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Epileptogenesis after traumatic brain injury in *Plau*-deficient mice

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ABSTRACT

Several components of the urokinase-type plasminogen activator receptor (uPAR)-interactome, including uPAR and its ligand sushi-repeat protein 2, X-linked (SRPX2), are linked to susceptibility to epileptogenesis in animal models and/or humans. Recent evidence indicates that urokinase-type plasminogen activator (uPA), a uPAR ligand with focal proteinase activity in the extracellular matrix, contributes to recovery-enhancing brain plasticity after various epileptogenic insults such as traumatic brain injury (TBI) and status epilepticus. Here, we examined whether deficiency of the uPA-encoding gene *Plau* augments epileptogenesis after TBI. Traumatic brain injury was induced by controlled cortical impact in the somatosensory cortex of adult male wild-type and *Plau*-deficient mice. Development of epilepsy and seizure susceptibility were assessed with a 3-week continuous video-electroencephalography monitoring and a pentylenetetrazol test, respectively. Traumatic brain injury-induced cortical or hippocampal pathology did not differ between genotypes. The pentylenetetrazol test revealed increased seizure susceptibility after TBI ($p < 0.05$) in injured mice. Epileptogenesis was not exacerbated, however, in *Plau*-deficient mice. Taken together, *Plau* deficiency did not worsen controlled cortical impact-induced brain pathology or epileptogenesis caused by TBI when assessed at chronic timepoints. These data expand previous observations on *Plau* deficiency in models of status epilepticus and suggest that inhibition of focal extracellular proteinase activity resulting from uPA–uPAR interactions does not modify epileptogenesis after TBI.

1. Introduction

The annual incidence of traumatic brain injury (TBI) is ~500 cases per 100,000 population in the USA and Europe [1,2]. Approximately 5% of patients with moderate and 16% with severe TBI develop epilepsy in a 30-year follow-up [3]. Altogether, 10% to 20% of symptomatic epilepsy is estimated to be due to TBI [4]. Why posttraumatic epilepsy develops in a subpopulation of subjects with TBI is poorly understood. Some evidence suggests that genetic factors make an individual more prone to posttraumatic epileptogenesis by directing the abnormal programming of tissue recovery. Consistent with this notion, mice with a CD1 background develop epileptogenesis after controlled cortical impact (CCI)-induced TBI more frequently than mice with a B6 background [5,6]. Studies in humans demonstrate that single nucleotide polymorphisms in genes encoding apolipoprotein E4, glutamic acid decarboxylase 1, A1 adenosine receptor, interleukin-1β, and methylenetetrahydrofolate reductase are associated with the development of posttraumatic epilepsy [7–11]. More candidate genes that increase the likelihood for developing posttraumatic epilepsy remain to be discovered. One strategy for identifying these genes is to assess whether the genes previously determined to associate with epilepsy or with compromised postinjury recovery make an individual susceptible to post-TBI epileptogenesis.

The urokinase-type plasminogen activator receptor (uPAR)-interactome comprises uPAR anchored to the plasma membrane, its lateral receptor partners, and uPAR ligands, including urokinase-type plasminogen activator (uPA), sushi-repeat protein X-linked 2 (SRPX2), kininogen, and vitronectin [12]. The binding of uPA to uPAR initiates a proteolytic cascade in the extracellular matrix via activation of the plasminogen system, which is linked to the regulation of neuronal migration, neurite outgrowth, and synaptogenesis [13–19]. In adulthood, constitutive expression of uPA in the brain is low, but it can be robustly induced by various types of experimental epileptogenic brain injuries such as status epilepticus (SE), blood–brain barrier damage, and hypoxic–ischemic injury [20–22]. Functional studies in experimental models revealed that a deficiency in *Plau*, a gene encoding uPA, is associated with compromise of both tissue repair and functional outcome after SE and CCI-induced TBI in mice [20–26].

In humans, a single nucleotide polymorphism in the *PLAU* gene (rs2227564) is associated with Alzheimer's disease, which may be comorbid with epilepsy, but there is no evidence to date that this single nucleotide polymorphism is associated with epilepsy itself [27–29]. As in animal models, uPA expression is increased in the brain tissue of patients operated on for drug-refractory epilepsy caused by various genetic or acquired pathologies such as hippocampal sclerosis, focal cortical dysplasia, tuberous sclerosis complex, and gangliogliomas

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[30]. Moreover, a functional analysis by Johansson et al. [31] demonstrated that high plasma uPA levels together with glycocalyx degradation markers in the vascular endothelium are associated with a 3-fold increase in mortality in human TBI.

Based on these different lines of evidence, regulation of uPA expression is involved in network reorganization and functional outcome after brain injury. Here, we tested the hypothesis that *Plau* deficiency results in worsened brain damage and functional outcome, particularly epileptogenesis, after experimental TBI. We induced TBI in wild-type (Wt) and *Plau*-deficient mice using CCI and assessed the development of tissue pathology and epileptogenesis for up to 8 months.

2. Materials and methods

The study design, which included two experiments, is summarized in Fig. 1.

2.1. Animals

Adult male mice (12–14-week-old) lacking the *Plau* gene in a C57BL/6J background (uPA −/−; B6.129S2-*Plau*^{tm1Mlg}/J from The Jackson Laboratory, Bar Harbor, ME, USA; [32]) (n = 36) and Wt mice (n = 32) were used. Mice were backcrossed to the C57BL/6 genotype (The Jackson Laboratory) for at least eight generations. Mouse genotypes were determined by polymerase chain reaction. Breeding was continued as Wt or uPA homozygous lines (maximum of 10 generations before backcrossing).

Mice were housed in individual cages under a controlled environment (temperature 22 ± 1 °C, humidity 50–60%, lights on 0700–1900) with free access to food and water. All animal procedures were approved by the Animal Ethics Committee of the Provincial Government of Southern Finland and carried out in accordance with the guidelines of the European Community Council Directives 86/609/EEC.

2.2. Induction of TBI with CCI

The Wt (n = 17) and uPA (n = 18) mice were subjected to unilateral cortical contusion using the CCI protocol described by Smith et al. [33]. Animals were anesthetized with sodium pentobarbital [60 mg/kg; single intraperitoneal (i.p.) injection] and placed in a stereotaxic frame. The skull was exposed and a craniotomy was performed using a 5-mm diameter trephine over the left parietotemporal cortex between the lambda and bregma (Fig. 2D). The bone was carefully removed without disruption of the underlying dura. Forty-five minutes after pentobarbital injection, TBI was performed with a CCI device (eCCI-6.3, VCU Health System, Department of Radiology, Virginia Commonwealth University) equipped with an electrically driven metallic piston controlled by a linear velocity displacement transducer. Briefly, the mouse was positioned in the stereotaxic frame on the adjustable table, and CCI was delivered using the following stroke (∅ 3 mm flat tip) parameters: depth, 0.5 mm from the dura; velocity, 5 m/s; and dwell time, 100 ms. Sham-injured Wt (n = 19) and uPA (n = 14) animals underwent identical anesthesia and craniotomy procedures but were not exposed to CCI. After the injury, a piece of plastic was placed over the craniotomy and the incision was sutured. Four groups of animals were included in the analysis: (1) Wt-sham (n = 19), (2) Wt-CCI (n = 17), (3) uPA-sham (n = 14), and (4) uPA-CCI (n = 18).

2.3. Video-electroencephalography (EEG) monitoring of spontaneous epileptiform activity

2.3.1. Electrode implantation

Electrode implantation was performed at 23 weeks (Experiment 1) or 31 weeks (Experiment 2) post-TBI (Fig. 1), as described previously by Bolkvadze and Pitkänen [34]. Briefly, the mice were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic frame. A stainless steel screw electrode (1 mm diameter) was placed ipsilaterally just rostrolateral to the midline of the craniotomy (Fig. 2D). Another recording electrode was positioned contralaterally to the region corresponding to the center of the craniotomy. A reference electrode was inserted into the skull above the right frontal cortex, and a ground electrode was inserted into the occipital bone over the cerebellum. All electrodes were connected to a plastic pedestal (MS 363 Plastics One, Inc., Roanoke, VA), which was cemented onto the skull with dental acrylic. The animals were allowed to recover for at least 1 week before starting the recordings. If the headset was lost during the experiment, it was re-implanted (no more than once), and monitoring was continued as scheduled.

2.3.2. Video-EEG

Continuous video-EEG monitoring (24 h/day, 7 days/week) was performed for 2 weeks at 6 (Experiment 1) or 8 (Experiment 2) months post-TBI, as previously described by Bolkvadze and Pitkänen [34]. Briefly, the mice were placed in Plexiglas cages (size 30 × 18 ×21 cm) where they could move freely (one mouse per cage) and connected to the recording system with commutators (SL6C, Plastics One Inc.). The EEG recording was performed using the Nervus EEG recording system connected to a Nervus magnus 32/8 amplifier (Taugagreining, Iceland) and filtered (high-pass filter cutoff, 0.3 Hz; low-pass filter cutoff, 100 Hz). The behavior of the animals was taped using a WV-BP330/GE video camera (Panasonic, Japan) that was positioned in front of the cages and connected to an SVT-N72P time-lapse VCR (Sony, Japan) and a PVM-145E video monitor (Sony, Japan). A wide-angle lens permitted simultaneous videotaping of up to eight animals. Type WFL-II/LED15W infrared light (Videor Technical, GmbH, Germany) was used at night to allow for continuous 24 h/day video-monitoring.

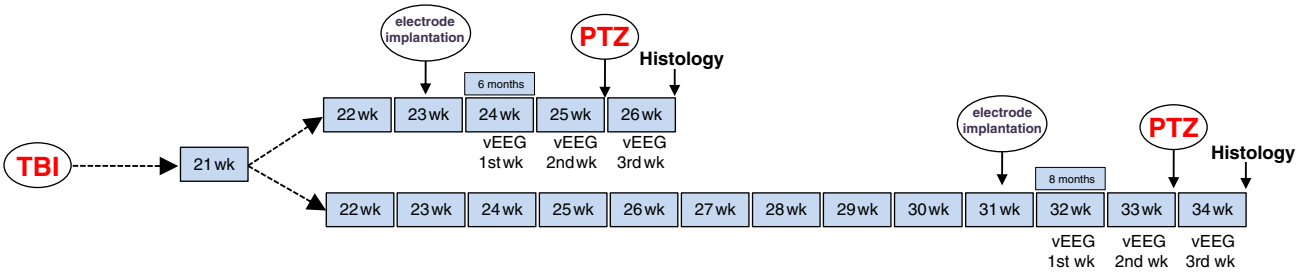

Fig. 1. Study design. Traumatic brain injury (TBI) was induced in Wt and *Plau*-deficient (uPA) mice using controlled cortical impact (CCI). In Experiment 1, 15 injured (10 Wt, 5 uPA) and 17 sham-operated mice (11 Wt, 6 uPA) were followed up for 6 months. In Experiment 2, 20 injured (7 Wt, 13 uPA) and 16 sham-operated mice (8 Wt, 8 uPA) were followed up for 8 months. Cortical electrodes were implanted into the skull at 23 weeks (Experiment 1) or 31 weeks (Experiment 2) post-CCI to monitor the development of epileptiform activity by continuous video-electroencephalographic (vEEG) monitoring. The first 2-week vEEG was started at 6 months (Experiment 1) or at 8 months (Experiment 2) post-CCI. Thereafter, mice were subjected to the pentylenetetrazol (PTZ) test to assess seizure susceptibility, and vEEG monitoring was continued for another 1 week. At the end of the monitoring period, mice were perfused for histology.