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ERRATUM

Neural protection by naturopathic compounds—an example of tetramethylpyrazine from retina to brain

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Abstract Given the advantages of being stable in the ambient environment, being permeable to the blood–brain and/or blood–eye barriers and being convenient for administration, naturopathic compounds have growingly become promising therapeutic candidates for neural protection. Extracted from one of the most common Chinese herbal medicines, tetramethylpyrazine (TMP), also designated as ligustrazine, has been suggested to be neuroprotective in the central nervous system as well as the peripheral nerve network. Although the detailed molecular mechanisms of its efficacy for neural protection are understood limitedly, accumulating evidence suggests that antioxidative stress, antagonism for calcium, and suppression of pro-inflammatory factors contribute significantly to its neuroprotection. In animal studies, systemic administration of TMP (subcutaneous injection, 50 mg/kg) significantly blocked neuronal degeneration in hippocampus as well as the other vulnerable regions in brains of Sprague–Dawley rats following kainate-induced prolonged seizures. Results from us and others also demonstrated potent neuroprotective efficacy of TMP for retinal cells and robust benefits for brain in Alzheimer's disease or other brain injury. These results suggest a promising prospect for TMP to be used as a treatment of specific neurodegenerative diseases. Given the assessment of the distribution, metabolism, excretion, and toxicity information that is already available on most neuroprotective naturopathic compounds such as TMP, preclinical data to justify bringing such therapeutic compounds to clinical trials in humans is feasible.

Keywords Naturopathic compounds · Tetramethylpyrazine · Retina · Brain · Neuronal degeneration

Introduction

Neuronal degeneration, i.e., neuronal cell death, underlies the pathology and malfunction of many different neurological diseases occurring in both animals and human beings. Progressive and selective neuronal cell death in the central nervous system (CNS) and/or the peripheral nerve network has been profoundly implicated in the pathogenesis of neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Lou Gehrig's disease or amyotrophic lateral sclerosis, multiple sclerosis, epilepsy, stroke, traumatic injury, age-related macular degeneration, glaucoma, prion diseases, infections, and so on [1, 2]. Evidence is rapidly accumulating to suggest that selective neuronal cell death through necrosis and/or apoptotic mechanisms contributes significantly to the functional anomalies of specific neurologic disorders [1]. Changes of genetic, epigenetic, metabolic, and environmental factors might directly or indirectly cause (1) massive DNA damage, (2) dysfunction of the ubiquitin-proteasomal system, (3) disruption of the axonal transport machinery, (4) abnormalities of mitochondrial structure and function, (4) disturbance of intracellular ionic homeostasis

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(particularly Ca2+ and Zn2+), and (5) accumulation of reactive oxygen species (ROS) in the neuronal cells. Accumulating intracellular stress subsequently results in (1) loss of spines and synapses, (2) fragmentation of neuronal processes and extended neuritic degeneration leading to demyelination, (3) global neuronal cell death leading to activation of signal transduction cascade for programmed cell death, (4) anomalies of microvasculature, and (5) provoked neuroinflammatory response leading to destructive pathogenic changes (see several representative reviews) [1–4]. Accordingly, the main aim of neural protection in neurodegenerative disorders is to retard progression by blocking the mechanisms that lead to neuronal cell death as well as associated neuroinflammatory events. Considerable efforts have been made in recent decades to discover new potential therapeutic compounds that can help to prevent the onset or to slow down the progression of such diseases. Equal attempts are in progress to improve the therapeutic efficacy of known medications through chemical modification. Given the evident advantages of being stable in the ambient environment, being permeable to the blood–brain and/or blood–eye barriers, and being convenient for administration (Fig. 1), naturopathic compounds have increasingly become suitable therapeutic candidates for neural protection from the sensory system including retina to the central nervous system (brain). Table 1 summarizes a group of selected herbal extracts that have demonstrated significant neuroprotective efficacy both in vivo and in vitro. Taking tetramethylpyrazine (TMP) as an example, the neuroprotective efficacy and related issues are discussed here.

Tetramethylpyrazine, an herbal extract showing multiple protective effects on cells and benefits on physiological function

As listed in Table 1, TMP, also designated as ligustrazine, is an alkaloid extracted from the Chinese herbal medicine, *Ligusticum wallichii* Franchat (*chuanxiong*) [5]. For hundreds of years, *chuanxiong* has been used as a traditional Chinese medicine for heart, kidney, and brain diseases [6, 7]. Experimental studies demonstrated that TMP treatments significantly improved cardiac and cerebral blood flow and elevated blood reperfusion as shown in the nail microcirculation [8, 9]. In an ex vivo study, a semi-synthetic form of TMP monomer induced a dose-dependent relaxation of human pulmonary and bronchial arteries [10]. TMP also exhibited a calcium antagonist role in vascular tissues [11]; functioned as a ROS scavenger to deactivate cytotoxic ROS such as superoxide anion (O2−), hydroxyl (OH−), and lipid peroxyl (LOO−) free radicals [12, 13]; and inhibited inflammatory events in vivo possibly through modulating secretion of specific cytokines and nitric oxide-related pathways [14–16].

About two decades ago, a study briefly reported that TMP alleviated ischemic retinal degeneration in vivo [17]. Recently, a different group of researchers demonstrated that systemic injection of TMP significantly protected retinal photoreceptors from loss induced by *N*-methyl-*N*-nitrosourea in rats [18]. Further, we demonstrated that TMP efficiently enhanced in vitro survival of cultured rat retinal cells and significantly attenuated cell damage in these cells exposed to hydrogen peroxide [19].

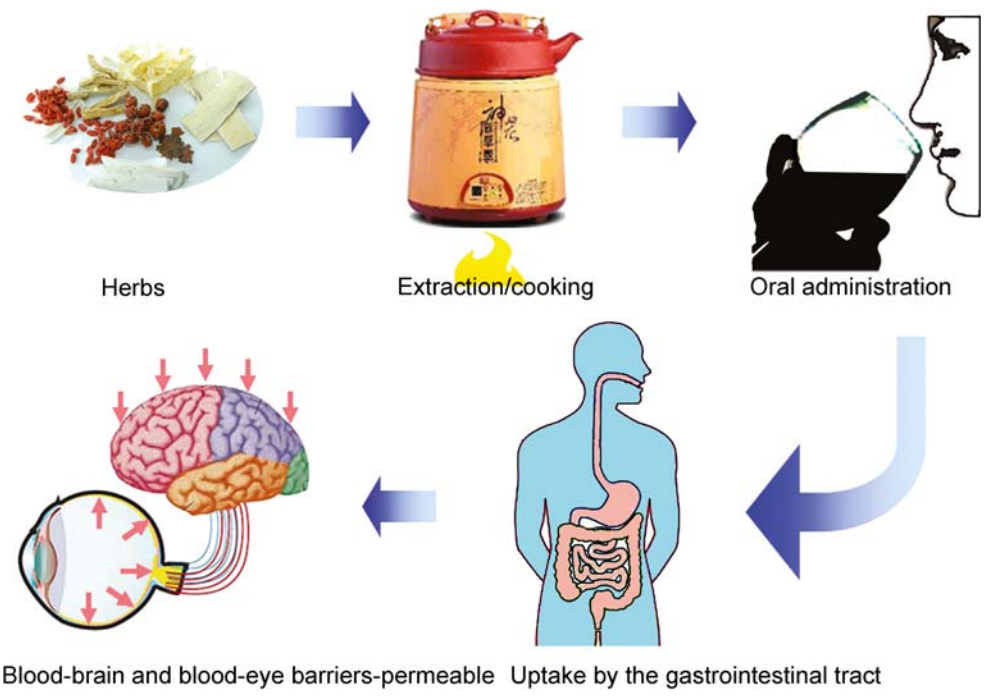

Fig. 1 The conventional route of most natural compounds from herbs to target organs. In the traditional Chinese medicine, the herbal therapeutic ingredients are extracted and prepared as herbal tea or soup with water through regular cooking and taken orally. The effective ingredients are absorbed by the gastrointestinal tract and successfully pass through the blood–brain and/or blood–eye barriers to reach the targets, brain and/or retinas (*graphic clips* were taken from the Internet free sources)

Table 1 List of selected naturopathic compounds that have demonstrated neuroprotective efficacy in both retina and brain

Name	Natural sources (selected)	Structural classification	Selected studies
Baicalein	*Radix scutellariae*	Alkaloids	C [49, 50]; R [51, 52]; B [53, 54]
Chlorogenic acid	*Eucommia* or other plants	Polyphenolics	C [55]; R [56]; B [57, 58]
Curcumin	*Curcuma longa*	Alkaloids	C [59, 60]; R [59, 61]; B [60, 62, 63]
Emodin	Leguminosae seed	Alkaloids	C [64, 65]; R [66]; B [65, 67]
Fisetin	*Rhus cotinus* bark	Polyphenolics	C [68, 69]; R [70]; B [71]
Kaempferol	*Euonymus alatus* or *Impatiens balsamina*	Flavonoids	C [72, 73]; R [70, 74]; B [75]
Ligustrazine	*Ligusticum wallichii* roots	Alkaloids	C [19]; R [76]; B [23, 77]
Morin	The Moraceae family, e.g., mulberry	Flavonoids	C [78]; R [79]; B [80, 81]
Myricetin	*Myrica rubra*	Flavonoids	C [78]; R [74]; B [82]
Naringenin	*Satureja obovata*	Flavonoids	C [83]; R [84]; B [85]
Paeoniflorin	Paeony roots	Polyphenolics	C [86]; R [87]; B [88–90]
Puerarin	*Pueraria lobata* roots	Alkaloids	C [91, 92]; R [93, 94]; B [95, 96]
Pycnogenol	Pinus maritime bark	Flavonoids	C [97]; R [98, 99]; B [100]
Quercetin	*Euonymus alatus*	Flavonoids	C [101, 102]; R [84, 103]; B [104, 105].
Resveratrol	Grapes	Flavonoids	C [106, 107]; R [108]; B [108–110]
Rutin	Buckwheat	Flavonoids	C [111]; R [84]; B [104]
Wogonin	*Scutellaria baicalensis*	Flavonoids	C [112, 113]; R [114]; B [115, 116]

C, in vitro studies conducted in cultured cells; *R*, in vivo studies showing retinal protection; *B*, in vivo studies demonstrating neuroprotective efficacy in the brain

In the CNS, TMP significantly suppressed oxidative stress and attenuated neuronal cell death in neuronal cultures following iron-mediated oxidative damage and glutamate-mediated excitotoxicity [20–22]. Systemic administration of TMP protected neuronal cells against ischemic or traumatic brain or spinal cord injury and promoted functional recovery in rodents and rabbits [23, 24]. Interestingly, systemic administration of TMP also attenuated impairment of learning and memory performance in rodents following D-galactose- or ischemia-induced brain injury [25, 26]. The potential therapeutic efficacy of TMP for AD is further supported by our recent observations about significant improvement of cognitive function as well as cerebral amyloid pathology in the demented Alzheimer's triple transgenic (3xTg-AD) mice (Tan *et al*., unpublished observations). These findings suggest that TMP has potent neuroprotective efficacy.

TMP protects neuronal degeneration in rat brain against excitotoxicity—an experimental study

Systemic administration of the excitotoxin, kainic acid (KA), a glutamate analog, causes prolonged seizures resulting in massive neuronal cell death in rat brain. KA-induced neuronal degeneration is one of the most common animal models for excitotoxic neuronal cell death, which is apparently involved in the pathogenesis of multiple neurodegenerative disorders [27–29]. One-month old male Sprague–Dawley rats were housed and treated according to the National Institutes of Health guidelines for the care and use of laboratory animals and a protocol approved by the UCI Institutional Animal Care and Use Committee. One hour after the onset of seizures following subcutaneous (s.c.) injection of KA (10.5 mg/kg), rats received TMP (50 mg/kg, s.c.) or an equal volume of vehicle. Untreated animals, which received neither KA not TMP, were used as blank controls. All the animals (N=6 each group) were decapitated 24 h after TMP or vehicle injection under CO_2 gas-induced deep anesthesia. The brain was rapidly harvested, frozen, and cryosectioned in the coronal plane at 10 μm. Adjacent sections from each brain were stained with hematoxylin–eosin (H & E) and in situ cell death assay kit for terminal deoxytransferase-mediated dUTP nick end labeling (TUNEL; Roche, Indianapolis, IN, USA), respectively, as described in our previous work [30, 31].

Systemic administration of KA resulted in a well-described pattern of behavioral seizures including wet dog shake at the beginning stage and progressed to tonic–clonic activity [27]. Seizures typically occurred intermittently during the first 6–8 h and yielded about 10% or less mortality. As observed in this study, TMP treatments did not alter the pattern and the severity of the seizures. Brain sections from the animals that received "KA + vehicle" demonstrated robust neuronal degeneration in KA-vulnerable regions in brain as revealed by both H & E staining and TUNEL labeling (Fig. 2a (f–j)) whereas the controls showed no eosinophilic

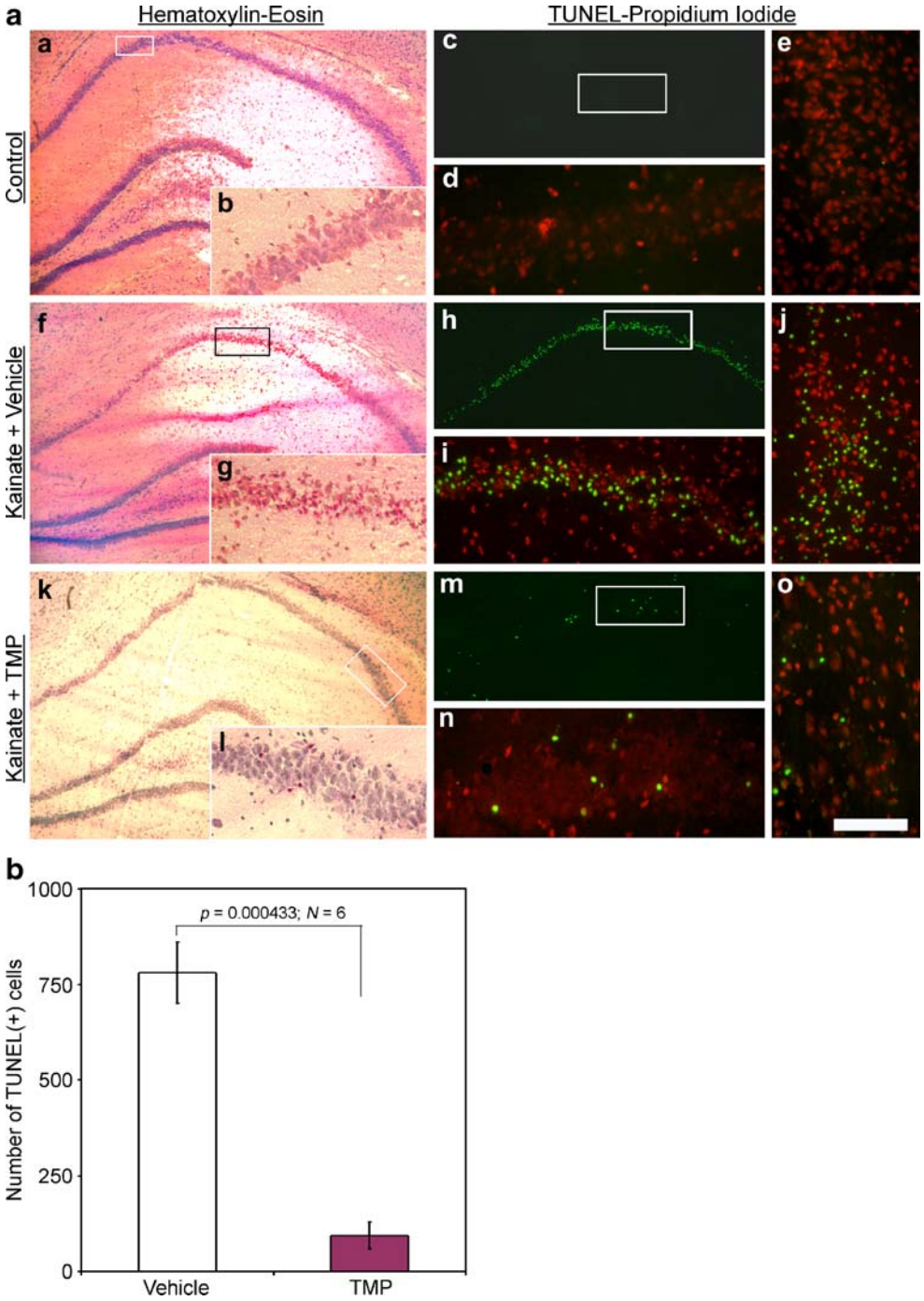

Fig. 2 Systemic administration of TMP attenuates neuronal degeneration in rat brains following kainate-induced seizures. **a** Both hematoxylin–eosin (H & E) and TUNEL staining demonstrate massive neuronal damage shown as *pink* in H & E staining (*f*, *g*) and *green* in TUNEL (*h*, *i*, and *j*) in both hippocampus (*f*, *g*, *h*, and *i*) and piriform cortex (*j*) regions. In contrast, the animals received TMP only exhibited a few sporadic eosinophilic (*l*) and TUNEL-positive cells in the corresponding regions (*m*, *n*, and *o*). **b** Quantification of TUNEL-positive cells according to our previous methods reveals a significant decrease in the number of damaged neurons in the hippocampal regions with TMP treatment. *Scale bar*= 100 μm in *b*, *d*, *e*, *g*, *i*, *j*, *l*, *n*, and *o*; and 400 μm in *a*, *c*, *f*, *h*, *k*, and *m*

or TUNEL-positive damaged cells (Fig. 2a (a–e)). In contrast, given 50 mg/kg TMP following the onset of seizures, animals showed markedly fewer damaged cells in the corresponding regions in the brain (Fig. 2a (k–o)). Quantification of TUNEL-positive cells conducted as described in our previous work [32, 33] revealed a statistically significant difference between "KA + vehicle" and "KA + TMP", suggesting remarkable neuroprotective efficacy of TMP in the CNS under excitotoxic attack.

In cultured neural cells, TMP treatments significantly reduced the generation of lipid peroxidation products, malondialdehyde, induced by hydrogen peroxide [19, 20]. These observations are also in agreement with increased levels of glutathione in 3xTg-AD mouse brains following TMP treatments (data not shown). In addition, the benefits of TMP treatments preserved high levels of MAP2 and rattin, two molecules that play important roles in cell growth and function [19, 34, 35]. Taken together, TMP may target

multiple cell signal transduction pathways to contribute to the survival of neural cells and the normal function of the nervous system.

How far is TMP from the next phase for clinical applications?

As a naturopathic compound isolated from a Chinese herbal medicine, TMP has been the subject of many pharmacological and toxicological studies. The solubility of purified TMP in crystal form is relatively low in neutral aqueous solution (~10 μg/ml) and dramatically increases in an acidic environment (>40 mg/ml, pH <4), in which TMP is stable and active [36]. Preclinical assessment of the distribution, metabolism, excretion, and toxicity (ADMET) of TMP has been performed in animals and in vitro for over 20 years [37–41]. Pharmacokinetic studies demonstrated that TMP was efficiently permeable to the blood–brain barrier in multiple animal models [39, 40]. Toxicity assays revealed a very low level of toxicity in animals with an oral LD_{50} of about 1,910 mg/kg in rats and 1,436 mg/kg in mice [42, 43]. Of significance, practitioners of traditional Chinese medicine have continued to use TMP as a treatment for inflammatory or degenerative diseases, usually in combination with other medications [6, 8, 44–46]. In this regard, both TMP tablets and TMP–HCl injectable solution are prescription-available for the treatment of cardiovascular diseases in China [47, 48].

Given the favorable ADMET outcomes on TMP in animals, and the longstanding clinical use of TMP in China, the path is set for preclinical data to justify clinical trials of TMP in humans. This proof-of-concept proposal would provide sufficient preclinical evidence to justify studying TMP in patients with neurodegenerative disorders in a double-blind placebo-controlled manner. Our understanding of the molecular basis for TMP-mediated pharmacological actions remains limited. Similarly, there are a lack of well-designed preclinical studies of TMP efficacy for neurological disorders. Such studies are critical prior to studying TMP in humans.

Summary

In addition to TMP, there are groups of naturopathic compounds that have been purified from related herbal medicines and identified as efficient neuroprotective ingredients as mentioned. Resembling TMP, many of these compounds have demonstrated remarkable neuroprotective efficacy in experimental studies conducted in cell cultures and/or live animals. Some of them are also used in clinics as treatment for specific neurological disorders. Therefore, once further studies are warranted to decipher the molecular basis of related pharmacological efficacy, many of such naturopathic molecules would move to clinical assessments for neural protection in humans.

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