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# Intraocular administration of tetramethylpyrazine hydrochloride to rats: a direct delivery pathway for brain targeting?

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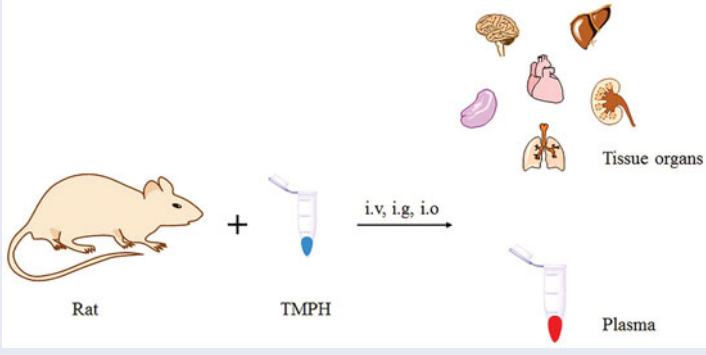
**ABSTRACT**

The purpose of this study was to compare the pharmacokinetic profile of tetramethylpyrazine hydrochloride (TMPH) in rat plasma and tissues following intravenous (iv), intragastric (ig) and intraocular (io) administration. After io, ig and iv administration of a single dose at 10 mg/kg, tissue and plasma samples drawn from the femoral artery were collected at timed intervals. The concentration of TMPH in the samples was analyzed using high-performance liquid chromatography (HPLC). The area under the concentration–time curve (*AUC*) and the drug targeting efficiency percentage (*DTE*(%)) were calculated to evaluate the targeting efficiency of the drug with the three different administration routes. After io administration, TMPH was rapidly absorbed to reach its peak plasma and brain concentration within 5 min. The systemic bioavailability obtained with io administration was greater than that obtained through the ig route (63.22% vs. 16.88%). The $AUC_t$ rank order of the iv administration group was $AUC_{kidney} > AUC_{heart} > AUC_{liver} > AUC_{brain} > AUC_{spleen} > AUC_{lung}$; that of the ig administration group as $AUC_{kidney} > AUC_{liver} > AUC_{heart} > AUC_{spleen} > AUC_{brain} > AUC_{lung}$; while that of the io administration group was $AUC_{kidney} > AUC_{brain} > AUC_{heart} > AUC_{liver} > AUC_{spleen} > AUC_{lung}$. The ratio of the $AUC_{brain}$ value between the io route and iv injection was 1.05, which was greater than that obtained after ig administration (0.30). The *DTE* after io administration was calculated: brain (165.72%), heart (97.76%), liver (113.06%), spleen (105.31%), lung (163.40%) and kidney (135.31%). The io administration group showed obvious drug transport to the brain. These results indicate that TMPH is rapidly absorbed from the eye into the systemic circulation, and there may be a direct translocation pathway for TMPH from the eye to the brain. Therefore, io administration of TMPH could be a promising alternative to intravenous and oral approaches.

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Tetramethylpyrazine hydrochloride; intraocular administration; bioavailability; pharmacokinetics; tissue distribution

## 1. Introduction

Ophthalmic drug administration has a long history and has always played an important role in the clinic. However, research into ophthalmic drug delivery systems has mainly focused on the treatment of eye diseases (Yellepeddi & Palakurthi, 2016), including ocular infections, dry eye, glaucoma, and retinal lesions. The absorption of drugs through the ophthalmic route includes corneal penetration and conjunctival penetration (Mannermaa et al., 2006). Corneal penetration is the major route for drugs to exert local action. However, the conjunctiva is rich in blood and lymphatic vessels, the drug can also be absorbed by the conjunctiva through these vessels.

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Based on the special structural characteristics of the eye and the fact that drugs can enter the rest of the body through a variety of routes after io administration (Hornof et al., 2005), we believe that drugs can be absorbed directly into the systemic circulation through io administration and therefore treat systemic diseases. Previous studies (Baba et al., 1983; Kumar et al., 1985; Novack et al., 1987; Lahdes et al., 1988, 1990; Kaila et al., 1989; Lahdes et al., 1994; Lanzl et al., 2016) have shown that the blood concentration of many eye drops, such as betamethasone and atropine, are high after io administration, which indicates that the drug can enter the systemic circulation through the eyes. Chiou (Chiou, 1994) explored the possibility of the systemic administration of insulin via io administration. Therefore, io administration may be a new way to achieve systemic administration, which can avoid the first pass effect of the liver. It is therefore of practical value to study the *in vivo* pharmacokinetic properties of a drug following io administration.

Ligustrazine, also known as tetramethylpyrazine (TMP), is the main active component in the Chinese herbal medicine *Ligusticum wallichii*. Tetramethylpyrazine hydrochloride (TMPH) is a fully synthetic version of this compound. Pharmacological studies have shown that TMPH is a calcium antagonist, which has many functions such as anti-platelet aggregation, stimulating the medullary respiratory center and the vasomotor center, dilating vascular and bronchial smooth muscle, and improving microcirculation (Wang et al., 2011). At present, it is widely used in the treatment of acute occlusive cardio-cerebrovascular and ischemic cardio-cerebrovascular diseases such as coronary heart disease, cerebral thrombosis, and cerebral artery embolism. The pharmacological effect is specific and the side effects are small (Ding, 2007). The commonly used dosage forms are injection and tablet. As the half-life of TMPH is short achieving sustained effects is inconvenient (Yan et al., 2015). When administered orally, the bioavailability of TMPH is decreased because of the first-pass effect of the liver (Feng et al., 2009). As a mucosal administration route, io administration is convenient, facilitates patient compliance, and can avoid gastrointestinal degradation and the first-pass effect. Therefore, I consider that tetramethylpyrazine's target organs (brain and heart) and the ocular administration herein proposed can be advantageous in relation to classic routes. In the current study, the *in vivo* pharmacokinetics, including plasma pharmacokinetic and tissue distribution, of TMPH after io administration were studied to investigate the potential of the drug to exert therapeutic effects.

## 2. Materials and methods

### 2.1. Chemicals and reagents

TMPH was purchased from Yuancheng Technology co., Ltd. (Wuhan, China). Carbamazepine (internal standard, I.S.) was supplied by DeSite Biotechnology Co., Ltd. (Chengdu, China). Methanol (high-performance liquid chromatography [HPLC] grade) was purchased from sigma scientific (USA). Water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and reagents used were of analytical grade.

### 2.2. Animals

Male Sprague Dawley rats weighing 200–250 g were obtained from the Experimental Animal Center of Anhui Medical University and maintained on a light/dark cycle. Temperature and relative humidity were maintained at 25 °C and 50%, respectively. The rats were fasted overnight (approximately 12 h) before each experiment. And they were under awake during drug administration.

### 2.3. Preparation of TMPH solution

A dosing solution of 100 mg/mL was prepared by dissolving TMPH powder in physiological saline for the io administration, and a dosing solution of 2 mg/mL was prepared by dissolving TMPH powder in physiological saline for the ig and iv administration routes. The preparations were made immediately prior to drug administration. All animal experiments were approved by the Animal Management and Ethics Committee of Anhui University of Chinese Medicine.

### 2.4. Animal experiment

#### 2.4.1. Experiment design

A total of 135 Sprague-Dawley rats randomly divided into three groups were used in this study ($n = 3$), including an iv administration group, an ig administration group and an io administration group. TMPH solution was administered at a single dose of 10 mg/kg body weight to each rat (Meng et al., 2014).

#### 2.4.2. Biological sampling and treatment for pharmacokinetic studies

For iv injection, dosing solutions were delivered using a 1-mL syringe into the tail vein. For io delivery, dosing solutions were delivered using a micro syringe into the conjunctival sac. Oral gavage of TMPH was performed using a stainless-steel feeding needle attached to a syringe containing the oral formulation.

At predetermined time points (2, 5, 10, 30, 60, 90, 120, 180 and 210 min) after TMPH dosing (five animals per time point, $n = 5$), rats were sacrificed through femoral artery bleeding and the blood was immediately collected into heparinized polystyrene tubes while the tissues (brain, heart, liver, spleen, lung and kidney) were quickly removed and weighed. Blood samples were centrifuged at 4 °C and 3500 rpm for 10 min to obtain plasma supernatants that were stored at −20 °C prior to analysis. The tissues were homogenized in three times their weight of physiological saline. Tissue homogenates were centrifuged at 3500 rpm for 10 min (4 °C) and the resultant supernatants were also frozen at −20 °C until analysis.

Plasma and tissues samples were processed through the following steps: a 100 μL aliquot of plasma or tissues sample

Table 1. Validation parameters of the HPLC method employed for the quantification of tetramethylpyrazine hydrochloride in plasma and tissue homogenate supernatants ($n = 3$).

| Validation parameters | Plasma | Brain | Heart | Liver | Spleen | Lung | Kidney |
|---|---|---|---|---|---|---|---|
| Linearity range (μg/mL) | 0.1–10[a] | 0.05–5[b] | 0.05–5[b] | 0.05–5[b] | 0.05–5[b] | 0.05–5[b] | 0.05–5[b] |
| $r^2$ | 0.9979 | 0.9984 | 0.9989 | 0.9992 | 0.9967 | 0.9976 | 0.9983 |
| LLOQ (μg/mL) | 0.1[a] | 0.05[b] | 0.05[b] | 0.05[b] | 0.05[b] | 0.05[b] | 0.05[b] |
| Intra-day precision (%CV) | 1.79–3.82 | 0.78–6.52 | 1.52–6.11 | 1.11–7.68 | 1.30–3.92 | 1.03–5.76 | 0.53–5.70 |
| Inter-day precision (%CV) | 2.91–5.74 | 2.03–7.09 | 1.03–8.45 | 2.96–7.64 | 1.03–5.68 | 1.58–6.07 | 1.21–6.35 |
| Intra-dayaccuracy (%Bias) | −3.97–3.65 | −6.65–7.46 | −5.43–6.61 | −8.26–6.82 | −4.08–3.74 | −5.26–6.16 | −3.94–6.53 |
| Intra-dayaccuracy (%Bias) | −6.60–6.98 | −9.77–8.74 | −10.26–11.95 | −8.19–12.26 | −7.97–7.03 | −6.53–9.38 | −8.34–7.91 |
| Absolute recovery (%) | 83.45–101.71 | 84.48–94.17 | 83.31–98.03 | 85.45–100.71 | 86.40–103.38 | 87.42–101.99 | 91.95–102.19 |

[a]Values expressed in μg/mL.
[b]Values expressed in μg/g.
Coefficient of determination ($r^2$); Lower limit of quantification (LLOQ); Coefficient of variation (CV); Bias (deviation from nominal value).

and a 10 μL I.S. (10 μg/mL) working solution were added in turn. Samples were then vortex-mixed for 1 min and extracted with 300 μL of methanol through vortex mixing for 3 min. After centrifugation at 12,000 rpm for 10 min, a 40 μL aliquot of the supernatant was injected into the HPLC system for analysis.

### 2.5. Analytical method

Plasma and tissue concentrations of TMPH were measured using a Shimadzu Liquid chromatographic 20 system equipped with a DAD(Diode Array Detector) (Shimadzu Corporation, Japan). Separations were carried out using an Agilent TC-$C_{18}$ column (250 mm × 4.6 mm, 5 μm, Agilent, USA). The mobile phase consisted of water-methanol (45:55, v/v) at a flow-rate of 1 mL/min and the detector wavelength was set at 279 nm. Chromatographic separation was performed at 30 °C. The retention times of TMPH and the I.S. were 6.1 min and 10.5 min, respectively. The main validation parameters of the analytical method are in agreement with the international bioanalytical guidelines (European Medicines Agency, 2011; Food and Drug Administration, 2018) and summarized in Table 1. For plasma analysis, the lower limit of quantification (LLOQ) for TMPH was 0.1 μg/mL and the linear range was 0.1–10 μg/mL in rat plasma. The LLOQ value of the tissue samples was 0.05 μg/mL and the method was linear over a concentration range of 0.05 to 5 μg/mL.

### 2.6. Pharmacokinetic analysis

The results obtained from the HPLC analyses were plotted as concentration–time curves for plasma and tissues. The pharmacokinetic parameters were estimated through a noncompartmental pharmacokinetic analysis employing the DAS 2.0 software. The mean area under the curve ($AUC_t$) was calculated using the trapezoidal method; the *AUC* from time zero to infinite ($AUC_{inf}$), calculated by $AUC_t + (C_{last}/k_{el})$, where $C_{last}$ is the last quantifiable concentration and $k_{el}$ is the apparent elimination rate constant, estimated by log-linear regression of the terminal segment of the concentration–time profile; and the percentage of *AUC* extrapolated from $t_{last}$ to infinity [$AUC_{extrap}$(%)], where $t_{last}$ is the time of the $C_{last}$. The apparent terminal elimination half-life ($t_{1/2el}$) and the mean residence time (MRT) were additionally determined. The maximum concentration ($C_{max}$) and the time to reach peak concentration ($t_{max}$) were the observed values. Results are presented as mean ± SD.

The absolute bioavailability [*F*(%)] of TMPH after io and ig administration was calculated as follows (Equation (1)):

$$F(\%) = \frac{(AUC_{i.o.} \times Dose_{i.v.})}{(AUC_{i.v.} \times Dose_{i.o.})} \times 100$$
$$F(\%) = \frac{(AUC_{i.g.} \times Dose_{i.v.})}{(AUC_{i.v.} \times Dose_{i.g.})} \times 100 \quad (1)$$

where $AUC_{io}$ and $AUC_{iv}$ are the areas under the drug concentration–time curves from time zero to the last following io and iv administration, respectively; $Dose_{iv}$ and $Dose_{io}$ are the values of the TMPH dosage (mg/kg) administered through the iv and io route to rats.

To assess the overall tendency of io administered TMPH to accumulate in tissues, especially the brain, the drug targeting efficiency (*DTE*) index was calculated (Kozlovskaya et al., 2014). The *DTE* index represents the tissue-to-plasma partitioning ratio of the drug administered through the io or ig route compared with that administered through iv injection. The higher the *DTE*, the higher the expected degree of TMPH tissue targeting. *DTE* was calculated using the following equation (Equation (2)):

$$DTE_{i.o.}(\%) = \frac{\left(AUC_{tissue}/AUC_{plasma}\right)_{i.o.}}{\left(AUC_{tissue}/AUC_{plasma}\right)_{i.v.}} \times 100$$
$$DTE_{i.g.}(\%) = \frac{\left(AUC_{tissue}/AUC_{plasma}\right)_{i.g.}}{\left(AUC_{tissue}/AUC_{plasma}\right)_{i.v.}} \times 100 \quad (2)$$

In order to estimate the drug fraction that is transported directly into brain through io administration, the direct transport percentage (DTP) was calculated by subtracting the contribution of the indirect pathway (via absorption into the systemic circulation) from the total io brain *AUC*, in accordance to following equation (Equation (3)) (Gonçalves et al., 2019):

$$DTP(\%) = \frac{AUC_{brain(i.o.)} - \left[\frac{AUC_{brain(i.v.)}}{AUC_{plasma(i.v.)}} \times AUC_{plasma(i.o.)}\right]}{AUC_{brain(i.o.)}} \times 100 \quad (3)$$

Hence, the smaller the ratio of iv brain *AUC* to iv plasma *AUC* is, the larger *DTP* values are. Consequently, *DTP* values higher than 0 indicate the presence of brain targeting through direct pathways, in opposition to values from $-\infty$ to 0, which indicate a more efficient brain targeting by iv route (Katare et al., 2017).

### 2.7. Statistical analysis

The data were expressed as means ± S.D. Statistical analyses were assessed using ANOVA test. Statistically significant differences were for a *p*-value lower than 0.05 ($p < 0.05$).

## 3. Results

### 3.1. Pharmacokinetics of TMPH after iv, ig and io administration

The mean concentration-time profiles of TMPH obtained in rat plasma and tissues after a single dose administration of the drug (10 mg/kg) are depicted in Figures 1 and 2, respectively. The main plasma pharmacokinetic parameters estimated using a noncompartmental analysis are summarized in Table 2.

Although $C_{max}$ of TMPH reached in the plasma after io administration lower than iv injection ($p < 0.05$); however, the plasma $C_{max}$ following io administration was 2.37 times higher compared with that following ig administration, and the bioavailability was 63.22%, 3.75 times higher than that following ig administration ($p < 0.05$). Interestingly, the plasma half-life of TMPH after io administration was significantly higher than iv and ig administration, which was beneficial for prolonging the duration of drug action ($p < 0.05$).

### 3.2. Biodistribution studies

Figure 2 shows the mean tissue concentration–time profiles of each tissue for the three routes of administration. The corresponding main pharmacokinetic parameters estimated using a non-compartmental analysis are summarized in Table 3.

The $C_{max}$ of TMPH achieved in each tissue after io administration was almost lower than that of iv injection; however, regarding the total tissue exposure of the drug being evaluated by the $AUC_{inf}$ parameter, it is interesting that the higher values of total brain exposure were assigned to the io route. The $C_{max}$ in each tissue following io administration was higher than that following ig administration, except in the liver and lungs. The ratio of $C_{max}$ in the io administration group to that in the ig administration group in the brain, heart, liver, spleen, lung and kidney was 1.96, 1.86, 0.86, 2.03, 0.80 and 1.63, respectively. The $AUC_t$ values after iv administration were in the rank order of $AUC_{kidney} > AUC_{heart} > AUC_{liver} > AUC_{brain} > AUC_{spleen} > AUC_{lung}$; the $AUC_t$ values after ig administration were in the rank order of $AUC_{kidney} > AUC_{liver} > AUC_{heart} > AUC_{spleen} > AUC_{brain} > AUC_{lung}$; however, the same figures after io administration of TMPH were in the rank order of $AUC_{kidney} > AUC_{brain} > AUC_{heart} > AUC_{liver} > AUC_{spleen} > AUC_{lung}$. The ratio of $AUC_t$ in the io administration group to that in the iv administration group was higher than the ratio of $AUC_t$ in the ig administration group to that in the iv administration group, in the brain, heart, liver, spleen, lung and kidney. Moreover, the ratio of $AUC_t$ in the io administration group to that in the iv administration group in the lung and brain was more than 1. After ig administration, the $AUC_t$ in the brain was 21.14 μg·min/mL, whereas the $AUC_t$ after io administration was a surprising 3.54 times higher at 74.79 μg·min/mL. In addition, the $t_{1/2el}$

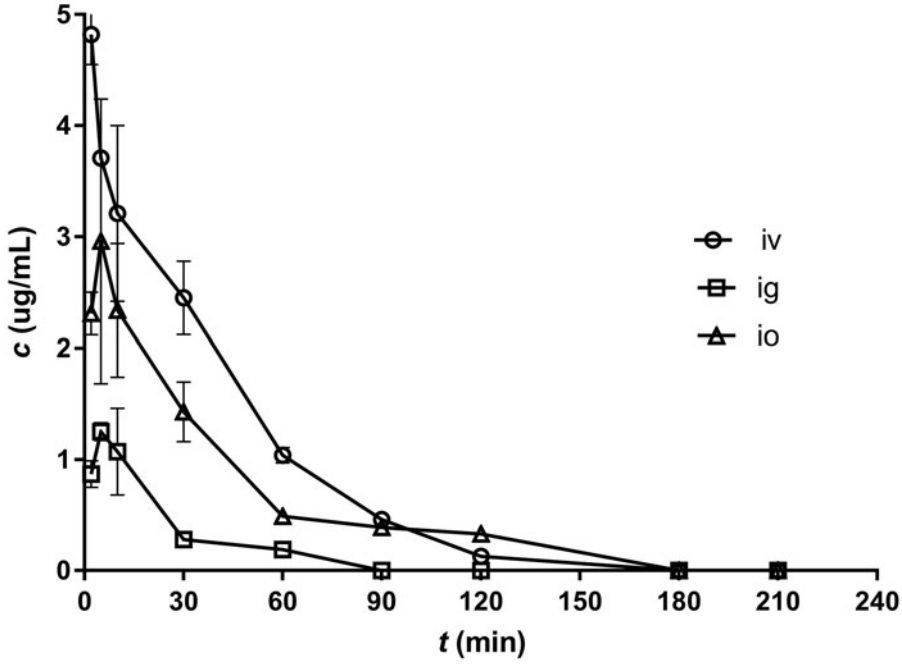


Figure 1. Concentration–time curve of tetramethylpyrazine hydrochloride in plasma with different administration routes.

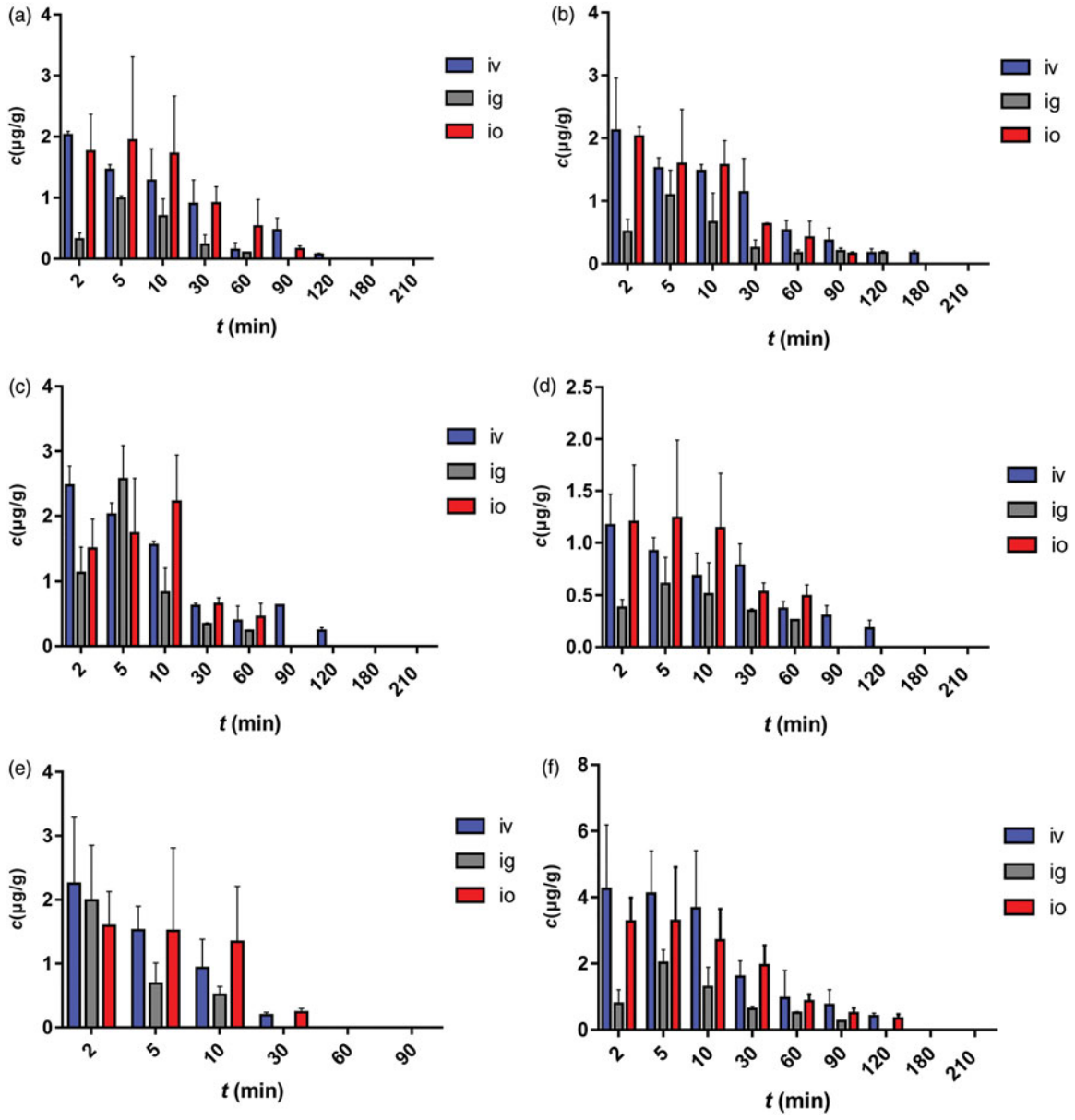


**Figure 2.** Concentration of TMPH in tissue samples following different routes of administration (Brain (a), Heart (b), Liver (c), Spleen (d), Lung (e), Kidney (f)).

**Table 2.** Plasma pharmacokinetic parameters of TMPH after intravenous, intragastric and intraocular administration at a dose of 10 mg/kg ($n = 5$, Mean ± SD).

| Parameters | iv | ig | io |
|---|---|---|---|
| $t_{max}$ (min) | – | 5 | 5 |
| $C_{max}$ (μg/mL) | 4.82 ± 0.27 | 1.25 ± 0.30 | 2.96 ± 0.43*△ |
| $k_{el}$ ($min^{-1}$) | 0.03 | 0.03 | 0.02 |
| $t_{1/2el}$ (min) | 24.75 ± 1.36 | 20.16 ± 2.74 | 31.20 ± 1.85*△ |
| $AUC_t$ (μg·min/mL) | 180.20 ± 5.71 | 30.41 ± 3.29 | 113.93 ± 18.93*△ |
| $AUC_{inf}$ (μg·min/mL) | 187. 20 ± 6.53 | 35.94 ± 2.79 | 122.88 ± 15.78 |
| $AUC_{extrap}$ (%) | 3.74 | 15.39 | 7.28 |
| [a]*F*/% | – | 16.88 ± 1.29 | 63.22 ± 8.51* |

*Significantly different from ig group, $p < 0.05$.
△Significantly different from iv group, $p < 0.05$.
$t_{max}$: Time to achieve the maximum peak concentration; $C_{max}$: Maximum peak concentration; $k_{el}$: Apparent elimination rate constant; $t_{1/2el}$: Apparent terminal elimination half-life; $AUC_t$: Area under the concentration time–curve from time zero to the last quantifiable drug concentration; $AUC_{inf}$: Area under the concentration time–curve from time zero to infinite; $AUC_{extrap}$: Extrapolated area under the drug concentration time–curve.
[a]Absolute bioavailability (*F*) was calculated based on $AUC_t$ values.

of the drug in the tested tissues was found to be higher in the io administration group than in the ig administration group, except in the spleen and lung.

The $AUC_t$ and $AUC_{inf}$ values of TMPH in rat tissues are shown in Figure 3. And $AUC_{extrap}$ % was calculated by them, the results are all below 20%, it suggest that the collection

Table 3. Pharmacokinetics parameters of TMPH in different tissues ($n = 5$, Mean ± SD).

| | | | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tissue | Routes | $t_{max}$ (min) | $C_{max}$ (μg/g) | $K_{el}$ ($min^{-1}$) | $t_{1/2}$ (min) | $AUC_t$ (μg·min/mL) | $AUC_{inf}$ (μg·min/mL) | $AUC_{extrap}$(%) | $AUC_{ig(io)}/AUC_{iv}$ (%) |
| Brain | iv | 2 | 2.03 ± 0.06 | 0.03 | 25.62 ± 1.03 | 71.38 ± 2.17 | 74.02 ± 3.56 | 3.57 | – |
| | ig | 5 | 0.99 ± 0.04 | 0.06 | 12.03 ± 0.35 | 21.14 ± 1.01 | 21.84 ± 2.13 | 3.21 | 29.62 |
| | io | 5 | 1.94 ± 0.37 | 0.02 | 29.45 ± 3.25 | 74.79 ± 6.39 | 85.94 ± 5.78 | 12.97 | 104.78 |
| Heart | iv | 2 | 2.12 ± 0.84 | 0.01 | 37.57 ± 5.37 | 100.34 ± 13.12 | 118.44 ± 10.35 | 15.28 | – |
| | ig | 5 | 1.09 ± 0.40 | 0.05 | 13.34 ± 1.23 | 33.98 ± 3.46 | 36.16 ± 1.58 | 6.42 | 33.86 |
| | io | 2 | 2.03 ± 0.15 | 0.03 | 25.03 ± 4.79 | 62.02 ± 10.03 | 67.83 ± 11.23 | 8.57 | 61.81 |
| Liver | iv | 2 | 2.47 ± 0.30 | 0.02 | 69.12 ± 3.06 | 85.87 ± 5.48 | 96.52 ± 4.36 | 11.03 | – |
| | ig | 5 | 2.57 ± 0.52 | 0.04 | 16.13 ± 0.78 | 35.56 ± 2.31 | 37.75 ± 3.17 | 5.80 | 41.41 |
| | io | 10 | 2.22 ± 0.72 | 0.05 | 30.99 ± 3.21 | 61.38 ± 12.15 | 70.69 ± 15.13 | 13.17 | 71.48 |
| Spleen | iv | 2 | 1.17 ± 0.30 | 0.02 | 61.26 ± 7.35 | 64.37 ± 8.76 | 79.05 ± 12.34 | 18.57 | – |
| | ig | 5 | 0.61 ± 0.25 | 0.05 | 47.20 ± 2.58 | 22.83 ± 1.35 | 26.45 ± 2.63 | 13.69 | 35.47 |
| | io | 5 | 1.24 ± 0.75 | 0.05 | 40.67 ± 6.12 | 42.86 ± 5.21 | 51.46 ± 3.56 | 16.71 | 66.58 |
| Lung | iv | 2 | 2.25 ± 1.04 | 0.08 | 8.43 ± 1.48 | 32.34 ± 2.17 | 32.53 ± 3.18 | 0.58 | – |
| | ig | 2 | 1.99 ± 0.86 | 0.05 | 14.68 ± 1.16 | 17.36 ± 2.30 | 18.42 ± 1.78 | 5.75 | 53.68 |
| | io | 2 | 1.59 ± 0.54 | 0.05 | 14.31 ± 1.59 | 33.41 ± 4.35 | 36.48 ± 5.49 | 8.42 | 103.31 |
| Kidney | iv | 2 | 4.26 ± 1.93 | 0.02 | 34.89 ± 3.43 | 174.97 ± 21.57 | 195.78 ± 19.48 | 10.63 | – |
| | ig | 5 | 2.02 ± 0.40 | 0.02 | 28.60 ± 2.14 | 62.11 ± 11.21 | 72.62 ± 10.47 | 14.47 | 35.50 |
| | io | 5 | 3.29 ± 1.62 | 0.02 | 36.64 ± 4.89 | 149.68 ± 15.13 | 167.96 ± 13.85 | 10.88 | 85.55 |

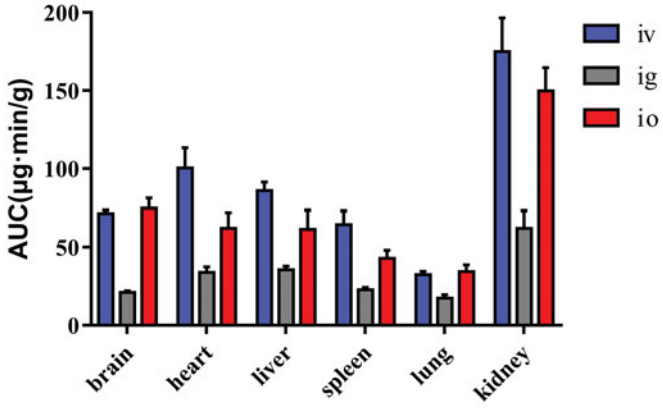


Figure 3. $AUC_t$ of tetramethylpyrazine hydrochloride in rat tissues after intravenous, intragastric and intraocular administration at 10 mg/kg.

time of biological samples was enough. It can be seen that TMPH is widely distributed in blood-rich tissues, such as the heart, liver and kidney; it can also penetrate the blood-brain barrier, and substantial levels of the drug were detected in the brain; but in the lung and spleen, the distribution was relatively small

### 3.3. Drug targeting evaluation

As shown in Table 3, the io administration group demonstrated a high drug concentration in the heart, kidney and brain tissues; the brain tissue in the io administration group showed the maximum drug concentration enhancement compared with that in the ig administration group, except the kidney. Table 4 shows the *DTE* of TMPH obtained after io and ig administration. For io administration, the maximum *DTE* value was observed in the brain, while the *DTE* of the lung was largest for ig administration. Consistently, the value of *DTE* in the brain was 165.72% and *DTP* was 39.99%. The results demonstrate that a greater portion of TMPH targets brain tissue than other tissues following io administration.

Table 4. Comparison of drug targeting parameters of TMPH in different tissues after intravenous, intragastric and intraocular administration at a dose of 10 mg/kg ($n = 5$).

| $AUC_t$ ratios | io | ig | iv | $DTE_{io}$/% | $DTE_{ig}$/% |
|---|---|---|---|---|---|
| $AUC_{brain}/AUC_{plasma}$ | 0.66 | 0.70 | 0.40 | 165.72 | 175.50 |
| $AUC_{heart}/AUC_{plasma}$ | 0.54 | 1.12 | 0.56 | 97.76 | 200.67 |
| $AUC_{liver}/AUC_{plasma}$ | 0.54 | 1.17 | 0.48 | 113.06 | 245.39 |
| $AUC_{spleen}/AUC_{plasma}$ | 0.38 | 0.75 | 0.36 | 105.31 | 210.17 |
| $AUC_{lung}/AUC_{plasma}$ | 0.29 | 0.57 | 0.18 | 163.40 | 318.09 |
| $AUC_{kidney}/AUC_{plasma}$ | 1.31 | 2.04 | 0.97 | 135.31 | 210.35 |

*DTE* (%): Drug targeting efficiency percentage.

## 4. Discussion

The treatment of biological samples prior to the determination of drug levels is critical. We attempted to extract TMPH from plasma and tissue samples using ethyl acetate 10%, perchloric acid and acetonitrile (Liu et al., 2014, 2017; Li et al., 2016), but the extraction rate was low. Subsequently, the organic phase was concentrated in a water bath at 40 °C under a nitrogen atmosphere; however, the drying process caused a significant loss of TMPH because of its volatility. After several attempts, methanol was chosen as a protein precipitator to prepare the samples, and the results show that the method is simple and reliable. For the selection of an I.S., we assessed nitrendipine, coumarin, and carbamazepine (Yao et al., 2010; Li et al., 2011; Han et al., 2014). The results show that carbamazepine was the most suitable.

The pharmacokinetic results showed that the bioavailability of TMPH was very low following ig administration (16.88%), which is consistent with the literature (Meng et al., 2014); but its absolute bioavailability increased significantly after io administration (63.22%). In addition, the plasma half-life of TMPH was 31.20 min after io administration, which was longer than that following iv administration (24.75 min) and ig administration (20.16 min). These results suggest that TMPH can be absorbed into the blood following io administration and it is feasible for systemic therapy. The peak concentration of TMPH in plasma was reached approximately

5 min after io and ig administration, but the $C_{max}$ following io administration was significantly higher than that following ig administration, which indicated that io TMPH administration could avoid the first-pass effect of the liver and improve the bioavailability of the drug. These results for io administration approximate the law of systemic transportation of drugs after nasal administration (Yao et al., 2010; Yan et al., 2015). Eye drops can quickly enter the blood, which can meet the requirement for rapid effects in the treatment of cardiovascular and cerebrovascular diseases.

In terms of tissue distribution, regardless of the route of administration, the drug was detected in all tissues. As TMPH is mainly used for the treatment of cerebral ischemia, it is expected that it will be more distributed in brain tissue. As shown in Figure 2, TMPH can be absorbed into the brain and reaches a peak concentration 5 min after io and ig administration, but the $C_{max}$ following io administration was significantly higher than that following ig administration, and similar to iv administration. The ratio of the $AUC_{brain}$ value between the io administration route and the iv injection route was 1.05, which is greater than that obtained after ig administration (0.30). The *DTE* of the io administration route in the brain was 165.72%, which is close to that of the ig administration route (175.50%). This is consistent with a study by Meng et al., but the *DTE* values calculated in that study were all less than 1. This may be caused by individual variability between distinct experimental animals since each rat was assigned to one of the three administration groups (io, iv or ig) and also to a unique sampling time point. However, the results demonstrate that a substantial fraction of the drug has effectively been absorbed from the eye into the systemic circulation and has gained access to the central nervous system by crossing the blood-brain barrier. The *DTE* values greater than 1 demonstrated in the current study may indicate a direct eye-brain pathway that is involved after io administration. And at the same time, *DTP* was estimated, and revealed that 39.99% of TMPH that reached the brain underwent direct eye-to-brain delivery.

In the current study, the total brain tissue was evaluated for brain targeting; however, the specific distribution of TMPH in cerebral areas, such as in the cerebellum, striatum, and olfactory bulb, requires further study. And, comparing to iv route, intraocular present lower $C_{max}$ values, we will further verify whether TMPH can achieve the expected therapeutic effect through the efficacy test in the latter study. More importantly, due to metabolic differences between human and rat, the results of the study cannot be easily extended to humans, requiring a large number of experimental arguments (Yang et al., 2018).

## 5. Conclusion

To the best of our knowledge, a comprehensive characterization of the pharmacokinetic behavior and tissue distribution profile of TMPH following io administration in rats has not been reported previously. A method for the determination of TMPH levels in rat plasma and tissue was established. The specificity, linear relationship, intra-day and inter-day precision, recovery rate and detection limit of the chromatographic analysis method were investigated. Then, the drug concentration data were processed using a pharmacokinetic procedure, and related pharmacokinetic parameters, such as $AUC_t$, $AUC_{inf}$, $AUC_{extrap}$, $t_{max}$, $C_{max}$, $t_{1/2el}$ and the *DTE* index were calculated and *DTP* was calculated. The results show that TMPH was rapidly absorbed into the systemic circulation and its distribution in the brain was significantly increased following io administration, which provides a theoretical basis for further study of the preparation.

In conclusion, compared with ig and iv administration, io administration can lead to rapid TMPH targeting in brain tissue and the $AUC_{brain}$ values far exceed those of ig administration, suggesting that some drugs may enter the brain directly through the eye. Therefore, io administration is a promising alternative route for the administration of TMPH for brain therapy. The method is also noninvasive, permits the rapid onset of therapeutic effects and avoids first-pass hepatic metabolism.

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## Disclosure statement

No conflict of interest exits in the submission of this manuscript, and the manuscript is approved by all authors for publication.

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