

Metabolic manipulation of glioblastoma in vivo by retrograde microdialysis of L-2, 4 diaminobutyric acid (DAB)

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Abstract

Objectives To study the metabolic effects in vivo of L-2, 4 diaminobutyric acid (DAB) administered by retrograde microdialysis in glioblastoma and to evaluate the feasibility of the technique.

Methods In 10 patients with glioblastoma, a stereotactic biopsy was performed followed by implantation of microdialysis catheters. One or two catheters were implanted in tumor tissue and two reference catheters were implanted in normal brain tissue and subcutaneous abdominal tissue, respectively. Tumor catheters were perfused with 80 or 120 mmol/l DAB and reference catheters were perfused with a Ringer solution, all with a flow rate of 2.0 µl/min. Treatment was given for at mean 9.1 (5–19) days.

Results The treatment was well tolerated by the patients with the exception of two patients in whom a

transient brain edema appeared. No complications related to the technique were encountered. During treatment, an increase in the extracellular amino acids alanine, glycine, glutamate, aspartate, serine, threonine, and taurine was found demonstrating a significant influence on the intracellular pool of free amino acids induced by DAB. No change in glucose metabolism or glycerol was evident. The metabolism in normal brain was unaffected during treatment.

Conclusions Retrograde microdialysis is a feasible method for intracerebral administration of drugs to tumor tissue in patients with glioblastoma. We found it possible to deliver DAB to glioblastoma tumors in fully mobilized patients and to assess the metabolic effects induced by the treatment. The changes in extracellular amino acids were in concordance to what was expected from in vitro studies. Elevation of glutamate and taurine may be regarded as markers for an induced cellular toxicity while the unchanged level of glycerol may indicate no direct effects on phospholipase activity and membrane phospholipid composition. The effects were restricted to the tumor compartment. Although an improved survival could possibly be suspected no dramatic effect on outcome could be detected. However, the series was small and, most probably, the time for treatment was too short.

Keywords Glioblastoma · Microdialysis · Diaminobutyric acid · Local regional treatment

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Introduction

It has become more and more obvious that the treatment of glioblastoma will need a multimodality

approach in order to reach a significant result. Chemotherapy is one of the options for treatment but, although some improvements have been reached recently, the prognosis is poor and there are still major obstacles to overcome. One issue is the difficulty to reach significant cytotoxic concentrations of the chemotherapeutic drug used throughout the tumor mass and surrounding infiltrated brain. The blood–brain (tumor) barrier may be disrupted in the central and often necrotic part of the tumor but tumor cells infiltrating the surrounding brain are probably protected by the barrier [1, 2]. It has been clinically confirmed that drug levels are significantly lower in peritumoral areas [3, 4] and it is usually in this peritumoral area, within 2 cm, where 90% of the recurrences will appear [5].

To improve the drug delivery to tumor cells several methods have been developed to overcome the blood–brain barrier. Intratumoral administration of various drugs using surgically implanted polymer matrices is one technique which has been demonstrated to achieve some advantages although not dramatically [6–9]. Other techniques have utilized the implantation of different types of catheters allowing a drug to be locally administrated into the tumor bed [10–12].

The technique of microdialysis was developed in order to provide a method for studying low molecular weight biomolecules in various tissues [13]. Initially it was adopted for the use in animals but later it was also adopted for humans and human brain [14–16]. In the very same way that microdialysis allows “collection” of substances from the extracellular space it is possible to add a substance or a drug to the dialysis fluid and by

“retrograde” microdialysis deliver it to the extracellular space in the preferred tissue [17]. This technique with retrograde microdialysis was first explored by Ronquist et al. in 1992 when they treated three patients with glioblastoma adding L-2, 4 diaminobutyric acid (DAB) to the dialysis fluid [17]. DAB has been found in vitro to exert cytotoxic effect on several types of tumor cells, including glioma cells [18]. DAB is a non-physiologic amino acid that is taken up in huge amounts in tumor cells by the transmembrane amino acid transport system, resulting in an irreversible cellular injury leading to cell death [18–20]. In this study we have extended this work now comprising 10 patients with special emphasis on some aspects of intermediary metabolism and amino acid fluxes in tumor and normal tissue induced by DAB. The feasibility of the technique and treatment was also evaluated.

Patients and methods

Ten patients with suspected glioblastoma multiforme scheduled for a stereotactic biopsy were included in the study (Table 1). The mean age was 59.1 (46–74) years and the male/female ratio was 5/5. Seven patients were therapy-naïve and three others had previously undergone treatment, resection or biopsy and radiotherapy, for a high-grade astrocytoma. For all patients with newly detected tumors, the tumor was centrally located and not suitable for debulking surgery. During the DAB treatment and microdialysis no additional therapy was given. All patients gave their full informed consent to participate and the Ethics Committee of

Table 1 Characteristics of the patients

Pat. no	Age (yrs)	Primary or recurrent	Localisation	Size (cm)	WHO perf. status	Side effects	CTCAE	Additional treatment	Best resp.	Surv. months
1	50	rec	Right front/central	2.4	0	paresis seizures	3	RT ^a , chemo ^a	PD	4.5
2	67	prim	left front/central	4.0	0	no	–	RT	CR	8
3	67	prim	right temp/central	3.8	1	no	–	RTchemo	PR	11
4	48	rec	left temp/central	5.7	1	dysphasia paresis	2	RT ^a , chemo ^a	PD	2.5
5	59	prim	left front/central	5.6	3	no	–	RT	PD	6
6	45	prim	left central	3.0	2	no	–	RT	PR	8
7	64	prim	left central	2.5	1	no	–	RTchemo	SD	12
8	73	prim	right central	5.1	1	no	–	RT	PR	3 ^b
9	52	rec	left occipital	6.0	0	no	–	RT ^a chemo	PD	9
10	62	prim	left central	7.7	2	no	–	RT	SD	3

Abbreviations. Size—largest diameter in cm; CTCAE—Common Terminology Criteria for Adverse Events v3.0, RT—radiotherapy; chemo—chemotherapy

^aTreatment given before DAB; Best response according to the Macdonald criteria, CR = complete regression, PR = partial regression, SD = stable disease, PD = progressive disease

^bNot tumor related death

Umeå University approved the study. The study was also approved by The Medical Products Agency which is the Swedish national authority for drug approvals.

The surgical procedure is described in detail in a previous paper [21]. Briefly, before surgery, patients underwent a CT for determination of stereotactic targets for the biopsy and catheter implantation. The Laitinen stereoguide and the Laitinen stereotactic frame were used for the stereotactic surgical procedure [22, 23] and the biopsy was mostly performed under general anesthesia. After a confirmation of the diagnosis glioblastoma by frozen section, one or two catheters were implanted into tumor tissue depending on the size of the tumor, and in 7 out of 10 patients one catheter was implanted in normal brain, outside the contrast-enhanced tumor volume. To minimize the tissue destruction, the same trajectory as for the biopsy was used. The catheters used for tumor tissue had a 100 mm shaft with a 30 mm semipermeable membrane (CMA 70, CMA Microdialysis, Stockholm, Sweden). The catheters implanted in normal brain had a 10 mm long membrane (CMA 70, CMA Microdialysis). All catheters had a semipermeable membrane with a cut-off of 20 kDa allowing a transmembrane passage of molecules smaller than 20 kDa. The catheters were tunneled away from the burr hole and brought out through a small incision in the skin. All patients were given betamethason starting at least the day before surgery and continuing during the DAB treatment. One patient (no5) that has had previous seizures were on carbamazepin during the treatment. Another patient (no1) that was on phenytoin before DAB treatment received carbamazepin in addition after having a seizure during the treatment.

Immediately after surgery all brain catheters were perfused with CNS-Ringer (Perfusion fluid CNS, CMA Microdialysis). The dialysis catheters were perfused with the aid of small battery-driven non-peristaltic pumps hidden in the dressing of the patient's head and the flow rate was 2.0 µl/min in all catheters (CMA 107, CMA Microdialysis). Surgery was always performed in the morning and ended before 12 a.m. Twelve hours, or more, were allowed for normalization of the tissue milieu before 8 h sampling to establish baseline levels of the metabolites. Treatment with DAB, via the microdialysis device, was started in the morning 1 or 2 days after surgery. The day after surgery all patients were fully mobilized and allowed to move freely at the neurosurgical or oncology ward and all of them had full normal enteral nutrition. According to the protocol, treatment was stopped on day 10 if no dysfunction of the catheters had been interfering earlier. However, some patients

demanded a prolonged therapy and in one patient treatment was actually given for 19 days. Hence, all 10 patients were treated for at least 5 days and 5 of them were treated for at least 10 days.

L-2,4-diamino-*n*-butyric acid (dihydrochloride) (DAB) was from Sigma Chemical Company, ST Louis, MO, USA. Two solutions were made: 80 mmol/l of DAB (first two patients) and 120 mmol/l of DAB (remaining 8 patients). The DAB solutions were neutralized and buffered at pH 7.55 by adding TRIS (Sigma). The first solution contained 45 mmol/l NaCl and the second 30 mmol/l. The solutions were slightly hyperosmolar, 380–400 mOsmol/l and they were freshly prepared by the local Pharmacy that made the necessary controls of sterility and possible presence of unacceptable levels of endotoxins. The prepared solution was subdivided and frozen at -70°C in small vials until being administered via the microdialysis device maintaining a flow of 2 µl/min. Measurement of substances in the low-protein environment of the extracellular fluid was carried out on microdialysis samples collected every second hour during the time from surgery until the end of DAB treatment. They were kept at -70°C until analysis. At the end of treatment the catheters were brought out simply by pulling them out through the small cutaneous incisions.

Biochemical analyses

Glucose and its metabolites, lactate and pyruvate, as well as glycerol were analyzed using the CMA 600 analyzer (CMA Microdialysis). These assays were based on auxiliary enzyme and photometric readings at 546 nm.

Samples intended for amino acid analysis were investigated with high performance liquid chromatography (HPLC). Amino acids were detected fluorometrically following precolumn derivatization with orthophthaldialdehyde, via a modification of Lindroth and Mopper's method [24], as described by Reid et al. in the presence of 2-mercaptoethanol at alkaline pH [25].

Statistical analysis and follow-up

Comparisons of metabolites at different time points in relation to baseline level were performed using Wilcoxon signed rank test for paired samples. For all metabolites, the mean concentration of samples collected between 0.00 at night and 8.00 in the morning was calculated and used for statistical comparison. All patients were followed until death.

Table 2 Drug penetration from microdialysis catheters delivering DAB into tumor tissue to catheters outside of tumor region

Distance outside of tumor in mm (No. of patients)	Maximal DAB concentration in percent of that in delivering catheter. Not corrected for recovery	DAB concentration (mmol/l) in tumor corrected for recovery	DAB concentration (mmol/l) in normal brain tissue corrected for recovery
0 (1)	7.0	46.1	6.1
10 (4)	4.2 (0.1–11.7)	22.0 (1.3–51.2)	3.7 (0.1–10.2)
15 (1)	1.0	0.5	0.1
25 (1)	0.1	1.6	0.1

The maximal values obtained during the treatment are reported as mean and range. Recovery of the delivering tumor catheter was estimated to 73% and in the assessing normal brain catheter to 33%

Results

In general the patients tolerated the treatment and procedure well. They were all mobilized and could freely move around at the ward. A few patients, actually, could also take a walk in the hospital park during treatment. Eight patients had no side effects from the treatment and did not suffer from any complication. In one patient, his right-sided arm paresis did deteriorate after 2 days of DAB treatment and he had a general seizure. The treatment was withheld and the paresis improved promptly. After another 2 days the treatment was restarted but unfortunately the catheter broke down shortly thereafter. Additionally, one patient suffered from a deterioration of a right arm paresis and worsening of speech. In this patient the treatment could be continued according to the protocol and the patient's wish. He improved slowly after termination of treatment. One patient developed fever and leucocytosis without any obvious cause. Since the catheters could not be ruled out as a source of infection those were taken out after 6 days of treatment. However, culture of the catheters was negative. Ten patients underwent at least 5 days of treatment and 5 of them were treated for 10 or more days. Mean time for treatment was 9.1 (5–19) days. When treatment was ended before the tenth day it was due to malfunction of the catheters except in one case reported above. When occurring, the malfunction presented a gradual decrease in dialysate from the catheters. In those cases an amorphous deposition was noted covering the semi-permeable membrane.

The drug penetration through the interstitial space from catheters delivering DAB in tumor tissue to sampling catheters in normal brain outside the contrast enhanced region was analyzed when possible. One patient had his reference catheter tip immediately outside the tumor, four patients had it 10 mm outside, one patient had it 15 mm outside, and one patient had

it 25 mm outside the tumor margin. The results are presented in Table 2 and Fig. 1. To get realistic figures we have made the assumption that the recovery of DAB in the treatment catheters was 73% (30 mm membrane) and that of the reference catheters in normal brain was 33% (10 mm membrane) [21]. It seemed that the tissue penetration of DAB is reasonable for at least 10 but not 15 mm.

The results from analysis of extracellular levels of amino acids in tumor tissue and normal brain are reported in Table 3, 4, 5, 6. In general there was an increase in alanine and glycine in tumor tissue (Fig. 2 a, b), the two amino acids that utilize the same membrane transport system, System A, as DAB does. The corresponding increase in normal tissue was slight and only significant at one time point. The excitatory acidic amino acids glutamate and to a minor degree aspartate

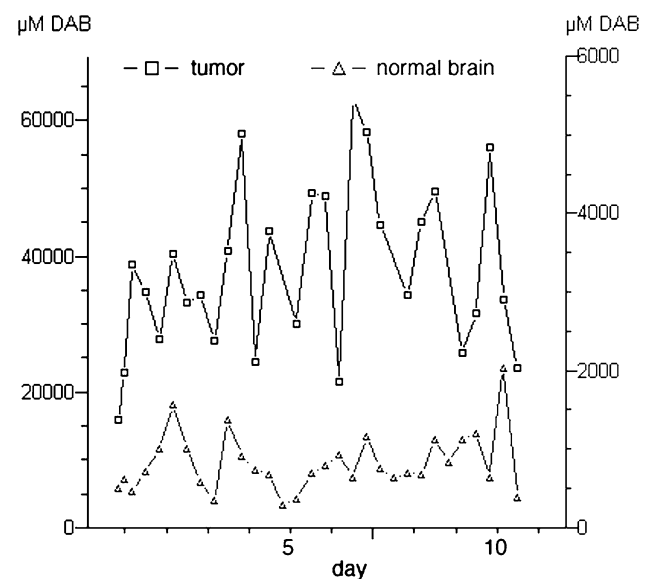


Fig. 1 Representative graph demonstrating the extracellular concentration of DAB in glioblastoma tissue (left y-axis) and normal brain (right y-axis) during DAB treatment by retrograde microdialysis

Table 3 Amino acids, glucose metabolites, and glycerol in tumor tissue in 10 patients with glioblastoma multiforme before and during retrograde microdialysis with DAB

		Baseline	Day 1	Day 2	Day 5
System A	Alanine	110.5	130.6	156.9	170.8 ^{0.0593}
	Glycine	57.5	80.8	104.3	101.7 ^{0.0593}
System L	Leucine	52.7	70.6	88.5	128.7
	Glutamate	16.7	29.1	74.3*	50.9**
Excitatory	Aspartate	3.6	4.9	18.8*	9.6**
	Serine	47.1	60.3	74.3	58.4
Neutral/OH containing	Threonine	38.9	57.0*	72.6*	66.3
	Tyrosine	27.6	26.9	34.5	36.4
Aromatic	Asparagine	20.8	21.5	24.4	26.8
	Taurine	15.0	37.3*	44.7*	33.3*
Substrate/metabolites	Glucose	0.5	0.3	0.2*	0.4
	Lactate	5.3	4.7	6.5	6.5
	Pyruvate	96.6	79.9	97.2	130.5
	Lactate/pyruvate	55.3	59.2	67.0	49.9
	Glycerol	43.9	39.8	48.5	47.2

Figures are given as mean values in $\mu\text{mol/l}$, except for glucose and lactate which are given in mmol/l . Statistical analysis with Wilcoxon signed rank test; * $P < 0.05$, ** $P < 0.01$

were significantly elevated with increase over time in tumor tissue but not in normal brain (Fig. 3). The efflux of serine and threonine, two neutral amino acids containing also a hydroxy group, were affected as well with increase in extracellular concentration, especially in tumor tissue. Tyrosine, leucine and asparagine remained stable during the course of the treatment, both in tumor and normal brain tissue. Extracellular taurine showed an evident increase during the treatment in tumor tissue but not in normal brain.

The glucose homeostasis is presented in Table 3–6. Extracellular level of glucose was mostly slightly lower in tumor tissue compared to normal brain while lactate and pyruvate were considerably higher. The differ-

ences for glucose will be even more pronounced if data are corrected for recovery, i.e. 73% for tumor catheters and 33% for reference catheters (data not shown). In general, glucose decreased the first, second and/or the fifth day of treatment in tumor tissue and normal brain. However, the change was small and with the exception of a decrease in lactate/pyruvate ratio day five in normal brain the glucose metabolism was unaffected.

The median overall survival of all the patients was 6.7 (2.5–12) months. In those patients with centrally located tumors undergoing a stereotactic biopsy and DAB followed by radiotherapy, 56–60 Gy, the median survival was 7.3 (3–12) months, while in patients with recurrent tumors it was 5.3 (2.5–9) months.

Table 4 Amino acids, glucose metabolites, and glycerol in normal brain in 7 patients with glioblastoma multiforme before and during retrograde microdialysis with DAB

		Baseline	Day 1	Day 2	Day 5
System A	Alanine	20.0	24.9	46.2*	20.7
	Glycine	12.5	12.9	22.7*	9.0
System L	Leucine	7.0	12.2	27.9	12.7
	Glutamate	3.3	7.8	15.0*	5.1
Excitatory	Aspartate	1.2	1.0	2.0	0.9
	Serine	15.0	16.1	19.9	12.9
Neutral/OH containing	Threonine	10.3	10.3	19.1*	11.9
	Tyrosine	7.5	6.5	13.6	6.9
Aromatic	Asparagine	4.4	4.7	8.4	6.9
	Taurine	7.0	6.5	6.7	4.7
Substrate/metabolites	Glucose	0.7	0.4	0.3*	0.3*
	Lactate	1.5	0.9	1.0	0.8
	Pyruvate	27.0	28.2	23.7	31.0
	Lactate/pyruvate	41.7	36.6	43.8	16.0*
	Glycerol	22.7	13.0	14.7	5.4 ^{0.07}

Figures are given as mean values in $\mu\text{mol/l}$, except for glucose and lactate which are given in mmol/l . Statistical analysis with Wilcoxon signed rank test; * $P < 0.05$

Table 5 Amino acids, glucose metabolites, and glycerol in tumor tissue in 5 patients with glioblastoma multiforme before and during retrograde microdialysis with DAB

		Baseline	Day 1	Day 2	Day 5	Day 10
System A	Alanine	64.6	66.2	80.1	147.4*	117.8
	Glycine	50.1	69.4	92.5	107.0*	97.6*
System L	Leucine	42.6	73.9	46.1	66.7	29.5
	Glutamate	24.2	38.8*	67.2*	63.8*	117.8*
Excitatory	Aspartate	5.2	7.2	11.3*	9.0*	9.8*
	Serine	31.5	46.4	67.1	56.2*	49.5
Neutral	Threonine	33.3	50.1*	73.0*	62.1*	49.9
	Tyrosine	20.5	23.9	35.6	32.5	25.8
Aromatic	Asparagine	14.5	14.8	17.4	19.7	14.6
	Taurine	14.7	30.4 ^{0.068}	42.9 ^{0.068}	29.8 ^{0.068}	29.4
Substrate/metabolites	Glucose	0.5	0.1*	0.1*	0.1	0.2
	Lactate	4.3	5.2	7.0	6.3	5.5
	Pyruvate	61.0	56.4	78.0	130.3	91.4
	Lactate/pyruvate	70.0	93.0	89.9	48.6	59.6
	Glycerol	27.2	52.5	52.1	39.4	36.1

Figures are given as mean values in $\mu\text{mol/l}$, except for glucose and lactate which are given in mmol/l . Statistical analysis with Wilcoxon signed rank test; * $P < 0.05$

Table 6 Amino acids, glucose metabolites, and glycerol in normal brain in 5 patients with glioblastoma multiforme before and during retrograde microdialysis with DAB

		Baseline	Day 1	Day 2	Day 5	Day 10
System A	Alanine	22.5	21.4	34.7	18.6	36.3
	Glycine	15.9	12.7	22.8	9.5	14.2
System L	Leucine	7.9	11.0	23.8	12.7	12.6
	Glutamate	2.7	9.0	13.1	5.0	6.3
Excitatory	Aspartate	1.3	1.0	1.2	0.9	1.4
	Serine	14.1	13.4	20.6	12.2	16.4
Neutral	Threonine	11.3	11.1	21.5*	12.2	18.2
	Tyrosine	8.2	5.4	11.3	5.1	6.0
Aromatic	Asparagine	5.0	4.1	8.4*	4.3	5.7
	Taurine	7.4	3.1	5.2	1.7	3.0
Substrate/metabolite	Glucose	0.4	0.4	0.3	0.2	0.2
	Lactate	1.1	0.9	1.0	0.7	0.8
	Pyruvate	23.9	23.7	24.2	24.6	22.5
	Lactate/pyruvate	45.5	39.6	42.4	30.4	33.8
	Glycerol	10.3	12.0	11.3	5.7	10.9

Figures are given as mean values in $\mu\text{mol/l}$, except for glucose and lactate which are given in mmol/l . Statistical analysis with Wilcoxon signed rank test; * $P < 0.05$

Discussion

By performing this study in patients undergoing a stereotactic biopsy only, we could minimize the influence of metabolic changes induced by the surgical trauma. We believe that this is very important since we demonstrated previously that even minimal surgical trauma can alter amino acid and glucose levels for up to 8–12 h after a minor surgical procedure, such as stereotactic biopsy, and catheter implantation [21]. The baseline was established after at least 12 h of postoperative stabilization. Also, from a pathophysiologic point of view, it should be most interesting to study effects and events in solid tumor tissue and not in tu-

mor remnants or effects in tissue at the border of a resection cavity. The high lactate levels and the differences found in tumor tissue versus normal brain are indicative of so called aerobic production of lactate that is typical for malignant tissue in general [26]. These findings are in concordance with what we found previously [21] also suggesting that the catheters were located in the intended tissue compartments. This was also confirmed by the histopathology taken at the same target spot as the tumor catheters were introduced. Altogether, this makes the tumors studied and the observed metabolic alterations highly representative.

DAB is a non-physiological amino acid which is transported into human cells via system A in a markedly

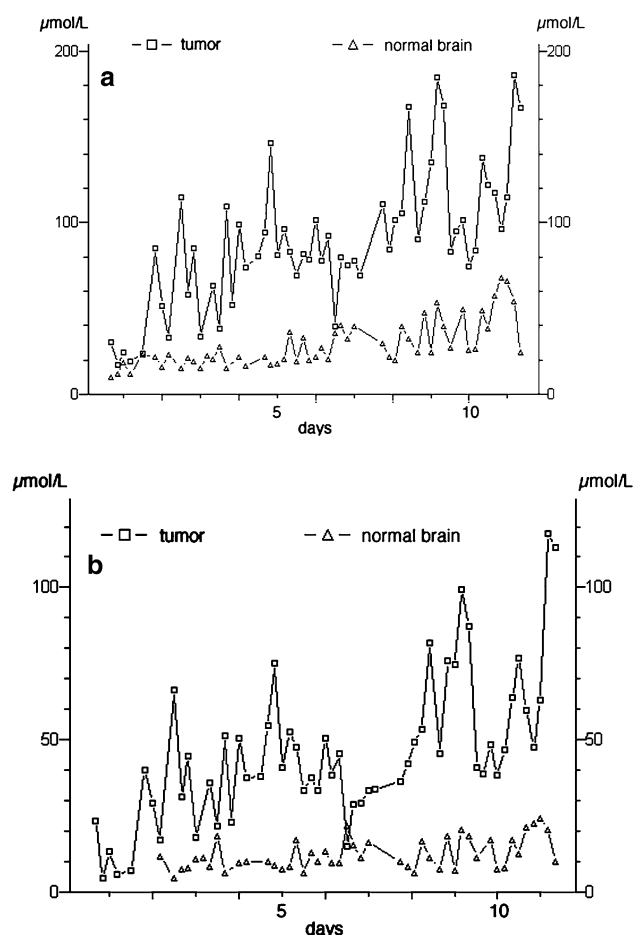


Fig. 2 Representative examples of the extracellular concentrations of the amino acids alanine (**a**) and glycine (**b**) during DAB treatment by retrograde microdialysis. Concentrations in glioblastoma tissue and normal brain are demonstrated

asymmetric way, i.e. great intracellular gradients of DAB can be generated [19]. In vitro studies on glioma cells have demonstrated that system A is much more expressed in malignant human glioma cells than in normal human glial cells [20]. Accordingly, human malignant glioma cells incubated with DAB were completely destroyed due to this very high uptake capacity of DAB at a concentration that did not affect concomitantly cultured glial cells, that escaped destruction due to their weakly expressed system A and therewith low uptake capacity [18].

The results described in this study give strong evidence that DAB was taken up by tumor cells in vivo. Analyzing the output dialysate from the catheters delivering DAB demonstrated that the tumor cells took up a significant part of the DAB administered. This tumor cell uptake in vivo of DAB was reflected by the reciprocal efflux of alanine together with glycine, serine, and threonine. According to the analyses from

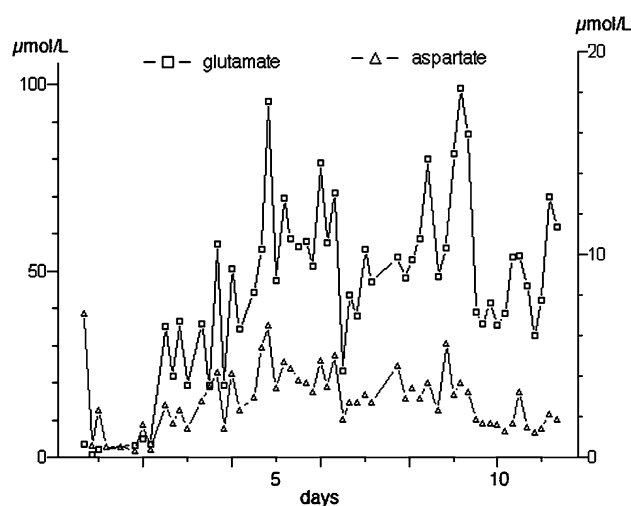


Fig. 3 Representative example of the extracellular concentrations of the amino acids glutamate and aspartate during DAB treatment by retrograde microdialysis. Concentrations in glioblastoma tissue are demonstrated

normal tissue, it seems that DAB does not diffuse in the extracellular compartment for a longer distance than 10 mm outside the tumor.

The increase in efflux of taurine during treatment with DAB was in line with this reasoning. The amino acid taurine is found in high concentrations in cardiac cells and also in brain cells and is suggested to serve as an important osmoregulator [27, 28]. The intracellular concentration of taurine, therefore, has a major influence on cell volume regulation, and the glioma cells are supposed to release taurine in response to a change in osmotic conditions. DAB, due to a supposedly huge intracellular uptake, which is sodium ion-dependent and therefore ATP-requiring via the Na⁺/K⁺ATPase, will become decisive for the tumor cell outcome. Sodium ions serve normally as the primary extracellular osmolytes to balance the osmotic effects of intracellular proteins, metabolites, and electrolytes. However, the huge DAB uptake dramatically alters the intracellular osmolyte content. Breakdown of a single molecule of ATP to 1 molecule of ADP and 1 molecule of orthophosphate transiently doubles the osmotic pull of the original single nucleotide molecule (in addition to the effect of intracellular DAB itself). Furthermore, stoichiometric amounts of sodium ions to the DAB molecules inside the cells will lead to at least a transient fall in the transmembrane sodium gradient. These factors cause the malignant tumor cells to swell.

Cell swelling with subsequent membrane failure has been proposed to be a major mechanism of cell death under such perturbed conditions (also illustrated by the exodus of the excitatory amino acids glutamate and

aspartate) [29]. If cells are subjected to such an osmotic insult, they respond by a substantial release of taurine [30, 31]. This movement of taurine out of the cell is an important process in the regulatory volume change that accompanies these insults. The acute taurine loss of tumor cells observed by us may be beneficial in these exposed malignant cells to reduce the osmotic pressure inside the cell therewith reducing the prospects of membrane rupture. This might be an explanation to the less successful effects on survival in our patients. Another possible explanation could be the augmented release of glutamate. There are reports claiming that release of glutamate is directly related to tumor growth: the more glutamate the tumor release, the larger the tumor mass. The authors speculate that glutamate carves an excitotoxic path of destruction through brain tissue, thus explaining the particularly invasive and destructive nature of gliomas [32].

In patients with unresectable, often centrally located, glioblastoma the median survival has been reported to be 13–32 weeks after biopsy and radiotherapy [33–36]. In our series the overall median survival was 8.0 (2–11) months for the seven patients with newly detected unresectable tumors. This may be an improvement, but our series was small and therefore no safe conclusions can be made. Additionally, one must also keep in mind two things. Firstly, the mean time for DAB treatment was only 9.1 days, which is probably not sufficient. Unfortunately, in a few patients the catheters started to malfunction early making the time for treatment shorter than planned. Secondly, all patients in this study had centrally located tumors with a historically poor prognosis whatever treatment given. The catheters used in this study were made for diagnostic purposes and were not intended to use for delivery of drugs, especially not drugs increasing the general level of amino acids in the extracellular compartment. However, it is our experience that the catheters do function for a longer time if used in non-tumor tissue, e.g. in patients suffering from severe brain injury.

It should be kept in mind that the principle of a continuous administration of a non-metabolizable amino acid with a steep uptake pattern in glioma cells is novel and not shared by other known chemotherapeutic principles. Therefore, this mechanism of action does not profit from the other known principles, meaning that DAB treatment should be additive and complementary to the other known mechanisms of action, when given in accordance with a multimodality approach, which could be a further developmental step.

The main contribution of this study is that it demonstrates that metabolic manipulation of malignant glioma is possible in vivo by retrograde microdialysis. Actually, the metabolic alterations induced by DAB in tumor tissue of the patients demonstrated grossly the same pattern as could be predicted from previous studies in vitro. Interestingly, the effects induced by the treatment were restricted to tumor tissue. The study also demonstrates that the technique with retrograde microdialysis in fully mobilized patients is feasible without any complications during the studied time span of up to 19 days. Any dramatic improvement in survival of the patients could not be established. However, the series was small and the time for treatment was probably too short. A further development of the catheters together with combining DAB administration with other drugs should be the next step in improving this approach to treatment of malignant glioma.

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