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Pharmacological blockade of IL-1β/IL-1 receptor type 1 axis during epileptogenesis provides neuroprotection in two rat models of temporal lobe epilepsy

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ABSTRACT

We studied whether pharmacological blockade of the IL-1β-mediated signaling, rapidly activated in forebrain by epileptogenic injuries, affords neuroprotection in two different rat models of status epilepticus (SE). As secondary outcome, we measured treatment's effect on SE-induced epileptogenesis. IL-1β signaling was blocked by systemic administration of two antiinflammatory drugs, namely human recombinant IL-1 receptor antagonist (anakinra), the naturally occurring and clinically used competitive IL-1 receptor type 1 antagonist, and VX-765 a specific non-peptide inhibitor of IL-1β cleavage and release. Antiinflammatory drugs were given 60 min after antiepileptic (AED) drug-controlled SE induced by pilocarpine, or 180 min after unrestrained electrical SE, for 7 days using a protocol yielding therapeutic drug levels in brain. This drug combination significantly decreased both IL-1β expression in astrocytes and cell loss in rat forebrain. Neuroprotection and the antiinflammatory effect were more pronounced in the electrical SE model. Onset of epilepsy, and frequency and duration of seizures 3 months after electrical SE were not significantly modified. Transcriptomic analysis in the hippocampus showed that the combined treatment did not affect the broad inflammatory response induced by SE during epileptogenesis. In particular, the treatment did not prevent the induction of the complement system and Toll-like receptors, both contributing to cell loss and seizure generation.

We conclude that the IL-1β signaling represents an important target for reducing cell loss after SE. The data highlight a new class of clinically tested agents affording neuroprotection after a delayed post-injury intervention. Earlier blockade of this rapid onset inflammatory pathway during SE, or concomitant treatment with antiinflammatory drugs targeting additional components of the broad inflammatory response to SE, or co-treatment with AEDs, is likely to be required for optimizing beneficial outcomes.

Introduction

Increased levels of inflammatory molecules and upregulation of their cognate receptors in glia, neurons and microvessels have been demonstrated in human brain specimens of drug-resistant forms of epilepsy, thus suggesting that proinflammatory pathways are activated in seizure foci (Aronica et al., 2012; Choi et al., 2009; Vezzani et al., 2011a). Induction of the same inflammatory molecules occurs after epileptogenic injuries and during recurrent seizures in epilepsy models (Aronica et al., 2012; Ravizza et al., 2011; Vezzani et al., 2013). Inhibition of experimental seizures and neuroprotection have been attained by pharmacological blockade of specific proinflammatory signalings (Aronica et al., 2012; Friedman and Dingledine, 2011; Kwon et al., 2013; Maroso et al., 2011). In particular, the activation of the IL-1 receptor 1 (IL-1R1)/Toll-like receptor (TLR) signaling in glia and neurons by the endogenous ligands IL-1β and High Mobility Group Box 1 (HMGB1) protein plays a key role in ictogenesis. In fact, treatment with the IL-1 receptor antagonist (Ra), TLR4 blockers, or specific inhibitors of Interleukin Converting Enzyme (ICE), the biosynthetic enzyme producing the releasable form of IL-1β, results in drastic reduction of acute or chronic seizures in various epilepsy models (Akin et al., 2011;

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Auvin et al., 2010a; Marchi et al., 2009; Maroso et al., 2010, 2011; Ravizza et al., 2006, 2008b; Vezzani et al., 1999, 2000, 2002). Supportive evidence is provided by studies in transgenic mice with perturbed IL-1R1/TLR4 signals (Dubé et al., 2005; Maroso et al., 2010; Ravizza et al., 2006; Spulber et al., 2009; Vezzani et al., 2000, 2011b). There is compelling evidence that the activation of the IL-1R1/TLR4 signaling is also involved in excitotoxicity since its pharmacological blockade affords significant neuroprotection following various injuries (Allan et al., 2005; Ross et al., 2007; Vezzani et al., 2011b, 2013). In particular, IL-1β and HMGB1 enhance NMDA-induced hippocampal cell loss by increasing receptor-gated Ca^{2+} influx into neurons (Bernardino et al., 2008; Iori et al., 2013; Maroso et al., 2010; Viviani et al., 2003), a mechanism also involved in their proictogenic effects (Balosso et al., 2008; Maroso et al., 2010). Neuroprotective effects were observed using antagonists of the IL-1β/IL-1R1 axis in organotypic cell cultures exposed to AMPA (Bernardino et al., 2008).

The activation of the IL-1R/TLR signaling is pivotal for initiating the complex brain inflammatory response to various CNS injuries, including status epilepticus (SE) (Bartfai et al., 2007; Clausen et al., 2009; Dinarello, 2011; Ravizza et al., 2008a). This occurs by induction of NF-kB- and AP-1-dependent transcription of a large array of inflammatory genes in target cells (O'Neill and Bowie, 2007; Vezzani et al., 2011b). IL-1R1/TLR4 signaling induction after chemical or electrical SE in rodents is rapid (<2 h) and persists until the onset of spontaneous seizures (De Simoni et al., 2000; Dhote et al., 2007; Librizzi et al., 2012; Marcon et al., 2009; Maroso et al., 2010; Ravizza et al., 2008b; Vezzani et al., 2011b; Voutsinos-Porche et al., 2004).

Beneficial outcomes, such as decreased cell loss and reduced spontaneous seizure frequency/severity, are induced by non-steroidal antiinflammatory drugs blocking pathways downstream IL-1R/TLR, when administered to rodents after SE (reviewed by Gao et al., 2012; Löscher and Brandt, 2010; Pitkanen, 2010; Ravizza et al., 2011). This evidence suggests that the various inflammatory pathways activated during epileptogenesis contribute in concert to the adverse outcomes. Preventing the activation of this inflammatory cascade by blockade of the upstream IL-1R/TLR pathway, therefore, represents a promising strategy to attain improved therapeutic effects.

In this study, we used a novel treatment combination of clinically tested and safe antiinflammatory drugs (Dinarello et al., 2012; Vezzani et al., 2010), namely human recombinant (hr)IL-1Ra (anakinra) and the ICE inhibitor VX-765, in order to block the IL-1β/IL-1R1 axis in two SE rat models. Each drug, individually, mediates neuroprotection in acute injury models (Allan et al., 2005; Ross et al., 2007), displays anti-ictogenic properties (Akin et al., 2011; Maroso et al., 2010, 2011; Ravizza et al., 2006) and inhibits kindling progression (Auvin et al., 2010b; Ravizza et al., 2008b).

Our primary endpoint was to assess whether the combined treatment affords neuroprotection by preventing IL-1β actions during epileptogenesis. As a secondary outcome, we evaluated the effect of treatment on epilepsy development.

Materials and methods

Animals

The experiments were carried out in two distinct laboratories: rats exposed to electrically-induced SE were prepared in Milano (male Sprague-Dawley rats, 275–300 g; Charles-River, Calco, VA, Italy) while rats exposed to lithium/pilocarpine were prepared in Hannover (female Sprague-Dawley rats, 250–275 g; Harlan, Horst, The Netherlands). Rats were housed at constant temperature (23 ± 1 °C) and relative humidity (60 ± 5%) with free access to food and water and a fixed 12 h light/dark cycle. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L.n.116, G.U., Suppl. 40, February 18, 1992; German Tierschutzgesetz, Dec. 9, 2010) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Experimental groups

We compared drug combination effects in two experimental models of SE induced by either hippocampal electrical stimulation or systemic administration of the chemoconvulsant pilocarpine. The models were established in either gender by the two experimental groups. The intent was to exclude model specific treatment effects.

Electrically-induced SE

Animals were randomly divided into 3 experimental groups: 1. sham-operated rats implanted with electrodes but not electrically stimulated (*Sham*); 2. rats experiencing SE and treated with vehicle (*Vehicle*); 3. rats experiencing SE, and treated with a combination of anakinra and VX-765 (*Treatment*). Drugs were administered for 5 or 7 consecutive days starting 3 h after the end of electrical stimulation (Supplementary Fig. 1, see later for details).

Experiment 1 measured the human recombinant (hr)IL-1Ra (anakinra) concentration in rat blood and cerebrospinal fluid (CSF) after 7 consecutive days of antiinflammatory treatment (n = 5 rats in each vehicle and treatment group).

Experiment 2 assessed the effect of antiinflammatory treatment on IL-1β induction in the hippocampus and frontoparietal and entorhinal cortices 7 days post-SE by immunohistochemistry (n = 5 sham rats; n = 5 rats in each vehicle and treated group). This time point was chosen since it represents the minimum number of days preceding the onset of spontaneous seizures as indicated by our previous experience with this model (Noé et al., 2008).

Experiment 3 studied the differential expression of inflammatory genes during antiinflammatory treatment by microarray analysis of gene transcription, 5 days post-SE (n = 5 sham rats; n = 5 rats in each vehicle and treated group). This time point is within the temporal window of the plateaux increase of IL-1β after SE in this model, then decline afterwards as assessed by immunohistochemistry (data not shown). Moreover, at this time VX-765 has already reached its maximal antiinflammatory effect (Maroso et al., 2011).

Experiment 4 studied the effect of antiinflammatory treatment (i) on forebrain cell loss, and (ii) on epileptogenesis using as outcome measures the onset of spontaneous seizures (n = 11 rats in each vehicle and treated group) and their frequency and duration, as assessed 3 months post-SE by EEG analysis. Six out of 11 chronic epileptic rats in vehicle or treatment group were EEG recorded at 3 months, because of the loss of the EEG implant before analysis in some rats. Neuropathology and glia activation were assessed by quantitative immunohistochemistry in brain sections of epileptic rats at the end of the EEG evaluation. Sham rats (n = 5) were used as controls for immunohistochemical analysis.

Pilocarpine-induced SE

In *Experiment 5*, rats were divided into 3 groups: 1. rats implanted with electrodes receiving vehicle (*Sham*, n = 4); 2. rats implanted with electrodes and exposed to SE receiving vehicle (*Vehicle*, n = 12); 3. rats implanted with electrodes and exposed to SE receiving a combination of anakinra and VX-765 (*Treatment*, n = 7). Drugs or vehicle was administered for 5 or 7 days starting after 1 h of SE (see later for details). For histological analysis, we included naive rats (n = 6) without any treatment. In this model, we studied specifically the effect of treatment on both IL-1β induction and forebrain cell loss by quantitative immunohistochemistry. Although chronic epilepsy was not assessed in this model, the same rats were evaluated for early seizure occurrence during the first week post-SE.

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