Monitoring of brain interstitial total tau and beta amyloid proteins by microdialysis in patients with traumatic brain injury

Clinical article

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Object. Damage to axons contributes to postinjury disabilities and is commonly observed following traumatic brain injury (TBI). Traumatic brain injury is an important environmental risk factor for the development of Alzheimer disease (AD). In the present feasibility study, the aim was to use intracerebral microdialysis catheters with a high molecular cutoff membrane (100 kD) to harvest interstitial total tau (T-tau) and amyloid beta 1–42 (Aβ42) proteins, which are important biomarkers for axonal injury and for AD, following moderate-to-severe TBI.

Methods. Eight patients (5 men and 3 women) were included in the study; 5 of the patients had a focal/mixed TBI and 3 had a diffuse axonal injury (DAI). Following the bedside analysis of the routinely measured energy metabolic markers (that is, glucose, lactate/pyruvate ratio, glycerol, and glutamate), the remaining dialysate was pooled and two 12-hour samples per day were used to analyze T-tau and Aβ42 by enzyme-linked immunosorbent assay from Day 1 up to 8 days postinjury.

Results. The results show high levels of interstitial T-tau and Aβ42 postinjury. Patients with a predominantly focal lesion had higher interstitial T-tau levels than in the DAI group from Days 1 to 3 postinjury (p < 0.05). In contrast, patients with DAI had consistently higher Aβ42 levels when compared with patients with focal injury.

Conclusions. These results suggest that monitoring of interstitial T-tau and Aβ42 by using microdialysis may be an important tool when evaluating the presence and role of axonal injury following TBI. (DOI: 10.3171/2008.9.JNS08584)

Key Words • amyloid beta • diffuse axonal injury • microdialysis • tau protein • traumatic brain injury

Widespread traumatic damage to axons and white matter tracts, referred to as DAI, may be an important contributor to functional and behavioral deficits following TBI. Although originally described in severely brain injured patients, damage to axons is increasingly being observed also in cases of mild TBI. It has become evident that axonal injury does not occur exclusively at the time of injury. Instead, there may be a progressive axonal swelling continuing as long as several days postinjury, ultimately causing disconnection of the injured axons. Conventional clinical neuroimaging is relatively insensitive in detecting axonal injury following TBI, and understanding of the temporal progression of human DAI is lacking. Thus, in addition to improved neuroradiological techniques, clinically available biomarkers for axonal injury are urgently needed.

Microdialysis is the only bedside technique that can monitor the focal neurochemistry of the injured human brain, and it is increasingly being used as a routine clinical monitoring method in many neurointensive care units worldwide. Until recently, the limited membrane pore size of microdialysis catheters has permitted sampling of only LMW compounds (< 20 kD), including for example glucose, lactate, pyruvate, glutamate, and glycerol. The recent development of commercially available microdialysis catheters with a membrane pore size of 100
kD has allowed the sampling of small proteins, providing a tool for the evaluation of novel biomarkers in the injured brain. The Aβ peptides are cleaved by proteolysis from the APP and constitute the major components of amyloid plaques in AD. Due to both disruption of the normal axonal transport and a TBI-induced upregulation, a marked accumulation of APP has been observed in damaged axons following TBI in both animal models and in humans. Accordingly, extensive coaccumulation of Aβ peptides with APP was observed in swollen axons within days after injury in a porcine model of DAI, and widespread axonal Aβ accumulation associated with Aβ plaques was also observed in brain-injured humans with DAI. Tau protein is a microtubule-associated protein that is involved in microtubule assembly and stabilization and is predominantly present in neurons and axons. These observations suggest that damaged axons may be an important source of Aβ and tau following TBI. In addition, there is an increased risk for AD in patients who in adulthood sustained a severe head injury. Amyloid plaques and neurofibrillary tangles, formed by abnormal tau filaments, are pathological hallmarks of AD.

The aim of this pilot study was to evaluate whether T-tau, Aβ40, and Aβ42 could be detected in the injured human brain by using microdialysis, and we correlated our findings to the clinical course and the cerebral energy metabolic pattern. In particular, we were interested in T-tau and Aβ42 as potential biomarkers for axonal injury.

Methods

All research procedures described herein were approved by the Regional Research Ethics Committee at Uppsala University, and informed consent was obtained from the patient’s closest relative.

Patient Population

The study included 8 consecutive patients with a severe TBI (5 male and 3 female patients, mean age 35 ± 18 years [range 15–67 years]) who presented in our NICU with a postresuscitation GCS score of 4–8 (RLS score of 3–7). The median GMS score for all patients was 4, corresponding to a median RLS score of 5 (Table 1). All patients underwent monitoring of ICP, for which either an intraparenchymatous ICP monitor (Codman, Johnson & Johnson; 4 patients), an external ventricular drain (2 patients), or both an external ventricular drain and an intraparenchymatous monitor (2 patients) were used. The patients were treated in the NICU at Uppsala University Hospital according to an ICP-guided protocol with mild hyperventilation (PaCO₂ 30–35 mm Hg; 4.0–4.5 kPa), head elevation (30°), and careful volume expansion to obtain normovolemia. All patients had a decreased level of consciousness, were intubated, and received artificial ventilation. Propofol sedation was used initially in all patients, but due to refractory elevations of ICP, high-dose sodium pentobarbital infusion was initiated in 2 patients (Cases 1 and 3; Table 1). Surgery for the evacuation of space-occupying focal mass lesions was performed in 3 patients, and a bilateral decompressive frontal craniec-
Monitoring of interstitial axonal injury markers using microdialysis

### TABLE 1: Demographic and injury characteristics of 8 patients with TBI*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Group</th>
<th>Age (yrs), Sex</th>
<th>Cause of Injury</th>
<th>Associated Injuries/Conditions†</th>
<th>GMS/RLS Scores on Arrival</th>
<th>Pupils</th>
<th>MD Duration (hrs);‡</th>
<th>LOS (days)</th>
<th>GMS/RLS Scores on Departure</th>
<th>eGOS Score at 3 Mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DAI</td>
<td>21, F</td>
<td>MVA</td>
<td>lung contusion</td>
<td>4/5 bilateral dilated</td>
<td>13–180</td>
<td>17 NA</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DAI</td>
<td>15, M</td>
<td>moped accident</td>
<td>femur fx, facial fx</td>
<td>27 bilateral nonreactive</td>
<td>15–133</td>
<td>12 6/2</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>DAI</td>
<td>18, F</td>
<td>MVA</td>
<td>alcohol</td>
<td>5/3 normal</td>
<td>17–177</td>
<td>14 6/1</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>focal/mixed</td>
<td>31, M</td>
<td>fall</td>
<td>alcohol</td>
<td>4/4 normal</td>
<td>19–125</td>
<td>13 6/2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>focal/mixed</td>
<td>41, M</td>
<td>bike accident</td>
<td>alcohol</td>
<td>5/3 normal</td>
<td>14–121</td>
<td>10 6/2</td>
<td>6</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>focal/mixed</td>
<td>33, M</td>
<td>abuse</td>
<td>alcohol, skull fx</td>
<td>2/7 normal</td>
<td>17–89</td>
<td>9 6/2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>focal/mixed</td>
<td>67, M</td>
<td>fall</td>
<td>alcohol</td>
<td>3/6 lt dilated</td>
<td>26–70</td>
<td>4 5/3</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
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<td>focal/mixed</td>
<td>55, F</td>
<td>fall</td>
<td>none</td>
<td>5/4 normal</td>
<td>16–41</td>
<td>2 5/3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* eGOS = extended GOS; fx = fracture; LOS = length of stay; MD = microdialysis; MVA = motor vehicle accident; NA = not available.
† “Alcohol” denotes high serum ethanol levels on arrival at the primary hospital.
‡ Number of hours postinjury at which the microdialysis was begun and then stopped; the interval between the numbers represents the duration of microdialysis.
§ Transferred to another neurosurgical unit.

until further analyzed. Urea was monitored to control probe performance.31 Glutamate and glycerol were analyzed retrospectively (CMA model 600). The Analyzer was automatically calibrated when started, as well as every 6th hour thereafter by using standard calibration solutions from the manufacturer. Quality controls at 2 different concentrations for each substance were performed every weekday. The imprecision value for between-assay coefficient of variation was < 10% for all analytes.

Based on previous data30 and personal experience, the following values for the LMW microdialysis analytes were considered critical, with potential pathological implications: glucose < 1 mmol/L; LPR > 30; pyruvate < 120 μmol/L; glutamate > 15 μmol/L; and glycerol > 100 μmol/L.

### Analysis of T-tau, Aβ40, and Aβ42

After bedside analysis, the remaining hourly microdialysis samples (~14 μl) were pooled into 12-hour fractions and used to analyze T-tau, Aβ40 (MW 4330 D), and Aβ42 (MW 4514 D) according to previously published methods.

The level of T-tau (measuring all isoforms of tau, independently of phosphorylation state), and the monoclonal antibody 3D6, which is specific to the N terminus, was used as the detector. Detection limits for T-tau, Aβ40, and Aβ42 were 75, 156, and 15.6 pg/ml, respectively.

### Statistical Analysis

All statistical analyses were performed using Statistica software (StatSoft, Inc.). The data were normally distributed and comparisons between the groups with diffuse or focal injuries were performed using a repeated-measures analysis of variance. The AUC values were calculated. These data were not normally distributed, and pairwise comparisons were made using the Mann-Whitney U-test. The T-tau, Aβ40, and Aβ42 data and LMW microdialysis analytes were correlated from 27 to 99 hours postinjury, and a probability value of < 0.05 was considered statistically significant. Data are presented as the mean ± standard deviation.

### Results

### Microdialysis Protocol

The mean duration from time of accident to start of microdialysis sampling was 17.1 ± 4 hours, and the mean duration of microdialysis sampling was 119.5 ± 50 hours (Table 1). No signs of bleeding around the microdialysis probe were seen on CT scans.

### Analyses of LMW Substances Using Microdialysis

Urea levels in microdialysis samples were stable, indicating adequate probe performance, with a gradually increasing trend during the course of the monitoring period as previously described.31

### Glucose

Glucose levels in microdialysis samples showed a decreasing trend during the monitoring time for both injury groups, and there were no significant differences between the groups. In the focal injury group, 123 (39.9%) of 308 samples had a glucose value of < 1 mmol/L. In the DAI group, 238 (56.8%) of all 419 microdialysis samples showed a glucose value of < 1 mmol/L. Particularly at the later part of the microdialysis monitoring time, there were low glucose levels in the DAI group (data not shown).
The LPR. In the focal injury group, 51 (17.2%) of 296 samples had an LPR of > 30. One patient (Case 7) showed a markedly elevated LPR (range 44–69) in 36 of 37 samples. In the DAI group, 29 (7%) of 413 microdialysis samples showed an LPR of > 30. There were no significant differences in the LPR among the injury groups. The LPR was significantly positively correlated to glutamate levels from 27 to 63 hours postinjury (p < 0.05; r = 0.98–0.99; data not shown).

Glutamate. In the focal injury groups, 94 (29.6%) of 318 microdialysis samples had glutamate levels > 15 μmol/L (56 of 318 were > 40 μmol/L). In the DAI group, 21 (5.2%) of 402 samples showed a glutamate level of > 15 μmol/L (0 of 402 were > 40 μmol/L). There were no significant differences in glutamate levels among the injury groups (data not shown).

Glycerol. In the focal injury group, 50 (17.3%) of 289 samples had a glycerol level of > 100 μmol/L. In the DAI group, 118 (27.7%) of all 426 microdialysis samples showed a glycerol level > 100 μmol/L. There were no significant differences in glycerol levels among the injury groups (data not shown).

In conclusion, these data suggest that there was no significant difference in energy metabolic status between the patient groups at the site of axonal injury marker sampling.

Interstitial T-tau, Aβ40, and Aβ42 Levels on Microdialysis

The T-tau. In all patients and samples, the T-tau was above the detection limit (mean 2881 ± 1774 pg/ml, range 121–6500 pg/ml) up to 173 hours postinjury. There were gradually decreasing T-tau levels during the monitoring time (Fig. 1A). The average T-tau levels were consistently higher in patients with a focal/mixed TBI when compared with patients who had DAI (Fig. 1A), being significantly higher when comparing the AUC calculations from 27 to 75 hours postinjury (p < 0.05; Fig. 1B).

The Aβ42. Using microdialysis sampling, Aβ42 was detected in all patients except for one with a focal TBI (Case 7; Table 1) who had high T-tau levels (up to 5700 pg/ml), and in whom the microdialysis catheter was placed in pericontusional tissue. Initial Aβ42 levels were consistently higher in the DAI group when compared with the focal/mixed injury group (Fig. 1C), although without reaching statistical significance. There was no statistically significant difference in the AUC calculations (data not shown). At 27–38 hours, although not at later postinjury time points, a negative correlation between T-tau and Aβ42 levels was found using microdialysis (p < 0.05; r = −0.95).

The Aβ40. The Aβ40 levels were above detection levels (> 156 pg/ml) in 4 patients (3 with focal/mixed TBI and 1 with DAI; Cases 2, 4, 5, and 6 in Table 1). In the patient with DAI (Case 2), Aβ40 levels were high (mean 5421, range 2761–8629 pg/ml) throughout the monitoring time. In the patient in Case 4, 6 of 8 samples were over the detection limit (mean 1149, range 651–1493 pg/ml); in the patient in Case 6, 2 of 6 were over the detection limit (mean 694, range 419–970 pg/ml); and in the patient in Case 5, 3 of 9 were over the detection limit (mean 1316, range 327–2651 pg/ml).

Illustrative Cases

Case 1

This patient was involved in a car accident and pre-
Monitoring of interstitial axonal injury markers using microdialysis

Fig. 1. A: Graph showing interstitial T-tau levels obtained in 8 severely brain injured patients up to 123 hours (h) postinjury. The patients with focal/mixed disease (open squares; 5 patients) had consistently higher T-tau levels on microdialysis sampling than those with DAI (open triangles; 3 patients). B: Bar graph showing the AUC calculations for T-tau from 27 to 75 hours postinjury. The AUC levels were significantly higher (*p < 0.05) in patients with focal/mixed disease (stippled bar; 3 patients) compared with those who had DAI (black bar; 3 patients). C: Graph showing interstitial Aβ42 levels from 8 severely brain injured patients. Due to missing data, no patient with a focal disease had Aβ42 levels available beyond 99 hours postinjury. The patients with DAI (open triangles; 3 patients) had higher Aβ42 levels than those with focal/mixed disease during the first 75 hours of microdialysis monitoring (open squares; 5 patients).

In the present report, which is the first to evaluate markedly reduced from elevated levels (Fig. 2C and D). The LPR was > 20–25 during the course of the disease, and following craniectomy it gradually decreased to 15–20 (data not shown). The T-tau levels gradually decreased to low levels (< 1000 pg/ml) at ~ 100–110 hours postinjury. In the sample obtained immediately prior to the craniectomy, a marked elevation of T-tau is observed that decreased following surgery (Fig. 2B). In the same microdialysis samples, Aβ40 levels were below the detection limit and Aβ42 levels did not change from stable and relatively low levels (31–67 pg/ml; data not shown). At follow-up the patient was severely disabled (extended GOS Score 3).

Case 2

This patient was involved in a moped accident and presented in our unit with bilaterally dilated and unresponsive pupils with GCS score of 4. The initial CT scan (not shown) demonstrated diffuse brain swelling and minor, central contusions indicative of a DAI. The ICP remained relatively stable with occasional plateau waves during the first 3 days, and the patient was treated with controlled ventilation and propofol sedation for 9 days. The neurochemical findings in the right frontal lobe are shown in Fig. 3. The energy metabolic status was unremarkable, showing an LPR of < 30 for all samples. Glutamate levels never exceeded 28 μmol/L initially, and then decreased to low levels (data not shown). The glycerol levels were normal except for occasional elevations to > 100 μmol/L, and glucose values showed a gradually decreasing trend, reaching < 1 mmol/L during the later course of the disease. This patient also had high Aβ42 (mean 253, range 155–420 pg/ml) and Aβ40 levels (mean 5421, range 2761–8629 pg/ml), although the T-tau levels were the lowest among all patients in this series (mean 1753, range 1206–1856 pg/ml). This patient made a good recovery (extended GOS Score 6).

Case 7

After a fall, this patient presented in the emergency department with a GCS score of 14–15, but rapidly deteriorated to a GCS Score of 5–6 with a dilated left pupil; an emergency CT scan revealed a large intracerebral hemorrhage and an acute SDH on the left side (Fig. 4A). Both the ICHs and SDHs were immediately evacuated, and the microdialysis probe was placed through the craniotomy in pericontusional tissue. Postoperatively, the patient improved to a GMS score of 5 (RLS 3). The results of neurochemical investigations are shown in Fig. 4B–D. The LPR was consistently elevated (> 30) and glutamate was very high and increasing (Fig. 2C and D). In contrast, the glycerol levels were normal and never exceeded 100 μmol/L, and glucose levels were < 1 mmol/L from 46 hours postinjury and onward (data not shown). The T-tau levels showed high initial values that gradually decreased (Fig. 2B). No Aβ40 or Aβ42 levels exceeding the detection limit were observed. This patient never regained consciousness and died soon after the stay in our unit.

Discussion

In the present report, which is the first to evaluate
intracerebral microdialysis for the monitoring of interstitial T-tau and Aβ42, 8 severely brain injured patients were analyzed. Our findings indicate that T-tau and Aβ42 levels vary in relation to the clinical status of the patients. The T-Tau levels were significantly higher in patients with focal TBI in contrast to Aβ42 levels, which were consistently higher in patients with DAI, without reaching statistical significance. The Aβ42 levels were negatively correlated to T-tau levels during the initial postinjury days. These pilot data suggest that the recent development of microdialysis catheters with a high-MW cutoff may provide an important tool to evaluate and monitor novel biomarkers of importance for the understanding of the role of axonal injury in clinical TBI. Our working hypothesis is that the clinical value of biomarkers may increase when microdialysis is used to harvest the biomarkers directly in the injured brain parenchyma, because of an improved spatial and temporal resolution in comparison with CSF and blood analysis.

**Fig. 2.** Case 1. Neurochemical and CT findings in a patient with severe diffuse brain swelling causing ICP elevations refractory to medical therapy and pentobarbital infusion. At Day 5 postinjury, a bilateral frontal decompressive craniectomy (indicated by an arrow on graphs) was performed, with marked improvement in ICP. The preoperative CT scan is shown (A). Changes in T-tau (B), glycerol (C), and glucose (D) levels detected using microdialysis sampling are shown in the graphs. The Aβ42 levels remained at low and stable levels (31–67 pg/ml; data not shown) throughout the monitoring time.

**Interstitial Aβ42 and Aβ40 in TBI**

We observed that brain interstitial Aβ42 was detected with microdialysis sampling following mixed/focal or diffuse TBI in 7 of 8 patients. Because an uninjured control group was not available, interstitial Aβ42 levels in uninjured patients remain unknown. In the CSF, levels of Aβ42 were increased following TBI, although a contradictory report exists. The Aβ42 levels observed in the present study (31–295 pg/ml) were comparable to those previously observed in the ventricular CSF following TBI (median levels ~ 20–100 pg/ml), with initial CSF levels < 20 pg/ml. In a previous report, patients with TBI had lower Aβ42 levels in the lumbar and ventricular CSF compared with patients with hydrocephalus and headache, although their Aβ42 levels were similar to those in the present report (median 167 pg/ml). In the study by Olsson et al., plasma levels of Aβ42 were in the normal range, ~ 50 pg/ml, and that did not change following TBI, even in the presence of marked elevation of
Aβ42 in the CSF (> 1000 pg/ml). These data may suggest that our observed interstitial Aβ42 levels may be elevated and that the analyte was not derived from plasma through a disturbed blood-brain barrier post-TBI. We argue that microdialysis sampling may be a better alternative for the detection of Aβ42 post-TBI than CSF, because of the common difficulties in obtaining CSF in the initial postinjury phase due to brain swelling and/or shift.

Following transmembrane cleavage and metabolism of APP mediated by β-site APP cleaving enzyme (β-secretase) and presenilin-1(γ-secretase), Aβ peptides may be produced. Due to both disruption of the normal fast axonal transport and TBI-induced upregulation, a marked accumulation of APP has been found in damaged axons following brain trauma in several experimental animal models. Accordingly, extensive coaccumulation of Aβ together with APP was observed in axons within days postinjury in a pig model of DAI and in rodent TBI models. Clinical studies have shown that the increased APP expression is associated with the extent of axonal injury in patients with TBI, and numerous studies with survival times between 4 hours and many years postinjury have demonstrated accumulation of Aβ peptides in damaged axons post-TBI. (however, see Adle-Biassette et al.).

The principal source of Aβ is probably peptide release from the presynaptic ending of the axon (however, see Siman et al.). and axonal damage may be the source of increased production and/or accumulation of APP and Aβ. In the present report, interstitial Aβ42 levels were slightly higher in the DAI group when compared with patients who had focal disease. These findings are similar to those following acute ischemic stroke, where levels of Aβ42 in ventricular CSF were unchanged, supporting the view that axonal injury and not focal neuronal damage is the most important contributor to interstitial Aβ42 following TBI. In the present report, we also analyzed brain interstitial Aβ40 levels that were above the detection limit in only 50% of patients. These data are in accordance with previous postmortem series or resection studies showing a preponderance of Aβ42 over Aβ40 following TBI. The increased Aβ42 levels over Aβ40 may be of importance; it is plausible that, similar to plaque genesis in AD, Aβ42 is more prone to aggregation and may initiate plaque formation. In a resection series, occasional patients showed increased Aβ40 depositions. It is yet unclear, similar to the patient in our series, whether high Aβ40 levels postinjury are of pathophysiological importance in clinical TBI.

**Interstitial T-tau in TBI**

Human brain tau protein has 6 isoforms that are mainly found in neurons, and tau mRNA is also expressed predominantly in neurons, particularly in their axons. Tau proteins are microtubule associated and contribute to axonal transport and the maintenance of the cytoskeleton. In patients with TBI, there may be an enhanced risk for developing diffuse Aβ deposits and neurofibrillary tangles due to abnormal tau filaments, both of which are pathological hallmarks of AD (see Introduction).

We observed that interstitial T-tau was detected using microdialysis in all patients with TBI that we evaluated. Interstitial levels may not be directly compared with those in CSF, where various forms of tau proteins have previously been evaluated post-TBI. The T-tau levels observed in the present study (range 121–6500, median 2170 pg/ml) were comparable to those previously observed when analyzing T-tau in the ventricular CSF following TBI. Additionally, in patients with normal-pressure hydrocephalus, high CSF levels (> 702 pg/ml) of tau protein correlated with a poor clinical outcome.

Increased tau levels are generally believed to represent axonal injury in various neurodegenerative diseas-
Fig. 4. Case 7. Neurochemical and CT findings following surgery in a patient with a severe focal TBI that was immediately evacuated. The preoperative CT scan is shown (A). Changes in T-tau (B), glutamate (C), and LPR (D) levels detected using microdialysis sampling are shown in the graphs. The T-tau levels were high initially and then gradually decreased. Interstitial Aβ40 and Aβ42 were below the detection limit.

Following axonal injury, microtubules will be damaged, releasing tau proteins into the interstitial space and the CSF. In our patient series, patients with a focal/mixed TBI had significantly higher T-tau levels compared with patients with DAI. Although tau protein was found to accumulate in both axons and neuronal cell bodies post-TBI, our present findings are consistent with those from a resection series in which temporal cortex was surgically excised early post-TBI. Here, diffuse tau immunostaining was observed in most patients in neuronal cell soma, in axon and glial cells, whereas only 2 of 18 patients showed tau-positive neurofibrillary tangles. These data and ours suggest that although tau is present in axons, this protein may also be released from neuronal and glial cell damage.

Methodological Aspects

In the present report, we used a 4% albumin solution to increase the colloid osmotic pressure of the perfusate to minimize fluid loss. However, standard perfusate (without albumin or dextrane) has been used safely in patients, in whom it was demonstrated that dialysate volume was maintained during passage through the CMA 71 catheter (with a 10-mm membrane length) despite the omission of dextrane. The addition of albumin did not appear to change the recovery of standard metabolites (urea, glycerol, glutamate, lactate, and pyruvate), which is similar to numerous reports from our group and others and in accordance with a recent study comparing the commercially available CMA 70 and 71 catheters. Since the termination of this study, our own clinical experience and bench testing suggested that omission of albumin was safe in patients with TBI (CMA 71 catheter, 10-mm membrane). Future testing using standard perfusate will allow comparisons to the data in our current report.

Following its introduction into clinical neurosurgery in 1990, the majority of reports on clinical intracerebral microdialysis has focused on LMW molecules such as lactate, pyruvate, glutamate, and glycerol, indicating impending or manifest ischemia (see Hillered et al.). In the present study, no in vivo calibration of the microdialysis catheters was performed. Instead, we used urea as an endogenous reference compound. In the evaluated patients, interstitial urea levels did not change markedly during the course of
Monitoring of interstitial axonal injury markers using microdialysis

the disease, except for a gradually increasing trend in most patients as previously described, presumably reflecting a general catabolic state of the patients in the NICU. Although microdialysis catheters were used for up to 9 days postinjury, the urea levels indicated a correct performance of these devices. In addition, we cannot exclude the possibility that insertion of the microdialysis catheters per se induced tissue damage that may influence the levels of the evaluated markers. However, a previous study evaluating the neuropathological effects of microdialysis probe insertion in sheep did not find evidence for microdialysis-induced axonal injury.21 In our present series, 4 of 5 patients with focal TBI had their microdialysis catheters placed ipsilateral to the injury. Further studies in which (for example) bilateral microdialysis probes are used may be necessary to evaluate the influence of microdialysis catheter placement on interstitial biomarker levels.

Presently, the relative recovery of the evaluated injury biomarkers by using the new microdialysis catheters is unknown. When evaluating interleukin-1b and interleukin-6 by using the 100-kD cutoff catheter, a recovery of 7–10% (for interleukin-6) and 20–30% (for interleukin-1b) was suggested.23 These values are considerably lower and more variable than those reported for membranes with a 20-kD cutoff, in which the recovery of the routinely measured LMW substances was consistently ~ 70% with the microdialysis system used in this study.23 The variability of small peptide recoveries in particular remains an issue that warrants further elucidation.

Even though both Aβ40 and Aβ42 (MW ~ 4.5 kD) could theoretically be evaluated using the 20-kD cutoff microdialysis catheter, the use of a catheter with a 100-kD cutoff membrane enabled us also to evaluate concomitant changes in T-tau (MW 40–60 kD). Thus, we suggest that the 100-kD cutoff microdialysis membrane may be recommended for the evaluation of axonal injury markers using microdialysis.

Conclusions

Our results provide novel information on T-tau and Aβ42, which are important biomarkers for axonal injury and AD. Due to the limited number of patients in the study, our results must be interpreted with caution. The correlation of these biomarkers to long-term outcome and use of enhanced radiological methods such as diffusion tensor imaging and/or electrophysiological measures are needed and should be evaluated in future studies. However, our results indicate that the interstitial levels of T-tau were higher in patients with focal disease and that the Aβ42 levels were higher in patients with DAI, indicating that Aβ42 may be a more sensitive biomarker of axonal injury than T-tau.

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Monitoring of interstitial axonal injury markers using microdialysis


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