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The Distribution of [^{3}H]-Dibromodulcitol in the Central Nervous System of Patients with Brain Tumour

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Abstract—*The uptake of* [^{3}H]-*dibromodulcitol* ([^{3}H]-*DBD*) *into glioblastomas, white matter and cerebrospinal fluid was studied in* 10 *patients. Single-tissue samples were taken from different subjects at* 4, 15 *and* 24 hr *after* [^{3}H]-*DBD administration. The level of* ^{3}H-*compounds in the central nervous system was similar after a single* (400 mg/m^2), *or* 3 *smaller daily oral doses of* 150–180 mg/m^2 *of* [^{3}H]-*DBD. The distribution of radioactivity was uniform in the tumour, white matter and muscle. Between* 3 *and* 15 hr *after administration of DBD the concentration of radioactivity did not change significantly and was between* 5 *and* 13 μg *of DBD/g tissue wet wt. At the same time the level in the cerebrospinal fluid (CSF) remained between* 1 *and* 4 μg/ml. *Meanwhile, the average concentration of radioactivity in the plasma fell from* 11 *to* 3 μg/ml. *The elimination half-life of the labelled compounds from the tissues was about* 1 day *as judged from the limited number of non-serial data obtained* 4 *and* 24 hr *after the last dose of repeated drug administration.*

INTRODUCTION

DIBROMODULCITOL (DBD, Mitolactol®, NSC-104800) is one of the alkylating hexitol compounds developed in Hungary. It was first introduced for the treatment of different human malignancies by Sellei *et al.* [1], and was later extensively studied by other investigators [2–4].

Distribution studies demonstrated a high uptake of the drug into the central nervous system (CNS) of animals [5]. Belej *et al.* [6] and Horváth *et al.* [7–9] also found significant amounts of DBD and its metabolites in different human organs and cerebrospinal fluid (CSF), demonstrating the rapid penetration of the drug through the cell membranes and the blood–brain barrier (BBB). DBD and its main metabolite, dianhydrogalactitol (DAG), were found to be among the most active drugs out of the 177 cytostatic agents screened by Geran *et al.* [10] on the mouse ependymoblastoma. Merker *et al.* [11], using a transplantable dog brain tumour model, showed DBD to be very effective in prolonging survival times.

In adjuvant treatment of supratentorial human glioblastomas and malignant astrocytomas DBD exhibited no activity if given alone. Irradiation resulted in a median survival of 40 weeks, while the combination of DBD therapy with irradiation increased it to 57 weeks [12, 13]. Similarly, the combination of BCNU and DBD gave satisfying results in the treatment of malignant glioma recurrencies [14]. Various dose schedules had been applied in the clinical trials: daily doses of 120–180 mg/m^2 for 10 days or longer, and single doses of 350–500 mg/m^2 every 5th or 7th day respectively. Both schedules were found to be active and well tolerated [3, 15].

In the present study the time course of the uptake and elimination of [^{3}H]-DBD into and from the human CNS, malignant gliomas and CSF were investigated by collecting single tissue samples from different patients at various time points after [^{3}H]-DBD administration. In addition, the pharmacokinetic basis of various administration schedules were studied after the administration of DBD in a single dose and 3 smaller daily dosages.

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MATERIALS AND METHODS

The studies on the pharmacokinetics of DBD were carried out using [^{3}H]-DBD labelled in the C-1 position with a specific activity of 7.77 MBq/mg (210 μCi/mg), provided by the CHINOIN Chemical and Pharmaceutical Works, Budapest. The material proved to be chromatographically 97% pure. The ^{3}H-atom is firmly bound to the carbon skeleton, i.e. it is also present in all the metabolites [8].

The study was performed according to the guidelines of the Hungarian Human Research Ethical Committee, limiting the total amount of ^{3}H given to each individual patient to 22.2 MBq (600 μCi). Therefore [^{3}H]-DBD was mixed with unlabelled DBD before use in order to achieve the required specific activity. An aliquot was taken to determine the actual amount of radioactivity.

The [^{3}H]-DBD was given orally to 10 patients, all harbouring malignant gliomas. Histologically all tumours investigated were uniformly glioblastomas (Kernohan IV). Samples were taken at surgery. Two different dose schedules were applied. A group of 6 patients received a single dose of 400 mg/m^2 of [^{3}H]-DBD. The time interval from the drug administration until collection of specimen was 3.5–4.75 hr in 3 patients and 13.3–15.25 hr for another 3 cases. To the next 4 patients the drug was given in daily doses of 150–180 mg/m^2 for 3 subsequent days prior to surgical intervention. Materials were taken at 4.25 (2 patients) and at 24 hr (2 patients) following the last [^{3}H]-DBD dose.

From each patient a single sample of plasma, CSF, brain tumour and white matter was collected. From 2 patients a piece of temporal muscle was also obtained. An assay for radioactivity in each specimen was performed as described previously [16]. Measurements were performed in a liquid scintillation counter (LKB-Wallac, 81000) and in Carbon–Tritium Automatic Gas Analyser (CHINOIN Chemical and Pharmaceutical Works, Budapest).

RESULTS

The ^{3}H-content of the samples following a single oral dose of 400 mg/m^2 of [^{3}H]-DBD expressed as μg of DBD/ml or g tissue wet wt is summarized in Table 1. The radioactivity levels measured approximately 4 hr after drug administration in white matter and tumour tissue were similar, with mean amounts of DBD/g tumour tissue of 8.3 μg (range 5.6–12) and 9 μg (range 6.2–13) respectively. The mean plasma concentration at the same time was slightly higher, whereas the concentration in the CSF was much lower, namely 2.1 μg/ml (range 1.5–3.5). In the muscle specimen of patient 3 13.4 μg/g was found. No significant changes in DBD concentrations in CSF, tumour and white matter could be observed at approximately 14 hr after drug intake. However, the plasma DBD concentration fell significantly from 10.8 μg/ml (range 8.8–12) to 3.4 μg/ml (range 2.1–4.1).

*Table 1. ^{3}H levels after a single oral dose of 400 mg/m^2 of [^{3}H]-DBD at the time of surgery**

Patient No.	Time after dose (hr)	Plasma	CSF	Tumour	White matter
1	3.5	11.6	1.2	6.2	5.6
2	4.5	8.8	1.5	7.6	7.2
3	4.75	12.0	3.5	13.0	12.0
Mean		10.8	2.1	9.0	8.3
4	15.16	4.1	3.3	8.3	8.0
5	13.5	3.9	1.4	5.9	7.0
6	15.25	2.1	4.3	4.6	5.3
Mean		3.4	3.0	6.3	6.7

*Total radioactivity expressed as μg of DBD/ml or g wet wt. Each figure represents a single measurement in 1 patient.

In the patients who received approximately the same total DBD dose but in 3 divided portions, the amounts of radioactivity in the tissue samples were similar to those obtained after a single dose (Table 2). Four hours after the last DBD intake, the concentrations of radioactive materials were between 7.8 and 12.0 μg/g wet wt in the tumour, 7.5–10.7 μg/g in the white matter samples, 5.7 μg/ml in the plasma and 4.2 μg/ml in the CSF. The levels of radioactivity in the tumour and plasma specimens were 3.9–6.7 and 5.4–7.9 μg/g respectively 24 hr after the last dose. The drug concentration was 2.4–2 μg/ml in the plasma, 3.8 μg/ml in the CSF and 4.8 μg/g in the muscle specimen of patient 10.

*Table 2. ^{3}H levels following 3 repeated oral doses of 150–180 mg/m^2 of [^{3}H]-DBD at the time of surgery**

Patient No.	Time after the last dose (hr)	Plasma	CSF	Tumour	White matter
8	4.25	5.6	—	7.8	7.5
8	4.25	—	4.2	12.0	10.7
9	24	2.4	—	3.9	7.9
10	24	2.0	3.8	6.7	5.4

*Total radioactivity expressed as μg of DBD/ml or g wet wt. Each figure represents a single measurement in 1 patient.

DISCUSSION

As generally believed, drugs with high lipid solubility and low molecular weight cross the BBB and easily reach the target in the CNS [11, 17, 18]. The low molecular weight of DBD (308) and its amphiphilic property due to the C-Br and hydroxyl groups facilitate its penetration through both lipophilic and hydrophilic phases. Previous human studies using radiolabelled drug proved that orally administered DBD was easily and completely absorbed from the gastrointestinal tract [6–9]. It rapidly appeared in the CSF, where approximately 30% of the radioactivity was present in the form of pharmacologically active, bifunctional alkylating compounds, such as DBD, bromoepoxygalactitol (BrEpG) and DAG, 6 hr after drug administration [6, 7].

Following the two different drug dose schedules no consistent differences appeared between the radioactivity levels in brain tumour and white matter, except for in patient 9. Moreover, similar amounts of radioactivity were found in the temporal muscle biopsies from patients 3 and 10. These data clearly indicate that 3H-DBD easily penetrates the BBB of humans, confirming comparable results in experimental animals presented by Institoris and Dzurillay [5]. Our data are in marked contrast to previously reported results with DAG [16], showing a tumour: white matter ratio of 2.66 for glioblastomas and much higher differences for the more differentiated astrocytomas.

Two factors might be primarily responsible for this difference: (i) both DBD and DAG easily penetrate the BBB; however, the more hydrophilic DAG can hardly enter the white matter; (ii) studies of Institoris *et al.* [19] 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 has no significant interaction with proteins. However, the DBD–DNA interaction is delayed by an early association of DBD with chromosomal proteins. Consequently, any difference in the DNA alkylation between tumour and white matter might be concealed by the large amounts of protein-bound DBD.

The disappearance of radioactivity from the plasma was much more rapid than from the compartment inside the BBB. According to earlier investigations on [³H]-DBD in patients [6–9], the plasma half-life of ³H-elimination was around 6 hr. The very limited number of non-serial data suggests that the biological half-life of [³H]-DBD may be at least 24 hr.

The adminisration of DBD for longer periods of time may therefore lead to drug accumulation in the CNS. The slow elimination might be explained by hexitol moieties that are covalently bound to the target DNA and to other macromolecules [8]. From pharmacokinetic and hematologic points of view, both schedules are acceptable for the treatment of gliomas.

The low amount of radioactivity permitted to be used did not allow us to determine the fraction of unchanged drug and active metabolites including BrEpG and DAG. However, earlier measurements of Horváth [7] showed that 6 hr after drug administration about 30–40% of the calculated drug concentration was actually unchanged DBD or active metabolites. In this study the minimal effective drug concentration in contact with the tissues can be thus estimated to be around 2–3 μg/ml, which kills about 50% of HeLa or Chinese hamster cells in tissue culture [20]. Comparing the uptake of the amphiphilic DBD and the more hydrophilic DAG on the basis of total administered dose, 3–5 times more DBD enters the CNS. Moreover, the alkylating action of DBD *in vivo* is a slow process because it is mediated by BrEpG and DAG, involving a slow local release of these potent cross-linking agents. Consequently, high alkylating activity is maintained for a more prolonged time [9]. Hence pharmacokinetically, DBD should be more suitable for brain tumour treatment than DAG.

REFERENCES

1. SELLEI C, ECKHARDT S, HORVÁTH IP, KRALOVÁNSZKY J, INSTITORIS L. Clinical and pharmacologic experience with dibromodulcitol (NSC-104800), a new antitumor agent. *Cancer Chemother Rep* 1969, **53**, 377–384.
2. CHIUTEN DF, ROZENZWEIG M, VON HOFF DD, MUGGIA FM. Clinical trials with the hexitol derivatives in the U.S. *Cancer* 1981, **47**, 442–451.
3. ECKHARDT S. Ten year international experience with DBD in the treatment of malignant diseases. In: ECKHARDT S, ed. *Dibromodulcitol*. Budapest, Medicina, 1982, 174–189.
4. MISCHLER NE, EARHARDT RH, CARR B *et al.* Dibromodulcitol: a review. *Cancer Treat Rev* 1979, **6**, 191–121.
5. INSTITORIS L, DZURILLAY E. Comparative studies on the *in vivo* distribution pattern of dibromodulcitol and diepoxydulcitol. *Z Krebsforsch* 1973, **79**, 49–59.

6. BELEJ MA, TROETEL WM, WEISS AJ, STAMBOUGH JE, MANTJEL RW. The absorption and metabolism of dibromodulcitol in patients with advanced cancer. *Clin Pharmacol Ther* 1972, **13**, 563–572.
7. HORVÁTH IP. Pharmacokinetics and metabolism of DBD in man. In: ECKHARDT S, ed. *Dibromodulcitol.* Budapest, Medicina, 1982, 148–165.
8. HORVÁTH IP, CSETÉNYI J, KERPEL-FRONIUS S, HINDY I, ECKHARDT S. Metabolism and pharmacokinetics of dibromodulcitol (DBD, NSC-104800) in man—I. Metabolites of DBD. *Eur J Cancer* 1979, **15**, 337–344.
9. HORVÁTH IP, CSETÉNYI J, KERPEL-FRONIUS S, INSTITÓRIS L, HEGEDÜS L, ECKHARDT S. Metabolism and pharmacokinetics of dibromodulcitol (DBD, NSC-104800) in man—II. Pharmacokinetics of DBD. *Eur J Cancer Clin Oncol* 1982, **18**, 1211–1219.
10. GERAN RI, CONGLETON GF, DUDECK LE, ABBOTT BJ, GARGUS JL. A mouse ependymoblastoma as an experimental model for screening potential antineoplastic drugs. *Cancer Chemother Rep* 1974, **4**, 53–87.
11. MERKER PC, WODINSKY, GERAN RI. Review of selected experimental brain tumor models used in chemotherapy experiments. *Cancer Chemother Rep* 1975, **59**, 729–736.
12. ÁFRA D. Treatment of gliomas by DBD. In: ECKHARDT S, ed. *Dibromodulcitol.* Budapest, Medicina, 1982, 189–199.
13. ÁFRA D, KOCSIS B, DOBAY I, ECKHARDT S. Combined radiotherapy and chemotherapy with dibromodulcitol (Mitolactol®, NSC-104800) and CCNU in the postoperative treatment of malignant gliomas. *J Neurosurg* In press.
14. ÁFRA D. Treatment of supratentorial glioma recurrencies with reoperation and chemotherapy, Vol. 1. In: BROCK M, ed. *Modern Neurosurgery.* Berlin, Springer, 1982, 137–143.
15. HINDY I, SZÁNTÓ J, BODROGI I *et al.* Forms and results of Mitolactol therapy. *Oncology* 1980, **37** (Suppl. 1), 115–117.
16. ECKHARDT S, CSETÉNYI J, HORVÁTH IP *et al.* Uptake of labelled dianhydrogalactitol into human gliomas and nervous tissue. *Cancer Treat Rep* 1977, **61**, 841–847.
17. EDWARDS MS, LEVIN VA, WILSON CB. An evaluation of agents in current use for phase II and III trials. *Cancer Treat Rep* 1980, **64**, 1179–1205.
18. WALKER MD, WEISS HD. Chemotherapy in the treatment of malignant brain tumours. In: FRIEDLANDER WJ, ed. *Neurology.* New York, Raven Press, 1975, 149–175.
19. INSTITORIS E, SOMFAI S, VARGAI Z, HOLCZINGER L. Interaction of dibromo-and dianhydrodulcitol with chromatin constituents of tumor cells. In: SIEGENTHALER W, LÜTHY R, eds. *Current Chemotherapy.* Washington, American Society for Microbiology, 1978, Vol. II, 1305–1307.
20. PÁLYI I. Effects on cells *in vitro.* In: ECKHARDT S, ed. *Dibromodulcitol.* Budapest, Medicina, 1982, 69–72.