Brain Extracellular Interleukin-6 Levels Decrease Following Antipyretic Therapy with Diclofenac in Patients with Spontaneous Subarachnoid Hemorrhage

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In patients with aneurysmal subarachnoid hemorrhage (aSAH), increased brain extracellular interleukin (IL)-6 levels measured by cerebral microdialysis (CMD) were associated with disease severity, early brain injury, delayed cerebral infarction, and axonal injury. In this study, we analyzed brain extracellular IL-6 levels of aSAH patients following parenteral diclofenac. Twenty-four mechanically ventilated poor-grade aSAH patients were included. Changes in cerebral metabolism, brain/body temperature, and CMD-IL-6 levels following intravenous diclofenac infusion (DCF; 75 mg diluted in 100 cc normal saline) were retrospectively analyzed from prospectively collected bedside data (at 1 hour before DCF = baseline; and at 2, 4, and 8 hours after DCF). Statistical analysis was performed using generalized estimating equations. Seventy-two events in 24 aSAH patients were analyzed. Median age was 60 years (interquartile range [IQR]: 52–67), admission Hunt & Hess grade was 4 (IQR: 3–5), and modified Fisher grade (mFisher) was 4 (IQR: 3–4). Higher CMD-IL-6 levels at baseline were linked to fever, higher mFisher, delayed cerebral infarction, and metabolic distress ($p < 0.05$). CMD-IL-6 levels at baseline were 281.4 pg/mL (IQR: 47–1866) and significantly ($p < 0.001$; Wald- $X^2 = 106$) decreased at 2 hours to 86.3 pg/mL (IQR: 7–1946), at 4 hours to 40.9 pg/mL (IQR: 4–1237), and at 8 hours to 53.5 pg/mL (IQR: 5–1085), independent of probe location or day after bleeding. Parenteral diclofenac may attenuate brain extracellular proinflammatory response in poor-grade aSAH patients.

Keywords: neuroinflammation, interleukin-6, microdialysis, temperature management, subarachnoid hemorrhage, diclofenac

Background

ANEURYSMAL SUBARACHNOID HEMORRHAGE (aSAH) accounts for 2–16% of all strokes and is associated with a high socioeconomic burden and poor quality of life among survivors (Connolly *et al.*, 2012).

While the primary impact of the bleeding contributes to the high mortality, secondary brain injury largely accounts for the high morbidity (Park *et al.*, 2004; Carteron *et al.*, 2017). Pathophysiologic mechanisms of secondary brain injury such as delayed cerebral ischemia (Chung *et al.*, 2016) are multifactorial, which renders counteracting treatment strategies difficult. One of these concepts is based on the proinflammatory response, including higher levels of the cytokine

interleukin (IL)-6 (Thelin *et al.*, 2017). IL-6 triggers important repair functions on the one hand, but may also induce an overwhelming and potentially detrimental neuroinflammatory response after acute brain injury (Spooren *et al.*, 2011). Brain extracellular IL-6 controls the integrity of the blood–brain barrier, plays a critical role in the evolution of cerebral edema, regulates brain and body temperature, and is mainly released by astrocytes (Spooren *et al.*, 2011).

Cerebral microdialysis (CMD) allows quantification of brain extracellular proteins, including cytokines. Increased brain extracellular IL-6 levels were detected in aSAH and traumatic brain injury (TBI) patients (Winter *et al.*, 2002; Sarrafzadeh *et al.*, 2010; Møllergård *et al.*, 2011; Perez-Barcena *et al.*, 2011; Helbok *et al.*, 2015, 2017; Hutchinson

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et al., 2015). Excessive brain extracellular IL-6 release was associated with elevated TAU-protein levels, suggesting an interference between neuroinflammation and axonal damage (Schiefecker *et al.*, 2017). Brain extracellular IL-6 might play a crucial role in the pathophysiology of delayed cerebral infarction (Sarrafzadeh *et al.*, 2010; Helbok *et al.*, 2015). Attenuating the cerebral proinflammatory response might potentially serve as a treatment option for counteracting mechanisms of secondary brain injury in aSAH. Anti-inflammatory and neuroprotective treatment strategies have been investigated in observational trials in aSAH patients (Broessner *et al.*, 2010; Muroi *et al.*, 2014) and in experimental studies indicating decreased systemic IL-6 levels following antipyretic treatment (Park *et al.*, 2004; Altay *et al.*, 2016).

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac have been shown to effectively reduce fever in neurocritical care patients (Cormio *et al.*, 2000; Cormio and Citerio, 2007).

In this study, we aimed to investigate the effect of parenteral diclofenac infusion as routine fever treatment on brain extracellular IL-6 levels.

Materials and Methods

Study setting

As part of an ongoing prospective collaboration for improving patient care technology of the Neurologic Intensive Care Unit of the Medical University of Innsbruck with the Institute of Biomedical Informatics UMIT- (University of Health

Sciences, Medical Informatics and Technology) (Hackl *et al.*, 2015) prospectively recorded multimodal neuromonitoring data of consecutive aSAH patients were retrospectively analyzed.

Between September 2010 and May 2015, 24 patients receiving parenteral diclofenac infusion during neuromonitoring, including CMD, following aSAH were included (for the detailed inclusion algorithm, see Fig. 1). Criteria for invasive neuromonitoring, including measurement of brain tissue oxygen tension (PbtO₂), intracranial pressure (ICP), and cerebral metabolites (glucose, lactate, pyruvate, glutamate) were as follows: (a) Glasgow Coma Scale ≤ 8 , (b) poor likelihood for regaining consciousness within the following 48 hours, (c) high likelihood for surviving at least 48 hours, and (d) older than 18 years.

Ethical guidelines

Written informed consent was obtained according to Austrian law. The study was approved by the Review Board of the Medical University of Innsbruck, Austria: neuromonitoring, collection of data, including CMD and leftover storage (Internal Review Board ID: UN3898-285/4.8), and a prospective database (Internal Review Board IDs: AN3898-285/4.8, AM4091-292/4.6). The study was conducted in accordance to the ethical guidelines of the Declaration of Helsinki 1975 (and as revised in 1983).

General management

Critical care management followed recent guidelines (Connolly *et al.*, 2012; Steiner *et al.*, 2013). aSAH was diagnosed

FIG. 1. Illustrating inclusion algorithm: Between September 2010 and May 2015, 24 patients receiving parenteral diclofenac infusion during neuromonitoring, including cerebral microdialysis, following aneurysmal subarachnoid hemorrhage, were included.

using computed tomography (CT) scan and if necessary lumbar puncture. The patients were referred to the NICU either from the emergency department or from other hospitals in Tyrol. The aneurysm was assessed using digital subtraction angiography (DSA) or CT-angiography and was secured by coiling or clipping. An external ventricular drain was placed in case of intraventricular blood and signs of hydrocephalus. All included patients were mechanically ventilated, received appropriate sedation/analgesia, and had a continuous invasive mean arterial pressure (MAP) monitoring. As a modification to recent guidelines, nimodipine (1–2 mg/h) was routinely applied parenteral in all included patients. A Lindegaard Ratio >3 assessed by Doppler sonography (TCD, DWL Doppler-Box system; Compumedics, Singen Germany) was defined as sonographic vasospasm that was further investigated by cerebral DSA if clinically necessary. All patients were treated with vasopressors to achieve a cerebral perfusion pressure (CPP) >50–80 mmHg guided by the clinical situation and multimodal neuromonitoring parameters. Delayed cerebral infarction was defined according to the radiologic criteria as defined by a previous work (Vergouwen *et al.*, 2010) as presence of cerebral infarction on CT or MR scan of the brain within 3 weeks after SAH, or on the latest CT or MR scan made before death within 3 weeks, or proven at autopsy, not present on the CT or MR scan between 24 and 48 hours after early aneurysm occlusion, and not attributable to other causes such as surgical clipping or endovascular treatment (Vergouwen *et al.*, 2010). Anemia requiring transfusion was defined as any hemoglobin level <7–8 g/dL which was treated with packed red blood cells. Pneumonia was defined as a new or progressive radiologic infiltrate, consolidation, or cavitation and at least one of the following: elevated or decreased white blood cell count (<4000 and >12,000 WBC/mm³) or fever with no other recognized cause (Klompas *et al.*, 2012).

Clinical and radiologic grading

Initial Hunt & Hess grade (H&H) was assessed based on preclinical information and reevaluated on admission. The first available cerebral CT-scan was defined as admission CT-scan and analyzed using the modified Fisher Scale (Frontera *et al.*, 2006). Admission CT scans were evaluated by the study team for the presence of global cerebral edema (GCE) (Claassen *et al.*, 2002). CMD probe location was confirmed on axial postsurgical CT-scans by a member of the study team. “Perilesional” probe location was defined as CMD gold tip located within 10 mm to radiologically damaged brain tissue (lesion), whereas probe location within normal-appearing brain tissue was graded as “healthy” probe location (Helbok *et al.*, 2017).

Outcome was evaluated 3 months after aSAH by a study nurse blinded to monitoring data using the modified Rankin Scale (mRS) with scores ranging from 0 (fully independent without any deficits) to 6 (deceased). Unfavorable outcome was defined as mRS >3.

Monitoring and data acquisition

Neuromonitoring probes were implanted into the hemisphere deemed at greatest risk for secondary brain injury using a frontal approach. CT-scan was performed within 24 hours after surgery to verify correct placement of neuromonitoring probes. P_{bt}O₂ was measured using Licox™ (Integra LifeSciences Corporation®), ICP and brain temperature by a pa-

renchymal probe (Neurovent-P-temp; Raumedic®, Germany). CPP was calculated by MAP, measured on the level of Foramen interventricular (Monroi), minus ICP. CMD was performed using a 100 kDa cutoff-microdialysis catheter (CMA-71; µDialysis, Stockholm, Sweden), membrane length 1 cm. The CMD catheter was rinsed with artificial cerebrospinal fluid (Isotonic, sterile; Contents: NaCl 147 mmol/L, KCl 2.7 mmol/L, CaCl₂ 1.2 mmol/L, MgCl₂ 0.85 mmol/L (total chloride 153.8 mmol/L); Osmolality 305 mOsm/kg; pH ~ 6; Perfusion fluid CNS; µDialysis AB, Sweden) at a perfusion rate of 0.3 µL/min. Samples were collected hourly and frozen at –80°C after bedside analysis for CMD-lactate, CMD-pyruvate, lactate/pyruvate ratio (LPR), CMD-glucose, and CMD-glutamate using a point-of-care analyzer (ISCUS^{flex}; µDialysis AB, Sweden) (Hutchinson *et al.*, 2015). Body temperature was measured using a temperature sensor of the bladder catheter.

Fever definitions and management

Fever was defined as body temperature >38.0°C. The routine fever treatment threshold was >38.3°C. Parenteral diclofenac (75 mg; maximum dosage, 150 mg/day; Ratiopharm, generic Diclobene; drug approval no.: 1-19719) or parenteral paracetamol (acetaminophen) (1000 mg; maximum dosage, 3000 mg/day) were used as first-line antipyretic therapy. DCF was administered at a dosage of 37.5 mg/h, to minimize known hemodynamic side effects, including P_{bt}O₂ and CPP drops (Schiefecker *et al.*, 2013). The choice of routine antipyretic treatment was at the discretion of the treating neurointensivist. In case of ineffectiveness or contraindications against the first-line antipyretic treatment, meperidine (Pethidine; 100 mg; maximum, 400 mg/day) as second-line therapy was given.

Diclofenac treatment

Routine antipyretic infusions of parenteral diclofenac for fever treatment without other concomitant antipyretic treatment (within 4 hours prior and after DCF infusion) or without targeted temperature management were included in analysis. The exact starting and end of dose time were identified from the electronic chart.

Laboratory methods

CMD-IL-6 levels were analyzed using enzyme linked immunosorbent assay (ELISA; Custom Chemiluminescent Array Kit; Aushon BioSystems).

After thawing the CMD samples at room temperature, 50 µL calibrated protein standards or 3 µL of cerebral microdialysate diluted in 50 µL diluent were added to precoated wells and incubated for 3 hours. After a washing step, the wells were incubated for 30 minutes with biotinylated antibodies, again washed, and then streptavidin-horseradish peroxidase conjugate was added and the samples were again incubated for 30 minutes. Following this step, the Super-Signal® Chemiluminescent Substrate was added and immediately analyzed using a charge-coupled device imaging system. All incubation steps were performed on a shaker at room temperature. The concentration of each sample was quantified by comparing the spot intensities to the corresponding standard curves calculated from the standard sample results using SearchLight® Analyst Software. The IL-6 detection limit was 0.4 pg/mL.

Data management and statistics

Patient data management system (Centricity® Critical Care, General Electric Company®) was used to store digital data of ICP, MAP, CPP, $P_{bt}O_2$, and brain and body temperature every 5 minutes from the monitoring device (Carescape B650, General Electric Company). Monitoring data were averaged over the collection time of the corresponding CMD sample used for protein analysis. Baseline was defined as CMD-collection interval (~1 hour) before diclofenac treatment.

CMD-IL-6 levels were analyzed at baseline and at 2, 4, and 8 hours after DCF. Fever within the first 72 hours after ICU admission was defined as “central fever” if cultures remained negative, and there was no clinical or radiologic suspicion of infection and no antibiotic treatment was prescribed (Hocker *et al.*, 2013). Temperature response was defined as a decrease in body temperature of at least 0.5°C following DCF. Brain tissue hypoxia was defined as $P_{bt}O_2 < 20$ mmHg (Ko *et al.*, 2011; Schiefecker *et al.*, 2013). Metabolic distress was defined as LPR >40 (Hutchinson *et al.*, 2015). An abnormal neuronal redox state was defined as elevated LPR (>25) (Hillered *et al.*, 2005; Hutchinson *et al.*, 2015). Renal failure was defined as an increase in serum creatinine ≥ 4 mg/dL or glomerular filtration rate $\geq 75\%$ (Bellomo *et al.*, 2004). Ventriculitis was defined as decreased serum/CSF glucose ratio <0.5, increased CSF white cell count, increased CSF lactate, and/or detection of bacteria in CSF. Any clinically relevant increase in subarachnoid hemorrhage volume was defined as rebleeding. Complications were stated after adjudication of all relevant information in weekly meetings by the study team.

IBM SPSS™ 23.0 was used for statistical testing. Time series data were analyzed using generalized estimating equations with an autoregressive matrix of the first order to account for repetitive testing (four time-points) within subjects (Gosho, 2016; Liu, 2016). Multivariate analysis was adjusted for probe location and initial H&H, because previous studies revealed higher CMD-IL-6 levels in patients with worse initial H&H (Sarrafzadeh *et al.*, 2010) and perilesional probe location (Schiefecker *et al.*, 2017). Wald- X^2 was used to reflect the strength of a test result. Nonparametric variables were log-transformed for statistical analysis.

$p < 0.05$ was considered statistically significant. Time series data in the figures are presented as mean ± 1 standard error of mean, nonparametric data within the text are presented as median with interquartile range (IQR).

Results

Patients

Baseline characteristics, clinical, and outcome data of the study population and complications are shown in Table 1. Neuromonitoring was initiated at day 1 (IQR 1–2) and maintained for 12 days (IQR 8–14).

Brain and body temperature following diclofenac

Seventy-two events in 24 aSAH patients were analyzed. Three (IQR 2–5) DCF infusions per patient were administered during neuromonitoring at a median of 9 days (IQR 6–12) after ictus. Body temperature decreased by more than 0.5°C in 70% and below 38°C in 50% of events. Baseline body ($38.3^\circ\text{C} \pm 0.1^\circ\text{C}$) and brain temperature ($38.6^\circ\text{C} \pm 0.1^\circ\text{C}$; Fig. 2) decreased to a minimum value within 4 hours ($37.4^\circ\text{C} \pm 0.1^\circ\text{C}$

TABLE 1. DEMOGRAPHIC, RADIOLOGIC, AND CLINICAL CHARACTERISTICS

Basic clinical information	Median (IQR) or N (%)
Age (years)	60 (52–67)
Female gender	15 (63)
Admission H&H	
2–3	9 (38)
4–5	15 (63)
Relevant comorbidities	
COPD	2 (8)
Cancer	1 (4)
Immunosuppressive therapy	1 (4)
Statin medication	2 (8)
Admission radiologic data	
mFisher scale score	
1	0 (0)
2	2 (8)
3	3 (13)
4	19 (79)
Aneurysm size (mm)	7 (5–9)
Generalized cerebral edema	9 (38)
Intracerebral hematoma	10 (42)
Surgical procedures	
Hydrocephalus requiring EVD	21 (88)
Clipping	15 (63)
Complications	
Pneumonia	15 (63)
Delayed cerebral infarction	5 (24)
Renal failure	0
Rebleeding	2 (8)
Anemia requiring transfusion	8 (33)
Ventriculitis	1 (4)
Outcome characteristics	
Length of hospital stay (days)	37 (25–45)
Three-month mRS	
0–1	6 (25)
2–3	4 (17)
4–5	9 (38)
6	5 (21)

Data are presented in median with IQR or count (N) with percentage (%).

COPD, chronic obstructive pulmonary disease; EVD, external ventricular drainage; H&H, Hunt and Hess grade; IQR, interquartile range; mFisher scale score, modified Fisher scale score; mRS, modified Rankin scale.

[$p < 0.001$, Wald- $X^2 = 17$] and $37.6^\circ\text{C} \pm 0.1^\circ\text{C}$ [$p < 0.001$, Wald- $X^2 = 43$], respectively). Seven patients had no temperature response to DCF. There was no difference in temperature response during episodes with central or infectious fever ($p = 0.7$).

Brain extracellular IL-6 levels following diclofenac

Higher CMD-IL-6 levels measured during baseline and after diclofenac application were associated with higher mFisher scale ($p = 0.034$; Wald- $X^2 = 5$; Fig. 3), DCI ($p = 0.018$; Wald- $X^2 = 6$), focal or GCE ($p = 0.038$; Wald- $X^2 = 4$), but not initial H&H ($p = 0.8$), CRP levels ($p = 0.5$), or unfavorable outcome ($p = 0.7$). CMD-IL-6 levels significantly ($p < 0.001$; Wald- $X^2 = 106$) decreased following parenteral diclofenac (Fig. 4 and Supplementary Fig. S1): CMD-IL-6

FIG. 2. Baseline brain temperature ($38.6^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$) decreased to a minimum value within 4 hours ($37.6^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$); mean ± 1 standard error.

levels at baseline were 281.4 pg/mL (IQR: 47–1866), and significantly decreased at 2 hours to 86.3 pg/mL (IQR: 7–1946; $p=0.042$; Wald- $X^2=4$), at 4 hours to 40.9 pg/mL (IQR: 4–1237; $p=0.005$; Wald- $X^2=8$), and at 8 hours to 53.5 pg/mL (IQR: 5–1085; $p=0.008$; Wald- $X^2=7$), independent of probe location or day after bleeding. All patients without temperature decrease following diclofenac treatment developed delayed cerebral infarction ($p=0.009$). Baseline CMD-IL-6 levels were higher ($p<0.001$; Wald- $X^2=26$) in diclofenac infusions without subsequent decrease in body temperature (3221 pg/mL ; IQR: 1769–6210) compared to baseline levels of interventions followed by a decrease in temperature (130 pg/mL ; IQR: 26–1089). The maximum decreases in CMD-IL-6 levels (by 64% and 65% from baseline values, respectively) were observed at 4 hours following diclofenac in interventions with ($p=0.017$) and without ($p<0.001$) consecutive temperature decrease.

CMD-IL-6 levels during diclofenac application were correlated with the percentage of time with fever during neuromonitoring ($\rho=0.5$; $p=0.03$). CMD-IL-6 levels in patients with central or infectious fever during DCF did not differ ($p=0.5$). Higher CMD-IL-6 levels were associated with an increased probability of metabolic distress ($p<0.001$; Wald- $X^2=32$; odds ratio; OR=5.1 [2.9–9.1] per pg/mL) and abnormal neuronal redox state (LPR >25 ; $p=0.001$; Wald- $X^2=10$; OR=3.7 [1.7–8.2] per pg/mL), but not with brain tissue hypoxia ($p=0.27$).

Discussion

The main finding of this study is that brain extracellular IL-6 levels significantly decreased following antipyretic treatment with parenteral diclofenac infusions in patients with aSAH.

Cytokines are a heterogeneous group of proteins which orchestrate the inflammatory response following a wide range of diseases. In peripheral tissue, T-helper cells secrete cytokines associated with cellular immunity, including

Interferon-gamma, IL-2, IL-4, IL-5, IL-6, and IL-10 (Mahdy *et al.*, 2002). In the human brain, astrocytes and microglia release IL-6 into the extracellular space (Spooren *et al.*, 2011), which is triggered by several factors, including the neuroendocrine stress response, amount of subarachnoid blood, or ischemia (Mahdy *et al.*, 2002; Tam *et al.*, 2010).

Recent studies reported an association between CMD-IL-6 levels and biomarkers of secondary brain injury after aSAH, including brain metabolic distress (increased LPR) (Sarrafzadeh *et al.*, 2010) and axonal damage (Schiefecker *et al.*, 2017). Brain extracellular IL-6 levels might be highest in the initial phase after SAH and may play a role in early brain injury mechanisms (Park *et al.*, 2004; Helbok *et al.*, 2015;

FIG. 3. Brain extracellular interleukin-6 levels (measured at baseline, 2, 4, and 8 hours after diclofenac) were associated with higher modified Fisher score at admission ($p=0.034$); mean ± 1 standard error.

FIG. 4. Brain extracellular interleukin-6 levels significantly decreased following diclofenac ($p < 0.001$), independent of probe location, or day after bleeding; mean $\pm$ 1 standard error.

Altay *et al.*, 2016; Carteron *et al.*, 2017). A sustained pro-inflammatory response might further contribute to secondary brain injury and unfavorable functional outcome in aSAH (Sarrafzadeh *et al.*, 2010; Helbok *et al.*, 2015). Spreading depolarizations, slow depolarization waves spreading over mainly cortex, have been associated with the occurrence of delayed cerebral ischemia and infarction following aSAH (Dreier *et al.*, 2006). Case studies in patients with intracerebral bleeding suggested an association between brain extracellular IL-6 levels and the initiation of spreading depolarizations (Schiefecker *et al.*, 2015).

The results of our study clearly demonstrated a link between CMD-IL-6 levels and the amount of subarachnoid blood, which has been partially shown in previous studies (Sarrafzadeh *et al.*, 2010; Helbok *et al.*, 2015; Schiefecker *et al.*, 2017).

DCF is a cyclooxygenase (COX) inhibitor decreasing prostaglandin synthesis and IL-6 release (Gan, 2010). DCF can further inhibit the thromboxane-prostanoid receptor, affect the arachidonic acid release and uptake, inhibit platelet function and lipoxygenase enzymes, activate the nitric oxide-cGMP antinociceptive pathway, and influence peroxisome proliferator-activated receptor gamma and acid-sensing ion channels and *N*-methyl-D-aspartate receptors (Gan, 2010). DCF was found to decrease IL-6 levels in rodents via the toll-like receptor-nuclear factor kappa-B pathway, which controls the expression of inflammatory cytokine genes (Vieira *et al.*, 2016; Barcelos *et al.*, 2017). These experimental findings are supported by clinical data in aSAH (Broessner *et al.*, 2010; Muroi *et al.*, 2014).

The data of our study showed that brain extracellular IL-6 levels might be linked to higher body and brain temperatures during diclofenac application. IL-6 is an important humoral mediator of fever and activates the COX within the brain tissue, mediated by a STAT-dependent pathway (Rummel *et al.*, 2006). Any increase in the COX-mediated PGE₂ syn-

thesis of the hypothalamic thermoregulatory center is leading to an increase of the body set-point temperature (Gan, 2010). There was a clear decrease in brain extracellular IL-6 levels after DCF treatment, which might affect neuroinflammation. Although absolute CMD-IL-6 levels were lower when temperature decreased following diclofenac infusions compared with fever episodes refractory to diclofenac, we found a consistent decrease of CMD-IL-6 levels by ~65% in both groups. In parallel, the antipyretic action of DCF reached the maximum at 4 hours after infusion, which would bring up the hypothesis that brain extracellular IL-6 orchestrates the COX-dependent PGE₂ pathway. In contrast, CMD-IL-6 levels were higher during diclofenac infusions without temperature decrease. One explanation could be that refractory fever might be induced by a profound cytokine activity due to neuronal damage. In line with that, we found an association between a lack of temperature response to diclofenac and the occurrence of delayed cerebral infarction, which was linked to higher CMD-IL-6 levels in the current and previously published study (Sarrafzadeh *et al.*, 2010). The concept of so called “central fever” has been early established in neurocritical care patients (Hocker *et al.*, 2013). Central fever is not related to infection, but rather to a neurohumoral stress response due to injury of the hypothalamic axis (Hocker *et al.*, 2013). Although the study was designed to analyze CMD-IL-6 levels only several hours after DCF treatment, no association could be established between the occurrence of “central fever” and CMD-IL-6 levels.

DCF has well known hemodynamic side effects (Cormio *et al.*, 2000; Cormio and Citerio, 2007), including a drop in $P_{br}O_2$ (Schiefecker *et al.*, 2013), which might be mainly driven by blood pressure and consecutive CPP decreases and may counteract the benefit gained from its anti-inflammatory capacity. Importantly, we observed no changes in brain metabolism following DCF (Schiefecker *et al.*, 2013). A randomized prospective controlled clinical trial showed that

parenteral, continuous low-dose DCF for fever management after TBI and aSAH effectively decreases core body temperature without negative hemodynamic side effects (Cormio and Citerio, 2007), which might offer a new neuroprotective treatment strategy in aSAH patients.

The data of this study create the hypothesis that DCF decreases brain extracellular IL-6 levels, a process which might have a protective effect on the evolvement of brain edema and/or delayed cerebral infarction.

This study has important limitations. A causal relationship between DCF treatment and CMD-IL-6 levels cannot be certainly proved based on retrospective data. Although the sample size is limited to poor grade aSAH patients, the study delivers exclusive pathophysiologic insights of proinflammatory cytokine expression following NSAIDs application. Although several factors which may influence neuroinflammation were included in multivariate models, we cannot exclude that unrecognized events may have contributed to the decrease in brain extracellular IL-6. Although specific anesthetic agents may also affect IL-6 release, no clinically relevant modulation of the inflammatory response was observed in previous studies (Mahdy *et al.*, 2002). A clinical microdialysis study suggested an association between CMD probe location and CMD-IL-6 levels (Schiefecker *et al.*, 2017). We could not replicate a link between probe location and inflammatory response following DCF in this study, which can be explained by a general decrease in CMD-IL-6 levels independently from CMD probe location. We did not measure serum IL-6 levels in our patients, however, as serum IL-6 levels correlate with CRP levels following aSAH (Sarrafzadeh *et al.*, 2010), we analyzed daily CRP levels and found no correlation between brain extracellular IL-6 levels during the observation period and daily serum CRP levels. Importantly, the results of this study might serve as hypothesis generating and cannot be generalized to good-grade aSAH patients. Prospective trials are necessary to implement new treatment strategies in aSAH patients.

Nevertheless, this study addresses a potentially novel treatment option for attenuating neuroinflammation in aSAH.

Conclusions

Parenteral diclofenac may attenuate brain extracellular proinflammatory response in poor grade subarachnoid hemorrhage patients. The finding of this study has to be confirmed in prospective trials.

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Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1

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