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In vivo Characterization of Traumatic Brain Edema Formation by Free Water Diffusion MRI and 2-Photon Microscopy

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List of abbreviations

ASIC	Acid-sensing ion channels
BBB	Blood-brain barrier
CBE	Cytotoxic brain edema
CCI	Controlled cortical impact
CNS	Central nervous system
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid
DWI	Diffusion weighted imaging
ECS	Extracellular space
FW	Free water
FWI	Free water imaging
ICP	Intracranial pressure
MD	Mean diffusivity
TBI	Traumatic brain injury
VBE	Vasogenic brain edema
VEGF	Vascular endothelial growth factor
2-PM	2-Phonton microscopy

List of publications

A: Original manuscripts that are part of the dissertation

1. Characterization of Vasogenic and Cytotoxic Brain Edema Formation after experimental TBI by Free Water Diffusion MRI.

Senbin Hu, Carina Exner, Rebecca Isabella Sienel, Antonia Clarissa Wehn, Fatma Burcu Seker, Fanni Magdane Boldoczki, Yinghuimin Guo, Marco Duering, Ofer Pasternak, Nikolaus Plesnila and Susanne M Schwarzmaier

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2. Acid-ion sensing channel 1a deletion reduces chronic brain damage and neurological deficits after experimental traumatic brain injury.

Shiqi Cheng, Xiang Mao, Xiangjiang Lin, Antonia Wehn, **Senbin Hu**, Uta Mamrak, Igor Khalin, Maria Wostrack, Florian Ringel, Nikolaus Plesnila, and Nicole A. Terpolilli

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B: Additional original manuscripts

1. Perfusion pressure determines vascular integrity and histomorphological quality following perfusion fixation of the brain

Susanne M. Schwarzmaier, Maximilian R.O. Knarr, **Senbin Hu**, Ali Ertürk, Farida Hellal, Nikolaus Plesnila

J Neurosci Methods, 2022 Apr 15;372:109493. doi: 10.1016/j.jneumeth.2022.109493

2. Characterization of cytotoxic brain edema in vivo (manuscript in preparation)

Senbin Hu, Malo Gaubert, Severin Filser, Fanni Magdane Boldoczki, Xiangjiang Lin, Marco Düring, Ofer Pasternak, Nikolaus Plesnila and Susanne M Schwarzmaier

C: Poster presentations:

1. Characterization of Vasogenic and Cytotoxic Brain Edema Formation after experimental TBI by Free Water Diffusion MRI

Senbin Hu, Carina Exner, Fanni Magdane Boldoczki, Yinghuimin Guo, Marco Duering, Nikolaus Plesnila and Susanne Schwarzmaier

15th International Neurotrauma Symposium, Berlin, 2022

1. Your contribution to the publications

1.1 Contribution to paper I

Title: Characterization of Vasogenic and Cytotoxic Brain Edema Formation after experimental TBI by Free Water Diffusion MRI.

Authors: Senbin Hu, Carina Exner, Rebecca Isabella Sienel, Antonia Clarissa When, Fatma Burcu Seker, Fanni Magdane Boldoczki, Yinghuimin Guo, Marco Duering, Ofer Pasternak, Nikolaus Plesnila and Susanne M Schwarzmaier

For this publication, Mr. Senbin Hu helped designing the experiments. He then performed the experiments and analyzed the majority of the data. He interpreted the data with his co-authors and wrote the first draft of the manuscript.

1.2 Contribution to paper II

Title: Acid-ion sensing channel 1a deletion reduces chronic brain damage and neurological deficits after experimental traumatic brain injury.

Authors: Shiqi Cheng, Xiang Mao, Xiangjiang Lin, Antonia Clarissa Wehn, Senbin Hu, Uta Mamrak, Igor Khalin, Maria Wostrak, Florian Ringel, Nikolaus Plesnila, Nicole Angela Terpolilli

For this publication, Mr. Senbin Hu performed and analyzed the experimental series investigating the effect of Acid-ion sensing channel 1a on brain edema formation after trauma by determining brain water content in ASIC1a knockout and wild-type mice.

2. Introduction

Brain edema formation is a critical factor in the pathogenesis of secondary brain injury following traumatic brain injury (TBI), resulting in impaired vascular perfusion, dysfunction of brain cells and is closely linked to poor patient outcomes [1-3]. Current treatments for brain edema are still limited to symptomatic strategies. This is also because the methods to investigate brain edema formation *in vivo* are technically limited. Therefore, a detailed characterization of brain edema formation following trauma was not possible until now, and the investigation and verification of causal treatment strategies *in vivo* was very difficult. In my thesis, I present a novel *in vivo* technique that can be used to advance our understanding of brain edema formation following TBI.

2.1 Epidemiology of TBI

Traumatic brain injury is a major public health problem worldwide with a persistent increase in the incidence by 3.6% over the course of nearly three decades [4]. Based on the Global Burden of Diseases Study 2016, there are approximately 27 million new TBI cases each year, with incidence rates of 369 per 100 000 population worldwide [4].

For the European countries, studies estimate that at least 2.5 million new cases of TBI occur annually, with around 1.5 million hospitalizations and 57,000 fatalities [5, 6], and a hospital admission incidence rate of 235 per 100,000 people [7]. During the period of 1990-2014, the mechanism of injury appears to be shifting from traffic-related to fall-related injuries due to the aging population [8].

In the developing countries, the burden of TBI is particularly high, owing to the rising numbers of injuries associated with the increased vehicle usage, as well as the deficiencies in traffic regulations, healthcare policy, and clinical management [9]. However, the mean incidence rates of TBI vary in different regions, from 326 (per 100 000 population) in Sub-Saharan Africa to 495 (per 100 000 population) in central Asia [4]. Due to the lack of precise epidemiological data, these numbers most likely underestimate the actual incidence of TBI [5, 10].

The estimated financial costs associated with TBI worldwide are estimated to be around 400 billion US$ annually [5]. Unfortunately, the overall mortality of severe TBI patients is still up to 30-40%. Even after receiving emergency neurosurgery, 18% of severe TBI patients die within 14 days. For survivors, including those with mild/moderate TBI, up to 30% experience a substantial disability that can last for years, leading to enormous physical and emotional suffering for the patients and their families [4, 5, 11].

2.2 Pathophysiology of TBI

TBI is a multifaceted pathological process caused by external mechanical forces that result in structural and functional damage of the brain. TBI is a complex process that involves a primary injury and multiple secondary injury cascades. The primary injury consists of the irreversible damage to the cerebral tissue by the initial mechanical trauma, whereas the secondary injury refers to a cascade of cellular and molecular events that are triggered by the initial trauma and lead to further brain damage and/or neurological deficits [12].

The cranium functions as a rigid container, maintaining a constant intracranial volume comprising brain tissue, cerebrospinal fluid (CSF), and blood under physiological conditions. The equilibrium

between these volumes is maintained through various mechanisms ensuring a stable intracranial pressure (ICP) and cerebral perfusion pressure (CPP) thereby providing a proper cerebral perfusion for the metabolic demands of the brain [13, 14]. The correlation between ICP and intracranial volume is non-linear. Initially, an increase in intracranial contents, for example by brain edema formation, triggers compensatory mechanisms, such as draining of venous blood and CSF, maintaining a physiological ICP. However, with a further volume increase, the compensatory mechanisms exceed. In that case, even a minor increase in intracranial volume can lead to a rapid elevation in ICP and significant decrease in CPP, and thus cause brain ischemia [14]. After TBI, both the primary injury and the following secondary injuries contribute to brain swelling, increased ICP, and impaired cerebral perfusion [15].

2.2.1 Primary and Secondary Injury

The traumatic injury is not a single event, but a highly dynamic process set in motion by the initial mechanical impact, i.e. the primary injury, and the following secondary injury cascades [12, 16].

Primary injury after TBI is the brain damage that results from the direct impact of the external forces. Traumatic events include open and closed contusions, rapid accelerations or decelerations, penetrating injuries (such as gunshots), and blast injuries [17]. Based on the pathoanatomical features, primary injuries can be classified as focal or diffuse [18, 19]. Focal injuries include contusions and hematomas with localized and visible lesions, while diffuse axonal injuries are characterized by multiple microscopic lesions on the level of the axon that may globally affect brain function [20].

Secondary injuries are delayed mechanisms of brain damage, which may develop already within hours but also up to years after trauma. At the cellular level, secondary processes include neurotransmitter release, free-radical generation, calcium-mediated damage, gene activation, mitochondrial dysfunction, and inflammatory responses [2, 12, 17]. These cellular mechanisms then contribute to blood-brain barrier (BBB) breakdown, brain edema formation, intracranial hypertension, ischemia, cell apoptosis and necrosis. Secondary brain damage can be determined histologically, i.e. the tissue volume that is not affected by the primary insult but where cell death occurs during the following hours and days. It can also be interpreted functionally, including long-term alterations and dysfunctions, which might even exceed the histologically detectable damage.

For the model used for this thesis, Controlled Cortical Impact (CCI), primary and secondary injury have been described in detail [21-23]. Following CCI, secondary injury occurs mainly in the traumatic penumbra. Similar to the concept of ischemic penumbra observed in ischemic stroke, the term ‘traumatic penumbra’ can be defined as the surrounding cerebral parenchyma adjacent to the contusion site that initially survives the primary injury but subsequently undergoes secondary injury [24, 25]. Following the primary injury, brain tissue within the lesion area experiences immediate energy failure, membrane depolarization and infarction, resulting in an irreversible tissue damage [26]. In contrast, brain tissue in the surrounding penumbra area only undergoes functional alterations secondarily due to the compromised energy metabolism [25, 26]. Following trauma by CCI, the tissue in the traumatic penumbra becomes necrotic within 24 to 48 hours [21, 23, 27]. Considering the potentially reversible progression of secondary injury, there is a time window of about 12-24 hours for clinical interventions. Therefore, further studies investigating the mechanisms and identifying potential therapeutic approaches are urgently needed.

2.3 Brain Edema Formation Post TBI

Brain edema, characterized by the accumulation of excess fluid in the brain, is a critical factor in the pathogenesis of secondary brain injury following TBI. This pathological process can lead to intracranial hypertension, resulting in impaired vascular perfusion, dysfunction of brain cells, and further cell death and is closely linked to poor patient outcomes [1, 3, 14, 28-30].

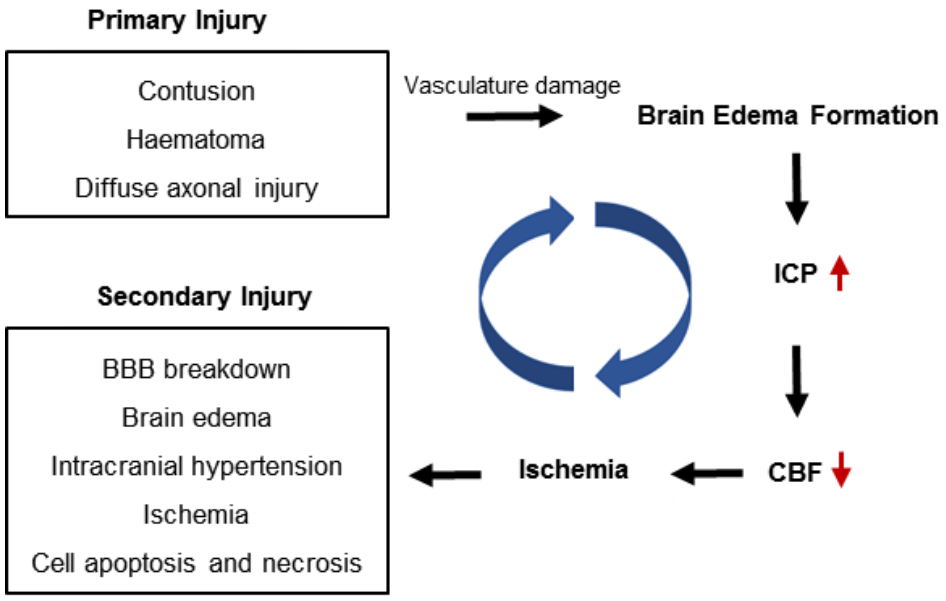

Figure 1: Brain edema formation is a critical factor in the pathophysiology of TBI.

Classically, brain edema post TBI is classified into two major categories: vasogenic and cytotoxic [31, 32]. Vasogenic edema occurs when the blood-brain barrier is disrupted, resulting in the accumulation of fluid in the extracellular space. By contrast, cytotoxic edema is characterized by the swelling of neurons and glial cells, which is caused by an influx of osmolytes and water from the extracellular space into the cells due to the disruption of energy metabolism [1, 29, 33].

Current treatments for brain edema formation after TBI are still limited to symptomatic strategies, while causal therapies are still not available [34]. Thus, there is an urgent need for further research to illuminate the underlying mechanisms of brain edema formation and identify potential therapeutic options.

2.3.1 Vasogenic Brain Edema (VBE)

The blood-brain barrier refers to a multicellular vascular structure that separates the cerebral interstitium from the peripheral blood circulation [35]. This barrier dynamically modulates its properties to regulate the influx and efflux of molecules and to protect the brain against potentially harmful substances, which is fundamental to maintain the cerebral homeostasis [35]. Impairment of the BBB is associated with neuronal loss and alterations in multiple brain functions, including reduced consciousness, impaired memory, and motor dysfunction [36].

After TBI, multiple mechanisms might damage the BBB, such as the initial mechanical impact, brain tissue hypoxia or ischemia, neuroinflammation, and excitotoxicity [1, 2, 33, 37, 38]. This leads to the extravasation of water and plasma into the cerebral interstitial space, resulting in fluid accumulation and the subsequent formation of VBE.

The molecular mechanisms underlying VBE are not fully understood. It is widely believed that VBE primarily occurs through paracellular transport across endothelial cells [38]. Cerebral ischemia and inflammation following TBI can induce endothelial cell retraction, leading to increased permeability of BBB [39]. The expression of vascular endothelial growth factor (VEGF) has been shown to down-regulate tight junction proteins, further compromising BBB integrity [40]. Moreover, increased activity of matrix metalloproteinases after TBI can degrade endothelial basement membrane proteins and tight junction proteins, contributing to BBB dysfunction [41]. Bradykinin was shown to be involved in vasogenic edema formation [42]. Another mechanism focusses on neuroinflammation, a process that includes the cytokine-mediated interaction of inflammatory cells with the cellular endothelium and the migration of leukocytes into the injured brain, which is discussed to be associated with BBB disruption after TBI [43, 44]. As a consequence, fluid can permeate into the brain parenchyma through these compromised channels. This leads to an influx of water from the vasculature into the cerebral extracellular space and therefore brain swelling.

In previous studies from our group, the formation of VBE following brain trauma was investigated by a specifically developed 2-photon microscopy approach: These studies demonstrated that a) trauma indeed induces VBE, b) vascular leakage is most pronounced in the penumbra, c) extravasation occurs mainly in capillaries, d) VBE is visible until 72 hours following CCI, and e) BBB permeability shows a biphasic pattern [45, 46]. That means the permeability of BBB undergoes a rapid onset, reaching its peak in the first couple of hours, and then shows a second, prolonged phase of extravasation at 48-72 hours after trauma. While the first peak is most likely attributed to the direct impact caused by the primary injury (e.g. sheer stress), the second peak might be caused by secondary injury mechanisms, such as neuroinflammation [47]. Further studies on the role of inflammation, such as the role of peripheral leukocytes and cerebral microglia for edema formation following brain trauma, are needed to follow up this hypothesis [48, 49].

2.3.2 Cytotoxic Brain Edema (CBE)

Cytotoxic edema is characterized by astrocytic swelling in various pathological conditions [1, 50]. Astrocytes are the main supportive cells of the brain parenchyma, playing a crucial role in maintaining the homeostasis of the extracellular space and in providing a stable microenvironment for brain cells [51]. Astrocytic swelling is a physiological response that occurs as a consequence of the active regulation of ions, neurotransmitters, protons and other neuroactive molecules in ECS [33, 38]. However, in pathological conditions, this physiological mechanism may become overactivated, leading to the development of cytotoxic edema [38, 52].

Following TBI, the primary injury initiates a series of cascades, which involves exposure to endogenous toxins (e.g. K^+, glutamate, H^+), intracellular ATP depletion, and disturbance of intracellular Na^+ transport. As a result, various ion channels are activated, facilitating the transfer of osmolytes and water from the extracellular space into astrocytes [29, 38, 53]. This process leads to astrocytic cellular swelling and a subsequent reduction in the extracellular space, accompanied

by impairment in extra-synaptic transmission, ultimately contribute to neurofunctional deficits and neuronal cell death [51].

One potential pathway is based on acid-sensing ion channels. Acid-sensing ion channels (ASIC) are cation channels that respond to changes in extracellular pH [54-56]. Among the ASIC isoforms, ASIC1a is highly expressed in the central nervous system (CNS), which can be activated by a decreased extracellular pH, leading to increased permeability to sodium (Na^+) and calcium (Ca^{2+}) ions. In the context of TBI, ATP depletion triggers anaerobic metabolism and subsequent tissue acidosis [54]. The activation of ASIC1a in response to the acidic environment contributes to Na+ influx and excitotoxicity, leading to the development cytotoxic edema [29, 38, 54]. Therefore, in a collaboration, I investigated the effect of ASIC1a on brain edema formation following brain trauma.

2.4 Measurement of Brain Edema Formation *in vivo*

Unfortunately, the available methods for brain edema investigation lack temporal resolution, e.g. Evan's Blue extravasation, have only a limited spatial resolution, e.g. epifluorescence microscopy, or lack specificity for the different forms of brain edema, e.g. conventional diffusion weighted magnetic resonance imaging (DWI)[2, 34]. Only very recently, an *in vivo* 2-photon microscopy approach was developed to investigate VBE dynamically following trauma [45, 46]. While this method allows great spatial and temporal resolution *in vivo*, the area that can be covered by one scan is quite small, i.e. 425*425*300 μm^3 (x*y*z). Even though several areas can be scanned in one animal, an investigation of the whole brain is not possible. Another disadvantage is the invasive implantation of a cranial window for 2-PM.

Current available clinical methods for detection of BBB disruption rely on non-invasive imaging methods such as contrast-enhanced CT and MRI, in combination with contrast agents [37]. These non-invasive imaging methods offer the advantage of repeated measurements *in vivo*, but are limited in their spatial resolution. The assessment of the presence of specific biomarkers, such as albumin and S100 β, in cerebrospinal fluid (CSF) or blood samples can provide indirect insights into BBB permeability, however the specificity and sensitivity of these biomarkers is still not fully understood [57].

Taken together, the development and optimization of the methods to investigate brain edema formation *in vivo* are essential for advancing our understanding of BBB function and its implications in various neurological conditions. In this thesis, I used two novel *in vivo* imaging techniques that can advance our understanding of brain edema formation:

1. A novel diffusion-weighted MRI approach, called "free water diffusion MRI (FWI)", which allows to differentiate "free", i.e. non-restricted water, which is mainly in the extracellular space from water, that is restricted in its movement by cell membranes [58, 59].
2. Validation of MRI measurements mentioned above by *in vivo* 2-photon microscopy, i.e. the visualization of VBE formation by measuring the extravasation of a tracer across the disrupted BBB [45, 46].

2.4.1 Characterization of Brain Edema Formation by Free Water Diffusion MRI

Diffusion-weighted imaging (DWI) using magnetic resonance provides valuable insights into the diffusion properties of water molecules. However, the sensitivity to very subtle pathologies is

limited [60]. Importantly, a differentiation between extracellular and intracellular water is not possible because one voxel might contain extracellular and intracellular water and only mean values can be recorded. Free water diffusion MRI is an advanced technique originally developed to correct the partial volume effects caused by cerebrospinal fluid in DWI [59, 61]. By explicitly modeling a free water compartment, representing freely moving water molecules (mainly in the extracellular space), and a tissue compartment, representing water molecules which are restricted by cellular membranes (mainly intracellular water), FWI enables the quantification of the free water fraction and the estimation of microscopic measures without the influence of extracellular free water [62]. Thus, changes in the free water fraction can indicate the presence of vasogenic (high free water fraction) or cytotoxic (low free water fraction, reduced diffusivity due to restricted water movement) edema. With FWI, vasogenic and cytotoxic brain edema formation was investigated following experimental brain trauma *in vivo* until seven days following injury and the results were validated by *in vivo* 2-photon microscopy.

2.5 Research Objective

To date, current techniques for assessing brain edema in TBI are limited both in experimental and clinical settings. These limitations can be overcome by the implementation of free water diffusion MRI and 2-Photon microscopy, which allow a detailed and dynamic *in vivo* investigation of the brain edema formation following TBI.

Aim of the project:

1. To characterize VBE and CBE up to one week following brain trauma *in vivo*

2. To investigate the role of acid-sensing ion channel 1a for edema formation after brain trauma

2.6 Methods

Study 1:

Male C57/Bl6 mice were randomly subjected to Controlled Cortical Impact or sham surgery and investigated by FWI at 4, 24, 48, 72 hours and 5 and 7 days thereafter. Mean Diffusivity (MD) and Free Water (FW) were determined in the contusion, the peri-contusional area, and the ipsi- and contralateral brain tissue. *In vivo* imaging by 2-Photon microscopy at 48 hours after TBI was used to validate the data obtained in the peri-contusional area. Nissl staining and Luxol fast blue staining at 7 days after TBI were performed to determine lesion area and white matter injury. All procedures and analyses were conducted by a researcher who was blinded to the group allocation.

Study 2:

Male ASIC1a$^{-/-}$ and WT littermates were randomly assigned to CCI or sham surgery. Brain water content was measured at 24 hours after TBI by the wet-dry method. Repetitive MRI was performed on days 14, 60, 90, and 180 days after TBI and histological analyses were performed at 180 days after TBI to assess posttraumatic brain damage, respectively. The Beam walk test, the tail suspension test and the Barnes maze test were used to evaluate neurological function. All procedures and analyses were conducted by a researcher who was blinded to the group allocation and genotype.

2.7 Results

2.7.1 Characterization of VBE and CBE following Brain Trauma by Free Water MRI

In the first part of this project, I characterized vasogenic and cytotoxic brain edema formation up to 7 days following experimental TBI by free water diffusion MRI. The study shows that CBE formation occurs for 48 hours following TBI and is restricted to the contusion, while VBE forms in the peri-contusional tissue up to 7 days after TBI. Additionally, the data suggested the occurrence of demyelination in the peri-contusional region. The data obtained by FWI were then verified by two methods: *in vivo* 2-photon microscopy and histological analysis. The formation of VBE was confirmed by 2-photon microscopy via the extravasation of a fluorescent tracer. The demyelination was confirmed by histological staining with Luxol fast blue.

These results were accepted for publication in the Journal of Neurotrauma ("Characterization of vasogenic and cytotoxic brain edema formation after experimental TBI by free water diffusion MRI") in September 2023.

2.7.2 ASIC1a Deletion Attenuates Brain Edema and Chronic Brain Damage following TBI

The role of ASIC1a in the formation of brain edema was investigated in a chronic TBI model. ASIC1a-deficient mice and wild-type (WT) littermates underwent controlled cortical impact (CCI) or sham surgery, and brain water content was used to assess brain edema. MRI and histology were performed to determine the brain tissue damage. We found a significant reduction in brain edema formation 24 hours after TBI in ASIC1a knockout mice, as well as improved posttraumatic brain damage and neurological function in the chronic phase until 6 months after trauma. The results of this study were published in the Journal of Neurotrauma with the following title: "Acid-ion sensing channel 1a deletion reduces chronic brain damage and neurological deficits after experimental traumatic brain injury" [63]

2.8 Discussion

2.8.1 Vasogenic and Cytotoxic Brain edema Formation following Experimental TBI

Until now, it is discussed controversially, which type of edema occurs when and where after brain trauma. Previous studies have presented conflicting findings. The occurrence of CBE was described by MRI already in the 1990s [64, 65]. Some reviews consequently focus on CBE as the main type of edema which is relevant following brain trauma [28, 66, 67]. By contrast, others also describe VBE as a relevant factor in the pathophysiology following brain trauma [1-3]. This is in line with data from our own group, which described the occurrence of VBE from 4 hours until 72 hours after experimental brain trauma by *in vivo* 2-PM [45, 46]. Since the pathophysiology and, thus, potential therapies of VBE and CBE differ significantly [1], it is crucial to know which type of edema occurs when after trauma to develop causal therapeutic strategies and to determine therapeutic windows.

The first study employed the novel method of free water diffusion MRI to simultaneously measure VBE and CBE *in vivo*, providing a detailed characterization of brain edema formation after experimental TBI. The results revealed the presence of cytotoxic edema primarily in the contusion area and vasogenic edema predominantly in the penumbra area. This is the first time that CBE and VBE were investigated simultaneously, *in vivo*, longitudinally, and with high spatial resolution. These results can explain the contradictory observations from earlier studies. The occurrence of CBE within the contusion area matches the descriptions of CBE by Barzo et al. [64, 65], while the description of VBE in the peri-contusional area is in line with the data obtained by 2-PM in the penumbra by our laboratory [45, 46]. Additionally, we observed demyelination in peri-contusional brain tissue at 7 days after TBI by FWI and histological analysis, which also corresponds to previously published data [68].

The non-invasive and reproducible nature of free water diffusion MRI makes it a powerful tool for studying the dynamics of brain edema formation. We have validated this method by two other techniques, i.e. *in vivo* 2-PM and histological analysis. Its future application holds promise in advancing our understanding of brain edema dynamics and facilitating the development of targeted interventions for improved TBI management.

2.8.2 The role of Acid-sensing Ion Channel 1a for Brain Edema Formation after TBI

ASIC1a is associated with various pathological processes following TBI, such as neuronal excitotoxicity, neuroinflammation [69]. However, its role of brain edema formation and further chronic brain damage is unclear. In the second study, our findings demonstrated that the absence of ASIC1a reduced the brain edema formation and resulted in significant improvements in both structural and functional outcome following TBI. One of the underlying mechanisms might be the ASIC1a deficiency resulted in a reduced post-traumatic inflammatory reaction by attenuating microglia activation. Thus, targeting ASIC1a channels might be a promising pharmacological approach for the management of chronic post-traumatic brain damage.

3. Summary

TBI is a significant global health issue, characterized by a range of clinical manifestations and potential long-term disabilities. TBI causes a primary injury, which is irreversible, and secondary injuries that occur during the following hours and days. This leads to various neurological impairments, ranging from mild concussions to severe brain damage. Due to the time delay, the secondary injury is principally susceptible to treatments.

Brain edema, the accumulation of fluid in the brain parenchyma, plays a critical role in the pathogenesis of secondary brain damage and significantly affects the outcome of patients with TBI. The development of brain edema leads to brain swelling. Once the intracranial compliance mechanisms are exceeded, this results in intracranial hypertension. Despite the clinical significance of brain edema in TBI, current therapeutic options are limited to symptomatic strategies, while no causal therapy is available. One problem is that the methods to investigate brain edema formation *in vivo* are technically limited. So far, it remains unclear which type of edema occurs when and where following trauma. Novel methods, which allow an investigation of cytotoxic and vasogenic brain edema formation with sufficient detail *in vivo* are urgently needed to unravel the underlying pathophysiological mechanisms and verify therapeutic approaches for TBI-induced brain edema.

One such method is free water diffusion MRI (FWI). This novel MRI technique allows the differentiation between extracellular and intracellular water, i.e. the assessment of vasogenic and cytotoxic brain edema, respectively.

In the first part of the project, we investigated the formation of vasogenic and cytotoxic edema from 4 hours to 7 days following experimental TBI by FWI. The data was verified by *in vivo* 2PM and histological analysis. The results showed that CBE occurs mainly within the primary contusion, while VBE was the predominant form of edema in peri-contusional tissue. This was consistent with the results obtained by 2-PM. In conclusion, the study demonstrated that CBE formation occurs for 48 hours after trauma and is restricted to the contusion, while VBE forms in the peri-contusional tissue up to 7 days after TBI. Furthermore, the results indicate that free water MR imaging may represent a promising tool to investigate vasogenic and cytotoxic brain edema in the laboratory and in patients.

In the second publication, the role of ASIC1a in brain edema formation and chronic brain damage after trauma was investigated. ASIC1a knockout and wide-type mice were subjected to CCI or sham surgery. Brain water content was measured 24 h thereafter, repetitive magnetic resonance imaging and histology was performed to assess the chronic brain tissue damage, and behavior tests were used to evaluate neurological function. The study demonstrated that the ASIC1a deficiency significantly decreased brain edema formation and resulted in less brain damage and functional impairments after experimental TBI.

Taken together, we used and verified a novel method, i.e. free water diffusion MRI, to assess the formation of vasogenic and cytotoxic brain edema following brain trauma. Furthermore, we explored the role of acid-sensing ion channels after TBI. The results contribute to a better understanding of the pathophysiological processes of brain edema in secondary brain injury following TBI and are highly relevant for the development of therapeutic strategies.

4. Zusammenfassung

Das Schädel-Hirn Trauma (SHT) ist eine der häufigsten Ursachen für Todesfälle oder eine schwere Behinderung weltweit. Es verursacht einen primären Hirnschaden, der irreversible ist, und sekundäre Hirnschäden, die innerhalb der folgenden Stunden und Tage entstehen. Das klinische Bild reicht von der milden Commotio bis zur schweren Contusio. Aufgrund der zeitlichen Verzögerung ist der sekundäre Hirnschaden prinzipiell behandelbar.

Das Hirnödem, d.h. die Akkumulation von Wasser im Parenchym, spielt eine wichtige Rolle für die Pathogenese des sekundären Hirnschadens und für den Verlauf der Erkrankung. Das Hirnödem führt zur Schwellung des Gehirns. Nach Ausschöpfen der intrakraniellen Compliance-Mechanismen führt das zum Anstieg des intrakraniellen Drucks. Leider gibt es bislang nur symptomatische Behandlungsoptionen für das Hirnödem und keine kausale Therapie. Ein Grund hierfür liegt darin, dass die Methoden zur Untersuchung des Ödems *in vivo* aufgrund technischer Limitationen bislang unzureichend sind. Bis heute ist deshalb nicht geklärt, welche Form des Ödems wann nach Trauma wo im Gewebe entsteht.

Neue Methoden, die eine Untersuchung des zytotoxischen und des vasogenen Ödems (CBE und VBE) *in vivo* und mit suffizienter Detailtiefe ermöglichen, werden dringend gebraucht um die pathophysiologischen Mechanismen zu klären und therapeutische Ansätze zu prüfen.

Eine solche Methode ist das “Free water diffusion MRI” (FWI). Diese neue MRT Technik ermöglicht die Differenzierung von extrazellulärem und intrazellulärem Wasser, d.h. die Bestimmung von VBE bzw. CBE, *in vivo*.

Im ersten Teil des Projekts wurde die Entstehung des vasogenen und zytotoxischen Ödems im Zeitraum von 4 Stunden bis 7 Tage nach Trauma mittels FWI untersucht. Die Ergebnisse wurden mit *in vivo* 2-Photonenmikroskopie (2PM) und histologischen Schnitten verifiziert. Die Daten zeigen, dass das CBE hauptsächlich in der Kontusion auftritt, während das VBE vor allem im Gewebe um die Kontusion herum entsteht. Das konnte mit 2PM bestätigt werden. Die Studie demonstriert, dass sich das CBE bis 48 Stunden nach Trauma bildet und hauptsächlich auf die Kontusion beschränkt ist, während das VBE bis 7 Tage nach Trauma vor allem im peri-kontusionellen Gewebe entsteht. Zudem deuten die Daten darauf hin, dass das Free water diffusion MRI ein vielversprechendes Model zur Untersuchung von VBE und CBE ist, sowohl experimentell wie auch klinisch.

In der zweiten Publikation wurde die Rolle von ASIC 1a (acid-sensing ion channel 1a), einem Säure-sensitivem Ionenkanal, für die Ödementwicklung und den Schaden nach Trauma untersucht. ASIC1a Knockout und Wildtyp Tiere erhielten ein experimentelles Trauma oder eine Scheinoperation. Der Hirnwassergehalt wurde 24 Stunden danach gemessen. Zudem wurde der Hirnschaden mit MRT und Histologie quantifiziert und die neurologische Funktion bis 6 Monate nach Trauma untersucht. Die Studie zeigte, dass ein ASIC1a Mangel die Ödembildung und den sekundären Hirnschaden reduzierte und die neurologische Funktion verbesserte.

Kurz zusammengefasst wurde in dieser Arbeit eine neue Methode zur Untersuchung des Hirnödems verifiziert, nämlich FWI. Die Entstehung von VBE und CBE wurde hiermit bis 7 Tage nach Trauma charakterisiert. Zudem wurde die Rolle von Säuresensitiven Ionenkanälen für die Ödementstehung nach Trauma untersucht. Die Ergebnisse tragen signifikant dazu bei, die Pathophysiologie der Ödementstehung nach Trauma besser zu verstehen und sind für die Entwicklung klinischer Therapien hochrelevant.

5. Paper I

Characterization of Vasogenic and Cytotoxic Brain Edema Formation after experimental TBI by Free Water Diffusion MRI

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6. Paper II

Acid-Ion Sensing Channel 1a Deletion Reduces Chronic Brain Damage and Neurological Deficits after Experimental Traumatic Brain Injury

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