



Intratumoral retrograde microdialysis treatment of high-grade glioma with cisplatin

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Abstract

Purpose This study evaluates the application of a microdialysis technique for interstitial chemotherapy using cisplatin in high-grade glioma.

Method An in vitro study demonstrated that cisplatin can be administered through retrograde microdialysis and defined the recovery for cisplatin. In a subsequent phase I study, 1–4 microdialysis catheters were implanted in tumor tissue, brain adjacent to tumor (BAT) tissue, and subcutaneous tissue in 10 patients with recurrent high-grade glioma. Cisplatin was administered continuously in daily doses between 0.3 and 3.9 mg for 4 to 12 days. Microdialysis samples were continuously collected and analyzed for glucose metabolites, glutamate, glycerol, and cisplatin concentrations. Treatment tolerability was evaluated through clinical monitoring. Quality of life was assessed using the EORTC-QLQ-C30 questionnaire for up to 3 months after treatment.

Results This in vitro study showed that cisplatin could be administered with a recovery of 41–97%, depending on flowrate, type of catheter, and cisplatin concentration. During the treatment, patients were exposed to a total dose of 1.2–36.8 mg cisplatin. The concentration of cisplatin in BAT, serum, and subcutaneous tissue was close to detection level in all but two patients. A transient neurologic deterioration due to edema was commonly observed, but no systemic side effects were recorded. After onset of treatment, concentrations of glutamate and glycerol were significantly increased in tumor tissue but not in BAT, with a peak after 3 days, and consistent for the rest of the treatment. Five of the patients survived between 153 and 492 days after treatment.

Conclusion This phase I study demonstrates that retrograde microdialysis can be used to administer cisplatin interstitially into high-grade glioma tissue. A high cytotoxicity was detected in tumor tissue, but not in the surrounding brain. Retrograde microdialysis appears to be a clinically useful method for intratumoral drug administration in high-grade glioma.

Keywords Retrograde microdialysis · Malignant glioma · Cisplatin · Brain microdialysis · Interstitial · Chemotherapy

Introduction

Although chemotherapy is an important component of the primary treatment of glioblastoma [20], no standard second

line treatment has significantly improved survival. There are two major obstacles to overcome in the development of new chemotherapeutic regimens for this disease. First, the intrinsic chemoresistance of glioblastoma cells is a limiting factor in chemotherapy of brain tumors. Second, the blood–brain barrier (BBB) prevents most hydrophilic drugs and drugs of higher molecular weight from entering the brain compartment. During the last decades, several advances have been made in the delivery of drugs locally to brain tumors. Presently, five potential approaches exist for achieving high intratumoral drug concentrations without the associated systemic toxicities: enhancing drug permeability through the BBB, temporary disruption of the BBB, interstitial delivery of drugs via catheters, convection-enhanced delivery of drugs to the CNS, and the use of polymers or microchips to directly deliver medical therapy [13]. These five advances have increased the interest

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in developing new systems to interstitially deliver potent drugs into brain tumors in hopes of increasing therapeutic efficacy and reducing systemic side effects. This study shows that there could be another approach, retrograde microdialysis.

Originally, microdialysis was used to monitor and measure the chemical composition of interstitial tissue by sampling soluble molecules from interstitial spaces using a semipermeable membrane at the tip of a microdialysis probe. Stereotactic microdialysis allows precise and accurate placement of catheters, making it possible to study the metabolic and immunological events taking place within a specific area of the brain. In earlier studies, we used stereotactic microdialysis to investigate the basic metabolism in glioblastoma by establishing baseline levels for metabolites in malignant glioma and brain adjacent to tumor [16]. In addition, we have used this technique to measure glucose metabolites and cytokines to investigate changes in tumor tissue following radiotherapy [21, 22, 26].

In addition, we have successfully adopted the technique to deliver an antimetabolite, diaminobutyric acid; however, in the present study, we use retrograde microdialysis to deliver a more potent chemotherapeutic agent, cisplatin [2, 19]. We hypothesize that interstitial delivery of drugs to brain tumors increases the therapeutic efficacy and reduces systemic side effects. Cisplatin, a small molecule and highly active anticancer agent, is widely used for treatment of different solid tumors [4, 15, 24]. Studies suggest that systemically administered cisplatin has a beneficial effect in the treatment of brain tumors [14]. However, cisplatin's significant systemic toxicity and inability to pass the BBB have limited its clinical application in glioblastoma [4, 19]. Cisplatin, usually administered intravenously, displays toxic effects on kidney, bone marrow, and neuronal tissue [3, 25].

In this phase 1 study, we aimed to administer cisplatin locally in tumor tissue and simultaneously monitor metabolic effects of the treatment in tumor as well as in surrounding brain tissue and systemic compartments. By using this approach, we aimed to administer clinically significant doses of cisplatin without the adverse systemic side effects.

Methods

In vitro

An in vitro study was performed to test whether the administration of cisplatin is possible using microdialysis catheters and to define the recovery for cisplatin. The microdialysis catheters used had 30-mm-long semipermeable membranes with a 20-kDa or 100-kDa cut-off (CMA 70), placed in a buffer solution prepared in house to resemble the extracellular fluid in the brain (NaCl 147 mmol/L, KCl 2.7 mmol/L, CaCl 1.2 mmol/L, and MgCl 0.85 mmol/L). The microdialysis

system was perfused with Perfusion Fluid CNS® (NaCl 147 mmol/L, KCl 2.7 mmol/L, CaCl 1.2 mmol/L, and MgCl 0.85 mmol/L prepared by M Dialysis AB) and contained 0.1, 0.5, or 1.0 mg/mL of cisplatin using flow rates of 0.5, 2.0, and 5 uL/min. In one catheter, Plasmadex Meda (1000 ml containing dextran-60 30 g, sodium chloride 5.9 g, sodiumacetat 4.1 g, kaliumchlorid 300 mg, calciumchloriddihydrat 295 mg, and magnesiumchloridhexahydrat 200 mg) was added to the dialytic fluid to prevent ultrafiltration (22). Thereafter, cisplatin concentrations in the buffer solution were analyzed to quantify the amount of cisplatin transferred to the buffer solution through microdialysis.

In vivo

Patients

This study included 10 patients with relapsing high-grade glioma not suitable for further surgery, re-irradiation, or other established treatments. However, neither patients with multifocal relapses nor patients with severe organ dysfunction, including renal impairment, were included in the study.

After providing written informed consent, the patients were included in the study and a baseline MRI was obtained prior to intervention. Nine patients had a histologically verified glioblastoma and one had an anaplastic oligoastrocytoma. Two of the ten patients had a secondary glioblastoma primarily diagnosed as a low-grade glioma. These two patients had mutations in the gene encoding isocitrate dehydrogenase (IDH1, R132H). The study included five males and five females with a mean age of 54.3 years (range 40–80). The median time from diagnosis to inclusion was 20.3 (4.7–153) months (Table 1).

All patients had primarily been treated with surgery, radiochemotherapy, and adjuvant temozolomide chemotherapy according to national guidelines. Two of the patients also received second line treatment with bevacizumab-based chemotherapy at first recurrence [17]. At the time of inclusion, all patients exhibited disease progression and therefore were not suitable for further surgery or any conventional chemotherapy. All patients entering the study had, by our clinical impression, an estimated survival of at least 3 months. The two patients who had the longest survival after the microdialysis treatment received either irinotecan/bevacizumab (patient 4) or reirradiation 3.4 Gy × 10 (patient 10) as second line treatment. A baseline MRI was obtained for all patients.

Surgical implantation of catheters

Eight patients received stereotactic implantation of microdialysis catheters under general anesthesia with the aid of a Leksell stereotactic frame (Elekta, Stockholm, Sweden). After mounting the frame, a stereotactic CT was performed

Table 1 Patients characteristics, survival, and overview of catheters and drug delivery

	Sex (M/F)	Age	WHO performance status	Tumor location	Diagnosis	Treatment catheters	BAT catheter	S.C. catheter	Survival (days)
1	F	52	1	Frontal dx	GBM	1 × 100 kDa/40 mm	1 × 100kDa	1 × 100 kDa/30 mm	153
2	M	48	1	Frontal sin	GBM	3 × 100 kDa/40 mm	–	1 × 100 kDa/30 mm	34
3	M	46	2	Frontal sin	GBM	3 × 100 kDa/40 mm	–	1 × 100 kDa/30 mm	72
4	M	50	1	Frontoparietal dx	GBM	2 × 20 kDa/30 mm	1 × 20 kDa/30 mm	1 × 20 kDa/30 mm	492
5	M	44	1	Temporal dx	GBM	3 × 20 kDa/10 mm	1 × 20 kDa/10 mm	1 × 20 kDa/30 mm	179
6	F	72	2	Temporal dx	GBM	3 × 20kDa/30 mm	1 × 20 kDa/30 mm	1 × 20 kDa/30 mm	30
7	F	60	1	Frontal sin	GBM	3 × 20 kDa/30 mm	1 × 20 kDa/30 mm	1 × 20 kDa/30 mm	188
8	M	80	2	Temporal sin	GBM	2 × 20kDa/30 mm	1 × 20 kDa/30 mm	1 × 20 kDa/30 mm	10
9	F	51	1	Temporal sin	GBM	3 × 20 kDa/30 mm	1 × 20 kDa/30 mm	1 × 20 kDa/30 mm	47
10	F	40	2	Temporal dx	Oligoastro III	2 × 20 kDa/10 mm	1 × 20 kDa/10 mm	1 × 20 kDa/30 mm	383

GBM glioblastoma multiforme, *Oligoastro III* oligoastrocytoma grade III, Dx dexter, Sin sinister

for target calculations. A previous study has described stereotactic introduction of microdialysis using catheters [16]. In two patients, the catheters were implanted using neuronavigation. The number of catheters used was based on the volume and configuration of the tumor. From previous studies and the first patients treated, we estimated that cisplatin had a tissue penetration of about 10 mm around the catheters, knowledge used to optimize the positioning of the catheters in the following patients [2] (Table 2). One catheter was placed in brain adjacent to tumor (BAT) approximately 10 mm outside of the contrast-enhancing lesion. The intracranial catheter Dyphylon Cardiac Catheter® (100 kDa) was used in patients 1–3, CMA 62® (100 kDa) in the patient 4, and CMA 71® (20 kDa) in patients 5–10. Systemic reference catheters were used: CMA 71® (100 kDa) in patients 1–3 and CMA 60® (20 kDa) in patients 4–10, all placed in the abdominal subcutaneous tissue. All catheters used the same semipermeable polyamide membrane.

Treatment

After surgery, the patients were monitored in the neurological intensive care unit. A postoperative CT scan confirmed the position of the catheters before treatment was started (Fig. 3A, B). Immediately after surgery, microdialysis was started to establish baseline levels of analyzed metabolites. The day after surgery, the patients were mobilized and moved to an ordinary ward. Cisplatin treatment was started in one of the catheters, usually the most centrally located, allowing pharmacokinetic analysis of cisplatin in the other catheters. On the second day after surgery, treatment was started in the other catheters implanted in tumor tissue.

The plan was to administer cisplatin in a dose-escalated manner, starting at 1 mg/day in patients 1–4 for 12 days. In

the following patients, the dose was planned to be increased to 3 and later to 5 mg/day. The aimed daily doses were based on previous studies that used local infusion of cisplatin [6, 12]. The concentration of cisplatin in the dialytic fluid and the flow rate in each catheter were based on our in vitro experiment and depended on the planned daily dose and the number of catheters used. Commercially available cisplatin was used in a 1-ml solution of 1-mg cisplatin in 9-mg/ml NaCl (Cisplatin Meda® (patients 1–5), Cisplatin Ebewe® (patient 6), Cisplatin Accord® (patients 7–9), or Cisplatin Hospira® (patient 10). The solution used for patient 10 also included 1-mg/ml mannitol, which was added by the manufacturer.

Evaluation

Daily clinical examinations were used to evaluate the neurological status of the patients. Fasting blood samples, used to analyze blood cell count, electrolytes, liver enzymes, creatinine, and C-reactive protein (CRP), were taken in the morning before treatment and after 3, 6, and 12 days of treatment and when clinically needed. Blood samples for pharmacokinetic analysis were collected on the same days.

The patient's quality of life (QoL), cognitive status, and neurologic function were evaluated with the self-reported questionnaire EORTC-QLQ-C30 before treatment and after 1 and 3 months, depending on the condition of the patient [1, 23].

Microdialysate

The collected microdialysate was analyzed for cisplatin concentration and as potential marker for cytotoxicity, glutamate, and glycerol. Total platinum content was determined using inductively coupled plasma mass spectrometry (ICPMS).

Table 2 Overview of intratumoral delivery of cisplatin by microdialysis catheters. In all patients with more than one tumor catheter, the treatment started in catheter I 24 h before the other tumor catheters. The concentration of cisplatin was analyzed after 12 h in the non-drug-delivering tumor catheters if present and after 12 h, 3 days, 6 days, and 7–9 days in BAT catheters if present and functioning

Patient no	Tumor catheter I			Tumor catheter II			Tumor catheter III			BAT catheter			Catheter in subcutaneous tissue				Blood	
	Membrane (mm)	Cisplatin (mg/mL)	Flow rate (μL/min)	Distance* (mm)	Cisplatin (μg/mL) 12 h	Distance (mm)	Cisplatin (μg/mL) 12 h	Distance (mm)	Cisplatin (μg/mL) 12 h	Distance (mm)	Cisplatin (μg/mL) 12 h	Cisplatin (μg/mL) 3 days	Cisplatin (μg/mL) 6 days	Cisplatin (μg/mL) 7–9 days	Cisplatin (μg/mL) 12 h	Cisplatin (μg/mL) 6 days	Cisplatin (μg/mL) 12 h	Cisplatin (μg/mL) 5–7 days
1	30	1.0	2.0	–	–	–	–	14.8	0.114	0.704	0.717	1.19 ⁹	0.009	0.002 ⁶	< 0.02	< 0.02	< 0.02	0.07 ⁶
2	30	1.0	0.5	16.0	< 0.02	21.3	< 0.02	–	–	–	–	–	< 0.001	< 0.001 ⁶	< 0.02	< 0.02	< 0.02	0.08 ⁷
3	30	1.0	1.0	30.5	< 0.02	40.3	< 0.02	–	–	–	–	–	0.0010	0.0013 ⁶	< 0.02	< 0.02	< 0.02	0.09 ⁷
4	30	1.0	0.5	20.5	0.24	–	–	20.1	0.26	0.19	1.68	4.41 ⁷	< 0.02	< 0.02 ⁶	< 0.02	< 0.02	< 0.02	0.04 ⁷
5	10	1.0	1.0	9.6	< 0.02	17.6	< 0.02	34.0	< 0.02	< 0.02	< 0.02	< 0.02 ⁷	< 0.02	< 0.02 ⁶	< 0.02	< 0.02	< 0.02	0.02 ⁷
6	30	1.0	1.0	36.9	0.68	13.9	0.80	27.5	< 0.02	0.04	0.05	0.04 ⁹	< 0.02	< 0.02 ⁶	< 0.02	< 0.02	< 0.02	0.10 ⁷
7	30	1.0	1.0	17.6	< 0.02	17.2	< 0.02	29.6	0.06	0.14	0.22	0.46 ⁹	< 0.02	< 0.02 ⁶	< 0.02	< 0.02	< 0.02	0.17 ⁷
8	30	1.0	1.0	15.0	0.01	–	–	31.2	1.60*	1.40	7.4	–	0.40	< 0.02 ⁵	< 0.02	< 0.02	< 0.02	0.05 ⁴
9	30	1.0	1.0	15.7	7.70	33.0	4.1	17.8	< 0.02	0.70	1.9 ⁵	–	5.80	0.10 ⁵	< 0.02	< 0.02	< 0.02	0.09 ⁴
10	10	1.0	0.5	14.7	2.08	–	–	24.0	18.4*	54.5	–	–	1.30	3.80 ⁵	< 0.02	< 0.02	< 0.02	< 0.02 ⁴

*Distance = distance to catheter I in which the treatment was started 24 h before catheters II and III. Measurements between the midpoint of the semipermeable membrane of the catheters. Superscript numbers report after how many days the samples were analyzed
BAT brain adjacent to tumor

Before analysis, sample solutions were diluted to a suitable concentration range with a 3% HCl solution, and indium was added as an internal standard. If cisplatin concentration needed to be quantified, hydrophilic interaction chromatography (HILIC) coupled to ICPMS was used [7]. Glutamate and glycerol were analyzed with a standard enzymatic analyzer (CMA 600, CMA Microdialysis, Stockholm). These samples collected between 4:00 and 6:00 a.m. and 4:00 and 6:00 p.m.

Follow-up

The patients were evaluated at 1 and 3 months with clinical evaluation, MRI, EORTC-QLQ-C30, and EORTC-BN20. Additional follow-up was performed if the treatment or the condition of the patient warranted.

Statistical analyses

Statistical evaluation was performed using IBM SPSS Statistics 26 (IBM, Armonk, NY). Non-parametric statistics were applied since data could not be considered to be normally distribute due to the limited number of observations. Hypothesis testing was performed using Wilcoxon signed rank test. A $p < 0.05$ was considered significant.

Ethics and permissions

The study was approved by the Swedish Medical Products Agency (EU no. 2010-018281-23) and the Ethics Committee of Umeå University Medical Faculty (ethical permission: 09-199M). The patients were fully informed of the experimental nature of the treatment both orally and in writing to ensure they understood the experiment and their rights as participants. All patients participated voluntarily and provided their informed consent.

Results

In vitro

The in vitro study clearly showed that delivery of cisplatin to the buffer solution, which resembled the extracellular fluid of the brain, was possible using microdialysis (Fig. 1). The obtained data made it possible to calculate the concentration of cisplatin and the flow rate of the dialysate for the patients.

Treatment tolerability

MRIs or CT scans during the treatment confirmed that all patients developed some edema around the catheters (Fig. 3G and H; Table 3). Edema resulted in transient neurological deterioration in six patients, who were treated with an

increased dose of corticosteroids (six patients) together with a decreased cisplatin dose (five patients). In four patients, the treatment was aborted before the planned 12 days of treatment. In all cases, the edema regressed after the treatment. The observed side effects are presented in Table 3.

Patient 8 died 10 days after start of treatment. This patient had deteriorated just before treatment, and the condition progressed after the treatment was started. As with the other patients, this patient's radiological control showed nothing more than edema around the catheters. Furthermore, the edema regressed as the therapy was aborted and steroids were given. Despite this, the patient did not recover, dying shortly after being transferred to a local hospital.

Pharmacokinetics

As presented in Table 2, we found that microdialysis successfully administered cisplatin into the tumor tissue but with varying concentrations. The drug penetrated the tumor tissue at measurable levels up to 37 mm from the supplying catheter after 12 h. In nine of the patients, we also found detectable but low concentrations of cisplatin in the BAT catheter 12 h after administration and up to 30 mm from the delivering catheter. During the treatment, the cisplatin concentrations in the BAT catheters increased, with the highest concentrations on days 7, 8, and 9. In patient 10, the cisplatin concentration in BAT was higher than in tumor catheter II. Furthermore, cisplatin was below or close to the level of detection in the subcutaneous tissue at 12 h and after 5–6 days in most patients. In blood, the level of cisplatin was below the level of detection after 12 h of treatment; however, very low concentrations were detected after 4–7 days of treatment.

For the entirety of the treatment, the total dose delivered to tumor tissue was 1.2–36.8 mg. The daily dose delivered ranged between 0.3 and 3.9 mg/day. We did not find any correlations between the doses of cisplatin, side effects, and survival time (Table 3).

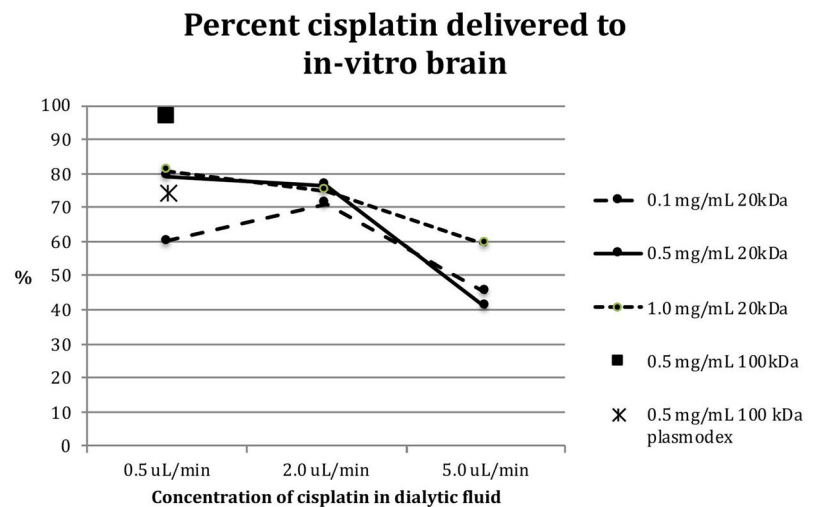
Glutamate/glycerol

We observed a significant increase in glutamate and glycerol in tumor tissue compared with BAT (Fig. 2). This increase took place gradually after the onset of treatment and peaked after 3 days of treatment. For the rest of the treatment, the elevated levels of the metabolites were fairly consistent. In BAT, glutamate was unchanged and glycerol decreased, but not to the level of statistical significance. Figs. 3E, F, 4, and 5 show an example of the resulting cytotoxicity manifesting as necrosis as visualized by MR.

Quality of life/functional status and survival

The reported quality of life is presented in Table 4. Only 8 of the 10 patients in the study could answer the life quality

Fig. 1 Examination of the percentage cisplatin delivered to an in vitro brain. The relationship between concentration of cisplatin in dialytic fluid (mg/mL), flow velocity in the microdialysis catheters (uL/min), and the cut-off of the semipermeable membrane (kDa). As the figure indicates, the maximum percent cisplatin is delivered with a high concentration of cisplatin in the dialytic fluid, low velocity of fluid in the catheter, and a cut-off of 100-kDa membrane



questionnaires at the first follow-up. One patient died 10 days post treatment, and one patient was unable to answer the questionnaires. Three patients experienced a pronounced decline in their functional status and QoL, although the majority had only a minor decline. However, in patients who had only a minor decline, a slight improvement was noted shortly after treatment and improvement was more evident at the one-month follow-up. Five patients survived more than 179 days, and one of those died of a cause unrelated to the disease. Five of the ten patients did not survive 3 months after the therapy (Table 3).

Discussion

This study confirms that retrograde microdialysis is a promising new method for interstitial intratumoral administration of cisplatin. Cisplatin were detected in tumor tissue in possibly clinically relevant concentrations, while only low or very low concentrations were observed in BAT, blood, and subcutaneous tissue. We detected high degree of cellular toxicity as assessed by glutamate and glycerol in tumor tissue but not in BAT or in the systemic compartments. This local toxicity was also confirmed by a different analytical method in which the metabolome was investigated [5]. No systemic toxicity was noted, but increased edema was a common local sign of brain toxicity.

Table 3 Side effects, survival, and total dose of cisplatin administered to tumor tissue. Edema and neurological symptoms are graded slightly, moderate, or severe

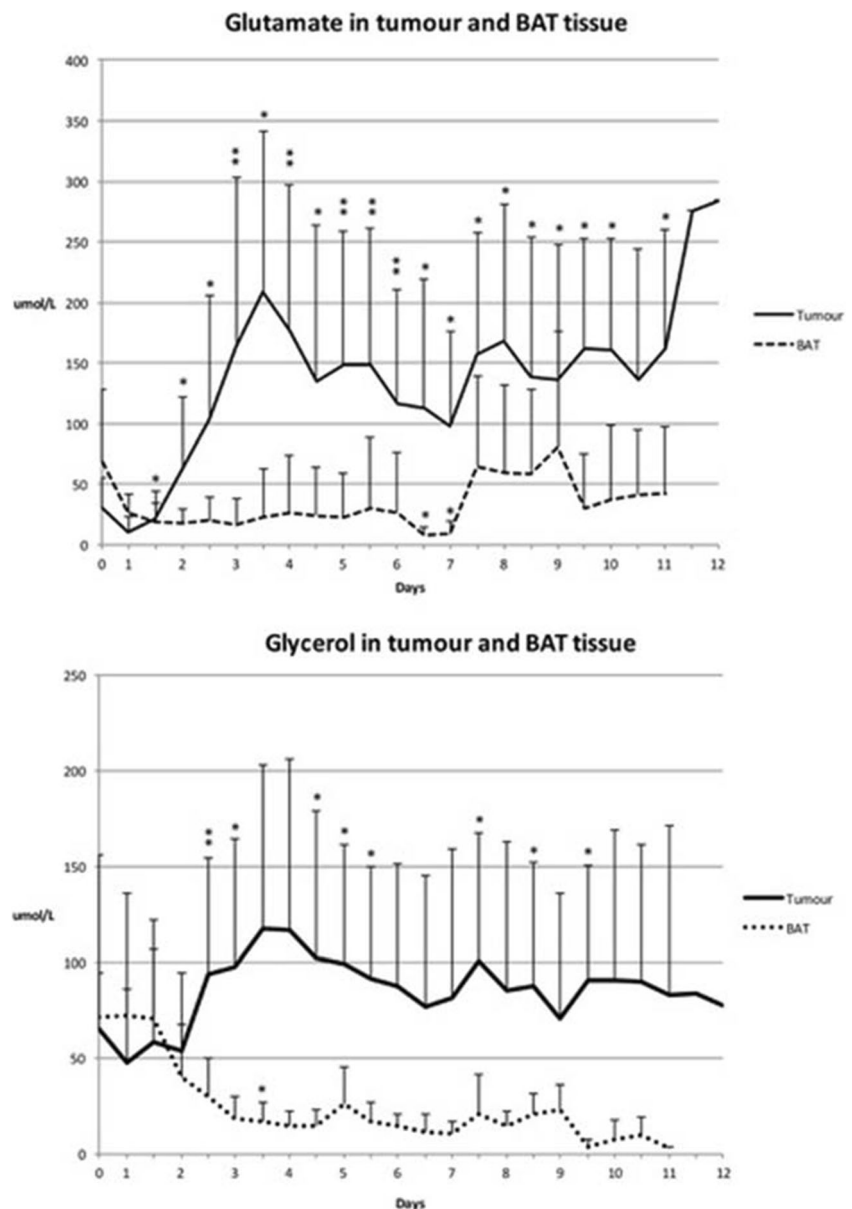
Pat. no.	Days of treatment	Flow speed (uL/min)	Cath. A (mg/day)	Cath. B (mg/day)	Cath. C (mg/day)	Total dose of cisplatin (mg)	Mean dose (mg/day)	Side effects Edema	Neurological symptoms*	Days of treatment	Survival (days)
1	8.5	2.0	1.95	—	—	16.56	1.95	Slightly	No	8	153
2	5–6	1.0	0.72	0.56	0.72	10.59	1.76	Severe	Moderate	6	34
3	5.5–7	1.0	—**	1.44	1.34	17.46	2.78	Moderate	Moderate	7	72
4	7–11	0.5	0.38	0.29	0.39	9.50	1.06	Moderate	Moderate	10	492
5	7–11	1.0	0.76	0.57	0.78	19.00	2.11	Slightly	Slightly	12	179
6	9–10	1.0	1.24	1.26	1.41	36.76	3.94	Slightly	Slightly	9	30
7	9–10	1.0	1.16	1.12	1.21	32.56	3.49	Slightly	No	8	188***
8	4.5–5.5	1.0	1.25	0.96	—	10.88	2.20	Severe	Severe	5	10
9	3–4	1.0	1.28	1.19	1.21	12.28	3.69	Severe	Moderate	4	47
10	4–5.5	0.5	0.13	0.17	—	1.23	0.30	Severe	Moderate	5	383

*Observed neurological symptoms: headache, vomiting and nausea, epileptic seizures, disorientation, paresis, dysarthria, and dysphagia

**This catheter was never used due to a small hemorrhage showing in the microdialysate

***This patient died from an unrelated cause

Fig. 2 Analysis of glutamate and glycerol in microdialysis from tumor tissue and BAT. The two graphs show the levels of glutamate and glycerol before and during the treatment with intratumoral cisplatin. Both markers of cell damage increase abruptly in tumor tissue as the treatment is started 24 h after implantation of the catheters. Statistical analyses were performed with Wilcoxon signed-rank test. * $P < 0.05$; ** $P < 0.01$



We chose cisplatin, a drug with strong cytotoxic effects [3], for several reasons. Cisplatin is a small compound, 298.9 Da, with bipolar property, so in vitro we could show that it readily passed the semipermeable membranes in the microdialysis catheters. The clinical use of cisplatin for treatment of brain tumors is limited by its systemic toxicity and inability to cross the BBB, which makes it a good candidate for intratumoral microdialysis treatment [4, 15, 18, 24].

This in vitro study confirmed that microdialysis could be used for administration of cisplatin. The delivery mainly depended on the membrane cut-off, the concentration of cisplatin in the dialysate, and the flow rate within the catheters. However, the polarity of cisplatin might have impacted its ability to permeate the catheters' semipermeable membranes. The semipermeable catheters were available with two

different mass cut-offs, 20 kDa and 100 kDa. Initially, we chose 100-kDa catheters as we were concerned about the risk for insufficient delivery of cisplatin molecules and we wanted to analyze cytokines during treatment. However, this decision showed to have disadvantages as patients 1–3 who received the 100-kDa catheter deteriorated neurologically due to an excessive edema developed around the catheters. To address this problem, we changed to 20-kDa catheters for patients 4–10. Thereafter, we observed less edema and the patients tolerated the therapy better. Another possibility to prevent ultrafiltration from the 100-kDa membranes could be the use of plasmadex in the dialytic fluid [9]. However, this would result in lower concentration of cisplatin in the dialysate and therefore a lower dose administered. Again, because our main

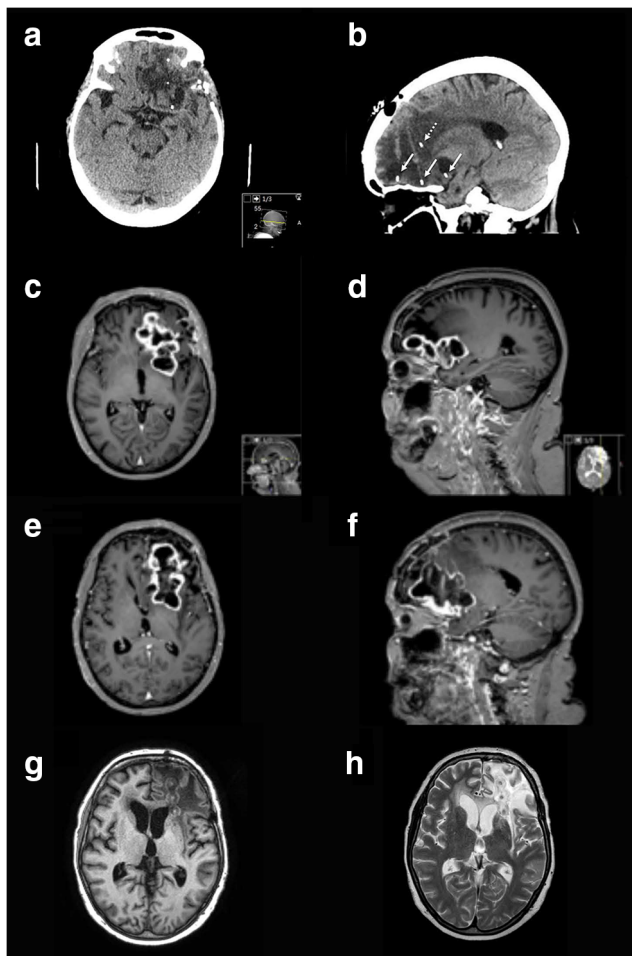


Fig. 3 A–H Patient 7 with three catheters for treatment and one catheter in BAT. **A** and **B** The tips of the catheters are demonstrated on a non-enhanced CT investigation. Solid arrows = tumor catheters, dashed arrow = BAT catheter. **C** and **D** Before treatment. **E** and **F** Progressive necrosis after 12 days of treatment. **G** and **H** Edema reaction along the catheters outside of the tumor and proximal of the semipermeable membranes after 12 days of treatment

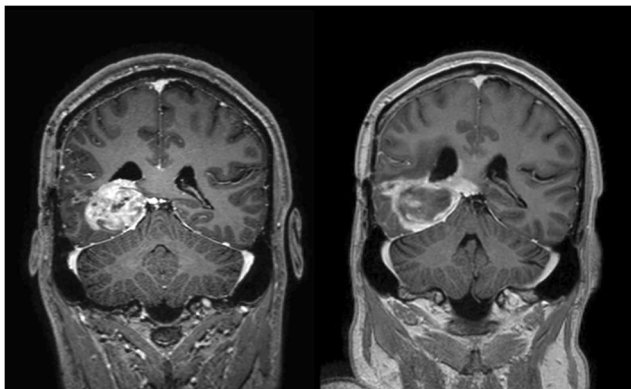


Fig. 4 MR images before and after 10 days of treatment in patient 5. Extensive necrosis in previous contrast enhancing tumor tissue is demonstrated

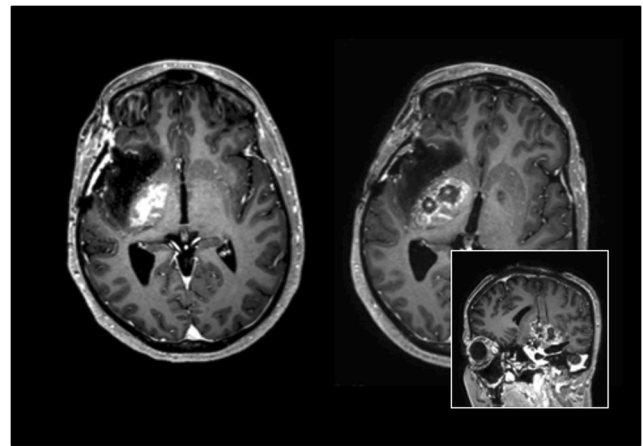


Fig. 5 Patient 10. MR images before and after 5 days of treatment. Catheters are removed, but very localized fibrosis and circular necrosis were visible where the catheters were inserted

concern was to administer a significant dose of cisplatin to the tissue, we chose to omit plasmodes in the dialysate.

The mean duration of treatment was 7.4 days (3–11) days. The reasons for the widely different treatment periods were mostly technical issues or a patient's tolerability. In five patients, obstruction in one or several of the catheters made it impossible to continue with the therapy as planned. All patients developed some edema around the catheters as visualized by MRI (Fig. 3G and H). In four patients, the edema resulted in severe neurological deterioration. In two other patients, the edema resulted in moderate neurological deterioration. Of these, all six patients with neurological deterioration were treated with an increased dose of corticosteroids and five also had their cisplatin dose decreased. Two of these patients had their cisplatin dose temporarily stopped for 2 to 3 days. Despite these measures, four patients did not respond to corticosteroids or/and dose reduction, developed excessive edema around the catheters, and deteriorated significantly during the therapy. In these cases, the treatment was aborted before the planned 12 days. In all cases, the edema regressed after the treatment period. No other side effects such as nausea or dizziness were noted.

The total dose of cisplatin delivered to the patients ranged between 1.2 and 36.8 mg during the treatment. The daily dose delivered ranged between 0.3 and 3.9 mg/day, which was roughly in agreement with what we had planned according to the protocol. By starting the active treatment with cisplatin in one catheter (usually the most centrally located catheter) 24 h before the other tumor catheters, we obtained data on drug penetrance in the tissue. We found that the penetrance of cisplatin differed between the patients, and we could detect cisplatin as far as 36.9 mm from the supplying catheter after 12 h. The pharmacokinetic data together with CT and MRI studies performed during and after the treatment gave us some further understanding regarding the penetrance of the drug. We believe that it is possible to achieve a clinically sufficient

Table 4 EORTC C30 in 10 patients treated with intratumoral cisplatin administered with retrograde microdialysis. Mean and SD are given as well as the number of patients who answered the questionnaire

	Before treatment (<i>n</i> = 10)	7 days (<i>n</i> = 8)	After treatment (<i>n</i> = 8)	1 month (<i>n</i> = 6)	3 months (<i>n</i> = 4)
EORTC C30					
Global health status/QoL	52.6	57.8	58.3	51.4	62.5
Functional scale					
Physical functioning	63.3	42.5	49.6	42.2	56.7
Role functioning	45.0	35.4	28.6	36.1	54.2
Emotional functioning	68.3	78.1	81.3	62.5	70.8
Cognitive functioning	71.7	69.6	80.4	69.4	75.0
Social functioning	55.0	59.5	61.9	58.3	75.0
Symptom scale/items					
Fatigue	58.9	66.7	58.3	51.9	43.5
Nausea and vomiting	8.3	25.0	18.8	19.4	4.2
Pain	18.3	35.4	25.0	30.6	12.5
Dyspnea	23.3	29.2	25.0	33.3	25.0
Insomnia	16.7	25.0	29.2	33.3	25.0
Appetite loss	10.0	29.2	20.8	16.7	0.0
Constipation	10.0	29.2	20.8	11.1	8.3
Diarrhea	0.0	0.0	0.0	0.0	0.0
Financial difficulties	23.3	20.8	20.8	50.0	25.0

concentration of cisplatin about 15 mm (\pm 5 mm) around the catheters. This information will be important for future studies when deciding how far apart catheters should be placed to ensure the drug penetrates as large a volume of the tumor as possible. Although the dose could be calculated with fairly good accuracy, the distribution within the tumor tissue and BAT was difficult to predict, an unsurprising result as the heterogeneity of this tumor has been established.

We also analyzed the concentration of cisplatin in BAT, blood, and subcutaneous tissue. In BAT, we analyzed cisplatin at 12 h, day 3, and days 6–9. At 12 h, the concentration of cisplatin in BAT was close to or below detection limit in all but one patient. In addition, in all but one patient concentration of cisplatin increased with time, demonstrating a certain degree of accumulation in the tissue. In total, the distribution of cisplatin in compartments outside the tumor was low, indicating that this method for administration results in a desired local delivery.

Patient 10 showed a high concentration of cisplatin in tumor catheter II and exceptionally high concentrations in BAT at the different times, concentrations even higher than the first measurement in tumor tissue. Patient 10, who developed neurological symptoms after just a few days, had treatment suspended as this patient had a stronger penetrance of cisplatin into normal brain than predicted. This unpredictable finding could have been due to the mannitol the manufacturer added to the cisplatin solution. During this project, there was a change in the supplier of

cisplatin. In all patients except patient 10, the cisplatin solution was identical with cisplatin dissolved in NaCl 9 mg/ml. However, patient 10 received a dose with added mannitol (1 mg/ml). As mannitol can disrupt the blood brain barrier, we believe that this together with an osmotic effect could explain the high penetrance of cisplatin into the BAT region [10].

In the subcutaneous tissue, we found very low or no detectable concentrations of cisplatin in all patients except for patient 10. In blood, we found no detectable concentrations of cisplatin at 12 h, but low concentrations were found after 5–7 days. These results might indicate that cisplatin passes the blood brain barrier only to a lower degree or more probably that cisplatin is diluted in the systemic compartments to such a degree that it does not cause any systemic toxicity.

Since a microdialysis catheter allows for collection of metabolites in a specific tissue, it allows for the analysis of events in the extracellular space of a specific tissue. In this series, we found a distinct cytotoxic response as detected by glutamate and glycerol in tumor tissue, which reached its peak as early as day 3. These findings correspond well with other studies that suggest glycerol and glutamate are potential markers for cellular damage in the brain [8, 11]. During treatment, glutamate and glycerol did not increase in BAT, supporting our hypothesis that the cytotoxicity of cisplatin was restricted to tumor tissue.

As expected, we did not see any correlations between survival and the doses given to the patients. However, the

small number of patients in this pilot study makes it difficult to perform any reliable statistical analysis. All the patients were in an advanced stage of their lethal disease with an overwhelming progressive burden of the tumor. Although the patients were in a poor prognostic state, five of the patients survived between 153 and 492 days, which might suggest that the treatment had a clinically positive effect in a subgroup of patients. However, the main purpose of this study was to evaluate the method as such along with toxicity to the treatment.

In all the patients, quality of life was assessed with EORTC C30 (Table 4) before the therapy, during the therapy (at day 7), and 1 month and 3 months after the therapy. We found large differences in life quality from the start of the treatment, during the treatment, and 1 and 3 months after the treatment. This might reflect the experimental pilot character of this study: a study of patients with a very poor prognosis. In patients not responding to the treatment, there was a significant drop out of patients during the follow-up period. Five of ten patients did not survive 3 months after treatment. This fact makes the results somewhat uncertain. However, four of the five patients with longer survival completed their questionnaires at 3 months and had a fairly good QoL as well as neurologic function at 3 months. The fifth patient with longer survival was in such poor condition at the time of the 3-month follow-up that she could not complete the questionnaire. Although not all patients could participate throughout the follow-up, it is interesting to note that the eight patients that responded to the questionnaire directly after treatment had a fairly stable self-evaluated quality of life.

The primary objective with this study was to evaluate whether retrograde microdialysis is a feasible method for locoregional administration of drugs and specifically cisplatin into glioblastoma tissue. This phase I pilot study aimed to investigate the feasibility of the method and possible toxicity using this method to administer Cisplatin. To fulfill the aim of the study, we have been forced to customize and adapt details in the protocol to cope with obstacles and challenges presented in the clinical setting. This resulted in a heterogeneous material with different tumor locations and different cut-offs of the catheters. Due to different sizes and locations of the tumors, we had to use different numbers of intracranial catheters and consequently different flow-rates of the dialysate. During the study, there was also a change in manufacturers of cisplatin. Due to variation in response and side effects in the individual patients as well as technical issues, the number of days of treatment was not the same for all patients. The above-mentioned circumstances as well as the heterogeneity and small number of patients make it very difficult to draw any conclusions concerning treatment efficacy and outcome in terms of survival. However, in spite of the limitations of this

study, we believe that we have reached our primary goal, to evaluate retrograde microdialysis as a method of intracranial drug delivery and state that the method may be evaluated further for administration of drugs, including cisplatin, locoregionally into tumor tissue in brain.

Conclusions

This experimental phase I study demonstrates that microdialysis can be used to administer cisplatin locoregionally into high-grade glioma tissue. We detected signs of cytotoxicity in tumor tissue but not in the surrounding brain. A transient minor or more evident neurologic deterioration due to edema was observed, but no systemic side effects were recorded. In five of the ten patients, survival was longer than expected. We believe that intratumoral retrograde microdialysis is a promising new method for interstitial delivery of drugs in patients with high-grade glioma.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study was approved by the Swedish Medical Products Agency (EU no. 2010-018281-23) and the Ethics Committee of Umeå University Medical Faculty (Ethical permission: 09-199M).

Informed consent Informed consent was obtained from all participants included in the study.

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