Cancer Chemother Pharmacol (1982) 8: 125–131

Cancer Chemotherapy and Pharmacology

Chemotherapeutic Approaches to Brain Tumors

Experimental Observations with Dianhydrogalactitol and Dibromodulcitol

Victor A. Levin and Kenneth T. Wheeler*

Departments of Neurological Surgery, Pharmacology, and Pharmaceutical Chemistry, Schools of Medicine and Pharmacy, University of California, San Francisco, CA 94143, USA

Summary. *Dianhydrogalactitol (DAG) or its active cell-killing moiety has a relatively long biological half-life in 9L cells cultured in vitro. The shape of the DAG dose-response curves was similar to that of those observed for most oncolytic agents. The prominent shoulder on the 24-h dose-response curve indicates that 9L cells can accumulate a reasonable amount of DAG-induced sublethal damage before they are killed. The appearance of 9L colonies in petri dishes was delayed 3–5 days after a DAG treatment that killed more than 99% of the cells, an observation not previously made with radiation, hyperthermia, the nitrosoureas, or other chemotherapeutic agents.*

Comparison of the in vitro exposure integral and the in vivo tumor tissue integral indicated that DAG would have to be administered at a dose in excess of its LD_{10} to achieve an in vivo 2 log cell kill. The lack of a significant increase in lifespan after a LD_{10} dose confirmed this prediction. While DAG alone is active against IC ependymoblastoma, it had very limited activity against IC glioma 26; however, the combination of DAG with BCNU was curative in 85%–100% of animals at 120 days. BCNU alone achieved no more than a 4%–16% survival at 120 days. The combination of DBD and BCNU was not consistently better than BCNU alone against IC glioma 26.

It appears that DAG may have a limited place in CNS chemotherapy for specific kinds of tumors. BCNU-DAG combination studies suggest that we may, under the right conditions, enhance the antitumor activity of the hexitol epoxides by drug combination therapies, although the mechanism for this enhanced antitumor activity is presently unknown.

Introduction

Our initial interest in the hexitol epoxides was stimulated by the studies of Geran et al. [2], who screened 177 drugs and showed that dianhydrogalactitol (DAG: NSC-132313; 1, 2, 5, 6-dianhydrogalactitol) was the single most active drug against the experimental intracerebral [IC] ependymoblastoma tumor model in C57/BL6 mice. Subsequently, we reconfirmed their findings in the ependymoblastoma tumor model but found that activity against a sister tumor, the murine glioma 26, and also the IC 9L rat brain tumor was far less impressive [7]. This differential activity of IC tumors to DAG remains unsolved to date.

In this paper we will consider: (1) the effects of DAG on cell kill and division delay of 9L tumor cells in vitro; (2) a comparison of in vitro and in vivo exposure doses as an explanation for the lack of DAG activity against the IC tumor; and (3) insights gained by evaluating DAG and dibromodulcitol (DBD: NSC-104800; 1,6-dibromo-1,6-dideoxydulcitol) in two drug combinations in IC tumor models.

Materials and Methods

Cell Culture. 9L Cells were grown in Eagle's Basal medium (BME) supplemented with 10% fetal calf serum, twice the usual concentration of essential amino acids and vitamins, plus 87.0 μg streptomycin/ml and 87 units of penicillin. Under these culture conditions, 9L cells have a doubling time of 18–20 h and a colony-forming efficiency of 60%–80% in a humidified 5% CO_2 environment [12]. In these experiments, approximately 2×10^6 trypsinized cells from an exponentially growing culture were seeded into 75 cm^2 tissue culture flasks 24 h before DAG treatment.

Reprint requests should be addressed to V. A. Levin

* *Present address:* Department of Radiation Oncology, Rhode Island Hospital, Providence, RI 02902, USA

This paper was presented at the Hungarian Cancer Society, International Symposium on Cytostatic Galactitol Derivatives, Budapest, 24–26 September 1977

0344-5704/82/0008/0125/$ 01.40

DAG was weighed and dissolved in Hank's balanced salt solution, then diluted in complete medium; of the final dilution, 1.5 ml was added to each flask, which now contained $3-4 \times 10^6$ exponentially growing cells in 13.5 ml complete medium. All flasks were maintained at 35–37° C and pH 7.2–7.4 during treatment. After a 1-h or 24-h exposure, the cells were washed twice with Hank's solution and immediately trypsinized, counted, diluted, and plated into 60-mm petri dishes containing 5×10^4 heavily irradiated (4,000 rads) 9L feeder cells. After various periods of incubation (7–24 days, depending on the type of experiment), the medium was removed from the plates, and the colonies fixed with methanol, stained with Giesma, and counted. Only groups containing more than 50 cells were counted as colonies. Each data point on a DAG dose-response curve is the average from five plates at each of two or three different cell dilutions. In all other experiments, at least ten dishes of one dilution, usually containing more than 25 colonies, were analyzed. Two-dimensional size measurements were made on colonies with the aid of a magnifying eyepiece containing a micrometer calibrated in tenths of a millimeter.

Antitumor Studies. The tumor lines used were the glioma 26 and ependymoblastoma lines in C57/BL6 mice and the 9L line in F344 rats. All tumors were implanted similarly. For the mouse tumors a brei of subcutaneous tumor was prepared; for the 9L tumor cells from culture were harvested. Approximately 4×10^4 cells/0.01 ml were injected into the centrum ovale of the parietal lobe of the mice and stereotactically into the left cerebral hemisphere of the rats. The techniques have been well documented [6, 7]. Tumor takes exceeded 99% and control animals were all dead within 6 days of the median.

Dianhydrogalactitol (DAG) and dibromodulcitol (DBD) were both stored at −60° C, weighed immediately before each injection period, and dissolved in normal saline for injection. The volume administered was 0.1 ml/100 g rat and 0.1 ml/10 g mouse. BCNU (NSC-409962) was dissolved in ethanol and diluted to a 10% solution with water for injection.

Median survival of treated animals/median survival of control untreated animals (T/C), reported as a percent age, was used to compare treatments; the Gehan modification of the Wilcoxon rank sum analysis was used to provide statistical verification of the results.

Results

The shape of the in vitro dose-response curves for 9L cells exposed to various concentrations of DAG for 1 h or 24 h was similar to that observed for most oncolytic agents (Figs. 1 and 2). The D_o^* and D_q^* for the 1-h exposure were 4.6 μg/ml and 2.6 μg/ml, respectively; for the 24-h exposures they were 0.21 μg/ml and 0.082 μg/ml. The appearance of colonies in the petri dishes was delayed to a greater extent as the survival of clonogenic cells decreased, i.e., as a function of increasing DAG exposure dose (Fig. 3). This delay in the appearance of colonies was not caused by slower growth of the clonogenic survivors of the DAG treatment, because the colonies, once formed, grew at about the same rate as those surviving a similar BCNU or X-ray treatment where no delay in the appearance of colonies was observed

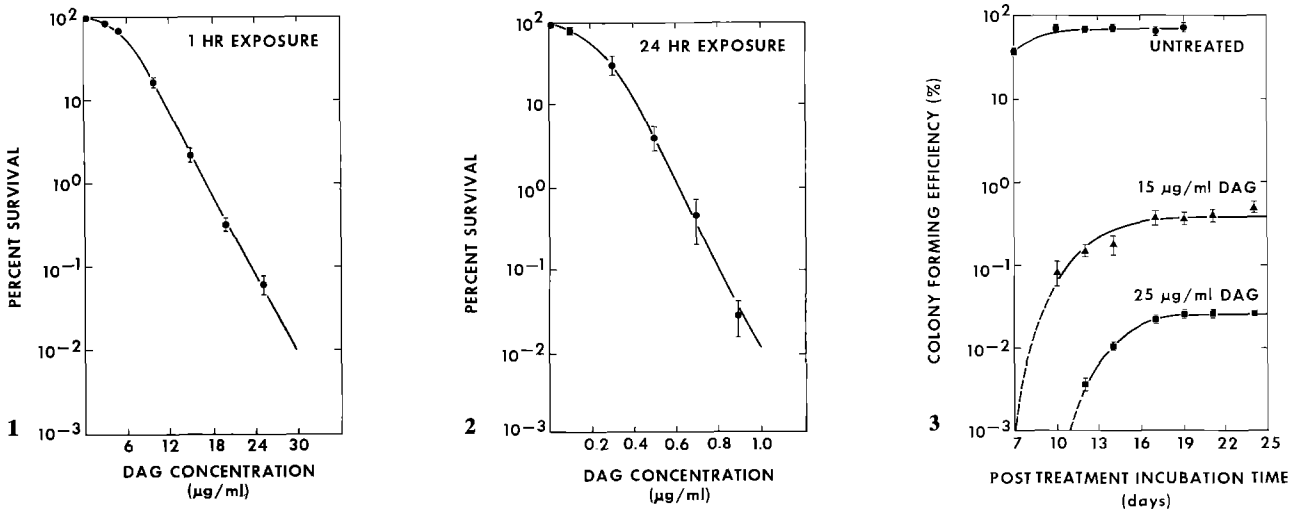

Fig. 1. Dose-response curve of 9L cells following a 1-h in vitro exposure. Only groups containing more than 50 cells were counted as colonies. Each data point represents the average (± SD) of five plates at each of two or three different cell dilutions

Fig. 2. Dose-response curve of 9L cells following a 24-h in vitro exposure. Conditions were the same as in Fig. 1

Fig. 3. The colony-forming efficiency (%) of 9L cells in vitro measured at various days following no DAG treatment (●), 15 μg/ml for 1 h (▲), or 25 μg/ml for 1 h (■). The *lines* were fitted by eye. Each *point* represents the mean (± SD) for at least 10 dishes of one dilution containing more than 25 colonies

* D_0 is the dose required to reduce the number of clonogenic cells by a factor of 0.37. D_q is the dose at which the extrapolated straight portion of the dose-response curve cuts the dose axis

Table 1. Comparison of colony growth rate determined from a regression analysis of the natural logarithm of the square of the average radius of the colonies as a function the posttreatment incubation time[a]

Treatment	Survival (%)	Dose (rads)	Concentration (μg/ml)[b]	Slope[c]	Ratio (treated/untreated)
None	70			0.488	1.00
BCNU	0.033		10	0.352	0.72
BCNU	0.0016		15	0.373	0.76
None	65			0.507	1.00
X-Ray	1.30	1,500		0.303	0.60
X-Ray	0.160	2,000		0.293	0.58
None	70			0.454	1.00
DAG	0.38		15	0.294	0.65
DAG	0.025		25	0.396	0.87

[a] All correlation coefficients were greater than 0.99
[b] All drug treatments were for 1 h
[c] The square of the colony radius is proportional to the area of the colony, which in turn is approximately proportional to the number of cells in the colony. The slope of this regression analysis, therefore, approximates the rate of growth of the cells

(Table 1). The close correspondence between the log cell kill achieved after 1 h or 24 h at comparable in vitro DAG exposure doses, calculated by assuming no significant decrease in DAG concentration over a 24-h exposure period, indicated that DAG had a very long half-life in this tissue culture system (Table 2).

The antitumor activity of DAG was minimal against the IC 9L gliosarcoma and IC glioma 26 models, but very effective against IC ependymoblastoma (Table 3). The antitumor activity of DBD against IC glioma 26 was also minimal (Table 4). When DAG or DBD was combined with BCNU to treat IC glioma 26, a substantial improvement in therapy occurred for the DAG-BCNU combination (Table 5), but less improvement in therapy was evident for the DBD–BCNU combination (Table 6). The DAG-BCNU therapy schemes were essentially as effective in treating IC glioma 26 as DAG alone was in treating IC ependymoblastoma (Tables 3 and 5).

Table 2. Relationship of 1-h and 24-h in vitro exposure to ^{14}C-DAG and single-dose pharmacokinetics of ^{14}C-DAG to cell kill and survival of 9L cells

In vitro						In vivo		
Concentration (μg/ml)		Exposure dose (μg · min/ml)		Log kill		Concentration (mg/kg)[a]	Exposure dose (μg · min/g)[b] 24 h	T/C %
1 h	24 h	1 h	24 h	1 h	24 h			
5	0.2	300	288	0.4	0.4	2	266	122
10	0.4	600	576	0.9	1.0	4	532	113
15	0.6	900	864	1.8	1.9	6	798	125
20	0.8	1,200	952	2.6	2.9	8	1,064	Toxic
25	1.0	1,500	1,240	3.4	3.7	10	1,330	Toxic

[a] Single dose $LD_{10} = 6$ mg/kg
[b] Assuming 200 g rats; integral was computed for brain tissue from the pharmacokinetic parameters previously determined [7]

Table 3. Activity of dianhydrogalactitol against IC tumors [7]

Tumor	Best dose[a] mg/kg	Schedule days	T/C[b] %	Survivors	
				60 Day	110 Day
Glioma 26	2–5 (2–10)	12	119	1/20	0/20
	3 (1–3)	1–5	153[c]	0/10	0/10
	3 (1–3)	5–9	130[c]	1/10	0/10
	3 (0.2–3)	12–16	128[c]	1/10	0/10
Ependymo-	1–2 (1–3)	1–5	>440[c]	18/18	18/18
blastoma	2–3 (1–3)	12–16	>440[c]	16/20	13/20
9L	6 (1–4)	12–16	107	0/30	0/30
	6 (2–10)	12	125	0/10	0/10

[a] At least 10 animals per dose group; 3–5 dose groups for each range
[b] The best median survival treated/control animals at dose(s)
[c] Statistically different than controls, $P < 0.01$
( ) Dose range tested

Table 4. Activity of dibromodulcitol against IC tumors

	Best dose[a] mg/kg/injection	Schedule days	T/C[b] %	60–Day survivors
Glioma 26	400 (150–600)	1	128	0/14
	20–100	13–17	96	0/42
Ependymo-	400 (300–600)	1	130	
blastoma [2]	128 (128–1,024)	1	126	
	500 (62–500)	1–5	158	
	400 (300–600)	1–5	255	

[a] At least 13 animals/dose for glioma 26 tumors
[b] Best median survival treated/control animals at dose(s)
( ) = Dose range

Table 5. Percent survival of glioma 26 tumor mice at 120 days [6][a]

BCNU[b]	DAG[c]					
	Day 11		Day 12.2		Day 13	
	2 mg/kg	4 mg/kg	2 mg/kg	4 mg/kg	2 mg/kg	4 mg/kg
20 mg/kg	100[d]	100[d]	100[d]	92	85	100[d]
30 mg/kg	92	85	50	92	02	100[d]

[a] 20 animals in control group; 13–15 animals per treatment group
[b] IP on day 12. No DAG alone survivors at 120 days and only 3%–16% BCNU alone survivors on day 120. LD_{10} BCNU = 34 mg/kg
[c] IP on days designated; dose/injection; single dose LD_{10} = 5.5 mg/kg
[d] Best at days 45, 75, and 120 by an analysis of variance at $P = 0.01$. All combinations better than BCNU alone at $P = 0.001$

Table 6. % T/C and 60–day survivors for BCNU-DBD combination therapy for IC glioma 26

BCNU[a] mg/kg	DBD[b], mg/kg per injection each day						
	0	150	200	300	20 × 5	30 × 5	100 × 5
0	[c]	89 (0/14)	96 (0/27)	103 (0/14)	92 (0/14)	96 (0/14)	95 (0/14)
20	128[d] (3/42)	89 (0/13)	134[d] (3/27)	117 (0/14)	140[d] (3/14)	134[d] (2/14)	100 (0/14)
30	144[d] (2/28)	89 (0/13)	109 (1/28)	90 (0/14)	180[d] (5/14)	182[d] (3/14)	76 (1/14)

( ) = 60-day survivors/number of animals
[a] Day 12, IP
[b] Day 13, IP
[c] Median = 26 days (n = 44)
[d] Different from controls, $P < 0.01$

Discussion

Pharmacokinetics of Galactitol Epoxides

The empiric requirement for effective therapy against solid CNS tumors is the ability of a drug to cross the normal blood-brain barrier (BBB). Even though the tumor has a defective BBB (e.g., a capillary endothelial barrier) to large macromolecules, there are many areas with neoplastic or potentially neoplastic cells that have normal brain capillaries (e.g., infiltrative regions in the brain adjacent to tumor).

Institoris et al. were the first to demonstrate that DBD and DAG crossed the mouse BBB [3]. We have quantitated this further and measured the capillary permeability coefficient for both DAG and DBD, and have found the value for DBD was slightly higher than that for DAG [5, 8]. The ability of DAG to cross brain capillaries is not surprising even though the drug is hydrophilic, since it has a molecular weight of 146. On the other hand, DBD has a molecular weight of 308 but is more lipophilic than DAG, which accounts for the fact that it too crossed brain capillaries with greater ease.

Using radiolabeled DAG, we showed that entry into normal brain and tumor tissue followed a simple two-compartment catenary model with $t_{1/2}$ for equilibrium in the two compartments of 22 and 105 min in the brain and 4 and 56 min in the IC 9L tumor [9]. In addition, drug entry into the intracerebral compartment was quite rapid, as was binding to nucleic acids. Binding to RNA was linear with time and was six-fold greater than for binding to DNA.

More recent studies of Kimura et al. [4] have shown DAG penetration into normal dog nervous system and CSF, and Eckhardt et al. [1] have shown DAG penetration into the human CNS, brain tumors, and CSF.

The second prerequisite for activity against brain tumors is a mode of action that produces cytotoxicity in tumor cells at achievable tumor exposure doses. To determine whether this condition existed in the 9L

tumor system, 9L cells were treated in vitro and the subsequent dose response data were used as models for the effects expected in vivo.

Effect of DAG on 9L Cells in vitro

Although complete pharmacokinetic information for DAG in our 9L tissue culture system is unavailable at this time, and the D_q and D_o for the 1-h exposure (4.6 μg/ml and 2.6 μg/ml, Fig. 1) and for the 24-h exposure (0.21 μg/ml and 0.082 μg/ml, Fig. 2) dose response curves cannot be compared directly with the values obtained for other chemical and physical agents, there are three general observations that can be made.

First, DAG or its active cell-killing moiety has a relatively long half-life in this tissue culture system, which coincides with data obtained by Munger for DAG stability in buffered solutions [10]. Second, the kinetics of cell kill are similar to those observed for most oncolytic agents. Finally, the prominent shoulder on the 24-h dose-response curve indicates that 9L cells can accumulate a reasonable amount of DAG-induced sublethal damage before they are killed. At this time we have no information about the ability of 9L cells either to remove this sublethal damage or to recover from DAG-induced potentially lethal damage.

It was also observed that the appearance of colonies on the petri dishes was delayed considerably after some DAG treatments (Fig. 3). Most colonies from untreated 9L cells were present on day 7 after treatment, and all colonies were present by day 10 (Fig. 3). When the survival of DAG-treated cells was above 10%, the kinetics of colony formation were identical with those of the untreated cells (data not shown). However, no colonies were apparent until day 10 on dishes containing 9L cells treated for 1 h with 15 μg DAG/ml (survival 0.025%), and no colonies were apparent until day 12 on dishes containing 9L cells treated for 1 h with 25 μg DAG/ml (survival 0.025%). Thus, a delay of 3–5 days in the appearance of colonies occurred at these low survival levels. No similar delay in the appearance of colonies at any level of survival has been observed for these cells treated with X-rays, hyperthermia, BCNU, CCNU, PCNU, or procarbazine.

Two mechanisms could account for this effect: There could be a long delay before division of the clonogenic survivors begins, or the clonogenic survivors could simply grow and divide much more slowly than normal. To determine which of these hypotheses was more plausible, we measured the dimensions of the colonies as a function of posttreatment incubation time. Since the square of the average colony radius is proportional to the number of cells in the colony, the slope, determined by a regression analysis of the natural log of the radius squared vs posttreatment incubation time, approximates the posttreatment growth rate of the cells. Table 1 shows that the slope of the DAG-treated 9L cells, relative to the slope of their untreated controls, is certainly no less than the slope observed for BCNU and X-ray-treated cells, relative to their untreated controls. Since (1) BCNU and X-ray-treated cells produced colonies at all survival levels by day 7 after treatment; (2) the maximum number of colonies from BCNU and X-ray-treated cells was reached by days 11–12 after treatment; and (3) there was no difference in the rate of growth of the 9L colonies after DAG, BCNU, or X-ray treatment, the delay in the appearance of DAG colonies at the low survival levels (< 1.0%, Fig. 3) is probably due to a very long delay in the first, or one of the first, four or five generations after treatment.

There are two important ramifications of this later observation. First, from a methodological point of view, accurate dose response curves with a shoulder and exponential region, which determine inherent DAG sensitivity parameters, must be obtained with different incubation times employed at different levels of survival; otherwise, continuously bending curves are obtained. Second, increases in the lifespan of animals bearing 9L brain tumors after DAG treatment may be due to both the cell division phenomenon and DAG-induced cell kill.

Nomura and Hoshino [11] have studied the effects of doses of 1 μg and 5 μg DAG/ml on cultured 9L cells and have made a variety of observations. they found a prolongation of S-phase, partial G_1-S block, and an unusual accumulation of cells in G_2 with random release into mitosis 24–36 h after exposure. These delays may be mechanistically related to the delay described above for the formation of 9L cell colonies following DAG exposures of 1 h.

Our colony-forming experiments demonstrate a significant delay (3–5 days) in cell division after DAG doses that result in reasonable amounts of cell kill, but unfortunately, increases in lifespan caused predominantly by this delay in cell division cannot be expected to result in eventual cures unless accompanied by more than a 3 log cell kill. The lack of a significant increase in the lifespan of rats bearing IC 9L tumors (Table 1) suggests that even with DAG doses in excess of the LD_{10} in situ tumor cells did not receive an exposure dose adequate to produce significant amounts of either cell kill or dividion delay.

Comparison of the DAG Exposure Dose Received by 9L Cells in vivo and in vitro

Comparing the in vitro exposure integral ${}_0\int^t C\,(t)\,dt$ for DAG, following a 1-h or 24-h exposure, to its antitumor activity against 9L cells, we found that doses over 15 μg/ml for 1 h or approximately 0.6 μg/ml for 24 h would be necessary to achieve a 2 log cell kill. In Table 6 we compare the log cell kill to the exposure integral for DAG in culture, assuming no breakdown of drug over 1–24 h. Clearly, this is a modest overestimate for the 24-h exposure, since degradation occurs slowly ($t_{1/2} \geq 7$ h in buffer or rat microsomes) [10]. By assuming no drug breakdown, however, we were able to compare the in vitro exposure integral to the in vivo tumor tissue integral based on the pharmacokinetic parameters measured previously [7]. From these data it is apparent that to achieve an in vivo 2 log cell kill the drug would have to be administered at a dose in excess of 6 mg/kg, the LD_{10} in these rats.

At present the cellular or molecular mechanisms for the apparent resistance of in situ 9L cells to DAG in comparison to the sensitivity of in situ ependymoblastoma cells have not been elucidated. Among the possibilities that have not been investigated are: (1) that the in situ 9L cells are not as susceptible to DAG damage as the in vitro 9L cells, and (2) that they can repair this damage more rapidly. Cells existing in three-dimensional spheroids have been shown to be more resistant to both drugs and radiation than the same cells grown in two-dimensional monolayer cultures, probably because of an increase to their ability to repair their molecular damage. Regardless of the mechanism for resistance, one possible way of improving the limited response of some tumors to the hexitol epoxides used as single agents is to combine them with other drugs.

Drug Combinations: DAG and DBD in IC Tumor Model Systems

The activity of DAG in combination with BCNU in the IC glioma 26 tumor model system is a particularly important interaction, since DAG and DBD alone had very limited and irregular activity against glioma 26 (Tables 5 and 6). Yet in combination with BCNU, DAG is curative in 85%–100% of the animals at 120 days (Table 5); BCNU alone achieved no more than a 4%–16% survival at 120 days with the doses used in this study. Of interest is the fact that DAG was active before, during, and after BCNU chemotherapy [6]. These data suggest that DAG failure as a single agent against glioma 26 was not due to its inability to enter tumor cells, because DAG given before BCNU improved survival markedly. We presume that the reason for DAG resistance in the 9L and glioma 26 models may be related to the ability of glioma 26 cells to rapidly repair DAG-induced damage; in contrast, the capacity of the ependymoblastoma cells (line EP-A) [2] to repair this damage may be slow or nonexistent.

When DAG was combined with BCNU in the IC 9L tumor system, the results were not as dramatic as those observed with glioma 26. The combination of DAG (2 mg/kg on day 12) with BCNU (5–10 mg/kg dose on day 12) showed only additive activity and no long-term survivors.

Combination of DBD and BCNU in the IC glioma 26 tumor model produced a modest improvement in survival compared with BCNU alone treatment. DBD produced an increase in % T/C from 144–180 and an increase in 60-day survivors from 7% for BCNU (30 mg/kg) to 21%–36% when combined with DBD (20 and 30 mg/kg per injection each day for 5 days). While better dose schedules for DBD may improve the benefit of the two agents in combination, the trend is less dramatic than that apparent in the DAG-BCNU data.

It appears that DAG, and possibly DBD, a precursor of the DAG epoxide, may have a limited place in CNS chemotherapy for specific kinds of tumors. Our original studies with three different IC tumors showed that, unlike the nitrosoureas, DAG cannot be expected to have widespread antitumor activity against CNS tumors. It is important for the future that we investigate the reason for tumor cell resistance to the hexitol epoxides, to put ourselves in a better position to prevent this resistance either by changing the molecular structure of the parent compound, which may not produce the desired result, or more importantly, by using DAG in combination with a second cytotoxic drug. Our BCNU-DAG studies suggest that we may, under the right conditions, enhance the antitumor activity of the hexitol epoxides by drug combination therapies with nitrosoureas.

Acknowledgements: This work was supported by NIH Grants CA-13525, CA-15435, and RCDA NS-70739 (KTW), and American Cancer Society Faculty Research Award FRA-155 (VAL).

We acknowledge the technical help of M. E. Williams, S. Miranda, B. Usog, and C. Morton.

References

1. Eckhardt S, Csetengi J, Horvath IP et al. (1977) Uptake of labeled dianhydrogalactitol into human gliomas and nervous tissue. Cancer Treat Rep 61: 841
2. Geran RI, Congleton GF, Dudeck LE (1974) A mouse ependymoblastoma as an experimental model for screening potential antineoplastic drugs. Cancer Chemother Rep 4: 53
3. Institoris L, Dzurillay E, Pethos G (1973) Comparative studies on the in vivo distribution pattern of dibromodulcitol and diepoxydulcito. 1. Z Krebsforschung 79: 49
4. Kimura T, Sternson LA, Stella VJ, Higuchi T (1977) A preliminary pharmacokinetic study of dianhydrogalactitol (NSC-132313) disposition in the dog. J Natl Cancer Inst 58: 311
5. Levin VA (1980) Relationship of lipophilicity and molecular weight to brain capillary permeability. J Med Chem 23: 682
6. Levin VA, Wilson CB (1978) Correlations between experimental chemotherapy in the murine glioma and effectiveness of clinical therapy regimens. Cancer Chemother Pharmacol 1: 41
7. Levin VA, Freeman-Dove MA, Maroten CE (1976) Dianhydrogalactitol (NSC-132313): Pharmacokinetics in normal and tumor-bearing rat brain and antitumor activity against three intracerebral rodent tumors. J Natl Cancer Inst 56: 535
8. Levin VA, Landahl H, Freeman-Dove MA (1976) The application of brain capillary permeability coefficient measurements to pathologic conditions and the selection of agents which cross the blood-brain barrier. J Pharmacokinet Biopharm 4: 499
9. Levin VA, Patlak CS, Landahl HD (1980) Heuristic modeling of drug delivery to malignant brain tumors. J Pharmacokinet Biopharm 8: 257
10. Munger D, Sternson LA, Repta AJ, Higuchi T (1977) High-performance liquid chromatographic analysis of dianhydrogalactitol in plasma by derivation with sodium diethyldithiocarbamate. J Chromatogr 143: 375
11. Nomura K, Hoshino T, Deen DF, Knebel K (1978) Perturbed kinetics of 9L rat brain tumor cells following dianhydrogalactitol (NSC-132313). Cancer Treat Rep 62: 2055
12. Wheeler KT, Tel N, Williams ME, et al. (1975) Factors influencing the survival of rat brain tumor cells after in vitro treatment with 1,3 bis(2-chloroethyl)-1-nitrosourea. Cancer Res 35: 1464

Received September 17/Accepted December 17, 1981