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Toxicity, Antitumour and Haematological Effects of 1,2-Anhydro-6-bromogalactitol and D-Mannitol: A Comparison with the Related Dibromo- and Dianhydro-derivatives*

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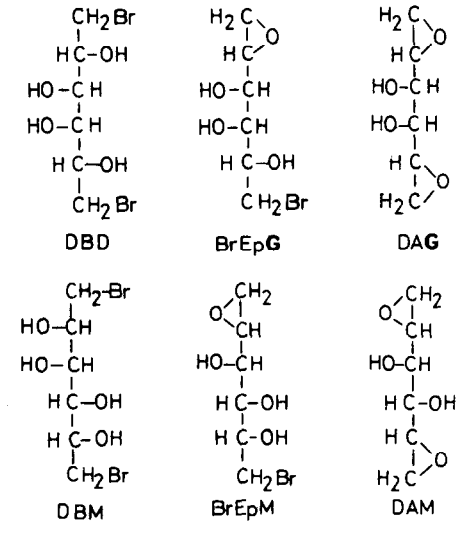
Abstract—*1,2-Anhydro-6-bromo-6-deoxygalactitol (BrEpG) and its D-mammitol analogue (BrEpM) intermediary metabolites in the conversion of dibromodulcitol (DBD) and dibromomannitol (DBM) into dianhydrogalactitol (DAG) and dianhydromannitol (DAM) have been prepared. The three types of derivative of each hexitol have been compared in their toxicities towards mice, tumour inhibitory activities against the Walker carcinosarcoma and haematological effects in rats. The bromoepoxides showed intermediate potency in all tests. The galactitol derivatives were always more potent than their mannitol counterparts. The mannitol derivatives were selectively myelosuppressive, being twice as toxic towards granulocytes as towards lymphocytes. The lymphotoxic activity of DBM, in particular, relative to its other toxic effects was particularly mild. These differences have been ascribed principally to the more rapid reactivity of DAG compared with DAM towards target nucleophiles, modulated by the influence of the bromine substituent on the transport properties of the dibromo- and bromoepoxy-derivatives.*

INTRODUCTION

1,6-DIBROMO-1,6-DIDEOXYGALACTITOL (dibromodulcitol, DBD) and 1,6-dibromo-1,6-dideoxy-D-mannitol (dibromomannitol, DBM) are myelotoxic alkylating agents which have been used clinically in the treatment of granulocytic leukaemia [1–3] and squamous cell carcinoma [4]. Following administration of DBD to patients, three bifunctional alkylating agents were present in plasma and urine [5], namely the starting dibromo-derivative and the bromoepoxy- and diepoxy-derivatives (respectively BrEpG and DAG). The diepoxide, and its D-mannitol analogue (DAM) can also be prepared from the dibromo-analogues by titration with alkali at near neutral pH [6, 7]. An analogous preparation of the bromoepoxides (BrEpG and BrEpM) is described in the present study, which aims to assess the relative roles of the bromo- and epoxy-functions as well as the influence of the hexitol configuration (galactitol or D-mannitol) on the biological effects of the three types of derivative. Their toxicities towards mice, their activities against the Walker carcinosarcoma in rats and their haematotoxicities have therefore been assessed.



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MATERIALS AND METHODS

Synthesis of 1,2-anhydro-6-bromo-6-deoxygalactitol (BrEpG) and 1,2-anhydro-6-bromo-6-deoxy-D-mannitol (BrEpM)

The bromoepoxides were prepared from the corresponding diepoxides by treatment with hydrobromic acid, essentially by the procedure used to convert DAG into the corresponding chloroepoxide using hydrochloric acid [8]. Thus 1,2:5,6-dianhydrogalactitol [6] (DAG, 1.2 g; 8.2 mmol) afforded BrEpG (0.4 g, 21%), m.p. 118–120°C. (Found: C, 31.8; H, 4.9; Br, 35.2%. $C_6H_{11}BrO_4$ requires C, 31.7; H, 4.9; Br, 35.5%.) 1,2:5,6-Dianhydro-D-mannitol [6] (DAM, 0.3 g; 2.05 mmol) afforded BrEpM (0.065 g, 14%), m.p. 114–116°C, $[\alpha]_D^{20} = +11.5°C$ ($c = 2$ in water). (Found: C, 31.7; H, 5.0; Br, 35.2%.)

Biological testing

Bromoepoxides and diepoxides were stored at 4°C and were stable for 3 months. Solutions of each compound in saline were administered to the animals within a few minutes.

For comparative toxicity studies Swiss/H-Riop mice were used. For anti-tumour tests Walker carcinosarcoma (2×10^7 ascites cells) was transplanted intramuscularly into the right hind legs of Wistar/H-Riop rats. Single-dose treatments were given intraperitoneally 24 hr after tumour inoculation, blood was sampled on the 4th day and tumour growth inhibition evaluated on the 10th day. Lymphocyte and granulocyte counts and tumour weight were compared with contemporary tumour-bearing control groups. For each dose level, groups of 6 animals were used.

RESULTS

Table 1 shows the lethal, tumour-inhibitory and haematotoxic effects of the compounds. In Table 2 the relative magnitude of these various biological effects is compared for each compound. Figures 1 and 2 show the dose–response relationships for the galactitol and the mannitol analogues respectively in the test against the Walker 256 carcinosarcoma in rats.

Toxic and tumour-inhibitory effects

The bromoepoxides were intermediate in toxicity (LD_{50} in mice, LD_{10} in rats) between the dibromides and the diepoxides, and the galactitol analogues were more toxic than their mannitol congeners. In the antitumour tests, doses giving respectively 50% (Tu_{50}) and 90% (Tu_{90}) regression of the Walker tumour in rats were calculated from the dose–response curves for the galactitol analogues (Fig. 1) and their mannitol congeners (Fig. 2). The dose–response curves at higher doses were less steep for the bromoepoxides than for the dibromides and the diepoxides. Thus, the therapeutic index for BrEpG, measured at 50% tumour inhibition ($LD_{10}/Tu_{50} = 35$) was *ca.* 17-fold greater than the corresponding index for 90% inhibition ($LD_{10}/Tu_{90} = 2.1$), whereas the corresponding ratios (Tu_{90}/Tu_{50}) for DBD and DAG were only *ca.* 3 and 2 respectively. At all dose levels, galactitol derivatives were more tumour-inhibitory than their mannitol congeners.

Haematological toxicity

Haematological toxicities are expressed in Table 1 as the doses giving 50% reduction in the count for granulocytes (Gr_{50}) and lymphocytes (Ly_{50}) in the tumour-bearing rats. The values demonstrate the selective myelosuppressive action of the mannitol derivatives. Two-fold higher doses are required to induce a 50% depression in the lymphocyte count than in the granulocyte count, as illustrated by the Ly_{50}/Gr_{50} ratios (Table 2). Relative to their lethal and tumour-inhibitory effects, the lymphotoxicity of the mannitol derivatives was milder than that of their galactitol congeners, as reflected in the ratios (Table 2) LD_{10} to Ly_{50} and of Ly_{50} to Tu_{90}. This mild lymphotoxicity was particularly striking for DBM. Thus DBD was about twice as toxic towards mice (LD_{50} values, Table 1) and rats (LD_{10}) as DBM, and was similarly more tumour inhibitory (Tu_{50} and Tu_{90} values), but DBD was much more markedly myelotoxic (Gr_{50} ratios 4.0) and lymphotoxic (Ly_{50} ratios 7.1) than was the mannitol analogue.

DISCUSSION

DNA is presumably the target for the dibromo- and diepoxyhexitols (and implicitly for the bromoepoxides), since the extent of tumour inhibition correlates with binding to DNA [9]. Interaction of DBM and DBD with DNA *in vitro* resulted in significant cross-linking only if the medium was sufficiently alkaline to convert a part of the drug into diepoxide. Moreover, the rate of cross-linking by DBD was similar to the rate of its conversion into DAG [10]. These results imply that the cytostatic effects of both dibromo- and bromoepoxyhexitols are due principally to their conversion into diepoxides *in vivo*, the less reactive precursor drugs serving as depots from which the diepoxide is gradually released.

However, the bromine substituent(s) also modulated the biological effects. Thus the bromoepoxides, particularly BrEpG, showed a much slower increase in anti-tumour activity

Table 1. *Activity against the Walker* 256 *carcinoma and haemopoietic effects in rats of dibromo-, bromoepoxy- and diepoxy-derivatives of galactitol and* D-*mannitol (expressed in* μmol/kg)

	Tu_{50}	Tu_{90}	Gr_{50}	Ly_{50}	LD_{10} (rats)	LD_{50} (mice)
DBD	125	370	610	673	2000	2922
BrEpG	6	100	71	116	210	350
DAG	4.3	9	18.8	24.9	67	100
DBM	290	650	2425	4800	3650	5520
BrEpM	45	200	255	600	800	1010
DAM	10	27	45	110	150	137

Tu_{50}, Tu_{90} = dose producing respectively 50% and 90% tumour regression relative to untreated control animals.
Gr_{50}, Ly_{50} = dose producing 50% fall in granulocyte and lymphocyte count respectively compared with control animals.
LD_{10} = dose lethal to 10% of animals.

Table 2. *Indices relating toxic, tumour-inhibitory and haemotological effects of dibromo-, bromoepoxy- and diepoxy-derivatives of galactitol and* D-*mannitol*

	LD_{10}/Tu_{50}	LD_{10}/Tu_{90}	LD_{10}/Gr_{50}	Ly_{50}/Tu_{90}	LD_{10}/Ly_{50}	Ly_{50}/Gr_{50}	Gr_{50}/Tu_{90}
DBD	16	5.4	3.3	1.82	3.0	1.1	1.7
BrEpG	35	2.1	3.0	1.16	1.8	1.6	0.7
DAG	15.6	7.4	3.6	2.8	2.7	1.3	2.1
DBM	12.6	5.6	1.5	7.38	0.76	2.0	3.7
BrEpM	17.8	4.0	3.1	3.0	1.3	2.4	1.3
DAM	15	5.6	3.3	4.1	1.4	2.4	1.7

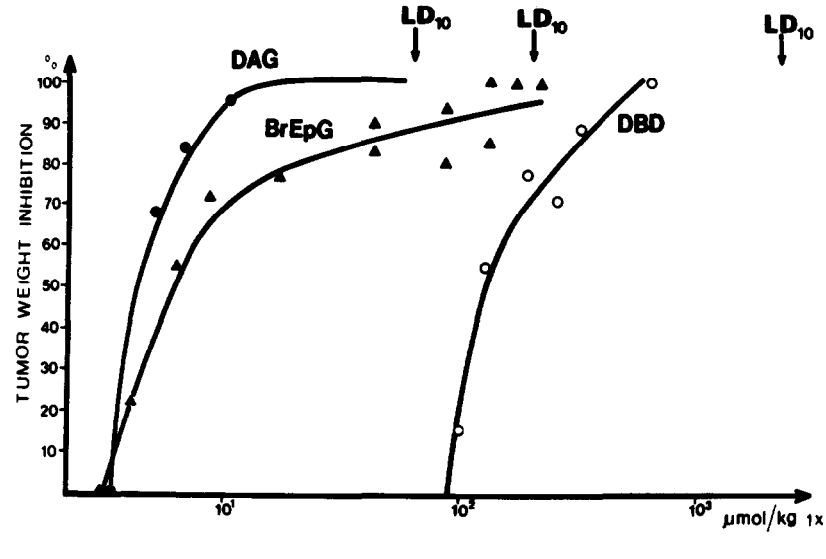

Fig. 1. *Tumour growth inhibitory curves for DBD, BrEpG and DAG. Each point represents a single experiment carried out on a group of* 6 *Wistar/H-Riop rats which were treated with the intraperitoneally designated dose* 24 hr *after intramuscular injection of* 2×10^7 *ascites cells of the Walker carcinosarcoma. They were sacrificied on day* 10, *the tumours excised and weighed and the weights for each animal averaged.*

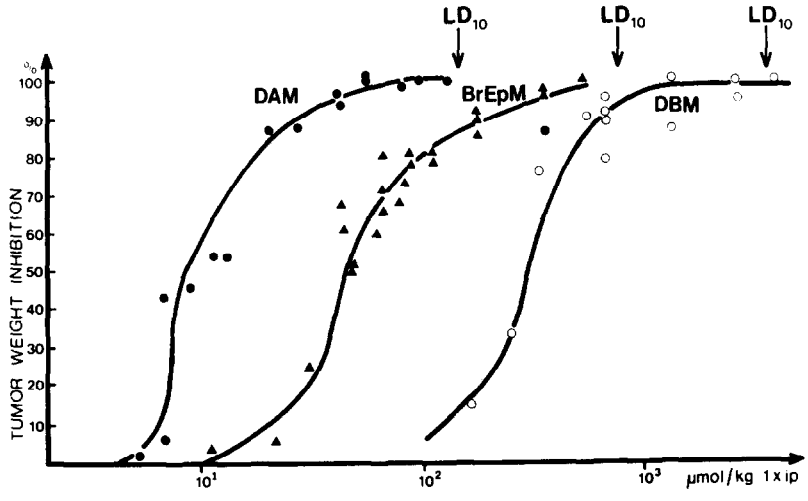

Fig. 2. Tumour growth inhibitory curves for DBM, BrEpM and DAM. Details as for Fig. 1.

with increasing dose than did the dibromides or the diepoxides for levels of tumour inhibition exceeding *ca.* 70%. Possibly, there is a mixed population of cells in the Walker tumour with markedly different sensitivities to BrEpG (cf. reference [11]). Alternatively, the difference may relate to the enhanced probability of alkylation via direct nucleophilic displacement of bromide ion from the C–Br linkage by, for example, guanine residues on the opposing strand of the DNA double helix following rapid monofunctional reaction by the epoxide moiety in the bromoepoxides.

The bromine substituent(s) may also influence transport properties since DBD and DBM bind reversibly to serum albumin, and the C–Br function appears to stabilize this binding during drug transport in plasma [12]. Studies on the interaction of DBD and DAG with chromatin constituents of tumour cells *in vivo* have shown that DAG is bound rapidly to DNA, but interacts negligibly with proteins, whereas the DBD–DNA interaction is delayed by an early association of DBD with chromosomal proteins [13].

Biological activity was also dependent on the configuration of the hexitol moiety, the galactitol configuration always conferring greater potency. This difference could relate to chemical reactivity, since the 1,6-disubstituted galactitols react about twice as rapidly as the mannitol analogues with water and other nucleophiles [6, 14]. Also, in the cross-link between guanine residues in DNA, the C–OH group at C_3 of the mannitol residue adopts an unfavourable conformation [15], further accentuating the intrinsic difference in cross-linking reactivity between the galactitol and the mannitol derivatives based on their relative affinities for nucleophiles. However, there is only a small difference between the reaction rates of DAG and of DAM with thiosulphate [16], implying that detoxification by thiols, for example the reaction between epoxides and glutathione [17], should show little dependence on hexitol configuration. Hence the galactitol derivatives may more readily undergo reactions producing toxicity, but be equally susceptible as their mannitol congeners to reactions resulting in detoxification. This difference between toxification and inactivation could account for some of the detailed differences between the biological effects of the mannitol and galactitol series. Thus the selective myelotropic action of the mannitol derivatives, which are *ca.* two-fold more toxic to granulocytes than lymphocytes (Table 2), indicates a possible defence mechanism for the lymphoid elements against slowly reacting alkylating agents such as DAM. This defence might be less efficient in preventing the interaction of the more reactive DAG and DNA. The exceptionally mild lymphotoxicity of DBM in relation to its other toxic effects may have a similar explanation.

In summary, the higher biological potency of the galactitol derivatives is consistent with the higher DNA cross-linking ability of DAG compared with DAM. However, the undoubtedly major role of diepoxide formation in determining the various biological activities of the dibromo-derivatives and the bromoepoxides is modulated by the contribution of the bromine substituent, which confers protein-binding affinity and can therefore affect drug transport.

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