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Metabolism and Pharmacokinetics of Dibromodulcitol(DBD, NSC-104800) in Man—II. Pharmacokinetics of DBD

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Abstract—*Dibromodulcitol (DBD), labelled with [^{3}H] at position C-1, was administered orally to 6 patients in a single dose of 15 mg/kg. Kinetic parameters were calculated for the effective drug (DBD + BrEpG + DAG), protein-bound hexitol moieties and free metabolites. Approximate values were estimated for the oral bioavailability of DBD. Disposal of the drug by metabolism and excretion was described by a simplified catenary model. The results indicated that 8–20% of the drug became firmly bound to macromolecules, probably by alkylation. The slow rate of alkylation in vivo (half-life 14 hr) may imply conversion of DBD into epoxides and their alkylating interaction with the target nucleofiles. The long retention of the firmly bound hexitol moieties in the body may be an indicator of the cumulative potency of DBD and must be taken into consideration by developing dosage schedules.*

INTRODUCTION

THE ANTICANCER drug, DBD, is a compound of weak alkylating capacity [1]. At slightly alkaline pH it is converted into epoxides which are more reactive alkylating agents than the parent compound. Thus the cytostatic activity of DBD seems to be mediated by the diepoxide 1,2:5,6-dianhydrogalactitol (DAG, NSC-132313) [2].

Absorption and metabolism of DBD has been studied by Belej *et al.* [3] in patients with advanced cancer after oral administration of [^{14}C]-DBD in a single dose of 15 mg/kg body weight. Absorption of the drug from the gastro-intestinal tract was rapid and almost complete. Plasma half-life of [^{14}C]-compounds was 8 hr. The plasma level approached background activity after 48 hr. The drug entered spinal, pleural and ascitic fluids. DBD was taken up into both normal and malignant tissues. In autopsy specimens, retention of radioactivity equivalent to 1–4 μg/g wet weight of tissue was detected 6 days after administration. Renal excretion was the only apparent route of elimination of [^{14}C]-DBD and its metabolites. Neither faeces nor expired air contained significant amounts of radioactive material.

The purpose of our study was to calculate kinetic parameters for the drug and its metabolic products, including the drug fraction which became firmly bound in the tissue compartment after administration of a single dose of DBD to patients. Clinical relevance of this fraction is due to the fact that the cytostatic action of DBD may be exerted mainly by alkylation of DNA and other cellular macromolecules [4, 5]. Although a great part of the drug may be wasted by binding to sites of no vital importance, calculation of the intracellularly bound quantity gives information about the upper limit for the acting drug fraction. The rate of elimination of the firmly bound hexitol moiety may reflect the repair or renewal of damaged macromolecules and might serve as an indicator of the cumulative potency of DBD.

For this reason, drug kinetics was investigated in 6 patients with advanced malignant disease for 4 days following drug administration. [^{3}H]-DBD was given orally in a

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single dose of 15 mg/kg body weight. The methods and the results concerning the metabolism of DBD have already been described in the first part of this report in detail [6]. The results indicated that the biotransformation of DBD implies two processes: (i) activation by alkaline solvolysis, yielding highly potent alkylating agents, such as the monobromoepoxide (1,2-anhydro-6-bromo-6-deoxy-galactitol, BrEpG) and a diepoxide DAG; (ii) formation of metabolites by degradation and/or alkylation. Process (i) leads to the simultaneous presence of 3 bifunctional alkylating agents, DBD, BrEpG and DAG. Their sum is considered as the 'effective drug'. Process (ii) includes covalent binding of the drug to macromolecular substances and degradation to cytostatically inactive free metabolites in urine.

Pharmacokinetic data on DBD and its metabolic products are now presented in this part of the report.

MATERIALS AND METHODS

[^{3}H]-DBD labelled at the C-1 position was administered orally to 6 patients in a single dose of 15 mg/kg body weight. Blood samples were taken 1, 2, 4, 8, 24, 48, 72 and 96 hr after treatment. Total urine was collected in the same time intervals. Cerebrospinal fluid (CSF) and tumor biopsy specimens were taken from a few cases. Plasma samples were deproteinized with a 9-fold volume of ethanol prior to chromatography. Chromatographic assay of radioactive and alkylating metabolites has been described in the first part of this report [6].

The mathematical methods involved in the evaluation of the results are described in the appendix.

RESULTS

The curve for the concentration of total radioactivity in plasma (Fig. 1) shows that the orally administered [^{3}H]-DBD was rapidly absorbed from the gastro-intestinal tract. The average absorption rate constant was 1.7 ± 0.7/hr (half-life 25 min). The plasma level attained a peak value of $2.2 \pm 0.6\%$ of the dose per 10^3 ml. equivalent to about 22 μg DBD/ml, at approximately 2 hr after dosing. The average plasma half-life of [^{3}H]-compounds was 4.6 hr (3.6–6.7 hr) during the first day, but became longer than 48 hr (37–99 hr) between the second and fourth days after administration. Chromatographic analysis of deproteinized plasma samples showed that the effective drug content reached a peak value of 1.2% of the dose per 10^3 ml at 2 hr, then decreased with a half-life of 2 hr during the first day, followed by a slow decrease with a half-life of 11 hr in the terminal phase. The alcoholic precipitate of the plasma proteins contained

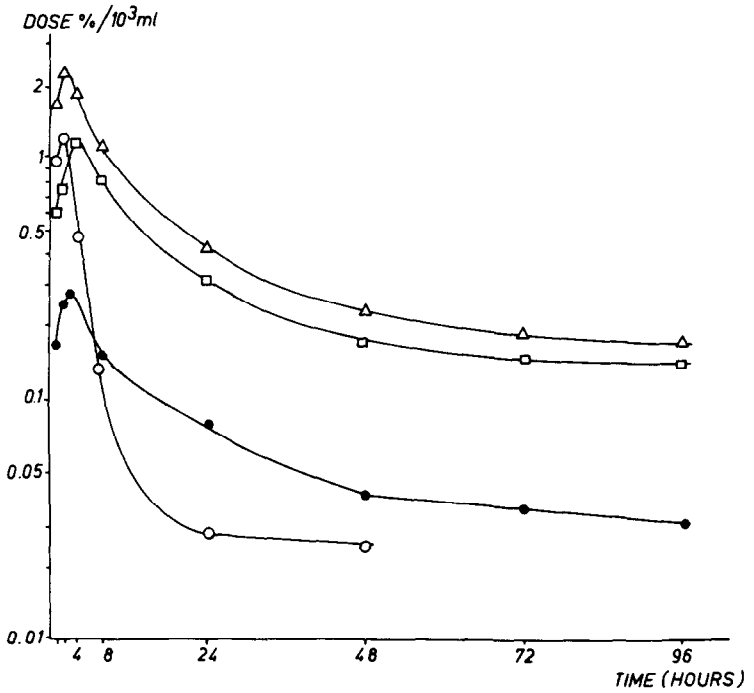

Fig. 1. Plasma levels of [^{3}H]-compounds after oral administration of [^{3}H]-DBD, 15 mg/kg. Each point represents the mean value of 5 patients. Patient G.P. was excluded because the lack of data for the effective drug content. △——△, Total [^{3}H]-level; □——□, free metabolites; ○——○, effective drug content; ●——●, protein-bound [^{3}H]-level.

some firmly bound radioactivity [6]. Its proportion gradually increased from 10 to 20% of the plasma radioactivity during the first 24 hr and remained nearly constant in the subsequent days. Protein binding may be the result of drug adsorption to albumin [7] and also of covalent binding via alkylation [6].

Distribution studies in whole blood (Table 1) indicated a rapid uptake and prolonged retention of the drug in the red blood cells (RBC). The RBC to plasma ratio of specific radioactivities was initially near 1 but rose to over 2 when the plasma level declined.

DBD easily penetrated the blood–brain barrier and entered the CSF. Metabolic degradation and removal of the drug was far slower from the CSF than from the plasma (Table 2).

Tumor biopsy specimens indicated that DBD rapidly entered the tissues and was retained there while the plasma level declined (Table 3).

The cumulative urinary recovery curves (Fig. 2) show that 17 ± 3% of the dose was excreted as effective drug during the observation period.

Table 1. Distribution of [^{3}H]-compounds in whole blood; time course of the red blood cells (RBC) per plasma ratio of the specific radioactivities

Time, hr	RBC/plasma, mean ± S.D.
1	0.96 ± 0.40
2	0.97 ± 0.14
4	1.09 ± 0.09
8	1.23 ± 0.17
24	1.67 ± 0.73
48	1.91 ± 0.78
72	2.55 ± 0.82
96	2.24 ± 0.96

Figures represent average values in 4 patients.

Table 3. Distribution of radioactivity in tumour and plasma following oral administration of [^{3}H]-DBD, 15 mg/kg

Time, hr	Patient M.J. Tumour*	Plasma	Patient G.P. Tumour†	Plasma
2	—	—	2.120	2.218
4	1.138	2.574	—	—
26	—	—	0.857	0.389

Values are expressed as % of dose per 10^3 ml plasma or g wet tissue weight.
*Carcinoma planocellulare keratoides.
†Angiosarcoma.

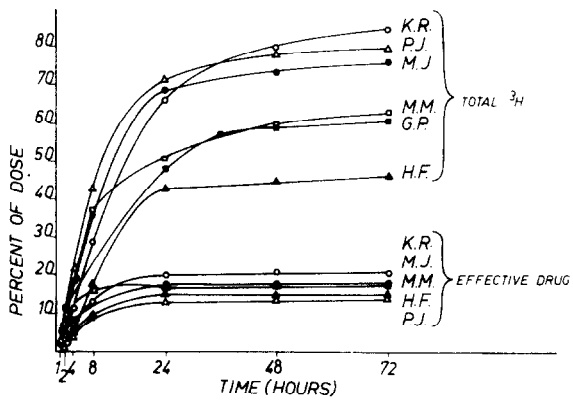

Fig. 2. Cumulative urinary excretion of effective drug and total amounts of [^{3}H]-compounds in patients after oral administration of [^{3}H]-DBD, 15 mg/kg. Data are expressed as % of the [^{3}H]-dose.

Large individual differences were found in the excretion of metabolites. The total urinary recovery of [^{3}H]-compounds ranged from 47 to 84% of the dose by 96 hr.

The renal clearance of DBD rose to peak values within the 4 to 8, and 8 to 24-hr periods, then declined exponentially (Fig. 3).

Pharmacokinetic parameters of DBD were calculated from the equations fitted to the concentration curves of effective drug (Table 4), total radioactive materials (Table 5) and

Table 2. Total [^{3}H]-compounds and effective drug concentrations in plasma and CSF

Patient	Total [^{3}H]-level 2 hr	6 hr	24 hr	30 hr	Effective drug level 2 hr	6 hr	24 hr	30 hr
CSF:								
P.J.	—	0.78	—	0.41	—	0.341	—	0.026
K.R.	—	0.74	—	0.53	—	0.324	—	0.031
M.M.	—	—	0.67	—	—	—	0.220	—
Plasma:								
P.J.	2.06	1.26	—	0.27	0.916	0.245	—	<0.01
K.R.	2.25	1.52	—	0.49	1.160	0.390	—	<0.01
M.M.	3.31	—	0.49	—	1.204	—	<0.01	—

Values are expressed in % of ^{3}H-DBD dose per 10^3 ml.

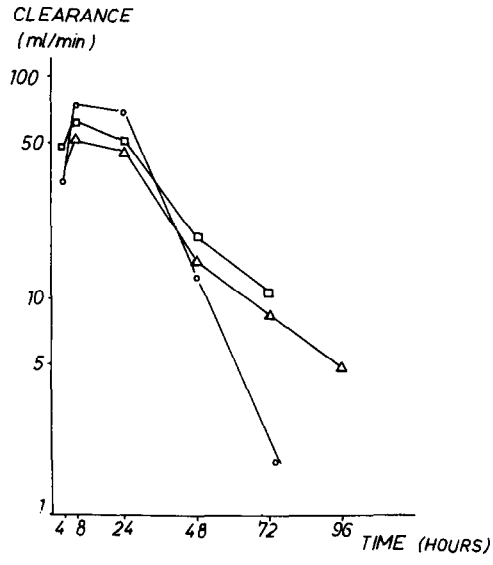

Fig. 3. Renal clearance of [^{3}H]-compounds derived from [^{3}H]-DBD. Mean values in 5 patients expressed as ml/min *in time intervals indicated at the time axis.* △——△, *Clearance of total [^{3}H]-materials;* ○——○, *clearance of the effective drug;* □——□, *clearance of the free metabolites.*

protein-bound [^{3}H]-compounds (Table 6) in plasma, and from the urinary recovery values of effective drug and metabolites extrapolated to infinity (Tables 4 and 5). The calculations (see Appendix) proved that the absorbed fraction of the DBD dose must be greater than would have been expected from the extrapolated value of the cumulative urinary excretion. The difference probably represents the drug fraction that became attached to macromolecules and could not be directly excreted in urine. It is impossible to calculate absolute oral bioavailability of the drug conventionally because it cannot be injected intravenously due to its poor solubility in the usual pharmaceutical solvent systems. Approximate values were estimated for the absorbed fraction of the dose (F.D. in Table 5) by supposing that the firmly bound fraction (F_BD in Table 6) may be correlated with the plasma level curve of protein-bound drug. The average amount of the bound drug for 6 patients would be 12 ± 5% of the dose.

Pharmacokinetic parameters for the disposal of the absorbed drug were calculated according to a catenary model (Fig. 4), which implies urinary excretion of effective drug and two types of metabolic processes: (i) degradation of DBD to free metabolites which are then excreted in the urine; (ii) binding to macromolecules by adsorption and alkylation.

Values from 5 patients showed, on average, that elimination of the effective drug from the plasma with a half-life of 2.2 hr would be the result of urinary excretion (half-life 11.6 hr), a rapid degradation into free metabolites (half-life 3.4 hr) and a slow binding to macromolecules, with a half-life of 13.7 hr (Table 7).

Fitting of the plasma level curve of protein-bound [^{3}H]-compounds to the two-compartment open model indicated that the firmly bound drug content of the tissues would rise slowly to a peak value of 6 ± 4% of the dose by 24 hr, and 3 ± 2% of the dose would be still retained there at 96 hr after dosing. Approximate values for the total radioactive material in the tissue compartment culminated earlier with 20 ± 8% of the dose at 8 hr, and still amounted to 4 ± 2% of the dose by 96 hr after administration of [^{3}H]-DBD (Fig. 5).

DISCUSSION

In the study on [^{14}C]-DBD-treated patients, complete absorption of the oral dose has been proved by the lack of radioactivity in the faeces.

Table 4. Pharmacokinetic parameters for the effective drug after oral administration of [^{3}H]-DBD in a single dose of 15 mg/kg

Parameters	Patients M.J.	P.J.	K.R.	M.M.	H.F.
A, % of dose/10^3 ml	4.788	3.139	3.630	1.569	9.600
α, hr^{-1}	0.526	0.587	0.448	1.138	1.200
B, % of dose/10^3 ml	0.158	0.248	0.240	1.368	0.165
β, hr^{-1}	0.048	0.105	0.020	0.109	0.038
C, % of dose/10^3 ml	4.946	3.387	9.580	2.937	9.765
k, hr^{-1}	3.13	1.28	1.43	1.99	1.68
AUC_D, area under the plasma level curve, % of dose · hr/10^3 ml	10.807	5.063	13.403	12.417	6.502
U_D^∞, urinary recovery of effective drug extrapolated to infinite time, % of dose	18.12	13.72	21.11	17.47	15.00

The plasma level curve was fitted by the equation $y_D = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} - C \cdot e^{-kt}$.

Table 5. Pharmacokinetic parameters for the total radioactivity after oral administration of [^{3}H]-DBD in a single dose of 15 mg/kg

Parameters	Patient M.J.	P.J.	K.R.	M.M.	H.F.	G.P.
A, % of dose/10^3 ml	3.656	3.062	2.512	2.554	1.349	2.687
α, hr^{-1}	0.159	0.227	0.185	0.115	0.107	0.103
B, % of dose/10^3 ml	0.552	0.317	0.667	0.382	0.277	0.722
β, hr^{-1}	0.0141	0.0068	0.0161	0.0068	0.0100	0.0235
C, % of dose/10^3 ml	3.906	3.379	4.425	2.936	1.832	10.171
k, hr^{-1}	3.130	1.280	1.593	1.991	0.847	1.300
AUC_{tot}, area under the plasma level curve, % of dose · hr/10^3 ml	60.921	57.160	52.174	76.987	38.164	48.971
V_p, apparent volume of distribution in plasma, 10^3 ml	24.06	35.84	34.32	27.03	41.26	24.53
F · D, absorbed fraction, % of dose	97.07	101.6	95.56	75.35	59.09	72.29
a, hypothetical maximum plasma level, % of dose/10^3 ml	4.0342	2.8346	2.7840	2.7880	1.4320	2.9474
K_{eltot}, rate constant of elimination from plasma, hr^{-1}	0.0662	0.0496	0.0534	0.0362	0.0375	0.0602
U^∞, urinary recovery of [^{3}H]-material, extrapolated to infinity, % of dose	77.43	82.98	88.15	65.50	51.45	63.00

The plasma level curve was fitted by equation $y_{tot} = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} - C \cdot e^{-kt}$.

Table 6. Pharmacokinetic parameters for the protein-bound radioactivity in plasma after oral administration of [^{3}H]-DBD in a single dose of 15 mg/kg

Parameters	Patients M.J.	P.J.	K.R.	M.M.	H.F.	G.P.
A, % of dose/10^3 ml	1.8463	0.3620	0.2327	0.3612	0.1360	0.3503
α, hr^{-1}	0.4224	0.2020	0.0307	0.1884	0.1630	0.1052
B, % of dose/10^3 ml	0.1344	0.0480	0.0000	0.0954	0.0740	0.0873
β, hr^{-1}	0.0144	0.0068	—	0.0133	0.0150	0.0186
C, % of dose/10^3 ml	1.9200	0.4100	0.2327	0.4556	0.2100	1.9262
k_B, formation rate constant, hr^{-1}	0.650	0.651	0.425	0.752	0.935	1.584
AUC_B, area under the plasma level curve, % of dose · hr/10^3 ml	10.663	8.370	7.033	8.483	5.992	6.808
F_BD, firmly bound fraction, % of dose	19.64	18.62	7.41	9.85	7.64	9.29
a_B, hypothetical maximum plasma level, % of dose/10^3 ml	0.8162	0.5193	0.2159	0.3644	0.1851	0.3789
Two-compartment model transfer rate constants						
K_{elB}, elimination, hr^{-1}	0.0765	0.0620	0.0307	0.0430	0.0309	0.0557
K_{-1B}, from tissues to plasma, hr^{-1}	0.0795	0.0222	—	0.0583	0.0791	0.0351
K_{1B}, from plasma to tissues, hr^{-1}	0.2808	0.1246	—	0.1004	0.0680	0.0330

The concentration curve was fitted by the equation $y_B = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} - C \cdot e^{-k_B t}$.

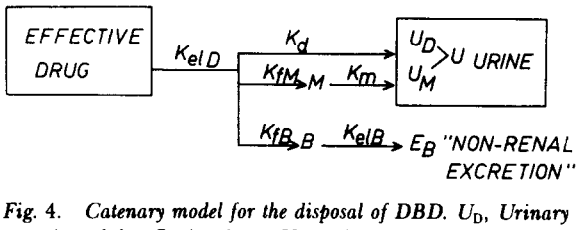

Fig. 4. Catenary model for the disposal of DBD. U_D, Urinary excretion of the effective drug; U_M, urinary excretion of the free metabolites. For explanation of other signs see Table 7.

Urinary excretion amounted to 58–79% of the dose within 48 hr [3]. Our calculations indicated almost complete absorption of [^{3}H]-DBD in the patients whose excretion was in the same range. On the other hand, incomplete absorption was calculated in a patient (H.F.) who vomited a part of the DBD dose soon after administration. Thus the results supported the view that the protein-bound drug fraction in the plasma cannot be excreted in the urine. Initially, when the plasma contained unchanged DBD in a high concentration the plasma protein precipitate still contained adsorbed DBD [5]. Later on, covalent binding of hexitol moieties may be predominant, due to the alkylating action of DAG [8] and, possibly,

Table 7. Apparent rate constants and half-lives for the disposal of DBD in a catenary system (Fig. 4), implying urinary excretion of the effective drug and the formation of two types of metabolite: free, M, and bound, B

Process	Rate constant, mean ± S.D. hr^{-1}	Mean half-life, hr
Elimination by urinary excretion and metabolism, $K_{elD} = K_d + K_{fM} + K_{fB}$	0.317 ± 0.135	2.2
K_d, urinary excretion of the effective drug	0.060 ± 0.011	11.6
K_{fM}, formation of free metabolites	0.207 ± 0.094	3.4
K_{fB}, formation of 'covalent linkage'	0.050 ± 0.033	13.7
K_m, urinary excretion of free metabolites	0.047 ± 0.013	14.8
K_{elB}, elimination of 'covalently bound' metabolites	0.049 ± 0.018	14.3

Figures represent mean values of 5 patients. See Appendix for the method of calculation.

of BrEpG. Detailed investigation of the protein binding of DBD is now in progress.

It is an approximation to consider the protein-bound [^{3}H]-compounds of the plasma as being representative of the drug fraction used up for alkylation and to apply it in calculating the rate constant of alkylation in the catenary model. The model presents only a simplified pattern for the rather complicated metabolism of DBD. In reality, the composition of both the effective drug content and the free metabolites change with time [6]. Each component has rate constants of its own. The apparent rate constants in the model are the results of individual rate constants averaged over infinite time. The clearance of the drug changes with time (Fig. 3), but the proportion of excretion and metabolism in the disposal is nearly constant. Therefore the ratios of the apparent rate constants (Table 7) may indicate the relative participation of covalent binding, degradation and urinary excretion in the disposal of DBD.

The slow apparent rate of alkylation with DBD *in vivo* (half-life 14 hr) implies at least two processes: conversion of DBD into BrEpG and DAG, and interaction of these epoxides with the target nucleophiles. Studies on the binding of DBD *in vivo* in rats to chromatin have revealed, however, a more intricate pattern [4, 5] involving an early association of high amounts of DBD to chromosomal proteins, which almost completely disappeared by 24 hr, and slow alkylation of DNA in increasing amounts till 72 hr after administration of DBD. These observations suggested that the effective drug is preserved in the cells far longer than in the plasma [9].

It is supposed by the catenary model that the bound metabolites would leave the cells as macromolecular fragments which could be co-precipitated with the plasma proteins. The model suggests that the bound metabolites are removed from the plasma by some kind of 'non-renal' excretion. This may occur with faeces, expired air, sweat or, later on, with urine at such a slow rate that its radioactivity would not significantly surpass the background.

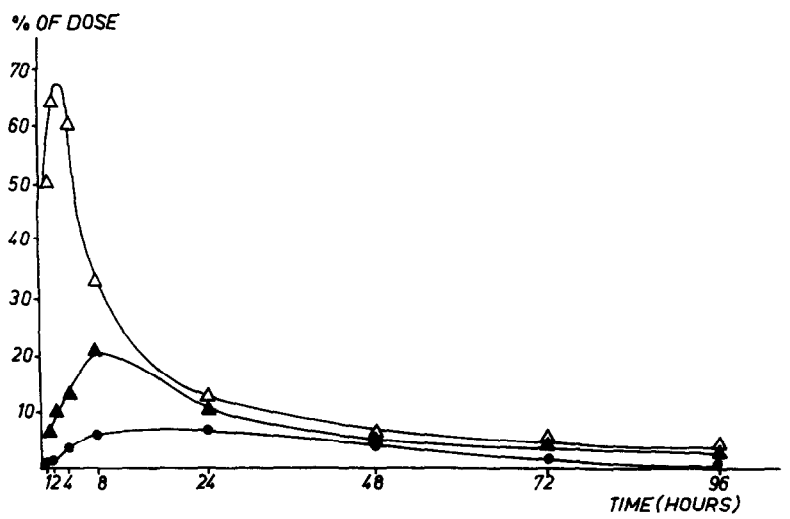

Fig. 5. Distribution of [^{3}H]-DBD and its metabolites in the plasma and tissue compartments. Mean values in 5 patients. △——△, Total [^{3}H]-compounds in plasma; ▲——▲, total [^{3}H]-compounds in tissues; ●——●, 'covalently bound' drug in tissues.

Studies on the tissue distribution of [^{14}C]-DBD in cancer patients [3] have shown retention of radioactive materials in the same range as the values indicated by our pharmacokinetic calculations.

In conclusion, investigation of the metabolism and pharmacokinetics of [^{3}H]-DBD in patients with cancer and interpretation of the data by a catenary model indicated that 8–20% of the drug would be consumed by alkylation of macromolecules. Alkylating action of DBD *in vivo* is a slow process (half-life 14 hr) because it is mediated by the epoxides BrEpG and DAG and implies the slow release of these potent alkylating agents. It is suggested that the slow elimination of the covalently bound hexitol moieties from the body should be taken into consideration by developing dosage schedules because it may reflect the rate of renewal of damaged macromolecules and is an indicator of the cumulative potency of the drug.

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APPENDIX

Calculation of parameter a *and* K_{el}

The curves for the concentration of the effective drug y_D, protein-bound drug y_B and total radioactivity y_{tot} in plasma showed a triphasic pattern within 96 hr after oral administration of [^{3}H]-DBD. Using the method of least squares, the curves were fitted by tri-exponential equations like equation (1) (Tables 4, 5 and 6):

$$y = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + C \cdot e^{-kt}. \quad (1)$$

The initial absorptive and distributive phases of the plasma level curve can be described by a two-exponential equation, equation (2), which gives a good approximation for values observed within the first 8 hr:

$$t = 0 \rightarrow 8\text{ hr}, \quad y = (A + B) \cdot e^{-Kt} - C \cdot e^{-kt}. \quad (2)$$

If $A + B = C$, equation (2) can be rewritten according to the one-compartment model [10], as $t = 0 \rightarrow 8$ hr, as

$$y = \frac{k}{k - K} \cdot \frac{F \cdot D}{V_p} (e^{-Kt} - e^{-kt}), \quad (3)$$

where F is the absorbed fraction of the dose D and V_p is the apparent distribution volume of the labelled drug and its metabolites in the plasma. In equation (3) the quotient

$$\frac{F \cdot D}{V_p} = a \quad (4)$$

represents the maximum hypothetical concentration the drug would have reached in the plasma after ending the absorption period if no drug would have been eliminated in the meantime. Its value may be calculated from equations (2) and (3). If $A + B = C$,

$$a = (A + B)\frac{k - K}{k}. \quad (5)$$

If the onset of drug absorption has been delayed, $A +$

$B \neq C$ and the plasma level curve intersects the axis t at a time $t = t_0$. In this case

$$a = \frac{(k - K) \cdot (A + B) \cdot e^{-Kt_0}}{k}. \quad (6)$$

The elimination rate constant K_{el} can be calculated generally as

$$K_{el} = \frac{F \cdot D}{V_p \cdot AUC} = \frac{a}{AUC}, \quad (7)$$

where AUC is the area under the plasma level curve from $t = 0$ to infinity. That is, AUC_D for the effective drug Table 4 and AUC_B for the protein-bound drug fraction Table 5. The elimination rate constant K_{elD} for the effective drug is then

$$K_{elD} = \frac{\mathbf{a}}{AUC_D}, \quad (8)$$

and for the total radioactivity derived from [^{3}H]-DBD the elimination rate constant K_{eltot} is

$$K_{eltot} = \frac{\mathbf{a}}{AUC_{tot}}. \quad (9)$$

The elimination rate constant K_{elB} for the protein-bound radioactivity in plasma is

$$K_{elB} = \frac{\mathbf{a}_B}{AUC_B}, \quad (10)$$

where

$$\mathbf{a}_B = \frac{F_B \cdot D}{V_p}. \quad (11)$$

Here $\mathbf{a}_B$ is the hypothetical maximum concentration that the bound fraction of the drug $F_B \cdot D$ would reach in the plasma if no elimination had occurred during its formation (Table 6). In the case of the protein-bound drug, the transfer rate constants of a two-compartment open model with first-order absorption were calculated using the relationships [10]

$$\alpha \cdot \beta = K_{elB} \cdot K_{-1B}$$

and

$$\alpha + \beta = K_{elB} + K_{1B} + K_{-1B},$$

where K_{1B} is the rate constant for the transfer of the bound radioactivity from plasma to the tissue compartment, and K_{-1B} from the tissue compartment to the plasma. Attempts for applying a two-compartment model to the effective drug concentration were unsuccessful.

Calculation of the volume of distribution for [^{3}H]-DBD in plasma and of the absorbed fraction of the oral dose

Urinary excretion has been reported as the only detectable route of elimination of DBD and its metabolites [3]. However, if the absorbed fraction of the oral [^{3}H]-DBD dose was considered to be equal to the radioactive material excreted in urine to infinite time U^∞, the V_p values derived from $V_p = U^\infty \mathbf{a}$ were so large that the apparent drug content of the plasma surpassed the quantity remaining in the whole body at many time points. Thus $F \cdot D$ was greater than U^∞.

It is a reasonable assumption that the drug fraction which became firmly bound to plasma proteins cannot directly be excreted in the urine. According to this assumption,

$$U^\infty = F \cdot D - F_B \cdot D. \quad (12)$$

Considering equations (4) and (11), it follows that

$$U^\infty = V_p \cdot (\mathbf{a} - \mathbf{a}_B). \quad (13)$$

Consequently,

$$V_p = \frac{U^\infty}{\mathbf{a} - \mathbf{a}_B}. \quad (14)$$

The absorbed fraction of the oral dose would be then

$$F \cdot D = V_p \cdot \mathbf{a}. \quad (15)$$

Catenary model for DBD

A simplified catenary model (Fig. 4) was applied to describe the disposal of [^{3}H]-DBD. Drug elimination implies 3 processes: renal excretion of the effective drug (DBD + BrEpG + DAG); degradation to free metabolites which can be excreted in the urine; and firm binding by adsorption and alkylation to proteins and other macromolecules which cannot be excreted in urine and are supposed to be removed via 'non-renal' excretion. There is no interchange between the free and the firmly bound metabolic products. Models with such an interchange did not fit the experimental data.

According to the catenary model, the rate constant of elimination of the effective drug K_{elD} has 3 components,

$$K_{elD} = K_d + K_{fM} + K_{fB}, \quad (16)$$

where K_d is the rate constant for urinary excretion of the effective drug, K_{fM} is the rate constant for the conversion into free metabolites, and K_{fB} is the rate constant for binding to macromolecules by adsorption and alkylation. From equations (8) and (16), it follows that

$$(K_d + K_{fM} + K_{fB}) \cdot AUC_D = \mathbf{a} = \frac{F \cdot D}{V_p}. \quad (17)$$

In the model, all the free metabolites were formed from the effective drug and would be recovered in urine by infinite time. Therefore

$$(K_d + K_{fM}) \cdot AUC_D \cdot V_p = U_D^\infty + U_M^\infty = U^\infty, \quad (18)$$

where U_D^∞ and U_M^∞ are urinary recovery for the effective drug and free metabolites, respectively, in infinite time. From equations (13) and (18), it follows that

$$(K_d + K_{fM}) = \frac{\mathbf{a} - \mathbf{a}_B}{AUC_D} \quad (19)$$

and

$$K_d = \frac{U_D^\infty}{V_p \cdot AUC_D}. \quad (20)$$

The rate constant for the interaction of DBD with macromolecules is

$$K_{fB} = \frac{\mathbf{a}_B}{AUC_D}. \quad (21)$$

The rate constant of the urinary excretion of free metabol-

ites, K_m, is calculated as

$$K_m = \frac{U_M^\infty}{V_p \cdot AUC_M}. \quad (22)$$

Mean values for the rate constants and half-lives in 5 patients are presented in Table 7.

Distribution of [^{3}H]-compounds derived from [^{3}H]-DBD in the plasma and in the tissues

The plasma level curve of protein-bound drug was successfully fitted to a two-compartment open model with first-order formation. Its distribution was calculated in the usual way [10].

During the terminal phase of elimination, the amount of effective drug and free metabolites still remaining in the body, R_F, can be estimated from the quantity still to be excreted in the urine between t to infinite time, adding to it the amount of firmly bound metabolites yet to be formed, ΔB. If at time t the cumulative urinary recovery of [^{3}H]-compounds is U^t, and the area under the plasma level curve of effective drug is AUC_D^t, then

$$\Delta B = K_{fB} \cdot (AUC_D - AUC_D^t) \cdot V_p \quad (23)$$

and R_F would be

$$R_F = U^\infty - U^t + \Delta B. \quad (24)$$

ΔB is an approximate value because we supposed that the difference between K_{fB} and the real rate constant can be neglected after 24 hr following drug administration. The quantity of the drug and free metabolites in the 'tissue' compartment T_F, would be at time $t > 24$ hr

$$T_F = R_F - V_p \cdot (y_{tot} - y_B). \quad (25)$$

The total amount of the radioactive material in the tissue compartment T_{tot} is the sum of T_B and T_F, where T_B represents the bound metabolites.

In the first 8 hr after dosing, approximate values for T_{tot} were calculated by subtracting the amount of radioactivity in plasma from the amount absorbed but still not excreted by urinary and non-renal excretion from the body. Mean values for drug distribution in 5 patients are presented in Fig. 5.