

# Clinical Microdialysis in Glioma

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## 1. Introduction

Microdialysis is a technique that may be used to directly investigate brain chemistry in-vivo. Although initially developed over 35 years ago (Ungerstedt and Pycock 1974), it is only relatively recently that studies have begun to utilise microdialysis in patients with glioma and other brain tumours. In this chapter we will review the general principles of microdialysis, the use of the technique to investigate glioma pathogenesis and evaluate chemo- and radiotherapy, and the potential utilisation of retrograde microdialysis to administer chemotherapeutic agents directly to the tumour bed.

## 2. Microdialysis

Present-day microdialysis is the result of several decades of technological advancement. An understanding of the principles underlying the technique is an essential prerequisite to appreciating its potential uses and limitations.

### 2.1 Principles, uses and limitations

#### 2.1.1 Principles

Microdialysis enables sampling of the extracellular fluid (ECF). A microdialysis catheter or probe with a semi-permeable membrane at its tip is placed into the tissue of interest. Perfusate with a similar composition to the ECF is then slowly and continuously infused through the catheter. Substances of interest diffuse across the semi-permeable membrane into the catheter, and the resulting dialysate is collected in microvials, which are changed at regular intervals and subsequently analysed (see Figure 1).

Diffusion of substances from the ECF, across the membrane, and into the flowing perfusate, is often incomplete. Thus, the concentration of a substance within the dialysate represents a fraction of that in the ECF. The *extraction fraction* or *relative recovery* is defined as the ratio of a substance's concentration in the dialysate ( $C_{dialysate}$ ) compared to the actual concentration in the ECF ( $C_{ECF}$ ).

$$\text{Relative Recovery} = C_{dialysate} / C_{ECF} \times 100\%$$

A number of variables may influence the relative recovery including the flow rate, the semi-permeable membranes length and pore size, and the properties of the substance of interest itself (see Table 1) (de Lange et al. 1997, de Lange, de Boer and Breimer 1999, Hutchinson et al. 2000, Benjamin et al. 2004, Helmy et al. 2009, Chefer et al. 2009, Blakeley et al. 2009).

Reducing the perfusate flow rate increases the time available for diffusion of substances across the semi-permeable membrane, and in turn increases the relative recovery of a substance (Tossman and Ungerstedt 1986, Hutchinson et al. 2000). This must be balanced against the reduced dialysate volumes obtained over time, which usually necessitate longer sampling intervals. Increasing the length of the semi-permeable membrane along which diffusion can occur also increases the relative recovery of a substance (Tossman and Ungerstedt 1986, Hutchinson et al. 2000) but the dimensions of the tissue being probed may limit this. Increasing the pore size of the semi-permeable membrane increases the size of molecules that are able to diffuse across it. Most microdialysis catheters used clinically are low molecular weight cut-off (LWCO) with a membrane pore size permitting molecules of approximately 20kDa (such as glucose and its metabolites) to diffuse across them. Recently high molecular weight cut-off (HWCO) catheters have been utilised with a larger membrane pore size permitting molecules of approximately 100kDa (such as cytokines) to diffuse across them. There are a number of methodological difficulties with using such catheters to measure the concentration of macromolecules (Helmy et al. 2009). One concern is that the increased membrane pore size used may lead to net efflux of fluid from the perfusate into the ECF thus influencing the composition of the ECF itself and compromising the validity of data obtained. There have been efforts to counter this net fluid efflux with the addition of a colloid to the perfusate. Various properties of the molecule being measured may also influence its relative recovery such as its shape, charge, hydrophobicity or hydrophilicity, hydrodynamic radius, and interaction with other molecules, such as dimerisation. The effect of these factors is that even molecules of a similar molecular weight may have considerably different relative recoveries in-vivo. Other factors may also alter the relative recovery. The diffusion coefficient has been estimated to increase by 1-2% for every degree Celsius increase in temperature. The diffusion coefficient within an aqueous solution is almost always greater than in tissue due to the increased diffusional path (or "tortuosity") of the latter (Blakeley and Portnow).

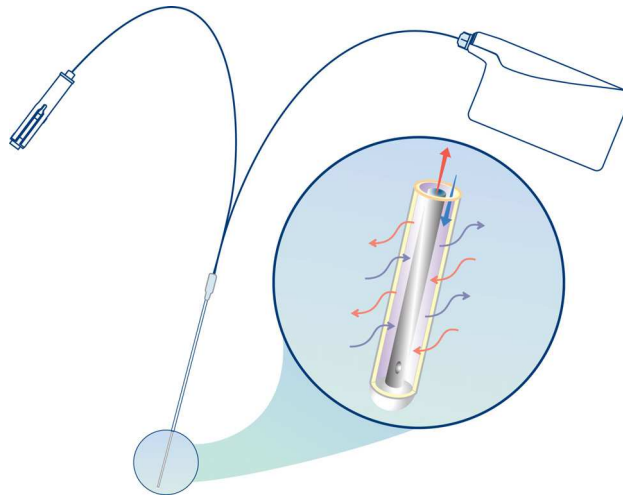


Fig. 1. Microdialysis components. microdialysis catheter is seen on the right, microdialysis pump in the centre, and microvials on the left.

Factor	Effect
Microdialysis dependent	
Perfusate flow rate	Decreasing recovery with faster flow rate
Membrane length	Increasing recovery with larger membrane
Membrane pore size	Larger molecules recovered with increasing pore size
Context dependent	
Analyte properties	Recovery of molecules of similar size may be very different
Solution properties	Recovery in-vitro and in-vivo may be very different
Temperature	Increasing recovery with temperature

Table 1. Factors affecting relative recovery

In-vitro studies have calculated the relative recovery for specific molecules under different experimental conditions in which the concentration of a substance in the external medium is known or directly measurable. Using such methods the in-vitro recovery for glucose and its metabolites using a LWCO catheter with a 10mm membrane at a flow rate of 0.3microl/min has been estimated at between 70-100% (Hutchinson et al. 2000, Blakeley and Portnow). The in-vitro recoveries of macromolecules such as cytokines using similar methods with a HWCO catheter are variable but usually far lower (Helmy et al. 2009). Although some investigators have used these calculated relative recoveries to correct dialysate concentrations measured, this has proved unreliable because, as mentioned previously, diffusion within aqueous test solutions differs significantly from diffusion within tissue in-vivo.

Several methods of determining relative recovery in-vivo have been described in attempt to overcome the shortcomings of in-vitro estimates (see Table 2) (Benjamin et al. 2004, Chefer et al. 2009, Blakeley and Portnow). These methods include the no-net-flux method, the flow-rate method, and the use of standards whose concentration is known (both exogenous and endogenous). In the no-net-flux method, perfusate containing several different concentrations of the analyte of interest (both above and below the anticipated concentration in the ECF) is perfused through the microdialysis probe and the amount of this analyte gained or lost from the probe is determined. Using this method the relative recovery may be calculated as the gradient of the linear regression that describes the dialysate concentration of the analyte being studied as a function of experimenter controlled variations in the perfusate concentration. In the flow-rate method, it is assumed that at a flow rate of zero (i.e. stasis) equilibrium between perfusate and the ECF is eventually achieved and that increasing the flow rate leads to a reduced relative recovery in a predictable but non-linear fashion. By infusing at different flow-rates and measuring the concentration of the analyte of interest, it is therefore possible to calculate the relative recovery (Hutchinson et al. 2000). Other methods rely on the use of an internal standard to estimate in-vivo relative recovery. Often, the perfusate contains a known concentration of a radiolabelled molecule similar to the analyte of interest. By determining the loss of this molecule during microdialysis it is possible to calculate its relative recovery. Alternatively, some investigators have made use of urea - which is assumed to have the same concentration throughout all water compartments in the body - as an endogenous standard. By determining the difference

between the concentration of urea in plasma, and the concentration in dialysate collected, an estimate of the relative recovery of similar small molecules may be obtained (Brunner et al. 2000, Sorg et al. 2005).

Method	Principle
No-net-flux	When analyte concentrations within perfusate and ECF are equal, there is no-net-flux
Variable flow rate	When flow rate is zero (i.e. stasis) equilibrium occurs between perfusate and ECF
Internal standard (Exogenous e.g. radiolabelled)	Fraction of exogenous standard lost from perfusate is equal to fraction of analyte extracted from ECF
Internal standard (Endogenous e.g. urea)	Fraction of endogenous standard and analyte extracted from ECF is equal

Table 2. In-vivo methods of determining relative recovery

There are a number of methodological difficulties in estimating relative recovery using these described in-vivo techniques, particularly in the context of glioma research. The no-net-flux method requires an accurate estimation of the concentration of analytes in-vivo but the concentration of the cytokines and growth factors involved in gliomagenesis can vary by several orders of magnitude. The flow-rate method requires very slow flow rates to increase the accuracy of the regression analysis, which in turn necessitates long collection periods to obtain sufficient sample volume. The use of an internal standard relies on the assumption that it has a similar relative recovery to the analyte of interest, which, for the reasons mentioned above, may not be valid. These methodological difficulties in estimating relative recovery using in-vivo techniques have led some commentators to the conclusion that the ratio of the concentration of related physiological substances (such as the ratio of lactate/pyruvate, or pro-/anti-inflammatory cytokines) may be a more robust and valuable measurement than attempts to determine the absolute concentration of these molecules in the ECF (Helmy et al. 2009).

### 2.1.2 Uses

Until relatively recently few studies had applied microdialysis to patients undergoing surgical biopsy or resection of their brain tumours. To this end, clinical studies using microdialysis in patients with brain tumours offer a number of potential advantages over other methodological approaches. First, in contrast to traditional in-vitro studies, clinical microdialysis studies permit the assessment of brain tumours in-vivo, recognising the complex interactions between tumour- and host-related factors, and the role these interactions play in tumourogenesis. Second, by applying microdialysis to patients with brain tumours, rather than animal models of such tumours, clinical microdialysis eliminates the possibility of erroneous interpretation of interspecies differences or of limitations of the brain tumour model itself. Third, clinical microdialysis provides a direct measure of analytes within the ECF when compared with imaging techniques. Fourth, microdialysis easily allows repeated evaluation over an extended time course. Microdialysis therefore

provides a unique method of continuously measuring brain and tumour chemistry allowing investigation of metabolites and macromolecules involved in tumourogenesis, the dynamic changes in the concentration these molecules over time, and their response to chemo- and radiotherapy. Finally, retrograde microdialysis offers the potential for the direct administration of chemotherapeutic agents to brain tumours.

### 2.1.3 Limitations

Several confounding factors must be considered when performing or interpreting studies that utilise microdialysis to investigate brain tumours. First, although microdialysis is a direct measure of analytes within the ECF, the concentration of a substance within the dialysate still represents only a fraction of that in the ECF. As discussed above, this relative recovery depends upon a large number of variables and estimation by in-vitro and in-vivo techniques has proved unreliable. Second, the invasive nature of microdialysis probe insertion may result in trauma artefact. A recent consensus meeting on microdialysis in neuro-intensive care recognised that data was unreliable for at least one hour after insertion (Bellander et al. 2004). In patients with brain tumour undergoing resection or debulking, the trauma artefact may be considerably longer, particularly if the macromolecules such as growth factors and cytokines are being monitored. Third, the precise location of the catheter tip may greatly influence the data obtained by microdialysis. Studies that have applied microdialysis to patients with brain tumour have demonstrated significantly different metabolic profiles at the tumour centre, tumour periphery or border, and grossly normal peri-tumoural tissue (Roslin et al. 2003, Marcus et al.).

These confounding factors are at least partially mitigated by the use of physiologically meaningful ratios (rather than absolute concentrations), the omission of the first few hours of data obtained post-insertion, and the careful note of catheter locations intra-operatively and using post-operative imaging (see Table 3). The combination of microdialysis with other research methods such as animal studies, in-vitro techniques and imaging provides a powerful research paradigm.

Limitation	Strategy
Relative recovery variable	Use physiological ratios rather than absolute concentrations
Trauma artefact	Minimise trauma and wait for data to normalise
Location of probe	Note location intra-operatively and image post-operatively to confirm

Table 3. Limitations of microdialysis and strategies to avoid

## 2.2 Equipment and technique

### 2.2.1 Equipment

The equipment required for microdialysis includes perfusion fluid, microdialysis syringes, microinfusion pumps, microdialysis catheters, and microvials (See Figure 1). Not all commercially available microdialysis equipment is suitable or certified for human use and this must be carefully considered before selecting study apparatus. Perfusion fluid should be as close to the cerebral ECF as possible and CMA CNS perfusion fluid composed of NaCl

(147 mM), KCl (2.7 mM), CaCl<sub>2</sub> (1.2 mM), and MgCl<sub>2</sub> (0.85 mM) in water, is often used. Perfusion fluid is contained in microdialysis syringes with a capacity of approximately 2.5ml. Microinfusion pumps are portable battery driven pumps that compress syringes at a slow predefined rate, which is usually fixed (0.3µmol/min) but may be adjustable (0.1µmol/min to 5µmol/min). Microdialysis catheters vary in their membrane length (10-30mm) and pore size (LWCO/20kDa or HWCO/100kDa), and by their physical properties (such as shaft size). Conventional clinical microdialysis uses LWCO catheters. Clinical microdialysis studies investigating macromolecules such as cytokines or growth factors require HWCO catheters to maximise recovery of these substances. Fluid is collected in microvials designed to collect micro volume samples and minimise evaporation.

### 2.2.2 Technique

All patients must be thoroughly counselled beforehand about the potential (but very low) additional risk of haemorrhage and infection, and written informed consent obtained. Pre-operatively all the microdialysis equipment should be checked. Particular attention must be paid to the microdialysis catheter, syringe and perfusion fluid to ensure that their packaging remains intact and sterility maintained. Many clinicians advocate priming the catheter so that the system is already functional prior to insertion. This ensures constant fluid flow at the catheter tip and theoretically reduces sedimentation and non-specific binding of proteins to the catheter membrane. The microdialysis syringe is filled with CNS perfusion fluid, and connected to the microdialysis catheter using strict aseptic technique. The microdialysis syringe is placed in the microinfusion pump and a microvial placed at the distal end of the microdialysis catheter to collect the dialysate. Upon closing the lid of the microinfusion pump a 5-minute flush cycle is initiated followed by an automatic decrease to the preset flow rate.

Operative insertion of the microdialysis catheter into cerebral parenchyma may be via a closed or an open technique following tumour biopsy or resection respectively. In the closed technique stereotactic biopsy of brain tumour tissue is performed and then the microdialysis catheter inserted so that the catheter tip lies in the region of interest. Multiple catheters can be placed through a single burr hole using multiple different trajectories. The advantage of this technique is that traumatic artefact is minimised. In the open technique the brain tumour is resected and then the microdialysis catheter placed into the region of interest. Intra-operative real-time three-dimensional ultrasound probes have been used to assist catheter placement (Homapour et al.). Although there is greater traumatic resection artefact associated with open placement the risk of inadvertent complications, such as intracerebral haematoma, is theoretically lower because the catheter is inserted under direct visualisation and blood vessels can be avoided. The precise position of catheters within the brain is critical to interpreting clinical microdialysis studies but a number of terms have been used in the literature with conflicting and overlapping definitions. To avoid confusion during subsequent discussion we will define catheter locations in the following way: Tumour (T) catheters are either within grossly affected tumour tissue, or within 5mm of the resection margin of such tissue; Peritumour (PT) catheters are within 5mm-20mm of the tumour or resection margin; Brain Around Tumour (BAT) catheters are within grossly unaffected brain at least 20mm away from the tumour or resection margin. Once in place the catheter may be secured using a commercial "bolt" or by tunnelling the catheter and stitching it into place, depending on whether a closed or open approach insertion technique is used respectively.

Term	Definition
Tumour (T)	Within grossly affected tumour tissue, or within 5mm of the resection margin of such tissue
Peritumour (PT)	Within 5mm-20mm of the tumour or resection margin
Brain Around Tumour (BAT)	Within grossly unaffected brain at least 20mm away from the tumour or resection margin

Table 4. Definitions on the location of microdialysis catheters

Imaging should be performed to confirm the catheter position post-operatively. Most commercially available microdialysis catheters are fashioned with a “gold-tip” that is visible on CT to facilitate their identification. The initial microvial (containing flush) is not analysed. Subsequent microvials are numbered and exchanged sequentially at predefined intervals. Microvials are either analysed immediately or stored in -80c for subsequent analysis. Studies have shown that the concentration of glucose and its metabolites within microdialysate is equivalent with measured immediately or after storage in this manner (Hutchinson et al. 2000).

Glucose and its metabolites are frequently measured using commercial point-of-care analysers such as the ISCUS or CMA 600 (CMA Microdialysis AB, Solna, Sweden), which employ an enzyme-kinetic technique. Several techniques can also be used to analyse the macromolecules present within dialysate. Because the volumes of liquid are small and the concentrations of substances very low, techniques such as Enzyme-Linked Immunosorbent Assay (ELISA), High Performance Liquid Chromatography (HPLC), or Mass Spectroscopy (MS) are often employed.

### 2.2.3 Troubleshooting

Although microdialysis is generally a robust technique if difficulties do arise and dialysate is not obtained, a systematic approach is advocated. The micropump should be examined and new batteries placed (if not already done). The catheter insertion site should be examined to check that the catheter remains secure and is not obviously displaced. The microvials can be removed and replaced to ensure that they click into place appropriately. Once confident these components are satisfactory the system may be flushed by re-opening and then closing the lid of the micropump.

## 3. In vivo assessment of pathogenesis

To date, almost a dozen clinical studies have utilised microdialysis in patients with cerebral tumours; with approximately half of these devoted to investigating gliomagenesis, and the remaining to evaluating treatment with chemo- and radiotherapy. Studies investigating gliomagenesis may be further subdivided into those utilising LWCO or HWCO microdialysis catheters.

### 3.1 Low molecular weight cut off

In a landmark Swedish study in 2003 Roslin et al studied the baseline concentration of glucose and its metabolites, glycerol and glutamate in patients with high grade glioma

(HGG) (Roslin et al. 2003). The group performed an in-vitro recovery experiment, which confirmed relative recoveries of greater than 90% of the substrates of interest. Fifteen patients with HGG undergoing brain biopsy were recruited and two LWCO catheters were placed stereotactically: one within the tumour (T), and one 10mm outside the contrast enhancing region in the peritumour region (PT). Surprisingly, the only significant difference between dialysates obtained from T and PT was lactate, which was more concentrated in T than PT ( $p < 0.05$ ). This is in contrast to in-vitro studies (Klegeris, Walker and McGeer 1997), animal studies (Behrens et al. 2000), and subsequent clinical microdialysis studies (Marcus et al.), all of which demonstrate an increased concentration of glutamate and other metabolites within tumour cell lines and tissue respectively. The possible reasons for this incongruity are discussed below (see Section 3.2)

Investigators in Italy also used LWCO microdialysis catheters to establish the baseline concentration of other small molecules including amino acids, adenosine, and choline in 21 patients with HGG (Melani et al. 2003, Bianchi et al. 2004). An in-vitro recovery experiment was carried out and demonstrated the relative recovery of adenosine estimate to be  $43.4 \pm 5.1\%$  (relative recovery was not calculated for amino acids and choline). Unlike the Swedish study patients underwent tumour resection and three microdialysis catheters were placed using an open technique: one into the tumour resection margin (T), one into peritumoural tissue 10mm away from the resection margin (PT), and one into grossly normal brain around the tumour at least 20mm away from the resection margin (BAT). Samples were analysed using various methods including HPLC. The group found that concentration of adenosine and glutamate were significantly reduced in T compared with BAT ( $p < 0.05$ ), the concentration of aspartate was unchanged, and the concentration of the remaining amino acids and choline were all significantly increased in T compared with BAT ( $p < 0.01$ ). Interestingly epilepsy, which occurs in approximately a third of patients with brain tumours (Villemure and de Tribolet 1996), was found to be an important confounding variable when the concentrations of aspartate, glutamate and GABA were considered.

### 3.2 High molecular weight cut off

Flannery et al were the first group to take advantage of HWCO catheters to assess the cysteine protease Cathepsin S (CatS) in gliomagenesis (Flannery et al. 2007). In total 11 patients with suspected HGG were recruited. Of these 11 patients, one was subsequently found to be a low-grade glioma (LGG), 2 were cerebral metastases, and the remaining 8 cases confirmed HGGs. A further patient with suspected hydrocephalus that was undergoing intracranial pressure monitoring was also included as a control. All patients underwent tumour resection with insertion of a single microdialysis catheter at the tumour resection margin (T). Analysis of CatS was by activity and ELISA concentration assays. Unfortunately, the absence of paired catheter data makes interpretation of the study's findings difficult but there was no significant relationship between CatS concentration and function, and the grade of brain tumours investigated.

A more recent study utilising HWCO catheters in patients undergoing surgery for intrinsic brain tumours set out to first to repeat earlier measurements of glucose and its metabolites, glycerol and glutamate, and second to assess the concentration of macromolecules such as growth factors, cytokines and other proteins involved in the pathogenesis of HGGs (Marcus et al.). Eight patients with suspected HGG were recruited. Of these 8 patients, one was found to have a lymphoma, and the remaining 7 cases confirmed HGGs. A further patient



with traumatic brain injury was included as a non-tumour control. All but one of the patients with cerebral tumours underwent surgical resection with the first microdialysis catheter placed at the tumour resection margin (T) and the second inserted at least 20mm away in macroscopically unaffected brain around tumour (BAT). The remaining one patient had an image-guided biopsy of their tumour with stereotactic insertion of a catheter into the tumour margin (T). Microdialysates were first assessed for small molecules using the CMA 600 or ISCUS analyser. Tumour microdialysates were found to have a significantly lower glucose, higher lactate/pyruvate (L/P) ratio, higher glycerol and higher glutamate compared to the brain around tumour. These findings suggest that the tumour margin of HGGs is particularly metabolically active and are consistent with previously published *in-vitro* and animal studies, but differ from the previous clinical microdialysis study by Roslin et al. There are a several reasons that may account for the discrepancy between these studies. First, the small number of cases in both studies necessitates cautious interpretation of their findings as variation in patients, their pathology and tumour heterogeneity may all have influenced the concentration of glucose and its metabolites, glycerol and glutamate. Second, Roslin et al introduced catheters following biopsy using a closed stereotactic technique while Marcus et al introduced catheters after tumour resection using an open technique. Trauma artefact may therefore have influenced findings (though in the Marcus et al study measurements were taken at least 4 hours post-operatively to try and reduce this effect). Third, while Roslin et al placed the tumour catheters in the tumour centre, Marcus et al placed tumour catheters at the tumour resection margin or tumour periphery. It is possible that the core of the tumour, which is often necrotic, may be less metabolically active than the brain-tumour interface.

In the same study Marcus et al analysed all the remaining microdialysate samples for macromolecules using a sandwich ELISA like procedure. There was great variability in the dialysate concentrations of the various growth factors (TGF- $\alpha$ , VEGF, EGF), cytokines (IL-1a, IL -1b, IL-1ra, IL-6, IL-8) and matrix metalloproteases and their tissue inhibitors (MMP2, MMP9, TIMP1, TIMP9). Nevertheless, microdialysates were found to have significantly raised MMP2/TIMP1 and IL-8 in T compared to BAT samples suggesting an environment favouring invasion and angiogenesis respectively.

#### **4. In vivo assessment of therapies**

In addition to using microdialysis to evaluate the baseline concentration of molecules involved glioma pathogenesis, several studies have also made use of the technique to investigate the response to treatment with chemo- and radiotherapy.

##### **4.1 Chemotherapy**

Microdialysis may be used to evaluate both chemotherapeutic pharmacokinetics and pharmacodynamics. Interestingly, the earliest example of clinical microdialysis in patients with brain tumours to investigate a drug's pharmacology focused not on chemotherapy but on the antimicrobial rifampicin. Mindermann et al recruited 5 patients with HGG and 3 patients with LGG (Mindermann 1999). All patients received a single pre-operative dose of 600mg rifampicin 3 hours before skin incision. Patients then underwent craniotomy and tumour resection with a single microdialysis catheter placed distantly from the resection margin in grossly unaffected brain around tumour (BAT). A LWCO catheter was infused

with two solutions of different concentrations of rifampin at a rate of  $3\mu\text{l}/\text{m}$ . The loss or gain of rifampin from the two solutions was determined and rifampin concentration then calculated using the no-net-flux method. Intra-operatively solid tissue samples were also taken from tumour tissue, peri-tumour tissue and unaffected brain around tumour tissue. The concentration of rifampin was greatest within solid tissue samples from tumour and peritumour, followed by BAT microdialysates, and then solid tissue samples from unaffected brain around tumour. The rifampin concentration in all compartments exceeded the minimum inhibitory concentration (MIC) for staphylococci and streptococci.

Blakeley et al used clinical microdialysis to investigate the pharmacokinetics of high dose methotrexate ( $12\text{g}/\text{m}^2$ ) (Blakeley et al. 2009). The group performed an in-vitro recovery experiment, which demonstrated a relative recovery of  $43.6 \pm 2.6\%$ . Four patients with recurrent HGG were recruited and underwent biopsy or resection as clinically indicated. A LWCO microdialysis catheter was then placed into either the contrast-enhancing or non-enhancing residual tumour (T). Samples were analysed using liquid chromatography/mass spectroscopy. Methotrexate penetration in T was found to be variable with the highest concentrations measured within the contrast-enhancing regions. Nevertheless, the concentration of methotrexate in all regions exceeded the minimum concentration required for 50% cell kill against glioma cell lines in vitro.

Portnow et al used clinical microdialysis to investigate the pharmacokinetics of another chemotherapeutic drug temozolamide (TMZ) (Portnow et al. 2009). Contemporary post-operative management of patients with a HGG is with daily TMZ tablets and concurrent radiotherapy. Phase I studies of TMZ suggested that peak levels in blood occurred approximately an hour after ingestion and patients are therefore typically instructed to take their tablets an hour prior to radiotherapy to potentiate their oncotoxic effects (Dhodapkar et al. 1997). The group first performed an in-vitro recovery experiment, which demonstrated a relative recovery of  $87 \pm 5.5\%$ . Portnow et al then recruited 9 patients of which 6 patients had HGG, and 3 had non-small cell lung cancer. All patients underwent resection and a single LWCO microdialysis catheter was placed within 5mm of the tumour resection margin (T). Post-operatively one patient refused TMZ, and in another the microdialysis catheter was occluded. In the remaining 7 patients microdialysates were analysed using HPLC to determine the concentration of TMZ. Concentrations of temozolamide in the brain measured in their study were consistent with previous studies but it was noted that the mean time to reach peak level in the brain was  $2.0 \pm 0.8$  hour. The clinical corollary of their findings is that current chemoradiation regimens may be improved by advising patients to take their tablets 2 hours before radiotherapy sessions.

## 4.2 Radiotherapy

A series of studies by a group in Sweden have used microdialysis to monitor patients undergoing radiotherapy. The focus of their first study was Boron Neutron Capture Therapy (BNCT), an experimental technique in which patients are injected with boron, which preferentially binds to tumour cells, and then treated with neutron beam radiotherapy generating oncotoxic alpha particles and Lithium ions. The technique is theoretically attractive because of the short path length of alpha particles (approximately one cell diameter) compared to conventional gamma radiation. Bergenheim et al used clinical microdialysis to determine the pharmacokinetics of boronophenylalanine (BPA) with a view to optimising the timing of radiation (Bergenheim et al. 2005). An in-vitro

experiment determined a mean BPA recovery of  $66.8 \pm 8.8\%$ . The group also monitored glucose and its metabolites, glutamate and glycerol throughout the procedure. Four patients with WHO grade IV glioblastoma multiforme (GBM) were recruited. One patient underwent gross total resection, one a subtotal resection, and two stereotactic biopsies. In the patients that underwent resection a microdialysis catheter was placed within 5mm of the resection margin (T), and in the patients undergoing biopsy catheters were placed within viable tumour tissue (T). In all patients a second catheter was placed at least 20mm outside of the radiological bulk of the tumour in macroscopically normal brain around tumour (BAT). Microdialysates were analysed using the CMA 600 analyser, except for boron that was measured using an inductively coupled atomic emission spectrometer. The concentrations of boron varied considerably depending on the tissue sampled: in T samples the pharmacokinetic profile of BPA followed that of blood, while in BAT uptake was generally very low with a delay of up to 8 hours in relation to blood levels. No significant changes in glucose and its metabolites were noted during BNCT treatment. An increase in the concentration of glycerol was noted in T and PT 1-3 days after BNCT treatment while BAT levels were low and unchanged. Glutamate also showed high levels in PT compared to BAT, although no obvious changes were observed over time.

In their second study the Swedish group evaluated the levels of glucose and its metabolites, glycerol and glutamate in patients with HGG undergoing conventional post-operative radiotherapy (Tabatabaei et al. 2008). Thirteen patients with HGG were recruited with one catheter placed within the tumour (T), and a second 10mm outside the contrast-enhancing area in the peritumour region (PT). Samples were obtained at least 20 hours before radiotherapy commenced, and then continued for at least 20 hours after the fifth radiotherapy session. Baseline levels of glucose were significantly lower, and the L/P ratio significantly higher, in dialysates from T compared with PT. Radiotherapy did not influence glucose and its metabolites, or glycerol or glutamate.

Bergenheim's group subsequently extended their approach to utilise HWCO catheters enabling evaluation of macromolecules during conventional radiotherapy (Wibom et al.). Eleven patients with HGG were underwent stereotactic biopsy with insertion of two microdialysis catheters: one placed into the contrast enhancing tumour (T), and a second outside it in the peritumour region (PT). Reference samples were also collected subcutaneously from patients' abdomen. Microdialysates were analysed using gas chromatography - time-of-flight mass spectroscopy. Marked differences in metabolomic patterns were noted between T and PT, and between brain and abdominal microdialysates. In addition, dynamic changes occurred with radiotherapy in T and PT microdialysates.

## 5. Retrograde microdialysis

A novel use of clinical microdialysis is to deliver chemotherapeutic agents through a process termed retrograde microdialysis. The technique offers a number of potential advantages. First, the precise placement of catheters allows chemotherapy to bypass the blood-brain barrier and be administered directly to the tumour bed. Second, equilibration occurs across a semi-permeable membrane ensuring the therapeutic concentration is maintained. Third, simultaneous measurement of metabolism allows direct assessment of a drug's effects. The therapeutic principle was first explored by Ungerstedt's group in Sweden who treated three patients with GBM by adding the oncotoxic non-physiological amino acid L-2, 4

daminobutyric acid (DAB) to perfusate (Ronquist et al. 1992). This was extended by Bergenheim et al who recruited 10 patients with GBM that underwent stereotactic biopsy with the insertion of two microdialysis catheters: one in the contrast enhancing tumour (T), and the second 10mm outside the contrast enhancing region in the peritumour region (PT) (Bergenheim et al. 2006). Catheters inserted into T were LWCO with 30mm membranes, and were perfused with 80 or 120mmol/l DAB at a rate of 2 $\mu$ l/m. Samples were analysed for metabolites using a CMA 600 analyser, and for amino acids using HPLC. During treatment with DAB a significant increase in a number of amino acids including glutamate was observed suggesting cellular toxicity. PT samples were unaffected suggesting treatment effects was localised to the tumour compartment. Although the sample size was too small to determine whether there was an effect on clinical outcome, the study nevertheless provides evidence to support the feasibility of the technique.

## 6. Conclusion

In the last decade there has been a surge of interest in the application of clinical microdialysis to neuro-oncology. In this chapter we have reviewed the principles of microdialysis, and systematically appraised studies on the use of the technique to investigate gliomagenesis, the effect of treatment with chemotherapy and radiotherapy, and the potential for administration of drugs with retrograde microdialysis. The utility of the technique lies in its use alongside other methods such as in-vitro, animal and imaging studies.

## 7. Acknowledgments

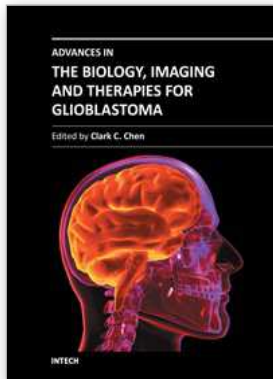
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