



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

Journal homepage: www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60082-1

Protective effect of topiramate on hypoxic–ischemic brain injury in neonatal rat

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ARTICLE INFO

Article history:

Received 10 December 2013

Received in revised form 15 January 2014

Accepted 15 May 2014

Available online 20 June 2014

Keywords:

TPM

Brain Injury

NSE

GABA

ABSTRACT

Objective: To explore protective effect of topiramate (TPM) on hypoxic–ischemic brain injury.**Methods:** A total of 360 neonatal rats were selected then randomly divided into sham operation group, ischemia and hypoxia group, conventional treatment group and degradation therapy group ($n=90$). After surgical treatment, sham and ischemic hypoxia group were treat with normal saline; conventional treatment group was received TPM solution 100 mg/kg, 2 times/d; degradation therapy group received TPM solution 150 mg/kg, 2 times/d, per 3 d treatment each dosage was reduced 50 mg/kg, the lowest reduced to 50 mg/kg. Four groups received continuous treatment for 10 d. After treatment for 1 d, 4 d, 7 d, 10 d the cerebral edema, neuron–specific enolase (NSE) and γ –aminobutyric acid (GABA) levels and cognitive abilities of four groups were observed.**Results:** After 1 d, 4 d of treatment, the brain water content and NSE levels in ischemia and hypoxia group, the conventional treatment group and the degradation therapy group were significantly higher than that in sham group ($P<0.05$), the brain water content and NSE levels of the conventional treatment group and the degradation therapy group were significantly lower than that in the ischemic hypoxia group ($P<0.05$). GABA levels and learning ability of the ischemia and hypoxia group, the conventional treatment group and degradation therapy group were significantly lower than the sham group ($P<0.05$), the GABA levels and learning ability of the conventional treatment group and degradation therapy group were significantly higher than the ischemia and hypoxia group ($P<0.05$). After 7 d, 10 d of treatment, the brain water content and NSE levels in the sham operation group, the conventional treatment group and degradation therapy group were significantly lower than the ischemia and hypoxia group ($P<0.05$), while the GABA levels and learning ability of these three groups were significantly higher than that in the ischemia and hypoxia group ($P<0.05$), the GABA levels in the conventional treatment group were significantly higher than degradation therapy group ($P<0.05$); After 10 d of treatment, the GABA levels of the conventional treatment group were significantly higher than the sham group, the learning ability of the degradation therapy group and sham operation group were significantly higher than the conventional treatment group ($P<0.05$). **Conclusions:** The correct amount of short–term TPM has protective effect on hypoxic–ischemic brain injury, but long–term or excessive use may cause new damage to the brain and reduce the cognitive ability.

1. Introduction

Neonatal hypoxic–ischemic brain damage (HIBD) is a relatively common malignant complications caused by clinical perinatal asphyxia[1,2]. After brain injury, there will

be brain edema, functional damage of nerve cells and nerve cell apoptosis[3,4] and other serious consequences. Improper treatment will become a major obstacle to the development of the nervous system of the neonatal, resulting in children mental retardation, epilepsy and cerebral palsy and other serious neurological disease. Serious patients will lead to death[5]. How to protect the cranial nerve of neonatal hypoxic–ischemic brain injury and promote the recovery of nerve cell function has been a research topic for medical workers. But HIBD pathogenesis is still inconclusive and its treatment is still not effective[6], the effect is not ideal. In

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Foundation project: It is supported by Social Development Science and Technology Research Projects of Shaanxi Province (2011 ks–30).

this study, we observed the effect of TPM different dose on hypoxic–ischemic brain injury in neonatal rats, in order to seek more effective treatment for HIBD.

2. Materials and methods

2.1. Animal grouping

A total of 360 7 d neonatal rats provided by the Experimental Animal Center of Medical College of Xi'an Jiaotong University were selected, weighting 12–16 g. They were randomly divided into sham operation group, ischemia and hypoxia group, the conventional treatment group and degradation therapy group ($n=90$).

2.2. Methods

2.2.1. Established models

Rats in the ischemia and hypoxia group, the conventional treatment group and degradation therapy group received 10% concentration of chloral hydrate 3 mg/kg intraperitoneal anesthesia and fixed in the operation board at the supine posture; under a 2 mm middle anterior neck incision and extended 1 mm to the right, after blunt separation of bilateral common carotid arteries and right commonjugular veins, proximal end of arteries and the distal end of the veins was ligated, and 2 mL 2.5 μ /mL heparin saline was infused. Then 20% blood in rats were drawn out to make ischemia, after 20 min they received blood infusion and the incision was sutured. The rats were exposed to 8% oxygen–92% nitrogen gas mixture for 2 h^[8]. In Sham group only the vascular was isolated after anesthesia without ischemia and hypoxia treatment.

2.2.2. Topiramate (TPM) treatment

After the treatment, the sham group and ischemic hypoxia group received saline by gastric perfusion. The conventional treatment group were given with 2% TPM (Xian–Janssen Pharmaceutical Ltd., batch number: 080121230, size: 25 mg/tablet) by gastric perfusion, 100 mg/kg, 2 times/d; degradation therapy group received TPM solution 150 mg/kg, 2 times/d, per 3 d treatment each dosage was reduced to 50 mg/kg, the lowest reduced to 50 mg/kg. Four groups received continued treatment for 10 d, with the same volume of gavage solution.

2.2.3. Specimen collection

After treatment of 1 d, 4 d, 7 d, 10 d, 20 rats in each group were randomly selected and Y–type water maze was used to test their learn ability, then the brain tissue was obtained to detect cerebral edema and neuron–specific enolase (NSE)

and γ –aminobutyric acid (GABA) levels.

Y water maze was applied to determine the ability of learning and memory. The water maze was consisted of a diameter 130 cm, height 50 cm circular pool and a “Y–type” device. “Y–type” device had a 40 cm long arm track and two short 15 cm arm, the top of one short arm was a resting platform for young rats, the other short arm had underground passage and the top was unobstructed. During the test, the depth was 30 cm and temperature was (25 ± 1.2) °C, the outer wall was pasted with reference. Rats were put into the water with the back towards of the “Y–type” device. Young rats climbed to the crossroad of “Y–type” device was recorded as one exercise, continue to exercise after break for 30 s. Rats had exercise 10 times in a group. When nine times of a group reached the standards of exercise, the total number of exercise minus 10 times was used as training times. All the training times were recorded to reach the required standard; the average number was used as training times. The less training times, the stronger learning ability of young rats^[9].

2.2.5. Determination of brain water contents^[10]

10 rats of each group received 10% chloral hydrate 3 mL/kg anesthesia by intraperitoneal injection^[11], the brains were removed after cardiac perfusion of saline. Rhinencephalon and hindbrain was removed, wet weight of brain tissue was measured by electronic scales (accurate to 1 mg, temperature 25 °C, humidity 65%). They were placed at 80 °C thermostatic drying chamber drying, constant weight (2 times measured value difference <0.2 mg) was measured dry weight, brain water content (%) = (wet weight – dry weight) / wet weight \times 100%.

2.2.6. NSE and GABA levels detection

Another 10 rats of each group were selected and the cardiac blood were collected after the anesthesia, the NSE serum levels^[12] were detected with double–antibody sandwich assay. NSE determination steps were as follows: Samples were fully integrated with NSE antibody on the coated ball, then combined with 125I anti–NSE to form immune complexes, determined the pulses of coated ball by immune counter as the NSE content. The animals were fixed by cardiac perfusion and the brain was obtained. Concentration 40 g/L paraformaldehyde was placed for 2 h and in distilled water for 4 h. Fixation, dehydration, transparency, waxing and embedding were carried out. GABA was detected by SABC markers^[13]. All I antibody, anti–II poly peptides were purchased from Shanghai Biological Technology Co. BX–51 optical microscope (Olympus, Japan produced) was used to observe hippocampus CA1 region slices. In each slice the average of positive cells was taken from 3 visions and expressed as positive neurons/high–power vision.

2.3. Statistical analysis

The database was built in Excel 2007. All data was analyzed with SPSS 18.0 software. Data were expressed as mean±SD values, Dunnett-*t* method was used for multiple comparison ($P<0.05$) was considered as statistical significant difference.

3. Results

3.1. Brain water content of four groups

By Dunnett-*t* test, after 1 d, 4 d of treatment, the brain water content and NSE levels in ischemia and hypoxia group, the conventional treatment group and the degradation therapy group were significantly higher than that in sham group ($P<0.05$) (Dunnett-*t* critical values were 2.647, 2.386, 2.388 and 4.256, 2.504, 2.501, respectively). The brain water content of the treatment group and the degradation therapy group were significantly lower than that in the ischemic hypoxia group ($P<0.05$); (Dunnett-*t* critical values were 2.164, 2.165 and 2.158, 2.153). There was no significant difference between the treatment group and the degradation therapy group (Dunnett-*t* boundary values were 0.938 and 0.940, $P>0.05$); After 7 d, 10 d of treatment, the brain water content in the sham operation group, the conventional treatment group and degradation therapy group were significantly lower than the ischemia and hypoxia group ($P<0.05$), (Dunnett-*t* critical values were 3.142, 3.139, 3.144 and 2.651, 2.674, 2.669). There was no significant difference between the treatment group, the degradation therapy group and the sham operation group (Dunnett-*t* critical values were 0.973, 0.985 and 0.986, 0.978, $P>0.05$).

3.2. NSE, GABA levels of rats in four groups

After 1 d, 4 d of treatment, the NSE levels in ischemia and

hypoxia group, the conventional treatment group and the degradation therapy group were significantly higher than that in sham group ($P<0.05$) (Dunnett-*t* critical values were 4.685, 3.572, 2.575 and 4.108, 2.856, 2.857 respectively), GABA levels were significantly lower than the sham group (Dunnett-*t* critical values were respectively 2.793, 2.438, 2.441 and 2.750, 2.194, 2.189, $P<0.05$); the NSE levels of the conventional treatment group and the degradation therapy group were significantly lower than that in the ischemic hypoxia group ($P<0.05$) (Dunnett-*t* critical values were 2.835, 2.837 and 3.485, 3.491); The GABA levels were significantly higher than that in the ischemia and hypoxia group (Dunnett-*t* critical values were respectively 2.167, 2.168 and 2.54, 2.55, $P<0.05$). After 7 d, 10 d of treatment, the NSE levels in the sham operation group, the conventional treatment group and degradation therapy group were significantly lower than the ischemia and hypoxia group ($P<0.05$) (Dunnett-*t* critical values were 4.089, 3.516, 3.514 and 3.927, 3.748, 3.742, while the GABA levels of these three groups were significantly higher than that in the ischemia and hypoxia group ($P<0.05$) (Dunnett-*t* critical values were 2.696, 2.625, 2.598 and 2.547, 2.634, 2.573). The GABA levels of the conventional treatment group were significantly higher than the degradation therapy group (Dunnett-*t* critical values were respectively 2.376 and 2.354, $P<0.05$). After 10 d of treatment, the GABA levels of the conventional treatment group were significantly higher than the sham group (Dunnett-*t* critical values was 2.093, $P<0.05$, Table 2).

3.3. Y-type maze experiment results of rats in four groups

After 1d, 4d of treatment, the learning ability of the ischemia and hypoxia group, the conventional treatment group and degradation therapy group were significantly lower than the sham group ($P<0.05$) (Dunnett-*t* critical values were 3.458, 2.934, 2.940 and 3.315, 2.574, 2.579), the

Table 1
Brain water content of four groups (mean±sd).

Groups	Brain water content (%)			
	1 d	4 d	7 d	10 d
Sham operation group	71.2±0.5	71.5±0.6	71.0±0.5	71.2±0.4
Ischemia and hypoxia group	79.4±0.8	85.6±0.9	81.7±0.7	75.2±0.5
Conventional treatment group	76.5±0.7	77.9±0.6	71.1±0.5	70.9±0.6
Degradation therapy group	76.6±0.6	77.6±0.6	70.8±0.6	71.0±0.5

Table 2
NSE, GABA levels of rats in four groups (mean±sd).

Groups	NSE (ng/mL)				GABA positive neurons(n)			
	1 d	4 d	7 d	10 d	1 d	4 d	7 d	10 d
Sham operation group	2.3±0.5	2.2±0.5	2.3±0.6	2.3±0.5	8.6±1.0	8.5±1.2	8.6±0.9	8.5±1.1
Ischemia and hypoxia group	8.7±1.2	7.8±1.0	7.3±0.9	6.5±0.7	5.1±0.6	5.3±0.7	5.4±0.8	5.8±0.9
Conventional treatment group	5.8±0.8	3.9±0.7	3.3±0.6	2.5±0.5	6.3±0.9	7.6±0.9	8.4±1.1	8.9±1.2
Degradation therapy group	5.9±0.8	4.1±0.7	3.3±0.7	2.4±0.5	6.4±0.8	7.5±0.9	7.9±0.9	8.4±1.0

Table 3

Y-type maze experiment results of rats in four groups.

Groups	Learning ability (times)			
	1 d	4 d	7 d	10 d
Sham operation group	24.7±2.5	25.0±2.3	24.8±2.4	25.2±2.5
Ischemia and hypoxia group	35.4±3.2	34.8±3.0	32.6±2.9	31.4±2.8
Conventional treatment group	30.2±2.6	28.5±2.5	28.0±2.5	29.3±2.7
Degradation therapy group	30.4±2.7	28.7±2.6	26.8±2.6	25.0±2.6

learning ability of the conventional treatment group and degradation therapy group were significantly higher than the ischemia and hypoxia group ($P<0.05$) (Dunnett- t critical values were 2.287, 2.293 and 2.352, 2.357). After 7d, 10d of treatment, learning ability of the sham operation group, the conventional treatment group and degradation therapy group were significantly lower than the ischemia and hypoxia group ($P<0.05$) (Dunnett- t critical values were 3.269, 2.476, 2.875 and 2.792, 2.271, 3.277), the learning ability of the degradation therapy group and sham operation group were significantly higher than the conventional treatment group ($P<0.05$) (Dunnett- t critical values were 2.376 and 2.354).

4. Discussion

Hypoxic-ischemic brain damage refers to neonatal perinatal asphyxia caused partial or complete anoxia, cerebral blood transport reduced or suspended, eventually lead to neonatal brain damage, which is the main reason of newborn deaths and nervous system development disorders. Brain damage can cause cerebral ischemia, hypoxia, vasospasm and other diseases, while ischemia and hypoxia will increase cerebral edema, which will further aggravate brain damage^[4].

TPM is a new antiepileptic drug which have a good effect on a variety of epilepsies, the possible mechanism maybe: enhance GABA-mediated nerve inhibition through the action the GABA-A receptor site^[15]; block Na⁺ channel voltage to restrict the sustaining discharge; inhibit excitatory glutamate by blocking the glutamate receptor and regulate the Ca²⁺ channel^[16]. In protecting brain injury, TPM as a neuroprotective agent has an effectively protective effect on neuronal injury after ischemia, its mechanism may be: TPM block Na⁺ channel voltage and glutamate (Glu) receptor isoxazole propionic acid (AMPA)/kainate (IAA) receptors' action, thereby inhibiting the release of excitatory amino acids to prevent intracellular imbalance which induced by the inflow of Na⁺^[17].

At present, there are lots of research of TPM treatment on brain injury and epilepsy, but it has not been determined. The study of Dong *et al*^[18] showed that during the PM treatment of epilepsy, it can also restrict the damage of brain cells caused by epilepsy and protect brain. In the study of Songxing *et al*^[19], TPM can stimulate the activity of GABA

and enhance GABA receptor frequency. The study of Liu *et al*^[20] showed TPM can increase brain GABA levels and further balance Glu excitability, and may have a role as neuroprotective agents. The study of Wang *et al*^[21] showed that TPM has a good effect in the treatment of epilepsy and can protect nerve function, which is the drug of choice to treat pediatric patients with epilepsy. The study of Zhang *et al*^[22] showed that TPM has significant neuroprotective effect in the treatment of brain injury caused by cerebral artery embolization, TPM can reduce neuronal death and apoptosis, ease glial cell proliferation. However, study of Sun *et al*^[23] showed that after TPM treatment, patients' comprehensive IQ, verbal IQ and performance IQ decreased significantly than before treatment, among them the verbal IQ decreased most significantly. There are similar studies abroad; Salazar *et al*^[24] believes that the side effects of taking anti-epileptic drugs is proportional to the time of taking TPM. The longer of treatment, the more serious effects on cognitive function in patients; It is also mentioned in Battaglia^[25] study, the verbal IQ was significantly reduced in patients with long-term TPM treatment.

Most studies domestic and foreign showed that TPM can reduce sodium levels of hypoxic-ischemic brain tissue, relieve cerebral edema, reduce death or apoptosis of brain neurons, effectively inhibited the generation of oxygen free radicals and toxic, have a good protective effect after ischemic brain damage. However, the study about the route of administration time targets, dose, its future impact and the brain protective effect is still less^[26], the conclusion is still not clear.

In this study, we observed the treatment effect of different doses at different time. After 4d of treatment, the brain water content, NSE, GABA of the conventional treatment group were (76.5±0.7)%, (3.9±0.7) ng/mL, (7.6±0.9)n, respectively, those in the degradation therapy group were (77.6±0.6)%, (4.1±0.7)ng/mL, (7.5±0.9)n, respectively, there was no significant difference between the two groups ($P>0.05$). That showed TPM can relieve cerebral edema, increased NSE, enhance GABA and increase the learning ability of rats. This is consistent with the results of most current researches and indicate that short-term TPM treatment have a protective effect on the brain. However, as the treatment time increases, especially after 10 d, the GABA and learning ability of conventional treatment group were (8.9±1.2) n, (29.3±2.7) times, while in the degradation therapy group were

(8.4 ± 1.0), (25.0 ± 2.6) times, the differences between the two groups have statistically significant ($P < 0.05$). That showed with the prolong of TPM treatment, DABA levels continue to increase, but the learning ability decreased. GABA is the neurotransmitter which closely linked with the cognitive function. The high GABA levels may have a negative effect on cognitive function, which is similar with the result of Li et al[9]. Its internal cause is still unknown.

In summary, the correct amount of short-term TPM have a protective effect on hypoxic-ischemic brain injury, but the long-term or excessive use may cause new damage to the brain, decreased the cognitive ability.

Conflict of interest statement

We declare that we have no conflict of interest.

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