ABSTRACT

Dissemination of tumor to the leptomeninges and cerebrospinal fluid represents a common pattern of metastasis for many cancers; however, few chemotherapeutic agents are available for intrathecal (i.t.) use and treatment results are often poor. We studied the neurotoxicity and pharmacokinetics of i.t. 4-hydroperoxycyclophosphamide (4-HC) in the rabbit and the activity of i.t. 4-HC in a VX2 rabbit model of leptomeningeal carcinomatosis to evaluate the potential use of 4-HC in the treatment of leptomeningeal tumors. Toxicity studies examined 4-HC doses ranging from 0.5 to 6.0 µmol administered by intraventricular injection weekly for 4 to 8 weeks. Clinical or histological neurotoxicity was not observed in rabbits treated with <1.0 µmol 4-HC for 4 weeks. Clinical toxicity, characterized by lethargy, weight loss, seizures, or death, was apparent at doses >2.0 µmol. Vasculitis of superficial arteries was observed in rabbits treated with >1.0 µmol 4-HC. In cerebrospinal fluid pharmacokinetic studies, the mean drug half-life after intraventricular or intralumbar administration was 24.3 and 18.2 min. Regional inequities in drug exposure were apparent as area under the clearance curve values for cerebrospinal fluid distant from the injection site were lower than those of proximate sites (P < 0.001). Weekly intraventricular treatment of VX2 leptomeningeal tumor-bearing rabbits with 0.5 or 1.0 µmol of 4-HC resulted in an increased life span of 22.5 and 35%, respectively. These results indicate that i.t. 4-HC, at doses lower than those producing neurotoxicity in the rabbit, is effective treatment for VX2 leptomeningeal carcinomatosis.

INTRODUCTION

The leptomeninges and CSF are common sites of metastasis for leukemias (1, 2) and solid tumors (3). Despite the recognized clinical importance of this problem, current treatment results for leptomeningeal tumor remain poor. Effective therapy has been limited primarily by three factors: (a) inadequate penetration of the CSF by conventional systemic chemotherapeutic agents; (b) unacceptable neurotoxicity associated with the intrathecal administration of many chemotherapeutic drugs; and (c) limited clinical activity against many solid tumors for drugs commonly available for i.t. therapy, including methotrexate, 1-β-D-arabinofuranosylcytosine, and thiopeta (4, 5).

Cyclophosphamide, an effective agent in the treatment of solid and hematological malignancies, is a prodrug; it requires activation by hepatic microsomal enzymes, which precludes intrathecal use. This problem may be circumvented by use of a preactivated cyclophosphamide analogue such as 4-HC (6). 4-HC is reduced rapidly to 4-hydroxycyclophosphamide and then converts spontaneously to the cytotoxic alkylating moiety, phosphoramid mustard (7). 4-HC has shown activity in vitro against a diverse group of human tumors, including medulloblastoma (8), L1210 murine leukemia (9), Burkitt’s lymphoma (10), and breast carcinoma (11). In a recent study, Fuchs et al. (12) reported significant therapeutic activity for i.t. 4-HC in human rhabdomyosarcoma (TE-671) and malignant glioma (D-54 MG) xenograft models of leptomeningeal tumor in the nude rat.

To further evaluate the clinical potential of i.t. 4-HC in the treatment of leptomeningeal tumors, we examined the behavioral and histological characteristics of neurotoxicity from IVent versus intralumbar 4-HC administration in rabbits. We used VX2 rabbit carcinoma, a well-characterized and reproducible model of leptomeningeal carcinomatosis in the rabbit (13), to further define the in vivo activity of i.t. 4-HC.

MATERIALS AND METHODS

Drug Synthesis

4-HC (M, 229,000) was synthesized by the ozonization of cyclophosphamide as described by Hohorst et al. (14). Purity of the samples was assessed by HPLC (Waters Associates, Milford, MA). Samples showing greater than 90% purity were washed in ether, dried by evaporation under a nitrogen stream, and stored at −20°C.

Drug Assay

CSF and plasma samples were placed immediately in an acidified m-aminophenol-hydroxylamine solution and stored in the dark at 4°C for no more than 7 days until assayed. Samples were heated to 100°C for 15 min and centrifuged at 3000 rpm for 5 min, and the supernatant was stored at 4°C until assayed for 4-HC concentration. The 4-HC assay was based on the method of Alarcon (15) and has been described previously (16). Briefly, methyl vinyl ketone was added to the acidified m-aminophenol-hydroxylamine solution. This produces a fluorescent 4-methyl-7-hydroxyquinoline which is detected as an internal standard. Acrolein, released from 4-HC and 4-hydroxycyclophosphamide by acidification, reacts with m-aminophenol to produce a fluorescent 7-hydroxyquinoline. The fluorescent products were identified by HPLC equipped with a fluorescent detector (330 nm excitation, 418 nm emission). The limit of sensitivity of the assay was 0.1 µM.

Rabbits

Intraventricular Catheter. Male New Zealand White (NZW) rabbits (Bunnyville, Littlestown, PA) were used for all studies. General anesthesia was induced in 3.0- to 3.6-kg rabbits with i.v. injections of acepromazine maleate (10 mg) and ketamine hydrochloride (150 mg), followed by i.v. sodium thiymal (Bio-Ceutic, St. Joseph, MO) to effect. Under sterile conditions, a 20-gauge Silastic catheter (Dow Corning, Midland, MI) was inserted into the right lateral ventricle through a parietal skull burr hole according to previously described techniques (17). The catheter was then connected to an injection reservoir which was buried under the skin overlaying the forehead. The
catheter position and patency were evaluated by fluoroscopy after the injection of metrizamide into the reservoir. At least 4 days were allowed for wound healing prior to the injection of drug or tumor. No neurological abnormalities were observed in any rabbits after catheter implantation.

Cisterna Magna and Lumbar Catheter Placement. Rabbits weighing 4.5 to 5.5 kg were anesthetized as described above and placed in a prone position with the back slightly flexed. The distance from the cisterna magna to the seventh lumbar vertebra (L7) was measured and a sterile polyethylene catheter (PE 10; Intramedic, Parsippany, NJ) was marked accordingly. The lumbarosacral area was then shaved, a midline skin incision (1 cm long) was made over the L7-S1 vertebrae, and a lumbar puncture was made in the L7-S1 interspace with a 20-gauge, 1.5-inch spinal needle (Becton-Dickinson, Rutherford, NJ). Once CSF was aspirated, the PE 10 catheter was threaded through the lumen of the spinal needle into the subarachnoid space and then advanced to the midlumbar region or the cisterna magna for subsequent drug injection or CSF sampling (see "Pharmacokinetic Studies"). After correct placement of the subarachnoid catheter tip, the spinal needle was removed. Since we did not use the same catheter for drug injection and CSF sampling, Group 4 rabbits in the pharmacokinetic study had two lumbar catheters placed at the L6-L7 interspace by the procedures described above. The position of the catheter tips was verified at autopsy.

Neurotoxicity Studies

Rabbits (3–3.6 kg) with chronic indwelling lateral ventricular catheters were treated with IVent injections of 4-HC at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, or 6.0 μmol doses. 4-HC was diluted in a 0.9% NaCl solution immediately before injection and 100 μl of drug was followed by 200 μl of Elliott's B CSF solution over 1 min. Control rabbits received 100 μl of a 0.9% NaCl solution followed by 200 μl of Elliott's B solution. IVent injections were repeated weekly for 4 or 8 weeks, during which time the rabbits were observed closely for weight loss, lethargy, seizures, ataxia, paralysis, or other signs of neurotoxicity. Rabbits showing evidence of severe neurotoxicity (e.g., paralysis or persistent seizures) were sacrificed before the completion of the fourth or eighth week; all other animals were sacrificed 1 week after the fourth or eighth injection. The brain and spinal cord were removed and fixed in 10% buffered formalin. Paraffin-embedded sections of the brain and cord were stained with Luxol fast blue or hematoxylin and eosin for histological examination by light microscopy.

CSF Pharmacokinetic Studies

The disposition of 4-HC in the CSF after IVent or intralumbar administration was studied in four groups of rabbits. Group 1 (n = 7), 4-HC administered through a lateral ventricular catheter, CSF sampled through a cisterna magna catheter; Group 2 (n = 5), 4-HC administered through a lateral ventricular catheter, CSF sampled through a lumbar catheter; Group 3 (n = 6), 4-HC administered through a lumbar catheter, CSF sampled through a cisterna magna catheter; Group 4 (n = 6), 4-HC administered through a lumbar catheter, CSF sampled through a separate lumbar catheter (see "Cisterna Magna and Lumbar Catheter Placement," above). All pharmacokinetic studies were performed with the rabbits under general anesthesia, and all animals were hydrated with an i.v. solution of 0.9% NaCl throughout the study. The 4-HC dose for all rabbits was 2.0 μmol diluted in 100 μl of 0.9% NaCl solution immediately prior to injection for all rabbits, followed by a 200-μl flush with Elliott's B solution. A small sample of the 4-HC to be injected was reserved for drug assay. After IVent 4-HC injection (Groups 1 and 2), CSF samples (10 to 50 μl) were obtained at 2, 10, 30, 60, 90, and 120 min. After intralumbar injection (Groups 3 and 4), CSF samples were obtained 2, 10, 30, 45, 60, 90, and 120 min after drug injection. The 4-HC concentrations in CSF samples were measured as described above.

Pharmacokinetic Calculations

4-HC disposition curves for each experiment were evaluated for suitable pharmacokinetic models by visual inspection. For all experiments, the data were best fit by a one compartment model with first order delivery of drug to the sampling site, first order elimination, and a lag time (when present) (18). The following kinetic parameters were estimated by use of nonlinear least squares analysis by using PCNONLIN (Statistical Consultants, Lexington, KY): the rate constant for drug delivery (k0); the elimination rate constant (k); the volume of distribution (V); and the delivery lag time (T). The area under the clearance curve (AUC) and clearance rate (Cl) were calculated by using the parameter estimates.

VX2 Leptomeningeal Tumor Model

VX2 rabbit carcinoma was obtained from the American Type Culture Collection (Rockville, MD) and maintained as a s.c. tumor in rabbits by passage every 2 to 3 weeks. For the VX2 leptomeningeal model, approximately 0.5 g of freshly harvested tumor was coarsely sectioned and then incubated for 18 h (5% CO2, 37°C) in 10 μl of dissociation medium consisting of 50 units/ml collagenase (Worthington, Freehold, NJ) and 0.005% DNase (Sigma Chemical Co., St. Louis, MO) in RPMI 1640 containing 10% fetal bovine serum and 60 μg (pH 7.3) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. The tumor was further dissociated by repeated pipetting, washed twice with RPMI 1640/10% fetal bovine serum, and then centrifuged and resuspended in Elliott's B solution. Viable cells, determined by trypan blue exclusion, were counted with a hemocytometer.

Tumor cells, 3.0 × 103, were suspended in 100 μl of Elliott’s B solution and injected into the s.c. reservoir of lateral ventricular catheters that had been implanted in 3.0- to 3.6-kg rabbits at least 5 days earlier. The reservoir was then flushed with 200 μl of Elliott’s B solution through the same needle. Previous studies in our laboratory have shown that signs of leptomeningeal carcinomatosis (e.g., anorexia, lethargy, cranial nerve palsies, or paraplegia) are apparent within 20 days after IVent injection of 3000 viable VX2 cells (19). Animals were sacrificed when severe neurological deficits developed (e.g., ataxia, seizures, or paraplegia), inasmuch as previous studies showed that these signs were followed invariably by death within 24 h.

Survival Studies

Rabbits with leptomeningeal VX2 were randomly assigned to 4-HC treatment or control groups. At 24 h after VX2 injection, rabbits were treated with 0.5 μmol (0.11 mg; n = 18) or 1.0 μmol (0.23 mg; n = 13) of 4-HC in 100 μl of 0.9% NaCl solution administered by IVent injection. Control animals received 100 μl of 0.9% NaCl solution. After all injections, the intraventricular reservoirs were flushed with 200 μl of Elliott’s B solution. 4-HC or NaCl solution injections were administered weekly for 4 weeks. Rabbits were observed daily for signs of leptomeningeal carcinomatosis. Upon the appearance of severe neurological deficits (see above), the rabbits were sacrificed by injection of i.m. acepromazine and ketamine followed by i.v. injection of euthanasia solution T-61 (Taylor Pharmacal, Decatur, IL). Rabbits with no signs of leptomeningeal carcinomatosis were sacrificed on day 60 after the first 4-HC treatment and were considered long-term survivors. The day of death or sacrifice was recorded as the study end point. For all rabbits, the brain and spinal cord were removed and fixed in 10% buffered formalin for at least 10 days. Paraffin sections of the brain and spinal cord were stained with Luxol fast blue or hematoxylin and eosin and examined by light microscopy. Differences in survival between treatment and control groups were analyzed by Student’s t test.

RESULTS

Neurotoxicity Studies. The maximum tolerated dose of 4-HC in the rabbit as defined by the absence of behavioral or clinical signs of neurotoxicity was 1.0 μmol weekly for 4 weeks (Table 1). However, in all rabbits treated at this dose, mild to moderate arteritis was apparent on histological examination. No behavioral or histological abnormalities were observed in rabbits treated with 0.5 μmol 4-HC for 4 weeks. All rabbits treated with 2 μmol of 4-HC weekly developed clinical signs of
Table 1  Intrathecal 4-hydroperoxycyclophosphamide neurotoxicity

<table>
<thead>
<tr>
<th>4-HC dose (mmol)</th>
<th>Clinical toxicity</th>
<th>Observed neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 (n = 6)</td>
<td>NS</td>
<td>0</td>
</tr>
<tr>
<td>1.0 (n = 6)</td>
<td>NS</td>
<td>6</td>
</tr>
<tr>
<td>2.0 (n = 4)</td>
<td>14.0</td>
<td>4</td>
</tr>
<tr>
<td>3.0 (n = 2)</td>
<td>17.5</td>
<td>2</td>
</tr>
<tr>
<td>4.0 (n = 1)</td>
<td>9.0</td>
<td>1</td>
</tr>
</tbody>
</table>

* NS, not seen.

Toxicity: all had progressive weight loss; one had seizures; and another had lower extremity weakness. One rabbit died from toxicity 3 days after the third 4-HC injection. All rabbits treated with >2 μmol of 4-HC weekly developed severe neurotoxicity and died before the fourth 4-HC dose.

On histological examination, a reactive gliosis that ranged from mild to severe was noted at the catheter implantation site in all rabbits. The most consistent histological abnormality was a dose-dependent vasculitis. Vasculitis was not observed in rabbits treated with 0.5 μmol of 4-HC for 4 or 8 weeks. In rabbits treated with 1.0 μmol of 4-HC, the arterial lesions were most prominent at the base and lateral surfaces of the medulla and were characterized by granulocytic and mononuclear infiltrates, mineralization, and fibrosis (Fig. 1, A and B). At higher doses, the severity and distribution of the vascular injury were greater. Vessels on the ventral aspect of the cerebellum, pons, medulla, and spinal cord were surrounded by a dense sheet of reactive connective tissue resulting in the entrapment of exiting cranial nerves. Similar vascular changes were observed in the spinal cords of these rabbits. In all rabbits treated with doses >2.0 μmol, the superficial blood vessels in the cerebellum and medulla were severely compromised by transmural and circumferential fibrinoid necrosis accompanied by small foci of hemorrhage.

Fig. 1. Vascular and cerebellar injury in a rabbit treated with three weekly intraventricular 2.0-μmol doses of 4-HC. A, large vessel at the base of the brain containing multiple areas of fibrinoid necrosis and infiltrations of inflammatory cells. ×400. B, small artery at the base of the brain with an intense arteritis characterized by karyorrhexis, endothelial necrosis, and infiltration of a mixture of inflammatory cells. ×400. In C, the Purkinje cells in the cerebellum are degenerating and shrunken, and there is a mild gliotic response. ×160.
In addition to the vascular lesions, vacuolization of the choroid plexus was noted in the majority of the rabbits treated with >1.0 μmol of 4-HC. At doses >2.0 μmol, parenchymal injury was also observed, predominantly in brainstem and cerebellar regions that appeared to be secondary to the vascular lesions. Leukomalacia, vacuolization of white matter, focal inflammation, edema, and hemorrhage were noted in the dorsal medulla. In the cerebellum, reactive gliosis and foci of inflammation in the molecular layer and Purkinje cell degeneration were found (Fig. 1C). Axonal degeneration, demyelination, and gliosis in the subjacent subpial region of the cerebellum and pons were also evident. Similar changes were observed in the spinal cord at doses >2.0 μmol of 4-HC.

Histological examination of 7 of 24 rabbits treated with 4-HC and 1 of 4 untreated controls revealed lesions consistent with infection by Encephalitozoon cuniculi, a ubiquitous and clinically indolent protozoal infection in rabbits which causes a characteristic granulomatous meningoencephalitis.

Pharmacokinetic Studies. For rabbits treated at the highest i.t. 4-HC dose (4 μmol), the concentration of 4-HC in plasma was below the limits of HPLC detection (1 μM). Plasma 4-HC values were not evaluated at lower i.t. 4-HC doses. Fig. 2 shows the mean 4-HC concentrations in CSF after IVent or intralumbar administration in Groups 1 to 4. Peak 4-HC concentrations were attained within 30 min of injection for all groups except Group 2 (ventricular injection, lumbar sampling) in which the peak value, attained at 45 min, was the lowest of all groups. In Group 4 (lumbar injection, lumbar sampling), the peak 4-HC concentration was highest and was attained within 2 min of injection. Table 2 summarizes the pharmacokinetic parameters for all treatment groups.

Survival Studies. Treatment of rabbits with VX2 leptomeningeal carcinomatosis with IVent 4-HC resulted in a dose-dependent increased life span compared to controls (Table 3). One cohort of rabbits did not have successful tumor implantation, evidenced by survival of all control and treated rabbits to Day 45, and was not included in the survival data analysis. The median survival for rabbits treated with 0.5 μmol of 4-HC was 24.5 days, representing a 22.5% increased life span over controls (P = 0.002). A longer median survival (27.0 days) was found in rabbits treated at the 1.0-μmol dose, resulting in a 35% increase compared with controls (P = 0.0004). IVent 4-HC treatment produced two prolonged survivors (>60 days) at the 0.5-μmol dose and one at the 1.0-μmol dose. These prolonged survivals were not due to failure of tumor implantation, because small foci of tumor were observed upon histological examination of the spinal cords. Although we cannot exclude the possibility of an error in the number of tumor cells injected, the survival data for control animals in this treatment cohort were similar to those for other control groups.

The types of clinical abnormalities observed in control and 4-HC-treated groups were different, suggesting different sites of treatment failure. Thirteen of 19 controls had signs that were exclusively or predominantly due to intracranial tumor (e.g., seizures, anorexia, focal cranial nerve abnormalities) and 6 had paraplegia as the only abnormal neurological sign. By contrast, 20 of 31 rabbits in the 4-HC-treated groups developed paraplegia as the only neurological abnormality. Histological examination at the time of death or sacrifice confirmed that the greatest tumor burden in control animals was found in intracranial regions close to the site of tumor injection, the cerebral leptomeninges and ventricles. In 4-HC-treated rabbits, however, tumor burden was greatest in regions furthest from the site of tumor and 4-HC injection, the thoracic and lumbar spinal cord (Fig. 3).

**DISCUSSION**

Leptomeningeal metastasis is an important cause of morbidity and mortality in cancer patients. Prior to the introduction of central nervous system prophylactic therapy in childhood acute lymphoblastic leukemia, the incidence of meningeal metastases exceeded 80% (2). For extraneural solid tumors, in which hematogenous spread represents the most probable mechanism of meningeal metastases (20), the incidence of leptomeningeal tumor may reach 46% in melanoma (21), 11 to 18% in small cell lung carcinoma (22), and 5% in breast carcinoma (23). Direct extension via CSF pathways is responsible for metastatic tumor spread in primary brain tumors, where leptomeningeal metastases have been identified in 19% of childhood brain tumor patients (24). Reported increases in the frequency of leptomeningeal metastasis (25) may be due to improved diagnostic tests, greater surveillance, or longer patient survival (3).

Despite advances in the treatment of various primary tumors, the chemotherapeutic options for patients with leptomeningeal metastases are limited and results are generally poor. Treatment failures after standard-dose systemic chemotherapy may
be due, in part, to blood-CSF barrier restrictions resulting in inadequate drug concentrations in the CSF. This problem may be circumvented by regional (i.e., intrathecal) drug administration, where higher drug concentrations can be achieved than by conventional systemic routes. Unfortunately, few chemotherapeutic drugs are available for intrathecal use because of neurotoxicity. Intrathecal methotrexate is the most active drug for the treatment of central nervous system leukemia (1, 26) and may be useful in leptomeningeal carcinomatosis from breast cancer (27, 28); however, intrathecal methotrexate is associated with significant neurotoxicity (29-31). Intrathecal 1-β-d-arabinofuranosylcytosine may be less neurotoxic than methotrexate but is ineffective against many solid tumors. Thiopeta may be active against solid tumors (32), but the rapid efflux of thiopeta from the CSF may limit its effectiveness (5).

Efforts to improve treatment results for patients with leptomeningeal tumor include the use of intraventricular (Ommaya) reservoirs (26, 33), the development of animal models necessary for the preclinical evaluation of intrathecal drug neurotoxicity and efficacy (12, 19, 34, 35), and the introduction of new antineoplastic agents for potential intrathecal use (36-38). Pharmacological studies have indicated advantages for intrathecal 4-HC over i.v. cyclophosphamide for the treatment of leptomeningeal disease (39). Fuchs et al. (12) reported that 4-HC is active against leptomeningeal rhabdomyosarcoma (TE-671) and malignant glioma (D-54) human xenografts at doses that do not cause neurotoxicity in nude rats. To extend the preclinical evaluation of intrathecal 4-HC, we modified several of the experimental conditions used by these investigators: (a) we used immunocompetent rabbits instead of nude rats, inasmuch as the full expression of neurotoxicity may require a functional immune system; (b) we administered 4-HC by an indwelling intraventricular rather than intralumbar catheter, because previous intrathecal drug studies have suggested a therapeutic advantage for this route (26, 40); (c) we used a weekly schedule of 4-HC administration instead of a single dose, as multiple doses more closely approximate the anticipated human clinical schedule; finally (d) to expand the range of preclinical activity studies, we used a rabbit carcinoma tumor model (VX2) with sensitivity to 4-HC in vitro that is 4-fold less than reported values for TE-671 (41) (90% inhibitory dose = 50 µm versus 12.8 µm for VX2 and TE671, respectively).

In the present study, weekly intraventricular 4-HC at doses >1.0 µmol produced a dose-dependent vasculitis that most often involved the superficial arteries of the pons and medulla. At higher 4-HC doses (>2.0 µmol), axonal degeneration, gliosis, and focal necrosis and hemorrhage were also observed. Demyelination and other signs of parenchymal injury have been reported following intrathecal administration of methotrexate (42, 43) or 1-β-d-arabinofuranosylcytosine (44) and may represent a nonspecific response to chemical injury. However, the development of vasculitis as the earliest histological evidence of neurotoxicity has not been reported in CSF studies for any antineoplastic drugs. It is possible that the observed vasculitis is caused by acrolein, the metabolite that may be responsible for cyclophosphamide-induced hemorrhagic cystitis (45). Alternatively, the higher rate of cell division for cerebral blood vessels (46, 47) may predispose the superficial arteries to injury from alkylating agents. The pontomedullary distribution of arteritis may occur because these vessels are exposed to a high concentration of 4-HC as CSF exits from the foramina of the fourth ventricle. The absence of detectable 4-HC plasma concentrations in animals treated at the highest intrathecal doses (6 µmol) excludes the possibility that the observed vasculitis is due to high intravascular drug concentration.

E. cuniculi is a ubiquitous, endemic parasitic infection of laboratory rabbits. This parasitic infection is typically found in immunocompetent rabbits and is not specifically associated with a compromised host. The incidence of E. cuniculi infection was apparently higher in the 4-HC-treated rabbits than in controls and it is possible that i.t. 4-HC treatment activated or facilitated the development of this clinically indolent, nonfatal infection. Although we cannot exclude a contributory role, it is unlikely that the arteritis was due to the coexistent E. cuniculi infection for the following reasons: (a) rabbits without evidence of encephalitozoonosis treated with 4-HC at doses >1.0 µmol developed arteritis; (b) rabbits with encephalitozoonosis not treated with 4-HC and those treated at doses <1.0 µmol did not develop arteritis; (c) the histological appearance of arteritis in animals with and without encephalitozoonosis was identical;

### Table 2 Pharmacokinetic parameters of 4-HC in cisterna magna and lumbar subarachnoid space

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Injection site</th>
<th>Sampling site</th>
<th>Peak 4-HC (µmol/liter)</th>
<th>AUC (mmol/min/liter)</th>
<th>t½ (min)</th>
<th>Vd (ml)</th>
<th>Clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 7)</td>
<td>Ventricular</td>
<td>Cisterna magna</td>
<td>500</td>
<td>18</td>
<td>21</td>
<td>5.0</td>
<td>0.17</td>
</tr>
<tr>
<td>2 (n = 5)</td>
<td>Ventricular</td>
<td>Lumbar</td>
<td>62</td>
<td>8</td>
<td>27</td>
<td>23.0</td>
<td>0.53</td>
</tr>
<tr>
<td>3 (n = 6)</td>
<td>Lumbar</td>
<td>Cisterna magna</td>
<td>223</td>
<td>11</td>
<td>23</td>
<td>17.0</td>
<td>0.22</td>
</tr>
<tr>
<td>4 (n = 6)</td>
<td>Lumbar</td>
<td>Lumbar</td>
<td>3684</td>
<td>100</td>
<td>13</td>
<td>0.5</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Intrathecal 4-HC dose of 2.0 µmol was used for all treatment groups.

### Table 3 Survival following intrathecal treatment for rabbits with VX2 leptomeningeal carcinomatosis

<table>
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<th>Intrathecal treatment</th>
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<th>Survivors (&gt;8 wk)</th>
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<tr>
<td>NaCl (0.9%) (n = 19)</td>
<td>20.0 ± 0.9</td>
<td>0</td>
</tr>
<tr>
<td>4-HC (0.5 µmol) (n = 18)</td>
<td>24.5 ± 2.6*</td>
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</tr>
<tr>
<td>4-HC (1.0 µmol) (n = 13)</td>
<td>27.0 ± 1.6b</td>
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</tr>
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</table>

* P = 0.002, Student's t test.

### Footnotes

1. 4-HC IN LEPTOMENINGEAL CARCINOMATOSIS

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<td>27.0 ± 1.6b</td>
<td>1</td>
</tr>
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</table>

* P = 0.002, Student's t test.

b P = 0.0004, Student's t test.
and (d) the granulomatous changes characteristic of *E. cuniculi* were not observed in blood vessels.

Our results contrast with intrathecal 4-HP neurotoxicity studies reported in the nude rat: (a) the maximum tolerated dose in the nude rat was 2-fold higher than in the rabbit (Table 4); (b) 4-HP neurotoxicity in the nude rat was characterized histologically by demyelination, most prominently affecting the dorsal columns of the thoracic and cervical spinal cord, and there was no evidence of arteritis or parenchymal inflammatory changes as noted in the rabbit. Differences in maximum tolerated dose and neuropathology between the nude rat and rabbit models may be due to the weekly for 4 weeks dose schedule in the rabbit compared with a single dose in the rat. Alternatively, the ability to mount an inflammatory response may be generally reduced in immunodeficient nude rats or the drug clearance rate may be higher in the rat.

In the present study, the pharmacokinetic parameters for intraventricular or intralumbar 4-HP administration in the rabbit were closely comparable to those reported by Arndt et al. in the rhesus monkey (16). Differences in peak 4-HP concentration and AUC between these studies reflect the smaller CSF volume in the rabbit. Following an intraventricular injection of 4-HP, the mean AUC for lumbar CSF was 55% lower than that for the cisterna magna. However, a greater difference in AUC was observed; the AUC for the cisterna magna was 89% lower than the lumbar AUC value. These results, suggesting that intraventricular 4-HP may provide better drug exposure to the entire CSF space than intralumbar administration, are similar to findings reported by Bleyer and Poplack (26) and Shapiro et al. (40) for intraventricular methotrexate.

Treatment of VX2 leptomeningeal tumor-bearing rabbits with four weekly doses of 4-HP produced a dose-dependent increased survival. Although survival in three rabbits was prolonged, these rabbits were not "cured" of tumor, as evidenced by minute foci of residual leptomeningeal tumor identified at electron microscopic examination. It is beyond the scope of this study to identify the optimal treatment schedule for clinical use. It is possible that continuation of weekly treatments, an increase in dose intensity, or other alterations may further prolong survival or yield cures. The improvement in survival after four 4-HP treatments in VX2 tumor-bearing rabbits was similar to that reported after single-dose 4-HP treatment in nude rats bearing human rhabdomyosarcoma or malignant glioma, both of which show greater in vitro sensitivity to 4-HP than does VX2 (12). An evaluation of leptomeningeal tumor at the time of death or sacrifice showed that the greatest residual tumor burden was located in intracranial and upper cervical spinal cord regions for untreated rabbits and in the lumbar region for 4-HP-treated rabbits. These findings suggest that the regional inequities in 4-HP distribution demonstrated in pharmacokinetic studies may have significant implications for clinical outcome. Recognizing that CSF dynamics are usually abnormal in patients with leptomeningeal metastasis and that CSF transit is often delayed, it is possible that effective therapy may require alternating intraventricular and lumbar administration routes.

Our results indicate that i.t. 4-HP, at doses lower than those associated with significant neurotoxicity, is an effective treatment for VX2 leptomeningeal carcinomatosis in the rabbit. This and other preclinical studies strongly suggest that i.t. 4-HP may provide an important addition to the small number of chemotherapeutic agents available for intrathecal use. Recently opened clinical trials for adults and children with leptomeningeal tumor will define the toxicity and clearance rates for i.t. 4-HP and provide additional information for optimal i.t. 4-HP dosing schedules.

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