Intrathecal Administration of 4-Hydroperoxycyclophosphamide in Rhesus Monkeys

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ABSTRACT

The preactivated cyclophosphamide analogue, 4-HC, does not require activation by hepatic microsomal enzymes to express its cytotoxic activity and therefore, unlike cyclophosphamide, may be useful for the regional therapy of cancer. In the present study, the pharmacokinetics and toxicology of 4-HC were studied following intraventricular administration of 0.4 mg to rhesus monkeys with chronic indwelling Ommaya reservoirs. 4-HC was measured in cerebrospinal fluid (CSF) and plasma with a high-performance liquid chromatography assay utilizing a fluorometric detector following derivatization with m-aminophenol. The mean peak level of 4-HC in ventricular CSF was 100 μM 5 min after administration. The drug was cleared rapidly and the elimination was monoexponential with a mean half-life of 22 min. The mean clearance from CSF (0.33 ml/min) was 10-fold higher than CSF bulk flow. The drug was distributed throughout the subarachnoid space with lumbar levels approaching ventricular levels by 60 min. Neither acute nor chronic neurotoxicity or systemic toxicity was observed during the 6-wk observation period.

Concentrations of 4-HC demonstrated to be cytotoxic in vitro against human breast cancer, lymphoid leukemia, and rhabdomyosarcoma were readily achieved in CSF following intraventricular administration. This study demonstrates that intraventricular therapy with 4-HC is feasible and suggests that further study of this approach in the clinical setting should be considered.

INTRODUCTION

CPA, an alkylating agent with a broad spectrum of antitumor activity, is actually a produg which, to be activated, must be converted by hepatic microsomal enzymes into 4-hydroxycyclophosphamide. This compound subsequently undergoes spontaneous decomposition to a variety of biologically active compounds. Because of its requirement for hepatic activation, CPA cannot be used for regional therapy. In contrast, 4-HC, a preactivated derivative of CPA, exhibits equal toxicity on a molar basis to cells in vitro as 4-hydroxycyclophosphamide, the principal cytotoxic metabolite of CPA (1). 4-HC has been shown to be active in vitro against L1210 murine leukemia (2), MX-1 human breast cancer (3), and Burkitt’s lymphoma (4) cell lines; and 4-HC is currently used clinically to purge tumor cells from bone marrow prior to autologous marrow transplantation (5, 6). Since 4-HC does not require hepatic activation, we have studied the possibility of its use for regional chemotherapy. The present study was performed to determine the feasibility and define the pharmacokinetics of intrathecal administration of 4-HC.

MATERIALS AND METHODS

Monkeys. Adult male rhesus monkeys (Macaca mulatta) weighing 8–10 kg were obtained from the NIH Primate Center. Each animal was kept in a separate cage and fed Purina monkey chow and water ad libitum. A silicone Pudenz catheter was surgically placed into the fourth ventricle and attached to a s.c. implanted Ommaya reservoir as previously described (7). This system permits repeated sampling of CSF in unanesthetized animals and provides mixing of administered drugs with ventricular and lumbar CSF (8). Lumbar CSF samples were also obtained in one animal using an indwelling lumbar catheter. Blood samples were drawn from a catheter inserted into the femoral vein. To evaluate possible delayed neurotoxicity of 4-HC, 3 rhesus monkeys were given intralumbar injections of 0.4 mg 4-HC weekly for 4 wk. These animals were closely observed for neurological symptoms (change in activity, behavior, lethargy, seizure activity, pupillary changes) and other clinical toxicity (alopecia, hematuria, change in appetite, vomiting, weight loss). CSF was obtained prior to each dose and analyzed for pleocytosis, protein, and glucose concentrations.

Drug Formulation and Administration. 4-HC was synthesized by Dr. Michael Colvin of The Johns Hopkins Oncology Center, Baltimore, MD, according to the method of Hohorst et al. (9). Prior to injection, the drug was reconstituted in normal saline to a concentration of 0.4 mg/ml and filtered for sterility. The drug was stable for at least 2 h at room temperature. Following reconstitution, the drug was analyzed by HPLC analysis. Immediately following reconstitution and filtering, a dose of 0.4 mg was injected into the Ommaya reservoir, and the reservoir was pumped 4–6 times to ensure adequate mixing throughout the cerebrospinal fluid. For cytotoxicity studies, the drug was dissolved in dimethyl sulfoxide at a concentration of 0.01 M and diluted in media to desired concentrations for incubation.

Sampling Times and Assay. Ventricular CSF was collected immediately prior to and at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min following drug administration and was immediately frozen in a mixture of methanol and dry ice. Recovery experiments showed that there was no loss of 4-HC or 4-hydroxycyclophosphamide as a result of short-term freezing at −70°C. Blood samples were collected at similar times, derivatized immediately with m-aminophenol in acid according to the method of Alarcon (10), and centrifuged, since in our experience 4-HC decomposes more rapidly in blood than in CSF. The supernatant was aspirated and refrigerated at 4°C until the time of assay. CSF was derivatized immediately after thawing. The acidification releases acrolein from both 4-HC and 4-hydroxycyclophosphamide, and the acrolein reacts with the m-aminophenol to produce fluorescent 7-hydroxyquinoline. Methyl vinyl ketone was added to the acidic m-aminophenol reaction mixture. Methyl vinyl ketone generates fluorescent 4-methyl-7-hydroxyquinoline. Methyl vinyl ketone generates fluorescent 4-methyl-7-hydroxyquinoline, which was the internal HPLC standard. 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 is related to 4-HC administration. Free acrolein in CSF and plasma would be measured by this method, but we and others have not been able to detect acrolein in CSF and plasma following CPA administration (11). The fluorescent products were measured by HPLC utilizing a fluorometric detector. Excitation occurred at 330 nm and a 418-nm emission filter was used. The column was a Bondapak phenyl column (No. 27198, Waters Associates, Milford, MA); elution was isocratic with a mobile phase of 10% acetonitrile in 0.15 M formic acid at a flow rate of 2 ml/minute. Retention times for 7-hydroxyquinoline and 4-methyl-7-hydroxyquinoline were 16 and 19 min, respectively. The limit of sensitivity for the assay was 0.2 μM.

In Vitro Cytotoxicity Study. MCF-7 (breast cancer), Molt-4 (human T-cell leukemia), and a rhabdomyosarcoma cell line (gift of Mark Israel, Pediatric Branch, National Cancer Institute) were exposed to varying concentrations of 4-HC for 1 h at a temperature of 37°C. The incubation medium consisted of Hank’s balanced salt solution without calcium or magnesium with 0.02% EDTA and 10% fetal bovine serum (heat
I.T. ADMINISTRATION OF 4-HYDROPEROXYCYCLOPHOSPHAMIDE

RESULTS

Pharmacokinetics and Toxicity of Intrathecal Administration. 4-HC was measured following IVT injection of 0.4 mg 4-HC in 3 rhesus monkeys. 4-HC was rapidly cleared from the CSF with a mean t1/2 of 22 min (range, 19–25 min) and a mean clearance of 0.33 ml/min (range, 0.24–0.39 ml/min). The mean peak IVT level was 100 µM (range, 72–149 µM) 5 min after the injection. The mean apparent volume of distribution was 10 ml (range, 7–13 ml), and the mean AUC was 3096 µM·min (range, 3476–5400 µM·min). Fig. 1 shows the mean ventricular 4-HC concentration-time curve following IVT 4-HC administration. Concentrations remained above 10 µM for 75 min. In the animal in which lumbar CSF was obtained, the lumbar CSF 4-HC level was 38% of the ventricular level at 30 min following drug administration, but lumbar and CSF levels had equilibrated by 1 h. The AUC for the lumbar CSF was 1457 µM·min (27% of the ventricular AUC). For reference, following i.t. administration of inulin, inulin clearance is 0.34 ml/min, its terminal half-life in CSF is 4.9 h, and its volume of distribution at steady state is 10 ml (14).

Blood levels of 4-HC were undetectable following IVT injection. No animal demonstrated evidence of neurotoxicity or other clinical toxicity following 4-HC administration weekly for 4 wk. Weekly analysis of CSF demonstrated no pleocytosis or abnormalities in protein or glucose concentrations.

Cytotoxicity. The results of in vitro cytotoxicity studies with 4-HC are shown in Fig. 2. A 90% inhibition of clonogenic survival was seen at 4-HC concentrations of 8, 9.5, and 2.5 µM (AUC 480, 570, 150 µM·min, respectively) for the rhabdomyosarcoma, MCF-7, and Molt-4 cell lines, respectively.

DISCUSSION

In the present study we have demonstrated in a rhesus monkey model that direct i.t. administration of 4-HC, an activated derivative of CPA, can achieve cytocidal drug concentrations in the CSF without producing toxicity. 4-HC is rapidly removed from the CSF with a clearance rate that is 10-fold greater than that of CSF bulk flow (0.33 ml/min versus 0.034 ml/min, respectively; Ref. 14). This suggests that the clearance of 4-HC from CSF occurs not only via CSF bulk flow but presumably also by other mechanisms such as transcapillary passage, metabolism, or spontaneous decomposition to other compounds (15). Blood levels of 4-HC following IVT injection were undetectable, consistent with the lack of systemic toxicity. In addition, there was no acute or chronic neurotoxicity observed in any of the animals that were treated either with a single IVT dose of 4-HC or on a weekly for 4 wk intralumbar dosing regimen. The observation period was continued for 2 wk following the weekly for 4 wk dosing regimen.

The in vitro studies demonstrate that 4-HC is active against several tumor types that commonly metastasize to the meninges. The 90% inhibitory concentrations for a 1-h exposure were 2.5, 8, and 9.5 µM for Molt-4, rhabdomyosarcoma, and MCF-7 cell lines, respectively (Fig. 2). As shown in Fig. 1, the 0.4-mg dose used provided levels of the active metabolite in ventricular CSF which were greater than 10 µM for 80 min. It appears, therefore, that cytocidal levels of drug can be achieved in the CSF at nontoxic doses.
Available information suggests that intra-CSF administration of 4-HC has distinct pharmacological advantages over systemic administration of CPA. Although there are no studies of CSF levels of active metabolite following systemic administration of CPA, plasma levels of active metabolite following a 2-h infusion of CPA at a dose of 50 mg/kg were found to be 15 μM (11). At this dose of CPA, which is associated with significant systemic toxicity, corresponding CSF levels would be expected to be significantly lower. In contrast, with direct IVT administration of 4-HC, cytotoxic levels of greater than 100 μM are obtained in the CSF without systemic toxicity.

There is a paucity of effective agents available for i.t. administration. The two most commonly used, methotrexate and cytosine arabinoside, are antimetabolites. When administered systemically, alkylating agents are effective against a wide variety of malignancies which can spread to the meninges, such as breast cancer, lung cancer, and leukemia. This study has demonstrated in the rhesus monkey that cytotoxic levels of 4-HC can be obtained in the CSF following IVT administration of nontoxic doses of 4-HC and suggests that further study of this approach in the clinical setting is warranted.

REFERENCES

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