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Research Report

Aspirin attenuates spontaneous recurrent seizures and inhibits hippocampal neuronal loss, mossy fiber sprouting and aberrant neurogenesis following pilocarpine-induced status epilepticus in rats

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ABSTRACT

Accumulating data suggest that inflammation may contribute to epileptogenesis in experimental models as well as in humans. However, whether anti-inflammatory treatments can prevent epileptogenesis still remains controversial. Here, we examined the anti-epileptogenic effect and possible mechanisms of aspirin, a non-selective Cyclooxygenase (COX) inhibitor, in a rat model of lithium-pilocarpine-induced status epilepticus (SE). Epileptic rats were treated with aspirin (20 mg/kg) at 0 h, 3 h, or 24 h after the termination of SE, followed by once daily treatment for the subsequent 20 days. We found that aspirin treatment significantly reduced the frequency and duration of spontaneous recurrent seizures during the chronic epileptic phase. Hippocampal neuronal loss five weeks after SE was also attenuated in the CA1, CA3 and hilus following aspirin administration. Furthermore, the aberrant migration of newly generated granule cells and the formation of hilar basal dendrites were prevented by aspirin. Treatment with aspirin starting at 3 h or 24 h after SE also suppressed the development of mossy fiber sprouting. These findings suggest the possibility of a relative broad time-window for aspirin intervention in the epileptogenic process after injury. Aspirin may serve as a potential adjunctive therapy for individuals susceptible to chronic epilepsy.

1. Introduction

Currently available antiepileptic drugs (AEDs) are mainly seizure suppressing, while they do not affect the underlying pathology or the progression of the disease (Pitkanen and Sutula, 2002; Rogawski and Loscher, 2004). Therefore, a medical need exists to develop alternative therapeutics that not only alleviate the symptoms, but also inhibit the process of epileptogenesis (Perucca et al., 2007; Vezzani et al., 2011). However, the details of the mechanisms underlying epileptogenic process remain largely unclear (Jensen, 2009; Rakhade and Jensen, 2009). This hampers the development of better preventive treatments and cures for epilepsy cases that prove resistant to current therapies.

Recent experimental and clinical evidence highlights that the activation of inflammatory pathways may contribute to the development of epilepsy (Aarli, 2000; Ravizza et al., 2008;

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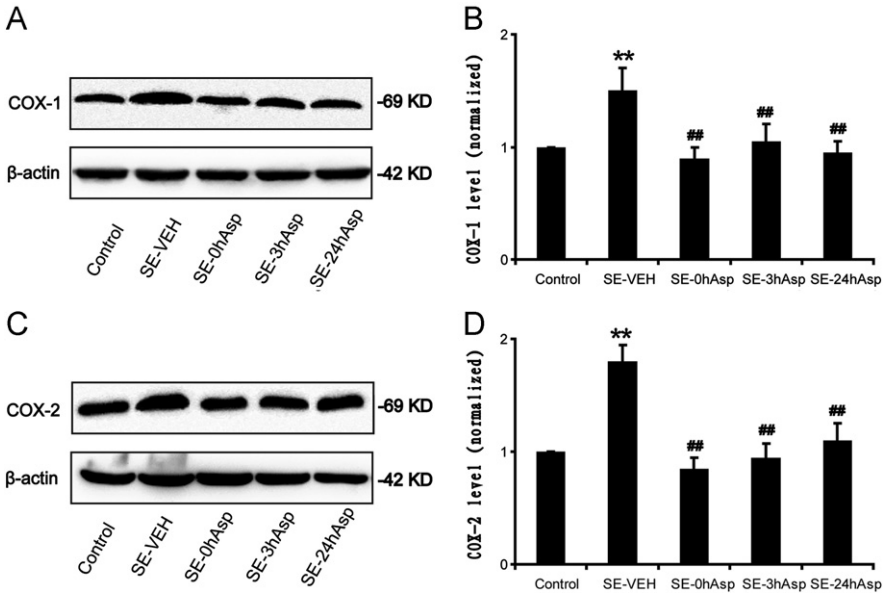

Fig. 1 – *COX enzymes expression in the epileptic rat hippocampus after chronic aspirin treatment*. Representative immunoblots of COX-1 (A) and COX-2 (C) from hippocampus of epileptic and control rats are shown. Increased level of COX-1 and COX-2 abundance was observed at 14 day after status epilepticus. Densitometric analysis revealed that aspirin administration significantly decreased the expression of COX-1 (B) and COX-2 (D) in the hippocampus of epileptic rats compared with untreated epileptic rats ($^{*}P<0.01$, $n=3$ for each group). Values are expressed as a percentage of the control value (mean ± SEM). The equivalence of loading protein in all samples was confirmed by immunoblots of β-actin.

Vezzani et al., 2011; Vezzani and Granata, 2005). The up-regulation of pro-inflammatory signals are observed during epileptogenesis in brain areas of seizure generalization (Friedman, 2011; Marchi et al., 2009). Furthermore, experiments in animal models suggest that antiepileptogenic effects might be achieved by pharmacological interventions that targeting of specific pro-inflammatory pathways (Ravizza et al., 2011).

Cyclooxygenases (COXs) are rate-limiting enzymes in the metabolic pathway that converts arachidonic acid to prostaglandins (PGs), which are potent mediators of inflammation (Simmons et al., 2004). A recent study by Jung et al. (2006) has demonstrated that chronic treatment with celecoxib, a selective COX-2 inhibitor, resulted in a remarkable decrease in the frequency of spontaneous recurrent seizures (SRS) after pilocarpine-induced prolonged seizures. This preclinical data support further attempts to use or development of various anti-inflammatory drugs for preventing seizures after epileptogenic injuries. However, long-term inhibition of COX-2 increases the risk of heart attacks, stroke, and related conditions (Flier and Buhre, 2008). These undesirable side effects may limit the use of celecoxib in the treatment of chronic epilepsy.

Aspirin, the most widely used medications in the world, represents one of the non-selective classical COX inhibitors (Vane and Botting, 2003). The drug is inexpensive and has been proved to be relatively safe in the long-term prevention of transient ischemic attacks, strokes, and heart attacks (Rothwell et al., 2011; Varughese, 1989). Previous studies have demonstrated that aspirin itself or in combination with AEDs dose-dependently decreased the incidence of seizures (Dhir et al., 2006; Srivastava and Gupta, 2001; Tandon et al., 2003; Wali and Patil, 1995). Although a large number of patients with epilepsy take aspirin while they are affected by a broad spectrum of other conditions, the effects of aspirin on the development of epileptogenesis have not been specifically addressed yet.

In the present study, we employed a Li-Pilocarpine rat model to investigate whether aspirin treatment after the induction of status epilepticus (SE) interferes with the development of SRS. The effects of aspirin on hippocampal neuronal loss, mossy fiber sprouting (MFS) and neurogenesis following SE have also been examined.

2. Results

2.1. *COX enzymes expression in the epileptic rat hippocampus after chronic aspirin treatment*

Representative immunoblots of COX-1 (Fig. 1A) and COX-2 (Fig. 1C) from hippocampus of epileptic and control rats are shown. Increased level of COX-1 and COX-2 abundance was observed at 14 day after status epilepticus compared with control rats. Densitometric analysis revealed that aspirin administration significantly decreased the expression of COX-1 (Fig. 1B) and COX-2 (Fig. 1D) in the hippocampus of epileptic rats compared with untreated epileptic rats ($*P<0.01$, $n=3$ for each group).

2.2. *Aspirin attenuated the development of spontaneous seizures following SE*

Eighty rats injected with lithium and pilocarpine developed SE and seventeen rats out of these died from SE. Seizures could be detected behaviorally within 15 min after lithium and pilocarpine injection and the duration of SE was