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Research Report

Topiramate attenuates early brain injury following subarachnoid haemorrhage in rats via duplex protection against inflammation and neuronal cell death

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ABSTRACT

Early brain injury (EBI) following aneurysmal subarachnoid haemorrhage (SAH) insults contributes to the poor prognosis and high mortality observed in SAH patients. Topiramate (TPM) is a novel, broad-spectrum, antiepileptic drug with a reported protective effect against several brain injuries. The current study aimed to investigate the potential of TPM for neuroprotection against EBI after SAH and the possible dose-dependency of this effect. An endovascular perforation SAH model was established in rats, and TPM was administered by intraperitoneal injection after surgery at three different doses (20 mg/kg, 40 mg/kg, and 80 mg/kg). The animals' neurological scores and brain water content were evaluated, and ELISA, Western blotting and immunostaining assays were conducted to assess the effect of TPM. The results revealed that TPM lowers the elevated levels of myeloperoxidase and proinflammatory mediators observed after SAH in a dose-related fashion, and the

Abbreviations: AED, antiepileptic drug; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; BBB, blood–brain barrier; EBI, early brain injury; GABA, γ-aminobutyric acid; ICA, internal carotid arter; IF, immunofluorescence; KCC2, K^+–Cl^- co-transporter 2; MPO, myeloperoxidase; NeuN, neuron-specific nuclear protein; NKCC1, Na^+–K^+–Cl^- co-transporter 1; NF-κB, nuclear factor-kappa B; SAH, subarachnoid haemorrhage; TPM, topiramate; ELISA, enzyme-linked immunosorbent assay; TUNEL, deoxynucleotidyl transferase-mediated dUTP nick-end labeling

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nuclear factor-kappa B (NF-κB) signalling pathway is the target of neuroinflammation regulation. In addition, TPM ameliorated SAH-induced cortical neuronal apoptosis by influencing Bax, Bcl-2 and cleaved caspase-3 protein expression, and the effect of TPM was enhanced in a dose-dependent manner. Various dosages of TPM also upregulated the protein expression of the γ-aminobutyric acid (GABA)-ergic signalling molecules, $GABA_A$ receptor ($GABA_AR$) α1, $GABA_AR$ γ2, and K^+–Cl^- co-transporter 2 (KCC2) together and downregulated Na^+–K^+–Cl^- co-transporter 1 (NKCC1) expression. Thus, TPM may be an effective neuroprotectant in EBI after SAH by regulating neuroinflammation and neuronal cell death.

1. Introduction

Subarachnoid haemorrhage (SAH), secondary to ruptured aneurysms, is a devastating subtype of stroke that can result in poor prognosis and high mortality and occurs in approximately 5–7% of all stroke cases (Ansar et al., 2011). According to previous studies, early brain injury (EBI) usually occurs within 72 h after SAH and may contribute to the poor outcomes observed in SAH patients (Cahill et al., 2006; Chen et al., 2014b). Several mechanisms are likely involved in the pathophysiological development of EBI after SAH, including increased intracranial pressure, reduced cerebral blood flow, blood–brain barrier (BBB) disruption, brain oedema, and neuronal cell apoptosis (Cahill et al., 2006; Hasegawa et al., 2011; Ostrowski et al., 2006). In addition, inflammation also contributes to the induction of brain oedema in EBI after SAH and other types of brain injury, which may be attributed to multiple pro-inflammatory cytokines (Chen et al., 2014a, 2014b.

Antiepileptic drugs have long been investigated for their possible neuroprotective effects in brain injuries, as their mechanisms of action are related to the regulation of cell death and neuroinflammation. Topiramate (TPM) is a broad-spectrum antiepileptic drug (AED) that exerts its anticonvulsant effect by enhancing γ-aminobutyric acid (GABA)ergic activity and by inhibiting kainite receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) glutamate receptors and voltage-sensitive sodium and calcium channels (Borowicz et al., 2003; Guerrini and Parmeggiani, 2006; Seckin et al., 2009). Prior research has verified the neuroprotective effects of TPM in excitotoxic brain damage, traumatic brain injury, periventricular leukomalacia and cerebral ischemia-reperfusion injury models in vivo (Follett et al., 2004; Gensel et al., 2012; Kouzounias et al., 2011; Mao et al., 2012; Seckin et al., 2009; Sfaello et al., 2005). Seckin et al. (2009) also showed that the post-injury administration of TPM can attenuate delayed hippocampal apoptosis and basilar artery vasospasm in an experimental SAH rabbit model. In addition, the early application of TPM induces a therapeutic effect on transient learning deficits after intracerebral haemorrhage (McDaniel et al., 2007). Given the above-described pharmacological properties, we hypothesized that TPM protects against EBI after SAH and explored its potential dose-dependency and mechanism of action based on an experimental rat SAH model.

2. Results

2.1. Mortality and SAH grade

There were no significant differences in mortality between the SAH+Vehicle group (26.67% [8 of 30]) and each of the SAH+TPM groups (20 mg/kg: 30.00% [9 of 30], $p=0.833$; 40 mg/kg: 23.33% [7 of 30], $p=0.833$; 80 mg/kg: 26.67% [8 of 30], $p=1.000$; all vs. SAH+Vehicle group), whereas none of the sham-operated rats died (Fig. 1A). In all the SAH groups that received vehicle or TPM, the SAH grading score indicated similar levels according to the results of the assessment of the perfused sample (Sham: 0; Vehicle: 14.55±2.22; TPM 20 mg/kg: 14.10±1.97, 40 mg/kg: 14.00±2.47, 80 mg/kg: 14.41±2.54) (Fig. 1B).

2.2. Neurological score and water content

Compared with the sham group (20.45±1.39), SAH induced a significant reduction in the neurological score in the SAH+Vehicle group (11.09±2.29) and the three SAH+TPM groups (20 mg/kg: 13.24±2.79; 40 mg/kg: 14.65±3.28; 80 mg/kg: 15.18±3.38) ($p<0.05$, vs. Sham) (Fig. 1C). Moreover, TPM treatment induced a gradual improvement after dosing. Marked increases were observed in the SAH+TPM 40 mg/kg group ($p<0.05$, vs. Vehicle) and the SAH+TPM 80 mg/kg group ($p<0.05$, vs. Vehicle) (Fig. 1C).

To assess brain oedema, the brain water content was measured 24 h after SAH. As shown in Fig. 1D, the brain water content significantly increased in the SAH+Vehicle group (Vehicle: 79.99±1.57%) ($n=6$, $p<0.05$, vs. Sham: 76.34±1.81%). Although low doses of TPM (20 mg/kg) slightly reduced this elevation induced by SAH (20 mg/kg: 79.92±1.71%) ($n=6$, $p>0.05$, vs. Vehicle), both the moderate and high doses (40 mg/kg and 80 mg/kg) induced more significant relief (40 mg/kg: 76.91±1.77%; 80 mg/kg: 76.94±1.84%) ($n=6$, $p<0.05$, vs. Vehicle).

2.3. TPM treatment dose-dependently influenced neuroinflammation at 24 h after SAH in rat brains through regulation of the NF-κB signalling pathway

As an indicator of the infiltration of neutrophils, MPO was selected to confirm the anti-inflammatory effect of TPM after SAH. The ELISA results illustrated that the MPO level in the