

Review Article

Theme: Integrating Microdialysis and Imaging Tools in Systems Pharmacology

Guest Editors: Robert E. Stratford, Nimita Dave, and Richard F. Bergstrom

Microdialysis Monitoring in Clinical Traumatic Brain Injury and Its Role in Neuroprotective Drug Development

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Abstract. Injuries to the central nervous system continue to be vast contributors to morbidity and mortality; specifically, traumatic brain injury (TBI) is the most common cause of death during the first four decades of life. Several modalities are used to monitor patients suffering from TBI in order to prevent detrimental secondary injuries. The microdialysis (MD) technique, introduced during the 1990s, presents the treating physician with a robust monitoring tool for brain chemistry in addition to conventional intracranial pressure monitoring. Nevertheless, some limitations remain, such as limited spatial resolution. Moreover, while there have been several attempts to develop new potential pharmacological therapies in TBI, there are currently no available drugs which have shown clinical efficacy that targets the underlying pathophysiology, despite various trials investigating a plethora of pharmaceuticals. Specifically in the brain, MD is able to demonstrate penetration of the drug through the blood-brain barrier into the brain extracellular space at potential site of action. In addition, the downstream effects of drug action can be monitored directly. In the future, clinical MD, together with other monitoring modalities, can identify specific pathological substrates which require tailored treatment strategies for patients suffering from TBI.

KEY WORDS: drug delivery; human; microdialysis; monitoring; pharmacokinetics; traumatic brain injury.

INTRODUCTION

Central nervous system (CNS) injuries, including conditions such as stroke, subarachnoid haemorrhage and traumatic brain injury, are great contributors of morbidity and mortality (1–3). As well as the primary injury or haemorrhage additional secondary injuries lead to deterioration with neuroinflammation, neurotoxicity and deranged metabolism causing further tissue swelling, distress and cellular death (4). While this underlying pathophysiology may overlap for different CNS conditions, there are no approved pharmacological treatments that have shown adequate efficacy in

targeting these conditions. Specifically in traumatic brain injury (TBI), physicians are left with the options of decompressive surgery (5), intravenous hyperosmolar therapy (6), sedative agents (7), hypothermia (8), hyperventilation (9) and anti-epileptic drugs (10) in order to treat the subsequent effects of affected tissue deterioration. The secondary insults which emerge as a result of these pathological derangements, including among others increased intracranial pressure (ICP) and decreased cerebral perfusion pressure (CPP), are closely monitored in the neuro-critical care unit (NCCU) as such derangements have been shown to lead to unfavourable outcome (11). Currently, ICP-targeted therapy is commonly used worldwide, as suggested by the updated Brain Trauma Foundation guidelines (12). However, ICP-targeted therapy has not been shown to improve outcome *versus* patients not monitored with ICP in a randomized control trial (13). That trial (BEST-TRIP) by Chesnut *et al.* concluded that care focused on maintaining ICP at 20 mmHg or less was not superior to care based on imaging and clinical examination. However, patients in both arms received tiered ICP-lowering therapies, and a decompressive craniectomy was performed in 30% of patients in each arm. Hence, the BEST-TRIP trial findings (13) do not challenge the belief that brain oedema and raised ICP should be actively managed after TBI (14).

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One likely reason that ICP monitoring in isolation does not improve outcome is that there are a number of paths to neuronal injury and it is only by considering the full scope of metabolic derangements that secondary injuries to the brain can be avoided and clinical outcome improved (15). Here, microdialysis (MD) is playing an important role in many NCCUs today for patients suffering from TBI and subarachnoid haemorrhage (SAH) (16), but is also actively researched in other CNS conditions such as bacterial meningitis (17, 18) and spinal cord injury (19).

Few pharmaceutical compounds diffuse freely from blood over the blood-brain barrier (BBB) into the brain parenchyma (20). While the blood-cerebrospinal fluid (CSF) barrier is more permissive, the brain-CSF barrier and BBB are a distinct and potent obstacle (at the cellular and molecular levels) for potential neurological drug candidates (21, 22). While the BBB provides important physiological benefits by excluding neuroactive substances, both endogenous and exogenous, from entering the brain, it creates an impediment for pharmacological development. This inherent barrier could explain why only 8% of drug candidates targeting CNS conditions become clinically available treatments, as compared to 15% overall (23). Thus, measuring drug concentrations in CSF is not representative of drug action; as even if it provides an estimate of CNS penetration, significant concentration gradients between the brain parenchyma (extracellular fluid, ECF) and CSF have been empirically demonstrated (24). To gain access to brain ECF for pharmacokinetic monitoring, the only currently available option is microdialysis (MD) (25, 26). Table I summarizes the beneficial effects of MD monitoring in pharmacological trials in TBI, as has been previously reviewed in the literature (27). Moreover, new clinical findings suggest that the MD technique could open up new routes of focal drug administration (28), bypassing innate barriers, although more research is necessary to fully validate the technique.

In summary, the microdialysis technique enables the clinician to monitor the metabolic state of the brain, to map pharmacokinetic concentration gradients of delivered drugs in the brain parenchyma as well as a potential route of drug administration focally, bypassing the BBB. The aims of this review are to elaborate on these aspects of MD monitoring and to provide the reader with the history, present application and future directions of clinical microdialysis in human TBI.

Table I. Advantages with MD Use in Pharmaceutical Trials

Advantages of MD monitoring in drug studies

Pharmacokinetics of drug concentration in brain ECF

Efficacy of bound/unbound drug concentration

Dose-response effects *in vivo*

Limit the effects of intra-patient heterogeneity by direct measurement of concentrations achieved

Analyse surrogate markers of drug efficacy in microdialysate

A list of benefits of MD monitoring in pharmacological trials of neuroprotective drugs

THE MICRODIALYSIS AS A CLINICAL MONITORING TOOL IN TRAUMATIC BRAIN INJURY

The History of Clinically Applied Microdialysis

Microdialysis (MD) was developed by Urban Ungerstedt at the Karolinska Institutet, Sweden, in the 1970s. MD was first described in 1974 (29), where the authors used a hollow dialysis fibre attached to a steel cannula implanted in rat brain to extract dopamine. While the device was novel, the concept of push-pull extraction, similar to MD, was first described in the early 1960s (30). The first MD devices were implanted horizontally through the brain (31), although Ungerstedt and co-workers later developed the first vertical probe containing both delivery and return cannulae arranged concentrically in the same housing in the 1980s (32). The MD catheters used then resemble the devices used today, consisting of a semi-permeable membrane which is inserted into the organ of interest through which a carrier fluid is slowly pumped. Substances will move from the extracellular fluid (ECF), through the pores of the membrane, into the carrier fluid, by diffusion, which is subsequently pumped out of the return catheter and is available for analysis. The first brain MD catheter implantation in humans was performed at the Karolinska University Hospital, Sweden, in 1990, where it was stereotactically inserted in patients with Parkinson's disease in order to study mono-amine concentrations in the basal ganglia (33). In the same year, researchers from Uppsala University, Sweden, monitored a patient undergoing tumour resection with MD and found brain lactate levels similar to what was seen in preclinical models of ischemia (34), thus suggesting brain extracellular fluid lactate to be a valid surrogate marker for cerebral metabolic disturbances. In 1992, the same group presented how they successfully integrated MD at the bedside in the neuro-critical care unit (NCCU) (35), thus providing the treating physician with the metabolic state of the cerebral extracellular environment. In 1995 and 2002, the MD technique became approved by regulating authorities for widespread clinical use in Europe and USA, respectively. During the latter part of the 1990s, the technology became more readily available for hospitals worldwide due to advancements in computing hardware (36). Since then, the technique has been extensively refined, and a multitude of methodological variants have been introduced to improve monitoring of the injured brain.

Microdialysis Monitoring of the Injured Brain

Through several consensus meetings, practising clinicians have contributed recommendations and expert opinion on how to best use, interpret and assess MD and MD findings (16, 37, 38). Clinically, the metabolites glucose, lactate, pyruvate, glycerol and glutamate are sampled at the bedside. The latest consensus meeting, 2014, identified the most important metabolites to measure based on the weight of evidence in the published literature, as surrogate markers of substrate delivery failure and the oxygenation state of the brain, namely glucose, lactate, pyruvate and the lactate/pyruvate ratio (LPR) (16). A brain ECF glucose <0.2 and a LPR >25 in TBI have both been suggested as thresholds as they have been found to correlate to an unfavourable long-

term outcome for patients (39, 40). A simplified schematic of major energy pathways in the brain is shown in Fig. 1. Through glycolysis, glucose will generate pyruvate. Furthermore, if there is an impairment in oxidative phosphorylation within the mitochondrion, either due to oxygen limitation or to mitochondrial dysfunction (41, 42), this will lead to an accumulation of lactate, and increased LPR in brain ECF. Lactate dehydrogenase (LDH) catalyses the biochemical conversion between lactate and pyruvate. Furthermore, the dynamic balance between lactate and pyruvate is determined by the cellular concentration of reducing equivalents (NADPH/NADH) that drive the flux towards lactate. In this way, LPR is a direct measure of the cellular redox state and is the single most biochemically useful metric for use as part of multi-modal monitoring in NCCUs.

The LPR is generally regarded as a more useful clinical marker than lactate concentration alone, although both lactate and pyruvate, along with glucose, also need to be reported individually for proper interpretation of bedside microdialysate monitoring as mentioned above, while glutamate and glycerol are considered of lesser priority (16). Lactate can be metabolised *via* the TCA cycle, and the astrocyte-neuron lactate shuttle hypothesis has been proposed whereby lactate produced by astrocyte glycolysis can be used as a fuel by neurons (43). This hypothesis has been subject to much debate and discussion over time. For a recent review on glycolysis and the significance of lactate in TBI, see (44). Interestingly, lactate can also spin out of the TCA cycle, a process termed cataplerosis (45). While this has previously been noted in experimental studies, our novel discovery of the TCA cycle as a source of lactate in human TBI brain calls

for revision of lactate's significance, hitherto regarded as simply glycolytic, with possible implications for other diseases too (28).

Challenges in Clinical MD Metabolic Monitoring

There are several inherent limitations to the MD technique, such as relative recovery (defined as the ratio of the concentration that is in the extracted dialysate to a true concentration of a given substance) (46) and the limited temporal resolution (by convention, 1 h per sample even if more frequent monitoring is possible), which have been previously reviewed (22, 47). In the following discussion, we have chosen to focus on specific aspects of clinical microdialysis which might cause difficulties in MD assessment and utilization.

The focal nature of microdialysis monitoring has been suggested as the key reason why the technique is struggling to gain ground in the field (48). As shown by several groups, catheters placed in 'peri-contusional' tissue have higher LPR compared to catheters placed in unaffected tissue, as defined by head computerized tomography (39, 49, 50). This is further supported by Nelson *et al.* which noticed differences in MD parameters between catheters placed two centimetres away from mass lesions, compared to catheters placed in closer proximity (51). The criticism is that it is difficult to extrapolate 1 cm³ of MD-monitored brain tissue to the metabolic state of the whole brain. In order to overcome this issue, Stein and co-workers have proposed a 'global metabolic crisis' (LPR > 25 with simultaneous brain ECF glucose levels < 0.8 mmol/L) if radiologically normal-appearing brain not close to focal lesions brain tissue is monitored (52), which was also shown to be independently correlated with long-term outcome. This is usually the most practicable option for MD catheter placement, as specifically directing the microdialysis catheter close to lesions is technically demanding and sometimes impossible. Another way to increase the spatial resolution is to monitor blood outflow from the brain using microdialysis placed in jugular venous efflux, where a higher LPR has been seen following experimental haemorrhagic shock (53). Alternatively by monitoring the CSF compartment, a more global measure of brain metabolism can be derived. By a modified set-up, consisting of a microdialysis catheter inserted into the ventricular CSF *via* an external ventricular drain, this has been performed and provides higher temporal resolution sampling of CSF metabolites by using the MD technique rather than intermittent CSF sampling (54). The converse argument is that by monitoring a larger compartment, the sensitivity of the technique is reduced as focal areas of metabolic crisis may be masked by dilution of the signal from areas of the brain that have a less severe injury. These arguments between focal and global monitors have been well rehearsed in the literature in relation to oxygenation parameters and jugular venous oximetry *vs* focal brain tissue oxygen monitors. In reality, there is no conflict in combining both focal and global monitoring techniques to enhance our understanding of the underlying pathophysiology as both approaches have their relative merits. Microdialysis, as a continuous extracellular monitoring technique, can complement evidence from scans such as positron emission tomography (PET) and magnetic

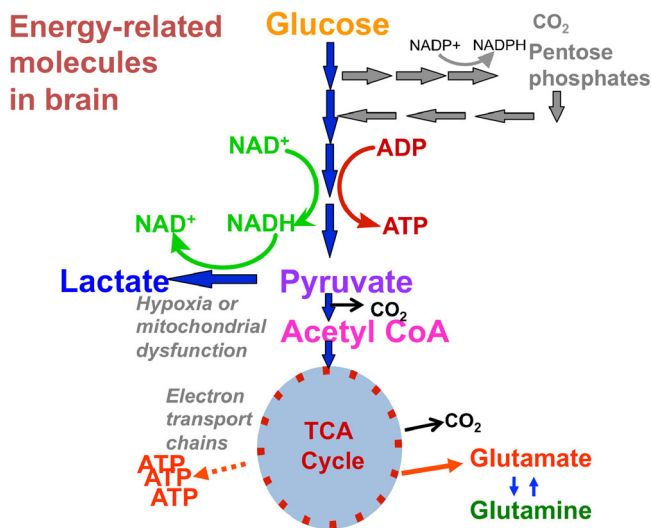


Fig. 1. Simplified schematic of major energy pathways in the brain includes glycolysis, which takes place in the cytosol and produces pyruvate, which enters mitochondria and is converted into acetyl CoA that enters the TCA cycle. Alternatively, pyruvate can stay in the cytosol and is converted into lactate that is exported out of the cell. The pentose phosphate pathway (PPP) takes place in the cytosol and is an alternative energy pathway that can be upregulated after injury; it is an important source of NADPH used to produce the reduced form of glutathione (GSH) for preventing oxidative stress. This figure was originally published by Carpenter *et al.* in Eur J Pharm Sci 57 (2014) 87–97. © 2014 The Authors. Published by Elsevier B.V. Open Access under a CC-BY licence

resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) that provide global 'snap-shots' of the brain tissue that predominantly reflect the intracellular environment (14).

However, there is evidence that following brain injury, the global metabolic state of the injured brain is altered, for example using PET monitoring of the utilization of glucose (55–57), revealing different metabolic derangements. These studies also show on the potential of MD as a focal but dynamic monitor while PET provides a cross-sectional whole brain snapshot and the combination of the two provides complementary data. Moreover, PET can also be used to provide focal data, as regions of interest can be evaluated as desired, during data processing. For example, studies by Hutchinson *et al.* evaluated oxygen metabolism and cerebral metabolic rate of glucose (CMR_{glc}) in regions of interest (ROIs) drawn around the MD catheter tips in triple-oxygen and labelled 2-fluoro-2-deoxy-D-glucose (FDG)-PET scans, respectively (58, 59). In a study by Timofeev and co-workers, up to 75% of the catheters were in radiologically (CT) 'uninjured' tissue; nevertheless, the LPR threshold of >25 correlated with clinical outcome (39). Moreover, different types of intracranial lesions impact on LPR to a greater degree, such as subdural hematomas and contusions (as high as a factor 10x) as compared to epidural hematomas and diffuse injuries (60). This reflects a difference in the metabolic sequelae of these patterns of TBI that is detected by MD monitoring and may carry additional prognostic information and guide therapy.

The MD technique in clinical use has shown high auto-correlation within the same patient (51, 61). This makes data trends as important as specific levels for the individual patient, stressing the need for good threshold levels in order to generalize the technique. In small studies, a useful approach is for each TBI patient to be used as his or her own control (28). Notably, TBI microdialysis data have been found to be highly auto-correlated even with samples at up to 30 h later, and subject identity alone explains 52 to 75% of microdialysis marker variance, determined in a large study (over 7350 hourly samples of complete microdialysis sets from 90 TBI patients) using statistical and pattern-recognition computer models (51). Thus, since microdialysate data are highly individualized, it is therefore logical to use each patient as his/her own control and examine temporal trends rather than comparing with a separate control group of separate TBI patients.

Another suggested limitation is the potential damage created by the insertion of the microdialysis catheter into brain parenchyma. While this has been shown in preclinical models (62), it is difficult to assess the extent of injury in the clinical monitoring in a disease such as severe TBI, and no studies have reported on any traumatic complications due to catheter insertion. However, it is recommended that the first 2 hours of MD sampling collected should not be used to guide care as these may represent unreliable results caused by the insertion and the pump-flush sequence (16).

While the frequency of haemorrhagic and infectious complications in patients with multimodality monitoring is low (63), the amount of MD catheters that either become

dysfunctional for unknown reasons or that are unintentionally removed due to the everyday care is fairly high (>10% in selected studies) (64, 65). The care provided in the NCCU requires patient turning, hoisting and transfer to neuroimaging, which may all result in unintentional MD catheter removal. A way to minimize the risk is to attach the catheter through a bolt where other devices, such as brain oxygen pressure and intracranial pressure probes, can also be inserted (66).

In aggregate, the MD technique presents both theoretical and practical limitations. However, steps can be taken in order to minimize these caveats in clinical practice.

Microdialysis to Monitor Drug Pharmacokinetics in the CNS

In the field of TBI, there are numerous substances that have shown neuroprotective effects *in vitro* as well as promising efficacy *in vivo* in small animal models. However, the transition to the clinic has proven difficult, and large, multicentre trials studying corticosteroid (67), erythropoietin (68) and progesterone (69), all showing promising preclinical efficacy, have failed to show adequate treatment efficacy in human TBI. One of the reasons why drug development in brain injury has been challenging is the heterogeneous nature of the disease, making it impossible to treat all TBI patients using a single 'golden bullet'. Another limitation is that none of these previously mentioned trials have measured the drug concentration in brain ECF (or CSF for that matter); thus, they go straight from preclinical efficacy in animal models to clinical outcome in patients and thereby overlook the putative desired pathophysiological effects these drugs might have in the patients, or if there is a dose-response correlation for unbound drug in the CNS (27, 70). Moreover, without measuring brain ECF concentrations of drugs, these studies have not established whether the drug has reached its target compartment. Inadequate pharmaceutical monitoring of brain ECF was also evident in the recent operation brain trauma therapy (OBTT) where a consortium of laboratories tested a number of different substances in rodent models of brain injury (71). While few drugs showed promising results (the best effect was shown by the anticonvulsant levetiracetam (72)), the authors did not measure CSF or brain ECF concentrations, thus limiting the understanding of dose-response, as well as if an adequate amount of drug even reached the CNS. Suffice to say, there is a need for better pharmacokinetic monitoring when developing studies analysing drug transportation in brain injury trials. In order to establish that a drug has reached the site of pharmacological action in the CNS, there are strong theoretical reasons to favour the brain ECF compartment where the MD technique is currently the only option in human patients.

This is further highlighted in studies analysing antibiotic concentrations (meropenem and doripenem) in the brain ECF compartment following TBI (73, 74). In patients with TBI, bacterial meningitis and other cerebral infectious complications occur in up to 17.2% of TBI patients (75, 76), and the risk increases the longer the period of external ventricular drain monitoring is (77). However, in the more general sense, TBI can provide a model for testing the penetration of these antibiotics into the brain ECF to inform dosing in other pathologies. These studies stress

the need to monitor the brain ECF compartment as it shows much lower antibiotic concentrations compared to serum, even if the extraction fraction (relative recovery) is taken into account, and to what has comparably been detected previously in the CSF (78). Microdialysis can thus provide important evidence as to whether the antibiotic minimum inhibitory concentration (MIC) can be attained in the brain. Treatment of cerebral infections is still a challenge, and that some of the dosages of antibacterial drugs may be inadequate for more resistant bacteria despite seemingly 'effective' plasma concentrations (79). Cerebral microdialysis pharmacokinetics studies of antibiotics can thus form the basis of more accurate dosage for patients with CNS infections (79).

Another pharmacokinetic study employing microdialysis in TBI patients has focused on vigabatrin (VGB), an anticonvulsant and putative neuroprotectant (80, 81). VGB, given enterally to severe TBI patients, crossed the BBB into the brain ECF, where it accumulated with multiple dosing. Pharmacokinetics suggest delayed uptake from the blood. Microdialysate VGB concentrations were higher close to focal lesions, possibly as a consequence of locally greater BBB permeability, than those in distant sites. Microdialysate GABA concentrations increased modestly in some of the patients after VGB administration, consistent with the mode of action of VGB as an inhibitor of GABA-transaminase, an enzyme responsible for degrading GABA. Analogous to the situation with antibiotics (above), microdialysis has a potential role to inform on dosing of anticonvulsants and neuroprotectants. For example, the causes of refractory epilepsy (occurrence of seizures despite anticonvulsant drug treatment) are not known, but inadequate drug concentration in crucial brain areas is a possible contributing factor. Knowledge about more appropriate dosing may thus ensue from microdialysis pharmacokinetic measurements.

Clinical microdialysis studies with small numbers of patients may be sufficient to show whether particular drug candidates might be worth pursuing further in larger clinical trials. A microdialysis study by Hutchinson *et al.* (82) investigated the potentially neuroprotective drug chlormethiazole in five TBI patients. This drug proved to be undetectable in brain microdialysates, suggesting that it did not reach the target site to exert its action. Interestingly, in a phase III clinical trial, chlormethiazole did not improve the outcome in patients with major ischemic stroke (83). This expensive, time-consuming and unsuccessful phase III trial could potentially have been avoided by first carrying out an *in vivo* clinical microdialysis study.

THE FUTURE OF CLINICAL MICRODIALYSIS IN TRAUMATIC BRAIN INJURY

Clinical Utility of the Lactate/Pyruvate Ratio

We acknowledge that monitoring of the biochemical state of the injured brain is complex, and that more research is necessary in order to fully understand intricate relationships between brain metabolites and proteins. Hence, we believe that there is a direction to facilitate monitoring using solely the LPR to monitor patients. While the LPR in theory

is a robust tool, we believe it is best together with other parameters.

Today, there are several different treatment strategies for a range of different intracranial conditions. Moreover, several monitoring modalities make it possible for the treating clinician to gain a better understanding of the ongoing pathological substrates in the injured brain. Different 'pathophysiological targets' have been suggested (15, 60, 84), including distinctive definitions of 'ischemia', 'hypoperfusion' and 'mitochondrial dysfunction'.

We suggest, based on the discussions and data presented during the latest consensus meeting (16), that LPR could act as guide as it would indicate deranged energy generation at the cellular level, creating a more informative monitor as compared to ICP, and that LPR could be used to further direct therapy based on other monitored modalities. Thus, we propose the following pathophysiological targets if the LPR is increased (>25) (Table II):

- If the LPR increase is concomitant with an increased ICP (>20 mmHg), the patient could be suffering from '*intracranial hypertension*'.

- If the ICP is normalized, but LPR is increased and the brain pressure oxygen is simultaneously affected (<15 mmHg). This may be due to '*delivery failure*' of oxygen if the cerebral perfusion pressure (CPP) is decreased and outside the auto-regulatory range (pressure reactivity index (PRx) >0.3) (85) or a '*diffusion barrier*' if the CPP is adequate (86).

- If both normal ICP and substrate delivery through adequate perfusion and oxygen pressures have been established, but the LPR increase remains and brain ECF glucose is low (<0.5–1.0 mmol/L), it could indicate low glucose in the affected brain tissue (87) (also referred to as '*neuroglycopenia*'). This could both be due to an increased diffusion path for glucose or downregulation of glucose transporters (88).

- If both oxygen and glucose delivery are adequate, together with a normal ICP, but the deranged LPR persists, this would fall under the definition of '*mitochondrial dysfunction*' (42), i.e. an inability of the brain tissue to meet energy demands despite adequate substrate and oxygen delivery.

Another pathophysiological state worth mentioning is hyperglycolysis (55, 56), where the utilization of glucose in the tissue increases, as visible by PET imaging, with a simultaneous increase in brain ECF lactate and pyruvate levels (59). The underlying pathophysiology is unknown and could be due to focal seizure activity, spreading depolarisation, and/or mitochondrial dysfunction. Subsequently, potential treatment should target these underlying conditions.

It is important to recognize that in human TBI, several pathological derangements coincide; nevertheless, the classification of specific patterns of injury is useful as a conceptual tool, a method of stratification of patients in research studies and a way of rationalizing treatments. We believe that microdialysis may be utilized, concurrent with a wider panel of multimodality parameters, to guide treatment as it opens up a wider path of potentially treatable pathological conditions than if ICP or other modalities are used in isolation.

Table II. Overview of the Suggested Pathophysiological Targets

Pathological target (if LPR > 25)	ICP (mmHg)	Auto-regulation (PRx)	PbO ₂ (mmHg)	Brain glucose (mmol/L)	Treatment
Intracranial hypertension	>20–25	X	X	X	Increase sedation, hypertonic saline, decompressive craniectomy etc.
Delivery failure	<20–25	>0.3	X	X	Increase CPP
Diffusion barrier	<20–25	<0.3	<15	X	Increase FiO ₂
Neuroglycopenia	<20–25	<0.3	>15	0.5–1.0	Increase systemic glucose levels
Mitochondrial dysfunction	<20–25	<0.3	>15	>1.0	None available

Definitions of intracranial hypertension, delivery failure, diffusion barrier, hypoglycaemia and mitochondrial dysfunction. ‘X’ indicates that this modality does not discriminate for the target of interest. ‘Treatment’ represents tentative treatment strategies for the different pathological targets

PRx pressure reactivity index, PbO₂ brain tissue oxygen pressure, as measured with intracranial oxygen pressure device, ICP intracranial pressure, CPP cerebral perfusion pressure, FiO₂ fraction of inspired oxygen

Pharmacokinetics of Neuroprotective Agents

One of the most important areas for pharmacological studies in TBI is the development and efficacy assessment of neuroprotective substances, where the MD technique has been a valuable addition in some studies.

Cyclosporin A, a calcineurin inhibitor, has shown good neuroprotective effect in preclinical animal studies of TBI (89–91), presumably by inhibiting the Ca²⁺-induced mitochondrial membrane permeability transition leading to less mitochondrial swelling and rupture of the outer mitochondrial membrane (92). In a randomized controlled trial treating severe TBI patients with cyclosporin A, monitored with MD, brain energy metabolism improved significantly in the treated group (25).

In a study by Helmy *et al.*, the authors treated severe TBI patients with interleukin-1 receptor antagonist (IL1ra) (Kineret®) in a double-blind randomized control trial (26). The innate immune system is a key player in the detrimental secondary injury cascades following TBI (93), and this provides the rationale as to why it would be beneficial to target pro-inflammatory interleukin-1 activity. All patients were monitored using MD, and not only were the authors able to accurately study the pharmacokinetics in the brain ECF of the subcutaneously administered drug, they also noted the changes of downstream chemokines and cytokines in the treated group *vs* placebo, extracted from the brain ECF as well (26, 94). Similarly, another randomized control trial analysing anti-platelet VAS203 (a nitric oxide synthase inhibitor) in TBI noted increased metabolites of the drug in the brain, extracted using MD, in the treatment group as compared to those in placebo (95).

In a recently published clinical study, patients suffering from severe spinal cord injury were monitored using MD implanted intradurally at the site of injury (19). This allowed the physicians to guide the spinal perfusion pressure and spinal ECF glucose, as deviations from 90–100 mmHg and 4.5 mmol/L, respectively, resulted in an increase of the LPR in the damaged area (19). The authors also noted that dexamethasone penetration into the tissue increased with increasing spinal perfusion pressure, prompting future trials in spinal cord injury to better monitor the affected area.

In summary, MD offers the potential to monitor the effect of delivered drugs into the injured CNS. Lessons from

previous studies show that pharmacokinetic monitoring is not adequately used and by using MD monitoring, future trials could be extensively improved.

Retrodialysis as a Route of Drug Administration

The design of the microdialysis allows not only substances to be extracted and analysed but also substances to be delivered through the membrane through a process referred to as retrodialysis, when the concentration of a molecule is of higher concentration within the microdialysis perfusate than the brain ECF. Delivery of ¹³C-labelled substrates focally into brain *via* the microdialysis catheter and simultaneous recovery of the ¹³C-labelled metabolites in the emerging microdialysate has been successfully performed in TBI patients (28, 96, 97). In preclinical experiments, retrodialysis has recently been shown to successfully deliver dexamethasone directly in both experimental TBI with ameliorating effects (98) and to transgenic animals expressing decreased microglia activity (99).

In their recently published study, Jalloh and co-workers delivered 2,3-¹³C₂ succinate through microdialysis catheters directly into the brain parenchyma of patients suffering from severe TBI (28), as succinate has been shown to act as energy substrate in affected mitochondria (100). Jalloh *et al.* demonstrated a decrease in LPR in patients with raised LPR at the time, supporting the thesis that succinate can normalize deranged energy metabolism and potentially ameliorate mitochondrial dysfunction by bypassing complex I of the mitochondrial membrane electron transport chain (ETC) (28). Succinate is energetically important as it is both a TCA cycle intermediate and a direct contact between the TCA cycle and the mitochondrial ETC complex II (succinate dehydrogenase (SDH)), on the mitochondrial inner membrane. An important feature of the study by Jalloh *et al.* was their use of double ¹³C-labelling, which proved unambiguously that the substrate molecules (2,3-¹³C₂ succinate) crossed from the microdialysis perfusate into the brain extracellular space, entered cells and were metabolised, and the metabolites exported into the extracellular fluid, and recovered by the microdialysis catheter. The characteristic NMR doublets arising from two adjacent ¹³C atoms prove cellular uptake and mitochondrial metabolism of 2,3-¹³C₂ succinate revealing downstream metabolites. Doublets are clearly distinct from native endogenous molecules, because

the probability of two endogenous ^{13}C atoms being adjacent to each other naturally is 0.01% (a 1 in 10,000 chance) and indistinguishable from baseline ‘noise’ in the NMR spectra. As SDH is exclusively mitochondrial, downstream metabolites of 2,3- $^{13}\text{C}_2$ succinate must be derived from pathways stemming from mitochondrial metabolism.

Not only does delivery by MD open up a new administration route for several promising neuroprotective agents for microdosing in a research setting but it also allows the possibility to assess efficacy (bypassing the BBB) in the treated area.

CONCLUSIONS

Clinical MD has emerged as an important addition to conventional intracranial monitoring of patients suffering from severe TBI. While the technique is associated with some caveats, new research further facilitates the usage and assessment of clinical MD. New pharmacological trials targeting the injured CNS will benefit from better monitoring, where the MD technique will be able to determine pharmacological concentration and dose-response effect in brain ECF. Finally, the MD, together with other monitoring modalities, may in the future help the clinician establish treatable pathophysiological targets.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The author(s) declare the following potential conflict of interest. PJH is a Director of Technicam, the company that manufactures the triple lumen cranial access device used in our microdialysis studies in Cambridge. The other authors (EPT, KLHC and AH) declare no conflicting interests.

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