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Metabolism and Pharmacokinetics of Dibromodulcitol (DBD, NSC-104800) in Man. I. Metabolites of DBD

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Abstract—*Dibromodulcitol (DBD) labelled with* ^{3}H *or* ^{82}Br *was administered orally to patients with advanced cancer. Chromatographic studies on plasma and urine showed the presence of unchanged DBD and* 10 *metabolites. Steady excretion of* 1,2:5,6-*dianhydrogalactitol (DAG) the most active alkylating derivative, as a minor component of the urine for a few days, indicates its persistence at low levels in the systemic circulation. In the urine,* 4% *of the drug was excreted as unchanged DBD,* 9% *as bromoepoxy-dulcitol* (Br*EpD*) *and* 25–30% *in the form of unidentified bromine-containing metabolites. Administration of DBD results in the simultaneous presence of* 3 *main alkylating agents (DBD, Bromoepoxide and DAG) with different transport characteristics. The less reactive parent drug and the bromoepoxide may reach different sites and serve as depots from which DAG is gradually released in low concentrations.*

INTRODUCTION

DIBROMODULCITOL or dibromogalactitol (DBD, Mitolactol) is an anticancer drug applied in the treatment of solid tumours of different types [1–6].

DBD is a compound of weak alkylating capacity. At slightly alkaline pH it is converted into epoxides (Fig. 1) which are more powerful alkylating agents than the parent compound. The main product of the reaction of DBD with alkali is 1,2:5, 6-dianhydrogalactitol (DAG, NSC-132313). It has been isolated and found to exert remarkable antitumour activity [7, 8]. DAG was the most effective of 177 drugs tested against intracerebrally implanted mouse ependymoblastoma [9].

On a molecular basis, DAG is a 30 times more active tumour growth inhibitor than DBD. Conversion of a few per cent of DBD into DAG would account for all the effects of DBD on transplantable tumours. Nevertheless, the biological properties of DBD and DAG are not identical in every respect, so the effects of DBD cannot be attributed to the *in vivo* formation of DAG alone.

Initial animal studies demonstrated that after i.v. administration of ^{82}Br-labelled DBD 60–80% of the radiobromine excreted in the urine was present in C–Br bond, partly as unchanged DBD, partly as metabolites [10]. Subsequent chromatographic investigation of the solvolysis products of DBD suggested that the conversion of DBD into DAG implies the intermediate formation of a bromoepoxide, i.e., 1,2-anhydro-6-bromo-dulcitol (BrEpD on Fig. 1). *In vitro*, at physiological pH in buffer solution or in human plasma DBD yielded 3 epoxides: BrEpD (R_F 0.67), DAG (R_F 0.47), and an other bromine-free one (R_F 0.57). After prolonged incubation at 37°C an alkylating degradation product (R_F 0.26) and an epoxide-free monobromo-derivative (R_F 0.72) could also be detected in traces [1, 11]. Presence of the same 3 epoxides and DBD has

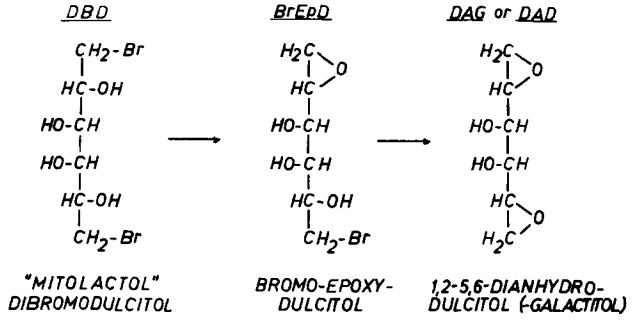

Fig. 1. Conversion of dibromodulcitol into bromoepoxide and diepoxide.

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been detected *in vivo* in plasma, ascites fluid and urine of DBD-treated rats [12].

Excretion of metabolites of ^{14}C-labelled DBD has been studied with the use of lyophilized urine specimens of cancer patients [13]. Radioscans of paper chromatograms indicated the presence of unchanged DBD and 7 transformation products: monobromo-dulcitol (R_F 0.71), 1,2-anhydro-dulcitol (AD, R_F 0.60), DAG (R_F 0.49), dulcitol (R_F 0.08), and 2 unidentified bromine-free alkylating agents (R_F 0.26 and 0.03). Except monobromo-dulcitol no other C–Br metabolite was detected.

According to the above quoted data, bromoepoxide has been found in large quantities in animal plasma and urine samples [12], but has not been positively identified in humans [13]. To elucidate its role as a mediator of therapeutic effects, the metabolite pattern was reinvestigated in patients, using ^{3}H-DBD of high specific activity, enabling the prolonged monitoring of the biotransformation products.

MATERIALS AND METHODS

Radiobromine-labelled and tritiated DBD were synthesized by L. Institóris (Chinoin Pharmaceutical and Chemical Works, Ltd, Budapest). The ^{82}Br–DBD was prepared from DAG [14], ^{3}H-DBD from galactitol labelled with tritium at position C–1, by treatment with hydrobromic acid [15]. The labelled compounds were triturated with the drug obtained from Chinoin.

The labelled drug was administered in single oral doses to cancer patients. ^{3}H–DBD was administered to 6 patients at a dose of 15 mg/22 μCi/kg body weight (Table 1). Plasma samples were taken 1, 2, 4, 8, 24, 48 and 72 hr after treatment. Total urine was collected in the same time intervals. Cerebrospinal fluid was withdrawn from a few patients at different times. Metabolites of ^{3}H–DBD were determined in the body fluids of 5 patients. Five patients received ^{82}Br–DBD (500 mg, 2 mCi) and urine was collected in the same way as above.

Analysis of body fluids was always performed in duplicate samples. Radioactive metabolites were separated by ascending chromatography on Whatman No. 1 paper Chromatograms were developed in *N*-butanol water (86:14 v/v). Terminally substituted epoxides were detected by spraying the chromatogram with a solution in acetone of the Epstein reagent, 4-(4-nitrobenzyl) pyridine (NBP) then heating above 90°C when a blue colour developed due to the alkali liberated on ring-opening of the epoxide. The halogenoderivatives lacking the epoxide substituent gave the blue colour only after subsequent spraying with a base (e.g., NaOH in ethanol). Epoxides were also detected by spraying with sodium thiosulphate and phenolphtalein (pink colour). The chromatograms were cut into 5 mm sections and their radioactivity was counted. Tritium was measured in an LKB-Wallace 81000 Scintillation Spectrometer using Aquasol universal LSC cocktail (New

Table 1. *Data of patients treated with a single oral dose of* ^{3}H–*DBD*, 15 mg/kg

Patient	Sex	Age	Weight	Diagnosis	Previous treatment
M. J.	male	72	58	Maxillary tumour	Tetramesyl-mannitol (Zitostop®)
P. J.	female	45	87	Mammary tumour	Cyclophosphamide + metothrexate
K. R.	female	65	70	Mammary tumour	Cyclophosphamide + metothrexate
M. M.	female	54	61	Mammary tumour	Irradiation testosterone
H. F.*	male	46	57	Pulmonary metastasis of unknown primary	Irradiation

*Unknown amount of drug was thrown up by the patient during the first day of study.

England Nuclear Corp). ^{82}Br was determined as described earlier [10]. Plasma proteins were precipitated with ethanol prior to chromatography. Some radioactive material, which had been bound irreversibly to the proteins, was removed by this treatment. The amount of the protein-bound material was estimated by measuring both the whole plasma and the precipitated protein after dissolving them in aquasol. The chromatographic results refer, therefore, to the deproteinized fraction of the plasma and the native urine and CSF.

When samples of plasma or CSF are applied on paper or thin layer plates some CO_2 is escaping and the increasing basicitiy enhances the conversion of DBD into BrEpD and DAG. Consequently in the case of DBD administration the apparent epoxide content of plasma and CSF will be increased relative to DBD. Therefore, the amount of DBD, BrEpD, and DAG should be considered together.

In order to ascertain that the epoxides found in urine are no mere artifacts, a sample of the radioactive DBD was dissolved in urine collected from the patient before administration of the drug. This solution (250 μg DBD per ml urine) served as a standard. It was co-chromatographed with the urine samples. This standard solution was kept at 4°C during the period of urine collection.

RESULTS

Chromatographic investigation of the body fluids showed that DBD was rapidly converted into epoxides and metabolized. Unchanged DBD, 3 epoxides (BrEpD, DAG and AD), 5 unidentified tritium-compounds (M-1, M-2, M-3, M-4 and M-5), and dulcitol were detected on the chromatograms of plasma, CSF and urine. A summary of the radioactive biotransformation products and their characterization by R_F values and colorimetric reactions for functional groups is given in Table 2.

Comparison of the urinary metabolites after administration of ^{3}H–DBD and ^{82}Br–DBD showed that M-1, M-2 and M-4 are bromine-containing alkylating compounds. BrEpD was identified by colour reactions of the epoxide ring, presence of radiobromine, and co-chromatography with a sample of the synthetic compound [16]. Traces of an epoxide-free monobromoderivative (R_F 0.72) were also detected but only after 48 hr.

Cumulative urinary excretion of radioactive metabolites after administration of ^{3}H–DBD and ^{82}Br–DBD is presented on Figs. 2 and 3. When comparing the excretion of radiobromine and tritium-labelled metabolites it has to be taken into account that ^{82}Br–DBD has two radiobromine atoms. The loss of one bromine atom yields monobromo-metabolites with one-half of the radioactivity of the parent compound. In ^{3}H–DBD there is one tritium atom in the hexitol molecule and, unless the carbon skeleton is degraded, each metabolite has the same molar radioactivity as the unchanged drug. Therefore, 1% of the administered ^{82}Br–DBD dose recovered

Table 2. Metabolites of DBD in patients treated with ^{3}H–DBD or ^{82}Br–DBD

		Detection				
				NBP		
Metabolic product	R_F	^{3}H	^{82}Br	alkali	alone	T
I. DBD	0.79	+	+	+	–	–
II. Monobromo dulcitol	0.72	+	+	+	–	–
III. Bromoepoxy dulcitol	0.67	+	+	+	+	+
IV. Anhydrodulcitol	0.57	+	–	+	+	+
V. DAG	0.47	+	–	+	+	+
VI. Unidentified M-1	0.38	+	+	+	–	–
VII. Unidentified M-2	0.26	+	+	+	–	–
VIII. Unidentified M-3	0.22	+	–	+	–	–
IX. Unidentified M-4	0.14	+	+	+	–	–
X. Dulcitol	0.08	+	–	–	–	–
XI. Unidentified M-5	0.04	+	?	+	–	–
XII. Bromide salt	0.05	–	+	–	–	–

R_F in *N*-butanol-water (86:14) ascending chromatography on Whatman 1 paper. NBP: 4-(*p*-nitrobenzil) pyridine in acetone, alone or followed by a treatment with alkali. T: sodium-thiosulfate and phenolphtalein.

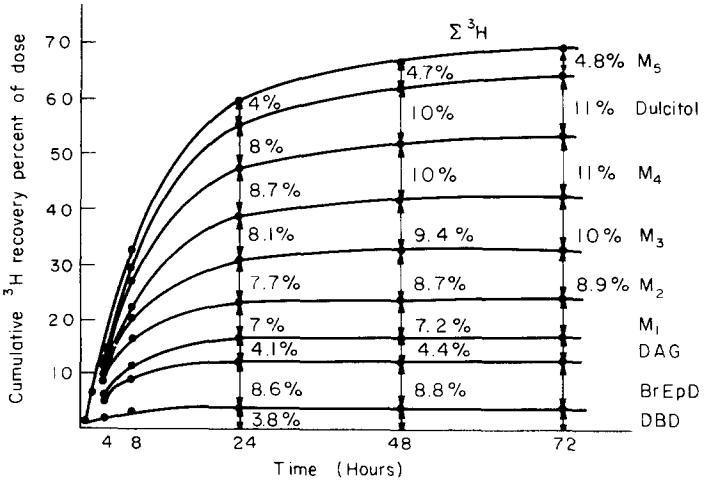

Fig. 2. Urinary recovery of radioactive metabolites after oral administration of ^{3}H–*DBD to patients. Mean values of 5 patients.*

as monobromo-derivative corresponds to 2% of the dose given as ^{3}H–DBD.

Interaction of epoxides with chloride ions yields chloro-substituted hexitol-derivatives. Chromatographic investigation of the metabolites of DAG showed that the R_F values of the analogous chloro- and bromo-derivatives are very close [17]. Radioactive peaks on the chromatograms of body fluids after ^{3}H–DBD treatment might be mixtures of the halogeno-

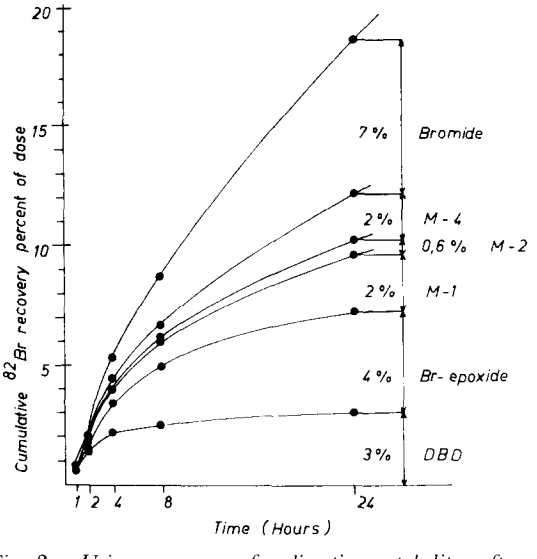

Fig. 3. Urinary recovery of radioactive metabolites after oral administration of ^{82}Br–*DBD to patients. Mean values of 5 patients.*

derivatives. Some information concerning their composition could be won by comparison with the urinary excretion of the metabolites after administration of ^{3}H–DBD and ^{82}Br–DBD. If the urinary recovery of a certain metabolite amounted to $T\%$ of the ^{3}H–DBD dose and to $B\%$ of the ^{82}Br–DBD dose, then a $T/B=1$ ratio corresponds to pure DBD and $T/B=2$ to monobromo-derivatives. Using the data presented in Figs. 2 and 3, average value of the T/B ratios found at 4, 8 and 24 hr has been calculated for each bromine-containing constituent of the urine (Table 3). The T/B ratios indicated no significant contamination of DBD and BrEpD with dichlorodulcitol (DCD) and chloroepoxydulcitol (ClEpD) within 24 hr. The major C–Br metabolites, M-1 and M-4 might contain considerable amounts of bromine-free material. M-2 (R_F 0.26) seems to be a mixture of a minor C–Br metabolite and a major bromine-free degradation product of DBD. The bromine-free component of M-2 may be identical with an alkylating compound detected among the reaction products of DBD with alkali [11]. A bromine-free compound of the same R_F value (0.26) has been reported to be the predominant urinary metabolite of ^{14}C–DBD in a patient who exhibited toxicity after repeated administration of the drug [13]. Urine of the patients was slightly acidic (pH

Table 3. Ratio of tritium and radiobromine excretion in urinary metabolites of DBD. Average of values found at 4, 8 and 24 hr after drug administration

Radioactive product	R_F	$T\%$ per $B\%$ Mean ± S.D.
DBD	0.79	1.2 ± 0.2
BrEpD	0.67	2.2 ± 0.5
M-1	0.38	3.8 ± 0.7
M-2	0.26	11.6 ± 3.0
M-4	0.14	5.7 ± 2.0

$T\%$ of dose is the amount of a metabolite excreted in the urine from ^{3}H–DBD; $B\%$ of dose is the excretion of

Table 4. Urinary excretion rate of ^{3}H–DBD and metabolites. Calculated from mean values of 5 patients

Metabolite	R_F	Per cent of dose excreted per hour			
		0–4 hr	4–8 hr	8–24 hr	24–48 hr
DBD*	0.79	0.63±0.37	0.39±0.22	0.03±0.02	0.002±0.002
BrEpD*	0.67	0.63±0.47	0.64±0.19	0.17±0.09	0.008±0.004
AD	0.57	0.13±0.13	0.07±0.07	0.03±0.03	0.004±0.004
DAG	0.47	0.19±0.17	0.26±0.15	0.06±0.04	0.008±0.003
M-1	0.38	0.52±0.30	0.69±0.28	0.13±0.08	0.008±0.008
M-2	0.26	0.17±0.12	0.68±0.33	0.27±0.26	0.04±0.04
M-3	0.22	0.32±0.27	0.32±0.31	0.35±0.35	0.05±0.02
M-4	0.14	0.40±0.24	0.90±0.36	0.25±0.26	0.06±0.04
Dulcitol	0.08	0.11±0.11	0.59±0.81	0.32±0.28	0.09±0.09
M-5	0.04	0.56±1.0	0.06±0.06	0.09±0.09	0.03±0.11
Total ^{3}H		3.66±1.7	4.60±0.8	1.70±0.57	0.30±0.18

*Values of DBD and BrEpD were corrected considering the rate of BrEpD formation from DBD added to urine.

5.5–6.9) with the exception of one patient, (pH 7.4, K.R.). Chromatography of standard solutions of labelled DBD in urine showed that 14–16% of the drug was converted *in vitro* into bromo-epoxide in the first 4 hr at room temperature. Further hydrolysis of DBD during storage of the urine at 4°C was very slow, at 24 and 48 hr the standards contained 67–73% unchanged DBD, 25–34% BrEpD, less than 1% DAG and traces of dulcitol and other degradation products.

During the first 48 hr after administration of ^{3}H–DBD, about 4% of dose was excreted as unchanged DBD and 9% as BrEpD (Fig. 2). Taking into account the *in vitro* degradation of DBD, 7–8% BrEpD might be excreted directly in the urine and 1–2% formed by subsequent solvolysis of DBD. Consequently, bromoepoxide was really present in the systemic circulation.

Time dependence of the excretion rate of ^{3}H–DBD and metabolites is presented in Table 4. Values for DBD and BrEpD are corrected, presuming that 20% of the originally excreted DBD has been degraded to bromoepoxide subsequently to urine collection.

Excretion of unchanged DBD was almost finished within 8 hr after administration of the drug. Bromoepoxide excretion was more prolonged. DAG was a minor but steady component of the urine during the whole period of observation. Traces of DAG and BrEpD were detected in the urine even after 48 hr. C–Br metabolite M-1 was excreted at rates similar to that of BrEpD. The relative amount of the other metabolites increased with time.

The standard deviation of the excretion rate values was very high because of large

Table 5. Urinary recovery of ^{3}H–DBD and metabolites in patients without previous chemotherapy and in patients who had been treated with alkylating agents; per cent of dose excreted in 48 hr

Compound \ Patients	After chemotherapy				After radiotherapy		
	M. J. %	P. J. %	K. R. %	Average %	M. M. %	H. F. %	Average %
DBD	4.4	0.6	4.4	3.3±1.8	4.2	5.0	4.6±0.4
BrEpD	10.8	3.8	11.8	8.8±3.6	9.0	8.4	8.7±0.3
AD	0.7	4.4	0.4	1.8±1.8	1.3	0.1	0.7±0.3
DAG	1.8	4.9	4.0	3.6±1.3	2.8	1.3	2.0±0.7
M-1	10.0	6.3	9.5	8.6±1.6	6.3	3.8	5.0±1.2
M-2	3.6	7.6	19.6	10.2±6.8	8.5	4.0	6.2±2.2
M-3	16.6	7.5	13.9	12.7±3.8	2.8	5.2	4.0±1.2
M-4	19.4	7.0	6.8	11.2±5.9	11.3	6.7	9.0±2.3
Dulcitol	4.6	21.2	8.0	11.3±7.2	4.8	9.0	6.9±2.1
M-5	0	14.1	0.7	4.9±6.5	7.9	0.1	4.4±3.4
^{3}H total	71.9	77.3	79.1	76.4±3.1	58.9	44.5	51.7±7.2

Table 6. Amount of ^{3}H–DBD and metabolites in plasma within the first 24 hr following drug administration, expressed as percentage of the area under the plasma level curve of radioactivity

Compound \ Patients	M. J. %	P. J. %	K. R. %	M. M. %	H. F. %	Average %
DBD and epoxides	21.0	23.9	24.9	37.6	26.0	25.7±5.1
M-1	5.3	5.5	4.5	5.4	17.7	7.6±6.5
M-2	12.0	2.5	19.4	14.4	16.9	13.0±5.8
M-3	21.0*	28.0*	18.0*	9.1†	7.8†	16.8±7.5
M-4	14.5	9.2	7.1	6.1	10.6	9.5±3.0
Dulcitol	3.7	4.6	1.5	0.0	10.9	4.2±3.7
M-5	8.3	13.7	8.7	15.4	1.4	9.5±4.8
Bound to protein precipitate	14.2	12.6	15.9	12.0	8.7	13.3±2.5
	100.0	100.0	100.0	100.0	100.0	

*Average % of M-3 in patients previously treated with alkylating agents: 22.3±4.2%.

†Average % of M-3 in patients previously treated with radiotherapy: 8.5 ±0.6%.

individual differences in the total radioactivity excreted. The patients seemed to belong to two groups: 3 patients excreted 70–80% of dose (M. J., P. J. and K. R.), the other ones (H. F. and M. M.) only 45 and 60% of dose within 48 hr (Table 5). Metabolites were not determined in the case of patient G. P.

There were essential differences neither in the renal or hepatic function nor in the supportive treatment of the patients with the exception of patient K. R. in whom increased activity of serum transaminases was noted. None of them had ascites. Case histories (Table 1) of patients M. J., P. J. and K. R. included treatment with cyclophosphamide or other alkylating agents alone or in combination with MTX 2–5 months before the administration of ^{3}H–DBD, whereas M. M. and H. F. had been treated with radio- and hormone therapy. The sum of DBD and epoxides, BrEpD, AD and DAG, was almost the same in the urine of all patients. The higher recovery of ^{3}H–compounds in the first 3 patients was due to the enhanced excretion of other metabolites—particularly of a bromine-free metabolic, M-3. On average, the three patients with previous chemotherapy excreted 13% of dose as M-3, while the other patients 4% only. Similar differences were found in the metabolite composition of plasma expressed in percentage of the area under the plasma level curve in 24 hr (Table 6). 22% M-3 metabolite was found in the first group and only 8% in the second one.

DISCUSSION

The results prove that:

1. Conversion of DBD into DAG implies, both *in vitro* and in humans, the intermediate formation of bromoepoxy-dulcitol.
2. Steady excretion of DAG as a minor component of the urine for a few days, indicates its persistence at low levels in the systemic circulation.
3. Besides the bromoepoxide there are at least 4 C–Br compounds among the metabolites: M-1, M-4, a part of M-2, and a monobromodulcitol derivative. (M-5 is located so close to the bromide ion that it could not be investigated for C–Br content).

Enhancement of formation and urinary excretion of a bromine-free metabolite, M-3, was observed in patients who had been treated with alkylating agents, like cyclophosphamide and Zitostop a few months before administration pf ^{3}H–DBD. Further studies on more patients are needed to confirm this observation.

Differences between our results and earlier findings in humans treated with ^{14}C–DBD [13] may be owed to differences in methodology. Probably, lyophilization and reconstitution of urine samples enhanced the hydrolysis of C–Br bond in metabolites M-1, M-2 and M-4, and diminished the concentration of the bromine-containing peaks beyond detection by the fluorescein reagent used by Belej and coworkers. Erroneous identifi-

cation of the bromoepoxide with monobromodulcitol might be due to close R_F values of the two compounds.

Recently, BrEpD has been synthesized and proved to exert remarkable cytostatic activity [16]. Its role as a mediator of the biological effects of DBD is to be considered since it may contribute to differences between the effects of DBD and DAG. After administration of DAG the body is exposed to one highly powerful cross-linking agent that is rapidly consumed by interaction with nucleophiles. Administration of DBD results in the presence of 3 alkylating agents with different transport characteristics. The less reactive parent drug and the bromoepoxide may reach different sites and serve as depots wherefrom DAG is gradually released in low concentrations. Milder side-effects of DBD as compared to DAG may be due to this slow process of *in vivo* activation.

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