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

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

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 cytotoxic 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 phosphoramidase mustard and nornitrogen 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 cytotoxic 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 in humans.

MATERIALS AND METHODS

Monkeys. Three adult male rhesus monkeys weighing 7–8.5 kg, with normal hepatic and renal function, were used in these experiments.

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.

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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,
and abnormal shaking of the extremities) following administration of IF. The levels of active metabolite observed in both blood and CSF in this animal were similar to those observed in the other animals.

**DISCUSSION**

Cyclophosphamide and ifosfamide are alkylating agents in clinical use for a wide variety of malignancies, many of which metastasize to the CNS. In addition CP has demonstrated activity against medulloblastoma (21). The data which are available on CSF penetration of CP and IF are scarce and restricted to analysis of parent compound or alkylating activity.

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 HI, 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 cytotoxic 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.

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