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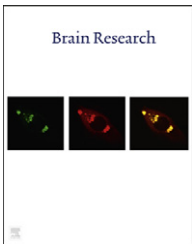

Research Report

Topiramate reduces blood–brain barrier disruption and inhibits seizure activity in hyperthermia-induced seizures in rats with cortical dysplasia

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ABSTRACT

We investigated the effects of topiramate (TPM), a novel broad spectrum anticonvulsant, on seizure severity, survival rate and blood–brain barrier (BBB) integrity during hyperthermic seizures in rats with cortical dysplasia (CD). Offsprings of irradiated mothers were used in this study. To show the functional and morphological alterations in BBB integrity, quantitative analysis of Evans blue (EB) extravasation, immunohistochemistry and electron microscopic assessment of horseradish peroxidase (HRP) permeability were performed. Rats with CD exposed to hyperthermia exhibited seizures with mean Racine's scores of 3.92 ± 1.2. Among the rats with CD pretreated with TPM, 21 of 24 rats showed no sign of seizure activity upon exposure to hyperthermia ($p < 0.01$). The immunoreactivity of occludin, a tight junction protein, remained essentially unaltered in capillaries of hippocampus in all groups. In animals with CD exposed to hyperthermia, the significantly increased p-glycoprotein immunoreactivity in hippocampus ($p < 0.01$) was slightly decreased by TPM pretreatment. Hyperthermic seizures increased BBB permeability to EB in animals with CD, but TPM pretreatment decreased the penetration of the tracer into the brain in these animals ($p < 0.01$). Ultrastructurally frequent vesicles containing HRP reaction products were observed in capillary endothelial cells in cerebral cortex and hippocampus of rats with CD subjected to hyperthermia-induced seizures, and TPM pretreatment prevented the development of HRP reaction products in these animals. The results of this study suggest that TPM inhibits seizure activity and maintains BBB integrity in the course of febrile seizures in the setting of CD.

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1. Introduction

Cortical dysplasia (CD), defined as a malformation of cortical development, is a common cause of developmental disorders or medically refractory epilepsy in both children and adults. The presence of preexisting brain injury such as CD renders the immature brain more susceptible to seizures (Germano et al., 1996; Gürses et al., 2009). The histological types of CD are increasingly considered to be associated with epileptogenesis in medically refractory patients (Cepeda et al., 2006; Porter et al., 2003). Animal models of in utero exposure to irradiation and methylazoxymethanol acetate are often used to induce cortical malformations and they are suggested to be the most similar models to human CD (Baraban and Schwartzkroin, 1995; Kaya et al., 2008; Wong, 2009).

Topiramate (TPM), an anti-convulsant with various known mechanisms of action, is shown more consistently to block AMPA-mediated currents than to have effects on voltage-gated sodium channels and GABA receptors. In addition, accumulated data indicate that TPM provides efficious anti-epileptic action on maximal electroshock (Rowley and White, 2010), amygdala kindling (Wauquier and Zhou, 1996), audiogenic convulsions (Rigoulot et al., 2003), and genetic models including DBA/2J mice, spontaneously epileptic rats (Nakamura et al., 1994) and genetic absence epilepsy (Rigoulot et al., 2003) in experimental animals. There are clinical studies showing the efficacy of TPM in patients with partial epilepsy (Ben-Menachem, 1996a) and generalized seizures (Montouris et al., 2000).

Human studies indicate that febrile seizures are the most common convulsive disorder in children between the ages of 6 months to 5 years, and seizures provoked by hyperthermia in animals share common mechanisms with febrile seizures seen in humans (Dubé et al., 2009; Hauser, 1994; Suenaga et al., 2008). Febrile seizures exert a strong destructive effect on the sensitive hippocampal neurons, and the neuroprotective properties of TPM were shown on this process (Sendrowski et al., 2007). In addition to its anti-epileptic effects, TPM also provides neuroprotective effects in models of neuronal damage, which are directly related to its inhibitory action on the mitochondrial permeability transition pore in the epileptic state (Kudin et al., 2004), traumatic brain injury (Kouzounias et al., 2011), and hypoxia (Mikati et al., 2011).

Endothelial cells of brain capillaries form the blood–brain barrier (BBB), which protects neuronal microenvironment and regulates the brain homeostasis under physiological conditions. Accumulated data indicate that neuronal morphology and vascular architecture are altered in cortical malformations (Chevassus-Au-Louis et al., 1998, 1999; Fan et al., 2008; Marchi et al., 2011). Aberrant vascular architecture in cortical malformations may contribute to the occurrence of seizures and the resultant increased BBB permeability (Chevassus-Au-Louis et al., 1999; Fan et al., 2008; Gürses et al., 2009; Kaya et al., 2008; Marchi et al., 2011). A recent study from our group showed that hyperthermic seizures disrupted BBB integrity in immature rats with CD (Ahishali et al., 2010). Furthermore, it is reported that TPM provided protective effects on the neuronal structures and BBB integrity in brain regions, including hippocampus during febrile seizures in intact rats (Lotowska et al., 2008; Sobaniec-Lotowska and Lotowska, 2011). However, the influence of TPM on the altered BBB components during hyperthermic seizures in the setting of CD is unclear. Therefore, the present study is intended to investigate whether TPM alters seizure severity and/or exerts protective effects on functional and structural properties of BBB in a "two-hit model" of CD and hyperthermic seizures.

2. Results

Baseline core (rectal) temperature values ranged between 36.1 and 36.7 °C in all rats. Following the maintenance of the rats with CD in the heat chamber for 10–15 min, rectal temperature values reached 41 °C. Elevation of rectal temperatures of these rats for 30 min resulted in hyperthermic seizures in 21 of 24 animals (88%; $p<0.01$), and the mean seizure score according to Racine's scale was 3.92 ± 1.2. The mean time from the initiation of hyperthermia to the onset of seizures was 5–6 min. Seizure duration averaged 14.2 ± 1.3 min and the animals had 1–2 seizure episodes during the hyperthermia period. TPM pretreatment prevented the occurrence of seizures in 21 of 24 rats with CD during hyperthermia ($p<0.01$). In the remaining 3 rats, the duration of the seizures was 2, 3 and 5 min and the Racine score of the seizures in these animals was 2, 2 and 4, respectively. TPM pretreatment significantly reduced the mortality rate in rats in CD plus hyperthermic seizures group from 19/24 (79%) to 1/24 (4%), ($p<0.01$).

The intensity of occludin immunostaining remained essentially unchanged in the brain capillaries in CA1 region of hippocampus of rats in experimental groups (Fig. 1A–D). In addition, the relative intensity of occludin immunoreactivity as assessed by image analysis did not differ significantly among the experimental groups (Fig. 1E). The intensity of immunostaining of p-glycoprotein (P-gp), which increased in the capillary wall in hippocampus of rats with CD upon exposure to hyperthermia-induced seizures, was observed to be slightly decreased by TPM pretreatment (Fig. 2A–D). The relative intensity of P-gp immunoreactivity, as assessed by image analysis, increased significantly in animals with CD plus hyperthermic seizures compared with that in control animals ($p<0.01$) and a slight decrease of relative intensity was found upon TPM pretreatment in this setting (Fig. 2E).

The EB dye content in left and right cerebral cortex, diencephalon and cerebellum regions was markedly increased in hyperthermic seizures in rats with CD compared with that in animals in control and CD groups ($p<0.01$; Fig. 3). In these animals, pretreatment of TPM significantly decreased EB dye content in all brain regions ($p<0.01$).

Macroscopic observation of Vibratome sections of brains revealed a widespread pattern of HRP extravasation in cortical and subcortical regions of rats with CD exposed to hyperthermic seizures, while the animals in control and CD groups as well as those in CD plus TPM plus hyperthermia showed no evidence of tracer extravasation (Fig. 4).

Ultrastructurally, no HRP reaction product was observed in the cytoplasm of endothelial cells of brain capillaries in the cerebral cortex and hippocampus of rats in control (Fig. 5A and B) and CD (Fig. 5C and D) groups. Hyperthermic seizures in animals with CD led to the observation of frequent vesicles containing HRP reaction products in the cytoplasm of endothelial cells in these regions (Fig. 5E and F). In these