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Research Report

Aquaporins and blood–brain barrier permeability in early edema development after traumatic brain injury

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ARTICLE INFO

Article history:
Accepted 2 March 2015
Available online 11 March 2015

Keywords:
Aquaporin
Blood–brain barrier
Brain injury
Brain edema
Rat
Brain trauma

ABSTRACT

Traumatic brain injury (TBI) is a major contributor to mortality and morbidity. The pathophysiology involves development of brain edema. Therapeutic options are limited as the mechanisms are not fully understood. Changes in the function of the blood–brain barrier (BBB), as well as variations in aquaporin expression, have been proposed to be involved in the development of the edema but the contribution of each factor has not been fully elucidated. In order to evaluate these mechanisms, in a potential window of opportunity, the early dynamic response was studied using an animal model causing a moderate TBI. Sprague-Dawley rats were subjected to blunt controlled head trauma and followed for up to four days by magnetic-resonance-imaging, immunohistofluorescence, immunohistochemistry, and quantitative protein analysis. Non-traumatized animals served as controls. TBI resulted in a midline shift and a decrease in Apparent Diffusion Coefficient, indicating a hemispheric enlargement due to cytotoxic edema. The tight junction protein Zona Occludens-1 was decreased (−25%) and associated with an increased IgG permeability (+20%) in the perilesional brain tissue in accordance with a BBB breakdown. The total amount of AQP4 protein decreased (−20%). The disruption of the BBB lasted for 4 days while the impact on AQP4 levels disappeared between day 1 and 4. Our findings shows that blunt focal brain injury results in an early development of brain edema involving both cytotoxic and vasogenic components, a persistent BBB breakdown and a temporary decrease in AQP4, and indicates that both types of edemas and mechanisms should be targeted in TBI treatment.

Abbreviations: ADC, apparent diffusion coefficient; AQP, aquaporin; AQP4, aquaporin-4; BBB, blood–brain barrier; C, contra; GFAP, glial fibrillary acidic protein; I, ipsi; IF, immunofluorescence; IgG, immunoglobulin G; MLS, midline shift; MRI, magnetic resonance imaging; RECA-1, anti-endothelial cell antibody; TBI, traumatic brain injury; WB, Western blot; ZO-1, zona occludens 1.

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http://dx.doi.org/10.1016/j.brainres.2015.03.004
0006-8993/

1. Introduction

Brain edema evolves as a consequence of several medical conditions such as traumatic brain injury (TBI), ischemic stroke and hyponatremia. The resulting increase in intracranial pressure can cause permanent brain damage and death (Masson et al., 2001; Marmarou et al., 2006). Globally TBI is a major cause of mortality and morbidity among children and young adults, responsible for more than 50 000 fatalities annually in the USA (Masson et al., 2001; Tagliaferri et al., 2006; Faul et al., 2007; Coronado et al., 2011). Although the clinical course of TBI is well described, the pathophysiology of brain edema following TBI is still not fully understood. TBI often results in secondary ischemia and increase in brain water content. The edema proceeds over days and, if uncontrolled, may reach a critical point where cerebral blood flow is compromised and ischemia worsens. This vicious circle can lead to brain herniation and death. Current medical treatment is directed towards limiting secondary brain injury (Clausen and Bullock, 2001; Marklund et al., 2006; Wang et al., 2006; Faul et al., 2007; Stein et al., 2010; Honeybul, 2011; Diaz-Arrastia et al., 2014). In humans, the resulting edema is fully developed within 2–3 days after brain trauma (Unterberg et al., 2004). Increased knowledge of the mechanisms involved in early edema development should be instructive for appropriate timing of, and new targets for, novel therapeutical approaches.

Treatment options directed towards traumatic brain edema are limited, partly due to lack of detailed insight into the pathogenesis and type of traumatic brain edema. In TBI, both vasogenic and cytotoxic edema is expected to coexist (Barzo et al., 1997; Marmarou, 2007; Hudak et al., 2014). Vasogenic edema results from trans-capillary movement of fluid due to disintegration of the blood–brain barrier (BBB) and hydrostatic pressure. The BBB normally restricts free transcellular water movement from the vascular compartment to the brain interstitium and thereby support the restricted and closely controlled environment necessary for brain survival (Chodobski et al., 2011). An important part of the BBB is the unfenestrated endothelium lined with tight junction (TJ) proteins. It has been shown that breakdown of the BBB occurs in TBI (Broman, 1955; Klatzo et al., 1958), promoting edema formation. However, there is still limited knowledge regarding the complex interaction between BBB damage, secondary brain injury and their temporal relationship. Cytotoxic edema is caused by an intracellular increase in osmotically active substances, usually due to ion-pump dysfunction or extracellular hypo-osmolarity (Kempski et al., 1987; Klatzo, 1987; Reinert et al., 2000; Signoretti et al., 2001). Water subsequently enters the cells, causing cellular swelling mainly in astrocytes (Gullans and Verbalis, 1993; Kimelberg, 2005; Sword et al., 2013). Water transport across the cell membrane is facilitated through specific water channels, aquaporins, which allow for bidirectional water passage depending on the osmotic gradient (Agre et al., 2002). Aquaporin 4 (AQP4) is the most detected aquaporin within the brain (Nielsen et al., 1997; Badaut et al., 2002; Nagelhus and Ottersen, 2013), primarily expressed on astroglial foot processes surrounding cerebral capillary vessels and facing cortical and ventricular surfaces. AQP4 has been shown to be involved in cerebral edema development in various pathological conditions but the role and expressional changes of AQP4 have been found to vary depending on the type of insult (Manley et al., 2000; Vajda et al., 2002; Amiry-Moghaddam et al., 2003; Amiry-Moghaddam et al., 2004; Papadopoulos and Verkman, 2005; Rama Rao and Norenberg, 2007). Several studies have described separate parts of the edema development in correlation to either BBB or AQP4. The aim of the present work was to study these mechanisms in relation to each other and to the formation and type of brain edema in a rat model of blunt head trauma. We focused on the early phase of edema development, as this phase clinically is associated with an opportunity for therapeutical interventions.

2. Results

All animals presented normal behavior without apparent neurological deficits after the brain trauma. Physiological parameters (body weight, temperature, saturation, heart rate and respiratory rate) were within the physiological range and did not differ among the groups (data not shown).

2.1. *Midline shift and ADC*

The MRI examination revealed a focal cortical contusion of similar extent in all TBI animals (Fig. 1). T2 weighted coronar MRI sections identified a visible edema in association to the contusion. To evaluate the time course of edema development and assess the type of edema following TBI in our rat model, we analyzed the MRI images with regard to midline shift (MLS) (Fig. 2A) and ADC values in the perilesional area

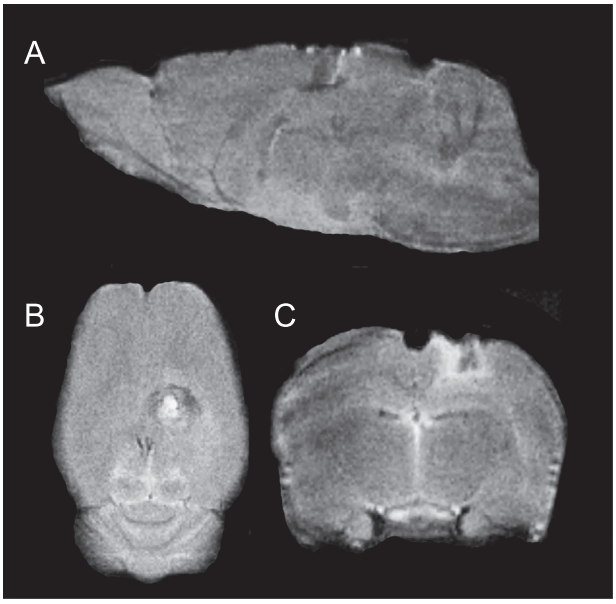

Fig. 1 – Representative T2 weighted MRI sections of an injured animal showing a hyper-dense area, indicating edema, in the area around the blunt injury. Sections; sagittal (A), axial (B), coronal (C).