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Effects of levetiracetam on blood-brain barrier disturbances following hyperthermia-induced seizures in rats with cortical dysplasia

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ABSTRACT

Aims: The mechanisms underlying the changes in blood-brain barrier (BBB) integrity and the generation of seizures in childhood associated with preexisting brain lesions like cortical dysplasia (CD) are poorly understood. We investigated the effects of levetiracetam (LEV) on BBB integrity and the survival during hyperthermic seizures in rats with CD.

Main methods: Pregnant rats were exposed to 145 cGy of gamma-irradiation on embryonic day 17. On postnatal day 28, hyperthermia-induced seizures were evoked in offspring with CD. To show the functional and morphological alterations in BBB integrity, quantitative analysis of sodium fluorescein (NaFlu) extravasation, immunohistochemistry and electron microscopy were performed.

Key findings: Seizure scores and mortality rates were decreased by LEV during hyperthermia-induced seizures in rats with CD ($P<0.01$). Increased NaFlu extravasation into brain by hyperthermia-induced seizures in animals with CD was decreased by LEV ($P<0.01$). While glial fibrillary acidic protein (GFAP) immunoreactivity slightly increased in brain sections of animals with CD during hyperthermia-induced seizures, LEV led to GFAP immunoreactivity comparable to that of controls. Decreased occludin immunoreactivity and expression in CD plus hyperthermia-induced seizures was increased by LEV. Opening of tight junctions and abundance of pinocytotic vesicles representing ultrastructural evidences of BBB impairment and severe perivascular edema were observed in animals with CD exposed to hyperthermia-induced seizures and LEV treatment led to the attenuation of these findings.

Significance: These results indicate that LEV may present a novel approach for the protection of the BBB besides its antiepileptic impact on hyperthermic seizures in the setting of CD.

Introduction

Febrile seizures are the most frequently seen convulsions in infants and young children. On the other hand, cortical dysplasia (CD), a developmental malformation of the neocortex, is recognized as one of the major causes of pediatric epilepsy. A number of studies indicate that the underlying pathology in CD may lower the threshold to seizures (Toth et al., 1998; Chen et al., 1999; Porter et al., 2003; Dube and Baram, 2006). Moreover, the presence of preexisting brain injury such as CD renders the immature more susceptible to seizures and susceptibility is shown to be more pronounced in kindled animals (Germano et al., 1996; Kaya et al., 2008; Lin and Roper, 2006).

Although many forms of epilepsy are successfully managed with antiepileptic drug therapy, epilepsy due to CD is markedly resistant to pharmacological treatment which is characteristic of these developmental lesions (Smyth et al., 2002; Wong, 2008). In recent human and animal studies focusing on the involvement of the efflux pumps in pharmacoresistance, overexpression of blood-brain barrier (BBB) efflux transporters in the brain capillary endothelial cells of the epileptic focus is reported (Sisodiya et al., 2003; Schmidt and Löscher, 2009; Kuteykin-Teplyakov et al., 2009). While a large number of studies have mainly focused on the susceptibility to seizure induction, accumulating data concerning seizure-induced cell damage and antiepileptic treatment in cortical malformations (Smyth et al., 2002; Aronica et al., 2003; Oh et al., 2008) are being obtained from

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recent studies concentrating on the BBB integrity in malformed brain (Marchi et al., 2006, 2007; Kaya et al., 2008; Gurses et al., 2009). Levetiracetam (LEV) is shown to be a highly effective antiepileptic drug against partial and generalized seizures in several animal models (Löscher et al., 1998; Klitgaard et al., 1998; Cilio et al., 2009). Although LEV has no effects on acutely generated seizures in animals (Löscher et al., 1998; Brandt et al., 2007), it markedly suppresses audiogenic seizures and kindling development (Löscher et al., 1998; Klitgaard and Pitkänen, 2003). In a recent study, it is shown that LEV may have inhibitory effects in hippocampus and attenuates other neuronal pathways to secondary generalization in Noda epileptic rats (Ishimaru et al., 2010). Meanwhile intravenous administration of LEV in a chemoconvulsant model of status epilepticus resulted in the attenuation of behavioral manifestations of seizure discharge and in the reduction of neuronal injury (Zheng et al., 2010).

Studies investigating the impact of febrile seizures on BBB integrity have shown disruption of BBB in humans and animals (van Eijsden et al., 2004; Suenaga et al., 2008). Because alterations in BBB integrity may precede neuronal damage in many neuropathologic conditions such as epilepsy, BBB impairment may also be involved in the initiation, progression and maintenance of seizures (Seiffert et al., 2004; Ransohoff, 2009). Two recent studies reported that antiepileptic drugs, LEV and topiramate, could exert protective effects on BBB components against febrile seizures and seizures induced by pentylenetetrazole in kindled animals with CD (Łotowska et al., 2008; Gurses et al., 2009). However, the impact of febrile seizures on BBB integrity in the setting of CD and the influence of LEV on the altered BBB components are unclear. The present study is therefore, designed to investigate whether LEV alters seizure severity and/or exerts protective effects on functional and structural properties of BBB during hyperthermia-induced seizures in rats with CD.

Materials and methods

Animal protocol and irradiation procedure

The experimental procedures were conducted in timed-pregnant Sprague–Dawley rats (n = 20) and their litters of both sexes. The day on which insemination was detected was determined as the embryonic day (E) 0. In order to induce CD, the animals were treated according to a protocol that was previously described (Roper et al., 1995). On E17, the pregnant rats were exposed to gamma-irradiation. For this purpose, the animals were anesthetized with sodium pentothal (35 mg/kg, i.p), and were placed in groups of ten, each in prone position on a wooden board. A polystyrene phantom was put under the board to achieve an acceptable backscatter. A nominal single dose of 145 cGy to mid-plane of the abdominal area was delivered by 6×27 cm posterior field using Co-60 tele-therapy unit (Alcyon II, General Electric, France). After irradiation, rats were taken back to the animal facility and cared for routinely until birth. After parturition, litters were allowed to live a 12-hour light/dark cycle with free access to food and water until the age of 28 days. The litters were randomly divided into the following experimental groups; CD, CD plus hyperthermia, CD plus LEV, and CD plus hyperthermia plus LEV, while in-utero untreated litters were randomly assigned to control, hyperthermia and hyperthermia plus LEV groups. The experimental protocols used in this study were approved by the Ethics Committee for Animal Experimentation of the Institute of Experimental Medicine, Istanbul University. All efforts were made to minimize animal suffering and reduce the number of animals used. Separate sets of experimental groups consisting of 8 rats were used for each experimental procedure except western blotting which was performed in experimental groups of 4 rats.

Administration of LEV and induction of hyperthermia

LEV (UCB Pharma, Belgium), dissolved in saline, was intravenously administered in a dose of 100 mg/kg through a catheter inserted into right femoral vein to 28-day-old rats under a mild dietyl ether anaesthesia. Twenty min later when the animals were fully awakened, severe hyperthermia with an average core (rectal) temperature of 40 °C (39.5–40.5 °C) was induced by placing the rats in a translucent fiberglass chamber (74 cm×42 cm×30 cm). A heated air flow system was adjusted to maintain the temperature of the chamber at 55–60 °C. Baseline core temperature was measured using a probe connected to a temperature indicator. To induce hyperthermia, the core temperature of animals was kept at 39.5–40.5 °C. Once seizures commenced, hyperthermia was continued, and core temperature was recorded every 2 min. The convulsive behavior was observed for 30 min and classified into the following stages as described by Racine (Racine, 1972): 0—no behavioral changes; 1—facial movements, ear and whisker twitching; 2—myoclonic convulsions without rearing; 3—myoclonic convulsions with rearing; 4—clonic convulsion with loss of posture; 5—generalized clonic–tonic seizures.

Measurement of arterial blood pressure and BBB permeability

The rats were anaesthetized with diethyl ether and polyethylene catheters (PE-10) were inserted into the right femoral artery and vein. The former catheter was connected to a pressure transducer which was interfaced to a data acquisition system (iWorx/ETH-256) to continuously monitor the mean arterial blood pressure by a personal computer, and the latter catheter was used to infuse sodium fluorescein (NaFlu; 2%, 5 ml/kg) tracer. BBB permeability was assessed by measuring the brain level of extravasated NaFlu. In animals which were exposed to hyperthermia, NaFlu was administered when core temperature reached 39.5 °C and allowed to circulate for 35 min. Then, rats were transcardially perfused with 100 ml of saline for about 15 min to remove intravascular NaFlu. After decapitation, brains were removed and dissected into four regions; left cerebral cortex, right cerebral cortex, diencephalon and cerebellum. Each brain region was weighed for quantitative measurement of NaFlu extravasation. Brain samples were homogenized in 2.5 ml phosphate-buffered saline and mixed with a vortex for 2 min after the addition of 2.5 ml 60% trichloroacetic acid to precipitate protein. Samples were later cooled for 30 min and centrifuged at 14000 g for 10 min. The concentration of tracer in the supernatant was measured at excitation wavelength of 440 nm and emission wavelength of 525 nm using a spectrophotofluorometer (Microplate Reader; DTX880 Multimode Detector, Beckman Coulter). NaFlu was expressed as ng/mg of brain tissue against a standard curve.

Immunohistochemistry

To demonstrate tight junction (TJ) protein, occludin, and glial fibrillary acidic protein (GFAP) immunoreactivity in the brain sections, rats were anaesthetized with diethyl ether. A bolus transcardial perfusion with 20 ml saline was administered at a pressure of 110 mm Hg for 15 s, followed by 100 ml fixative (4% paraformaldehyde in phosphate buffer; pH: 7.4) for 10 min. After the perfusion, brains were removed, immersed in the same fixative, kept for 24 h at 4 °C, and then embedded in paraffin. 5-μm thick sections were deparaffinized and incubated in 10 mM citrate buffer solution (pH: 6.0) at 1 atm pressure for 2 min for GFAP or incubated with protease (1 mg/ml; Sigma, USA) for 10 min for occludin to achieve antigen retrieval. Endogenous peroxidase activity was quenched using 0.3% hydrogen peroxide for 20 min. A nonspecific blocking reagent (Ultra-V-Block, Lab Vision, Westinghouse, CA) was used to prevent nonspecific binding. Monoclonal mouse anti-GFAP (Neomarker, Fremont, CA; 1/100, 60 min) and polyclonal rabbit anti-occludin (Zymed, CA; 1/50, 2 h) were used as primary antibodies. Secondary antibodies were biotinylated goat anti-mouse (Lab Vision, Westinghouse, CA) for GFAP and biotinylated goat anti-polyvalent for occludin. After washing, streptavidin–peroxidase complex was applied and aminoethyl carbazole chromogen was used. The sections were counterstained with Mayer's hematoxylin to enhance nuclear staining. Images were obtained from the hippocampus by