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Monitoring of β-Amyloid Dynamics after Human Traumatic Brain Injury

Niklas Marklund, Nina Farrokhnia, Anders Hänell, Eugeen Vanmechelen, Per Enblad, Henrik Zetterberg, Kaj Blennow, and Lars Hillered

Abstract

Epidemiological evidence links severe or repeated traumatic brain injury (TBI) to the development of Alzheimer’s disease (AD). Accumulation of amyloid precursor protein (APP) occurs with high frequency after TBI, particularly in injured axons, and APP may be cleaved to amyloid-β (Aβ) peptides playing key pathophysiological roles in AD. We used cerebral microdialysis (MD) to test the hypothesis that interstitial Aβ levels are altered following TBI and are related to the injury type, cerebral energy metabolism, age of the patient, and level of consciousness. In the present report, we evaluated 10 mechanically ventilated patients (7 male, 3 female, ages 18–76 years) with a severe TBI, who had intracranial pressure and MD monitoring. Each MD sample was analyzed for hourly routine energy metabolic biomarkers (MD-lactate, MD-pyruvate, MD-glucose, and MD-lactate/pyruvate ratio), cellular distress biomarkers (MD-glutamate, MD-glycerol), and MD-urea. The remaining MD samples were analyzed for Aβ1–40 (Aβ40; n = 765 samples) and Aβ1–42 (Aβ42; n = 765 samples) in pooled 2 h fractions up to 14 days post-injury, using the Luminex xMAP technique, allowing detection with high temporal resolution of the key Aβ peptides Aβ40 and Aβ42. Data are presented using medians and 25th and 75th percentiles. Both Aβ40 and Aβ42 were consistently higher in patients with predominately diffuse axonal injury compared with patients with focal TBI at days 1–6 post-injury, Aβ42 being significantly increased at 113–116 h post-injury (p < 0.05). The Aβ levels did not correlate with the interstitial energy metabolic situation, age of the patient, or the level of consciousness. These results support that interstitial generation of potentially toxic Aβ species may occur following human TBI, particularly related to axonal injury.

Key words: Aβ; APP; axonal injury; cerebral MD; energy metabolism; TBI

Introduction

Traumatic brain injury (TBI) frequently causes long-term sequelae such as cognitive impairment, personality changes, and depression leading to social problems and a reduced quality of life.1,2 The chronic disabilities associated with apparently mild TBI, occurring from some blast injuries sustained in the military setting or during sport activities, are also increasingly recognized.3,4 Epidemiological evidence links severe or repeated TBI to the development of Alzheimer’s disease (AD).5–10 Amyloid-β (Aβ) plaques, a hallmark pathological finding observed in the brains of AD patients, have been observed in a subset of TBI patients within hours post-injury.5,11,12 The membrane glycoprotein amyloid precursor protein (APP) is synthesized in the neuronal cytoplasm, and accumulates within injured axons following TBI.5,13–16 APP may be proteolytically cleaved by different enzymes including α-secretase, preventing the generation of Aβ peptides, or the β-site APP cleaving enzyme 1 (BACE1). BACE1-mediated APP cleavage, combined with γ-secretase-mediated cleavage of the remaining APP stub, leads to the generation of amyloid-β (Aβ) peptides of various lengths, including the 40 and 42 amino acid long Aβ peptides.17–20 BACE1 and presenilin, important for Aβ generation, have also been found to accumulate in injured and swollen axons post-injury.21,22 Although normal neuronal activity can produce Aβ via APP processing,23 these findings make the formation of Aβ peptides a likely consequence of axonal injury. Importantly, inhibition of APP β- or γ-secretases improves outcome in experimental TBI.24 As β- and γ-secretase-generated Aβ forms are released into the interstitial fluid, cerebral microdialysis (MD) is a preferred sampling method for these biomarkers.25,26
One predominant goal of neurocritical care (NCC) is the detection and avoidance of cerebral ischemia. Ischemic brain damage has long been considered a major secondary injury mechanism in TBI.\(^{27}\) In modern NCC, MD may be used to detect patterns suggestive of ischemia such as an elevated MD lactate/pyruvate ratio (MD-LPR)\(^{28-30}\) combined with a reduced interstitial glucose (MD-glce\(^{31,32}\)). In a recent study on 223 TBI patients, MD-LPR and MD-glce were found to be independent predictors of poor outcome.\(^{33}\) Experimental cerebral ischemia leads to increased APP production and upregulation of BACE activity.\(^{34-36}\) Additionally, cerebral ischemia has been associated with increased production of Aβ from APP, both in experimental models\(^{37,38}\) and in humans.\(^{39-41}\) These reports suggest a link between energy metabolic disturbance and Aβ peptide formation.

Experimentally, Aβ may also have direct neurotoxic properties,\(^{42-44}\) and, therefore, be important for the pathophysiology of TBI. In three recent publications,\(^{25,26,45}\) MD was used to detect interstitial Aβ formation early following severe TBI in humans. Using an Aβ(1-1x) enzyme-linked immunosorbent assay (ELISA) not directly measuring Aβ40 or Aβ42, MD- Aβ levels were found to correlate with the level of consciousness in neurovascular and TBI patients in an NCC setting.\(^{26}\) In another report, the temporal patterns of Aβ peptides could be studied in 12 h fractions using a sandwich ELISA.\(^{25}\) The results of this pilot study showed that Aβ42 was consistently higher in patients with diffuse TBI, calling for additional studies of this potentially useful biomarker of diffuse axonal injury (DAI). However, the sensitivity of the assay was limited. In the present report, we used the Luminox technique to improve the analytical sensitivity to enhance the temporal resolution of Aβ peptide dynamics in 10 patients with moderate to severe TBI. Our main hypotheses were that Aβ peptide levels would correlate with the presence of diffuse brain injury, indicating axonal injury, cerebral energy metabolic perturbation, patient age, and the level of consciousness.

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. This single center study was performed in a university hospital NCC setting.

Patient population and neurocritical care management

We included 10 patients (7 male, 3 female) conveniently recruited with a severe TBI, defined as a post-resuscitation Glasgow Coma Scale (GCS) score ≤ 8 at the primary hospital. On arrival in our unit, all patients were intubated and the motor component of the GCS (the Glasgow Motor Scale [GMS]) is presented in Table 1. No patient had a previous history of a neurodegenerative disease or Down’s syndrome. All patients required treatment in a NCC setting, including intubation and mechanical ventilation, and were managed according to a standardized brain injury protocol aiming to keep intracranial pressure (ICP) at ≤ 20 and cerebral perfusion pressure (CPP) at ≥ 60 mm Hg.\(^ {46,47}\) ICP was monitored in all patients, either with an intraparenchymal ICP monitor (Codman, Johnson & Johnson, six patients), an external ventricular drain (three patients) or both (one patient). We used a sedation protocol in which patients were sedated using continuous intravenous propofol infusion (1–4 mg/kg/h Propofol-Lipuro; B. Braun Melsungen AG, Melsungen, Germany) combined with intermittent intravenous morphine (1–3 mg Morfin Media; Media, Sollentuna, Sweden). When ICP elevations could not be controlled using standard therapy, continuous sodium pentobarbital infusion therapy was initiated in one patient. Volume substitution and/or inotropic agents (Dobutamine or norepinephrine) were administered when needed. Plasma glucose was frequently evaluated and maintained at 5–10 mmol/L where insulin was administered when plasma glucose levels reached >10 mmol/L. A neurological examination was performed regularly, up to six times daily, using the neurological wake-up test to register the motor component of the GCS (the GMS) and focal neurological signs on computerized observation charts.\(^ {48}\) For presentation of data, the patients were classified into conscious (GMS = 6) or not (GMS < 6) during the time for Aβ sampling (described subsequently). Additional injuries were scored according to the New Injury Severity Score (NISS), ranging from 1 to 75.\(^ {48}\) At ~6 months post-injury, patient outcome was assessed using the extended Glasgow Outcome Scale (eGOS).\(^ {49}\)

Radiological analysis

CT scans were frequently performed as part of the monitoring strategy. When analyzing each CT scan, the “worst” CT scan (i.e.,

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Group</th>
<th>Age (yrs), sex</th>
<th>Cause of injury</th>
<th>NISS/associated conditions(^ a)</th>
<th>GMS scores on arrival</th>
<th>Pupils</th>
<th>MD duration (h)</th>
<th>LOS extubation (days)</th>
<th>GMS score on departure eGOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Focal/mixed</td>
<td>76, M Fall</td>
<td></td>
<td></td>
<td>51</td>
<td>6</td>
<td>Normal</td>
<td>8–214</td>
<td>21/8</td>
</tr>
<tr>
<td>2</td>
<td>DAI</td>
<td>42, M MVA</td>
<td></td>
<td></td>
<td>50</td>
<td>2</td>
<td>Bilat small</td>
<td>17–346</td>
<td>17/7</td>
</tr>
<tr>
<td>3</td>
<td>DAI</td>
<td>52, F MVA</td>
<td></td>
<td></td>
<td>5</td>
<td>3</td>
<td>Rt dilated</td>
<td>19–145</td>
<td>8/6</td>
</tr>
<tr>
<td>4</td>
<td>Focal/mixed</td>
<td>19, M MVA</td>
<td></td>
<td></td>
<td>57</td>
<td>5</td>
<td>Normal</td>
<td>24–150</td>
<td>18/14</td>
</tr>
<tr>
<td>5</td>
<td>DAI</td>
<td>25, F MVA</td>
<td></td>
<td></td>
<td>38</td>
<td>5</td>
<td>Normal</td>
<td>14–164</td>
<td>7/6</td>
</tr>
<tr>
<td>6</td>
<td>Focal/mixed</td>
<td>31, M MVA</td>
<td></td>
<td></td>
<td>29</td>
<td>3</td>
<td>Rt dilated</td>
<td>7–147</td>
<td>10/9</td>
</tr>
<tr>
<td>7</td>
<td>Focal/mixed</td>
<td>43, M Tire explosion</td>
<td></td>
<td></td>
<td>30</td>
<td>4</td>
<td>Normal</td>
<td>13–58</td>
<td>6/2</td>
</tr>
<tr>
<td>8</td>
<td>DAI</td>
<td>35, F Fall</td>
<td></td>
<td></td>
<td>19</td>
<td>5</td>
<td>Rt dilated</td>
<td>7–188</td>
<td>11/8</td>
</tr>
<tr>
<td>9</td>
<td>Focal/mixed</td>
<td>55, M Fall</td>
<td></td>
<td></td>
<td>21(^ a)</td>
<td>3</td>
<td>Bilat dilated unresponsive</td>
<td>13–273</td>
<td>20/17</td>
</tr>
<tr>
<td>10</td>
<td>DAI</td>
<td>18, M MVA</td>
<td></td>
<td></td>
<td>29</td>
<td>4</td>
<td>Rt dilated</td>
<td>75–311</td>
<td>17/9</td>
</tr>
</tbody>
</table>

\(^a\)Isolated brain injury, NISS cannot be applied.
\(^b\)Alcohol indicates a high serum ethanol on arrival in the primary hospital.

Number of hours post-injury at which the microdialysis was initiated and stopped; the interval between the numbers represents the total duration of microdialysis.

DAI, diffuse axonal injury; eGOS, extended Glasgow Outcome Scale at 6 months post-injury; GMS, motor component of the Glasgow Coma Scale; NISS, New Injury Severity Score; LOS, length of stay; MD, microdialysis; MVA, motor vehicle accident.
the CT scan with the most intracranial lesions and/or mass effect during the course of the disease) was selected and analyzed for each of the 10 patients.\textsuperscript{20} We used the Marshall classification\textsuperscript{51} and divided the patients into having either a DAI or a focal/mixed TBI.\textsuperscript{25} Radiological characteristics of focal/mixed TBI included focal lesions such as epi- and subdural hemorrhages and lobar contusions. Criteria for DAI were diffuse brain swelling and/or small petechial hemorrhages located at the gray/white matter junction, corpus callosum, and/or brainstem. Compression of basal cisterns was determined by the following scoring system: 0 = normal, 1 = compressed yet visible, 2 = compressed. The midline shift was calculated at the level of the thalamus. The volume in milliliters of each mass lesion was calculated using the formula length $\times$ width $\times$ height/2.

\textbf{MD procedure}

The MD probe was inserted in conjunction with implantation of the ICP monitoring device, typically in the nondominant frontal lobe 1–2 cm anterior to the coronal suture ($n = 7$) or at the craniotomy site after evacuation of a focal mass lesion ($n = 3$). Care was taken to insert the MD catheter obliquely into the cortex using a non-traumatic technique.\textsuperscript{25}

We used 71 High Cut-Off Brain MD catheters with a membrane length of 10 mm and a 100 kDa nominal molecular weight cutoff polyarylethersulfone (PAES) membrane (M Dialysis AB, Solna, Sweden). The outflow hydrostatic pressure of the perfusion system was set at the zero midcranial reference level by taping the collecting vials at the bandage on the patient’s head to avoid additional hydrostatic effects on fluid recovery of the catheter. Perfusion of the catheters was performed using artificial cerebrospinal fluid (CSF) (Perfusion Fluid CNS, M Dialysis), containing NaCl 147 mM, KCl 2.7 mM, CaCl$_2$ 1.2 mM, and MgCl$_2$ 0.85 mM with the addition of 1.5% human serum albumin$,^20$ at a rate of 0.3 $\mu$L/min using a 106 MD pump (M Dialysis). At least 2 h passed after insertion of the MD catheter and start of sampling to allow for normalization of changes caused by catheter insertion. MD vials ($\sim$18 $\mu$L samples) were changed hourly. Samples were analyzed at the bedside using CMA microdialysis analyzers (CMA600 and ISCU5flex; M Dialysis) for concentrations of glucose (MD-gluc), lactate (MD-lact), and pyruvate (MD-pyr), and the LPR was calculated. Glutamate (MD-glut) and glycerol (MD-glyc) were evaluated on follow-up CT scans in all patients and checked for hemorrhages. The presence of any hemorrhage visible on T1 within 3 cm of the MD probe was analyzed on all CT scans and noted in Table 2.

Based on previous data$^{53,54}$ the following values for the routine biomarkers were considered critical: MD-gluc $<$ 1 mmol/L; LPR $>$ 30; MD-lact $>$ 3.8 mmol/L; MD-pyr $<$ 120 $\mu$mol/L; MD-glut $>$ 15 $\mu$mol/L; MD-glyc $>$ 100 $\mu$mol/L.

\textbf{Analysis of Aβ peptides}

After bedside analysis, the remaining hourly microdialysis samples ($\sim$14 mL) were pooled into 2 h fractions. Aβ42 and Aβ40 levels were measured using the INNO-BIA Aβ forms assay (Innogenetics, Ghent, Belgium), which is a multiplex microsphere-based Luminex xMAP technique. In this assay, the monoclonal antibodies 21F12 and 2G3, which specifically bind Aβ peptides ending at Ala42 and Val40, respectively, were used as capture antibodies, and the monoclonal antibody 3D6, which specifically binds Aβ peptides starting at Asp1, was used as detector antibody.\textsuperscript{55} All samples were analyzed in the presence of a mild detergent in the kit, which makes most of the Aβ accessible to the antibodies, although without using denaturing extraction protocols. Because of the small sample volume available, all samples were diluted 15-fold before analysis. The lowest standard point was 15 pg/mL for Aβ40 and 5 pg/mL for Aβ42. The lowest reported levels were set to half the value of the lowest standard point, which after correction for dilution gives a cutoff of 112 pg/mL for Aβ40 and 37 pg/mL for Aβ42. All analyses were performed by experienced and board-certified laboratory technicians. Serial samples from the same patient were analyzed on the same plate. Intra- and inter-plate coefficients of variation were below 10%.

\begin{table}[h]
\centering
\footnotesize
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Case no.} & \textbf{Group} & \textbf{Marshall classification} & \textbf{Lesion type} & \textbf{Hematoma vol (mL)} & \textbf{tSAH$^b$} & \textbf{Midline shift (mm)} & \textbf{IVH} & \textbf{Basal Cisterns$^b$} & \textbf{Hematoma location/MD probe location} \\
\hline
1 & Focal/mixed & EML & ICH & SDH & 51.4 & + & 7.5 & + & 2 & RF/RF cranio$^c$ \\
2 & DAI & DI II & Cps & 3.5 & SF & + & + & None & 0 & RFP/RF \\
3 & DAI & DI II & Cps & <0.1 & BP & + & + & None & + & 0 & LBG/RF \\
4 & Focal/mixed & EML & EDH & 24.9 & None & 4.5 & None & 1 & RFP/RF cranio$^c$ \\
5 & DAI & DI III & Cps & <0.1 & LTP & + & None & + & + & 1 & LF/RF \\
6 & Focal/mixed & non-EML & Cps & 10.0 & BFP & + & + & None & 0 & LF/RF \\
7 & Focal/mixed & EML & EDH & 54.9 & BSF & + & 2.0 & None & 2 & LT/RF cranio \\
8 & DAI & DI III & Cps & 0.3 & SF & + & None & + & 1 & LBG/RF$^e$ \\
9 & Focal/mixed & non-EML & ICH & SDH & 17.8 & Tent. & + & + & None & 0 & Bilat/F \\
10 & DAI & DI II & Cps & 0.6 & Occ. & + & none & + & + & 0 & RT/RF$^a$ \\
\hline
\end{tabular}
\caption{Radiological Analysis of the Worst CT Scan during the Course of the Disease (See text and Servadei et al.$^{50}$ for details)}
\end{table}

$^a$Semiquantitative scale; + = minimal, localized SAH; + + + = marked, widespread SAH.
$^b$Scores defined as follows: 0 = normal, 1 = compressed yet visible, 2 = compressed.
$^c$MD probe placed within 3 cm of a CT-verified hemorrhage/contusion.
$^d$DAI, diffuse axonal injury; Cps, parasagittal contusions; cranio, craniotomy; DI II/III, diffuse injury$^{51}$; EML, evacuated mass lesion; IVH, intraventricular hemorrhage; bilat., bilateral, BG, left basal ganglia; F, frontal; T, temporal; Tent, tentorium; FP, frontoparietal; Occ, occipital; tSAH, traumatic subarachnoid hemorrhage.
Statistical analysis

The microdialysis data was analyzed using the one sample Kolmogorov–Smirnov test and was found not to meet the assumption of normal distribution ($p < 0.05$ for all analytes). Therefore, pairwise comparisons between groups were analyzed using the Mann–Whitney $U$ test (Statistica software; StatSoft, Tulsa, OK). Nonparametric data are presented as medians and 75th percentile or individual values, whereas parametric data are presented using the means ± standard deviations (SD). Correlation analysis was performed on the first 48 h MD samples using Spearman’s rank correlation. For area under the curve (AUC) calculations, we focused our analysis on the initial 19–146 h (day 1–6) post-injury. A $p$ value $< 0.05$ was considered statistically significant.

Results

Patient characteristics and radiology

The mean age of the patients was 38.5 ± 18 years (range 18–76 years). The median GMS score on arrival in our NCC unit was 4 (range 2–6). Additional clinical characteristics including the NISS score are presented in Table 1. Based on the Marshall classification, the patients were divided into two groups: focal/mixed and DAI (Table 2). Five patients were categorized as having a DAI and five were categorized as having a focal/mixed injury. Radiological characteristics of each patient are presented in Table 2. Focal mass lesions were surgically evacuated in three patients (1, 4, and 7; Table 2).

Microdialysis procedure

The mean duration from time of accident to start of MD sampling was 19.7 h (range 7–75 h), and the mean duration was 179.9 ± 81 h (range 45–329 h; Table 1). Probe function was monitored using MD-urea levels, which were found to be stable, thus indicating adequate probe function, with a gradually increasing concentration (Table 2). MD-urea levels were consistently higher mainly because of one young patient with a focal injury group (Fig. 1A and B). We hypothesized that the energy metabolic situation could influence Aβ peptide levels. One patient in the DAI group (case 8, Fig. 4) had very high Aβ42 levels (for an illustrative patient example see Fig. 5). In most patients, there were only slight fluctuations in Aβ40 and Aβ42 levels. One patient in the DAI group (case 8, Fig. 4) had very high Aβ levels (Fig. 5).

Analyses of low-molecular weight MD markers

LPR. A total of 1582 MD samples could be analyzed for the LPR. LPR levels were significantly higher early post-injury in the focal injury group (Fig. 6A). Age (Fig. 6B) and the level of consciousness (data not shown) did not influence the LPR levels.

MD-glc. A total of 1582 MD samples were analyzed for glucose (MD-glc). Of these, 179 samples (11.3%) had a critical MD-glc value of < 1 mmol/L. Injury type (Fig. 6A), age (Fig. 6B), and level of consciousness (data not shown) did not significantly influence MD-glc levels.

MD-glut. We could analyze 1302 samples for glutamate (MD-glut). The MD-glut values did not markedly differ between the injury groups (Fig. 6A) and was not influenced by the level of consciousness (data not shown). In young patients, MD-glut levels were consistently higher mainly because of one young patient with high MD-glut levels (Fig. 6B).

MD-glyc. We could analyze 1657 samples for glycerol (MD-glyc). When compared with the focal TBI group, patients with diffuse TBI had consistently lower MD-glyc values ($p < 0.05$ in...
Discussion

The aim of this study was to evaluate the dynamics of the Aβ peptides Aβ40 and Aβ42 after TBI using intracerebral MD. The refined analysis method allowed for monitoring of both Aβ40 and Aβ42 in 2 h fractions in a small but thoroughly characterized TBI cohort. These results provide a unique data set for evaluation of Aβ peptide dynamics in the acute phase following injury, with higher temporal resolution and analytical specificity than in previously published studies.25,26,45 The Aβ results were also compared with injury type, biomarkers for cerebral energy metabolism (glucose, lactate, pyruvate), cellular distress (glycerol, and glutamate), and clinical factors including patient age and level of consciousness, to test our main hypotheses. Higher Aβ levels in patients with diffuse brain injury compared with focal TBI were observed, supporting our previous data suggesting that Aβ peptides may be valuable biomarkers for axonal injury.25 In contrast, the energy metabolic situation evaluated using MD biomarkers of ischemia and cellular distress in the same brain region only weakly correlated with Aβ peptide levels. Additionally, patient age or level of consciousness did not clearly influence Aβ levels in this patient cohort.

We observed that the levels of the Aβ peptides Aβ40 and Aβ42 were higher in patients with diffuse brain injury than in those with focal TBI, and there are several lines of evidence suggesting an increased Aβ formation following diffuse TBI. First, in approximately one third of TBI patients, Aβ plaques have been detected in the postmortem brain,56 in surgically resected tissue,11,12 and in injured axons of DAI patients dying <9 days post-injury.14 This TBI-induced Aβ pathology can remain for many years in the brains of TBI survivors.5,57 Second, following diffuse TBI in the pig, Aβ plaques were observed in the one third of animals displaying the most severe axonal injury.58 Importantly, such plaques occur not only in cortical tissue but also in white matter tracts.14 Finally, axonal accumulation of Aβ, APP, and BACE has been observed in...
FIG. 2. We correlated amyloid-β (Aβ)40 (A,C) and Aβ42 (B,D) to the lactate-pyruvate ratio (LPR; A,B), where an LPR > 30 was considered critical and to microdialysis (MD)-glucose levels (C,D), where a level <1.0 mmol/L was considered critical. We used the initial 48 h post-injury values and no correlation was found between Aβ40 or Aβ42 and LPR or any other marker of energy metabolic crisis.

FIG. 3. We analyzed the influence of age on amyloid-β (Aβ)40 (A,C) and Aβ42 (B,D) levels. Open circles represent the five patients <40 years of age and filled squares represent the five patients >40 years of age. Data are presented using means ± SD. (C,D) Area under the curve (AUC) Aβ40 and Aβ42 values, calculated for each traumatic brain injury (TBI) patient between 19 and 146 h post-injury and presented for patients <40 years (five patients, open circles) and >40 years old (five patients, filled squares). (A–D) Age defined as < or > 40 years old did not significantly influence Aβ levels.
both human and pig DAI up to 3 years post-injury.\textsuperscript{21,22,59} Therefore, it appears likely that a prolonged APP accumulation in swollen axons results in an increased formation of Aβ peptides\textsuperscript{5,56,60} released into the interstitial compartment following axonal rupture.

It should be noted that the classification of focal TBI versus DAI was solely based on the Marshall classification of CT scans, which is based on a 1991 analysis of the Traumatic Coma Data Bank. Although this classification was found to be predictive of a poor outcome, its ability to predict detailed neuropsychological deficits has not been established.\textsuperscript{61} MRI is clearly more sensitive than CT for small lesions such as those observed in DAI patients.\textsuperscript{62} In our material, no patient in the DAI group had a focal lesion, and the largest hematoma in this group was <4mL. However, with ever increasing sensitivity of MRI, it is becoming clear that a high proportion of patients with predominately focal TBI display components of axonal injury.\textsuperscript{63} As standard CT and MRI may underestimate the extent of white matter damage after TBI,\textsuperscript{64,65} additional neuroradiological tools such as diffusion tensor imaging (DTI) could further increase the detection of white matter damage post-injury.\textsuperscript{66} Ideally, MR scans following the implantation of the MD catheters could in more detail have evaluated the details of axonal injury in the vicinity of the probes in our present study.

The Aβ\textsubscript{40} and Aβ\textsubscript{42} levels did not markedly fluctuate during the initial post-injury days following human TBI, in contrast to previous MD studies in which they gradually rose over time.\textsuperscript{26,45} Our observations are also in contrast to experimental data analyzing Aβ levels using MD early following focal TBI in mice. In awake, moving mice, the hippocampal Aβ levels declined by up to 50% post-injury, depending upon injury severity. In animals with severe TBI, the Aβ decline was more marked and than in animals with a milder, focal TBI.\textsuperscript{67} In the present series, the pre-injury levels were unknown, and initial Aβ levels may have been different. It is likely that human TBI represents a less marked focal TBI than what is used in the rodent model,\textsuperscript{68} making direct comparisons difficult.

Although the evidence cited in the previous paragraphs suggest that Aβ levels may be increased post-injury, we must also consider that the Aβ levels presented in our study may actually be reduced in some TBI patients.\textsuperscript{25} In a previous study, patients with contusions had lower Aβ peptide levels than did DAI patients.\textsuperscript{26,45} findings similar to our present and previous report.\textsuperscript{25} In our focal TBI group, three patients had their MD probes close to an evacuated mass lesion, and the lower values observed in the focal TBI group may also represent reduced levels in the vicinity of contusion caused by Aβ consumption in the formation of plaques. Although the lack of histology makes any explanation hypothetical, early plaque formation is observed in only one third of TBI patients (see earlier description). Therefore, it is unlikely that the reduced Aβ levels in focal TBI patients are the result of plaque formation. Although CSF Aβ concentration may differ from the interstitial concentration,\textsuperscript{5} levels of Aβ\textsubscript{42} have been reported to increase following TBI.\textsuperscript{50,69–72}

Any biomarker may also have an effect on the injury process itself. Based on experimental data, interstitial Aβ may have deleterious effects following TBI.\textsuperscript{24} Neurotoxic and synaptotoxic effects of Aβ oligomers \emph{per se} have also been observed.\textsuperscript{62–64} Therefore, the interstitial Aβ could influence the secondary injury cascade after TBI. In addition, alternate splicing of the APP molecule may occur, and generation of other Aβ species than Aβ\textsubscript{42} evaluated in our present report is possible. The shorter Aβ\textsubscript{1–40} (Aβ\textsubscript{40}) makes ~80–90% of all generated Aβ peptides, although Aβ\textsubscript{42} is more hydrophobic and prone to aggregate into plaques.\textsuperscript{26} In

FIG. 4. (A,B) We evaluated the relationship between the level of consciousness and the interstitial amyloid-β (Aβ)\textsubscript{40} (A) and Aβ\textsubscript{42} (B) values, data presented as means±SD. Aβ levels were similar in conscious patients (defined as patients obeying command when assessed using the neurological wake-up test; open circles) or unconscious patients (filled squares). (C,D) Area under the curve (AUC) values for each patient in the conscious and unconscious groups. The AUC value for each patient was calculated for Aβ\textsubscript{40} (C) and Aβ\textsubscript{42} (D) between 19 and 146 h post-injury, presented with the median value (black line) and the individual values. There were no significant differences among the groups.
the pathophysiology of TBI, other Aβ subspecies than Aβ42 may be of interest, which could be evaluated using MD.73 The cutoff for the MD catheter used in the present study was 100 kDa, and although the molecular weight of Aβ42 is ~4.5 kD, longer peptides resulting from APP splicing or Aβ oligomerization, presumably a key event in the pathogenesis of AD, may not be detected using MD.74

As cerebral ischemia may influence Aβ levels,34–36 we evaluated biomarkers for energy metabolic perturbation obtained in the same location and brain compartment as Aβ. In contrast to a previous report,26 which found a positive correlation between MD-Aβ and MD-glc and a negative correlation with LPR, we observed only weak correlations. In our series, pathological LPR and/or MD-glc

**FIG. 5.** Microdialysis data from a patient who sustained a Marshall diffuse brain injury II traumatic brain injury after a fall during horseback riding. Graph showing changes in amyloid-β (Aβ)40 (A) and Aβ42 (B) levels detected using microdialysis. (C) An MR scan obtained at 2 days post-injury showing multiple hemorrhages at the gray–white matter interface, indicated with arrows, suggestive of diffuse axonal injury (DAI). The highest interstitial Aβ40 and Aβ42 levels of this patient series were observed in this patient. Despite gradually improving neurological status during the monitoring time, the Aβ did not change and remained at a high level. The time for interruption of sedation in order to evaluate the level of consciousness using the neurological wake-up test, shown with arrowheads, did not clearly influence Aβ levels. The time for extubation is shown with an arrow.
FIG. 6. A,B. Interstitial lactate-pyruvate ratio (LPR), glucose, glutamate and glycerol measured using microdialysis (MD), data presented as means ± SD. The energy metabolic markers showed similar values when the patients were divided into groups depending on injury type (A) and age (B). (A) Graph showing the LPR, MD-glucose, MD-glutamate, and MD-glycerol levels in the diffuse axonal injury group (DAI; open circles) compared with the focal injury group (filled squares) in MD samples from 21 to 145 h post-injury. Significant differences between the two groups indicated with an asterisk (*). (B) The energy metabolic situation, analyzed showing the LPR and MD-glucose in five patients >40 years old compared with the younger patients <40 years old. No significant differences were seen between the two groups. The dashed lines in A and B indicate critical cut-off levels for the LPR (>30), MD-glucose (<1.0 mmol/L), MD-glutamate (18 μmol/L) and MD-glycerol (100 μmol/L).
FIG. 6. (Continued)
values were rather infrequent and, at least for MD-glc, appeared less frequently than in the study by Brody and associates.\(^\text{26}\) Additionally, in that report, six patients with subarachnoid hemorrhage (SAH) were included, and it cannot be excluded that those patients had a different neurochemical profile. The patients in our series had a severe TBI requiring sedation and controlled ventilation. Despite the injury severity, ICP problems were rare, which likely prevented marked neurometabolic perturbation of the brain. It cannot be excluded that another injury severity may have caused a different correlation between Aβ peptide levels and the focal neurochemistry.

Previously, currently available ELISA methods were considered too insensitive to enable measurement of Aβ 1–42 levels, and in a previous report, an ELISA evaluating Aβ species from amino acid acid 1–28 or greater (Aβ1–x) was used.\(^\text{26}\) When using that analysis, the majority of the Aβ was neither Aβ40 nor Aβ42, and it was suggested that Aβ40 and Aβ42 concentrations were lower than the Aβ1–x concentrations by a factor of 35 and 2.5, respectively.\(^\text{26}\) In our previous study, a high sensitivity variant of a commercially available ELISA kit was used.\(^\text{25}\) In 12 h pooled samples, we were able to analyze Aβ40 in ~50% of the patients, and were able to analyze Aβ42 in seven out of eight patients. These levels were comparable to those obtained in the present report. However, in the present report, the analysis of Aβ40 and Aβ42 in 2 h samples was possible in all patients, indicating a superior sensitivity using the microsphere-based Luminex xMAP technique. The high specificity of the antibodies used in the present report and their application in the Luminex technique has previously been established.\(^\text{75–77}\) As long-term storage of CSF and plasma samples was found to influence the analysis of Aβ,\(^\text{78,79}\) consideration of pre-analytical factors was crucial. Prior to analysis, the MD samples in our present study were stored at -70°C, and every precaution was taken to handle and store the samples properly. We believe that the minor fluctuations of Aβ during the course of the disease for the individual patient reflected this effort. In addition, the MD membrane may serve as a barrier separating Aβ from degrading enzymes such as insulin and neprilysin,\(^\text{80}\) contributing to sample stability. Finally, other groups have observed long-term stability of Aβ in CSF samples for >2 years of freezing.\(^\text{81,82}\) Arguing that pre-analytical factors did not markedly influence our results. However, further studies are clearly needed to define the effect of pre-analytical factors such as long-term storage on analyte levels.

Although astrocytes, microglia, and endothelial and smooth muscle cells may release Aβ, neurons express the highest levels of Aβ peptides and may release Aβ into the extracellular space under normal cellular conditions.\(^\text{83}\) In one previous report using MD, the Aβ levels were dependent on the level of consciousness.\(^\text{26}\) In that report, studying a mixed TBI and SAH cohort, Aβ levels rose when the patient’s neurological status improved and vice versa. Here, we did not detect a similar correlation among Aβ40, Aβ42, and the level of consciousness in a pure TBI cohort. As an important part of routine NCC monitoring, we frequently evaluate the neurological status (the motor component of the GCS), using the neurological wake-up test\(^\text{46}\) requiring that the continuous sedation be interrupted. Despite the absence of sedation during the tests, interstitial Aβ levels were not markedly altered. There are several experimental reports suggesting a link between extracellular Aβ dynamics and neuronal activity.\(^\text{83–86}\) It is plausible that TBI-induced factors are responsible for regulating neuronal activity and, conversely, Aβ levels. The wake-up test and reduction of sedation may not be sufficient to overcome TBI-induced depression of neuronal activity. In addition, the neurological evaluation performed in the NCC setting provides only a crude measurement of neuronal activity, and may be influenced by many additional factors other than TBI severity. Therefore, the lack of correlation between Aβ levels measured using MD and the level of consciousness observed here may not be surprising. However, as there was a lack of pre-injury, baseline Aβ levels in the present data cannot resolve the possibility that our findings were the result of increased Aβ caused by axonal injury or a larger decrease in Aβ levels in focal TBI caused by a more marked regional decrease in neuronal activity. In addition, Aβ oligomers and prototibrils could be elevated after TBI, despite a reduction in MD Aβ levels. These issues should be addressed in future studies.

**Limitations**

There are obvious limitations to our study. The present cohort of 10 TBI patients is small, which may have prevented us from detecting significant correlations between Aβ levels and a number of clinical and radiological factors. Because of the heterogeneity of TBI, this number may be insufficient to enable firm conclusions, and even though each patient was thoroughly characterized, additional studies are needed. A routine use of MR imaging would more accurately have been able to determine the pathology and presence of axonal injury in the vicinity of the MD probe. Additionally, multimodal monitoring of each patient including electroencephalogram (EEG) and brain tissue oxygenation combined with, for example, positron emission tomography (PET) monitoring could have strengthened the interpretation of energy metabolic changes in relation to MD Aβ levels. The Aβ peptides evaluated in the present report may be relevant in the acute pathophysiology of TBI, and recent studies have indicated the importance of Aβ oligomers, not measured in the present study, in neurodegeneration.\(^\text{87}\) Even though Aβ is produced by normal neuronal metabolism,\(^\text{88}\) the normal extracellular Aβ levels using MD and our present analysis technique are unknown. Finally, the rates of enzymatic degradation, uptake into activated microglia, and receptor-mediated export of Aβ to other organs,\(^\text{73}\) perhaps influenced by TBI, were not measured, and may have modulated our MD Aβ levels.

**Conclusion**

Using a microsphere-based Luminex xMAP technique, we were able to analyze and monitor Aβ40 and Aβ42 levels in bi-hourly samples in severe human TBI. Our data support the notion that MD is a useful tool for studying Aβ dynamics following TBI. We also confirm our previous hypothesis that these Aβ peptides occur at higher concentration in patients with diffuse injury than in those with focal TBI. However, we could not find a correlation between Aβ levels and the focal energy metabolic situation, biomarkers of cellular distress, age of the patient, or the patient’s level of consciousness. Future studies using an increased number of TBI patients are needed to confirm these observations. Additional markers such as tau and neurofilament and/or Aβ subspecies including oligomers and prototibrils should also be used as previously suggested\(^\text{23}\) for elucidating the potential role of Aβ in the pathobiology of TBI as well as the complex relationship between TBI and AD pathology.

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Author Disclosure Statement

Analysis of Aβ peptides were done at the time Dr. Vanmechelen was employed at Innogenetics, and results were transferred to the Institute of Neuroscience and Physiology and Division of Neurosurgery. All subsequent analyses and interpretations were done after September 2011. No other competing financial interest exists.

References


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