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The enhancing effect of synthetical borneol on the absorption of tetramethylpyrazine phosphate in mouse

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Abstract

The main purpose of this study was to illustrate the effect of synthetical borneol (SB) on the plasma and brain concentration profile of tetramethylpyrazine phosphate (TMPP) in mice after oral administration of TMPP without or with different amounts of SB. The concentrations of TMPP on the plasma and brain in mice were determined by GC-FID. The pharmacokinetic parameters were computed by software program 3p97. Our data showed that after oral administration of 15, 30, 90 mg kg^{-1} of SB, oral bioavailability of TMPP in plasma was 1.52, 2.21, 2.95 times increase, respectively, than that without SB, and 1.12, 1.62, 1.93 times increase, respectively, in brain tissue. The pharmacokinetic data were simulated by non-linear least squares. The results showed that both open two-compartment model and one-order absorption were fitted to TMPP plasma and brain concentration-time course in vivo in mice. The MRT of TMPP showed same results under the conditions without or with SB. SB did enhance the oral absorption of TMPP and the concentration of TMPP in brain tissue, especially in the early period. But the use of SB did not change the behavior in vivo of TMPP.

Keywords: Tetramethylpyrazine phosphate; Synthetical borneol; GC-FID; Pharmacokinetics; Enhancement effect; Blood–brain barrier

1. Introduction

Tetramethylpyrazine (TMP) is a biologically active ingredient isolated from the traditional herbal medicine *Ligusticum wallichii* France or *Ligusticum chuanxiong* Hort, which is widely used in China for the treatment of cardiovascular and cerebrovascular disease (Guo et al., 1983). It was found to inhibit platelet aggregation in vitro, increase cerebral blood flow (Ojewole and Odebiyi, 1980; Feng et al., 1988; Liu and Sylvester, 1994; Liao, 2000) and ischemic attack (Ho et al., 1989) and improve blood viscosity (Watanabe, 1997). It has also recently been reported that TMP has appreciable blood–brain barrier (BBB) penetrability (Liang et al., 1999; Tsai and Liang, 2001). Nowadays, TMP phosphate (TMPP), molecular weight 234.2, as a new type of calcium blocker, is widely used in the medications such as TMPP tablets, capsules, injection and so on.

Borneol, molecular weight 154.24, a monoterpenoid alcohol is the main component of the medicinal plant. Some studies showed that borneol could improve some drugs oral bioavailability, accelerate the open of blood–brain barriers (BBB) and enhance the distribution of drugs in brain tissue (Liu et al., 1994; Chen and Wang, 2003, 2004). Synthetical borneol (SB) are widely used in medicine. SB includes d-borneol and isobrneol and natural borneol only contains d-borneol. Study showed that there were no differences between SB and natural borneol in pharmacodynamics (P. Wang et al., 2004; Y. Wang et al., 2004).

The Fufang TMPP tablets containing both TMPP and SB were developed for the treatment of cerebral thrombosis due to the effects of SB on increasing oral bioavailability of TMPP and enhancing TMPP distribution in brain tissue. The purpose of this study was to investigate the effect of SB on the concentration profile of TMPP in mouse plasma and brain after oral administration of TMPP without or with different amounts of SB. The chemical structures of d-borneol, isobrneol and TMPP are shown in Fig. 1.

2. Materials and methods

2.1. Materials

Tetramethylpyrazine phosphate reference (TMPP, 101.0% purity) was purchased from Beijing Yanjing Pharmaceutical

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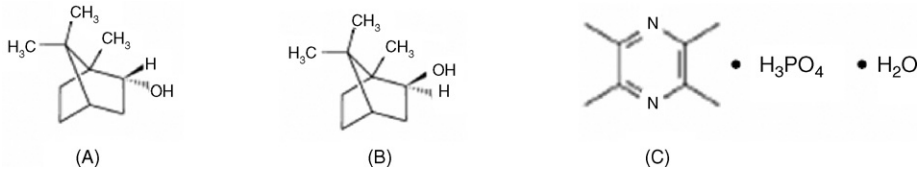

Fig. 1. The chemical structure of d-borneol (A), isobrneol (B) and TMPP (C).

Factory (Beijing, China). Synthetical borneol and dimethyl sulfoxide (internal standard, I.S.) were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). The I.S. solution was prepared in methanol at a concentration of 1 μg mL^{-1}. The other chemical reagents were of analytical grade or better.

2.2. Animals

Male mice (18–22 g body weight) were obtained from the Animal Center of China Pharmaceutical University (Nanjing, China). All the animals were clinically healthy and haematologically and biochemically normal throughout the experimental period. Food, but not water, was withheld for 24 h before and after drugs administration.

2.3. GC-FID assay method

The concentrations of TMPP in the plasma and brain tissue were determined by a gas chromatography by using flame ionization detector method (GC-FID). The GC-FID system consisted of an HP 4890 gas chromatographic system, a FID (Agilent 4890, USA) and a Agilent Chemstation software for data analysis. Separation of TMPP, SB and internal standard from endogenous substances was performed on a HP-5 MS capillary column (30.0 m×250 μm, 0.25 μm). Nitrogen, at a flow rate of 25 mL min^{-1}, was used as the carrier gas. The split mode with a split ratio of 10:1 was used in the study. Upon the injection of the sample to the chromatograph, firstly, the oven temperature was 100 °C for 1 min, then increased from 100 to 160 °C at a rate of 30 °C min^{-1}, then to 200 °C at a rate of 50 °C min^{-1}, and was thereafter maintained at 200 °C for 4 min. Injection port temperature were maintained at 180 °C; Detector temperature were maintained at 210 °C. 1 μL of samples was injected manually.

2.4. Plasma sample preparation and validity

The animal experiments had gained the China Ethic Committee's approval. The eyeballs of mice were peeled off and about 500 μL blood was collected. The plasma obtained after centrifugation (5 min, 4000 rpm) was stored at −20 °C until analysis. The plasma sample (100 μL) and phosphate buffer saline (pH8.0, 10 μL) transferred to a 1.5 mL polyethylene centrifuge tube were vortexed for 30 s and then mixed with internal standard working solution (150 μL) for 1 min. The precipitate of denatured proteins was separated by centrifugation at 12000 rpm for 10 min. An aliquot (1 μL) of supernatant was directly injected into the GC-FID apparatus for analysis. The method was validated by adding various quantities of TMPP to blank mice plasmas. The resulting concentrations of TMPP were 0.02, 0.1, 1, 3, 8, 12, 25 and 40 μg mL^{-1}. These calibrations were subjected to the entire analytical procedure, as well as to validate the linearity, precision and accuracy of the method.

2.5. Brain tissue sample preparation and validity

To investigate brain distribution of TMPP, male mice were sacrificed by decapitation after taking blood, and the brain tissues were taken out, washed by physiological saline and blotted by filter paper, then weighted. The brain tissues were homogenized with physiological saline (2 mL/1 g tissue) using a tissue homogenizer (Shanghai, China). The brain homogenate (200 μL) and phosphate buffer saline (pH8.0, 20 μL) transferred to a 1.5 mL polyethylene centrifuge tube was vortex-mixed for 30 s, then internal standard working solution (150 μL) was added and vortex-mixed for 1 min. The precipitate of denatured proteins was separated by centrifugation at 12000 rpm for 10 min. An aliquot (1 μL) of supernatant was directly injected into the GC-FID apparatus for analysis. The method was validated by adding various quantities of TMPP to blank homogenate of mice brain tissue. The resulting concentrations of TMPP were 0.02, 0.1, 1, 3, 8, 12, 25 and 40 μg g^{-1}. These calibrations were subjected to the entire analytical procedure to verify the linearity, precision and accuracy of the method.

2.6. Pharmacokinetic study of TMPP in mice

The animal experiments had gained the China Ethic Committee's approval. Two hundred and forty male mice (body weight 18–22 g) were divided randomly into four groups and fasted for 24 h, but allowed to take water freely. One group was orally administered 37.5 mg kg^{-1} of TMPP suspended in 0.5 mL of 0.5% CMC-Na solution. The other three groups were orally administered the mixture of TMPP and SB, suspended in 0.5 mL of 0.5% CMC-Na solution, which contains the same amount of TMPP and 15, 30, or 90 mg kg^{-1} of SB, respectively. After oral administration of TMPP or the mixture, the mice were sacrificed by picking off eyeball of mice at the time intervals of 2, 5, 10, 15, 20, 30, 40, 60, 90 and 120 min, respectively, and the cerebra were collected and weighted. At each time point, six mice were sacrificed and sampled.

Peak concentration (C_{max}) and peak times (T_{max}) of TMPP were derived directly from the experimental points. The other pharmacokinetical parameters were fitted by 3P97 pharmacokinetics program (the Section of Mathematical Pharmacology of Chinese Mathematical Pharmacological Society).

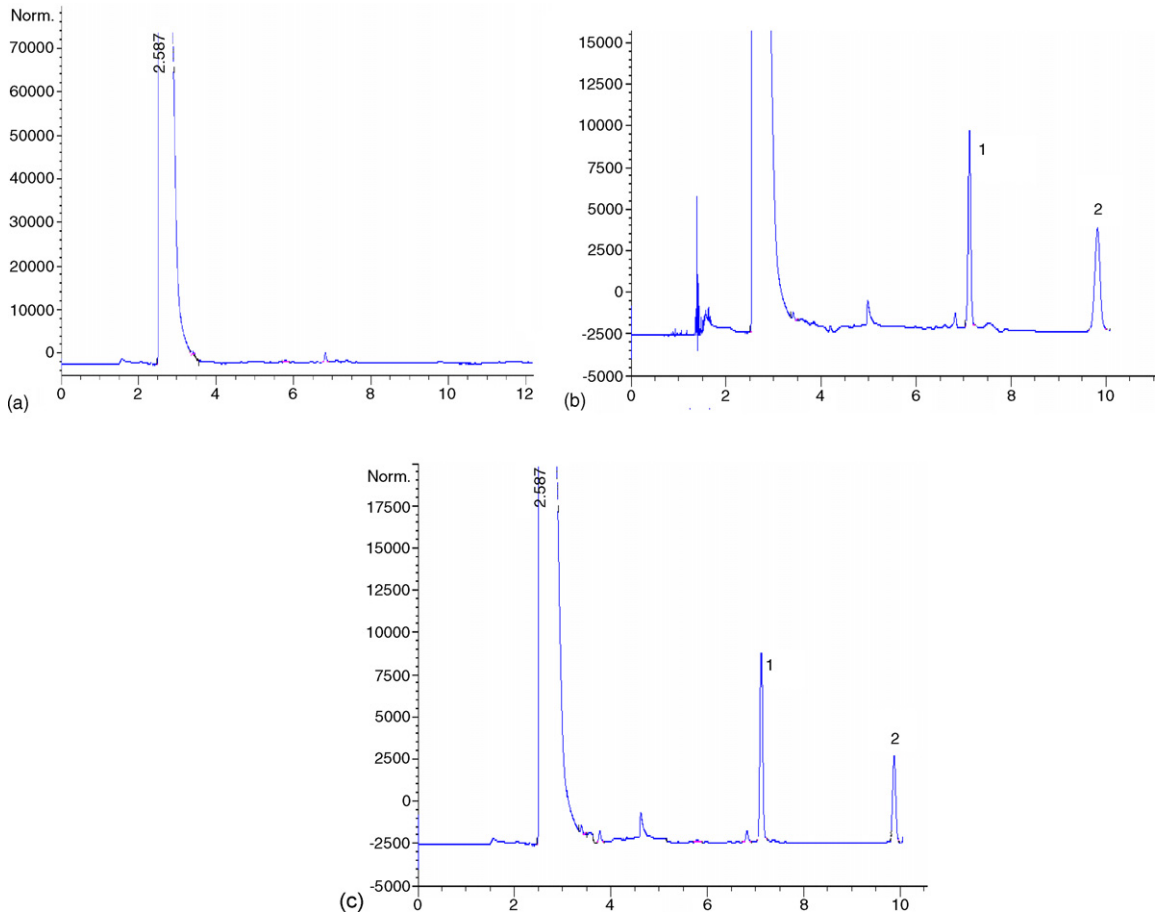

Fig. 2. Typical gas chromatograms of blank mice plasma (a), blank mice plasma spiked with 3 μg/mL TMPP and dimethyl sulfoxide (I.S.) (b), and plasma sample at 5 min after oral administration of 37.5 mg kg^{-1} TMPP and 30 mg kg^{-1} SB (c). TMPP, 1, I.S. (dimethyl sulfoxide), 2.

3. Results

Under the chromatographic conditions described above, optimized separation and detection conditions were achieved in both plasma and brain tissue. The retention time of TMPP and internal standard was found at 7.1 min and 9.8 min, respectively (Fig. 2.). The detection limit for TMPP at a signal-to-noise ration of 3:1, was 5 ng mL^{-1} in plasma. However, in brain tissue, it is 20 ng mL^{-1}.

The calibration curves of TMPP were linear in the range of 0.02–40 μg mL^{-1} plasma and brain tissue. Using the linear least squares regression, the calibration line of TMPP was $y = 0.0021x + 0.013$ with $r^2 = 0.9952$ in plasma and $y = 0.0034x - 0.0018$ with $r^2 = 0.9964$ in brain tissue. The mean relative recoveries of TMPP at high, middle, low concentrations were ranged from 96.45 to 99.13% in plasma and 95.32 to 98.76% in brain tissue. The intra-and inter-day precision (expressed as percent relative standard deviation, R.S.D.%) of TMPP were within 15.0% in plasma and brain tissue. The intra-and inter-day day accuracy (expressed as percent of nominal values) ranged from 93.13 to 100.52% in plasma and brain tissue. Those data showed recoveries and RSD in days or intra-days of TMPP in mice plasma and brain tissue were satisfying.

The plasma profile of TMPP with or without SB after oral administration to mice is shown in Fig. 3. The results showed that the average value of C_{max} was 9.09 μg mL^{-1} with a T_{max} at about 5 min after oral administration of TMPP without SB. However, the average value of C_{max} was 9.97, 15.26, 16.66 μg mL^{-1} after oral administration of TMPP with 15, 30 or 90 mg kg^{-1} SB, respectively. The T_{max} was at about 5, 5 and 10 min, respec-

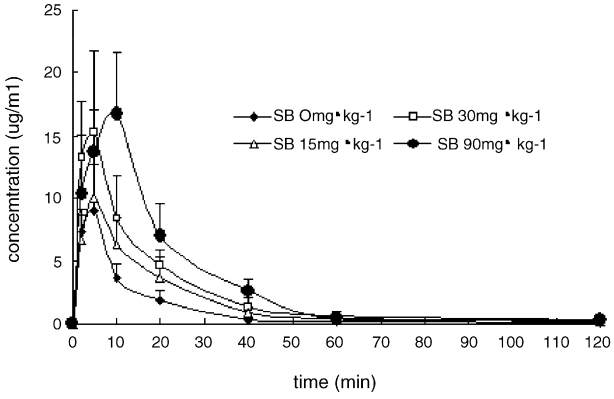

Fig. 3. Mean plasma concentration profile in mice after oral adminitration of TMPP without or with SB of 15, 30 and 90 mg kg^{-1} (six mice at each sampling time).

Table 1
The main pharmacokinetic parameters of plasma after oral adminitration of TMPP without or with SB of 15, 30 and 90 mg kg^{-1} in mice

Parameters	Unit	TMPP			
		0 mg kg^{-1} SB	15 mg kg^{-1} SB	30 mg kg^{-1} SB	90 mg kg^{-1} SB
A	μg mL^{-1}	20.23 ± 3.56	13.3 ± 1.64*	17.73 ± 4.79*	51.97 ± 8.64*
α		0.18 ± 0.091	0.077 ± 0.092*	0.089 ± 0.08*	0.092 ± 0.13*
B	μg mL^{-1}	2.39 ± 1.04	0.25 ± 0.98*	1.25 ± 1.27*	0.29 ± 1.02*
β		0.02 ± 0.009	0.035 ± 0.012*	0.013 ± 0.011*	0.0014 ± 0.008*
Ka	min^{-1}	0.66 ± 0.31	1.02 ± 0.11*	1.91 ± 0.17*	1.21 ± 0.14*
C_{max}	μg mL^{-1}	9.09 ± 2.56	9.97 ± 1.28*	15.26 ± 2.97**	16.66 ± 4.87**
T_{max}	min	5 ± 2.12	5 ± 1.54*	5 ± 3.21*	10 ± 2.69*
V/F(c)	mg μg^{-1} mL	0.98 ± 0.28	1.35 ± 0.32*	0.91 ± 0.19*	0.55 ± 0.23*
T1/2Ka	min	2.04 ± 1.02	0.68 ± 1.35*	0.36 ± 0.58*	3.27 ± 0.93*
T1/2α	min	3.76 ± 1.56	9.06 ± 1.46*	7.82 ± 0.94*	7.56 ± 0.91*
T1/2β	min	30.11 ± 5.78	35.48 ± 7.54*	54.72 ± 8.62*	50.49 ± 9.62*
K21	min^{-1}	0.045 ± 0.022	0.016 ± 0.019*	0.018 ± 0.014*	0.0092 ± 0.012*
K10	min^{-1}	0.094 ± 0.031	0.017 ± 0.061*	0.063 ± 0.038*	0.014 ± 0.045*
K12	min^{-1}	0.068 ± 0.026	0.073 ± 0.012*	0.021 ± 0.029*	0.089 ± 0.033*
CI/F(s)	μg mL^{-1} min	0.072 ± 0.021	0.023 ± 0.014*	0.057 ± 0.025*	0.077 ± 0.016*

$^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$.

Table 2
The main pharmacokinetic parameters of plasma after oral adminitration of TMPP without or with SB of 15, 30 and 90 mg kg^{-1} with non-model in mice

Parameters	Unit	TMPP			
		0 mg kg^{-1} SB	15 mg kg^{-1} SB	30 mg kg^{-1} SB	90 mg kg^{-1} SB
		T(0–120 min)	*T*(0–120 min)	*T*(0–120 min)	*T*(0-120 min)
AUC(s0)	μg mL^{-1} min	131.46 ± 15.76	199.98 ± 38.64**	289.82 ± 42.16***	388.13 ± 39.56***
AUMC(s1)		2429.2 ± 956.77	4352.3 ± 579.35**	6133.25 ± 864.98***	7930.15 ± 905.24***
MRT	min	18.48 ± 5.32	21.76 ± 4.38*	21.16 ± 6.81*	20.43 ± 5.46*

$^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$.

tively. The other parameters were obtained using 3p97 procedure (Tables 1 and 2).

The brain profile of TMPP after oral administration of TMPP with or without SB to mice is shown in Fig. 4. The results showed that the average value of C_{max} was 2.83 μg g^{-1} after oral administration of TMPP without SB with a T_{max} at about 5min. However, after oral administration of TMPP plus 15, 30 and 90 mg kg^{-1} of SB, the average value of C_{max} was 3.33, 4.38, 5.59 μg g^{-1} with a T_{max} at about 5, 5 and 10 min, respectively. The other parameters were obtained by 3p97 procedure (Tables 3 and 4).

The pharmacokinetic data of TMPP were simulated by non-linear least squares. The results showed that the kinetics of a two-compartment open model and first-order absorption were best fitted to TMPP both in mouse plasma and brain tissue concentration-time curves.

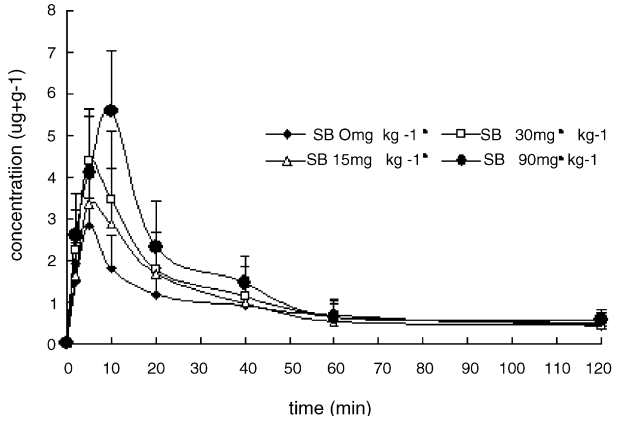

Fig. 4. Mean brian concentration profile in mice after oral adminitration of TMPP without or with SB of 15, 30 and 90 mg kg^{-1} (six mice at each sampling time).

4. Discussions

Sample preparation plays a key role for the determination of drugs in biological samples. At the beginning for this work, liquid-liquid extraction was tried and then the organic solvent was dried, but we found the drying process in liquid-liquid extraction caused a significant loss of the analytes due to their volatility. After several trials, a protein precipitation procedure was adopted and proved to be simple and reliable for the sample preparation in this work. Methanol other than acetonitrile and alcohol was selected as protein-precipitating solvent to produce the expected peak shapes of analytes. Internal standard is necessary for determination of analytes in biological samples and the determination of GC. In initial stage of our work, several compounds were tried and finally dimethyl sulfoxide was found to be optional for our work.

Table 3
The main pharmacokinetic parameters of brain in mice after oral adminitration of TMPP without or with SB of 15, 30 and 90 mg kg^{-1}

Parameters	Unit	TMPP			
		0 mg kg^{-1} SB	15 mg kg^{-1} SB	30 mg kg^{-1} SB	90 mg kg^{-1} SB
A	μg g^{-1}	2.91 ± 0.44	4.16 ± 3.56*	6.59 ± 1.71*	7.75 ± 5.98*
α		0.19 ± 0.05	0.07 ± 0.11*	0.12 ± 0.039*	0.065 ± 0.073*
B	μg g^{-1}	1.27 ± 0.17	0.69 ± 0.18*	1.41 ± 0.24*	0.52 ± 0.21*
β		0.0098 ± 0.009	0.004 ± 0.006*	0.011 ± 0.004*	0.0094 ± 0.003*
Ka	min^{-1}	2.72 ± 0.12	0.59 ± 0.13*	051 ± 0.17*	0.32 ± 0.13*
C_{max}	μg g^{-1}	2.83 ± 0.36	3.33 ± 0.41*	4.38 ± 1.24**	5.59 ± 1.01**
T_{max}	min	5 ± 1.67	5 ± 0.89*	5 ± 2.36*	10 ± 1.98*
V/F(c)	mg μg^{-1} g	4.16 ± 0.87	3.81 ± 0.54*	2.58 ± 0.46*	2.46 ± 0.16*
T1/2Ka	min	0.26 ± 0.71	1.17 ± 0.38*	1.36 ± 0.27*	2.15 ± 0.14*
T1/2 α	min	3.71 ± 1.03	9.58 ± 1.17*	5.65 ± 2.01*	10.74 ± 2.04*
T1/2 β	min	70.51 ± 21.56	165.47 ± 18.82*	69.15 ± 11.33*	60.18 ± 16.72*
K21	min^{-1}	0.067 ± 0.015	0.015 ± 0.008*	0.034 ± 0.009*	0.052 ± 0.007*
K10	min^{-1}	0.028 ± 0.002	0.021 ± 0.008*	0.036 ± 0.009*	0.008 ± 0.0024*
K12	min^{-1}	0.11 ± 0.069	0.041 ± 0.016*	0.063 ± 0.018*	0.059 ± 0.016*
CI/F(s)	μg g^{-1} min	0.12 ± 0.023	0.077 ± 0.015*	0.092 ± 0.014*	0.039 ± 0.004*

*$P > 0.05$, **$P < 0.05$, ***$P < 0.01$.

Table 4
The main pharmacokinetic parameters of brain after oral adminitration of TMPP without or with SB of 15 mg kg^{-1}, 30 mg kg^{-1} and 90 mg kg^{-1} with non-model in mice

Parameters	Unit	TMPP			
		0 mg kg^{-1} SB	15 mg kg^{-1} SB	30 mg kg^{-1} SB	90 mg kg^{-1} SB
		T(0–120 min)	T(0–120 min)	T(0–120 min)	T(0–120 min)
AUC(s0)	μg g^{-1} min	103.54 ± 11.23	116.17 ± 10.56**	168.15 ± 24.67***	200.26 ± 28.54***
AUMC(s1)		4395.05 ± 216.48	4352.21 ± 198.56**	5048.55 ± 612.73***	5887.35 ± 582.46***
MRT	min	42.45 ± 3.54	37.46 ± 4.62*	36.55 ± 2.35*	34.58 ± 3.78*

*$P > 0.05$, **$P < 0.05$, ***$P < 0.01$.

Several analytical methods were used to measure the concentration of TMPP in plasma and brain tissue such as HPLC (Liang et al., 1999), GC-MS (Lv et al., 2000), LC-MS (P. Wang et al., 2004; Y. Wang et al., 2004) and so on. The present study developed a simple and sensitive GC-FID method to measure the concentration of TMPP in mice plasma and brain tissue. The pharmacokinetic profile of TMPP as well as its brain tissue distribution, and the enhancement of absorbtion of TMPP by different amounts of SB in mice plasma and brain tissue after oral administration was investigated. The results showed that the absorption and the distribution, as well as the elimination of TMPP after oral administration was very rapid. The concentration of TMPP in mice brain tissue was parallel with the concentration of TMPP in plasma. The value of AUC of TMPP in plasma without SB is about 1 folds higher than that in brain tissue, which indicated TMPP had appreciable BBB penetrability. With the increase of the amount of SB, oral bioavailabilities of TMPP in plasma and brain tissue in mice were also increased. SB did increase the oral absorption of TMPP and this effect was markedly enhanced in early period. But the use of SB did not change the behavior in vivo of TMPP. (P. Wang et al., 2004; Y. Wang et al., 2004) reported that the effect of little amount of borneol was limit and the effect of large amount of borneol was not strong either. After oral administration of 15, 30, 90 mg kg^{-1} of SB, oral bioavailability of TMPP in plasma was 1.52, 2.21, 2.95 times respectively than that without SB. There were 1.12, 1.62, 1.93 times increase in brain tissue, respectively, based on the data of non-model. The results showed the enhancing effect of SB was not in proportion with the amounts of SB. (Chen and Wang, 2003, 2004) had explored the mechanism of borneol in opening the BBB. Using Matin-Darby canine kidney epithelium (MDCKE) cell line as in vitro BBB model, they observed that borneol could reduce intercellular tight junction (ICTJ) and caused increase of the number and enlarged the diameter of pinocytosis vesicles. The ICTJ was opened at 4 h after borneol treatment, then the pinocytosis was affected 24 h later. The effects disappeared at 24 h after removal of the borneol-contained serum, which indicated the effects were reversible. In this study, the MRT of SB in mice plasma and brain tissue were about 30 and 24 min, respectively, which showed SB were not detained and accumulated in vivo. According to that article, the mechanisms of SB in opening the BBB were it obviously loosen the intercellular tight junction in BBB, accelerate the transportation of substance through the intercellular passage and also increase the number and volumn of pinocytosis vesicles in BBB cells, thus to accelerate the transportation of substance by way of cell pinocytosis. Studies also showed that SB did can accelerate the opening of BBB, but that was the physiological open and did not hurt brain tissue and BBB (Zhao et al., 2002). Another phenomenon from Figs. 3 and 4, we know the absorp-

tion of TMPP material after oral administration was very rapid and T_{max} was 5 min in plasma and brain. When given SB and TMPP at the same time, T_{max} of SB was 10 min in plasma and brain (the data see my another article) and SB's function of penetratng the BBB needs some time to work, then it was difficult to show SB shorten the T_{max} of TMPP in the plasma and brain. But from those data we can see with the increase of the amout of SB, the function of SB became stronger and the T_{max} of TMPP in plasma and brain was delayed from 5 to 10 min which could show SB's function of penetration.

In conclusion, the analytical method described above was validated to meet the requirement of pharmaceutical investigation of TMPP. The pharmacokinetic results provide value information for studying the dosage of SB in the preparations containing TMPP and also indicate the potential use of SB in other drugs.

Acknowledgement

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