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Research Report

Effects of tetramethylpyrazine on nitric oxide/cGMP signaling after cerebral vasospasm in rabbits

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ABSTRACT

Tetramethylpyrazine (TMP), an ingredient of Chinese herbal Szechwan lovage rhizome, shows vasorelaxant effect. Cerebral vasospasm (CVS) after subarachnoid hemorrhage (SAH) is associated with high mortality and morbidity. Here, we evaluated the effect of TMP in a model of CVS and sought to identify the underlying mechanisms of action. A rabbit SAH model was established by injection of the autoblood via cisterna magna. Cerebral blood flow and arterial diameter were measured by Transcranial Doppler (TCD) and Computed Tomography Angiography (CTA). Expression of eNOS and PDE-V in basilar artery (BA) was assessed by western blots. Levels of nitric oxide (NO) in plasma and cerebral spinal fluid, and of intra-endothelium Ca^{2+} were measured. Significantly reduced diameter and accelerated blood flow velocity were detected in BAs of SAH animals ($P<0.05$ vs. sham group). Expression of eNOS and NO was increased, and PDE-V expression was reduced by TMP.TMP ameliorated cerebral vasospasm ($P<0.05$ vs. SAH group), and L-NAME (a NOS inhibitor) partly abrogated the effects of TMP. TMP induced a dose-dependent increase of intra-endothelium Ca^{2+}. The current results demonstrated that the vasorelaxant effect of TMP was at least in part via regulation of NO/cGMP signaling.

1. Introduction

Cerebral vasospasm (CVS) following subarachnoid hemorrhage (SAH) is associated with high mortality and morbidity (Kolias et al., 2009; Pyne-Geithman et al., 2009a,b). It has been reported that multifactorial mechanisms were involved in the occurrence of CVS (Rothoerl and Ringel, 2007; Gokce et al., 2010; Güresir et al., 2010). Recent studies indicated that dysfunction of eNOS may contribute to the development of CVS (Pyne-Geithman et al., 2009a,b; Osuka et al., 2009). As the key enzyme in nitric oxide (NO)/cGMP signaling pathway, eNOS was activated by binding with intra-endothelial Ca^{2+} and calmodulin. Endothelial NOS synthesis NO, the latter diffuses rapidly into adjacent vascular smooth muscle cells and combines with histidine ligand at β1 subunit of SGC, leading to the transformation of GTP to cGMP and vasodilatation (Murad, 2006; Denninger and Marletta, 1999). There is a close relationship between NO production and attenuation of CVS, and studies have revealed that delivery of NO ameliorates post-SAH vasoconstriction (Pradilla et al., 2004). However,

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evidences show that NO replacements were not always valid in treatment of CVS as being expected (Raabe et al., 2002).It means that NO/cGMP signal may not be the sole factor that is responsible for CVS.

The current therapy of CVS mainly depended on "triple-H therapy" (hypertension, hypervolemia, and hemodilution) and/or dihydropyridine calcium antagonists (Otten et al., 2008). Nevertheless, new medicines and methods are presented in recent years. Tetramethylpyrazine (TMP, Fig. 1), the active ingredient of Chinese herb Szechwan lovage rhizome, has been reported to possess therapeutic effects on vertebrobasilar ischemia (Ge et al., 2008). Our previous research found that TMP ameliorated CVS in SAH rats through the regulation of caspase-dependent apoptosis (Gao et al., 2008). However, the effects of TMP on NO/cGMP signal after SAH were not elucidated. Here, the effects of TMP on CVS and NO/cGMP signal were explored in SAH models.

2. Results

2.1. TMP attenuates CVS after SAH

All animals survived during the experimental period. Respiratory arrest occurred in four rabbits (12.5%) during autoblood injection. No neurological deficits were observed after external cardiac massage.

As compared with sham-operated animals, significant increase of mean MFV and PSV was detected in basilar arteries of SAH animals ($P<0.05$; Fig. 2). TMP (30 mg/kg, i.v.) resulted in significant reduction in both MFV and PSV compared with that of SAH animals (Figs. 2E and F). Animals treated with both TMP and L-NAME (inhibitor of NOS) had mean MFVs and PSVs similar to that of SAH animals (Figs. 2E and F).

We measured BA diameters using CTA. The schematics of scanning locations are shown in Figs. 3A and B, and C. Two rabbits (6.25%) suffered unilateral pinnal hematoma resulted from involuntary movements. Neither neurological deficits nor deaths were observed during and after the performance of CTA. Representative images of CTA were shown in Figs. 3D–G. Bead-like images and significant reduction of BA diameter were detected in SAH group as compared with that of sham group ($P<0.05$; Fig. 3E and H).This confirmed successful establishment of CVS models. Compared with SAH group administration of TMP induced marked increase of BA diameter ($P<0.05$; Fig. 3H), while subsequent application of L-NAME attenuated the vasorelaxant effect of TMP ($P<0.05$ vs. TMP group; Fig. 3H).

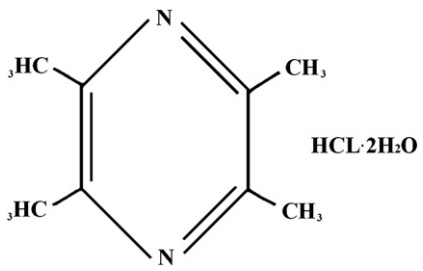

Fig. 1 – Chemical structure of tetramethylpyrazine hydrochloride (TMP).

2.2. TMP regulates NO/cGMP pathway

It was reported that TMP-induced relaxation in rat pulmonary artery was endothelium and NO-dependent (Peng et al., 1996), which prompted us to examine eNOS in basilar arteries of each group. As hydrolysis of cGMP by type-V phosphodiesterase (PDE-V) was the termination of NO/cGMP signal, PDE-V was determined as well. Western blot analysis of eNOS and PDE-V were shown in Fig. 4. In line with previous study (Shih et al., 2008), SAH caused significant reduction of eNOS when compared with the sham group in our study ($P<0.05$; Figs. 4A and C). TMP significantly increased the impaired expression of eNOS (Figs. 4A and C). In comparison to the results of TMP alone, L-NAME attenuated the effects of TMP by down regulation of eNOS ($P<0.05$; Figs. 4A and C). On the other hand, PDE-V expression was markedly increased in SAH group as compared with sham group. The level of PDE-V was reduced by TMP. In comparison to the results of TMP group, addition of L-NAME attenuated the effect of TMP by increasing PDE-V expression ($P<0.05$; Figs. 4B and D).

2.3. Identification of basilar arterial endothelium by immunocytochemistry and TEM

Freshly isolated endothelial cells exhibited a short, rounded appearance upon the attachment (Fig. 5A). After 5–7 days of incubation, the cells grew in confluent monolayer and became larger and polygonal. Factor-VIII related protein, an important antigenic marker of endothelial cells (Banerjee et al., 1985), was detected with immunological identification. About 89.13% of cultured cells were Factor-VIII positive as calculated from three independent immunohistochemical experiments (Figs. 5B–D). By using transmission election microscopy (TEM), the ECs were identified by the fusiform nucleus (lower arrow, Fig. 5E) and the flat body, as well as the sparing microvillus in cell membrane (upper arrow, Fig. 5E). Rod-shaped cytoplasmic inclusions (Weibel–Palade bodies), reported by Jaffe et al. (1973) to exist in endothelial cells, were observed in cytoplasm of cells evaluated (Fig. 5F).

2.4. TMP increased systemic NO production and endothelial NO production

As indicated by western blots, administration of TMP significantly increased the expression of eNOS. We therefore examined the levels of NO for further analysis of eNOS activity (Fig. 6). We found that the plasma NO was reduced in SAH group when compared with that of the sham ($P<0.05$). In contrast, NO levels increased in TMP group as compared with SAH group ($P<0.05$). Compared with TMP alone, L-NAME caused marked reduction of plasma NO ($P<0.05$). When compared with sham group, NO in CSF was reduced after SAH ($P<0.05$). The level of NO returned approximately to the value of sham group in animals treated with TMP ($P>0.05$). Animals treated with TMP+L-NAME had similar levels of NO in CSF compared with that of SAH group.