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Tetramethylpyrazine reduces cellular inflammatory response following permanent focal cerebral ischemia in rats

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ABSTRACT

Tetramethylpyrazine (TMP) has been used to treat ischemic stroke. However, scientific evidence related to its effectiveness or precise modes of neuroprotective action is largely unclear. This study provides evidence of an alternative target for TMP and sheds light on the mechanism of its physiological benefits. We report a global inhibitory effect of TMP on intracerebral cellular inflammatory response in a rat model of permanent cerebral ischemia. TMP exhibited a neuroprotective effect against ischemic deficits by reduction of behavioral disturbance, brain infarction, and edema. The results of immunohistochemistry, enzymatic assay, Western blot, real-time reverse transcriptase-polymerase chain reaction (RT-PCR), and flow cytometric analysis revealed that TMP reduced the percentages of activated macrophages/microglia and infiltrative lymphocytes, neutrophils, and macrophages and pro-inflammatory cytokine expression after cerebral ischemia. In parallel with these immunosuppressive phenomena, TMP also attenuated the activities of ischemia-induced inflammation-associated signaling molecules and transcription factors. Another finding in this study was that the anti-inflammatory and neuroprotective effects of TMP were accompanied by a further elevated expression of NF-E2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) in ipsilateral neurons and macrophages/microglia after cerebral ischemia. Taken together, our results suggest that both the promotion of endogenous defense capacity and the attenuation of the extent and composition percentage of the major cellular inflammatory responses via targeting of macrophages/microglia by elevating Nrf2/HO-1 expression might actively contribute to TMP-mediated neuroprotection against cerebral ischemia.

Introduction

Stroke is a leading cause of morbidity and mortality worldwide. Early reperfusion strategies remain the treatment of choice to protect the brain against ischemic stroke, but they can also initiate and augment an inflammatory response causing secondary brain damage (Jin et al., 2010; Moxon-Emre and Schlichter, 2010). Ischemic stroke triggers a complex cellular response which includes both the activation of local glial cells and the recruitment of inflammatory cells from the systemic circulation. Despite the potential contribution to neuroprotection and regeneration, the inflammatory response following ischemic brain injury is assumed to contribute significantly to the pathogenesis and outcome of stroke. There is a considerable body of evidence suggesting that the activation of resident glial cells, the infiltration of leukocytes, and the consequences of pro-inflammatory and neurotoxic mediator production in particular are detrimental for the injured brain and contribute to infarct evolution (Garcia et al., 1994; Gelderblom et al., 2009; Schilling et al., 2003; Stoll et al., 1998). Supporting evidence further shows that deletion or inhibition of leukocytes, depletion of adhesion molecules, or immunodeficiency leads to diminished infiltration of leukocytes, decreased pro-inflammatory mediator production, and reduced brain infarction (Connolly et al., 1996; Hurn et al., 2007; Satoh et al., 1999; Schuette-Nuetgen et al., 2012; Soriano et al., 1999; Yenari

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Table 1
Effects of TMP on physiological parameters.

	Sham		Ischemia	
	Vehicle	TMP	Vehicle	TMP
Before occlusion				
Body weight (g)	308 ± 10	309 ± 10	310 ± 9	307 ± 8
pH	7.32 ± 0.07	7.32 ± 0.06	7.31 ± 0.07	7.33 ± 0.05
pCO_2 (mm Hg)	64.4 ± 12.4	64.4 ± 9.9	66.6 ± 11.3	61.4 ± 7.9
pO_2 (mm Hg)	78.0 ± 9.2	74.3 ± 7.0	70.8 ± 7.8	73.6 ± 7.7
Hct (%)	40.9 ± 3.3	39.9 ± 2.0	40.0 ± 2.4	40.1 ± 1.5
Glucose (mg/dl)	135 ± 8	131 ± 10	136 ± 8	133 ± 16
3 days after occlusion				
Body weight (g)	326 ± 9	323 ± 7	301 ± 9**	306 ± 11*
pH	7.33 ± 0.06	7.32 ± 0.03	7.32 ± 0.04	7.29 ± 0.07
pCO_2 (mm Hg)	55.5 ± 14.3	59.9 ± 13.7	60.2 ± 8.3	63.5 ± 9.1
pO_2 (mm Hg)	73.0 ± 12.1	71.7 ± 6.1	72.4 ± 6.5	72.4 ± 7.5
Hct (%)	37.7 ± 4.4	39.8 ± 1.4	41.1 ± 0.9	41.8 ± 2.3
Glucose (mg/dl)	137 ± 6	132 ± 2	109 ± 9**	107 ± 6**

Rats were subjected to sham operation or permanent ischemia receiving saline vehicle or TMP administration (30 min before occlusion and 60 min after occlusion, 20 mg/kg). Physiological parameters were measured before occlusion and 3 days after occlusion. Data are expressed as means ± SD, n = 5 (each group). *p < 0.05 and **p < 0.01 *vs.* sham vehicle. Hct, hematocrit; glucose, plasma glucose.

et al., 1998). Besides, immunosuppressive pharmacological agents have the ability to attenuate ischemic brain injury (Kao et al., 2010; Lee et al., 2007; Li M et al., 2012). Therefore, inflammatory mechanisms which are activated after brain ischemia might play an important role in the pathogenesis of brain injury secondary to ischemia and represent a key target of current translational cardiovascular researches.

Nowadays, the use of medicinal plants is becoming an increasingly attractive approach as a complement and an alternative for treating acute and chronic inflammatory diseases. *Ligusticum wallichii* Franchat (Chuan Xiong) is one such plant. Both the herb and its extract are applied in the treatment of neurovascular and cardiovascular diseases. Additionally, 2,3,5,6-tetramethylpyrazine (TMP) is one of the most important active ingredients of Chuan Xiong, which is synthesized and widely used in the treatment of ischemic stroke (Lu et al., 1978). The beneficial effects of TMP against ischemic tissue injury including that in the brain have been demonstrated in several diseased animal models (Chang et al., 2007; Fan et al., 2006, 2011; Feng et al., 2011; Ho et al., 1989; Jia et al., 2009; Kao et al., 2006; Liao et al., 2004; Xiao et al., 2010). TMP has been reported to possess a diverse array of pharmacological functions in the modulation of arterial resistance, cerebral blood flow, platelet function, microcirculation, and capillary permeability (Dai and Bache, 1985; Feng et al., 1988; Tuttle et al., 1989). Moreover, TMP scavenges free radicals and down-regulates pro-inflammatory cytokine production (Chang et al., 2007; Fan et al., 2011; Feng et al., 2011; Li et al., 2009; Wu et al., 1999; Zhang et al., 2003; Zhao et al., 2003). In spite of the clinical applications and experimental demonstrations of TMP, however, the mechanisms by which it protects the brain are still not clear.

Traditional Chinese medicines have been used to treat stroke for years, but we still know very little about the mechanisms underlying their neuroprotective action. Consideration of the aforementioned properties of TMP leads to speculation that the anti-inflammatory effect might be a crucial mechanism of TMP in neuroprotection against stroke. To extend the scope of relevant studies, we therefore wanted to examine whether TMP treatment would alleviate post-stroke inflammation

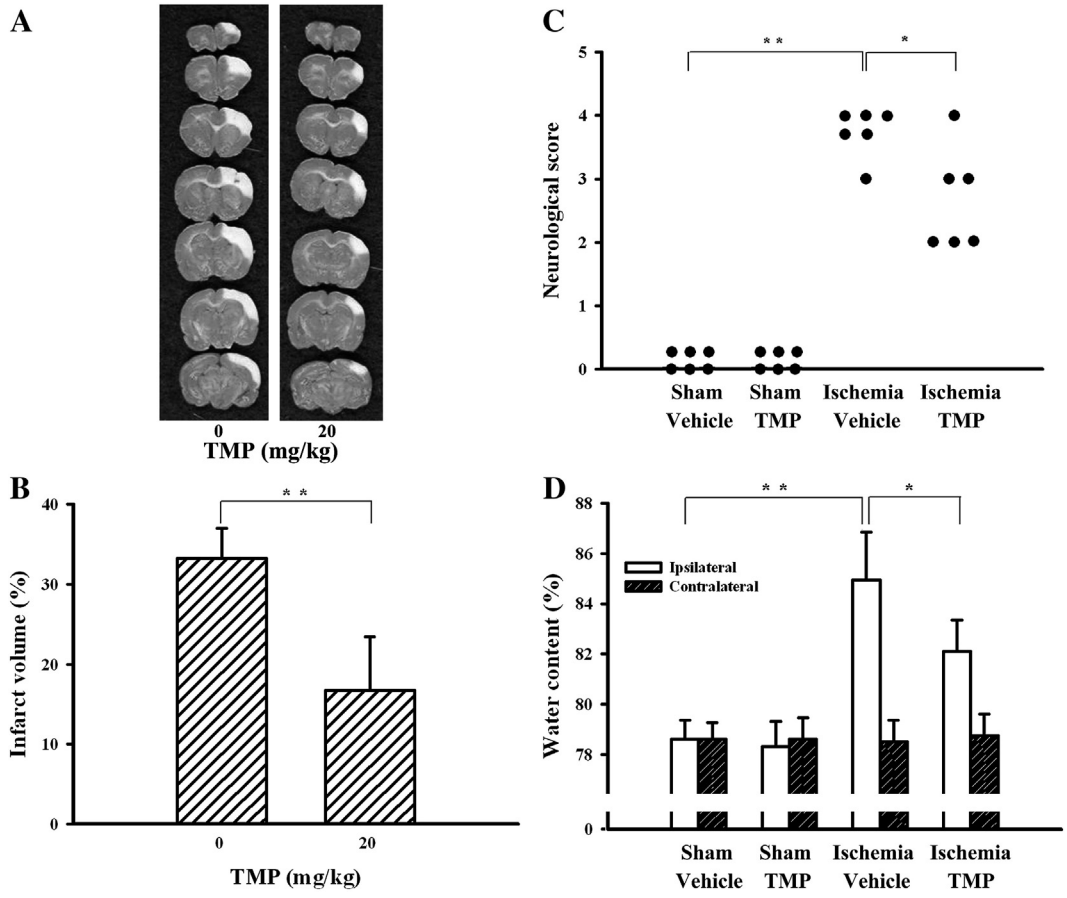

Fig. 1. TMP reduces cerebral ischemia-induced alterations. Rats given saline vehicle or TMP (20 mg/kg) pretreatment were subjected to sham and permanent cerebral ischemia operation for 3 days, respectively. Representative photographs show histological examination of brain infarction in ischemic animals (A). The average percentage of infarct volume in each ipsilateral hemisphere is depicted (n = 6/each group) (B). Neurological deficit was evaluated by neurological score (n = 6/each group) (C). Brain water content was measured in ipsilateral and contralateral cortexes obtained from sham and ischemic animals (n = 6/each group) (D). *p < 0.05 and **p < 0.01.