Tetramethylpyrazine for Treatment of Experimentally Induced Stroke in Mongolian Gerbils

Walter K.K. Ho, PhD, Hsiang Lai Wen, MD, and Chi Ming Lee, PhD

Tetramethylpyrazine, a drug originally isolated from the rhizome of *Ligusticum walliichi*, has been used routinely in China for the treatment of stroke and angina pectoris. We evaluated this drug by testing its effectiveness in increasing the survival rate in a stroke model using Mongolian gerbils. Our results indicate that tetramethylpyrazine can increase survival rate only if it is administered before the induction of cerebral ischemia. Since we administered the drug intraperitoneally, it is possible that pretreatment was necessary to increase its effective concentration in the blood. Receptor binding studies indicated that tetramethylpyrazine was inactive against a variety of pharmacologically active receptors. *(Stroke 1989;20:96-99)*

The rhizome of *Ligusticum walliichi* has long been used by practitioners of traditional Chinese medicine in treating blood stasis and vital energy stagnation. In recent years, the active ingredient of this herb has been characterized and its chemical structure has been worked out (Figure 1). This compound, tetramethylpyrazine (TMP), has been reported to be effective for the treatment of angina pectoris and cerebral thrombosis in China. Experimentally, TMP has been shown to induce vasodilation, to increase coronary blood flow, and to inhibit ADP-induced platelet aggregation. These properties of TMP apparently account for its efficacy in the treatment of disorders associated with blood vessel occlusion.

Although TMP has been used in a number of clinical trials for the treatment of cerebral thrombosis in China, its effectiveness in increasing survival rate has not been confirmed under stringent experimental conditions. As part of our overall effort to evaluate TMP, we tested it in a stroke model using Mongolian gerbils. The following is a report of our findings.

**Materials and Methods**

One hundred seventy-nine male Mongolian gerbils weighing 70–80 g were maintained in our animal house with a day/night cycle of 12/12 hours. Food and water were given ad libitum. Gerbils were stabilized for at least 1 week before the initiation of the experiment. Gerbils were anesthetized with 0.25 mg/kg pentobarbitol in 0.5 ml injection volume before unilateral cerebral infarction was induced by ligating one common carotid artery. A segment of the artery was tied doubly, and the artery was transected between the sutures. Gerbils were returned to their cages and observed approximately every 30 minutes for 3 hours. Gerbils dying within this period were excluded from the study; <2% of the gerbils died. At the end of the third hour, gerbils were injected intraperitoneally with either 2.5 mg TMP in 0.2 ml saline or 0.2 ml saline (vehicle). Signs of ischemia were then recorded by an independent observer.

We used two experimental paradigms. In the first, gerbils were pretreated with either TMP or vehicle for 7 days before the induction of stroke; in the second, gerbils were not pretreated. In both paradigms, 2.5 mg TMP or vehicle was administered intraperitoneally once daily in the morning for 4 days or until the gerbil died. Signs associated with ischemia (coma, motor function deficit, spiraling behavior, and eyelid movements) were scored by the same observer, who was blinded to the treatment conditions. Ratings were on a scale of 0 to 3, 3 being the most severe.

To quantify the degree of ischemic damage, the survivors were killed on the morning of Day 4 by spinal dislocation and decapitation. The brains from gerbils that died or were killed were dissected, sectioned, and stained with 2,3,5-triphenyltetrazolium. Coronal sections were made from the optic chiasm to the mamillary bodies. The infarct size
was scored as 1, 2, or 3, with 1 indicating an infarct encompassing one quarter, 2 an infarct encompassing approximately one half, and 3 an infarct encompassing the entire area.

Receptor binding was assessed by standard protocols using various radiolabeled ligands. Homogenates prepared from either the rat cortex or striatum were used as a crude preparation of the receptor.

TMP was obtained in sterile 25-mg/ml injection ampules from the Guangdong Province Lee Man Pharmaceutical Co. (Guangdong, China). This preparation was confirmed by thin-layer chromatography to be homogeneous, and its Rf was identical to that of a synthetic standard obtained from another source (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan). TMP was appropriately diluted with sterile vehicle before use. All radiolabeled ligands used in the binding studies were from either Amersham International (Amersham, UK) or New England Nuclear (Boston, Massachusetts).

Results

The results of five independent experiments using the first paradigm, each comprising 10 TMP-treated and 10 vehicle-treated (control) gerbils, are presented in Figure 2. Three hours after ligation, survival rates for the TMP-treated and control groups were similar (98% vs. 97.7%), suggesting that TMP has no effect in preventing acute death. Compared with controls, the TMP-treated group showed a significantly higher survival rate on Days 2, 3, and 4; at the end of the observation period, mean survival rate of the TMP-treated group was 69% while that of the control group was 42%. The most pronounced decrease in survival rate of the control group occurred between Days 1 and 2; the decrease in survival rate of the TMP-treated group was only one half this magnitude.

We also evaluated the frequency of occurrence of signs associated with ischemia. The percentage of surviving gerbils showing such signs was consistently higher in the control group (Table 1). Since the appearance of these signs correlates strongly with mortality (r=0.95, results not shown), the data in Table 1 further support our contention that the increased survival rate of the TMP-treated group was probably due to a reduction of ischemic brain damage.

A major problem in using Mongolian gerbils as a stroke model is the high incidence of unsuccessful

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Occurrence of Signs of Ischemia in Surviving Gerbils After Unilateral Common Carotid Artery Ligation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after ligation</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>First paradigm</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>26</td>
</tr>
<tr>
<td>TMP</td>
<td>20</td>
</tr>
<tr>
<td>Second paradigm</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>17</td>
</tr>
<tr>
<td>TMP</td>
<td>18</td>
</tr>
</tbody>
</table>

First paradigm, treatment for 7 days before and 4 days after ligation; second paradigm, treatment for 4 days after ligation; vehicle, 0.2 ml saline; TMP, 2.5 mg tetramethylpyrazine; n, gerbils showing at least one sign (coma, motor function deficit, spiraling behavior, eyelid movement), severity grade of 2 or higher in most; n, surviving gerbils.
FIGURE 3. Survival curve, effect of treatment with 2.5 mg tetramethylpyrazine (○) or 0.2 ml saline (●) on survival rate of 79 gerbils with cerebral ischemia induced by unilateral common carotid artery ligation. Gerbils were treated for 4 days after ligation. Results are mean±SD of three independent experiments (39 gerbils treated with tetramethylpyrazine, 40 treated with saline).

induction,\textsuperscript{5,6} documented to be 30–60%. To assure that our results were not due to background fluctuations, we performed histologic analysis on the brains of a few surviving gerbils. Of the 13 surviving TMP-treated gerbils examined, four (31%) had confirmed histologic damage in the brain; by contrast, only one of seven control gerbils examined (14%) exhibited such damage. Based on this somewhat limited data, it appears that TMP somehow increases the survival rate of gerbils with confirmed brain damage.

To assess the clinical use of TMP in the treatment of stroke, it is more logical to administer the drug after ligation (our second paradigm). The survival rate (Figure 3) and frequency of occurrence of signs of ischemia (Table 1) in the TMP-treated group was not different from control. Moreover, the frequency of gerbils that died with stainable infarction was similar in the two groups (Figure 4). The mean infarct size ratings of the TMP-treated and control groups were also indistinguishable (Figure 4, upper panel).

To evaluate the molecular properties of TMP and to assess its mechanism of action, we determined its binding affinity against 10 pharmacologically active compounds. TMP had low to no activity in competing against the ligands for receptor binding (Table 2). In view of this, TMP is unlikely to act on these receptor systems.

**Discussion**

Our objective was to evaluate the possible efficacy of TMP in the treatment of stroke. Our results cannot provide an unequivocal answer because TMP was effective only when administered 7 days before the induction of cerebral ischemia. Administration of TMP after induction of ischemia was unsuccessful in increasing survival rate maybe because of its slow increase to an effective concentration when administered intraperitoneally.

TMP in China has always been administered clinically by intravenous drip at 150 mg/day. The platelet aggregation inhibitory action of TMP and its dilation of blood vessels would probably account for some of its beneficial effects in cerebral ischemia.\textsuperscript{3} According to a study reported in the Chinese literature, TMP can disperse already-aggregated platelets\textsuperscript{3} and can prevent platelet aggregation. Apparently, if this mode of action is to be used effectively, TMP would have to be delivered intravenously. Experiments are currently in progress to alter our animal model so that intravenous administration of TMP can be evaluated.

Our goal was to evaluate the effect of TMP on survival over a relatively long period. We purposely
TABLE 2. Activity of Tetramethylpyrazine in Various Radioligand Binding Assays

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>[³H]ligand</th>
<th>IC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A₁</td>
<td>[³H]phenylisopropyladenosine</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Adenosine A₂</td>
<td>[³H]N-ethylcarboxamidoadenosine</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>α₁-Adrenergic</td>
<td>[³H]WB4101</td>
<td>NE</td>
</tr>
<tr>
<td>α₂-Adrenergic</td>
<td>[³H]p-aminoclonidine</td>
<td>6.8±0.5</td>
</tr>
<tr>
<td>β-Adrenergic</td>
<td>[³H]dihydroalprenol</td>
<td>NE</td>
</tr>
<tr>
<td>Serotonergic</td>
<td>[³H]spiperone</td>
<td>NE</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>[³H]flunitrazepan</td>
<td>7.1±0.3</td>
</tr>
<tr>
<td>Dopaminergic (DA₁)</td>
<td>[³H]spiperone</td>
<td>NE</td>
</tr>
<tr>
<td>Muscarinic/cholinergic</td>
<td>[³H]quinuclidinyl benzilate</td>
<td>NE</td>
</tr>
<tr>
<td>Calcium channel</td>
<td>[³H]nitrendipine</td>
<td>NE</td>
</tr>
</tbody>
</table>

NE, not effective, <20% inhibition at 10 mM tetramethylpyrazine.

excluded gerbils that did not show signs of recovery within 3 hours after surgery to avoid assessing gerbils with severe complications or complications not directly connected with cerebral damage (e.g., an overdose of anesthetic). On the other hand, ligation of the carotid artery may lead to acute ischemia, resulting in life-threatening seizure. Thus, it can be argued that TMP may increase survival rate by preventing complications arising from this cause. To test this possibility, we examined the records of our gerbils and found that only two had seizures within 3 hours after ligation. Moreover, only 2% of the gerbils died within 3 hours, and there was no difference in mortality between the TMP-treated and the control groups. In an independent experiment (data not shown), we evaluated the anticonvulsant activity of TMP and found it to be negative at a dose many times higher than the one we used here. In view of these observations, it is unlikely that the beneficial effect of TMP is mediated by an anticonvulsant mechanism.

Cerebral ischemia activates phospholipase, leading to the production of arachidonic acid. The conversion of arachidonic acid by the lipoxygenase pathway may increase the concentration of leukotriene, which can compound the damage generated. From our observations presented in Figure 2, pretreatment with TMP significantly increased the survival rate between Days 1 and 2 after the induction of ischemia. Since cerebral damage induced by ischemia may progress for 1–2 days before stabilizing, it is not unreasonable to expect that TMP may act by inhibiting the progression of damage. Measurement of leukotriene concentration after TMP treatment should provide a direct answer to this question.

Acknowledgment

The authors wish to thank Mr. S.H. Fong for his technical assistance.

References


KEY WORDS: cerebrovascular disorders • thrombosis • gerbils
Tetramethylpyrazine for treatment of experimentally induced stroke in Mongolian gerbils.
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Stroke. 1989;20:96-99
doi: 10.1161/01.STR.20.1.96

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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