

Post-resection Cavity Lavage of High Grade Glioma With a Novel Drug Combination: A Case Report

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Abstract. *Background: High grade gliomas are the most common and most lethal primary cancers of the central nervous system. Case Report: We herein present a case report of a long-term surviving 36-year-old female diagnosed with high grade glioma, for which she underwent neurosurgery with a gross total removal of the tumor. Shortly thereafter (<3 months) she was readmitted in a desolate state due to a large recurrence. After Ethical Committee approval, proper explanation, and consent from spouse, she was subjected to a reoperation involving a post-operative infusion into the excised tumor cavity, containing a mixture of a non-physiological amino acid in millimolar concentration and a proapoptotic drug in micromolar concentration. The patient tolerated the treatment well and was discharged in a stable state thereafter. A series of follow ups revealed successive clinical improvements and after 4-6 months, she had recovered with mild left hemiparesis, meaning that she was able to carry out activities of daily living independently. Now, 5.5 years later, after the recurrence and the infusion therapy, she continues to have a mild left hemiparesis and her MRI with contrast shows no evidence of tumor. Conclusion: Continuous intratumoral infusion therapy with an artificial amino acid combined with a proapoptotic drug results in complete glioma cell lysis both in vitro and in vivo.*

Glioblastoma multiforme (GBM) is the most common and the most aggressive primary malignant brain tumor, striking approximately 3 in 100,000 people. Patients with GBM are faced with a poor prognosis, despite the combined treatment with radiation therapy and chemotherapy after surgical removal (1).

All cells in our body need amino acids for survival. The malignant cells are no exceptions, instead their demand of amino acids is much larger. We have analysed in detail the uptake process of both normal (physiological) and artificial (non-physiological) amino acids into human glia (normal) and human glioma (malignant) cells (2). Although the uptake process is mechanistically similar between glia and glioma cells, there is still a significant difference regarding uptake capacity between the cell types, being almost four times higher in glioma cells (2). Amino acids are needed as building blocks for protein synthesis, which is an obligatory prerequisite for cell division. The principal supply of amino acids for cells is from blood circulation.

Chemotherapy, besides surgery and radiotherapy, is one of three options for treatment but so far there are still major obstacles to overcome, e.g., the inherent tendency of cancer cells to develop therapy resistance to cancer drugs. A conspicuously different antitumor effect kind was noted for the non-physiological amino acid L-2,4 diaminobutyric acid (DAB) against cultured human glioma cells (3). The mechanism of action of DAB involves transport of this cationic, non-physiological amino acid against its gradient into cells by the energy-requiring System A in an unusually asymmetric fashion, resulting in a huge gradient (4, 5). Thus, DAB is readily taken up in a defined mode by both normal and malignant cells through this specific transport System A, at the expense of the sodium ion gradient (symport), leading to a stimulation of the Na⁺ and K⁺ ATPase to restore the ion gradient (4, 5). This in turn means a strain on the energy economy of especially the glioma cells due to its enhanced uptake capacity.

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The high concentration of DAB inside the malignant cells, together with cellular energy crisis, result in the development of osmotic pressure leading to cellular swelling due to an uncontrolled water influx and subsequently to cellular lysis (3, 5). The uptake route of DAB in human glioma cells is identical to that for some of the physiological amino acids. Therefore, the uptake process can be inhibited and the lethal effects of DAB can be abolished *in vitro* by concomitant incubation with either of the physiological amino acids alanine or methionine (3). The authenticity of the identical transport for DAB and alanine was also proven *in vivo* by the sharp extracellular increase of alanine during ongoing DAB intra-tumoral infusion due to an exclusion effect of alanine by DAB (6, 7).

The only cellular organelle primarily involved in the function of DAB is the plasma membrane where the energy requiring transport System A for concentrative transport of some vital amino acids into normal and preferentially malignant cells against their gradient is localized. An additional inference is that the high intracellular concentration of DAB and the generated osmotic strength are not mutagenic since the genome is not involved. This is contrary to most other known anticancer drugs. It should be kept in mind that normal (glia) and malignant (glioma) cells cannot discriminate between physiological and non-physiological amino acids. Accordingly, during transport of amino acids into GBM-cells, there is no preference for either physiological or non-physiological amino acids apart from their actual extracellular concentration. Thus, if DAB is in higher concentrations extracellular, a preferential uptake of DAB into glioma cells will occur. Since there is no saturation point for intracellular DAB, high enough concentrations can be achieved intracellularly that will mediate the development of osmotic strength leading to influx of water, thus culminating in malignant cell lysis enhanced by an energy crisis as discussed above.

Cancer drug resistance significantly limits long term therapeutic efficacy (8). It is difficult to imagine how malignant cells can develop a drug resistance towards DAB, simply because the malignant cells cannot abandon the metabolic goal of their existence, which is to reproduce themselves, and for that, physiological amino acids are desperately needed. Accordingly, its mechanism of action, DAB is unique and does not interact with any other known anticancer drug, allowing an efficacious combination therapy with one or more other drugs.

Based on analysis of previous clinical experience with DAB administered by one (or more) analytical microdialysis probe(s) intratumorally (6, 7), and aiming to minimize the impact of tumor recurrence factors, we decided to replace the somewhat fragile microdialysis probe with a more robust one lacking a dialysis membrane for drug delivery. By doing so we were able to evacuate cells and cellular constituents as

well as extracellular vesicles (EVs) present in the tumor microenvironment (TME). Additionally, we combined DAB with another drug for better efficacy against glioblastoma initiating cells.

In our attempts to find an effective mixture, we carried out combined incubations *in vitro* of normal glia and malignant glioma cell lines with DAB in millimolar concentrations and Prazosin in micromolar concentrations. We found clear-cut potentiating effects by the combination of DAB and Prazosin over and above what was achieved through incubations with either DAB or Prazosin alone (Figure 1). Moreover, a dose-response relationship was achieved by the drug combinations. It should be noted that incubation of human microglial (normal) cells (HMC3) under conditions identical to those of malignant glioma cells (LN18) resulted in practically no harm at all for the (normal) glial cells (not shown in Figure 1). These *in vitro* results encouraged us to proceed with the clinical trial after Ethical Committee clearance and informed consent concerning the present patient.

Prazosin is a well-established α_1 - and α_2 -adrenergic receptor antagonist, with strong proapoptotic activity, which is a potent inducer of cell death of high-grade glioma, as demonstrated by the increased survival time in glioblastoma-bearing mice (9, 10). This is well in line with findings that Prazosin displays anti-growth activity with increased cell death in erythroleukemia (11), osteosarcoma (12), and medullary thyroid carcinoma (13).

Case Report

A 36-year-old female patient presented to the clinic with headache and vomiting. She had a limp to the left side and difficulty using her left hand. On examination she had features of raised intracranial tension (ICT) manifested by a grade 2 papilloedema in both eyes. She also had a grade 3 hemiparesis of both left upper and lower limbs.

Magnetic resonance imaging (MRI) was suggestive of an aggressive intrinsic lesion of the right parieto-occipital region of the brain (Figure 2). The lesion was hyperintense on MRI contrast with indistinct margins and multiple flow voids. In T2 flair sequence, there was significant perilesional oedema causing a mass effect and midline shift of over 1.7 cm.

The patient was counselled regarding the nature of the disease and the need for adjuvant therapy. She was taken up for a tumor decompression and tolerated the procedure well. Post-operative care with steroids and anti-convulsants was given as per the standard protocol and the hemiparesis and headache declined significantly. She was discharged walking and asked to review after 2 weeks to start adjuvant therapy. The patient was seen again after about 3 months in the outpatient clinic with headache, vomiting and hemiparesis like her initial presentation. MRI imaging showed a massive recurrence within 3 months after successful surgery (Figure

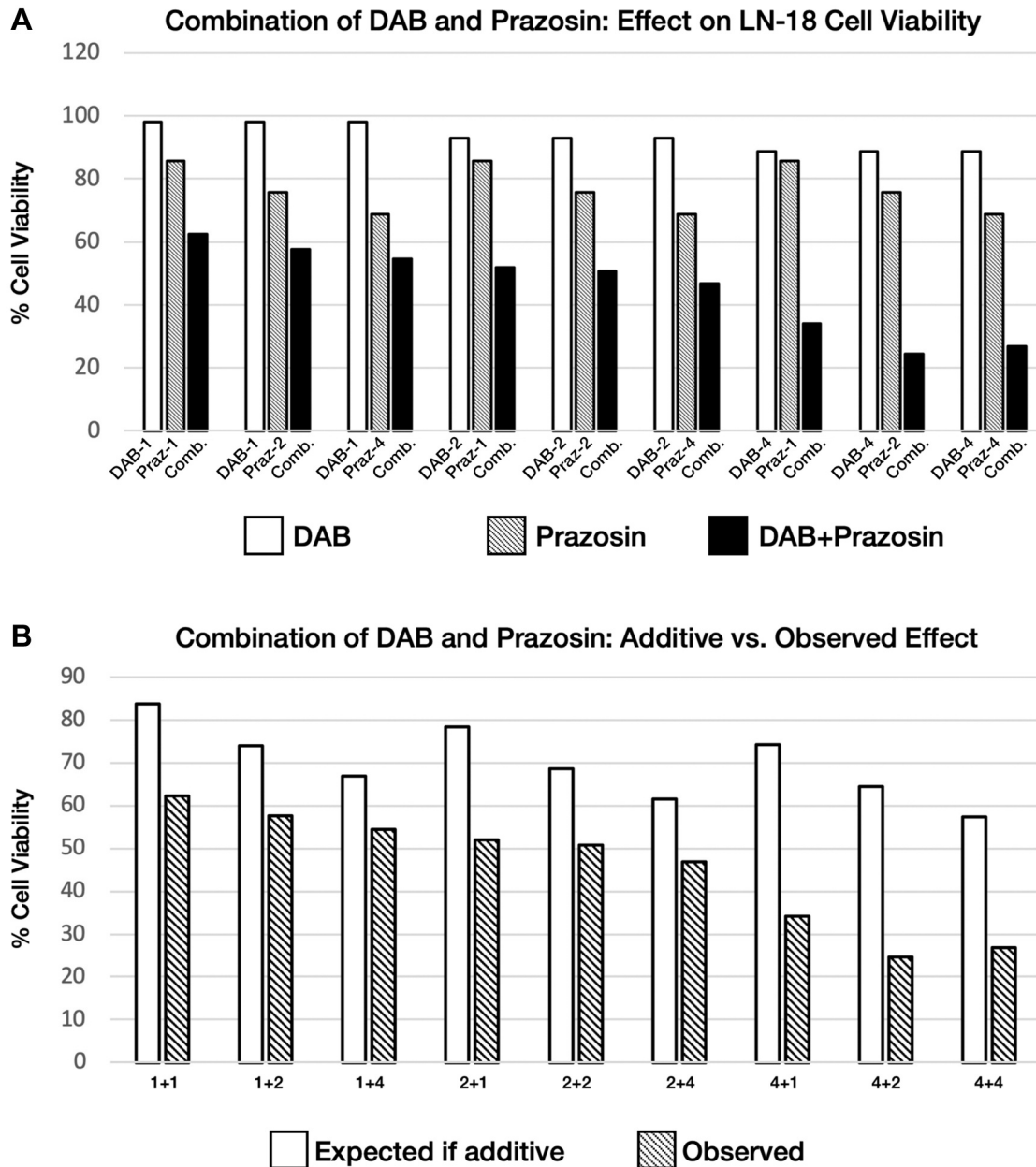


Figure 1. Viability of glioma cells in response to DAB and Prazosin treatment. A) Dose response effects on glioma cell (LN-18) survival following incubation with different concentrations of either DAB (1, 2 and 4 mmol/l, white column) or Prazosin (1, 2 and 4 μ mol/l, grey column) alone, compared with the combined lethal action of the two drugs (black column). Incubation time: 24 h at 37°C. B) Comparison between calculated lethal effect of drug combination (DAB, plus Prazosin; data picked up from Figure 1A) and observed lethal effect of the drug combination revealing a potentiating effect rather than a simple additive one.

3). The aggressive nature of the tumor coupled with short time to recurrence implied a very poor prognosis. At this juncture, the above-described experimental tumor cavity infusion therapy was considered for this patient.

After Ethical Committee's approval and explaining the experimental nature of the procedure to the patient and spouse,

a consent for surgery and local postoperative infusion was obtained. The patient was reoperated, and a gross total resection of the lesion was performed. Two external ventricular drain tubes (EVD) were placed in the tumor cavity (Figure 4). The tumor was infused for 90 hours with an isotonic, slightly alkaline drug solution of L-2,4-diaminobutyric acid (DAB) at

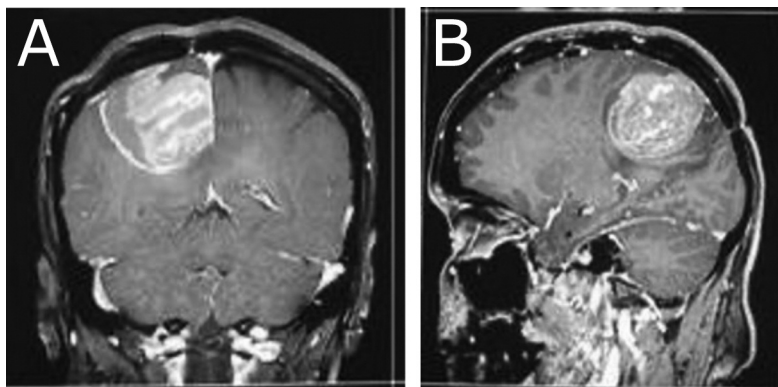


Figure 2. Coronal (A) and sagittal (B) view of a contrast MRI of the patient's brain showing a large intra axial lesion, with heterogeneous enhancement on contrast, causing mass effect and midline shift on the surrounding brain.



Figure 3. Images of the patient's brain after the re-emergence of the lesion showing a vascular recurrence, as visualized in an axial contrast magnetic resonance imaging image in (A), a T2 weighted image in coronal plane to show the perilesional reactive oedema in (B) and a sagittal contrast image in (C) showing a central area of necrosis.

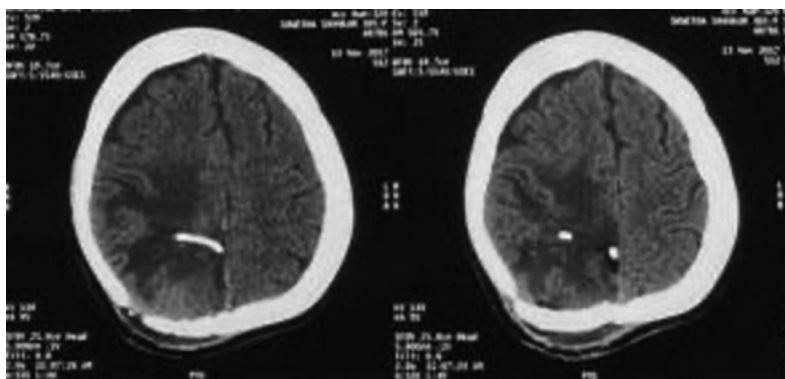


Figure 4. Computed tomography scan of the brain showing the catheter in situ in the excised tumor's cavity.

100 mmol/l and Prazosin at 25 μ mol/l, in a Tris-buffered solution at pH 7.55 and 37°C, with a flow rate of 3-6 ml/h.

At every 2-4 h, the infusion was paused to allow for drainage of the infusate/necrotic fluid from the cavity and prevent a rise

in ICP, as well as to carry out a lavage of remaining malignant cells and extracellular vesicles (EVs) present in the tumor microenvironment (TME). The patient was carefully monitored for seizures, features of raised ICT, or new onset of neurological

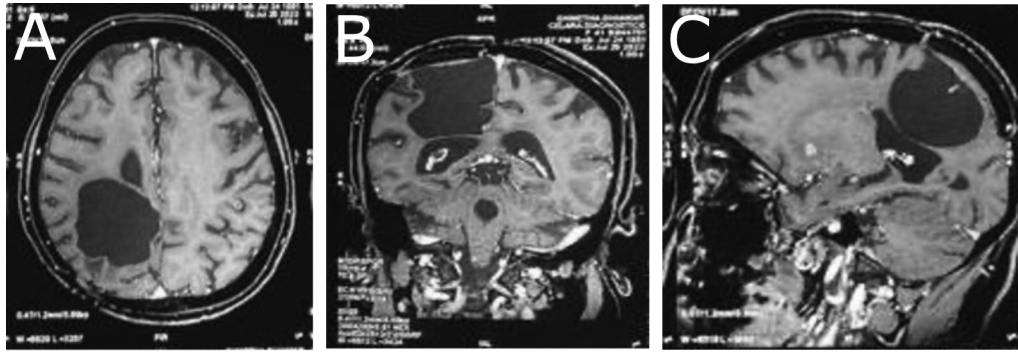


Figure 5. Five-year postoperative contrast magnetic resonance imaging (MRI) images of the patient's brain, axial (A), coronal (B) and sagittal (C), showing the excision cavity with absolutely no enhancement of the wall or surrounding tissue, indicating no recurrence.

deficits. The patient experienced multiple focal seizures which were controlled with anticonvulsants and eventually became stable and was discharged for follow up.

On review, she was improved dramatically, although her pre-existing left hemiparesis persisted. She complained of on-and-off mild focal seizures, which were controlled with antiepileptic medication. One month later she was started on adjuvant Temozolomide and radiation therapy and tolerated the sessions well. She gained weight and began attending her activities of daily living with minimal assistance (she used a walking stick to help her walk). Reviews yearly have been conducted for 5 years with MRI imaging (Figure 5).

At the end of 5 years, MRI revealed no tumor. However, over this period, she did show fluctuations of her hemiparesis, probably the result of a local ischemic insult caused initially by tumor compression. Thus, she is still undergoing rehabilitation therapy.

Conclusion

Intratumoral infusion therapy with millimolar concentrations of DAB and micromolar concentrations of Prazosin offers a ray of hope in the otherwise bleak prognosis of glioblastomas. The present case report may represent a first step in the development of a novel drug combination for postoperative intratumoral catheter enhanced delivery (CED) with robust *in vivo* efficacy.

Conflicts of Interest

None, apart from Christos Panotopoulos (CP): Founder and Director of Inderes Ltd. - owner of all intellectual property rights relative to this article.

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