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The Distribution of Bromine Content of Dibromodulcitol in the Central Nervous System of Patients with Malignant Gliomas

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Abstract—*The bromine content of human gliomas and white matter was determined by neutron activation analysis (NAA) following p.o. administration of a single dose of* 400–500 mg/m^2 *dibromodulcitol (DBD). In another group of patients with brain gliomas, the bromine content was measured subsequent to application of a single dose of* 334 mg/m^2 *of sodium bromide (equivalent dose regarding the bromine content of DBD).*

The bromine content of these two groups was compared to the values found in a third control group of untreated patients. The amount of bromine after DBD application was three to four times higher than in the untreated samples and the average accumulation ratio of 1.8 ± 0.4 *proved to be nearly identical both in tumour and white matter. The bromine values after* NaBr *treatment showed a different pattern of distribution. The accumulation was higher in the tumour tissue than in the normal white matter.*

These findings demonstrate that the pharmacokinetic properties of DBD- and NaBr*-derived bromine are different, suggesting that the increase of bromine after DBD administration could be due to covalently bound bromine in DBD.*

COMPARING the results of various treatment schedules, we believed that the concurrent use of DBD during irradiation might have been the main factor in improving survival times. Furthermore, it might have been supposed that DBD—probably due to its bromine content—might also have a certain potentiating or direct radiosensitizing effect [1, 2].

The uptake of [^{3}H]DBD into glioblastomas and adjacent white matter was demonstrated earlier in our experimental investigations. The ^{3}H atom is firmly bound to the carbon skeleton and the determination of radioactivity is suitable to give some information regarding the DBD molecule and its metabolites. However, it seemed to be necessary to obtain direct data about the bromine content of DBD in human brain tumours (malignant gliomas) and in the surrounding white matter. As the use of DBD labelled with radioactive bromine (^{82}Br) is rather difficult under clinical conditions, the neutron activation analysis (NAA) method was chosen as one of the most sensitive analytical procedures for the determination of bromine in brain samples.

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MATERIALS AND METHODS

DBD was given orally to nine patients at an average therapeutic dose of 400–500 mg/m^2 3–4 h before the operation, i.e. tumour removal. The total dose of the drug varied from 750 to 1000 mg. All investigated tumours were histologically anaplastic astrocytomas (grade 4 and 3). Two control experiments were performed. For the determination of normal bromine content, untreated tumour and white matter samples were taken from both tissues at surgery, in four patients operated on for malignant gliomas. In addition, sodium bromide in an equivalent dose (334 mg/m^2 of bromine) was given orally to another four patients at the same time interval before surgery. No samples of plasma and CSF were collected.

During the operation only a small but representative sample could be taken from each patient and the tissue samples (i.e. tumour and white matter) were divided into two parts and used for parallel measurements. Due to the small size of the samples available (20–30 mg for white matter and 30–50 mg for tumour), the bromine content of the two samples from each tissue for each patient was measured.

Immediately following the removal of the tumour, the samples were washed with double-distilled water in order to remove blood contamination. They were then put into carefully cleaned quartz ampoules, freezed and stored at −20°C until the lyophilization procedure.

The bromine content of the samples was determined by the non-destructive neutron activation analytical method using the

$$^{81}Br/n,\gamma/\,^{82}Br \quad t_{1/2}: = 35.6\ h \qquad E_\gamma: = 554.3\ keV,\ 776.6\ keV$$

nuclear reaction.

The brain tissue, as well as other tissues, contains relatively large amounts of sodium and chlorine; thus the resulting activities of the ^{24}Na ($t_{1/2}$ = 15.0 h, E_γ = 1368.5 keV) and ^{38}Cl ($t_{1/2}$ = 37.3 min, E_γ = 1642.0 keV) isotopes, especially the Compton edges of these gamma-radiations, creating a smooth background for the bromine peaks, usually interfere with the determination of bromine content of the brain tissues by NAA [3]. Using optimal irradiation (10 h) and decay time (10 and 12 days), this background was reduced in order to achieve the appropriate precision of ≤ 5% for the determination of bromine. Lyophilized samples of 6–12 mg white matter and 8–15 mg tumour were put into SUPRASILAN high purity quartz vials, heat-sealed and irradiated for 10 h. Irradiations were carried out in the vertical channel of the KFKI WWRS-M type reactor at a flux density of about 3.0×10^{13} n.cm^{-2}.s^{-1}. Samples and standards (10 μg Br) were irradiated together under strictly identical conditions to calculate the bromine content. Prior to irradiation, the quartz vials were rinsed with 40% hydrogen fluoride for 5 min, then they were washed with double-distilled water three times and dried. This procedure was repeated also after irradiation to remove external contamination and the samples were measured directly in the quartz vials. Blank vials were irradiated along with the samples but corrections for bromine turned out to be unnecessary.

Measurements were performed after 10 and 12 days decay time. For the measurement of the gamma-spectra an 80 cm^3 CANBERRA Ge/Li/type detector (with energy resolution of 1.82 keV for the 1332.5 keV ^{60}Co line), CANBERRA linear electronics and a KFKI-ICA-70 type 4096 channel analyser were used. Dead time corrections were taken into account with the aid of a pulse generator. The peak areas were determined by using the HYPERMET program [4]. The mean value was calculated from individual concentrations obtained by one irradiation with parallel samples and in two cycles of measurements, accepted as the most likely concentration of the element investigated.

Table 1. Bromine content (μg/g dry weight) of tumour and white matter of patients with untreated gliomas

Patients	Tumour	White matter	T/WM ratio
R.M.	12.7	7.9	1.6
T.G.	8.9	4.1	2.2
S.G.	15.9	8.4	1.9
D.F.	8.3	4.5	1.9
Average	11.5	6.2	1.9

RESULTS

The bromine content of human white matter and intracerebral gliomas was determined in four cases (Table 1). The bromine content of the tumours was always higher—on average 11.5 (8.3–15.9 μg/g)—than that of the white matter: 6.2 (4.1–8.4 μg/g). Following p.o. DBD administration, a considerably higher bromine content was found both in the tumours [on average 36.1 (19.9–60.5 μg/g)] and in the white matter: 21.2 (10.1–35.2 μg/g). The amount of bromine after a single DBD dose was 3.5–4 times higher than that of the untreated samples. Nevertheless, the average accumulation ratio—1.8 ± 0.4—was nearly identical in the untreated brains and subsequent to DBD administration (Table 2). When sodium bromide was given in an equivalent dose regarding the bromine content of DBD, a much more pronounced accumulation of bromine—on average 98.2 (75.1–121.8 μg/g)—could be observed in the tumour samples than after DBD administration (Table 2). However, the uptake in the white matter was nearly the same, 23.8 (16.5–30.9 μg/g), as following DBD. The ratios of the average values in μg/g are summarized as follows:

	Tumour	White matter
DBD/'control' Br	3.2	3.4
NaBr/'control' Br	8.4	3.8
NaBr/DBD-Br	2.6	1.08

This clearly shows that the bromine content of the white matter was identical subsequent to either DBD or NaBr application. On the other hand, approximately 2.5 times more bromine could be determined in the gliomas after giving NaBr.

DISCUSSION

Dibromodulcitol is rapidly converted into at least 12 transformation products in the body. Only five of these compounds do not contain bromine [5]. Consequently, excretion of free bromine into the urine accounts for only 5–7% of the DBD dose administered in 24 h [5, 6]. The distribution of bromine after DBD administration must therefore

Table 2. *Bromine content* (μg/g *dry weight*) *of tumour and white matter after a single DBD dose*

Patients	Tumour	White matter	T/WM ratio
M.J.	42.5	26.7	1.6
B.F.	39.5	27.6	1.4
F.J.	43.8	27.3	1.6
M.J.	34.5	21.7	1.6
D.F.	28.5	10.1	2.8
V.I.	25.5	14.4	1.8
G.G.	30.1	17.3	1.7
T.J.	19.9	10.9	1.8
M.I.	60.5	35.2	1.8
Average	36.1 ± 11.9	21.2 ± 8.9	1.8 ± 0.4

Table 3. *Bromine content* (μg/g *dry weight*) *of tumour and white matter after a single dose of* NaBr

Patients	Tumour	White matter	T/WM ratio
V.F.	75.1	17.9	4.2
M.Z.	87.2	29.9	2.9
C.L.	121.8	30.9	3.9
H.G.	108.8	16.5	6.6
Average	98.2	23.8	4.4

reflect mainly the distribution of bromine bound to the parent molecules and their transformation products. This conclusion is suggested by the earlier animal experiments of Institoris *et al.* [7], in which they showed that the pharmacokinetic properties of bromine were strikingly different depending on whether it was administered as NaBr or DBD.

The animal experimental results could be confirmed by our present observations in human beings: the tumour/white matter ratio varied also according to the mode of bromine intake. Following administration of an equivalent dose of NaBr we found a basically different distribution pattern of bromine. This can be explained by the fact that most of the bromine atoms of DBD follow the organic carrier molecule within the body and the increase in bromine content in the tumour and white matter must be due to the covalently bound bromine of DBD.

On the other hand, there must be some difference in the distribution of the transformation products according to their bromine content. The tumour/white matter ratio was about 1.0 when radioactivity was measured after administration of [^{3}H]DBD [8], compared to the presently found ratio of 1.8. This difference in the distribution pattern indicates a slightly preferential accumulation of bromine-containing DBD products in the tumour tissue.

Dibromodulcitol may be regarded as a bifunctional alkylating agent. It produces cross-links between nucleophylic centres when they are about 900 pm apart. In the antiparallel double helix of DNA, DBD cross links the N/7 atoms of the guanine bases [9]. This finding suggests that the bromine content of DBD may have a radiosensitizing effect only if the bromine containing molecule is incorporated into the DNA. It can be presumed that this mechanism of action is similar to that suggested by the observations of Sano *et al.* [10] and those of Szybalski [11] using the halogenated DNA precursor, bromodeoxyuridine (BUdR).

Our results show that the bromine content of brain tumours was increased 3–4-fold after DBD application. Whether this accumulation of DBD and its bromine-containing derivatives could be sufficient for causing any change in the radiosensitivity of human gliomas is not known at present. Nevertheless, in our ongoing experiments [12, 13] the combination of DBD and radiotherapy in the treatment of mouse ependymoblastomas results in a significant increase in the life span of the experimental animals.

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