Penetration of Clindamycin and Its Metabolite N-Demethylclindamycin into Cerebrospinal Fluid following Intravenous Infusion of Clindamycin Phosphate in Patients with AIDS

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Clindamycin, which is usually used in combination with pyrilmethamine, has been proven effective in the treatment of cerebral toxoplasmosis in human immunodeficiency virus-infected patients. However, it is not known if clindamycin achieves inhibitory concentrations at the site of infection. Also, it has been hypothesized that the activity of clindamycin against Toxoplasma gondii may be due, at least in part, to a metabolite. We evaluated the penetration of clindamycin and its major metabolite, N-demethylclindamycin (NDC), into cerebrospinal fluid (CSF) of AIDS patients undergoing lumbar puncture for diagnostic purposes. A single, 1,200-mg dose of clindamycin was administered as a 45-min intravenous infusion beginning at 1.5 or 2.5 h before CSF sampling. The concentrations of clindamycin in CSF ranged from 0.091 to 0.429 mg/liter at 1.5 h and from 0.120 to 0.283 mg/liter at 2.5 h following the beginning of the infusion. The concentrations of clindamycin in CSF were well above the 50% inhibitory concentration of 0.001 mg/liter and the parasiticidal concentration of 0.006 mg/liter. NDC was undetectable both in plasma and in CSF. Our study provides a pharmacokinetic rationale for the clinical efficacy of clindamycin in the treatment of cerebral toxoplasmosis.

Toxoplasmosis was recognized early as a significant cause of morbidity and mortality among patients with human immunodeficiency virus infection (12). The incidence of cerebral toxoplasmosis among AIDS patients in the United States varies from 5 to 10% (9, 12), while in western Europe it varies from 10 to 40% (2, 12). The activity of clindamycin for acute and maintenance therapy of cerebral toxoplasmosis in AIDS patients has been documented by several studies (3, 10). Clindamycin, usually administered in combination with pyrimethamine, has been shown to induce significant clinical and radiological improvement in such patients.

Up to a few years ago the mechanism of clindamycin activity against Toxoplasma gondii was highly controversial. In fact, it had been reported that the parent compound was active in vivo (8) in a murine model of toxoplastic encephalitis but was not active in vitro in cell cultures of T. gondii (7). This observation suggested that the apparent activity of clindamycin against T. gondii may be due, at least in part, to a metabolite. A more recent study (14) showed that clindamycin is extremely active against T. gondii in vitro, with a 50% inhibitory concentration (IC50) determined at 1 to 3 days of incubation, as low as 1 ng/ml and with a parasiticidal effect observed at a concentration as low as 6 ng/ml. Such delayed activity may represent an effect on parasite progeny after a replicative cycle caused by an impairment of mitochondrial protein synthesis of T. gondii rather than an effect on eucaryotic cytoplasmic protein synthesis.

The pharmacokinetic properties of drugs administered to AIDS patients may be altered because of altered gastrointestinal function, low body weight, or altered plasma protein concentrations (5, 11, 13). Therefore, it is important to characterize the values of relevant pharmacokinetic parameters, such as cerebral spinal fluid (CSF) penetration, in this specific patient population.

Our study aimed at the determination of the concentrations of clindamycin and its major metabolite, N-demethylclindamycin (NDC), in the blood and CSF of patients with AIDS following a single, 1,200-mg intravenous infusion.

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Patients. The study subjects were 10 patients with AIDS scheduled for lumbar puncture due to focal or generalized neurological abnormalities, such as seizure, paresis, or sensory loss. Toxoplasmic meningoencephalitis was excluded based on clinical signs and on the results of computed tomographic brain scanning performed before lumbar puncture. The study was approved by the hospital’s Committee for Human Research. Written informed consent was obtained from each patient before participation in the study. A complete physical examination, including patient history, vital signs, electrocardiography, and a panel of laboratory tests, consisting of a chemistry screening, a complete blood cell count with differential and platelet counts, and urinalysis, was conducted at the time of subject selection. CSF samples were subjected to standard examination for glucose, protein, and electrolyte levels as well as differential cell count. Smears and culture for bacteria, fungi, and parasites were performed on each CSF sample.

Patients were excluded from the study if they had received any investigational drug within 8 weeks prior to the beginning of the study. They were also excluded if they had hypersensitivity or allergy to clindamycin or lincomycin, history of antibiotic-associated colitis, bleeding tendencies, peptic ulcers, gastrointestinal bleeding, history of alcoholism, an absolute neutrophil count of ≤750 cells/mm³, a hemoglobin level of ≤7.5 g/dl, a platelet count of ≤50,000/mm³, or a bilirubin or serum creatinine level of ≥2 times the upper limit of the nor-
nal range. All patients had alanine aminotransferase levels not exceeding three times the upper limit of the normal range except for patient 8, who had a value of 256 U/liter.

**Drug administration and sample collection.** Each patient was hospitalized prior to participation in the study. Each patient received 1,200 mg of clindamycin (Dalacin C Phosphate) as an intravenous infusion over 45 min except patient 10, who received the same clindamycin dose but as a 60-min infusion. Eight milliliters of sterile 5% glucose injectable solution was withdrawn from a 100-ml bottle and replaced with 8 ml of a solution containing 1,200 mg of clindamycin. This final solution (containing 12 mg of clindamycin/ml) was infused with an infusion pump.

For five patients the clindamycin infusion was begun at approximately 1.5 h prior to CSF collection, and for the remaining five patients it was begun at approximately 2.5 h prior to CSF collection. CSF was collected by following standard clinical protocols. The lumbar puncture was made by placing the aspiration needle into the subarachnoid space of the lumbar area. Blood contamination of CSF was excluded by visual inspection and by cytochemical examination of CSF samples.

A blood sample (8 ml) was obtained from each subject prior to clindamycin administration, and an additional blood sample was obtained simultaneously with the CSF sample (6 ml). Blood samples were collected in tubes containing heparin, placed on ice, and centrifuged within 30 min after collection. Plasma and CSF samples were stored at −70°C until they were assayed for clindamycin and its metabolite NDC.

**Analytical methods.** The concentrations of clindamycin and NDC in plasma and CSF samples were determined by a high-performance liquid chromatography (HPLC) assay developed in our laboratory. Clindamycin and NDC powders were kindly provided by Pharmacia and Upjohn (Kalamazoo, Mich.).

Extraction of clindamycin and NDC from plasma was done by adding 300 µl of NaOH (1 N) and 4 ml of diethyl ether to 1 ml of plasma and vortexing for 20 s. The tubes were shaken for 10 min and then centrifuged for 15 min, and the upper organic layer was taken up and evaporated to dryness under a gentle stream of nitrogen. The pellet was then reconstituted with 200 µl of the mobile phase and injected into the column.

Separation of clindamycin and NDC from plasma and CSF was carried out with a Supelcosil ABZ-LC, C18 column (5-µm inside diameter, 15-cm height, and 4.6-mm outside diameter) (lot 250955AC; Supelchem, Milan, Italy) and acetonitrile-water (75:25), 15-cm height, and 4.6-mm outside diameter).

The standard curves for determination of clindamycin concentrations in plasma and CSF were obtained by using known standards prepared in the laboratory at 0.1, 0.2, 0.5, 1, 2, 5, and 10 µg/ml; quality control samples contained 0.25, 2.5, and 7.5 mg per liter of plasma blank. The lower limit of quantitation was 0.2 mg/liter. The interday (interval of not more than 4 days) (n = 6) and intraday (n = 6) precision values for clindamycin and NDC in plasma and CSF were determined at the quality control sample concentrations of 0.25, 2.5, and 7.5 mg/liter. The interday precision values for clindamycin at these concentrations in plasma blanks were 5.5, 2.2, and 7.5%, respectively; the intraday values were 7.6, 3.3, and 5.7%, respectively. The interday values for NDC in plasma were 20.3, 7.7, and 2.4%, respectively, and the intraday values were 7.8, 7.7, and 4.0%, respectively. The interday precision values for clindamycin concentration in CSF were 2.3, 4.7, and 7.4%, respectively, and the intraday values were 15.1, 4.2, and 5.0%, respectively. The overall accuracy (difference of the estimated mean concentration from the known standard concentration) for the standards and quality control samples used in the validation procedure (i.e., intra- and interday experiments) was less than 7% for clindamycin and NDC each in plasma and CSF except for the quality control samples for NDC at 0.25 mg/liter, which was 12%.

This HPLC assay presents improved characteristics compared to the HPLC assay previously employed for clindamycin (4). The present assay incorporates recent advances in column technology. The Supelcosil ABZ column allows reverse-phase chromatography without use of an organic modifier as a component of the mobile phase. This is because the column is prepared by a deactivation technique that allows reverse-phase chromatography without techniques involving silanol or competing amines to improve peak shape and separation. The assay resulted in improved peak definition and improved peak shape as well as simplicity of mobile-phase preparation.

The relationship of clindamycin concentrations in plasma and CSF with clinical parameters was evaluated by linear regression analysis.

**Analysis results.** The patient clinical profiles are shown in Table 1. Signs of very mild blood-brain barrier inflammation were evident only for patients 1 and 3, as can be seen from the CSF values for leukocyte count and levels of glucose and proteins. Patients 1, 3, 7, and 8 had cryptococcal meningitis. CSF culture yielded negative results for the remaining patients. The final diagnoses and clindamycin concentrations in plasma and CSF are also shown in Table 1. The concentrations of clindamycin in plasma ranged from 6.3 to 26.5 mg/liter at 1.5 h and from 10.6 to 19.6 mg/liter at 2.5 h following the beginning of the intravenous infusion. The concentrations of clindamycin in CSF ranged from 0.091 to 0.429 mg/liter at 1.5 h and from 0.120 to 0.283 mg/liter at 2.5 h following the beginning of the intravenous infusion. The CSF/plasma ratio ranged from 0.009 to 0.031 at 1.5 h and from 0.008 to 0.018 at 2.5 h following the beginning of the intravenous infusion. NDC was not detectable in any of the plasma or CSF samples.

The concentrations of clindamycin in plasma were within the expected range. In fact, simulation of plasma concentrations with the pharmacokinetic parameters obtained in a previous study in HIV-positive patients (5) resulted in mean concentrations of 16.2 mg/liter at 1.5 h and for the remaining five patients it was begun at approximately 2.5 h prior to plasma with saline solution (7.5:92.5), since the protein concentration at 1.5 h ranges from 5 mg/liter. The interday precision values for clindamycin and NDC in plasma and CSF were determined at the quality control sample concentrations of 0.25, 2.5, and 7.5 mg/liter. The interday precision values for clindamycin at these concentrations in plasma blanks were 5.5, 2.2, and 7.5%, respectively; the intraday values were 7.6, 3.3, and 5.7%, respectively. The interday values for NDC in plasma were 20.3, 7.7, and 2.4%, respectively, and the intraday values were 7.8, 7.7, and 4.0%, respectively. The interday precision values for clindamycin concentration in CSF were 2.3, 4.7, and 7.4%, respectively, and the intraday values were 15.1, 4.2, and 5.0%, respectively. The overall accuracy (difference of the estimated mean concentration from the known standard concentration) for the standards and quality control samples used in the validation procedure (i.e., intra- and interday experiments) was less than 7% for clindamycin and NDC each in plasma and CSF except for the quality control samples for NDC at 0.25 mg/liter, which was 12%.

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TABLE 1. Patient profiles and concentrations of clindamycin in plasma and CSF following a single, 1,200-mg intravenous infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Body wt (kg)</th>
<th>Height (cm)</th>
<th>CD4+ cell count (cells/μl)</th>
<th>Protein (g/liter)</th>
<th>Leukocytes (cells/μl)</th>
<th>Glucose (mg/dl)</th>
<th>Final clindamycin concentration in plasma (μg/ml)</th>
<th>Final clindamycin concentration in CSF (μg/ml)</th>
<th>CSF/plasma concentration</th>
<th>Final diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>M</td>
<td>80.0</td>
<td>179</td>
<td>25</td>
<td>83</td>
<td>0.53</td>
<td>16</td>
<td>0.191 mg/liter/kg at 1.5 h</td>
<td>0.091 mg/liter/kg at 1.5 h</td>
<td>0.21 mg/liter/kg at 1.5 h</td>
<td>Cryptococcal meningitis</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>M</td>
<td>57.2</td>
<td>170</td>
<td>55</td>
<td>58</td>
<td>0.63</td>
<td>12</td>
<td>0.143 mg/liter/kg at 1.5 h</td>
<td>0.011 mg/liter/kg at 1.5 h</td>
<td>0.13 mg/liter/kg at 1.5 h</td>
<td>Cryptococcal meningitis</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>M</td>
<td>50.5</td>
<td>173</td>
<td>6</td>
<td>78</td>
<td>0.13</td>
<td>47</td>
<td>0.131 mg/liter/kg at 1.5 h</td>
<td>0.018 mg/liter/kg at 1.5 h</td>
<td>0.07 mg/liter/kg at 1.5 h</td>
<td>Cysticercal brain cyst</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>M</td>
<td>46.3</td>
<td>180</td>
<td>5</td>
<td>64</td>
<td>0.13</td>
<td>40</td>
<td>0.113 mg/liter/kg at 1.5 h</td>
<td>0.008 mg/liter/kg at 1.5 h</td>
<td>0.006 mg/liter/kg at 1.5 h</td>
<td>Cerebral lymphoma</td>
</tr>
<tr>
<td>Mean</td>
<td>40</td>
<td>M</td>
<td>62.2</td>
<td>175</td>
<td>24</td>
<td>72</td>
<td>0.43</td>
<td>29</td>
<td>0.229 mg/liter/kg at 1.5 h</td>
<td>0.013 mg/liter/kg at 1.5 h</td>
<td>0.014 mg/liter/kg at 1.5 h</td>
<td>Cerebral lymphoma</td>
</tr>
<tr>
<td>SD (%CV)</td>
<td>11 (27)</td>
<td></td>
<td>11.9 (19)</td>
<td>175</td>
<td>24</td>
<td>72</td>
<td>0.43</td>
<td>29 (61)</td>
<td>0.26 (62) mg/liter/kg at 1.5 h</td>
<td>0.013 mg/liter/kg at 1.5 h</td>
<td>0.014 mg/liter/kg at 1.5 h</td>
<td>Cerebral lymphoma</td>
</tr>
</tbody>
</table>

- CD4+ cell count: Number of CD4+ cells in the blood.
- Protein: Protein concentration in the blood.
- Leukocytes: Number of leukocytes in the blood.
- Glucose: Glucose concentration in the blood.
- Final clindamycin concentration in plasma: Concentration of clindamycin in the blood at the end of the study.
- Final clindamycin concentration in CSF: Concentration of clindamycin in the cerebrospinal fluid at the end of the study.
- CSF/plasma concentration: Ratio of the concentration of clindamycin in the cerebrospinal fluid to that in the blood.
- Final diagnosis: Diagnosis at the end of the study.

Actual time from the beginning of infusion until the plasma and CSF sample collection was 1.5 h in patients 1 through 5 and 2.5 h in patients 6 through 10.

M, male; F, female.

The concentrations of clindamycin in the patients who had signs of mild blood-brain barrier inflammation did not appear to differ from the concentrations in other patients. There was a significant negative correlation between CSF clindamycin concentrations and CSF protein concentrations (r = 0.72, P = 0.018). Normalization of concentration in plasma to patient body weight resulted in the following values: 0.291 ± 0.191 mg/liter/kg at 1.5 h and 0.246 ± 0.246 mg/liter/kg at 2.5 h.

Although it is commonly thought that clindamycin penetration through the blood-brain barrier is poor (15) as a consequence of high protein binding and poor liposolubility, there are no data in the literature regarding such pharmacokinetic aspect of clindamycin in humans. The results of our study show that the proportion of clindamycin that penetrates the blood-brain barrier (penetration ratio) is low, given the mean concentrations obtained at the two time points following the beginning of infusion. If a significant system hysteresis underlies the kinetics of clindamycin penetration of the blood-brain barrier, a better approach to characterization of CSF penetration would have been the calculation of the ratio of the areas under the concentration-time curves for CSF and for plasma. However, in light of the low concentrations in CSF observed, such an approach would not have resulted in a substantial discrepancy with the penetration ratio observed in our study.

The clindamycin concentrations in the patients who had signs of mild blood-brain barrier inflammation did not appear to differ from the concentrations in other patients. There was a significant negative correlation between CSF clindamycin concentrations and CSF protein concentrations (r = 0.84, P = 0.005). The mechanism underlying this observation is unclear. Since our study was an open, prospective, single-dose, exploratory study we did not specifically evaluate the penetration of clindamycin in patients with cerebral toxoplasmosis. However, the data obtained in this study should be predictive of clindamycin penetration in patients with cerebral toxoplasmosis, since this pathology is not usually associated with significant blood-brain barrier disruption.

The concentrations achieved in CSF should be evaluated in light of the inhibitory concentration of clindamycin for T. gondii. In our study, clindamycin concentrations in CSF averaged approximately 0.2 mg/liter, a value several times higher than the IC50 of 1 ng/ml and the parasiticidal concentration of 6 ng/ml. Since clindamycin and pyrimethamine demonstrate synergistic activity in vivo against T. gondii, data on the IC50 of clindamycin for T. gondii in the presence of concentrations of pyrimethamine achievable in CSF should be obtained in the appropriate in vitro model.

It is not possible to establish whether the CSF concentrations of clindamycin found in our study are predictive of the concentrations in the cerebral parenchyma, which represents the site of infection by T. gondii in the central nervous system. A study of clindamycin concentrations in brain tissue may be of...
some help in this regard if evaluated in light of the concentrations in CSF found in our study. On the other hand, it is well known that studies of drug penetration in homogenized tissue are associated with serious problems of interpretation. It has to be considered that clindamycin is actively transported in various phagocytic cells by a cell membrane nucleoside (adenosine) transport system (6). Therefore, it may be possible that such cells serve as the drug vector and deliver clindamycin to the site of infection, resulting in a higher local drug concentration in the microenvironment of the intracranial mass lesions caused by *T. gondii*.

The absence of NDC from plasma was somewhat surprising. Only two reports are available in the literature regarding the concentrations of NDC in humans. One study (1) showed indirect evidence of the presence of NDC, although the compound was not recovered from urine in sufficient quantity for unequivocal identification. The other study (16) allows only indirect estimation of the NDC level in plasma expressed as the difference of the concentrations determined with a microbiological and a specific assay. Based on the results of these studies (1, 16) the expected NDC concentrations in the patients enrolled in our study would range between 0.3 and 0.6 mg/liter. Therefore, NDC should have been detectable, at concentrations close to the lower limit of detection of our assay. The observation of plasma NDC concentrations below the detection limit may be due to decreased rate and/or extent of metabolite formation in the AIDS patient population, leading to higher levels of protein binding and decreased intrinsic clearance (5). Also, assuming enterohepatic recirculation, a lower concentration of metabolite in plasma may be caused by a lower metabolism rate in the gut, which has been advocated as a possible underlying mechanism for the greater bioavailability of clindamycin observed in AIDS patients than in healthy volunteers (5). Considering that (i) the plasma NDC concentrations were <0.2 mg/liter in all the patients, (ii) the CSF/plasma concentration ratio for clindamycin averaged 2%, and (iii) NDC is more polar than the parent compound and therefore is expected to penetrate less freely across the blood-brain barrier, the CSF NDC concentrations in the patients in our study may be assumed to be <0.004 mg/liter, that is, 2% of 0.2 mg/liter. Therefore, even though we cannot exclude the possibility that NDC contributes to the overall activity of clindamycin against *T. gondii*, our study seems to confirm that this activity is due primarily to the parent compound.

In conclusion, our study supports the use of clindamycin in the treatment of cerebral toxoplasmosis, due to the favorable concentrations achieved in CSF.

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