Topiramate as a neuroprotectant in the experimental model of febrile seizures

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Abstract

Purpose: The aim of the study wad to estimate a potentially neuroprotective effect of topiramate (TPM) in the experimental model of FS.

Material and methods: 24 young male rats divided in 4 groups were involved in the study. Febrile seizures were induced by placing the animals in 45°C warm water bath for four consecutive days. TPM at the dose 80 mg/kg b.m. was administered: before the FS and immediately after the FS. FS group and control rats received only normal saline. Thereafter hippocampal slices were prepared to performing histological and morphometric examination.

Results: Morphometric investigations revealed that FS caused death of 60% of the neurons in sector CA1 and a half of them in sector CA3. Histological examinations of hippocampal slices showed that TPM at a dose of 80 mg/kg b.m., administered before the seizures, considerably improved CA1 and CA3 pyramidal cell survival. Similar neuroprotective effect, but in a markedly lesser degree was observed when TPM was administrated after the FS.

Conclusions: Our findings seem to confirm that FS exert a strong destructive effect on the sensitive hippocampal neurons and on the neuroprotective properties of TPM in this process, which may have practical implications. It can be assumed that in children with recurrent and prolonged FS, prophylactic drug administration could prevent hippocampal sclerosis and development of symptomatic epilepsy.

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Introduction

Febrile seizures (FS) occur between 6 months and 5 years of age at body temperature above 38.5°C, usually during infection with fever [1]. FS are the most common form of convulsions in childhood and have been associated with an increased risk of epilepsy in the future life. It has long been known that the risk of epilepsy in children with a past history of FS episodes is higher than in general population [2]. Temporal lobe epilepsy (TLE) due to its frequent drug-resistance remains a challenge for the epileptologist. TLE is the most prevalent type of epilepsy, but its origin is still not well understood. Intractable TLE is often associated with specific hippocampal cell loss termed mesial temporal sclerosis. This pathology is characterized by neuronal loss and gliosis, most prominent in the hippocampal CA1 and CA3 sectors [3]. A number of studies have shown a significant relationship between a history of FS, particularly of the complex type, in childhood and the presence of mesial temporal sclerosis, as identified on magnetic resonance imaging [4,5]. Some retrospective studies have shown that FS are the most frequently cited etiology of hippocampal sclerosis, especially when coexisting with additional risk factors [4,6]. Animal models support the association as well. For instance, immature rats exposed to hyperthermic seizures during infancy develop significantly reduced hippocampal seizure thresholds to chemical convulsants and electrical stimulation during adult life [7].

Pharmacological prevention of neuronal damage induced by various damaging factors (i.e. neuroprotection) has been intensively investigated in recent years. A search has been conducted for a neuroprotectant that would prevent hippocampal sclerosis caused by prolonged FS, and thus hinder epilepsy development in high risk patients. In experimental models, new generation antiepileptic drugs, e.g. topiramate (TPM), appear

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to be effective neuroprotectants [8-10]. TPM is a novel antiepileptic drug that is effective in the treatment of various types of epilepsy [11]. Its neuroprotective action has been described in many models of neuronal damage, among others epilepsy [12], epileptic state [13] or global ischemia [14]. Since there are no literature reports concerning the potential neuroprotective action of TPM in FS-induced damages, we decided to perform the current research.

The aim of the study was to estimate potentially neuroprotective effects of TPM in the experimental model of febrile convulsions.

Material and methods

Model of febrile seizures

The experiment used 24 young Wistar rats aged 22-30 days. The degree of brain maturity in such rats corresponds to that of 1- or 2-year-old children. Prior to the experiment, the animals were kept in cages (6 in one cage) with free access to food and water at 12-hour cycles of light and darkness. For the needs of the experiment, the animals were divided into 4 groups, 6 rats in each. Hyperthermia was induced by placing the animals in a 30x30x60 cm water bath filled with 45°C warm water to such a depth that a rat standing on its hind legs and leaning against a container wall had its head above water surface. Water temperature was maintained at the same level. Rats were put into water for 4 minutes or until convulsions appeared and then moved to a separate container lined with lignin [15]. The rats (except for control) were placed in water for four consecutive days. Topiramate (80 mg/kg b.m. dissolved in 2 ml normal saline) was administered with an intragastric tube, 90 minutes before the animals were placed in the water bath (group TPM+FS). In the FS+TPM group, the drug was administered in the same way and at the same dose, immediately after the each convulsion episode. Control rats and FS group received only normal saline. The dose of the drug was chosen according to literature references [13,16].

Histological analysis Seventy-two hours after the last convulsion episode, the rats were anesthetized with Nembutal (25 mg/kg b.w., i.p.) and transcardially perfused with 200 ml of 4% paraformaldehyde in phosphate-buffered saline under pressure of 80-100 mmHg [17,18]. The brains were removed from the skulls and fixed in the same fixative for 24 h. Next, the brains were embedded in paraffin and representative coronal sections (6- μ m thick), which included the hippocampus, were obtained with a rotary microtome. The sections were stained with hematoxylin and eosin and cresyl violet as well. A blinded investigator performed the histological examination.

Morphometric study Five randomly chosen non-overlapping high-power fields (original magnification x 400, light microscope) from the hippocampal CA1 and CA3 areas were examined separately from each section. Only normal-appeared neurons in the five high-power fields were counted and the number of cells per high-power field was calculated. All data are presented as the mean. Images were collected using an Olympus camera. Statistical analysis was conducted using the chi²-test. A level of p<0.05 was considered statistically significant.

Results

Histopathological findings

FS group Febrile seizures induced histopathological changes in three areas of the Ammonal cortex: CA1, CA3 and hilum. In half of all cases these changes were diffused, in the others showed a tendency to blend and involved extensive fragments of the hippocampal gyrus cortex. Neurons with features of chronic lesions and sclerosis were numerous, had sharpened profiles and were shrunken in appearance. They resembled dark polymorphous lumps with invisible intracellular structures (Fig. 2); dendrites of some of them had a wavy course. Degenerated cells represented even up to 30% of all neurons within these three areas. Distinct neuronal desertion areas were observed in the pyramidal layer (Fig. 2), being most pronounced in CA1 and less in CA3 sector. In the closest proximity, disintegrating neurocytes and shadows of disintegrated cells were seen (Fig. 3). Close to most damaged fragments of the hippocampal gyrus cortex, the structure of white matter was sometimes markedly loosened (Fig. 2).

FS+TPM group Macroscopically, the animals which after experimentally induced febrile convulsions received TPM had structural abnormalities qualitatively similar to those observed in group FS (*Fig. 4, 5, 6*). However, fewer cells were affected and the changes were more diffused. Degenerated, mainly sclerotic neurons were found in the pyramidal layer of the CA1 and CA3 sectors. Such cells accounted for approximately 25% of all neurons in the amonal cortex. The neuronal "balding" areas were visible but they were less common than in FS group. They were found mainly in the CA3 sector (*Fig. 5*).

TPM+FS group The histological picture of the hippocampal cortex of the animals receiving TPM prior to FS showed only slight abnormalities as compared to the control group. Besides well preserved neurons, few dark-staining and shrunken neurocytes could be seen, especially in the CA3 sector (*Fig. 7*). However, the neuronal "balding" areas were sporadic, indistinct and diffused.

Morphometric investigations Morphometric investigations of the hippocampus sections were conducted routinely, by assessing the number of neurons in the high power field in the CA1 and CA3 sectors separately in the control group and in each experimental group. Findings referring to the CA1 sector and CA3 sector are presented in Tab. 1. Statistical significances between control and experimental groups have been presented in the same Tab. 1. Experimentally induced febrile convulsions resulted in the death of 60% of the neurons in this Ammonal cortex area as compared to the control group. In the rats receiving TPM before FS, the number of survival neurons was markedly higher (the death of only 22% of neurons was observed). In the CA3 sector, febrile convulsions led to the death of 50% of neurons as compared to the control group. The loss of 28-43% of neurons was observed in the other two experimental groups.

Figure 1. Well-preserved neurons in the CA1 sector. Control group. Cresyl violet x400



Figure 3. Sector CA3. Substantial extension of desertion area enclosed by neuronal shadows. Group [FS]. Cresyl violet x400



Figure 5. Sector CA3. Distinct neuronal desertion areas. Single neurons are shrunken and very dark. Disintegrating shadows left after neurons are visible in the vicinity. Group [FS+TPM]. Cresyl violet x400



Figure 7. Sector CA1. Diffused degenerated neurons with features of sclerosis are visible among the population of relatively well preserved neurons. Group [TPM+FS]. Cresyl violet x400



Figure 2. Sector CA3. Picture of lethally damaged sclerotic pyramidal cells. Extensive neuronal desertion areas. Structural loosening of white matter. Group [FS]. H,E x400.



Figure 4. Sector CA1. Besides normal neurons, dark-staining neurons with features of chronic lesion and sclerosis are visible. Group [FS+TPM]. H,E x200



Figure 6. Sector CA3. In the vicinity of small areas of neuronal desertion areas, markedly degenerated cells with features of chronic lesion and dendrites taking a wavy course are observed. Group [FS+TPM]. Cresyl violet x400



Discussion

Recent researches have demonstrated that epilepsy and seizures (including FS) are often associated with neuronal lesions and neuronal cell loss [15,19]. It is believed that many different mechanisms are involved in the central nervous system damage. One of the crucial mechanisms leading to neuronal cell death is glutamate-induced excitotoxity [20]. The latest studies seem to verify the opinion that the mechanism of neuronal cell death occurs in the form of necrosis or apoptosis. It has been increasingly recognized that cell death molecular mechanisms are highly diverse. These two processes often occur simultane-

	Control group	TPM+FS group	FS+TPM group	FS group
CA1 sector	88	69	53*	36***
CA3 sector	68	49	39*	34**

Table1. Results of morphometric investigation in sector CA1 and sector CA3 of hippocampal cortex. The mean number of pyramidal neurons in the high power field (hpf) is presented

Statistical significance vs control group: *p<0.05; **p<0.01; ***p<0.001

ously and are referred to as aponecrosis [21,22]. The existence of multiple cell death pathways with both overlapping and distinct molecular mechanisms suggests that neuroprotective strategies should optimally be directed at multiple targets. Therefore, the choice of TPM, a compound with a potential neuroprotective effect, for our experiment was not incidental. TPM has several mechanisms of action that may contribute to its anticonvulsivant and neuroprotective activity [23], including antagonistic effects on glutamate receptors of the kainate/AMPA subtype, which play essential role in the excitotoxic neuronal damage [24,25].

It has been shown in experimental models that prolonged or recurrent convulsions may lead to the death of sensitive hippocampal neurons and that the risk of such complications correlates with the duration of convulsion incidents. It has been observed in the limbic model of the epileptic state in rats that the longer the epileptic state the more severe neuronal damage and that the greater loss of neurons the more frequent convulsion episodes [26].

In our experiment, extensive areas showing loss of pyramidal neurons was the predominant pathology in the histological picture, especially in the CA1 and CA3 sectors. In the morphometric investigations we demonstrated that hyperthermiainduced convulsions caused loss of 60% of neurons in the CA1 sector and death of 50% in the CA3 sector. In rats receiving TPM before FS, the loss of neurons was considerably smaller (20 and 28% of pyramidal neurons of CA1 and CA3 sectors respectively), which confirms the neuroprotective properties of the drug. Only very small neuroprotective effect was observed when TPM was administrated after the FS. Our results are comparable to the findings obtained by Rigoulot et al., who assessed neuroprotective properties of TPM in the experimental model of epilepsy [27]. In our study, neuronal abnormalities most frequently presented as dark shrunken pyramidal cells with features of sclerosis, more seldom with features of chronic lesion. Sclerotic neurons were most numerous in the animals not receiving the drug, while in the remaining two groups, such cells were fewer and more diffused. Hippocampal neurons were sometimes enclosed by white matter whose structure was loosened. Neuronal changes visualized in the current experiment are consistent with those described by Jiang et al. [15]. We also observed differentiated pathomorphological changes and lack of enhanced glial reactions in the CA1 and CA3 sectors of the hippocampal cortex. Similar changes were described by Gadamski and Lasocki in another model of hyperthermic damage in rabbits [28]. There are numerous reports on the neuroprotective effect of TPM in various models of neuronal damage, but not

to febrile seizures. The current study seems to be the first report on neuroprotective effects of TPM in the experimental model of febrile seizures.

Conclusions

Our findings indicate a very strong unfavorable effect of FS on the hippocampal cortical neurons in young rats. Induced FS caused advanced neurodegenerative changes in the pyramidal neurons of the hippocampal CA1 i CA3 sectors, with more than 50% neuronal loss. We also showed a beneficial effect of TPM on this process. TPM exerts a neuroprotective effect on the Ammonal cortex neurons in the rat, especially when administered before FS. Its beneficial action is mainly reflected in a higher number of survival neurons as compared to the untreated animals. Our results are very promising and may have clinical implications. Topiramate could be applied to prevent the effects of long-lasting and recurrent FS in children, thus extending the list of currently used preparations (benzodiazepine, phenobarbital).

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