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The enhancement mechanism of wine-processed Radix Scutellaria on NTG-induced migraine rats



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ABSTRACT

To elucidate the increasing dissolution and enhancement mechanism of wine-processed Radix Scutellaria (RS) by fractal theory in nitroglycerin (NTG)-induced migraine rats. We prepared three RS from the process with 10% (S1), 15% (S2), 20% (S3) (v/m) rice wine. Mercury intrusion porosimetry and scanning electron microscope were employed to explore the internal structure of RS and the components dissolution of RS was analyzed by HPLC. Rats were randomly allocated into following groups and orally given different solutions for 10 days: normal group (NOR, normal saline), model group (MOD, normal saline), Tianshu capsule group (TSC, 0.425 mg/kg), ibuprofen group (IBU, 0.0821 mg/kg), crude RS group (CRU, 1.04 mg/kg) and wine-processed RS group (WP, 1.04 mg/kg) followed by bolus subcutaneously injection of NTG (10 mg/kg) to induce migraine model except NOR. Biochemical indexes (nitric oxide-NO, calcitonin-gene-related peptide-CGRP, and endothelin-ET) and c-fos positive cells were measured with commercial kits and immunohistochemical method, separately. Total surface area significantly increased in wine-processed RS ($p < 0.05$) while fractal dimension markedly decreased ($p < 0.05$) compared with crude RS. Additionally, S3 owned the highest increase of dissolution including the percentage increase of total extract, total flavonoids and main compounds (all $p < 0.05$ vs S1 and S2). Pharmacodynamic data showed c-fos positive cells significantly decreased ($p < 0.05$) in WP compared with MOD and the level of NO, CGRP, ET in WP was better than that of CRU. Wine-processed RS could be a promising candidate medicine for migraine treatment due to its increased component dissolution.

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1. Introduction

Migraine is a primary episodic headache disorder of which affects up to 15% of the general population [1]. Although migraine pathogenesis is unclear, many researchers regard migraine as a neurological disorder in which CNS dysfunction plays a pivotal role [2]. Migraines are associated with cardiovascular disease and disabling medical illnesses. Two re-analyses from the Women's

Health and Physicians' Health Studies found that migraine were a risk factor for cardiovascular disease, especially death from ischemic cardiovascular disease [3,4]. Therefore, migraines greatly affect quality of life. Herbal therapies are a popular type of medicine used and evaluated in patients with migraines because of the inadequate therapeutic responses to current conventional treatments [5].

The dried root of *Scutellaria baicalensis* Georgi (Labiateae) or Radix Scutellaria (RS), one of the most widely used herbs in traditional Chinese medicines (TCM) [6], contains active ingredients such as wogonin, baicalein, wogonoside, baicalin, and scutellarin [7]. Wine-processed RS has been used traditionally to treat migraine in a traditional Chinese prescription of "Xiao Qingkonggao" while the crude was not. Wine-processed RS has been used traditionally to treat migraine in the traditional Chinese

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prescription of “Xiao Qingkonggao”, while crude RS has not. It has been proven that the component dissolution of wine-processed RS was better than that of the crude. RS is a porous material [8,9]. The mechanism of increasing dissolution may relate to changes of fractal structure [10,11]. The key parameter in fractal theory is the fractal dimension, which could provide a method for characterizing the overall pore structure of herbs. The scanning electron microscope (SEM) and mercury intrusion porosimetry (MIP) technique can be applied to describe fractal characteristics of pore-structure [12–14]. In fact, the fractal theory is usually used to characterize complex pore-structure such as mortars, sandstones, pulp and porcelain [12–15], but has not been applied in TCM. For the first time in this paper, fractal theory was used to analyze increases in component dissolution.

The levels of biochemical indexes, such as CGRP, NO and ET, will change during a migraine [16–18]. Moreover, the immediate early gene (IEG) *c-fos* has been used extensively to study inflammatory pathways and its expression in trigeminal ganglion neurons is an important signal in migraines [19]. In order to better define the synergistic mechanism of wine-processed RS, fractal theory and biochemical index were employed in our study.

2. Materials and methods

2.1. Plant material

RS was purchased from Xianglong Trading Co., Ltd. (Juxian, Shandong province, China) in August, 2015. RS samples were identified with respect to morphology by Professor Chun-Feng Zhang (China Pharmaceutical University). The voucher specimen was deposited at the State Key Laboratory of Natural Products and Functions, China Pharmaceutical University, China. After the drying process of steaming, the RS sample was cut into tiny slices with a width of 1–2 mm.

2.2. Animals

Male Sprague Dawley (SD) rats (150–180 g) purchased from Zhejiang Province Experimental Animal Center (Zhejiang, China) was used. Rats were housed in an animal room maintained under standard conditions of light and dark cycles with free access to a standard diet and drinking water. All the animal studies were performed in accordance with the Animal Ethics Committee of China Pharmaceutical University for animal experimentation. All efforts were made to minimize the number of animals and their suffering. Animals were randomly divided into six groups, namely the model group (MOD), crude group (CRU), wine-processed group (WP), Tianshu capsule group (TSC), ibuprofen group (IBU), normal group (NOR). Each group included 6 rats.

2.3. Chemicals and reagents

Ibuprofen tablets were purchased from Shandong Fangming Pharmaceutical Group Co., Ltd. (Dongming, China). Tianshu capsules were purchased from Jiangsu Kanion pharmaceutical Co., Ltd. (Lianyungang, China). Sodium carboxymethylcellulose (CMC-Na) and polysorbate-80 (Tween-80) were purchased from Anhui Sunhere Pharmaceutical Excipients Co., Ltd. (Huainan, China). Chloral hydrate and heparin were purchased from Nanjing Hanbang Chemical Reagent Company (Nanjing, China). CGRP, ET, NO kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Nitroglycerin (NTG) injections were obtained from Guangzhou Baiyunshan Mingxing Pharmaceutical Co., Ltd (Guangzhou, China).

Chinese rice wine (Huangjiu) as the solvent were acquired from Shaoxing, Zhejiang province, China. Distilled and deionized water

was produced by a Milli-Q water purification system (Millipore Co., Ltd., Bedford, MA, USA). Reference baicalin (purity >98.0%), baicalin (purity >94.0%), wogonin (purity >98.5%), wogonoside (purity >98.0%), and scutellarin (purity >99.5%) were purchased from National Institutes for Food and Drug Control (Beijing, China). All other chemicals and reagents were of HPLC or analytical grade.

2.4. Wine-processing of samples

Four RS samples were used in our study: the crude samples and the three other wine-processed samples, which were processed with different volumes of rice wine. RS samples were soaked with 10%, 15%, 20% (v/m) of the rice wine for 30 min, then were stir-fried for 5 min at 150 °C. The samples with 10%, 15%, 20% (v/m) of rice wine were respectively marked S1–S3.

2.5. Nitroglycerin-induced migraine

2.5.1. Preparation of drugs and NTG suspension

The powder of RS was set to a daily administration of 10 g for an adult weighing 60 kg. Thus the calculation of the dose for rats was 1.04 mg/kg. We used sonication to get a suspension which contained 1.04 g powder of crude or S3 sample and 10 ml 0.4% CMC-Na. Ibuprofen (0.7878 g three times a day) and a Tianshu capsule (4.08 g three times a day) were separately converted to a rats' dosage of 0.0821 and 0.425 g/kg. According to the classical method [20,21], the model group was subcutaneously injected with a NTG suspension (10 mg/kg) on the forehead area. The NTG suspension contained 1% Tween-80 while NTG was not present in the blank suspension.

2.5.2. Improving effect of wine-processed RS and crude sample for treating migraine

The normal and model groups were given equivalent volumes of 0.4% (w/v) CMC-Na. Other groups were treated with a gastric perfusion of drugs for 10 days (corresponding dosage). In addition to the normal group, each group was given a subcutaneous injection of the NTG suspension (10 mg/kg) before the last delivery; the normal group was given an equivalent volume of blank suspension.

After building for 30 min, 1 ml blood from rats' orbit were collected in a 1.5 ml heparinized tube, low temperature centrifugation for 10 min (4 °C, 4000 rpm), and the serum was used to determine the plasma level of NO by enzyme-linked immune sorbent assay (ELISA). Finally, all rats were anesthetized through intraperitoneal injection with 3.5% chloral hydrate. After building for 4 h, 4 ml blood from abdominal aorta was collected using heparinized blood vessel at a comprehensive necropsy, low temperature centrifugation 10 min (4 °C, 4000 rpm), and the serum was used to determine the plasma level of CGRP and ET by ELISA.

All animals were euthanatized after the collection of blood. The *c-fos* positive cells at the trochlear nucleus level in the rat periaqueductal gray matter were measured by means of immunohistochemical method. All biochemical assays were observed according to the manufacturer's instructions.

2.6. Extraction of samples

The RS slices weighed 10 g from the mentioned four samples, every sample added eight-fold mass of water to the extract for 1 h with the boiling conditions. The samples were extracted three times. The extracted liquid was filtered and the three filtrates were combined. Finally the extraction was concentrated and dried. The dissolution of active ingredients was determined by UV and HPLC separately.

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