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Pharmacokinetics and Metabolism of Dianhydrogalactitol DAG in Patients: a Comparison with the Human Disposition of Dibromodulcitol DBD

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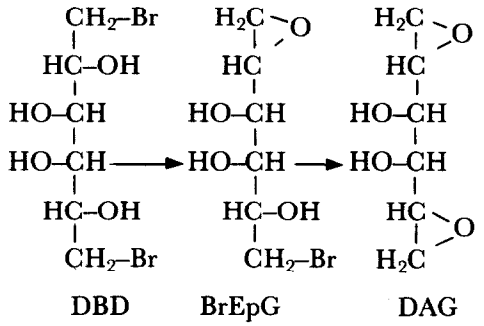
Abstract—*Dianhydrogalactitol (DAG), labelled with* ^{3}H, *was administered in single intravenous or oral doses to six patients (three in each group) with cancer. Kinetic parameters were calculated for the unchanged DAG and its biotransformation products. Elimination of the drug by metabolism and excretion was described by a catenary model. In order to elucidate the role of DAG as a mediator of the alkylating action of the cytostatic drug dibromodulcitol (DBD), the pharmacokinetic parameters of DAG and DBD were compared. The mean residence time for pharmacologically active molecules in the body was six times shorter for DAG* (1.9 hr) *than for DBD* (11.4 hr). *Alkylating action and metabolic degradation proceeded about* 8–9 *times faster for DAG than for DBD. The process of DBD alkylation implies a slow solvolytic conversion of the parent drug into the more reactive bromoepoxide and DAG. The preformed DAG would be rapidly consumed by intracellular alkylation and degradation, while unchanged DBD could form a depot in the cells and exert its cytostatic activity through the epoxides released* in situ *by solvolytic activation. Thus DBD entering the cells in unchanged form may have a more important role in its therapeutic effects than had been assumed earlier.*

INTRODUCTION

In 1979 and 1982 we reported studies on the metabolism and pharmacokinetics of the cytostatic drug dibromodulcitol (DBD, dibromogalactitol, mitolactol) in patients with advanced cancer [1, 2]. DBD is a weak alkylating agent, but at slightly alkaline pH it is converted into bromoepoxide (1,2-anhydro-6-bromo-6-deoxygalactitol, BrEpG) and a diepoxide (1,2-5,6-dianhydrogalactitol, DAG), which are more reactive alkylating agents than the parent drug. After administration of DBD *in vivo*, the effective drug content in the body is composed of these three compounds, and the final mediator of the cytostatic activity seems to be DAG [3]:



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Recently, 3,4-diacyl derivatives of DAG have been investigated for cytostatic activity and one of them, diacetyl-DAG, is already on clinical trial [4]. Administration of diacetyl DAG *in vivo* leads to the presence of DAG in body fluids [5]. Therefore, the pharmacokinetics and metabolism of DAG may be of interest in studying other hexitol derivatives.

The human pharmacokinetics of DAG have been investigated by Eagan *et al.* [6] in connection with phase II and III clinical studies. Disappearance of DAG from plasma was monitored by an analytical method using HPLC.

In the present study we used ^{3}H-labelled drug with the aim of setting up a total material balance of the drug and its metabolites, calculating the parameters of transfer, metabolic degradation and alkylating action after single intravenous and oral doses. Comparing the parameters of DAG to DBD, we tried to explain differences in the mode of action of the two drugs.

MATERIALS AND METHODS

DAG was labelled with ^{3}H at position C–1 and triturated with non-radioactive drug (obtained from the CHINOIN Chemical and Pharmaceutical Works) to a sp. act. of 666 MBq/mg (18 μCi/mg). It was administered intravenously or

orally to six patients — three patients in each group; (Table 1) with average doses of 0.7 mg/kg and 0.9 mg/kg, respectively.

Plasma samples and urine collected were measured as described earlier [1, 2]. Samples of cerebrospinal fluid (CSF) and tumor tissue were also taken in a few cases. Faeces was assayed in two orally treated patients (L.G. and B.S).

Radioactive metabolites in plasma, urine and CSF were separated by ascending paper chromatography then assayed for radioactivity and alkylating capacity, as in the case of the [^{3}H]DBD studies [1]. Attempts to use thin-layer chromatography gave no adequate results because of the low efficiency of ^{3}H-scanning. The plasma proteins were precipitated with ethanol prior to chromatography. The chromatographic data refer, therefore, to the deproteinized fraction of plasma, and to native urine and CSF [1, 2]. Some radioactive products were identified by co-chromatography of the authentic materials.

The mathematical methods and the catenary model used in the evaluation of the results has been described earlier [2].

RESULTS

By 30 min after a rapid intravenous injection of [^{3}H]DAG to three patients, the average plasma level of ^{3}H-compounds (Fig. 1) was 1.8 μg/ml but its DAG content was only 10–37%. The rest was bound to plasma proteins 8–25% or consisted of free metabolites 54–72% of the drug. Between 30 min and 8 hr the level of unchanged DAG decreased with half-life of 40 min, i.e. nearly the same value as the 'beta-period' half-life reported by Eagan *et al.* [6]. Considering that theoretically there was only DAG in the plasma at the time of injection (c_0 in Table 2), the equation fitted to the plasma level curve of unchanged DAG (Table 3) should include an exponential term with a very fast initial rate constant for the alpha or distribution phase.

After oral administration, the plasma level curves (Fig. 2) showed large individual differences. Peak levels of radioactivity corresponding to 0.8–2.8 μg/ml drug were attained between 15 and 200 min. The concentration of unchanged DAG reached a peak of about 0.1 μg/ml and decreased with a half-life of 80 min, in average, in patients L.G. and B.S. The lower oral DAG level was compensated by slower elimination. The area under the oral DAG level curves (AUC_D in Table 3) represented 5.1 ± 3.6% of the area under the curve of the total radioactivity (AUC_{tot} in Table 2). After intravenous treatment it was 6 ± 3%. The difference is not significant.

After oral dosing, the rate constant of absorption and the absorbed percent of dose (FD) varied in a wide range (Table 2). In the case of patient B.R., where the rate of absorption was extremely slow, no unchanged DAG was found in the plasma. The

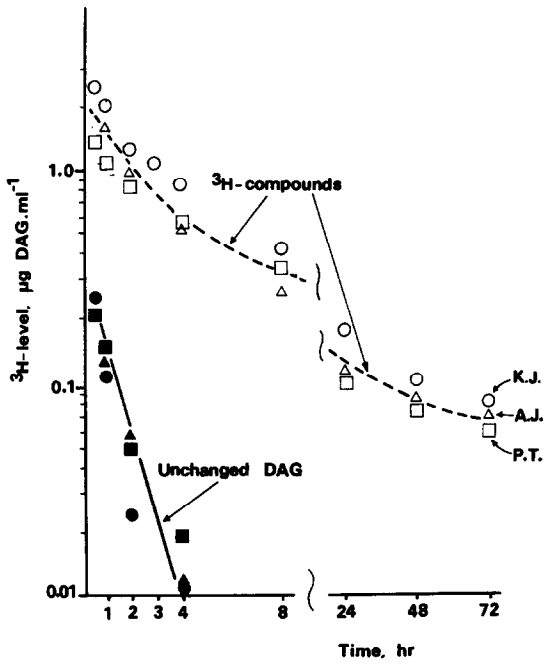

Fig. 1. Plasma levels of unchanged DAG (full marks) and total ^{3}H-compounds (empth marks) after a single intravenous dose of [^{3}H]DAG. Lines represent mean values of 3 patients.

Table 1. Data of patients treated with a single dose of [^{3}H]DAG

	Patient	Sex	Age (yr)	Diagnosis	Weight (kg)	Dose (mg)
Intravenously	A.J.	male	42	lymphosarcoma	75	42
	P.T.	female	68	mammary tumour, pleural effusion	50	46
	K.J.	female	49	mammary tumour	70	46.5
Orally	L.G.	female	34	soft tissue sarcoma	57	51
	B.S.	male	52	colorectal tumour	75	51
	B.R.	female	66	melanoblastoma	45	52

Table 2. Pharmacokinetic parameters for the total radioactivity after intravenous and oral administration of [^{3}H]*DAG*

Parameter	Patient				
	Intravenous			Oral	
	A.J.	K.J.	P.T.	L.G.	B.S.
A, % of dose/10^3ml	4.98	3.85	2.76	1.70	2.60
α, hr^{-1}	1.760	1.524	0.253	0.250	1.400
B, % of dose/10^3ml	2.84	3.50	0	0.32	0.99
β, hr^{-1}	0.200	0.246	—	0.0084	0.0616
C, % of dose/10^3ml	0.311	0.418	0.306	−2.020	−3.594
γ, hr^{-1}	0.0091	0.0076	0.0148	—	—
k, hr^{-1}	—	—	—	3.077	9.200
c_0, $A + B + C$, % of dose/10^3ml	8.131	7.768	3.066	0	0
AUC_{tot}, % of dose/hr/10^3ml	47.928	71.816	31.585	44.239	17.603
V_p, apparent volume of distribution, 10^3ml	12.30	12.87	32.61	21.94	30.50
FD, absorbed fraction, % of dose	—	—	—	41.27	97.31
a, hypothetical maximum plasma level, % of dose/10^3 ml	8.131	7.768	3.066	1.881	3.192
K_{eltot}, rate constant of elimination from plasma, hr^{-1}	0.1696	0.1082	0.0971	0.0725	0.1812
U^{∞}, urinary recovery of ^{3}H-compounds	93.05	80.58	47.00	37.19	47.72

The plasma level curves were fitted by equation $c_{tot} = A{\cdot}e^{-\alpha t} + B{\cdot}e^{-\beta t} + C{\cdot}e^{-\gamma t}$ after intravenous, and $c_{tot} = A{\cdot}e^{-\alpha t} + B{\cdot}e^{-\beta t} + C{\cdot}e^{-kt}$ after oral dose of [^{3}H]DAG. No adequate fitting was found for patient B.R.

Table 3. Pharmacokinetic parameters for the unchanged DAG after intravenous and oral administration of [^{3}H]*DAG*

Parmeter	Patient				
	Intravenous			Oral	
	A.J.	K.J.	P.T.	L.G.	B.S.
A, % of dose/10^3ml	8.027	7.418	2.881	2.500	1.050
α, hr^{-1}	1.927	6.300	4.225	2.420	3.436
B, % of dose/10^3ml	0.104	0.350	0.185	0.048	0.084
β, hr^{-1}	0.167	0.450	0.180	0.0153	0.500
C, % of dose/10^3ml	0	0	0	−1.548	−1.134
k, absorption rate constant, hr^{-1}	—	—	—	2.80	5.10
AUC_D, % of dose/hr/10^3ml	4.786	1.955	1.710	3.254	0.251
K_{elD}, elimination rate constant, hr^{-1}	1.699	3.973	1.793	0.578	12.699
K_1, from plasma to tissues,* hr^{-1}	0.206	2.063	2.187	1.793	−8.898
K_{-1}, from tissues to plasma,* hr^{-1}	0.190	0.714	0.424	0.064	0.135
U_D^{∞}, urinary recovery of DAG, % of dose	2.21	4.20	0	0	0
t, mean residence time, hr	1.23	0.98	3.43	—	—

The concentration curve was fitted by the equation $c_D = A{\cdot}e^{-\alpha t} + B{\cdot}e^{-\beta t} + C{\cdot}e^{-kt}$. No significant amount of unchanged DAG was found in the plasma of B.R.
*Two-compartment model transfer rate constants.

'unabsorbed' part of the dose could not be fully recovered in the faeces. A powerful first-pass effect might be acting there, and possibly some part of the DAG had been bound to the wall of the gastro-intestinal tract, if the absorption was slow.

Disappearance of the radioactive materials in plasma went on very slowly after the first day, due mainly to the slow elimination of the protein-bound ^{3}H-compounds (Table 4), which amounted to 20–90% of the plasma radioactivity by 72 hr, and represented 25–72% of AUC_{tot}.

The drug easily entered the CSF. Metabolism and removal of DAG was slower in the CSF than in the plasma (Table 5).

Tumour biopsy specimens indicated that DAG rapidly entered the tissues and was retained there,

while the plasma level declined. The very low DAG uptake into the fatty connective tissue of patient B.R. (Table 6) may be explained by the hydrophilic character of the drug.

Pharmacokinetic calculations showed (Table 3) that DAG entered tissue compartment with an apparent half-life of 27 min (mean K_1 for patients A.J., K.J., P.T. and L.G was 1.56 ± 0.79/hr). In patient B.S. the DAG concentration in plasma could not be adequately fitted by a three-exponential equation in the first 1-hr period, and therefore no acceptable values for K_1 could be derived. After intravenous administration, the radioactive materials in the tissue compartment reached a maximum of 36 ± 8% of the dose at 8 hr, as calculated by subtracting the plasma ^{3}H-content from the quantity not yet excreted from the body. In two orally treated patients (L.G., B.S). the maximum radioactivity amounted to 8–24% of the dose in the tissue compartment (Table 7).

Distribution of the firmly bound radioactivity between plasma and tissue compartments was calculated according to a two-compartment open model [7] and seemed to reach peak values of 9.4 ± 3.8% of the dose at 8 hr after intravenous [^{3}H]DAG dosing, still amounting to 5.7 ± 3.4% of the dose at 72 hr (Table 7).

The rapid clearance of DAG (Table 8) was mainly due to metabolism. Only a small percentage of the unchanged DAG was excreted in the urine. After intravenous treatment, 78–85% of the ^{3}H-dose was recovered in the urine of patients K.J. and A.J. within 72 hr (Fig. 3). The presence of pleural effusion in patient P.T. resulted in a slower

Table 4. Pharmacokinetic parameters for the plasma protein-bound radioactivity after intravenous and oral dose of [^{3}H]*DAG*

	Patient				
	Intravenous			Oral	
	A.J.	K.J.	P.T.	L.G.	B.S.
A, % of dose/10^{3}ml	0.960	1.338	0.302	0.138	9.800
α, hr^{-1}	0.900	0.496	0.251	0.131	1.720
B, % of dose/10^{3}ml	0.114	0.255	0.108	0.054	0.103
β, hr^{-1}	0.0098	0.0052	0.0148	0.0034	0.0497
C, % of dose/10^{3}ml	−1.074	−1.593	−0.410	−0.192	−9.903
k_B, formation rate constant, hr^{-1}	1.699	7.933	3.627	3.095	2.037
AUC_B, % of dose/hr/10^{3}ml	12.067	51.912	8.387	16.785	2.908
F_BD, firmly bound fraction, % of dose	6.94	19.42	53.00	4.08	49.60
a_B, hypothetical maximum plasma level, % of dose/10^{3}ml	0.564	1.51	0.39	0.19	1.63
K_{elB}, rate constant of elimination hr^{-1}	0.0468	0.0291	0.0463	0.0111	0.5590
K_1, from plasma to tissues,* hr^{-1}	0.6746	0.3844	0.1393	0.0384	1.0578
K_{-1}, from tissues to plasma,* hr^{-1}	0.1884	0.0881	0.0802	0.0403	0.1530

The concentration curve was fitted by the equation $c_B = A \cdot e^{-\alpha t} + B \cdot e^{\beta t} + C \cdot e^{-k_B t}$.
No adequate fitting was found for patient B.R.
*Two-compartment model transfer rate constants.

Table 5. Total ^{3}H*-compounds and unchanged* [^{3}H]*DAG concentrations in the CSF and in the plasma*

	^{3}H-level		[^{3}H]DAG level	
After intravenous dosing	1 hr	4 hr	1 hr	4 hr
CSF*	0.18 ± 0.05	0.22 ± 0.07	0.02 ± 0.004	0.04 ± 0.01
Plasma*	1.42 ± 0.29	0.69 ± 0.12	0.13 ± 0.025	0.02 ± 0.005
After oral dosing	27 hr		27 hr	
CSF†	0.126		0.013	
Plasma†	0.130		0	

Values are expressed in μg of DAG per ml. *Mean ± S.D. of 3 patients. †Data of patient L.G.

Table 6. *Distribution of radioactivity in tumour and subcutaneous connective tissue samples and in plasma at* 24 hr *after oral dose of* [^{3}H]*DAG*

Sample	^{3}H-level*	Tissue/ plasma
Patient B.S.		
Reactive lymph node	0.397	4.0
Florid tumour	0.158	1.6
Cyst in necrotic part	0.491	4.9
Subcutaneous connective tissue	0.161	1.6
Plasma	0.100	—
Patient B.R.		
Tumour	0.286	1.3
Subcutaneous fatty connective tissue	0.037	0.2
Plasma	0.229	—

*Values are expressed in μg of DAG per ml plasma or g wet tissue weight.

excretion and lower urinary recovery of ^{3}H-compounds (40%). The pleural fluid contained only 1% of the radioactive dose. After oral administration, an average of 42 ± 4% of the radioactive dose was recovered in the urine, and 1.5–15% was found in the faeces.

The metabolite pattern of DAG in plasma and in urine is shown in Table 9. Chromatographic investigations indicated the presence of DAG, five unidentified metabolites (M-1 – M-5), galactitol, minor quantities of chloroepoxygalactitol (ClEpG) and dichlorogalactitol (DClG). In contrast to BrEpG and DBD, the above chloro-derivatives exhibited no significant cytostatic effects [8, 9].

The main metabolites were M-4, M-5 and galactitol. These very polar substances represented altogether about 67% of the urinary ^{3}H-compounds recovered within 48 hr after intravenous dose. An almost similar pattern was observed in deproteinized plasma. The metabolites M-2 – M-5 are weak (probably mono-) alkylating agents. Oral administration did not alter significantly the metabolite pattern except the absence of DAG in the urine.

Pharmacokinetic parameters for the elimination of DAG were calculated according to the catenary model represented on Fig. 4. Rate constants are given in Table 10. The model implies a very slow or no urinary excretion of the unchanged DAG, and two types of metabolic processes: (i) degradation of DAG into diffusible free metabolites (M) which can be excreted in the urine; and (ii) covalent binding by alkylation of biopolymers (B). The model supposes that the covalently bound hexitol moieties would leave the cells as macromolecular fragments which could be co-precipitated with the

Table 7. *Distribution of the total and the covalently bound radioactivity in the tissue compartment,* T *and* T_B, *respectively: peak values and the quantities retained at* 72 hr *following* [^{3}H]*DAG administration*

Patient	Maximum ^{3}H-content* T (hr)	T_B (hr)	^{3}H-content at 72 hr T	T_B
Intravenously				
A.J.	45.6 (2)	5.1 (8)	9.55	2.61
K.J.	35.4 (4)	14.3 (8)	12.84	10.49
P.T.	26.8 (4)	8.8 (8)	7.42	4.06
Mean	36.2	9.4	9.90	5.70
Orally				
L.G.	8.27 (6)	2.3 (24)	0	2.10
B.S.	24.1 (4)	26.2 (4)	1.70	0.90

^{3}H-contents are expressed in % of the ^{3}H-dose. *Time indicated in parentheses.

Table 8. *Clearance of* [^{3}H]*DAG and* [^{3}H]*DBD*

	[^{3}H]DAG (intravenous)			[^{3}H]DAG (oral)		[^{3}H]DBD (oral)
	A.J.	K.J.	P.T.	L.G.	B.S.	Mean ± S.D. in 5 patients
Cl_D, total clearance	348	853	975	211	6457	171 ± 84
Cl_{RD}, renal clearance of the effective drug*	7.7	35.8	0	0	0	32.5 ± 7.9
Cl_{RM}, renal clearance of free metabolites	49.1	68.8	34.5	25.6	55.0	24.8 ± 6.1

Values are expressed in ml/min. *'Effective drug' means unchanged DAG or the sum of DBD + BrEpG + DAG, respectively.

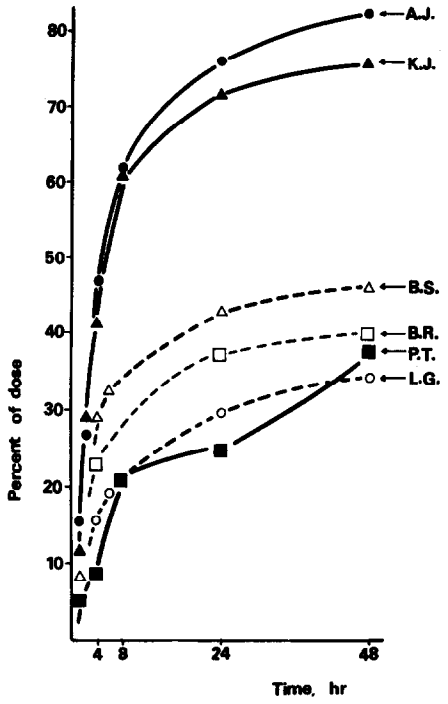

Fig. 2. Plasma levels of unchanged DAG (full marks) and total ^{3}H-compounds (empty marks) after a single oral dose of [^{3}H]DAG. No significant amount of unchanged DAG was observed in the plasma of patient B.R.

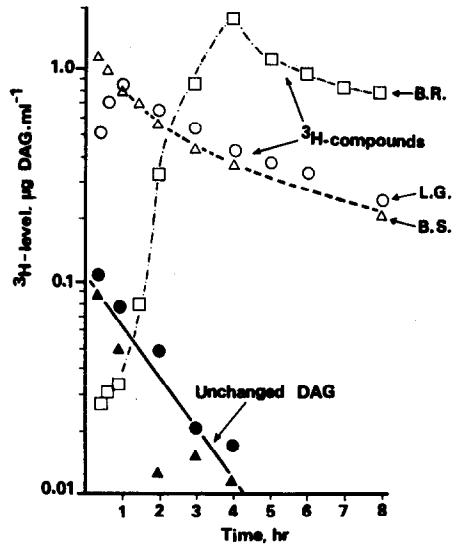

Fig. 3. Cumulative urinary recovery of ^{3}H-compounds after intravenous (solid lines) and oral (dotted lines) administration of [^{3}H]DAG.

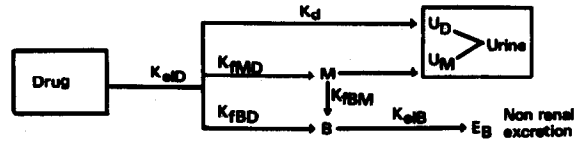

Fig. 4. Catenary model for the elimination of DAG. U_D, urinary excretion of unchanged DAG; U_M, urinary excretion of free metabolites; M, free metabolites; B, covalently bound hexitol moieties. For explanation of the rate constants see Table 10.

plasma proteins. This irreversibly bound fraction would then be removed from the plasma by some kind of non-renal excretion. This may occur with faeces, sweat, expired air or, later on, with urine at such a slow rate that the radioactivity would not significantly surpass the background value.

Some of the free metabolites are weak alkylating agents. Theoretically they can contribute to the alkylation of biopolymers. The reaction rate of this process seemed to be negligible in comparison to the rapid urinary excretion of the metabolites in most cases. However, if the excretion was slowed down, as occurred in patient P.T., the covalent binding of metabolites could not be neglected. In this patient nearly 80% of the radioactive dose was found in the plasma in the form of free metabolites by 1 hr after dosing, but only 40% of the dose was

Table 9. Metabolite pattern of DAG: distribution of radioactivity in the deproteinized plasma and in urine within 48 hr after intravenous dosing (mean ± S.D. in 3 patients)

Metabolite	R_f*	NBP†	Urine‡	Plasma§
DAG	0.47	+	3.1 ± 1.8	17.2 ± 7.5
M-5	0.04	+	42.0 ± 9.1‖	37.5 ± 5.3‖
Galactitol	0.08	−		
M-4	0.14	+	24.8 ± 4.4	14.0 ± 3.2
M-3	0.22	+	16.5 ± 4.9	16.8 ± 5.2
M-2	0.26	+	4.0 ± 2.8	4.0 ± 1.7
M-1	0.38	−	7.5 ± 2.6	6.2 ± 1.2
ClEpG	0.67	+	2.1 ± 1.8‖	5.0 ± 1.0‖
DClG	0.78	−		

*R_f in *n*-butanol–water (86:14), ascending chromatography on Whatman No. 1 paper.
†Alkylating reaction towards 4-(*p*-nitrobenzyl)pyridine (NBP).
‡Values are expressed in % of the urinary recovery of radioactive material.
§Values are expressed in % of $AUC_{tot} - AUC_B$.
‖Radioactive spots were partly overlapping.

Table 10. *Apparent rate constants for the elimination of* [^{3}H]*DAG in a catenary model (Fig.* 4*) implying urinary excretion of the unchanged drug and the formation of two types of metabolites: free, M, and covalently bound, B*

Rate constant (hr^{-1})	Patient				
	Intravenous			Oral	
	A.J.	K.J.	P.T.*	L.G.	B.S.
$K_{elD} = K_d + K_{fMD} + K_{fBD}$, elimination by urinary excretion and metabolism	1.6990	3.9734	1.7933	0.5780	12.7000
K_d, urinary excretion of DAG	0.0376	0.0669	0	0	0
K_{fMD}, formation of free metabolites	1.5431	3.0357	1.4053	0.5210	6.2280
K_{fBD}, alkylation by DAG	0.1180	0.7718	0.3880	0.0572	6.4710
K_{fBM}, alkylation by metabolites	0	0	0.0441	0	0
K_m, urinary excretion of free metabolites	0.2371	0.3288	0.0686	0.0719	0.1105
K_{elB}, elimination of covalently bound hexitol moieties	0.0468	0.0291	0.0463	0.0111	0.5589

*Patient with pleural effusion.

excreted in the urine within 72 hr. Extremely low urinary excretion in a patient with pleural effusion has been reported by Belej *et al.* [10] also after [^{14}C]DBD treatment. The equation fitted to the plasma levels of free metabolites in patient P.T. showed that a significant amount of free metabolites became firmly bound, in contrast to other cases (Table 11). In the other intravenously and orally treated patients the amount of free metabolites in the plasma ($V_p \cdot a_M$) was practically equal to the quantity recovered in the urine (U_M^∞). The rate constants of the catenary model are presented in Table 10.

DISCUSSION

Comparison of the pharmacokinetic parameters and metabolites of DAG with DBD may give some clues to differences observed between the therapeutic effects of these closely related drugs. The DBD data were taken from our earlier studies [1, 2] on five cancer patients treated with a single oral dose of 15 mg/kg [^{3}H]DBD.

Oral administration of [^{3}H]DBD resulted in a nearly complete absorption of the dose, with a half-life of 25 min. Absorption of [^{3}H]DAG showed great individual differences in the absorbed quantity (47–97% of the dose) and the absorption rate (half-life 5 min to more than 1 hr). Our results suggest that oral administration of DAG is not so reliable, as it proved to be in the case of DBD. Mean residence time of the drug molecules in the body (Table 3) was six times longer for DBD (11.4 hr; range: 6–46 hr) than for intravenously administered DAG (1.9 ± 1.1 hr).

Both DAG and DBD rapidly entered the CSF and were eliminated from there more slowly than from the plasma. If the respective drug concentrations in the CSF were expressed as a percentage of

Table 11. *Pharmacokinetic parameters for the free metabolites after intravenous dosing of* [^{3}H]*DAG in the plasma of patient P.T. with pleural effusion*

Concentration curve: $c_M = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} - C \cdot e^{-kt}$ where:	
A, % of dose/10^3ml	2.40
α, hr^{-1}	0.286
B, % of dose/10^3 ml	0.167
β, hr^{-1}	0.0119
C, % of dose/10^3 ml	2.567
k, formation rate constant, hr^{-1}	4.2
AUC_M, area under the plasma level curve, hr/% of dose/10^3 ml	21.814
a_M, hypothetical maximum plasma level, % of dose/10^3 ml	2.403
$F_M \cdot D = a_M \cdot V_P$, fraction formed, % of dose	78.38
U_M^∞, fraction excreted in urine, % of dose	47.0
$F_M \cdot D - U_M^\infty$, bound by alkylation, % of dose	21.62

Table 12. *Mean DAG/DBD ratios of the rate constants in the catenary model*

Rate constant	Ratio
K_{elD}, elimination by urinary excretion and metabolism	7.8
K_d, urinary excretion of the effective drug	0.8*
K_{fMD}, formation of free metabolites	9.7
K_{fBD}, covalent binding of the drug by alkylation	8.6
K_m, urinary excretion of the free metabolites	6.2*
K_{elB}, elimination of covalently bound hexitol moieties	0.8

Mean values calculated from 5 patients treated with [^{3}H]DBD [2] and 3 patients treated intravenously with [^{3}H]DAG (Table 10]; *except K_d and K_m, where the abnormal values of patient P.T. with pleural effusion were omitted.

the dose, about 2-fold higher DBD levels were observed than DAG.

Pharmacokinetic calculations showed that the transfer of DAG from the plasma to the tissue compartment (intracellular space) went on about five times faster (half-life 27 min) than that of DBD (half-life 126 min). Radioactive materials in the tissues attained their peak value by 8 hr, and this was slightly higher for DAG (36 ± 8% of the dose) than for DBD (20 ± 8% of the dose).

Alkylating agents exert their cytostatic effects by covalent binding to DNA and other biopolymers in the cells. Calculation of the firmly bound fraction in the tissue compartment gives information about the upper limit of the acting fraction. Calculations based on the two-compartment model indicated (Table 7) that the covalently bound drug fraction in the tissue compartment was only slightly higher after DAG treatment (maximum at 8 hr, T_B = 9.4 ± 3.8% of the dose) than after DBD (maximum at 24 hr, T_B = 6 ± 4% of the dose).

Total clearance of DAG was much more fast than that of DBD (Table 8), and was due mainly to metabolic processes. The contribution of the most polar products to the metabolite pattern was higher for DAG (Table 9) than for DBD.

The DAG:DBD ratios of the rate constants in the catenary model are summarized in Table 12. The higher reactivity of DAG manifested itself *in vivo* in both the alkylating action and metabolic degradation of the drug. The alkylating action of DBD *in vivo* is a very slow process, because it implies the conversion of the parent drug into the more powerfully alkylating BrEpG and DAG [1, 2]. *In vivo* alkylation itself proceeds about eight times faster than by DBD.

Removal of the covalently bound hexitol moieties from the body went on at the same slow rate for both DAG and DBD. Their long half-lives, of 14 and 17 hr respectively, may reflect the time needed for repair or removal of the damaged biopolymers and may be an indicator of the cumulative potency of the drugs.

In human plasma *in vitro* at 37°C the half-life of DAG decomposition was 17.7 hr [11]. The rapid *in vivo* degradation of DAG to free metabolites (half-life 22 min) was therefore the result of intracellular processes. For DBD this process was 9.7 times slower (K_{fMD} in Table 12). Consequently, an intracellular depot of unchanged DBD may be formed after its administration. Evidence to the intracellular persistence of unchanged DBD has been reported by Gáti *et al.* [12]. Recent studies on the action of rat liver 9000 ***g*** fraction on DBD and its solvolytic products showed also a rapid disappearance of DAG and BrEpG from the system, and a slow transformation of DBD itself into pharmacologically inactive mono-alkylating compounds [13].

DAG is the most powerful cytostatic agent among the solvolytic products of DBD [14], and it may be the final mediator of the cytostatic action of DBD. If DAG itself is administered to a patient, the organism will be exposed to high concentrations of the powerful alkylating agent which will soon be consumed by alkylation and metabolic degradation. On the other hand, administration of DBD results in the presence of three bifunctional alkylating agents: DBD, BrEpG and DAG. They can act on the cells simultaneously and differently, depending on their reactivities and transport characteristics. Pharmacological interpretation of our results suggests that the less reactive parent drug, DBD, may form intracellular depots and exert cytostatic activity through the epoxides released *in situ* by slow solvolytic activation in the immediate vicinity of the target. Thus DBD reaching the cells in unchanged form may have a more important role in the therapeutical effects of the drug than had been assumed earlier.

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