatments (29). Thus, although only moderate concentrations of acyclovir were achieved in the CSF, this level might be sufficient to prevent reactivation of latent infections caused by several species of herpesviruses.

Acyclovir has previously been shown to enter the CSF in experimental animals as well as in humans. This does not necessarily imply that similar concentrations are found in the brain parenchyma. However, in rats, acyclovir reaches levels in brain tissue about 20 to 30% of the concentrations in plasma (7, 22), and in three immunocompromised patients with fatal CMV pneumonia, a ratio of penetration into CSF of 25 to 70% of that in plasma was reported (27). The plasma protein binding of acyclovir has no marked influence on the ratios of the concentration in serum/concentration in CSF since it is limited to 9 to 33% (29). An important observation, often overlooked, is that levels in CSF vary considerably less over time than levels in plasma. This was one of the previous findings for acyclovir (14). Similar results for the human immunodeficiency virus protease inhibitor indinavir have been reported previously (16), and the same finding has also been made for zidovudine (19). Although CSF was sampled only twice in the present study, we assumed that the acyclovir concentration was essentially unchanged during the entire 8-h observation time. This assumption was supported by the consistency of stable acyclovir concentrations in the CSF at steady state both in the present study and in the previous study (14). In that study, stable CSF acyclovir levels were reported in three patients during oral treatment with acyclovir at 800 mg t.i.d. Lumbar punctures were performed on four different occasions during a study period of 12 months, and serum and CSF were sampled 1.5 h before or 1.5 h after dosing. We also collected CSF samples from two patients through a lumbar intradural catheter, which allowed us to simultaneously determine the concentration-time curve for acyclovir in serum and CSF over 8 h. Again, the CSF acyclovir level was essentially stable, while the concentration in serum fluctuated. Hence, even though only two CSF acyclovir concentrations were available for each patient, it seems possible to calculate the AUC for CSF from these values and more appropriate to use the ratio between the AUCs for CSF to the AUC for serum as a single number to indicate transport into the CSF and CNS compartments. In the present study we found a ratio of 20%, which is somewhat less than the ratio of 75% found for zidovudine (19) but similar to that found for indinavir (16).

Another interesting conclusion which can be drawn from the present data is that acyclovir must be actively transported out of the CSF compartment. The reason is that with completely passive transport, the AUCs for serum and CSF would be equal, while the pattern observed here is consistent with a slow diffusion across the blood-brain barrier and active transport out of the CSF (30).

Orally administered valacyclovir is generally very well tolerated, with few reported adverse events when it is administered at doses ranging up to 2,000 mg four times daily (30). Although the risk for neurotoxicity is mainly associated with intravenous acyclovir administration (18, 26, 28), these adverse effects have

also occurred during oral treatment and especially in patients with renal failure (6, 9, 11, 15, 25). The acyclovir concentrations have been reported for only a small number of patients with neurotoxicity. In one series, elevated peak levels in serum of 130 to 268 μM were found in four patients (28). In the present study, the serum acyclovir concentration was well below those previously reported for patients with neurotoxic adverse effects, and we found no signs of accumulation of acyclovir in the CSF after long-term treatment. It has been suggested that acyclovir neurotoxicity is also associated with increased CSF acyclovir concentrations. A dysfunction of the blood-CSF barrier, which is common during CNS infections, would then increase the risk for neurotoxicity. Further investigations are needed to establish the acyclovir level in CSF for neurotoxicity. It might be more appropriate to determine the CSF acyclovir level than to measure the concentration in serum for patients with suspected neurotoxicity. However, another possibility is that the carboxy metabolite of acyclovir is responsible for the neurotoxicity (A. Helldén et al., unpublished data).

It should be emphasized that although this investigation was performed with MS patients, they were healthy in all other respects, and none demonstrated any signs of a damaged blood-CSF barrier. Thus, we believe that our findings have a more general application for acyclovir and valacyclovir treatment.

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