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Review

Inflammation and reactive oxygen species in status epilepticus: Biomarkers and implications for therapy

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ABSTRACT

Preclinical studies in immature and adult rodents and clinical observations show that neuroinflammation and oxidative stress are rapid onset phenomena occurring in the brain during status epilepticus and persisting thereafter. Notably, both neuroinflammation and oxidative stress contribute to the acute and long-term sequelae of status epilepticus thus representing potential druggable targets. Antiinflammatory drugs that interfere with the IL-1β pathway, such as anakinra, can control benzodiazepine-refractory status epilepticus in animals, and there is recent proof-of-concept evidence for therapeutic effects in children with Febrile infection related epilepsy syndrome (FIRES). Inhibitors of monoacylglycerol lipase and P2X7 receptor antagonists are also promising antiinflammatory drug candidates for rapidly aborting de novo status epilepticus and provide neuroprotection. Antiinflammatory and antioxidant drugs administered to rodents during status epilepticus and transiently thereafter, prevent long-term sequelae such as cognitive deficits and seizure progression in animals developing epilepsy. Some drugs are already in medical use and are well-tolerated, therefore, they may be considered for treating status epilepticus and its neurological consequences. Finally, markers of neuroinflammation and oxidative stress are measureable in peripheral blood and by neuroimaging, which offers an opportunity for developing prognostic and predictive mechanistic biomarkers in people exposed to status epilepticus.

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1. Introduction

Status epilepticus (SE) is a serious and life-threatening condition with up to 40% of patients developing drug-refractory SE [1]. Refractory SE is associated with a worse prognosis in terms of mortality and morbidity. In particular, new onset (de novo) refractory SE in both children and adults may lead to deterioration of intellectual and cognitive functions. Chronic epilepsy occurs in up to 50% of adults after de novo refractory SE [2]. One predictor of poor outcome is the duration of SE, therefore, therapies are targeted to rapidly terminate seizures in order to prevent the pathologic sequelae.

Models of de novo SE are induced in immature or adult rodents by chemoconvulsive drugs or electrical stimulation of limbic areas, or by exposing neonatal/infantile rodents to hyperthermia to mimic febrile SE. These models are instrumental to describe the cellular and molecular changes occurring in the brain during SE. Some of these brain modifications may be causative for seizure recurrence and refractoriness to treatment and possibly implicated in epilepsy development, mortality, and comorbidities [3]. The availability of human brain tissue from patients who died in SE represents a highly valuable material for validation of animal findings [4].

The experimental studies may allow identifying potential targets for developing drugs against specific mechanisms underlying the pathophysiology of SE. Moreover, based on the identification of such mechanisms, prognostic and predictive biomarkers (*FDA-NIH Joint Leadership Council, 2015: Glossary for definitions*) may be developed in animal models for clinical translation.

This article will focus on neuroinflammation and oxidative stress, two phenomena described in various SE models, also occurring in humans exposed to SE [4–8], which have been both implicated in the acute and chronic sequelae of SE [4,8–14] (Fig. 1). First, we discuss the

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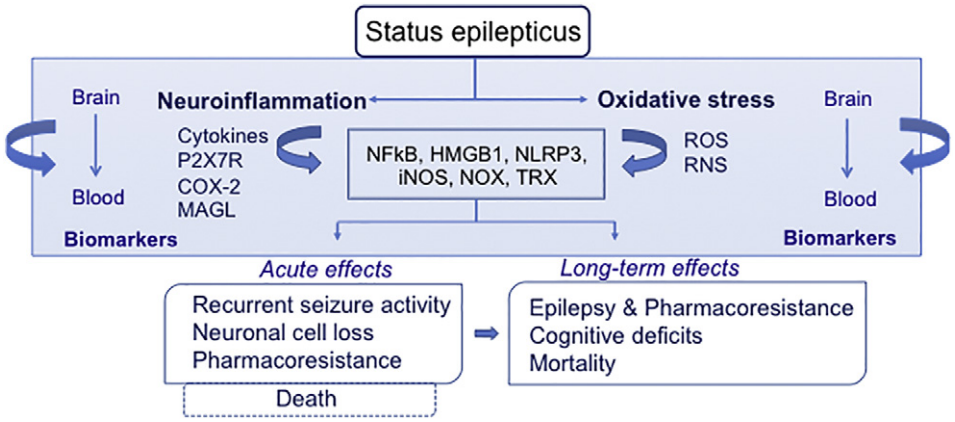

Fig. 1. Schematic representation of the chain of events triggered by SE. SE triggers both neuroinflammation (e.g., cytokines, P2X7R, COX-2, MAGL) and oxidative stress (ROS and RNS) in seizure-generating brain areas. The respective mediators (NfkB, HMGB1, NLRP3, iNOS, NOX, TRX) reciprocally reinforce both phenomena contributing to SE acute and chronic sequelae. Acute effects include the recurrence of seizure activity, neuronal cell loss and drug-refractoriness (e.g., to benzodiazepines), and death. Some of the acute effects may pave the way (arrow) to long-term effects. Long-term effects may also directly result from the cooperation of neuroinflammatory (e.g., reduced seizure threshold, poststranslational changes in ion channels, transcriptional activation of genes) and oxidative stress (e.g., protein, lipid and DNA damage, gene transcription) mechanisms. These mechanisms represent potential targets for pharmacological interventions apt to prevent acute or chronic sequelae, or both. Moreover, since inflammatory and oxidative stress *markers* mirroring the brain-born phenomena can be measured in blood and by neuroimaging, they represent potential mechanistic biomarkers with prognostic value and pharmacodynamic markers of drug's effects. TRX, thioredoxin; see abbreviations in main text.

data pertinent to the activation of these phenomena in the brain of animal models of SE and in patients; second, we describe the impact of targeted treatments on the acute and chronic SE sequelae; lastly, we report evidence for a potential biomarker role of inflammation and oxidative stress-related molecules for predicting SE outcomes and the response to treatments.

2. Neuroinflammation

2.1. Cytokines and COX-2

Animal models of de novo SE share a common activation of the neuroinflammatory response in brain areas involved in onset and spread of seizure activity. This response is induced rapidly during both febrile (immature animals) and afebrile (immature and adult animals) convulsive and nonconvulsive SE [15–21]. The inflammatory cascade involves the biosynthesis and release of various inflammatory mediators most prominently generated by activated glial cells. This is associated with induction of their cognate receptors and intracellular signaling in target cells. The released inflammatory molecules mediate pathophysiological interactions among neurons, reactive astrocytes, activated microglia, vascular endothelial cells and, eventually, neutrophils and monocytes infiltrating the brain from the blood [22]. The complexity and dynamics of the inflammatory response during SE are a matter of intense investigations: some cytokines and the prostanoid cascade have been studied in more detail [23,24]. In particular, as soon as 30 min–1 h of SE onset in rodents, IL-1β, TNF-α, and IL-6 levels are increased in forebrain glial cells together with a rise in neuronal COX-2 expression, and morphological evidence of activation of microglia, reactive gliosis, and infiltrating monocytes [23–26]. This induction outlasts the duration of SE for many days before declining to basal levels, then, a second surge occurs after the onset of epilepsy in animals experiencing recurrent seizures. Neuroinflammation in the immature brain generally displays a more rapid offset time that in the adult brain [15–18]. Glia activation and proinflammatory cytokines expression both show an age-dependent pattern of induction and precede neuronal cell loss in the hippocampus, thus suggesting that they may contribute to SE-induced neuronal damage [15].

Moreover, in the adult rodent brain, some inflammatory signalings significantly contribute to the acute SE and the long-term sequelae (*see later*); in immature rodents, IL-1β plays a permissive role in febrile SE since the threshold to seizures depends on the hippocampal levels of this cytokine [27]. Prolonged hyperthermic seizures in immature rats may lead to adult temporal lobe epilepsy (TLE) [28] and this occurs in animals with elevated IL-1β in the hippocampus [18].

The induction of inflammatory mediators during SE precedes and is causally associated with the development of neuronal cell loss, mortality, cognitive deficits, and the development of epilepsy. This was shown by pharmacological or genetic interference with specific receptor-activated inflammatory pathways, such as the IL-1 receptor type 1 (IL-1R1), activated by IL-1β, Toll-like receptor 4 (TLR4), and receptor for Advanced Glycation End product (RAGE), both receptors activated by High Mobility Group Box 1 (HMGB1), and EP2 receptors activated by prostaglandin E2 (PGE2) [23,29–33]. Moreover, the same pathways are involved in the breach of the blood-brain barrier (BBB) and the consequent extravasation of serum albumin into the hippocampus and cortex, normally observed within hours to days after SE [34–37].

These studies were done in naive animals exposed to SE without prior brain pathology, thus providing a demonstration that prolonged seizure activity per se is sufficient to activate neuroinflammation and ignite the pathologic outcomes. However, when SE is induced in a compromised brain, like rodents with induced heterotopia or exposed to a systemic inflammatory challenge mimicking infection, then a more severe acute course of SE and worsening of the long-term neuropathological consequences are observed [38–43]. This set of evidence highlights that specific inflammatory molecules and related pathways may be putative targets for novel therapeutic interventions to abort SE and prevent its consequences.

2.2. Antiinflammatory treatments

2.2.1. Acute sequelae

Pharmacological blockade of IL-1β/IL-1R1, HMGB1/TLR4, and COX-2/PGEs pathways has allowed demonstrating the contribution of these pathways to acute discrete seizures provoked by various convulsive stimuli or chronic seizures spontaneously recurring in rodents [33,44,45]. In general, interference with these inflammatory pathways neither delay SE onset nor reduced its duration or severity [29,32,46,47]. There are, however, a few exceptions: 1. Anakinra, the human recombinant endogenous competitive antagonist of IL-1β/IL-1R1 axis, is a drug (Kineret ®) in medical use for autoinflammatory and autoimmune disorders. Repetitive intracerebroventricular injections of anakinra after electrically-induced SE in rats provoked a decrease in spike frequency and reduced seizure generalization during convulsive SE [19]. Systemic administration of

anakinra before pilocarpine reduced the incidence of SE in rats. Moreover, in rats still developing SE, its onset was delayed, and both SE duration and severity were reduced [37]. 2. A monoclonal antibody directed against HMGB1 when administered to mice before pilocarpine delayed the onset and reduced the incidence of generalized seizures during convulsive SE; however, the treatment was ineffective when applied at the time of SE induction [48]. 3. To date, the antiinflammatory treatments most effective on the acute course of SE are the P2X7 receptor antagonists and the monoacylglycerol-lipase (MAGL) inhibitors which drastically reduce SE duration after 1-hour delay administration [49–51]. In particular, antagonism of P2X7 receptors expressed by microglia during SE inhibits the NLRP3 inflammasome activation, while MAGL inhibition decreases the brain levels of arachidonic acid. Both treatments reduced the ictogenic cytokine IL-1β in the hippocampus. These acute effects on SE were associated with neuroprotection of forebrain neurons, decreased glia activation, and reduced neuroinflammation [52–54]. Moreover, MAGL inhibition also rescued cognitive deficit related to SE [51].

Notably, anakinra or P2X7 receptor antagonists when coadministered with diazepam abrogated within 30 min benzodiazepine-resistant SE, and MAGL inhibition did so by 3 h [50,51,53,54]. The MAGL inhibitor CPD-4645 fully prevented SE development in mice under a ketogenic diet [51]. This evidence suggests that IL-1R1 and P2X7 receptors and MAGL are potential targets for adjunctive control of refractory SE.

2.2.2. Long-term sequelae

Antiinflammatory treatments, such as IL-1R1, EP2, or TLR4 antagonists, administered during and after SE mediate significant neuroprotection in the forebrain, reduce late mortality and accelerate functional recovery also improving cognitive deficits [29,32,46,47]. These

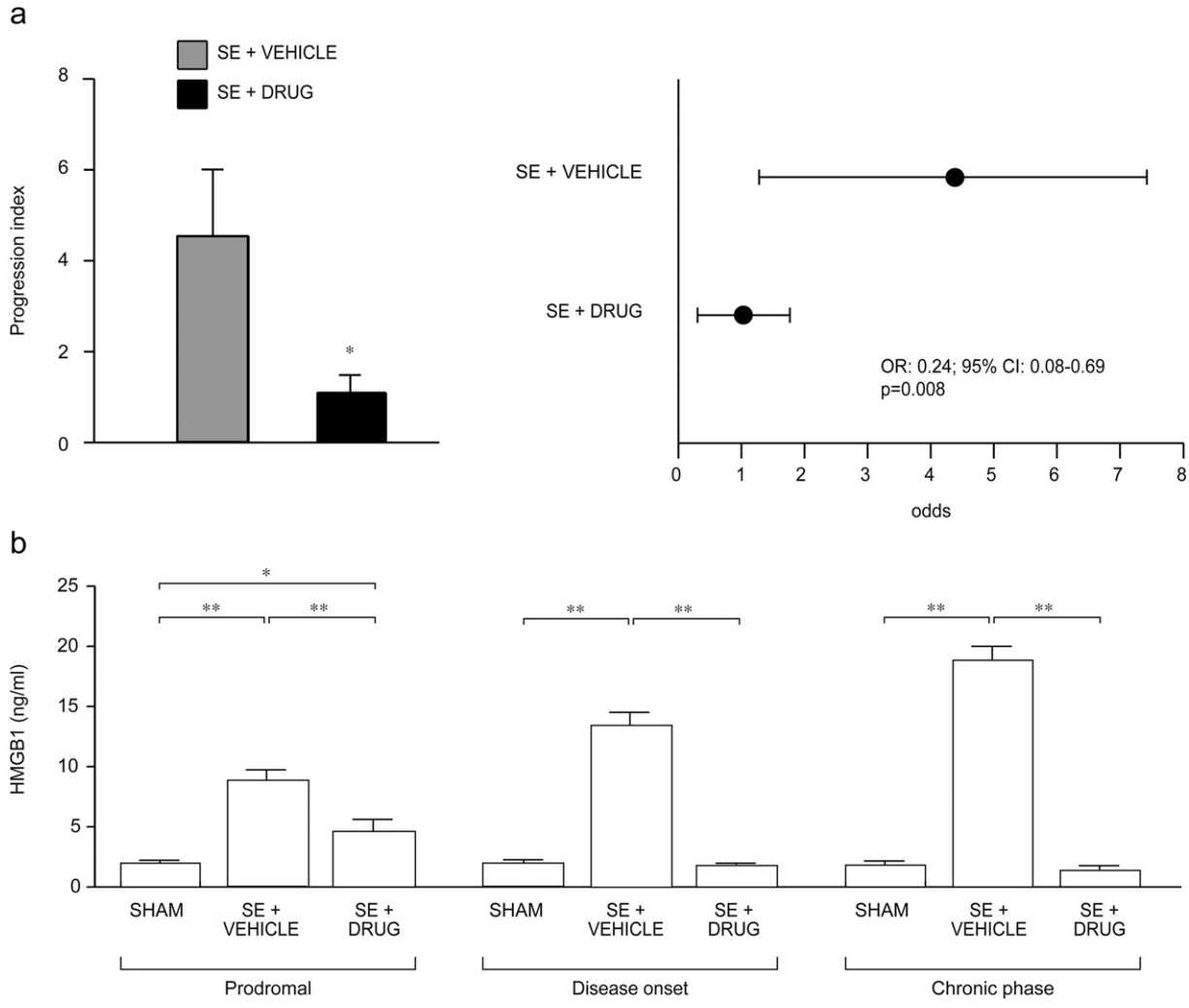

Fig. 2. Antiinflammatory drugs administered during epileptogenesis prevent disease progression in electrical SE-exposed rats. *Panel a:* Progression index (the ratio between the number of seizures at 4.5 months and at 2.5-months post-SE) in vehicle- and drug-treated rats. A value = 1 defines lack of progression. Treatment (SE + DRUG) protocol: 1 h after SE onset rats were injected with an intravenous (i.v.) bolus of anakinra (10 mg/200 μl sterile saline; Biovitrum AB, Stockholm, Sweden) which is the human recombinant form of the IL-1R1 antagonist and with BoxA (100 μg/200 μl sterile saline, HMGBiotech, Milano, Italy), a competitive HMGB1 antagonist [59]. A second i.v. injection of both drugs was done 5 h later, then once daily for a total of seven days, then treatment was stopped. The treatment schedule was designed based on previous evidence of drug's therapeutic effects on cell loss and seizures in animal models [46,59]. In this SE model, around 80% of rats show an average 5-fold increase in the frequency of spontaneous seizures over 4.5 months from the onset of SE, denoting disease progression. Sham-operated animals were implanted with electrodes but not electrically stimulated and were used as vehicle-injected controls for blood HMGB1 analysis (Panel b). Electroencephalographic (EEG) seizures were associated with generalized motor seizures. Video-EEG monitoring was done during SE, and at 2 and 4 months post-SE for 2 weeks (24/7) to determine spontaneous seizure frequency (see detailed Methods in Ref [4]). Data are the mean ± SEM (n = 9 rats each group); *p < 0.05 vs vehicle by Mann–Whitney test. Odds ratio (OR: 0.24; p = 0.008) indicates that drug-treated rats evaluated at 2.5 months have a 76% reduced risk of developing seizures at 4.5 months vs vehicle-treated rats. Panel b: Longitudinal analysis of total HMGB1 levels in blood serum at representative time points of disease development in the same rats described in panel a. Data are the mean ± SEM (n = 9 rats each group). *p < 0.05, **p < 0.01 by one-way Analysis of Variance (ANOVA).

therapeutic effects were observed also in the absence of drug's acute effects on SE severity or duration.

Moreover, targeting the IL-1R1/TLR4 axis [30,31], P2X7 receptors [55,56] or COX-2/PGE2 [57,58] inhibits epileptogenesis in SE-exposed animals, resulting in 50–90% reduction of spontaneous seizure frequency- or their severity- and IL-1R1/TLR4 blockade prevents seizure progression [30,31] (Fig. 2a).

The mechanisms by which inflammatory mediators modulate SE and affect epileptogenesis include the following: 1. neuroinflammation contributes to lower seizure threshold by inducing rapid posttranslational changes in neuronal ion channels and transporters, and by alterations in neurotransmitter release and uptake mechanisms [60–62]; 2. neuroinflammation affects the transcriptional activation of genes governing cellular, synaptic and molecular plasticity, promotes aberrant neurogenesis and BBB dysfunction [22,29,33,58,62].

3. Oxidative stress

3.1. Induction of reactive oxygen and nitrogen species (ROS and RNS)

This phenomenon consists in excessive generation of ROS and RNS due to mitochondrial dysfunction and increased activity of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, xanthine oxidase, and inducible nitric oxide synthase (iNOS). Oxidative stress is rapidly triggered by acute brain injuries, including SE [4,9–12], it is linked to neuroinflammation and the two processes reinforce each other [63]. We recently put forward the novel hypothesis, substantiated by experimental evidence, that oxidative stress promotes the nucleus-to-cytoplasm translocation, prodromal to extracellular release, of the inflammatory protein HMGB1 from neurons and glia (Fig. 3). This process leads to the HMGB1-TLR4 signaling activation in neurons resulting in hyperexcitability and excitotoxicity [64]. As for neuroinflammation, oxidative stress is rapidly induced by SE and persists in seizure generating brain areas during epileptogenesis [9,65]. Markers of oxidative stress (e.g., the cystine/glutamate antiporter system; iNOS, the transcriptional factor Nrf2 inducing antioxidant enzymes) are described in both neurons and astrocytes in animals exposed to SE and in patients who died in SE [4]. Markers of oxidative stress have also been measured in cerebrospinal fluid (CSF) of children with SE [7] and in blood of adult patients with SE [6].

3.2. Antioxidant treatments

There is evidence that by inhibiting ROS or RNS generation, neuronal damage in seizures and epilepsy is improved [66–69]. Although none of the antioxidant drugs so far tested in animal models affects the acute phase of SE, these treatments were shown to significantly alleviate the comorbidities and improve mortality, offering also neuroprotection. In particular, intervention with a catalytic antioxidant porphyrin in conjunction with diazepam 1.0–1.5 h after animals are exposed to pilocarpine significantly reduced both SE-related mortality during 60 h and hippocampal cell loss, and these effects were associated with reduction of oxidative stress and neuroinflammation in the forebrain [11]. Recent studies used either brain penetrant small molecules (i.e., RTA 408) able to uncouple the cytoplasmatic chaperon Keap1 from Nrf2 thus promoting Nrf2 nuclear translocation and transcription of antioxidant enzymes [14], or a combination of N-acetyl-cysteine (NAC), the precursor of glutathione (GSH), and sulforaphane (SFN) with acts as a Keap1-Nrf2 uncoupling molecule [4]. Treatments were initiated 1- to 2-h post-SE and continued for 3 days (RTA 408) or 14 days (NAC + SFN) in rats exposed to kainic acid- or electrical stimulation-induced SE, respectively. A common therapeutic outcome, also shared by antiinflammatory treatments, was full prevention of seizure frequency progression during epilepsy development. Moreover, the drugs reduced cell loss and the cognitive deficit occurring as a consequence of SE.

Notably, the administration of a superoxide dismutase mimetic antioxidant after the onset of epileptogenesis in rats previously sensitized by a stressful event (social defeat) and then exposed to SE, decreased spontaneous seizure frequency in epileptic animals and prevented depression-like symptoms and cognitive deficits [70].

In summary, the remarkable efficacy attained using antioxidant or antiinflammatory drugs on acute and long-term SE sequelae, even when the drugs are given to animals hours after the epileptogenic insult, markedly increase the translational value of these interventions. Moreover, RTA 408 is already undergoing clinical trials in other conditions [14], and both NAC and SFN are in medical use and showed therapeutic effects in neurological conditions at doses comparable to those used in SE-exposed animals [4].

4. Neuroinflammation and oxidative stress as potential biomarkers

Clinical translation of antiinflammatory or antioxidant treatments in refractory SE would be facilitated by the availability of biomarkers reflecting the occurrence of these processes in the brain. This would allow stratifying patients who might best benefit from the treatment and also provide a pharmacodynamic measure of drug's efficacy.

Moreover, the identification of biomarkers predicting worse outcomes in patients with SE, including comorbidity and epilepsy development or mortality, would be extremely helpful for preventative interventions in patients at high risk.

4.1. Inflammatory markers

Although inflammatory cytokines are often unstable in blood, there is clinical proof-of-concept validation for some inflammatory molecules, or their combination, with diagnostic, prognostic, or predictive value in epilepsy which might be applied for SE.

For example, pharmacoresistance in chronic epilepsy could be identified by circulating levels of soluble ICAM5 (sICAM5), an antiinflammatory protein only expressed in the CNS. Blood levels were significantly reduced in patients with drug-resistant focal epilepsy compared to healthy controls. In particular, the ratio sICAM5 to CCL17 was shown to have the best discrimination power [71].

In animal models, blood levels of HMGB1 are increased after SE in concomitance with its brain changes (nuclear-to-cytoplasmatic translocation) which are predictive of its extracellular release (Fig. 4a,b) [4]. This evidence suggests that changes in blood may reflect a brain-to-blood leakage. Notably, after SE, the blood HMGB1 levels are elevated and persist during the disease course but only in animals developing epilepsy (Fig. 5). Similarly, blood HMGB1 and IL-1β levels were increased in patients with severe neurotrauma compared to healthy controls and were predictive of mortality and unfavorable neurological outcomes [73]. In particular elevated CSF/serum IL-1β ratio predicted posttraumatic epilepsy in patients [74].

In the FEBSTAT study, significant increases in blood IL-1, IL-1RA, IL-6, and IL-8 were measured within 72 h in children with febrile SE compared with age-matched children with fever but no seizures nor CNS infection. Notably, a strong association between low IL-1RA/IL-6 ratio and T2 signal abnormality in the hippocampus was identified which is a potential predictor of the development of mesial TLE in these children [75]. IL-1β and HMGB1 were also increased in blood within 30 min in children with prolonged febrile seizures as compared with children with no febrile seizures [76]. Similarly, in an immature rat model of febrile SE, MRI T2* signal decreased in basolateral amygdala 6-h post-SE and positively correlated with IL-1R1 and COX-2 expression in the hippocampus and amygdala and increased HMGB1 cytoplasmatic staining in neurons, an index of the releasable pool. These changes were predictive of epileptogenesis in the animals [21].

HMGB1 blood levels during epileptogenesis are reduced by antiinflammatory treatments that block spontaneous seizures

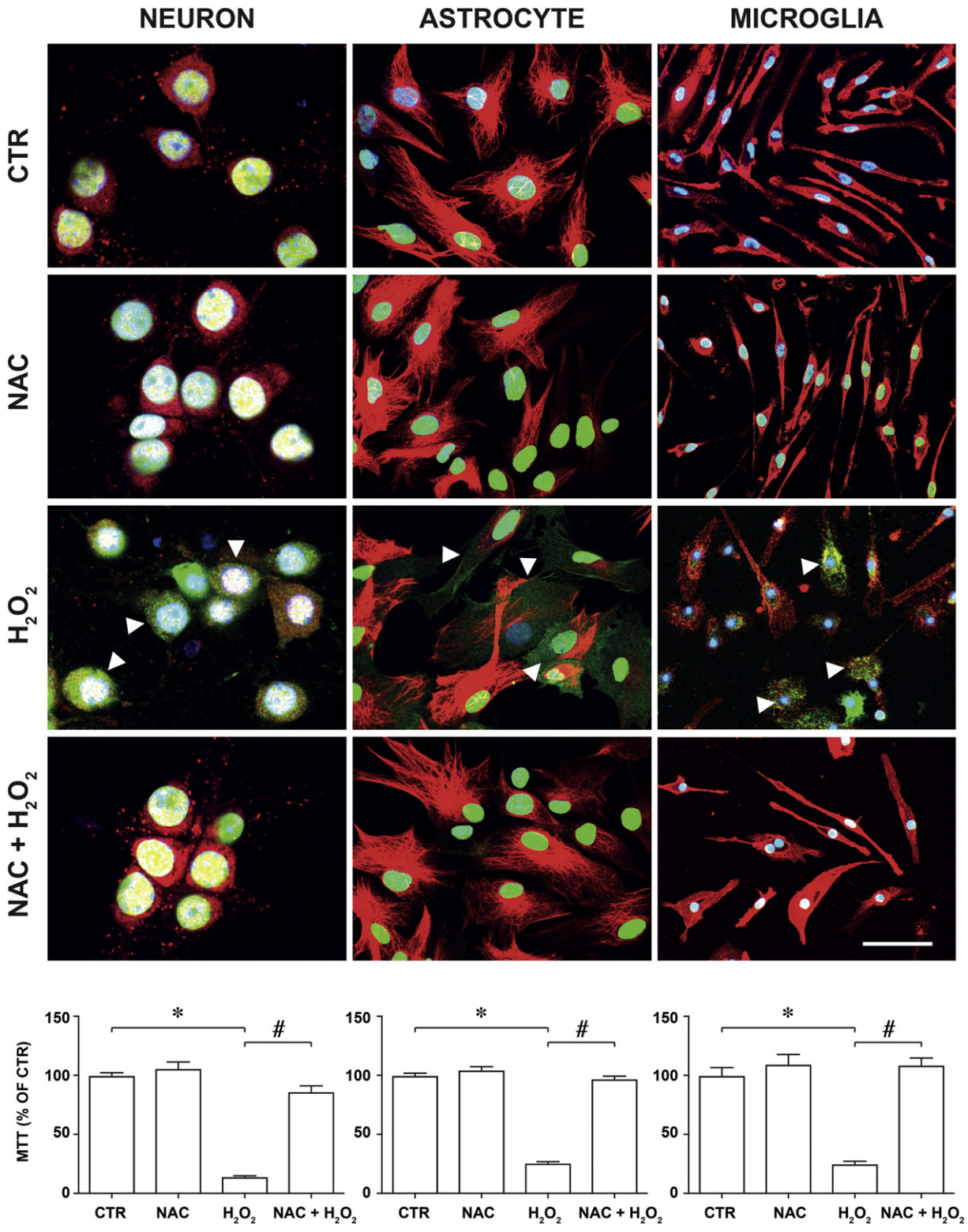

Fig. 3. Effect of oxidative stress on HMGB1 nuclear-to-cytoplasm translocation and cell damage in hippocampal primary cultures: rescue by NAC. Panels represent immunofluorescence colocalization of HMGB1 signal (green) with NeuN (neuron, red), glial fibrillary acidic protein (GFAP) (astrocyte, red), CD11B (microglia, red), and Hoecsht-positive nuclei (blue) in hippocampal primary cultures of neurons, astrocytes, and microglia. Cultures were treated with vehicle (control, CTR) or NAC (10 mM) or hydrogen peroxide (H_2O_2, 1 mM) for 1 h in neurons and astrocytes and for 3 h in microglia) or preincubated 30 min with 10 mM NAC followed by coincubation with 1 mM H_2O_2 (NAC + H_2O_2). HMGB1 immunoreactivity is localized in cell nuclei in CTR (physiological conditions) and in NAC-treated cultures while it is increased in cytoplasm of neurons, astrocytes, and microglia (white arrowheads) after H_2O_2 incubation, denoting its extracellular release (*see text for details*). Antioxidant treatment prevented HMGB1 translocation (NAC + H_2O_2) in all cell types. Scale bars: 50 μm (astrocyte and microglia); 25 μm (neuron). Bargrams below each column depict cell damage as assessed by MTT assay in neurons, astrocyte and microglia. Antioxidant treatment rescued neuronal, astrocytic, and microglia cell death induced by oxidative stress. Data are the mean ± SEM (n = 5–24 values from 2 to 3 independent experiments).*$p < 0.01$ vs CTR, #$p < 0.01$ vs H_2O_2 by two-way ANOVA followed by Sidak's test.

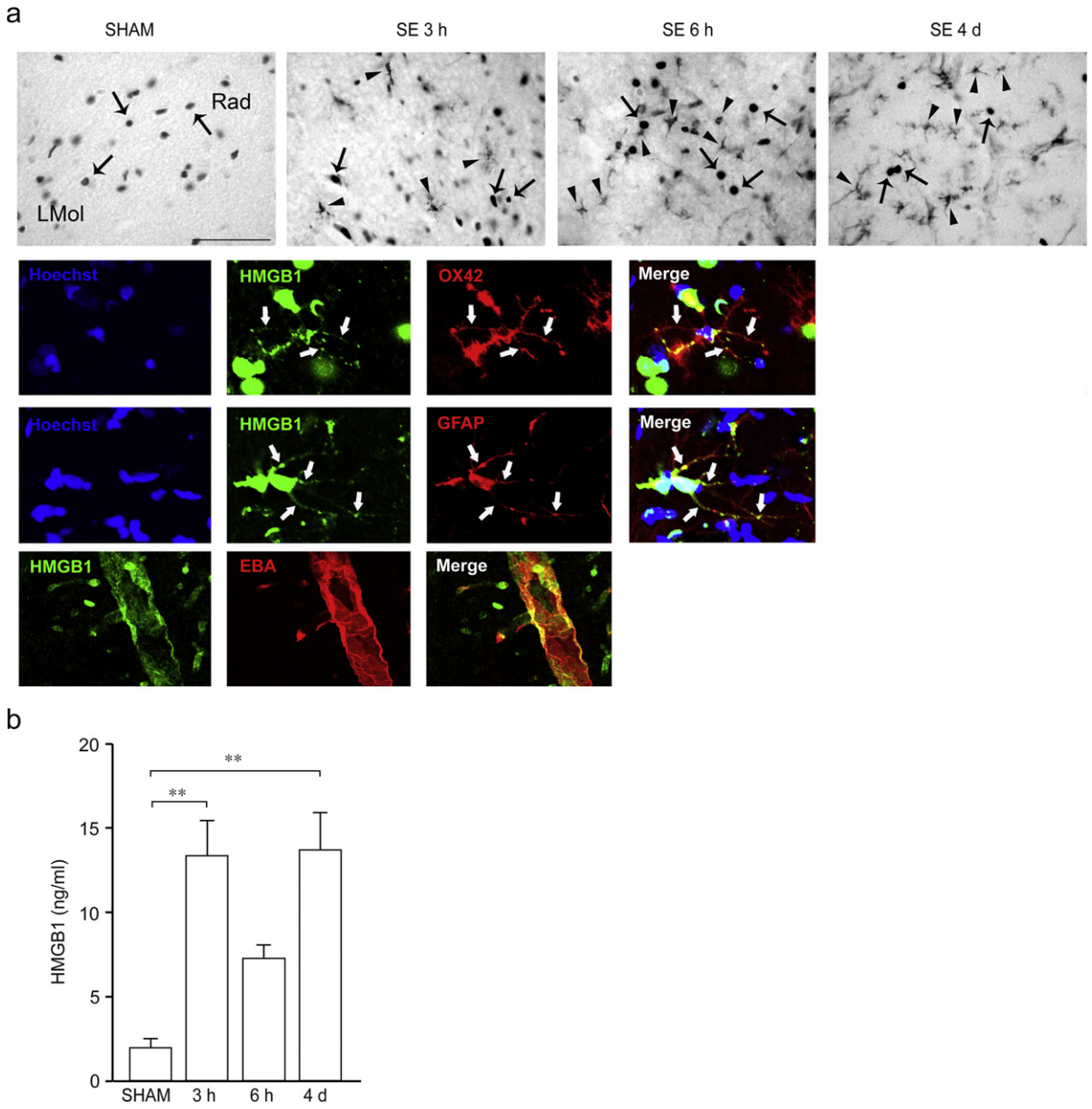

Fig. 4. Brain expression and blood levels of HMGB1 in adult rats exposed to electrical SE. *Panel a*: Representative photomicrographs of hippocampi from control rats (SHAM) or rats at 3 h, 6 h, and 4 days post-SE induced by electrical stimulation of the ventral hippocampus (SE; n = 5 each group; see methods in Ref [4]). *First row* shows HMGB1 immunoreactivity in cell nuclei (arrows) or in cytoplasm of glial cells (arrowheads). *Immunofluorescence panels* show localization of HMGB1 signal (green) in OX-42-positive microglia (red), GFAP-positive astrocytes (red), and EBA-positive endothelial cells (red); colocalization signal is depicted in merge panels. White arrows depict extranuclear staining. Hoechst-positive nuclei are shown in blue. Immunohistochemical methods are described in detail in Ref [4, 59]. Rad: stratum radiatum; Lmol: stratum lacunosum moleculare. Scale bar: first row 25 μm; immunofluorescence panels 20 μm. *Panel b*: HMGB1 was measured in corresponding blood of rats by a commercial ELISA kit (Shino-test Corp, Sagamihara, Japan) according to the manufacturer's guidelines [4]. Data are the mean ± SEM (n = 5 each group). **$p < 0.01$ by Kruskal-Wallis test.

progression during the disease course (Fig. 2b), thus supporting their potential predictive value for therapeutic outcomes.

4.2. Oxidative stress markers

Increased markers of oxidative stress were measured in blood of adult patients with SE compared to a control population. In particular, patients with SE displayed lower levels of superoxyde dismutase, catalase, GSH, and total antioxidant capacity, a measure of the antioxidant status [6]. No correlation was found with death or disability or with drug-resistance. However, the samples were collected only at the time of hospitalization, and a more extended post-SE time course is likely needed to determine potential correlations with the outcomes. Oxidative stress markers were increased in neocortical tissue from drug-resistant patients with epilepsy [77], and in blood of drug-resistant patients with complex partial seizures [78], or in temporal lobe epilepsy (TLE) while they were reduced in patients with TLE after surgically resection of the epileptic foci [79]. Notably, the frequency of seizures was inversely correlated with the levels of the antioxidant vitamin C and positively correlated with the levels of the oxidative damage marker 3-nitrotyrosine [78]. In patients with idiopatic generalized epilepsy, the increased markers of oxidative stress were not affected by anticonvulsant drugs [80]. Finally, the CSF levels of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, were increased in children with SE compared with healthy children [7].

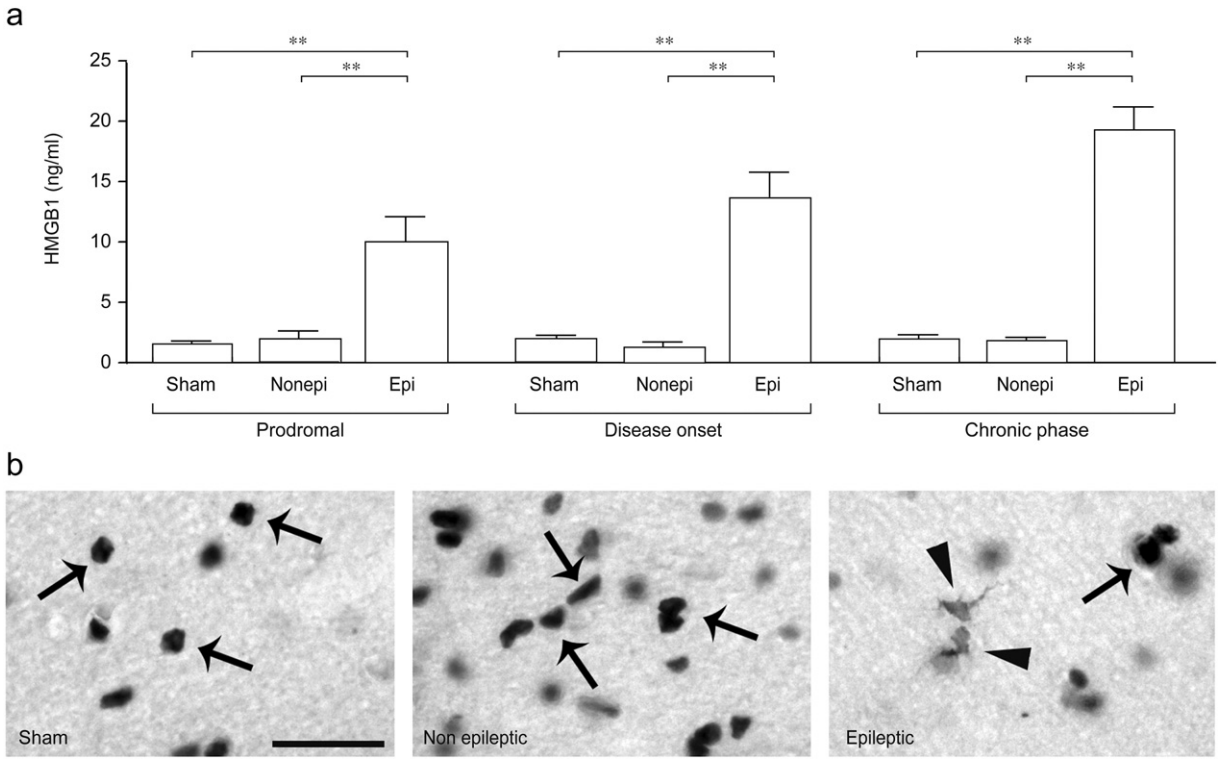

Fig. 5. Early prediction of epilepsy development by blood HMGB1 level in lithium + pilocarpine SE rats. *Panel a:* Longitudinal analysis of total HMGB1 level in blood at representative time points of disease development in 21 day-old rats exposed to SE evoked by lithium + pilocarpine. In this model, around 70% of rats develop epilepsy after an average time of 70 days after being exposed to SE of similar severity and duration (26.3 ± 0.5 h, n = 17). For details about this model see Ref [72]. Controls were age-matched rats injected with lithium chloride and with an equivalent volume of phosphate-buffered saline instead of pilocarpine (n = 7). Blood was drawn at 23 days (*epileptogenic phase* prodromal to epilepsy onset), 73 days (encompassing the *time of disease onset in 70% of rats*) and 7.5 months (chronic epilepsy). HMGB1 was measured in corresponding blood of rats by a commercial ELISA kit (Shino-test Corp, Sagamihara, Japan) according to the manufacturer's guidelines [4]. Rats (n = 17) were retrospectively divided between those developing (Epi, n = 12) or not developing (non Epi, n = 5) spontaneous seizures at 7.5 month (during 2 weeks 24/7 video-EEG recordings). In the Epi group, 5 out of 12 rats were randomly chosen to measure blood HMGB1. Data are the mean ± SEM; n = 7 sham; n = 5 epileptic (Epi); n = 5 non epileptic (non Epi) rats. **$p < 0.01$ by Kruskal-Wallis test. *Panel b:* Representative immunohistochemical pictures of CA1 stratum radiatum depicting HMGB1 staining (for methods see Ref [4, 59]) in control (SHAM, n = 7) and in SE rats with (Epi, n = 5 out of 12) or without (Non epi, n = 5) spontaneous seizures. Brains were harvested 7.5 months post-SE in the same rats described in *panel a*. Arrows: nuclear staining of HMGB1 in vehicle and nonepileptic rats; arrowheads: cytoplasmic HMGB1 staining in glia in epileptic rats. Scale bar: 15 μm.

Overall, the data indicate that peripheral markers of oxidative stress are increased in people with epilepsy [81] and after SE. These changes may reflect oxidative stress (and neuroinflammation) in the brain and should be further explored as potential prognostic biomarkers.

We recently reported that the GSSG/GSH ratio (an index of oxidative stress) increases in the brain and blood during epileptogenesis in rats exposed to SE [4]. Notably, this ratio was reversed to control value by antioxidant treatments which also prevent the raise in HMGB1 blood levels [4]. Since these drugs showed therapeutic effects on post-SE pathologic sequelae [4], circulating oxidative stress markers, and HMGB1, may represent a pharmacodynamic measure of neuroprotective and antiepileptogenic drug's effects in patients exposed to SE.

4.3. Molecular neuroimaging

Molecular imaging studies using magnetic resonance imaging (MRI), proton magnetic resonance spectroscopy (^{1}H-MRS), and positron emission tomography (PET) have provided tools for monitoring neuroinflammation and oxidative stress in animal models and in human epilepsy. Compatible with the clinical condition, some of these approaches may be considered also for patients with SE, for example to predict outcomes.

4.3.1. MRS

Astrocyte activation (involved in neuroinflammation and oxidative stress) can be monitored by measuring the brain levels of myo-Inositol (mIns) by ^{1}H-MRS after SE, which provides a prognostic biomarker for epilepsy development in animal models as well as an index inversely correlated with neuronal cell loss [72,82]. In the same study, GSH levels in the hippocampus are inversely correlated with frequency of seizures [82]. Magnetic resonance spectroscopy has been used to detect astroglia activation in human epileptic foci [83] and is a predictor of neurological dysfunctions after traumatic brain injury [84]. Electron paramagnetic resonance spectroscopy has been also considered to detect free radicals in vivo although the sensitivity of this method still needs improvements to be suitable for human studies [85].

4.3.2. MRI

BBB dysfunction is an indirect marker of neuroinflammation and a pathologic hallmark of SE in animals and patients [22,24,34]. Contrast-enhanced MRI (CE-T1 weighted) and T2 weighted signal protocols have been developed for monitoring BBB dysfunction a few days post-SE showing that MRI signal modifications in the piriform cortex network are sensitive and specific prognostic factors for development of epilepsy [86].

4.3.3. PET

Imaging studies monitored the expression of translocator protein (TSPO) in microglia/macrophages using various radioligands, providing a measure of cell activation. Increased uptake of radioligand was detected in the epileptic focus following a recent seizure and in the interictal phase, and also in the areas of seizure generalization [83]. This signal likely reflects microglia expressing an inflammatory phenotype [8,13]. TSPO signal measured 2–4 weeks after SE in rats was predictive of seizure burden and comorbidities in the epileptic animals [87].

PET tracers have also been developed to measure regional redox state in the brain, although these studies are not as yet fully validated for the human brain [88].

5. Conclusions

Preclinical studies in animal models and clinical observations show that both neuroinflammation and oxidative stress rapidly occur during SE and persist thereafter (Fig. 1). Pharmacological interventions in rodents exposed to SE show that antiinflammatory drugs interfering with the IL-1β pathway such as anakinra, or blocking P2X7 receptor or the MAGL enzyme, may help to control SE by reducing its duration and severity. Therapeutic effects of anakinra have been recently reported in children with FIRES [89,90]. Both antiinflammatory and antioxidant drugs significantly improve the neurological sequelae and the ensuing epilepsy in animals, also if they do not affect the acute SE.

Notably, some antiinflammatory and antioxidant drugs targeting the pathologic mechanisms ignited by SE are already in medical use [91–93] and have an acceptable safety profile, therefore they should be considered for improving SE and its neurological sequelae. Early interventions are suggested by the rapid onset of these phenomena during SE as indicated in the animal models. Finally, neuroinflammation and oxidative stress may be tracked with peripheral blood markers and molecular neuroimaging (Fig. 1) thus offering an opportunity for developing prognostic and predictive biomarkers in people exposed to SE.

Conflict of interest

There are no conflicts of interest to declare.

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