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Brain, CSF, and Tumor Pharmacokinetics of α-Difluoromethylornithine in Rats and Dogs

Victor A. Levin, Judit Csejtey, and Deborah J. Byrd

Brain Tumor Research Center of the Department of Neurological Surgery, School of Medicine, University of California, San Francisco, CA 94143, USA

Summary. *We have determined the pharmacokinetic parameters for diffusion of α-[5-^{14}C]-difluoromethylornithine (DFMO) from blood to brain, blood to cerebrospinal fluid (CSF), 9L rat brain tumor to adjacent brain, and blood to the subcutaneously-implanted 9L tumor in rats, and within the CSF of beagle dogs. DFMO diffusion across the blood-brain and blood-CSF barriers is quite restricted in both rats and dogs, but diffusion across the defective capillary system of both subcutaneous and intracerebral 9L tumors in rats is not. Under steady-state plasma conditions in rats, uptake of DFMO by the intracerebral 9L tumor and diffusion from tumor 5–6 mm into adjacent brain is not restricted; tissue/plasma ratios were approximately 1. Therapeutic efficacy will therefore not be limited by transport of DFMO into tumor or to the extracellular environment of tumor.*

Introduction

α-Difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase; by reducing intracellular levels of putrescine, DFMO treatment can slow or halt cell division [8, 12]. Because of our continuing interest in laboratory and clinical studies of agents that might improve the chemotherapy of brain tumors and of the relationship between brain tumors and polyamines [10], and because it has been shown that DFMO can significantly enhance the cytotoxicity of BCNU against the intracerebral (IC) 9L tumor in rats [3] and against 9L cells in vitro [9], we have determined brain and tumor capillary permeability coefficients, pharmacokinetics of tumor uptake and distribution of diffusion from tumor, and the CSF pharmacokinetics of DFMO in rats and beagle dogs.

Materials and Methods

DFMO and α-[5-^{14}C]-DFMO (specific activity, 60 mCi/mmol) were the generous gift of the Merrell-Dow Research Center, Cincinnati, OH, USA. ^{3}H-Inulin was purchased from New England Nuclear Corporation (Boston, MA, USA), and ^{3}H-dextran from Amersham-Searle Corporation (Arlington Heights, IL, USA). ^{3}H-Inulin was repurified immediately before use by Sephadex G-25 column chromatography.

F344 rats were obtained from the Simonson Company (Gilroy, CA, USA). Six- to nine-month-old beagle dogs were purchased from Marshall Research Corporation (North Rose, NY, USA).

DFMO levels in biological fluids were determined using a modification of the method of Grove et al. [2]. Radioactivity was determined after solubilization in NSC tissue solubilizer (Amersham, Palo Alto, CA, USA) and counted in Permablend flour (Packard, Palo Alto, CA, USA). Samples were counted in a Beckman LS-250 scintillation spectrometer, and quench corrections were made using the external standard method.

Permeability Determinations. Rat brain and tumor capillary permeability (P) measurements were made using published methods [5, 6]. Tumor plasma volume was determined by injecting ^{3}H-dextran 15 min before sacrifice; values were calculated using the formula

$$K_i = 0.93\ C\ [1\text{-}0.93\ PV - 0.85\ (BV - PV)f]/AUC\ ,$$

where K_i is the capillary permeability times the surface area coefficient (min^{-1}), C is the concentration (or dpm/g) of drug in tissue, PV is the tissue plasma volume (ml/g), BV is the tissue blood volume (ml/g), f is the red cell/plasma ratio of drug at the end of the experimental period, AUC is the integrated plasma drug or isotope exposure during the experimental period (dpm · min/ml), and 0.93 and 0.85 are correction factors for plasma and red cell water, respectively. P (cm/s) was calculated using the equation

$$P = 0.28\ (ICD)\ K_i / 60\ BV^{1/2}\ ,$$

where ICD is the intercapillary distance (cm), 0.28 is correction factor that accounts for capillary geometry in the tumor, and 60 is a conversion factor from min to s.

Tumor Uptake After a Bolus Injection of ^{14}C-DFMO. Under ether anesthesia, 14–18 days after subcutaneous (SC) implantation of the 9L rat brain tumor in F344 rats, the right carotid artery and jugular vein were catherized with PE 20 tubing. Labeled and unlabeled DFMO were mixed before intravenous (IV) injection; each rat received 200 mg DFMO/kg (total activity of 6 μCi). Fifteen minutes before sacrifice, ^{3}H-dextran (17 μCi) was injected. Arterial blood samples were obtained at various times. After sacrifice, tumor was rapidly removed, flash-frozen in liquid nitrogen, and sliced into 2 × 2-mm slabs cut across the length of the tumor. Slabs were then cut into 2 × 0.75-mm pieces using a specially designed knife. After

Reprint requests should be addressed to V. A. Levin

digestion and scintillation counting, counts per minute were converted to either nanograms per gram of tissue or nanograms per milliliter of plasma.

Tumor Uptake During Prolonged Administration of ^{14}C-DFMO. Eighteen days after the implantation of the IC 9L tumor, F344 rats had an Alzet minipump (Alza Corporation) containing 12 μCi DFMO implanted surgically in the peritoneum. One to four days after implantation of the pump, rats were anesthetized and sacrificed by decapitation; 2 h before sacrifice trypan blue dye was injected intraperitoneally (IP) to delineate tumor from normal brain, and 15 min before sacrifice each rat received ^{3}H-dextran IV to determine tumor plasma volume.

Rat heads were frozen in liquid nitrogen for 45 s and cut with a Stryker saw. A 1 × 2-mm slab of dye-stained tumor and adjacent brain was cut (coronal section), and then recut into 0.75-mm slabs, digested in tissue solubilizer, and counted. After correction for the plasma contribution to tissue ^{14}C-DFMO levels, the ratio of ^{14}C-DFMO in extravascular tissue to plasma was determined as a function of distance from tumor.

CSF/Plasma ^{14}C-DFMO Ratios in Rats and Dogs. Three rats with IP Alzet minipumps that contained 75–100 μCi ^{14}C-DFMO were anesthetized with ether and a 27 gauge needle was used to obtain CSF from the cisterna magnum at intervals through 7 days. If these taps were successfully performed – if they contained no blood – then a blood sample was obtained under direct vision from either the medial orbital sinus or jugular vein. The CSF/plasma ^{14}C-DFMO ratios were calculated.

In one dog, a Broviac catheter was tied into the right jugular vein and exteriorized to the interscapular region. An auto-syringe pump (Orange Medical Instruments) was used to deliver 200 mg unlabeled DFMO/24 h. At intervals of approximately 3 days the dog was anesthetized with pentothal and simultaneous cisternal CSF and peripheral venous blood were withdrawn. Plasma and CSF levels were measured with high-performance liquid chromatography [2] and the CSF/plasma DFMO ratio was determined.

CSF Pharmacokinetics in Dogs. Six dogs were anesthetized with pentobarbital and placed in a stereotaxic head holder. Using sterile techniques, either a 20 gauge spinal needle was inserted into the third ventricle, connected to polypropylene tubing, and fixed to the bone with dental acrylic, or a right ventricular Foltz reservoir (American Heyer-Schulte) was placed. The wound was closed and dogs were treated prophylactically with 600,000 IU of penicillin/day for 3 days.

After a 1-week recovery period, dogs were reanesthetized, placed in the lateral position, and a 22-gauge needle was placed percutaneously into the cisterna magnum. In a total volume of 0.5 ml vehicle, ^{14}C-DFMO (2 μCi) and ^{3}H-inulin (1 μCi) were injected into the third ventricle. Samples of cisternal CSF were removed at various times and the amount of radiolabel was counted.

The descending plot of dpm/ml 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 [1] these data were used to compute pharmacokinetic parameters.

Results

Brain and Tumor Permeability

Table 1 lists the calculated values of P for DFMO entry into rat brain and the K_i values for the 9L brain and SC tumors. The K_i for DFMO in 9L tumors varies considerably more than the K_i for brain, and the median value is higher than the value for brain.

Blood-CSF Transport

Figure 1 is a plot of the DFMO CSF/plasma ratio (corrected for the CSF/plasma water ratio by a factor of 1.05) vs time. For the continuous infusion studies, the half-life for entry of DFMO into CSF was 8.2 days for the rat, and for dogs was below the level of sensitivity of the assay; therefore, the value for dogs of 14.6 days is underestimated.

Table 1. Rat brain and subcutaneous 9L tumor permeability of ^{14}C-DFMO

	Permeability coefficient (cm/s)	K_i	
		Median (min^{-1})	95% limits (min^{-1})
Brain (n = 9)	3.9×10^{-7} (% SD = 14)	0.0026	0.0012–0.0040
Tumor (n = 48)	Not determined	0.083	0.035 –0.108

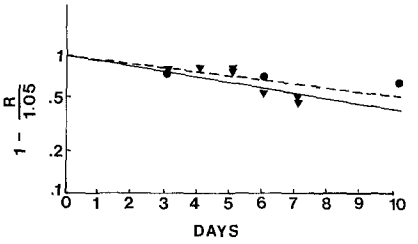

Fig. 1. The ratio (R) of CSF/plasma ^{14}C-DFMO radioactivity in dog (●– – –●) and rat (▼——▼). The factor in the denominator corrects for CSF/plasma water ratio [5]

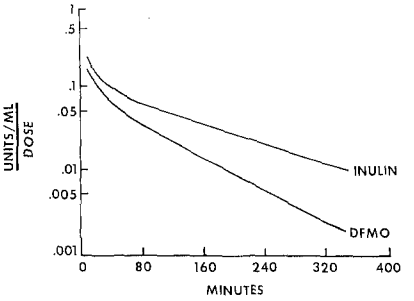

Fig. 2. Cisternal CSF levels of ^{14}C-DFMO and ^{3}H-inulin in a beagle dog after administration of isotopes in the third ventricle

Table 2. CSF pharmacokinetics in beagle dogs that received drug through the third or lateral ventricle and sampled at the cisterna magnum

	n	K_{12} (min^{-1})	K_{20} (min^{-1})	V_c (ml)	AUC (mg · min/ml)	CL_t (ml/min)	CL_{20} (ml/min)
^{3}H-Inulin	6	0.0122 (30%)	0.0044 (26%)	7.4 (4.0)	30.2 (14.2)	0.033 (0.015)	0.029 (0.017)
^{14}C-DFMO	6	0.0139 (22%)	0.0050 (37%)	11.3	12.4 (6.6)	0.080 (0.040)	0.057 (0.022)

V_c = dose/(A + B); CL_t = dose/AUC; CL_{20} = K_{20} V_P, where V_P is the volume of the peripheral compartment. A and B were obtained from the equation $C_{csf} = Ae^{-\alpha t} + Be^{-\beta t}$
Numbers in parenthesis are either ± % SD or ± SD

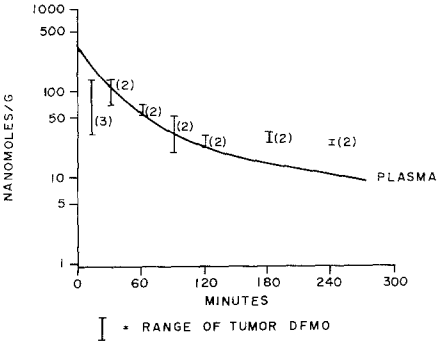

Fig. 3. Relationship of plasma ^{14}C-DFMO to SC 9L tumor uptake as a function of time after an IV bolus injection of 200 mg DFMO/kg. *Verticle bars* represent 95% confidence limits and the *number* in the *parenthesis* is the number of rats for each point. At least seven tissue samples were analyzed for each rat. The plasma clearance of DFMO was computer-fit using a nonlinear iterative least-squares program to the equation C_{pl} = 299 nmole $e^{-0.037t}$ + 39 nmole $e^{-0.0055}$

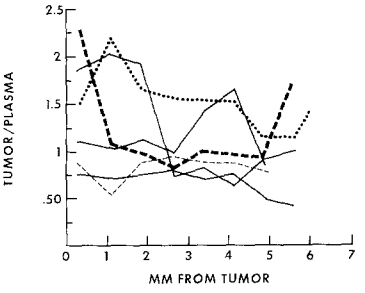

Fig. 4. ^{14}C-DFMO tumor/plasma ratios in brain adjacent to and distant from IC 9L tumors after 1–4 days of continuous infusion of ^{14}C-DFMO in rats. (———) 1 day; (- - - - -) 2 days; (- - - -) 3 days; (.....) 4 days

CSF Pharmacokinetics

Figure 2 is a typical plot of the clearance of DFMO and inulin vs time after the instillation of the two labeled compounds into the third ventricle. Table 2 summarizes pharmacokinetic parameters calculated using a two-compartment open model with elimination from the peripheral compartment. The first compartment is assumed to be related to equilibration among the CSF ventricles. The value of V_c of 7.4 ml calculated for inulin is consistent with the known volume of CSF in the dog [11]. The slightly higher value of V_c (11.3 ml) for DFMO probably reflects the slightly higher rate of DFMO transport across brain capillaries.

The transfer constant K_{12} approximates redistribution of the drug in the slowly equilibrating peripheral compartment, which probably reflects distribution in subarachnoid CSF. The values of K_{12} for inulin and DFMO are 57 and 50 min. K_{20} reflects elimination from the CSF to blood primarily by bulk CSF absorption through the arachnoid villi with a small amount of transcapillary diffusion. The values of K_{20} are equivalent to half-times of 158 and 139 min for inulin and DFMO, respectively. The total CSF clearance (CL_T) and clearance from the peripheral compartment (CL_{20}) are almost the same for inulin, but CL_T is greater than CL_{20} for DFMO; these data suggest that the majority of inulin is cleared from the second compartment by bulk flow, while some DFMO must either cross brain capillaries or bind to cells (such as ependyma or arachnoid cells) adjacent to CSF pathways.

Tumor Uptake and Diffusion from Tumor

Figure 3 shows the average plasma clearance for DFMO in rats compared with uptake of drug in SC 9L tumors. Vertical bars represent 95% confidence limits for DFMO levels in tumor.

Figure 4 shows plots of the DFMO tissue/plasma ratios as a function of distance from the IC 9L tumor in rats for 1–4 days during which plasma levels were held constant. Because in some tumors it was difficult to delineate the margins of tumors from 'normal' brain, the first 1–1.5 mm of normal brain may include some tumor.

Discussion

DFMO enhances the cytotoxicity of BCNU against 9L tumor cells in vitro [3] and IC rat tumors in vivo [9], which suggests that this agent might enhance the cytotoxic effects of these agents in human patients harboring solid tumors. Moreover, DFMO significantly affects tissue growth in vivo in a rat model [7]. Therefore, the agent could be used in human patients to slow tumor growth in the periods between courses of cytotoxic therapy. Because of these findings and because we are conducting a phase II trial of DFMO and another polyamine biosynthesis inhibitor, MGBG, in patients harboring primary brain tumors, we determined the pharmacokinetic parameters of DFMO in brain and CSF of rats and dogs, and in IC and SC 9L tumors in rats.

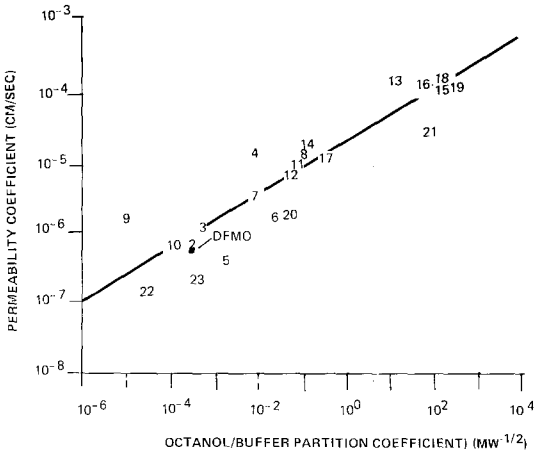

Fig. 5. Relationship of rat brain capillary permeability to molecular weight and lipophilicity. The permeability coefficient for DFMO is 3.9×10^{-7} cm/s (molecular weight = 237 and log $P = -2.45$). DFMO appears where expected on the in the plot of coefficient vs the product of the octanol/water partition coefficient and the square root of the molecular weight [4]

The results of these studies show that DFMO does not readily cross either the blood-brain or blood-CSF barriers, as predicted from a model of permeation based on lipophilicity and molecular size [4]. As can be seen in Fig. 5, a plot that shows the relationship of capillary permeability to molecular weight and lipophilicity for 23 compounds [4], the point for DFMO falls where expected. Experiments in which DFMO was continuously infused in rats and dogs show that the CSF/plasma ratios are quite low, even after 10 days of infusion. Data listed in Table 2 show that DFMO is cleared from CSF primarily by bulk flow through the arachnoid villi. Thus both blood-brain and blood-CSF, permeation is quite restricted.

Permeation of DFMO into either SC or IC tumors is approximately 30-fold higher than it is in brain. Within 30 min after IV administration, DFMO tumor levels are higher than plasma levels. Because the usual mode of administration of DFMO in human patients is multiple daily oral doses for at least 6 weeks, and because DFMO does not appear to undergo appreciable catabolism, DFMO can distribute into tumor and adjacent brain easily. As shown in Fig. 4, extravascular tumor/plasma levels of DFMO are generally greater than 1, even at distances of 5–6 mm from the 'leading edge' of the tumor.

These results show that DFMO can be delivered to solid tumors, including brain tumors, with ease. If adjuvant use of DFMO in a clinical setting is not efficaceous, it will be the result of factors other than distribution of the agent to target tissue.

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