Journal of Neuroimmunology 332 (2019) 57–63

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

The role of UCH-L1, MMP-9, and GFAP as peripheral markers of different susceptibility to seizure development in a preclinical model of epilepsy

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A B S T R A C T

In our study, we assessed the potency of the brain-derived proteins ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), matrix metalloproteinase 9 (MMP-9), glial fibrillary acidic protein (GFAP) and the immune activation indicators interleukin 1β (IL-1β) and interleukin 6 (IL-6) as peripheral biomarkers of different susceptibilities to kindling in a preclinical model. We observed increased plasma UCH-L1 levels in kindled vs. control animals. Furthermore, MMP-9 and IL-1β concentrations were the lowest in rats resistant to kindling. In summary, UCH-L1 is an indicator of neuronal loss and BBB disruption after seizure. MMP-9 and IL-1β may indicate resistance to kindling. UCH-L1, MMP-9 and IL-1β may have utility as peripheral biomarkers with translational potency in the clinic.

1. Introduction

Epilepsy, one of the most common neurological disorders, is characterized by recurrent and unprovoked seizures. Experimental and clinical data suggest that seizures can cause neuronal loss, gliosis and the rearrangement of neuronal circuits (Liu et al., 1994; Gorter et al., 2003; Pradhan et al., 2000). Furthermore, seizures lead to blood-brain barrier (BBB) disruption, which is believed to contribute to the pathogenesis and persistence of epilepsy (Marchi et al., 2007; Oby and Janigro, 2006). In recent years, substantial effort has been made to evaluate noninvasive, fast and specific assay methods to assess BBB conditions. Special attention has been paid to searching for brain-derived serum biomarkers to facilitate the diagnosis, treatment and prediction of epilepsy after the initial insult. To date, none of the proposed biomarkers have become clinically relevant.

Matrix metalloproteinase 9 (MMP-9) is a calcium-dependent protease that participates in extracellular matrix (ECM) remodeling in physiological and pathophysiological processes (Sternlicht and Werb, 2001). MMP-9 degrades not only ECM proteins, including type IV collagen, laminin or fibronectin, but also proteins that form tight junctions, which play a key role in regulating blood-brain barrier permeability. Thus, MMP-9 contributes to the regulation of BBB permeability (Chen et al., 2009). The role of MMP-9 has been studied in neurological disorders such as Alzheimer's disease (Asahina et al., 2001; Mizoguchi et al., 2009), ischemic stroke (Fujimura et al., 1999; Justicia et al., 2003) and epilepsy (Kim et al., 2009). Studies using MMP-9 knockout mice revealed a marked delay in seizure development after PTZ-induced kindling in MMP-9 knockout compared to wild-type mice (Mizoguchi et al., 2011). In contrast, rats overexpressing MMP-9 were more sensitive to chemical kindling (Wilczyński et al., 2008). In humans, BBB disruption caused by generalized tonic-clonic seizures increases the concentration of MMP-9 in cerebrospinal fluid (CSF) compared to levels in nonepileptic individuals (Li et al., 2013). Furthermore, serum MMP-9 levels are elevated in children with febrile seizures and convulsive status epilepticus (Suenaga et al., 2008).

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a 24 kDa protein that is predominantly expressed in the brain, comprising 1–5% of the total soluble proteins in neurons (Day and Thompson, 2010). Physiologically, UCH-L1 cleaves small peptide adducts from the C-terminus of ubiquitin and generates free monomeric ubiquitin from ubiquitin preproteins (Larsen et al., 1998). The faulty function of UCH-L1 has been associated with neurodegenerative disorders (Gong and Leznik, 2007). Elevated concentrations of UCH-L1 in serum have been observed after traumatic brain injury (TBI) (Blyth et al., 2011; Mondello et al., 2012a) and stroke (Ren et al., 2016), and epilepsy (Mondello et al., 2012b), in combination with BBB disruption.

Glial fibrillary acidic protein (GFAP) is a protein that is abundantly expressed in the brain and is recognized as a specific astrocyte marker. GFAP mainly plays a structural role, but it also coordinates mobility and signal transduction in the cell (Sofroniew et al., 2010). BBB disintegration and astrocyte damage may facilitate GFAP release into the bloodstream. The utility of peripheral GFAP as a marker of neurological

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https://doi.org/10.1016/j.jneuroim.2019.03.018
Received 16 January 2019; Received in revised form 6 March 2019; Accepted 28 March 2019

condition has been researched for years (Chmielewska et al., 2018). Despite some limitations, GFAP has been found to be a promising agent for distinguishing intracerebral hemorrhage from other types of stroke (Ren et al., 2016), for estimating infarct volume in TBI patients (Lei et al., 2015), and for diagnosing and prognosing glioblastoma multiforme (Tichy et al., 2016). In epilepsy, GFAP has not been widely researched; however, available data have revealed its value for the differential diagnosis of epileptic and psychogenic nonepileptic seizures (Simani et al., 2018).

The extent of the inflammatory response in susceptible brain regions may contribute to a decreased seizure threshold, spontaneous seizures and epileptogenesis (De Simoni et al., 2000; Vezzani and Friedman, 2011). It is unknown whether the activation of the immune system is a consequence of neuronal cell damage during seizures or whether it is a causative factor engaged in the etiopathogenesis of epilepsy. Accordingly, the expression of pro-inflammatory cytokines, such as interleukin 1β (IL-1β) and interleukin 6 (IL-6), has been shown to be affected in brain tissue and plasma in humans and various animal models of epilepsy (Uludag et al., 2015; Rijkers et al., 2009; Vezzani et al., 1999; Vezzani and Friedman, 2011). Many studies have revealed the ambiguous role of immune factors in seizures. IL-1β is perceived as one of the strongest immune modulators in epilepsy (De Simoni et al., 2000; Vezzani et al., 1999) and is believed to be associated with the activation of microglia, changes in astrocyte function, disruption of the blood-brain barrier and activation of the release of other cytokines, such as IL-6. However, some studies have demonstrated the neuroprotective, anticonvulsant and antiepileptogenic effects of IL-1β (Sayyah et al., 2005; Kołosowska et al., 2014). Despite these contradictory results that point to a complex role of immune factors in epilepsy, it is important to note that the increased expression of pro-inflammatory substances may be considered a marker of immune activation (Vezzani and Friedman, 2011). In physiological conditions, brain- and periphery-derived cytokines have limited ability to cross the BBB; however, an increase in the BBB permeability evoked by seizures may facilitate the transport of interleukins across the barrier.

The aim of this study was to determine the role of selected brain-derived proteins as peripheral indicators with potential prognostic value for specifying different susceptibility to kindling development. Therefore, the plasma concentrations of brain-derived proteins with a well-established role in neurological disorder etiology and with documented ability to cross the BBB and remain stable in the blood, i.e., MMP-9, UCH-L1, and GFAP, was assessed. Importantly, these proteins represent different components of brain tissue. Considering the engagement of pro-inflammatory cytokines i.e., IL-1β and IL-6 in epilepsy, their plasma levels in PTZ kindled rats, which were divided into groups with different susceptibility to seizure development, were also determined.

2. Materials and methods

2.1. Animals

The study was performed on 75 adult male Wistar rats, weighing 200 ± 20 g at the beginning of the experiment. The animals were housed in standard laboratory conditions under a 12 h light/dark cycle in a controlled temperature (20 ± 2 °C) at 50% humidity. All experiments were performed between 9.00 a.m. and 3.00 p.m. The animals had free access to food and water. The study was carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and was approved by the Committee for Animal Care and Use at the Medical University of Warsaw. All efforts were made to minimize suffering during the experimental procedures.

2.2. Drug

Pentylenetetrazole (PTZ, Sigma–Aldrich, Poland) was dissolved in 0.9% NaCl solution.

2.3. Kindling procedure

In the first stage of the procedure, experimental animals ($n = 43$) received a single i.p. injection of PTZ at a dose of 30 mg/kg or saline (control rats, $n = 32$). The drug was given in a 1 ml/kg volume of saline. Rats that reacted with tonic-clonic seizures after the first dose of PTZ were decapitated 24 h after the seizure. The remaining animals ($n = 34$) were subjected to kindling of seizure. The animals received repeated injections of either PTZ, i.e., kindled rats, or saline, i.e., control rats ($n = 32$), three times a week (Monday, Wednesday, Friday). After each injection, rats were singly housed in transparent Plexiglass cages (30 × 30 × 50 cm) and were observed for 30 min. The intensity of the convulsions was defined according to a modified, Racine's scale: 0 - no response; 1 - ear and facial twitching; 2 - myoclonic jerks without rearing; 3 - myoclonic jerks with rearing; 4 - turning over onto side position with clonic–tonic seizures; 5 - turning over onto back position with generalized clonic–tonic seizures (Becker et al., 1992). Rats were considered fully kindled in the case of two 5-degree seizures after two consecutive injections of PTZ. Rats were assigned to the groups according to their susceptibility to kindling: group that fulfilled the kindling criteria after 5 injections of PTZ ($n = 8$), after 10 injections of PTZ ($n = 10$), after 20 injections of PTZ ($n = 10$), and “resistant rats” ($n = 6$) that did not fulfill the kindling criteria after 20 injections of PTZ. The scheme of the experiment is shown in Fig. 1.

2.4. Determination of MMP-9, UCH-L1, GFAP, IL-1β, and IL-6 concentrations in the plasma

Kindled and control animals (with the same number of injections) were decapitated 24 h after the last PTZ and saline injections, respectively, and trunk blood was collected into heparinized tubes and centrifuged (2500 ×*g*, 15 min, 4 °C). The plasma was aliquoted and stored at −80 °C until further analysis. After thawing, to avoid unspecific signals from activated platelets, an additional centrifugation was applied (10,000 ×*g*, 10 min, 4 °C) to plasma for complete platelet removal.

The levels of MMP-9, UCH-L1, GFAP, IL-1β, and IL-6 were determined using commercially available sandwich ELISA kits for rats (Cusabio Biotech Co., Ltd., CSB-EL025541RA for UCH-L1, with a detection range of 0.78–50 ng/ml; R&D Systems, RMP900 for MMP-9, with a detection range of 0.156–10 ng/ml; MyBioSource Inc., MBS 2886354 for GFAP, with a detection range of 31.2–2000 pg/ml; Elabscience, *E*-El-R0012 for IL-1β, with a detection range of 31.25–2000 pg/ml; Elabscience, E-El-R0015 for IL-6, with a detection range of 62.5–4000 pg/ml). The assays were based on the quantitative sandwich enzyme immunoassay with double antibody sandwich technology. The optical density was determined using a microplate reader set to 450 nm.

2.5. Statistical analysis

The plasma levels of MMP-9, UCH-L1, GFAP, IL-1β, and IL-6 are expressed as the means ± SEM. Student's *t*-test for independent groups was used to compare data between animals that experienced tonic-clonic seizures after a single PTZ injection. To compare data between kindled and control groups at particular time points, one-way ANOVA followed by an LSD post hoc test was used. For all experiments, statistical significance was defined as $p < .05$. Statistical analyses were performed using Dell Statistica, version 13.1 (Dell Inc. (2016), USA).

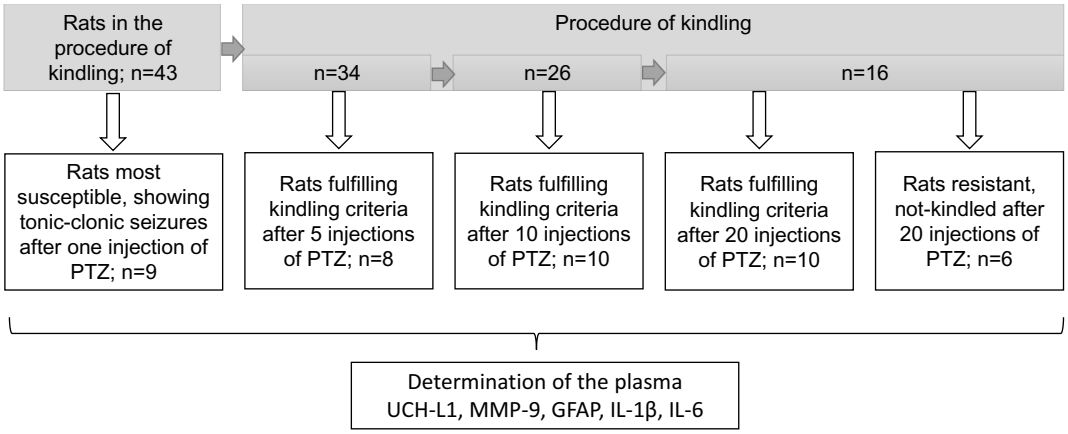

Fig. 1. Schematic presentation of the experimental design, which aimed to assign rats to groups based on different susceptibility to kindling. The rats were decapitated 24 h after the last PTZ injection after reaching kindling criteria (two consecutive stage 5 seizures after 5, 10 or 20 PTZ injections). Control rats (n = 32) were injected with saline to correspond with the PTZ injections (Sal - 1 inj., n = 8; sal - 5 inj., n = 8; sal – 10 inj., n = 8; and sal - 20 inj., n = 8).

3. Results

3.1. Changes in plasma levels of UCH-L1 in rats with different susceptibility to kindling (Figs. 2 and 3)

One-way ANOVA revealed significant differences in the UCH-L1 levels in rats with different susceptibility to kindling ($F = 5.44$, $p < .001$). An LSD post hoc test revealed that UCH-L1 levels were significantly higher in the animals that were kindled after 10 PTZ injections ($p < .01$) and 20 PTZ injections ($p < .001$) than in the control group. Moreover, there were significant differences between the group of resistant animals (which did not develop three consecutive stage 5 seizures after 20 PTZ injections) and animals that were kindled after 10 ($p < .05$) and 20 PTZ injections ($p < .05$).

UCH-L1 levels were not significantly different between animals kindled after 5 PTZ injections and control animals. However, a tendency toward increased UCH-L1 levels was observed in the PTZ kindled group ($p = .06$). In addition, Student's t-test indicated a significant increase in the plasma UCH-L1 level in the group of animals that developed stage 5 seizures after the first PTZ injection ($t = 4.17$; $p < .01$).

3.2. Changes in plasma levels of MMP-9 in rats with different susceptibility to kindling (Figs. 2 and 3)

One-way ANOVA revealed significant differences in MMP-9 levels in rats with different susceptibility to kindling ($F = 2.71$, $p < .05$). An LSD post hoc test revealed that MMP-9 levels in the resistant group were significantly lower than those in control animals ($p < .05$) or in those kindled after 5 ($p < .05$) and 20 PTZ injections ($p < .01$). The level of MMP-9 was significantly lower in the group kindled after 10 PTZ injections ($p < .05$). There were no significant differences between animals that were kindled after 5 PTZ injections and the control group. In the group of animals that developed stage 5 seizures after the first PTZ injection, MMP-9 plasma levels did not differ from those of the control group.

3.3. Changes in plasma levels of GFAP in rats with different susceptibility to kindling (Figs. 2 and 3)

There were no significant changes in the plasma GFAP level across experimental groups ($F = 0.90$, $p > .05$). Moreover, Student's t-test did not reveal a difference between control animals and animals that reacted with stage 5 seizures following the first PTZ injection; however, a tendency to show higher GFAP levels was observed in the PTZ group ($t = 2.07$; $p = .058$).

3.4. Changes in plasma levels of IL-1β in rats with different susceptibility to kindling (Figs. 2 and 4)

One-way ANOVA revealed significant differences in IL-1β levels in rats with different susceptibility to kindling ($F = 3.40$, $p < .01$). The LSD post hoc test revealed that IL-1β level in the resistant group of animals was significantly lower than that in the control animals ($p < .01$) or in animals kindled after 5 PTZ injections ($p < .05$). IL-1β levels in animals kindled after 20 PTZ injections were significantly lower ($p < .05$) than those in control animals and animals kindled after 5 PTZ injections ($p < .05$).

No significant changes in IL-1β plasma concentrations were found between animals kindled after 5 or 10 PTZ injections and control animals; however, animals kindled after 10 PTZ injections showed a tendency toward lower IL-1β levels ($p = .055$). Student's t-test did not reveal differences in IL-1β plasma levels between control animals and animals that reacted with stage 5 seizures following a single PTZ injection.

3.5. Changes in plasma levels of IL-6 in rats with different susceptibility to kindling (Figs. 2 and 4)

ANOVA did not show any significant differences in IL-6 plasma levels between animals with different susceptibility to kindling and the control group ($F = 1.71$, $p > .05$). However, a significant decrease in plasma IL-6 levels was observed in rats that developed stage 5 seizure after one PTZ injection compared to levels in the control group ($t = 3.55$; $p < .01$).

4. Discussion

In the present study, we assessed the plasma concentrations of UCH-L1, MMP-9, GFAP and the pro-inflammatory cytokines IL-1β and IL-6 in rats showing different susceptibility to kindling to assess their possible diagnostic value and translational potency in the clinic. We showed that there were increased plasma UCH-L1 concentrations in almost all kindled groups of rats compared to controls. Furthermore, lower levels of MMP-9 and IL-1β were found in rats resistant to kindling after 20 PTZ injections than in the control group and other experimental groups. Plasma GFAP did not differ between control and kindled animals.

UCH-L1, due to abundant neuron-limited expression in the CNS, has recently been proposed as a neuron cell body marker. Increases in UCH-L1 levels in CSF and blood have been observed in a number of neurological conditions (Mondello et al., 2012a,b; Ren et al., 2016). BBB leakage in epilepsy may increase peripheral concentrations of UCH-L1

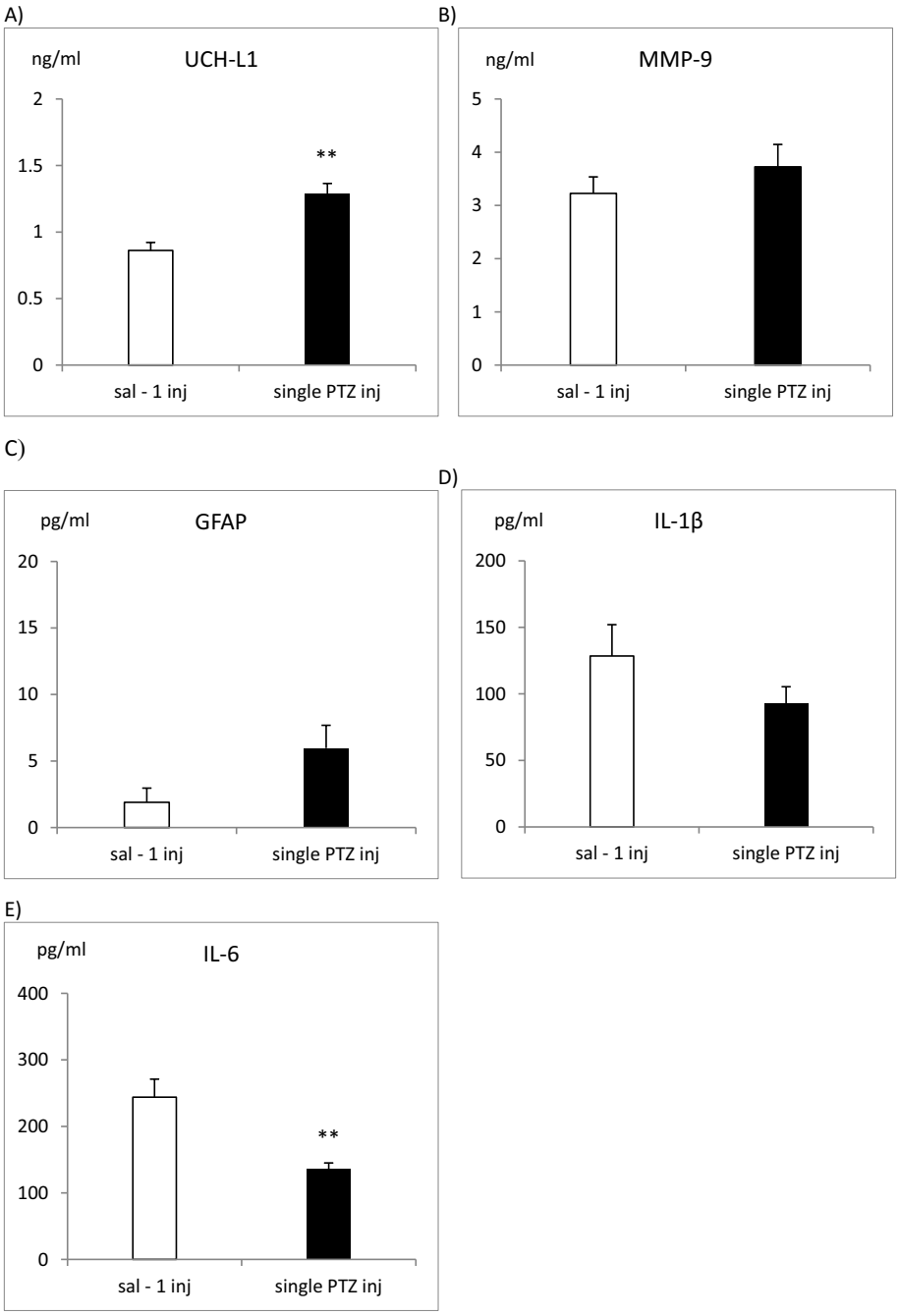

Fig. 2. Plasma UCH-L1 (A), MMP-9 (B), GFAP (C), IL-1β (D), IL-6 (E) levels in rats with tonic-clonic seizures after a single PTZ injection. The data are presented as the means ± SEM. Statistical significance: ** - $p < .01$, * - vs. control group; sal - 1 inj., $n = 8$ and single PTZ inj., $n = 9$.

(Glushakova et al., 2012; Mondello et al., 2012b; Asadollahi and Simani, 2019). In epileptic patients, plasma UCH-L1 has been found to be an early marker of neuronal damage (Mondello et al., 2012b). Available animal data from rats after kainic acid (KA) injections confirms UCH-L1 as an early, post-seizure peripheral marker of neuronal loss. However, its peak value at the earliest time point – 6 hours post-KA injection – was assessed only in CSF. In our study, plasma levels of UCH-L1 measured 24 h after the last kindling episode were increased in the group of animals kindled after 10 and 20 injections and in the rats that were most susceptible to seizure, i.e., rats that reacted with tonic-clonic seizures after one injection of PTZ. A tendency toward increased levels of plasma UCH-L1 was observed in the group of animals kindled after 5 injections of PTZ. Furthermore, the group of rats that was resistant to kindling after 20 injections of PTZ had a lower concentration of UCH-L1 than animals kindled after 10 or 20 injections of PTZ. These results emphasize differences in BBB permeability between kindled vs. resistant animals and suggest a role for the BBB condition in the development of kindling. We assume that the lack of a difference between particular groups of kindled animals (animals that were considered kindled after 5, 10 and 20 PTZ injections) may suggest that UCH-L1 is released postseizure, probably as a consequence of neuronal loss and BBB disruption rather than susceptibility toward kindling development. However, increased levels of UCH-L1 in kindled rats after 10 and 20 PTZ injections compared to levels in nonkindled animals suggests that high levels of this protein may be a marker of seizure.

MMP-9 is mainly synthesized by neurons and, to a lesser extent, by astrocytes in the CNS. MMP-9 plays a role in multiple neurological disorders, including epilepsy. Neuron remodeling, governed by MMP-9,

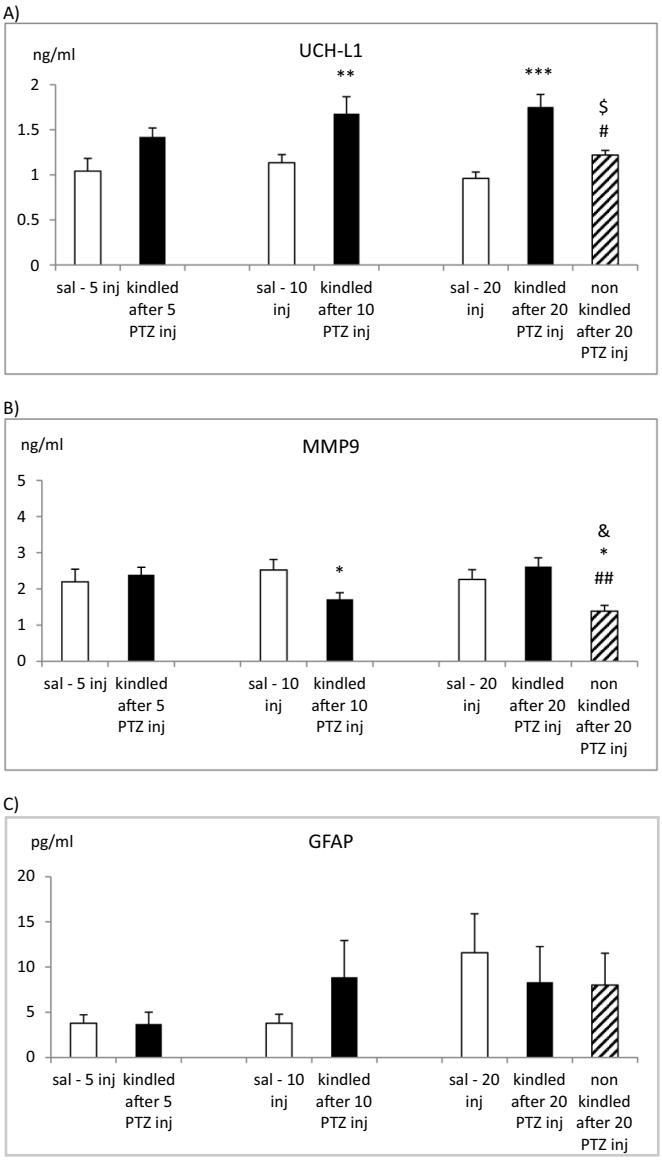

Fig. 3. Plasma UCH-L1 (A), MMP-9 (B) and GFAP (C) concentrations in groups of rats kindled after 5, 10, 20 injections of PTZ and rats resistant to kindling after 20 injections of PTZ. The data are presented as the means ± SEM. Statistical significance: * - $p < .05$; **,## - $p < 0{,}01$; ***$p < .001$, * - vs. control group, # - vs. group kindled after 20 injections of PTZ, $ - vs. group kindled after 10 injections of PTZ; & - vs. group kindled after 5 injections of PTZ; sal - 5 inj., $n = 8$; kindled after 5 inj., $n = 8$; sal – 10 inj., $n = 8$; kindled after 10 inj., $n = 10$; sal – 20 inj., $n = 8$; kindled after 20 inj $n = 10$; non kindled after 20 inj $n = 6$.

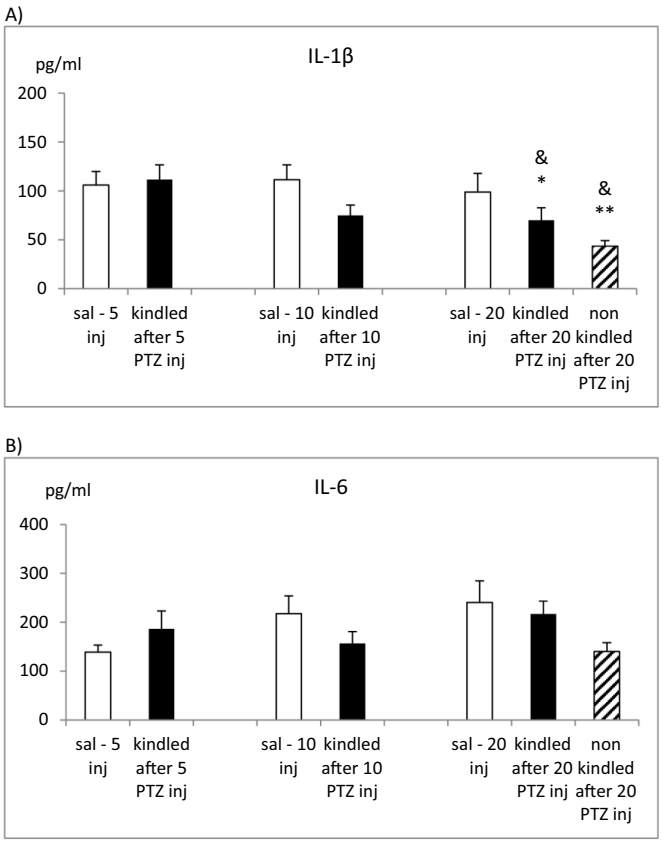

Fig. 4. Plasma IL-1β (A) and IL-6 (B) concentrations in groups of rats kindled after 5, 10, 20 injections of PTZ and rats resistant to kindling after 20 injections of PTZ. The data are presented as the means ± SEM. Statistical significance: * - $p < .05$; ** - $p < 0{,}01$; * - vs. control group; & - vs. group kindled after 5 injections of PTZ; sal - 5 inj., $n = 8$; kindled after 5 inj., $n = 8$; sal - 10 inj., $n = 8$; kindled after 10 inj., $n = 10$; sal – 20 inj., $n = 8$; kindled after 20 inj., $n = 10$; and not kindled after 20 inj., $n = 6$.

may lead to pathological synaptic rearrangement and promote epileptogenesis. The role of MMP-9 in the regulation of BBB permeability, particularly relating to open tight junctions and ECM degradation, has also been well described (Yang et al., 2007; Li et al., 2013; Cudna et al., 2017).

The seizure-induced injury of neurons and glia is a major source of MMP-9, and its release into the blood stream is facilitated by BBB leakage. Our study showed decreased plasma levels of MMP-9 in resistant rats and rats kindled after 10 PTZ injections compared to control animals. Furthermore, the level of MMP-9 in nonkindled rats was lower than that of kindled rats after 5 and 20 injections of PTZ. The results regarding rats kindled after 10 injections of PTZ are difficult to interpret. However, it is noteworthy, that no changes in the plasma levels of MMP-9 have been shown between the control group and other groups of kindled animals. The lowest values of MMP-9 were observed in resistant rats, in which a lower propensity to kindling may result from lower basal MMP-9 levels. Similar to our results, in MMP-9 knock-out mice, decreased sensitivity to PTZ kindling has been observed. Moreover, mice overexpressing MMP-9 have an increased sensitivity to kindling (Wilczyński et al., 2008). Considering the data in the literature and our results, we suggest that despite these ambiguous results regarding kindled rats after 10 injections of PTZ, decreased MMP-9 concentrations may have a protective influence against kindling. In this context, many mechanisms, such as increased activity of TIMP-1 (tissue inhibitor of metalloproteinase 1), which is an MMP-9 proteolytic activity inhibitor, may be related to this phenomenon. In neurons resistant to the excitotoxic effects of KA, TIMP-1 expression was found to be increased (Rivera et al., 1997). Although MMP-9 concentrations did not correlate with different susceptibility to kindling in our study, lower plasma levels of MMP-9 may contribute to resistance to kindling; however, more research is needed to confirm this hypothesis.

In our study, plasma GFAP levels did not differ between control and kindled animals or between groups of animals with different susceptibility to kindling 24 hours post-seizure. Similarly, in a group of 29 patients with epilepsy, only three patients suffering from status epilepticus had higher serum GFAP concentrations (Mayer et al., 2013). Furthermore, in children, only 7 out of 52 seizure patients showed increased CSF GFAP concentrations within 24 h, which may imply that astrocyte injury rarely occurs in this condition (Gurnett et al., 2003). However, serum GFAP levels measured 6 hours postseizure were found to be higher in patients with epileptic seizures than in patients with

psychogenic, nonepileptic seizures (Simani et al., 2018). This result suggests that an increase in GFAP levels resulting from astrocyte damage may be subtle and detectable in the periphery only shortly after seizure. Due to the pivotal role of GFAP in epileptic astrogliosis, its occurrence may be limited to the CNS in that condition (Steward et al., 1992). For this reason, GFAP as a peripheral marker may have limited relevance, if any.

A growing body of evidence suggests a link between inflammatory mechanisms and the pathogenesis and course of epilepsy. Animal data have demonstrated the immediate brain synthesis of proinflammatory cytokines during and after kindling (Pernot et al., 2011; De Simoni et al., 2000; Arican et al., 2006). IL-β, IL-6, and TNF-α were found to be increased in the acute phase after seizure in astrocytes and microglia in different brain regions. At low concentrations, IL-1β has mostly neuroprotective properties and participates in physiological processes such as memory formation and sleep (Huang and Sheng, 2010). However, interleukins are constantly released, and their action can become neurotoxic (Strijbos and Rothwell, 1995). Cytokines, such as IL-1β and TNF-α, may contribute to astrogliosis, loss of neurons, and BBB disruption (Arican et al., 2006; De Simoni et al., 2000).

Human and animal data report that in some types of epilepsy, inflammation extends beyond the CNS and may include the periphery (Rana and Musto, 2018). Considering this, since 2014, the National Institute of Neurological Disorders and Stroke, lists IL-1β as a potential biomarker of epileptogenesis (Galanopoulou et al., 2016). However, it is not certain that the immunological response takes part in all forms of epilepsy. In our study, decreased concentrations of plasma IL-1β were found in rats kindled after 20 injections of PTZ and in rats resistant to kindling. IL-1β promotes adult epileptogenesis after prolonged febrile seizures (Feng et al., 2016). Different variations in the IL-1β gene have also been shown to increase the risk of TLE (temporal lobe epilepsy) after TBI and cause a 2–3-fold enhancement of IL-1β synthesis induced by LPS (Diamond et al., 2014; Hall et al., 2004). Given these facts, we decided to test whether peripheral IL-1β could help identify different susceptibility to kindling. We found decreasing serum concentrations of IL-1β with increasing resistance to kindling. Accordingly, the level of IL-1β was the lowest in the nonkindled group of rats.

The only available data have shown that a higher IL-1β CSF/serum ratio predicted an increased risk of post-traumatic epilepsy over time in patients after TBI. Neither the serum nor CSF level alone has such a high prognostic value (Diamond et al., 2014). Similar to our results, other authors have shown a decreased concentration of plasma IL-1β in patients resistant to epilepsy (Diamond et al., 2014). This is consistent with previous works showing a proconvulsive role for IL-1β. Thus, lower IL-1β levels are correlated with resistance to seizure and may be an important mechanism of neuroprotection.

In sum, IL-1β levels in the blood seem to be dependent upon many factors, such as the progression of epileptogenesis, seizure phase, and the type and course of epilepsy. Therefore, it is suggested that the blood IL-1β concentration may serve as a biomarker and may have applications only in specific types of epilepsy.

Despite the well-established proinflammatory role of IL-6, the association of this cytokine with epilepsy is ambiguous. Some animal studies confirm a link between IL-6 release and a greater susceptibility to seizures (Arican et al., 2006; De Luca et al., 2004). In mice overexpressing IL-6, an increased sensitivity to excitatory input and a loss of GABA and parvalbumin neurons in the hippocampus have been found (Steffensen et al., 1994; Samland et al., 2003). On the other hand, its inhibitory role or limited role in seizure propagation has been described in different models of seizure (Fukuda et al., 2007; Kołosowska et al., 2014, 2016). We did not find differences in IL-6 between control animals and animals kindled after 5, 10 and 20 injections of PTZ or between control rats and rats resistant to kindling. Similarly, in electrically induced SE, the plasma concentration of IL-6 was not changed at any stage (Holtman et al., 2013). In the same study, after pilocarpine-induced SE, the levels of IL-6 were increased on the day of seizure but not one day after SE (Holtman et al., 2013).

In the plasma of the rats most susceptible to seizure, i.e., those showing stage 5 seizures after one injection of PTZ, decreased levels of IL-6 was observed. IL-6 has been found to be a factor contributing to GABA neurotransmission. In IL-6-deficient mice, diminished GABA neurotransmission correlated with greater susceptibility to seizures (De Luca et al., 2004). IL-6 may also be released by intensified skeletal muscle contraction, as observed during the acute stage after seizure (Pedersen and Febbraio, 2008). Most available human data showed increased IL-6 levels in the plasma of epileptic patients shortly after seizure (Uludag et al., 2013, 2015; Xia et al., 2018). This may be explained by the activation of the sympathetic nervous system during seizures and the release of catecholamines, which in turn, stimulate the synthesis of cytokines in peripheral blood cells (Meisel et al., 2005). The use of IL-6 as a peripheral biomarker in epilepsy may therefore have limited application.

Our study supports well-established findings that brain tissue damage and BBB disruption occur during kindling. Among all markers examined, UCH-L1 was found to be the most promising marker of seizure from the clinical point of view. Low plasma concentrations of MMP-9 and IL-1β were observed in seizure-resistant animals. The obtained results point to UCH-L1, MMP-9 and IL-1β as candidates for further research in other models of seizure kindling.

Acknowledgments

This study was supported by Grant No. 2014/13/B/NZ7/02277 from the National Science Centre, Poland. Project implemented with CePT infrastructure financed by the European Union – The European Regional Development Fund within the operational program “Innovative economy” for 2007–2013.

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