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CNS toxicity and CSF pharmacokinetics of intraventricular DFMO and MGBG in beagle dogs

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Summary. *We have developed a beagle dog model to study the pharmacology and toxicology of anticancer drugs administered through the 3rd or lateral ventricles. A Foltz-type reservoir was implanted SC and connected by tube into a cerebral ventricle. Drugs were administered directly into the reservoir; CSF sampling of drugs administered into the ventricle was achieved directly by tapping the reservoir or by percutaneous puncture of the cisterna magna. In the current study, we evaluated the CSF pharmacokinetics and CNS toxicity of two inhibitors of polyamine metabolism, α-difluoromethylornitine (DFMO) and methylglyoxal bisguanylhydrazone (MGBG). Both drugs were judged too toxic to justify intrathecal or intraventricular studies with these agents in patients.*

Introduction

α-Difluoromethylornithine (DFMO) is a new potential antineoplastic agent that binds irreversibly to the enzyme ornithine decarboxylase, inhibiting the production of putrescine from ornithine [9, 13]. Experimental activity of this agent alone [8, 13] and in combination with other inhibitors of polyamine synthesis [15] and alkylating agents [2, 6, 10, 11, 12] suggests potential clinical usefulness. Methylglyoxalbisguanylhydrazone (MGBG) is a competitive inhibitor of S-adenosylmethionine decarboxylase and inhibits the formation of spermidine and spermine (see reference [15] for review). A preliminary report indicates activity of DFMO in acute lymphocytic leukemia when DFMO is given with MGBG [15]. DFMO does not readily cross the blood-brain barrier (BBB) or achieve significant cerebrospinal fluid (CSF) levels after IV administration [4, 5, 7]. MGBG, while it can cross the BBB, does not achieve substantial CSF levels after IV administration under normal circumstances [14]. Since we considered that one or both these drugs might have a potential place in the treatment of meningeal neoplasia, we evaluated the pharmacokinetics of intraventricular (ICSF) DFMO [7] and, in this study, the ICSF pharmacokinetics of MGBG and the central nervous system (CNS) toxicity and pharmacology of chronically administered ICSF DFMO and MGBG.

Materials and methods

Chemicals. DFMO was supplied as a sterile, pyrogen-free stock solution of 100 mg/ml water by Merrell Dow Pharmaceuticals Inc. (Cinncinati, Ohio). The DFMO solution was pH 7.3 and 305 mosmol. MGBG was supplied as a sterile powder by the National Cancer Institute. MGBG was weighed into sterile containers and mixed with either sterile artificial CSF or saline for ICSF administration. The artificial CSF was pH 7.3 and 337 mosmol, while the sterile saline was pH 5.5 and 310 mosmol.

Experimental animals. Male grade 2 beagle dogs purchased from Marshall Research Animals (North Rose, NY) were used at 6–9 months of age. Grade 2 denotes congenital abnormalities, such as one detectable testicle, rectal prolapse (repaired), or lower incisors projecting severely over upper incisors. All dogs received parvo virus vacination upon arrival and were cared for in an approved animal facility sponsored by the University of California. All dogs were acclimated for at least 1 week before studies were initiated.

Implantation. Dogs were anesthetized with pentobarbital and placed in a stereotactic head holder (David Kopf instrument). Using sterile techniques, one of three methods was used for intrathecal catheter/reservoir implantation:

Method 1: A 20-gauge spinal needle connected to polypropylene tubing was inserted into the third ventricle and fixed to the bone with dental acrylic and then externalized with a heparin lock closure for sterility.
Method 2: A right-ventricular Foltz reservoir modified to our specifications and kindly provided by American Heyer-Schulte (Goleta, CA) was attached to the 20-gauge spinal needle in the third ventricle and fixed with dental acrylic, or the Foltz reservoir was connected to silastic tubing that had been stereotactically placed in the right lateral ventricle. This latter method is the current method of choice for drug delivery into the ventricle.
Method 3: The two-sided Foltz reservoir was placed into the lateral ventricle; a pediatric-size Broviac catheter was then attached to the opposite side and externalized with a heparin-lock closure.

Postoperative care included at least a 1 week recovery period, systemic treatment with 1,000,000 IU penicillin twice daily for 10 days, and intraventricular treatment with 0.5 mg gentamicin daily for 3 days.

Laboratory Tests. Routine blood, CSF work, and physical measurements were recorded. CSF sampling was performed by anesthetizing the dogs with pentothal and placing a 22-gauge spinal needle percutaneously into the cisterna magna. The

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following blood and CSF tests were carried out prior to surgery and in alternate weeks during and after drug treatments: WBC, differential, and hematocrit in blood; cell count, differential, glucose, and protein in CSF. CSF DFMO levels were measured by Milan Slavik, MD, using a modification of the method of Grove et al. [4].

For intraventricular or subarachnoid infections dogs were evaluated in the following manner: CBC, differential, and CSF culture, cell count, differential, protein, and glucose. Antibiotic treatment was then started pending culture results with IM penicillin or IM penicillin and IM gentamicyn and treatment through the reservoir with gentamicin and methicillin.

Drug administration. DFMO and MGBG were administered by three different methods, according to the surgical preparation.

Method 1: Third ventricle externalized catheter. An Auto syringe pump (Orange Medical Instruments) was used to deliver a constant infusion of DFMO for 72 h/week for 8 weeks. MGBG was given daily for 3 days/week by bolus administration for 8 weeks.
Method 2: Third or lateral ventricle with Foltz reservoir. The DFMO was given BID on 3 days/week for 8 weeks. The MGBG was given once/day on 3 days/week for 8 weeks.
Method 3: Lateral ventricle externalized Broviac catheter. The Auto syringe pump was used to deliver a continuous infusion of DFMO.

Post mortem studies. One month after the last dose of drug was given, dogs were sacrificed with Euthanol (concentrated phenobarbital solution). The brain and spinal cord were removed and fixed in buffered formalin; representative tissue blocks were taken and stained with hematoxylin-eosin and Luxol-fast PAS. Areas evaluated included dura, subarachnoid pia, cerebral cortex, cerebral white matter, caudate nucleus, putamen, globus pallidus, thalamus, hypothalamus, fornix, optic tract, lateral ventricle, third ventricle, aqueduct, fourth ventricle, midbrain, pons, medulla oblongata, cerebellum, spinal cord, spinal nerves, and pituitary gland.

CSF pharmacokinetics. Methylglyoxal-bis(^{14}C)guanylhydrazone dihydrochloride monohydrate (specific activity, 19 mCi/mmol) was supplied by the Research Triangle Institute through a contract with the National Cancer Institute. Radiopurity, as determined by thin-layer chromatography, was 98%−100%. ^{3}H-Inulin was purchased from New England Nuclear Corporation and was repurified before use by Sephadex G-25 column chromatography.

Dogs with reservoirs were reanesthetized, placed in the lateral position, and a 22-gauge needle was placed percutaneously into the cisterna magna. In a total volume of 0.5 ml saline, ^{14}C-MGBG (3 μCi) and ^{3}H-inulin (1 μCi) were injected into the ventricle. This was followed by 0.3 ml saline to wash the catheter line and reservoir clear. Samples of cisternal CSF were removed at various times and the amount of radioactivity was determined using a Beckman LS-250 scintillation spectrometer; quench correction was made by the external standards method.

The descending plot of dpm/ml of CSF(C_{csf}) against time was iteratively fit to the equation

$$C_{csf} = Ae^{-\alpha t} + Be^{-\beta t}$$

with a computerized nonlinear least-squares program. Using a two-compartment open model with elimination from the peripheral compartment [3], these data were used to compute pharmacokinetic parameters. The integrated area under the CSF curve was computed from both the ascending and descending points of the CSF drug level vs time curve.

Results

Ventricular reservoir technique

For intermittent intraventricular administration of DFMO two techniques were evaluated, needle placement in the third ventricle and Foltz reservoir placement in the lateral ventricle. Approximately four dogs were studied with each technique before facility with the preparation was achieved. Two problems arose that ultimately convinced us to use the lateral ventricle method 2. Method 1, with its exteriorized line, led to more ventricular infections and also more prevelant dehydration of the animals in study. Presumably because of damage in the hypothalamus due to infection or drug, animals frequently became dehydrated, a condition sometimes reversible by IV fluids.

Early in our studies it became clear that seizures occurred even in the absence of ventricular infection. We therefore treated our dogs prophylactically against seizures, with phenobarbital, 6 mg/kg, daily. Those animals that did develop transient bacterial ventriculitis were more likely to have seizures even when receiving phenobarbital. Of 13 dogs (15 dose schedules) treated with DFMO, nine developed seizures at one time or another and required supplemental doses of phenobarbital (Table 1). Of seven dogs treated with MGBG, four developed seizures (Table 2) requiring supplemental phenobarbital.

CNS toxicity

Tables 1 and 2 summarize the treatment plan and frequency signs, symptoms, and bacterial ventriculitis in the dogs receiving DFMO and MGBG, respectively. Clear evidence of intraventricular bacterial infection was noted in four dogs receiving DFMO and three receiving MGBG. The organisms were gram-positive cocci in all seven cases; all animals responded to antibiotic therapy. In none was infection associated with a lowered CSF glucose; the major manifestations of infection were a stiffly held neck, irritability, and increased numbers of CSF neutrophils and lymphocytes.

Table 3 summarizes the CSF cell count, protein, and glucose and the peripheral blood hematocrit, WBC, and platelet adequacy for DFMO and MGBG. Even in the absence of obvious intraventricular infection, the CSF WBC and protein were higher than normal, with greater variance than control dogs. The blood studies show no significant differences for hematocrit or WBC and the platelets were adequate by smear in the treated dogs.

Pathological examination of the brains of animals sacrificed 1 month after the completion of treatment was performed in all dogs except dog 1. Of 13 DFMO dogs, nine showed focal perivascular round cell infiltrates in and around the subependymal glia, often with a loss of ependymal lining cells and with focal supependymal gliosis. These changes involved primarily the lateral and third ventricles, although occasionally the aqueduct of Sylvius and the fourth ventricle were involved. Three animals with third ventricle reservoirs showed focal areas of hypothalamic necrosis and reactive changes around

Table 1. Doses, schedules, and complications of DFMO therapy

Dog	mg/week	Total weeks	Schedule	Infection	Seizure	Sali-vation	Vomit	Pacing	Irritable "mania"
1	32	8	3CT	+	+		+	+	+
2	60	8	3BL		+				
3	78	8	3CT						
4	84	8	3BL		+	+			
5	90	8	3CT				+		
6	120	8	3BL	+		+			
7	140	8	3CT	+	+				
8	150	8	3CT		+	+		+	+
9	200	8	3CT	+			+		
10	175	0.43	CL			+			+
11	259	0.26	CL			+	+	+	+
12	336	0.25	CL		+	+	+		+
13	525	0.11	CL		+		+	+	+
14	1050	0.14	CL		+	+			
15	1400	0.14	CL		+	+			

3, 3 days; B, twice/day bolus administration; C, continuous infusion; T, third ventricle infusion; L, lateral ventricle infusion

Table 2. Doses, schedules, and complications of MGBG therapy

Dog	mg/week	Total weeks	Schedule	Infection	Seizure	Salivation	Vomit	Circling/ ataxia
1	3	8	3FT	+		+		
2	3	8	3FT	+		+	+	
3	6	8	3FL			+	+	+
4	6	8	3FL	+	+			
5	6	8	3FL		+	+	+	
6	9	8	3FL		+	+	+	+
7	9	3	3FL		+	+	+	+

See abbreviations in Table 1; F, once/day bolus administration

Table 3. Comparison of pretreatment to DFMO or MGBG treatment for CSF and peripheral blood laboratory studies

	WBC	Cerebrospinal fluid		Peripheral blood		
		Glucose (mg/dl)	Protein (mg/dl)	HCT (%)	WBC (mm^3)	Platelets
Control	6.8 (1.3)	76 (1.6) $n = 29$	22 (3.4)	38 (1)	13350 (930) $n = 26$	Adequate
DFMO[a]	21.6 (3.8)	70 (2.9) $n = 33$	48 (15.5)	40 (1)	11170 (640) $n = 50$	Adequate
MGBG[a]	13.3 (2.3)	72.3 (1.6) $n = 15$	57.1 (9.9)	38 (1.4)	14700 (1,400) $n = 15$	Adequate

[a] In absence of obvious intraventricular infection
( ), ± SEM

the third ventricle. One case with a lateral ventricle reservoir showed extensive inflammation and scarring of the lateral ventricle. The inflammation was acute and chronic, with lymphocytes and extensive fibrosis. The process appeared to be localized to the shunt tubing. One case not included in the data analysis, developed, in response to pyrogens, capillary proliferation and endothelial necrosis in the thalamus and focally in the cerebral cortex, the condition being similar to that observed in Leigh's encephalopathy. Another dog not completing therapy and not included in the analysis had viral meningoecephalitis on postmortem examination. Thus, in the DFMO-treated dogs, the major pathologic features were

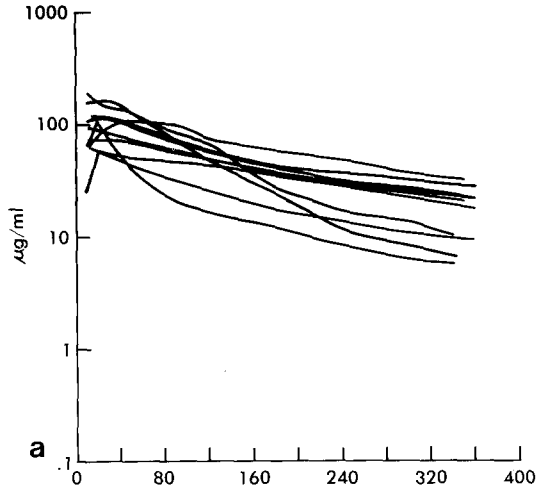

Fig. 1a.

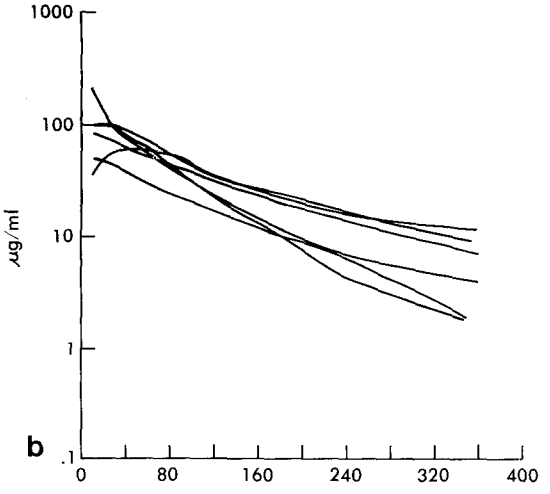

Fig. 1b.

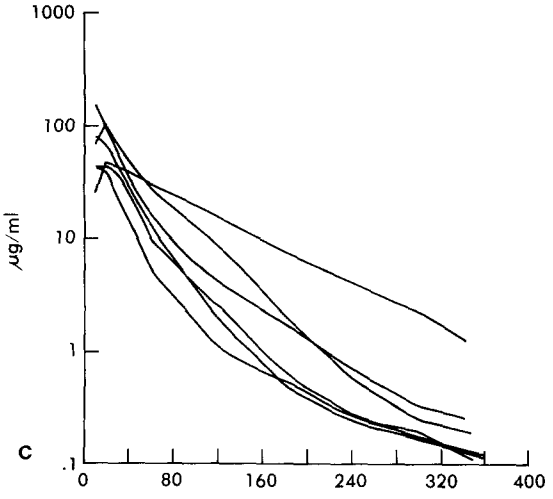

Fig. 1c.

Fig. 1a–c. The CSF levels of radiolabeled inuilin **(a)**, DFMO **(b)**, and MGBG **(c)** are shown for a unit dose of 1 mg administered into the 3rd or lateral ventricle and sampled at the cisterna magna

nonbacterial inflammation involving surfaces adjacent or subadjacent to the CSF. Parenchymal changes were generally absent.

All the MGBG-treated dogs had focal periventricular perivascular round cell infiltrates involving primarily the third or lateral ventricles, depending on where the reservoir tubing was located. In some animals this was accompanied by round cell infiltrates and gliosis of the periaqueductal gray matter and periventricular loss of lining cells. Some dogs showed perivascular round cell (lymphocytes, plasma cells, histiocytes) cuffing in brain parenchyma, and meninges suggesting a viral-like meningoencephalitis. Both of these animals also had severe inflammatory changes and necrosis of the temporal lobes on one side. In general, animals had varying degree of necrosis and inflammation associated with the shunt tubing. As in the DFMO-treated dogs, nonbacterial inflammation involving surfaces adjacent or subadjacent to the CSF was prominent.

CSF Pharmacokinetics

Figure 1 shows the inulin, DFMO, and MGBG CSF clearance curves from dogs after the instillation of radiolabeled compounds into the third or lateral ventricles. Table 4 summarizes pharmacokinetic parameters obtained from 27 animals (15 of these animals were from other studies) receiving radiolabeled inulin (15 in the third ventricle; 12 in the lateral ventricle) six receiving ^{14}C-DFMO, and six receiving ^{14}C-MGBG, using a two-compartment open model with elimination from the second compartment.

The volume of distribution of the central compartment, V_c, represents the hypothetical space or volume each isotope occupies in the ventricular CSF. For inulin, V_c should closely approximate the ventricular CSF volume. Since V_c for third ventricle injections is 8.8 ml, while that for lateral ventricles is 14.2 ml, it seems that, following injections into the third ventricular reservoir, there is little back flow into the lateral ventricles. The transfer constant K_{12} approximates redistribution of the drug between the ventricles and the more slowly equilibrating peripheral compartments of subarachnoid CSF. The K_{20} constant reflects elimination from the second CSF compartment and CL_T total drug clearance from the CSF. AUC is the computed exposure integral or concentration × time computed from the ascending and descending portions of the measured CSF drug level curves over time. Regardless of whether inulin was injected into the third or the fourth ventricles, AUC, K_{12}, K_{20}, and CL_T were similar. CL_T is, as expected, most rapid for MGBG, less for DFMO, and least for inulin. This was expected, since the molecular weight for MGBG < DFMO ≪ inulin, and MGBG crosses the BBB [14], DFMO is relatively restricted [7], and inulin is highly restricted in ability to cross the BBB.

CSF DFMO levels

Table 5 shows the measured DFMO levels. The wide variation probably reflects differing levels of CNS damage due to DFMO and coincident changes in CSF clearance of DFMO. While discrepancies exist, levels above 400 nmol/ml for 3 days each week for 8 weeks are clearly toxic.

Discussion

These studies served two purposes: the first was to develop a reproducible and reliable dog model with which to study chronic toxicity and pharmacology of interaventricularly

Table 4. Pharmacokinetics of intraventricular DFMO and MGBG

	n	K_{12} (min^{-1})	K_{20} (min^{-1})	V_c (ml)	AUC[a] (mg · min/ml)	CL_T (ml/min)
^{3}H-Inulin						
3rd ventricle	15	0.0137	0.0060	8.8	21.2	0.06
		(44%)	(37%)	(3.5)	(10.9)	(0.02)
Lateral ventricle	12	0.0086	0.0065	14.2	24.4	0.06
		(27%)	(44%)	(7.8)	(17.9)	(0.04)
Mean	27	0.0137	0.0062	11.2	22.6	0.06
		(37%)	(41%)	(6.3)	(14.2)	(0.03)
^{14}C-DFMO[b]	6	0.0133	0.0075	11.7	10.7	0.10
		(22%)	(28%)	(4.9)	(2.9)	(0.03)
^{14}C-MGBG[c]	6	0.0339	0.0138	15.4	3.4	0.36
		(22%)	(33%)	(6.7)	(1.6)	(0.19)

V_c, dose/(A+B); CL_T, dose/AUC; where A and B were obtained from the equation in *Materials and methods*. Numbers in parentheses are either ± %SD or ± SD. Results of the DFMO experiments are taken from Levin et al. [7]; the slight discrepancy in values from that report reflect a computer error

[a] Corrected for 1 mg dose

[b] Five 3rd ventricle and one lateral ventricle experiments

[c] Four 3rd ventricle and two lateral ventricle experiments

Table 5. CSF DFMO levels in beagle dogs

Animal	Dose schedule			Measured CSF[a] DFMO level (nmol/ml)
	Predicted mg/week	Actual		
		Doses/ week	Dose/ day	
8	150	3	50	93
1	32	3	11	127, 99
3	78	3	26	254, 79
5	90	3	30	–
7	141	3	47	430, 102
9	200	3	67	397
10	175	72 HR	25	202, 278
11	259	43 HR	37	956
12	336	42 HR	48	1419
13	525	19 HR	75	1973
14	1,050	24 HR	150	2410
15	1,400	24 HR	200	3272

[a] Taken at end of infusion or, for multiple injection sequences, on day 2 or 3

HR, total hours of infusion for dogs in which toxicity was too great for then to receive the full week of infusion

administered antineoplastic drugs; and the second was to evaluate the CNS toxicity of two inhibitors of polyamine metabolism, DFMO and MGBG. The models have undergone many modifications, mainly to reduce infections and to improve drug delivery. While some of our studies utilized a rigid needle in the third ventricle, we prefer a specially modified Foltz-type reservoir from American Heyer-Shulte implanted in a lateral ventricle. This is a completely SC system and can be used both for sampling of CSF and drug delivery. For constant daily infusions, the pediatric Broviac catheter connected to one side of the Foltz reservoir and exteriorized behind the nape serves our purposes well.

The pharmacokinetics of inulin administered in the lateral ventricle demonstrated a V_c that approximates the CSF volume in the dog (J. D. Fenstermacher, personal comunication); the difference between V_c computed from the third ventricle suggests that diffusion of tracer back-stream from the ventricle to the lateral ventricles did not occur to any great extent. The clearance and AUC for brain inulin in both cases were similar, implying that for the purposes of this study, pooling the pharmacokinetics of DFMO and MGBG by either route is reasonably satisfactory.

In terms of DFMO toxicity and efficacy, in a previous study we determined that DFMO will not achieve adequate CSF levels as a result of blood-to-CSF diffusion [7]. We found that, following intraventricular administration, DFMO CSF pharmacokinetics were similar to those of inulin, indicating primarily bulk absorption from CSF. Measurement of DFMO levels in the CSF on day 3 of 3-day treatments or at 19–72 h into the infusion of high-dose infusions of DFMO in experimental animals demonstrated that we could produce CSF levels comparable to that in plasma during chronic oral administration of DFMO [1]. Table 3 summarizes these findings. Dogs treated with DFMO and without concurrent ventricular infections had serious toxicity: 47% had seizures even though they were receiving phenobarbital; 27% had vomiting; 33% had serious irritability; and most had pathological changes at postmortem examination. This toxicity was most prominent in dogs with DFMO levels that exceeded 400 nmol/ml (123 μg/ml).

The pharmacokinetics of ICSF MGBG demonstrated that clearance from the CSF was faster than that of inulin and DFMO. This also resulted in a lower AUC for a comparable 1-mg dose of MGBG. This is in keeping with the lower molecular weight of MGBG and its ability to cross the BBB [14]. While the projected CSF AUC (concentration × time) of 6.1 mg · min/ml for each 1-mg dose given may be sufficient to achieve therapeutic effects, the acute toxicity of vomiting in 57%, seizures in 43%, ataxia in 43%, and the associated pathological findings severely reduce the potential clinical use of MGBG. Unfortunately, while the ICSF administration of

the polyamine inhibitors, DFMO and MGBG, would be of theoretical benefit, toxicity precludes further consideration of these agents for clinical practice.

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