Cerebrospinal Fluid Penetration of Active Metabolites of Cyclophosphamide and Ifosfamide in Rhesus Monkeys

Carola A. S. Arndt,1 Frank M. Balis, Cynthia Lester McCully, O. Michael Colvin, and David G. Poplack

Pediatric Branch, National Cancer Institute, Bethesda, Maryland 20892 [C. A. S. A., F. M. B., C. L. M., D. G. P.] and The Johns Hopkins Oncology Center, Baltimore, Maryland 21205 [O. M. C.]

ABSTRACT

The penetration of the active metabolites of cyclophosphamide (CP) and ifosfamide (IF) into cerebrospinal fluid (CSF) was determined in rhesus monkeys following an i.v. infusion of 1 gm/m² of CP and IF. Active metabolites were measured using a high-performance liquid chromatography assay with fluorometric detection following derivatization with m-aminophenol. CSF to blood ratios of the active metabolites of CP and IF were found to be 0.17 and 0.13 following systemic dosing of CP and IF, respectively. The levels achieved in the CSF, however, were equivalent to levels known to be cytotoxic to malignant cell lines derived from tumors which metastasize to the central nervous system. Only one animal demonstrated neurotoxicity with IF. CSF levels of active metabolite in this animal were similar to those observed in the other animals.

INTRODUCTION

CP2 and IF are alkylating agents which require activation by hepatic microsomal oxidases in order to exert their cytocidal effect. The active circulating metabolites of CP and IF appear to be 4-HCP and 4-HI, respectively (1-6). The pharmacology and pharmacokinetics of the parent compounds, CP and IF, and their mustard metabolites have been well characterized (7-12). However, despite the fact that phosphoramide mustard and nonrtnidostin mustard are highly reactive species, they are less cytotoxic than the primary metabolite (4-HCP) in vitro, probably because of poor penetration into cells (8). Alkylating activity following CP and IF administration has also been measured, but alkylating activity is very nonspecific and does not necessarily correlate with cytocidal activity (1).

Only limited information is available on the kinetic behavior of the active metabolites 4-HCP and 4-HI (13-15) despite the fact that the pharmacokinetic parameters of these compounds may correlate better with response or toxicity than the parameters of the parent compounds or alkylating activity. The primary active metabolites 4-HCP and 4-HI can be measured using a technique which measures acrolein released from 4-HCP or 4-HI and derivatized with m-aminophenol (16). The resulting compound, 7-hydroxyquinoline, can be quantitated by high-performance liquid chromatography. This method has not been applied previously to the measurement of active metabolites in the CSF.

This study determined the extent of penetration of the active metabolites of CP and IF into the CSF following systemic administration of CP and IF in a subhuman primate model which has previously been shown to reliably predict CSF penetration and pharmacokinetics of drugs administered in equivalent m² doses to humans (16, 17). Each animal was studied following 30-min infusions of 50 mg/kg (1 gm/m²) CP and IP. The order of administration of CP or IP was randomized, and each animal was studied at intervals of at least 2 wk. Blood and CSF samples were scheduled to be drawn before and at 5, 15, 30, 45, 60, 90, 120, 180, and 240 min following the start of the infusion. After procurement, samples were immediately placed into the reaction mixture used for derivatization.

Drug. Cyclophosphamide and ifosfamide were obtained in 1-g vials from the Pharmaceutical Resources Branch of the National Cancer Institute and were reconstituted with sterile water to a concentration of 20 mg/ml. The doses were administered as 30-min infusions through a catheter inserted into the femoral vein.

Drug Assay. CSF and blood samples were immediately placed into an acidified reaction mixture for derivatization with m-aminophenol according to the method of Alarcon (16). The acidification releases acrolein from 4-HCP, and the acrolein reacts with the m-aminophenol to produce fluorescent 7-hydroxyquinoline, which is then measured by high-performance liquid chromatography using a fluorometric detector (model 970; Kratos, Westwood, NJ). Excitation occurred at 330 nm and a 418-nm band pass emission filter was used. Derivatized samples were centrifuged and the supernatant was injected onto an aBondapak phenyl column (model 27198; Waters Associates, Milford, MA) and eluted isocratically with a mobile phase of 10% acetonitrile in 0.15 M formic acid at a flow rate of 2 ml/min. The retention time for 7-hydroxyquinoline was 16 min. The limit of sensitivity for the assay was 0.2 µg. This derivatization procedure will detect acrolein or any compound which releases acrolein. CSF and blood samples obtained prior to drug administration showed no peak corresponding to the retention time of 7-hydroxyquinoline, therefore we assume that any peak observed subsequently reflects the primary metabolites of CP or IF.

Although free acrolein in CSF and blood would interfere with this method, acrolein has not been detected in CSF and blood following CP administration (13), and, therefore the peaks observed are assumed to be from the 4-HCP or 4-HI.

Pharmacokinetic Calculations. The AUCs for 4-HCP and 4-HI were determined by using the linear trapezoidal rule (20) and summing the areas up to the last measured time point. CSF to blood ratio was calculated as the ratio of the AUC for active metabolite in CSF to the AUC of the active metabolite in blood. This ratio was used as a measure of CSF penetration of active metabolite.

RESULTS

Figs. 1 and 2 show the mean CSF and blood concentration-time curves of active metabolites for the three monkeys following administration of 1 gm/m² CP and IF, respectively. Peak blood levels were reached at the end of the infusions (30 min), and peak CSF levels were reached 51 and 58 min after the start of the infusions of CP and HI, respectively.

Table 1 shows pharmacokinetic parameters for active metabolites in CSF and blood following administration of CP and IF. CSF to blood ratios for the active metabolites of CP and IF were 0.17 and 0.13, respectively. One animal demonstrated evidence of neurotoxicity (tremors, abnormal eye movements, tremors, abnormal eye movements,
CSF LEVELS OF ACTIVE METABOLITES OF CYCLOPHOSPHAMIDE AND IFOSFAMIDE

Table 1 Pharmacokinetic parameters for active metabolites of CP and IF in CSF and blood. Values presented in table are mean values from three monkeys. Numbers in parentheses represent ranges.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Peak (μM)</th>
<th>AUC (μM-min)</th>
<th>Peak (μM)</th>
<th>AUC (μM-min)</th>
<th>(AUC)CSF: (AUC) blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>40 (37-45)</td>
<td>3500 (2800-3900)</td>
<td>5 (3-6)</td>
<td>600 (300-900)</td>
<td>0.17 (0.11-0.24)</td>
</tr>
<tr>
<td>IF</td>
<td>22 (14-28)</td>
<td>3100 (2200-3800)</td>
<td>2 (1-2)</td>
<td>400 (300-500)</td>
<td>0.13 (0.11-0.14)</td>
</tr>
</tbody>
</table>

Creaven et al. found CSF levels of IF which were 23–49% those of plasma 3 h following doses of 3.8–5 gm/m² in 3 patients, and negligible amounts of alkylating activity were detected in the CSF (11). Bahr et al. found a level of unchanged CP in the CSF of one patient which was 80% of that in the plasma (22). Egorin et al. also found excellent CSF penetration of unchanged cyclophosphamide but poor penetration of alkylating activity (23). Current understanding is that the therapeutically important circulating metabolites of CP and IF are the 4-hydroxy derivatives (1–6). 4-HCP and 4-HI, which have relatively low alkylating activity, are thought to serve as the transport forms of the ultimate cytotoxic intracellular alkylator, phosphoramide mustard (3, 8). The 4-hydroxylated derivatives are nonpolar and would be expected to enter cells more readily than phosphoramide mustard (8).

In light of the above information, this study examined the blood and CSF levels of 4-HCP and 4-HI. Following administration of 1 gm/m² CP or IF, the mean CSF to blood ratios for 4-HCP and 4-HI were 0.17 and 0.13 respectively. The finding of 13–17% penetration of the active metabolite into the CSF contrasts with the poor penetration of alkylating activity found by other investigators (11, 23). This could be explained by the nonpolar nature of 4-HCP, which would facilitate crossing the blood-brain barrier.

Of additional importance is that following administration of 1 gm/m² CP and IF, the AUCs of 4-HCP and 4-HI in the CSF approach or equal levels which are known to be cytotoxic in vitro. In previous work we demonstrated that 90% inhibition of clonogenic survival of Molt-4, rhabdomyosarcoma, and MCF-7 cell lines is obtained with an exposure of 150 μM·min, 480 μM·min, and 570 μM·min, respectively, to 4-hydroperoxycyclophosphamide (24). 4-Hydroperoxycyclophosphamide is a preactivated derivative of CP which exhibits equal cytotoxicity, on a molar basis, to 4-HCP (25). The mean AUCs for 4-HCP and 4-HI obtained in CSF in this study were 600 and 400 μM·min, respectively, indicating that cytocidal levels of active metabolite were obtained in the CSF.

Neurotoxicity has been reported in patients receiving ifosfamide (26) but is uncommon with cyclophosphamide. In this study, only one animal exhibited any evidence of neurotoxicity following the IF infusion, and the levels of active metabolite were no higher in this animal than in the other animals. There was also no difference in 4-HCP and 4-HI penetration into the CSF in our model.

REFERENCES

4. Cox, P. J., Phillips, B. J., and Thomas, P. The enzymatic basis of the selective...
CSF LEVELS OF ACTIVE METABOLITES OF CYCLOPHOSPHAMIDE AND IFOSFAMIDE


Cerebrospinal Fluid Penetration of Active Metabolites of Cyclophosphamide and Ifosfamide in Rhesus Monkeys

Carola A. S. Arndt, Frank M. Balis, Cynthia Lester McCully, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/8/2113

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.