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Research Report

Toll-like receptor 4 is associated with seizures following ischemia with hyperglycemia

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ABSTRACT

Seizures are a common sequel of cerebral ischemia, and hyperglycemia markedly increases the onset of seizures following an ischemic insult. However, the underlying mechanism of seizures is unclear. The toll-like receptor 4 (TLR4) pathway is known to be involved in temporal lobe epilepsy. The present study investigated the potential involvement of TLR4 in the pathogenesis of seizures following cerebral ischemia with hyperglycemia. Fifteen minutes of global ischemia was produced in adult Wistar rats using a 4-vessel occlusion method. Hyperglycemia was induced via an intraperitoneal injection of glucose 15 min prior to ischemia. We determined that 56.7% of the hyperglycemic rats, but none of the normoglycemic rats, developed tonic-clonic seizures within 12 h after ischemia. TLR4 was mildly expressed in a few cells in the control hippocampus, primarily in interneurons, and was localized in the cytoplasm. The TLR4-positive cells were significantly increased 3–12 h after ischemia. In the hyperglycemic ischemia group, TLR4-positive cells were further increased in quantity and intensity, with a peak at 3 h after ischemia relative to the normoglycemic group. There was no difference in the expression of TLR4 between the hyperglycemic ischemia and LPS groups or between the hyperglycemic non-ischemia and control groups. Western blot analysis consistently exhibited an increase in TLR4 protein levels in the CA3 region 3 h after hyperglycemic ischemia. High mobility group box 1 (HMGB1) (an endogenous ligand of TLR4) was localized in the nucleus of neuronal cells throughout the hippocampus in the control animals. We observed a dramatic decrease in HMGB1 immunostaining at 3 h after hyperglycemic ischemia that gradually returned to control levels. These results suggest that the TLR4 pathway is associated with seizures following global ischemia with hyperglycemia, which provides a new direction for the study of the pathogenesis of seizures that result from hyperglycemic ischemia.

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1. Introduction

Pre-ischemic hyperglycemia has been associated with worse outcomes in numerous animal models of global ischemia, and these outcomes typically manifest as an increased incidence of post-ischemic seizures and death. Although numerous studies have been conducted, the underlying causes of seizures following cerebral ischemia with hyperglycemia are not fully understood.

In the last decade, increasing clinical and experimental evidence has supported the involvement of inflammatory and immune processes in the etiopathogenesis of seizures and epilepsy (Vezzani and Granata, 2005; Vezzani et al., 2011a). Inflammatory responses induced by brain-damaging events, such as stroke, trauma, and infection, are associated with acute symptomatic seizures and a high risk of developing epilepsy (Bartfai et al., 2007; Ravizza et al., 2011; Vezzani et al., 2011b). The activation of specific proinflammatory signals, such as the Toll-like receptor 4 (TLR4) pathway, has been implicated in the precipitation and recurrence of seizures in experimental models (Maroso et al., 2010; Vezzani et al., 2011b).

The toll-like receptors (TLRs) are evolutionarily conserved protein receptors that are fundamental to the activation of the innate immune system. TLRs can be transmembrane or intracellular protein receptors, and thirteen homologues have been identified in mammals (Medzhitov et al., 1997). TLRs were initially found to recognize microbial components, such as lipopolysaccharide (LPS), termed "pathogen-associated molecular patterns" (PAMPs) (Mollen et al., 2006). PAMPs trigger inflammation by inducing the transcription of genes that encode cytokines, including IL-1β. Furthermore, increasing evidence indicates that certain TLRs also respond to endogenous molecules released from stressed or damaged cells, termed "damage-associated molecular patterns" (DAMPs), including the endogenous ligand high-mobility group box-1 (HMGB1) (Tsan and Gao, 2004; Bianchi and Manfredi, 2009). The activation of specific TLR pathways in animal models has been demonstrated to play a vital role in the pathogenesis of critical conditions, including ischemic stroke, brain trauma, "sterile" inflammation and tissue injury in the absence of pathogens. Among these TLRs, TLR4 has been the most extensively studied.

It is increasingly clear that post-stroke neuroinflammation from TLR4 signaling worsens stroke outcomes, as measured by infarct volumes, neurological function and inflammatory markers (Caso et al., 2007; Hua et al., 2009). Studies using different models of cerebral ischemia have elucidated the role of TLR4 signaling in mediating neuroinflammation and exacerbating stroke injury. Neurological outcomes after cerebral infarction are improved in mice with a TLR4 deficiency (Cao et al., 2007; Hua et al., 2007). Exercise therapy, electroacupuncture and certain medicines may play a protective role against ischemic injury via the downregulation of TLR4 expression (Ajamieh et al., 2012, Suzuki et al., 2012; Lan et al., 2013; Ma et al., 2013). Both in vivo and in vitro studies have demonstrated that hyperglycemia activates TLR4 expression and exacerbates the inflammatory response (Devaraj et al., 2009; Amir et al., 2011; Kaur et al., 2012). TLR4 gene silencing offers protection against hyperglycemia-induced cell apoptosis (Zhang et al., 2010).

Based on the evidence that the TLR4 pathway plays an important role in the precipitation and recurrence of seizures, post-stroke neuroinflammation and hyperglycemia-induced inflammation response, it is possible that the TLR4 pathway is involved in seizures following ischemia with hyperglycemia. However, there is little information available regarding the role of TLR4 in seizures following cerebral ischemia with hyperglycemia. The present study investigates the potential involvement of TLR4 in the pathogenesis of seizures following cerebral ischemia with hyperglycemia. Furthermore, the changes in the endogenous ligand ofTLR4, HMGB1, were also investigated.

2. Results

2.1. *Seizure rate and brain damage*

More than half of the rats (17/30, 56.7%) in the hyperglycemic group developed tonic-clonic seizures within 12 h after 15 min of ischemia (Fig. 2A). Most seizures (15/17, 88.2%) occurred within 3 h after ischemia. Every animal with seizures died of status epilepticus within 2 h after the onset of seizures. In contrast, no rats in the normoglycemic group developed seizures during the 12-h period after ischemia (Fig. 2A).

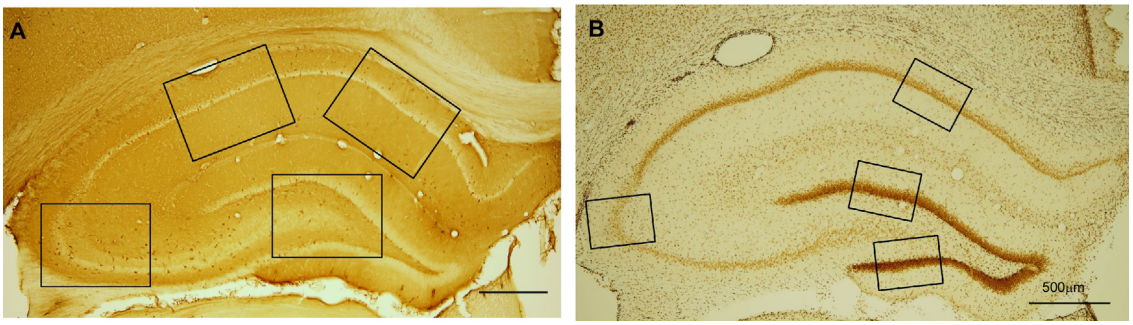

Fig. 1 – Fields chosen for TLR4 cell quantification analysis and HMGB1 immunoreactivity quantification analysis. (A) Fields chosen for TLR4 cell quantification analysis; each rectangular frame is 850 μm × 640 μm for 200 ×. (B) Fields chosen for HMGB1 immunoreactivity quantification analysis; each rectangular frame is 425 μm × 320 μm for 400 ×.