

Treatment of Malignant Glioma by a New Therapeutic Principle

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Summary

L-2, 4 diaminobutyric acid (DAB) is a non-physiological, cationic amino acid transported into cells by System A with potent antitumour activity in vitro against human glioma cells. This activity was the result of the pronounced concentrated uptake of DAB in glioma cells to the extent that a cellular lysis could occur due to osmotic reasons. We describe the treatment of 3 patients with inoperable malignant glioma by direct and continuous administration of DAB in tumour tissue employing a microdialysis technique.

One to three microdialysis probes were implanted in the tumour tissue through small dural incisions in 3 patients with inoperable malignant glioma. Micropumps charged with 3 ml a day of a Tris-buffered 0.125 M DAB solution made isotonic at pH 7.55 were adapted to the input channel of the probe and a sampling tube to the output for continuous flowing into tumour tissue. The patients were treated in this way for a total of 14–21 days without side effects assignable to DAB. Massive tumour necrosis occurred as judged by comparison of computed tomography performed before and after DAB treatment. The yield of the dialysis procedure with regard to DAB was estimated to be 40–50%. The dialysate concentration of arginine (a cationic amino acid considered to be transported mainly by system A) was high and increased nearly 4-fold from day 3 to day 6 of treatment. DAB administered in this way into malignant brain tumour tissue was well tolerated and showed promising antitumour activity in the 3 patients with inoperable malignant glioma. High and increasing concentrations of arginine in the dialysate during treatment indicated a displacement of this amino acid from the intracellular space of malignant tumour cells most probably as a consequence of huge intracellular accumulation of DAB preceding cellular lysis.

Keywords: Malignant glioma; microdialysis; L-2,4 diaminobutyric acid; cytotoxicity.

Introduction

Chemotherapy against malignant brain tumours has for well founded reasons been looked upon as rational treatment. More than 3 decades of non-success have, however, been discouraging. This in spite of all conceivable weak points within the neoplastic cell having been tried as targets^{3, 4}.

An antitumour effect of a different kind was noted for L-2, 4 diaminobutyric acid (DAB) against cultured human glioma cells⁷ and against an experimental tumour⁶. The mechanism of action of DAB was based upon the finding that this cationic, nonphysiological amino acid is transported into cells by the energy-requiring system A in an unusually asymmetric fashion creating a huge gradient¹. Furthermore, the V_{\max} -value of system A transport was much higher in glioma cells than in their counterparts, the normal glial cells⁵. Based on these findings, a theory was advanced suggesting a selective cytolytic action against glioma cells by DAB due to the much higher uptake capacity of these cells than of normal glial cells⁷. Herewith the accumulation of DAB in glioma cells would create an osmotic strength possibly combined with an energy crisis in these cells due to the large active uptake, ultimately leading to an irreversible cellular lysis⁷. Such an effect was not apparent in the normal glial cells due to their low capacity of system A transport⁷.

The DAB-induced lysis of glioma cells could be counteracted by the presence of certain physiological amino acids in equimolar concentrations⁷. Hence, it was important to keep the concentration of competing physiological amino acids at a low level for maximal cytolytic effect by DAB^{6, 7}. It should also be kept in mind that the cellular destruction was not complete until 20–24 h of incubation under in vitro conditions^{6, 7}. Accordingly, a prerequisite for DAB action to be accomplished in vivo seemed to be a mode of administration permitting the DAB concentration to be maintained reasonably high in the tumour for a certain period of time. Another requirement would seem to be the maintenance of as low a concentration as possible of competing physiological amino acids in the extra-

cellular space during DAB administration. Both of these demands were reasonably met by employing the microdialysis technique⁹, which offered a direct and continuous administration of DAB into the tumour along with the concomitant removal of low molecular compounds including physiological amino acids from the tumour cell environment.

The present investigation concerns the treatment of 3 patients with inoperable malignant glioma by a microdialysis device with DAB in the infusion fluid in order to estimate the value of our working hypothesis.

Methods

Microdialysis Device

Microdialysis was carried out by a microdialysis probe connected to a microsyringe pump maintaining a flow of 3 ml/24h. The microdialysis probe consisted of an outer flexible tube with a tubular dialysis membrane (diameter 0.6 mm) extending 20 or 30 mm from its tip. The membrane was sealed at its distal end. The perfusion fluid was brought to the distal end of the dialysis membrane by a thin inner tube. A similar outlet tube carried the dialysate from the proximal end of the membrane to a sampling tube.

Neurosurgical Procedure

A small craniotomy over the tumour region was made. Localization and extension of the tumour was established by an ultrasound sector scanning technique on the intact dura. Through small dural incisions the microdialysis probes were implanted in the tumour tissue and optimal placement was secured by the sonography. The micropumps, each charged with 3 ml of a Tris-buffered 0.125 M DAB solution made isotonic at pH 7.55 (Pharmacia AB, Uppsala, Sweden), were adapted to the input channel of the probe and a sampling tube to the output. These probes passed through a skin incision located at some distance from the craniotomy. They connected to the micropumps. The patients did not receive any radiotherapy or chemotherapy prior to or along with the DAB therapy.

Results

Three patients with inoperable malignant glioma were treated by DAB administered into the brain tumour with the microdialysis device. In the first patient one microdialysis probe was implanted in the tumour (astrocytoma grade III) and a micropump supplied the probe continuously with a total of 3 ml of 0.125 M DAB solution per day for 14 days. The second patient was treated for 21 days but she had two probes in the tumour (astrocytoma grade IV), each of them being supplied daily by 3 ml of the DAB solution. The third patient having astrocytoma grade IV received DAB similarly for a total of 15 days through 3 probes inserted in the tumour. DAB given in this way resulted in a massive necrosis of the malignant brain tumour in the 3 patients as judged by comparing computed tomography (CT scan) before and after DAB treatment.

The probe with intermediate position between the two others in patient 3 served as an analytical probe during the first 36 h. Hence, during this period only a physiological salt solution was given through this probe. The efferent fluid contained DAB, which means that a diffusion of DAB had taken place within the tumour tissue from the 2 other probes each administering 3 ml a day of the DAB solution at a distance of 11 mm from the probe used for analysis. After that all 3 probes were employed for administering purposes.

Post-treatment CT scan in patient 1 showed the position of the microdialysis probe and it was surrounded by a large tumour necrosis (Fig. 1). In order to verify this X-ray finding clearly, the patient was subjected to a craniotomy and the loose necrotic tissue could be seen and evacuated (about 12 ml). Figure 2 illustrates an evolving necrosing process around the 3 microdialysis probes in the tumour on CT scan in patient 3 during ongoing treatment 2 days after initiation of DAB administration. Patient 2 died 7 months after treatment because of gastro-intestinal rupture (presumably not related to DAB treatment) while patients 1 and 3 presented survival times that were about 3 times longer than the mean survival time for patients with malignant glioma receiving only radiotherapy – 16 respectively 17 months –. The treatment was well tolerated and there were no side effects assignable to DAB.

The fluid from the output channel of the microdialysis device of the 3 patients was sampled in 24 h

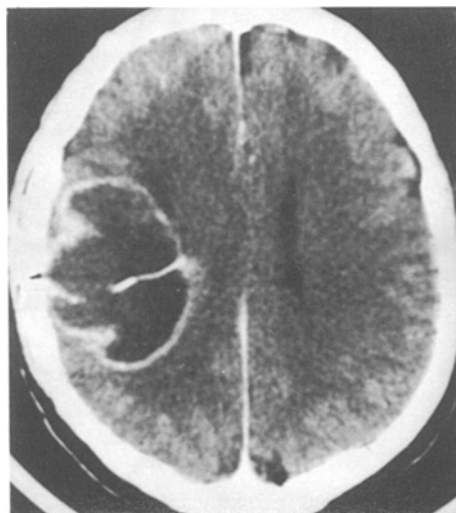


Fig. 1. CT scan of the brain of patient 1 after 14 days' treatment with DAB. The microdialysis probe in the tumour of the right parietal lobe is surrounded by a huge necrosis not observed before treatment. Survival time after DAB treatment: 16 months



Fig. 2. CT scan of the brain of patient 3 after 2 days' treatment with DAB. The 3 microdialysis probes are seen in the tumour together with an evolving necrosing process. Survival time after DAB treatment: 17 months

portions and analyzed with regard to some acidic, neutral and basic amino acids (Table 1). It is evident from Table 1 that the acidic amino acid (aspartate) remained rather low in the dialysis fluid of the patients during treatment. This was contrary to the very high concentrations found especially for arginine (a basic amino acid) and for alanine (a neutral amino acid) both of which being considered to be transported into cells mainly by system A (Table 1). The DAB concentration in the effluent fluid was estimated to be 60–75 mM, i.e. the yield of the dialysis procedure was 40–50% and these figures remained rather constant throughout the treatment period in the 3 patients.

Table 1. Microdialysate Content of Some Amino Acids During Treatment of Three Patients

Amino acid concentration, $\mu\text{mol/l}$

Day	Aspartate	Alanine	Arginine
3	6.3 \pm 4.1 (1.7–9.8)	166 \pm 65.4 (116–240)	191 \pm 230 (26–454)
6	11.2 \pm 7.4 (6.5–19.7)	394 \pm 176 (242–586)	717 \pm 52 (671–773)
12	14.9 \pm 10.7 (5.8–26.7)	362 \pm 143 (248–522)	782 \pm 28 (711–862)

Mean values \pm SD, range given in brackets.

Discussion

An unequivocal and extensive tumour necrosis was noticed on CT scans in the 3 patients treated with DAB. A causal relationship between this finding and the DAB treatment seemed evident although we did not prove by direct means an uptake of DAB in the brain tumour cells. A deposition of DAB in the brain tumour tissue was however apparent since the efferent fluid after dialysis contained only 60–75 mM DAB in contrast to the 125 mM DAB concentration given in the afferent fluid. Also, DAB was demonstrated in the dialysis fluid from the probe used initially for analytical purposes in patient 3 indicating a diffusion of DAB within the tumour tissue. Indirect evidence of a direct uptake of DAB in the tumour cells was provided by the biochemical investigation of the dialysis fluid. The free amino acids have been determined in cerebral astrocytoma tumour tissue by Shibasaki *et al.*⁸. Using their data the alanine/aspartate and arginine/aspartate ratios in tumour were 3.8 and 2.2, respectively. The corresponding ratios for dialysis fluid were as follows: 26 and 30 (day 3); 35 and 64 (day 6); 24 and 53 (day 12). Hence, much more alanine and especially arginine (transported by system A) in relation to aspartate (not transported by system A) were found in the dialysis fluid. This is consistent with the concentrated uptake of DAB in the tumour cells displacing intracellular arginine and alanine into the extracellular space preceding cellular lysis. Such a displacement is concordant with findings made in *in vitro* experiments on Ehrlich tumour cells incubated repeatedly with a high concentration of α -aminoisobutyric acid also transported by system A². A high concentration of arginine and alanine in the dialysis fluid due to other reasons, e.g. the small surgical trauma, seemed less probable since the acidic amino acid, aspartate remained rather low throughout the treatment period.

Another reason for tumour necrosis seen on CT scan could possibly be suspected to be an overload of DAB-containing solution. This is, however, contradicted by the inherent properties that govern the microdialysis principle which means that isovolumetric conditions are strictly maintained. We therefore conclude that our working hypothesis was valid also under *in vivo* conditions which means that DAB administered into brain tumour tissue by means of the microdialysis technique will result in a massive necrosis of the malignant brain tumour.

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