Biomedicine & Pharmacotherapy 96 (2017) 675–684

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Original article

Synergistic effect of mild hypothermia and the Notch inhibitor DAPT against post stroke seizures

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ARTICLE INFO

Keywords:
Notch 1
Notch 2
Stroke
Seizure
Mild hypothermia

ABSTRACT

Seizure is a serious complication of stroke, indicating poor prognosis. Notch signaling is associated with neuronal activity. Inhibition of Notch signaling suppresses seizure activity induced by kainic acid. The present study investigated the effect of the Notch inhibitor, DAPT, alone or in combination with mild hypothermia, on post-stroke seizures. A global cerebral ischemia (GCI) model was performed in Sprague Dawley (SD) male rats. Seizure activity was evaluated by the frequency of seizure attacks, seizure severity scores, and seizure discharges. Without any intervention, seizures occurred intensively between 24 h and 48 h following GCI. Seizure activity was confirmed using EEG monitoring. The expression of Notch intracellular domains (NICD) 1 and 2 were up-regulated in the cerebral cortex and hippocampus following GCI. DAPT was injected into the hippocampus of the rats to inhibit local Notch signaling. Active whole-body cooling was performed to maintain the core temperatures of rats at 33.5 °C (mild hypothermia). Mild hypothermia and DAPT synergistically inhibited NICD 1 and 2 up-regulation, and post-stroke seizures. GCI augmented excitatory synaptic neurotransmission by up-regulating glutamate receptor subunits (GluN2A, GluA1) and the cotransporter, NKCC1, but attenuated inhibitory synaptic neurotransmission by down-regulating gamma amino acid, butyric acid (GABA), and the co-transporter, KCC2. DAPT treatment normalized the homeostasis of excitatory and inhibitory synaptic neurotransmission, suggesting that aberrant activation of Notch signaling is involved in post-stroke seizures. The present study adds to the further understanding of the pathogenesis of post-stroke seizures and the improvement of the treatment provided with hypothermia.

1. Introduction

Cerebral ischemia is a high risk factor for the development of seizures. Seizure is a common neurologic disease that poses a serious threat, especially for neonates and elderly people [1–3]. The occurrence of seizures is commonly regarded as a poor prognostic factor for ischemia-induced brain injury. Approximately 20% of neonates with seizures die within the neonatal period, and 28% to 35% of the survivors later exhibit significant neurodevelopmental delay [3–5]. Elderly patients with post-stroke seizures show a higher short-term mortality (within a year) than patients who do not experience seizures [1]. The reasons for the increased mortality following post-stroke seizures are unclear. For elderly patients with epilepsy, neurological dysfunction, cerebrovascular disease, and neoplasias contribute to the excess mortality [6]. The imbalance between excitatory (glutamate) and inhibitory (gamma amino acid butyric acid, GABA) synaptic neurotransmission and altered ion channel (e.g. KCC2) function play critical roles in the pathogenesis of post-stroke seizures [2], however, the specific causes of these neurological dysfunctions are not well understood.

Cooling of the head or whole body after impairment of cerebral blood flow has been shown to reduce the seizure burden in neonates [3–5,7]. After therapeutic hypothermia, both video-electroencephalography (EEG) monitoring and magnetic resonance imaging (MRI) show reduced seizure activity in neonates with moderate hypoxic ischemic encephalopathy (HIE), while therapeutic hypothermia is not effective in severe HIE [3–5,7]. Additionally, mild hypothermia is an effective treatment for pediatric refractory status epilepticus [8]. A rodent model demonstrated that mild passive focal cooling of the perilesional neocortex, by 0.5–2 °C, inhibits the onset of seizures after head injury [9]. It is known that mild hypothermia reduces brain metabolism, oxygen utilization, and adenosine triphosphate (ATP) consumption. Furthermore, mild hypothermia activates GABA receptors and leads to the inhibition of glutamate release, mitochondrial dysfunction, and calcium overload [10–12]. These actions may be associated with the protective effect of mild hypothermia against seizures and epilepsy. However, without concomitant barbiturate (anti-seizure

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http://dx.doi.org/10.1016/j.biopha.2017.10.036
Received 6 June 2017; Received in revised form 9 October 2017; Accepted 9 October 2017

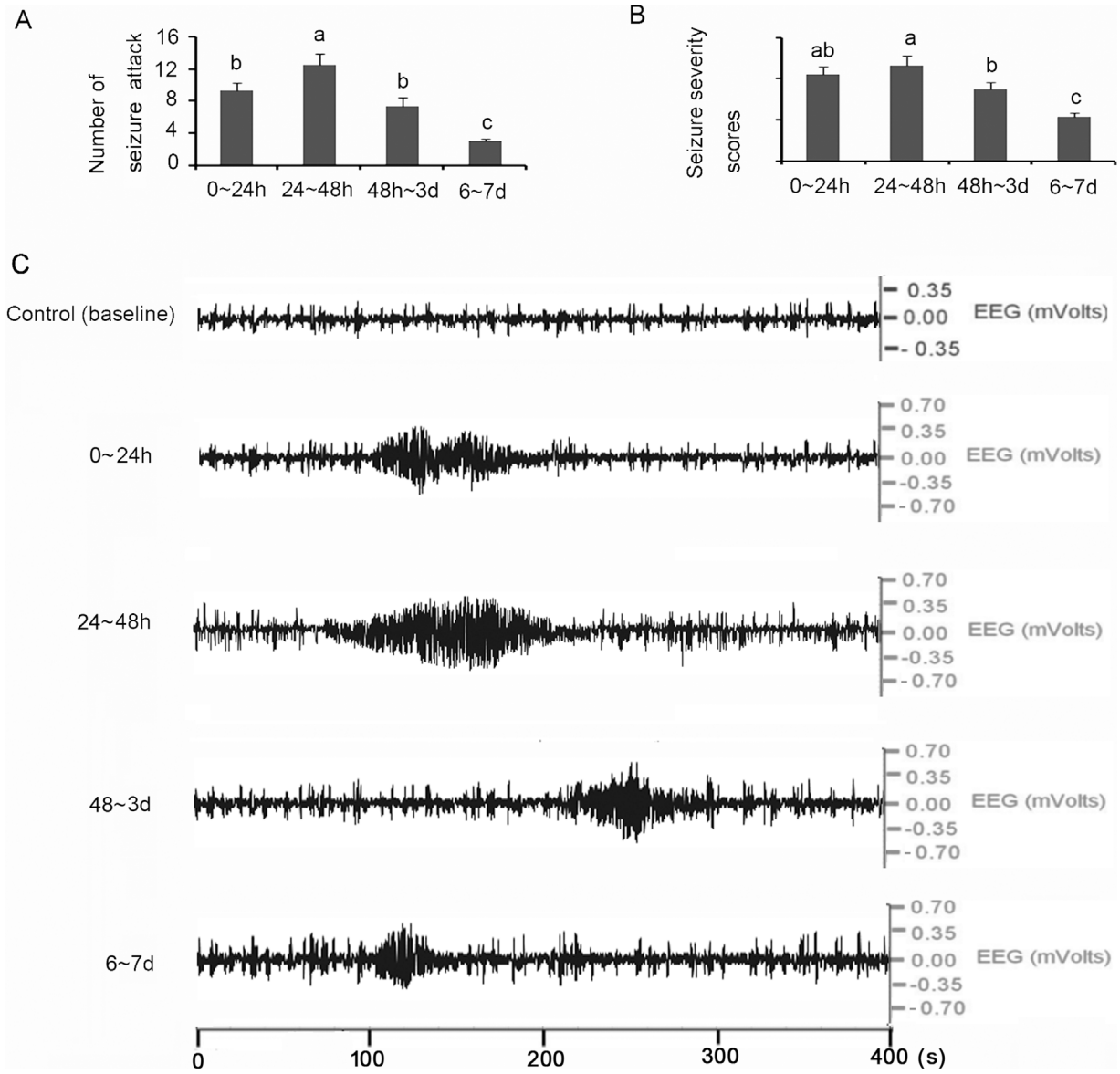

Fig. 1. GCI provoked seizures in rats.
The number of seizure attacks (A), seizure severity scores (B) and EEG recordings (C) were used to evaluate the intensity of seizures at different time periods or points after GCI. The mean values were significantly different between groups ($P < 0.05$, $n = 3$).

drug) treatment, hypothermia rarely enables the sustained control of ongoing status epilepticus. Seizures tend to recur after rewarming to normothermia [13]. Therefore, hypothermia is not a long-term effective option for clinical intervention in seizures.

The Notch pathway extensively controls neuronal proliferation, differentiation, apoptosis and discharges, and regulates arteriovenous differentiation. Therefore, Notch signaling is involved in various physiological and pathological functions of brain [14]. Mammalian Notch is known as a transmembrane receptor containing 4 receptor isoforms; Notch 1–4 [14]. Notch-ligand interactions induce activation of Notch signaling. The binding of ligands to the Notch receptors causes conformational changes in their intracellular domain that allows cleavage by γ-secretase, resulting in the release of the Notch intracellular domain (NICD) [14,15]. The NICD subsequently translocates to the nucleus where it activates the transcription of Notch target genes [15]. A recent report suggested that Notch signaling is implicated in temporal lobe epilepsy [16]. Treatment with exogenous Jagged 1, to increase Notch signaling, has a proconvulsant effect. In contrast, application of the Notch antagonist, DAPT, inhibits the epileptic seizures induced by kainic acid [16]. Currently, it is unclear whether DAPT has an inhibitory effect on the seizures caused by cerebral ischemia. The present study, thus, investigated the effect of the Notch inhibitor, DAPT, alone or in combination with mild hypothermia, on post-stroke seizures.

2. Materials and methods

2.1. Animals

Eight to ten-week-old Sprague Dawley (SD) male rats (Central South University, Changsha, China) were housed in vivariums that were maintained at 22–23 °C with a 12-h light on/off cycle. Food and water were provided ad libitum. All animal procedures were carried out in accordance with National Institutes of Health Guidelines for the care and use of animals. In line with the guidelines, rats with severe epilepsy were treated with clinically appropriate anticonvulsants. The animal study was approved by the Animal Care and Use Committee of the Central South University. Experiments were completely randomized and were analyzed by personnel kept blind to the treatment and the