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Microdialysis combined with UPLC–MS/MS method for determination of tetramethylpyrazine and ferulic acid in striatum of awake and anesthetic rats subjected to cerebral ischemia

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ABSTRACT

A rapid, sensitive and selective ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method has been developed for the simultaneous determination and pharmacokinetic investigation of Tetramethylpyrazine (TMP) and Ferulic acid (FA) in rat striatum. The method was validated over the concentration range of 1.15–505 ng/mL for TMP and 3.23–101 ng/mL for FA, with a lower limit of quantitation (LLOQ) of 1.15 ng/mL and 3.23 ng/mL, respectively. This method can be successfully applied in pharmacokinetic studies of TMP and FA in striatum of awake and anesthetic rats. The cerebral blood flow velocity (CBF) during middle cerebral artery occlusion (MCAO) was monitored by Laser speckle contrast imaging, to observe whether the compatibility of TMP and FA could improve CBF against cerebral ischemia/reperfusion (I/R) injury. Infarct volume was examined to evaluate severity of ischemic brain injury. The pharmacokinetic study indicated that $T_{1/2}$, C_{max}, MRT and $AUC_{0\text{-inf}}$ were changed after combined administration of TMP and FA, when compared with either drug alone both in awake and anesthetic groups. The pharmacodynamics results showed that co-administration of drugs could enhance the CBF during middle cerebral artery occlusion and reduced the infarct volume. Taken together, the compatibility treatment of TMP and FA might be a promising therapeutic strategy for ischemic stroke. Further study is required to optimize the compatibility proportion.

1. Introduction

Ischemic stroke is caused by the blockage of a blood vessel supplying the brain with oxygen and nutrients, impairing the blood flow to a particular region of the brain below a critical level which leads to a series of functional and structural changes resulting in death and disability [1]. Cerebral blood flow velocity (CBF) serves as an important physiological marker, which reflects the *in vivo* hemodynamic and cerebral circulation status. It has been reported that restored CBF to the ischemic region through collateral vasculature may save the brain tissue from death and lead to improved recovery [2,3]. Thus, studies establishing the drug's effects on the cerebral hemodynamic during an ischemic stroke would greatly improve our abilities to develop therapeutic interventions, aiming to improve vascular function and brain tissue survival after stroke. Laser speckle contrast imaging (LSCI), a non-contact full-field technique with high spatial and temporal resolution, has been reported that can enable rapid identification and determination of collateral blood flow, CBF and prediction of infarct area [4,5].

2,3,5,6-tetramethylpyrazine (TMP), one of the most important active ingredients isolated from the herb *Ligusticum wallichii*, demonstrates the effect of mediated neuroprotection in rat brain after focal ischemia [6] and reduces cerebral ischemic reperfusion damage [7], and thus widely used in the treatment of ischemic stroke. Ferulic acid (FA) also has long been used to treat ischemic stroke. It is the main water-soluble component of the roots of *Angelica sinensis*, showing a significant protective effect on the nerve injury of cerebral ischemia [8], and reduced cerebral infarction volume in MCAO rats [9]. However, TMP and FA displayed low bioavailability and metabolized rapidly, with a short half-life. Even though TMP and FA could traverse the blood brain barrier (BBB) quickly, they were eliminated from brain tissue rapidly [10,11]. Therefore, optimizing the bioavailability of TMP and FA to enhance their therapeutic efficacy is critical for application of drugs in the clinic. The conventional solution, raising the blood concentration of

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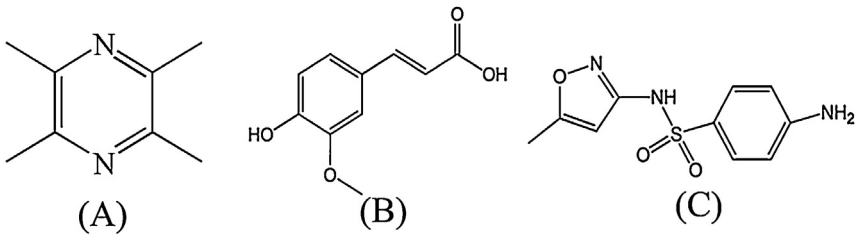

Fig. 1. Structures of tetramethylpyrazine(A), ferulic acid (B), and sulfamethoxazole (C).

Table 1
The SRM conditions of analytes in HESI mode in LC–MS/MS.

Analyte	Identity	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	T-Lens	Collision energy (eV)	Retention time (min)
TMP	$[M+H]^+$	137.130	55.430	60	24	1.34
FA	$[M-H]^-$	193.160	134.150	73	17	1.68
IS	$[M+H]^+$	254.080	92.150	74	27	2.03

drugs to a relatively high level, can produce undesired side-effects in patients (*e.g.* allergic reactions, angioedema, laryngeal edema, bronchial asthma or anaphylactic shock) [12].

Based on the remarkable cardiovascular and cerebrovascular effect, *Ligusticum wallichii* and *Angelica sinensis* are used in combination frequently. In addition, it has been reported that the plasma half-life time and the average residence time were extended after combined administration of TMP and FA, compared with either drug alone [13,14]. The present study was first carried out using microdialysis technique to investigate the brain pharmacokinetics behavior of TMP and FA monomers, and then the combination of drugs in awake and anesthetic rats, following intragastric administration. LSCI was used to determine whether TMP and FA could preserve CBF during MCAO at rats. The pharmacokinetics behavior and pharmacodynamics results of TMP and FA enable a better understanding of the complex mechanisms of absorption, distribution, metabolism, excretion and safety for future clinical applications; also partly clarifying the rationality and compatibility of the prescriptions containing *Ligusticum wallichii* and *Angelica sinensis*.

2. Experimental

2.1. Chemicals and reagents

Ferulic acid (Fig. 1; 99.7% purity) and Tetramethylpyrazine hydrochloride standard (65.2% of tetramethylpyrazine free base) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Tetramethylpyrazine (Fig. 1; 98.5% purity) was purchased from Nanjing Zelang Medical Science and Technology Co., Ltd (Jiangsu, China). Sulfamethoxazole (Fig. 1; internal standard, IS; 99.5% purity) was purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). 2,3,5-triphenyltetrazolium chloride (TTC, Mym Biological Technology, Andhra Pradesh, India). HPLC grade acetonitrile, methanol, and formic acid were provided from Thermo Fisher Company Inc. (Waltham, MA, USA). Ultra-pure water was obtained using a Millipore Milli-Q system (Billerica, MA, USA). All other reagents were purchased from Guangdong Guanghua Chenmical Factory Co., Ltd. (Guangzhou, China) of analytical grade.

2.2. Animals

Male adult Sprague-Dawley rats (weighting 265 ± 15 g) were obtained from the Laboratory Animal Center of Guangzhou University of Chinese Medicine (Guangdong, China) and housed at a temperature and light controlled environment with a 12 h light/12 h dark cycle for a week. The rats were then fasted with only access to water for 12 h prior to the experiment. All experiments were carried out in accordance with the NIH guidelines and approval by the Animal Care and Use Committee of the Guangzhou University of Chinese Medicine (Guangdong, China). All efforts were made to minimize animal suffering.

The rats were separated into two experiments. In the first experiment, rats (n = 36) were subjected to cerebral microdialysis study; all the rats were randomly divided into three groups: group 1 (Containing Awake and Anesthetic TMP group) was treated with TMP (4.0 mg/kg, ig [15]); group 2 (Containing Awake and Anesthetic FA group) was treated with FA (5.0 mg/kg, ig) and group 3 (Containing Awake and Anesthetic co-administration group) was treated with mixed FA (5.0 mg/kg, ig) and TMP (4.0 mg/kg, ig). In the second experiment, rats (n = 24) were subjected to cerebral ischemic model. All rats were randomly divided into four groups: group 1 (MCAO group) was treated with saline; group 2 (FA group) was treated with FA (5.0 mg/kg, ig); group 3 (TMP group) was treated with TMP (4.0 mg/kg, ig); group 4 (co-administration group) was treated with mixed FA (5.0 mg/kg, ig) and TMP (4.0 mg/kg, ig).

2.3. Liquid chromatography and mass spectrometric conditions

The UPLC–MS/MS system consisted of an UPLC system (Thermo Fisher Scientific, San Jose, CA) tandem with TSQ Vantage triple-quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA), equipped with a heat electrospray ionization (HESI) interface. Chromatographic separation was carried out at 40 °C on an Kinetex C_{18} column (50 × 2.1 mm, 2.6 μm, Phenomenex Company Inc., Guangzhou, China) with a C_{18} security guard cartridge (UHPLC C_{18} for 2.1 mm ID Columns). The autosampler was maintained at 4 °C and programmed to draw 5 μL of sample for chromatographic separation. An isocratic mobile phase of acetonitrile: 0.005% formic acid in water (13:87, v/v) was applied at a flow rate of 0.4 mL/min. The column temperature was kept at 40 °C. The total analytical run time was 2.4 min for each sample.

A TSQ Vantage mass spectrometer was applied for the quantitative analysis of target analytes after chromatographic separation. In order to achieve fast polarity switching, the mass spectrometer was operated in positive and negative ionization selective reaction monitoring (SRM) mode. The compound dependent parameters including Tube-Lens (T-Lens) and collision energy (CE) are listed in Table 1. Instrument parameters optimized were spray voltage, 3 kV; sheath gas and auxiliary gas pressures were 40 and 25 arb, respectively; capillary temperature 270 °C; vaporizer temperature, 350 °C; ion sweep gas pressure, 10 arb; and argon (collision gas) pressure, 1.5 mTorr. Data acquisition and processing were accom-