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Development of two step liquid–liquid extraction tandem UHPLC–MS/MS method for the simultaneous determination of Ginkgo flavonoids, terpene lactones and nimodipine in rat plasma: Application to the pharmacokinetic study of the combination of Ginkgo biloba dispersible tablets and Nimodipine tablets

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ABSTRACT

A sensitive, reliable and accurate UHPLC–MS/MS method has been firstly established and validated for the simultaneous quantification of ginkgo flavonoids, terpene lactones and nimodipine in rat plasma after oral administration of Ginkgo biloba dispersible tablets, Nimodipine tablets and the combination of the both, respectively. The plasma samples were extracted by two step liquid–liquid extraction, nimodipine was extracted by hexane-ether (3:1, v/v) at the first step, after that ginkgo flavonoids and terpene lactones were extracted by ethyl acetate. Then the analytes were successfully separated by running gradient elution with the mobile phase consisting of 0.1% formic acid in water and methanol at a flow rate of 0.6 mL/min. The detection of the analytes was performed on a UHPLC–MS/MS system with turbo ion spray source in the negative ion and multiple reaction monitoring (MRM) mode. The calibration curves for the determination of all the analytes showed good linearity ($R^2 > 0.99$), and the lower limits of quantification were 0.50–4.00 ng/mL. Intra-day and inter-day precisions were in the range of 3.6%–9.2% and 3.2%–13.1% for all the analytes. The mean extraction recoveries of the analytes were within 69.82%–103.5% and the matrix were within 82.8%–110.0%. The validated method had been successfully applied to compare the pharmacokinetic parameters of ginkgo flavonoids, terpene lactones and nimodipine in rat plasma after oral administration of Ginkgo biloba dispersible tablets, Nimodipine tablets with the combination of the both. There were no statistically significant differences on the pharmacokinetic behaviors of all the analytes between the combined and single administration groups. Results showed that the combination of the two agents may avoid dosage adjustments in clinic and the combination is more convenient as well as efficient on different pathogenesis of cerebral ischemia.

1. Introduction

Cerebral ischemia is one of the most common cerebrovascular diseases with the characteristics of high morbidity, high mortality and high recurrence rate. It will initiate serious threat to human health. Meanwhile, considering of varied aetiological agent, complex pathogenesis, and the wide harmful range of cerebral ischemia [1,2], taking the combination of drugs with different mechanisms could depress or cure cerebral ischemia markedly [3–5]. Among them, the combination of traditional Chinese medicine and chemical medicine gets general application [6].

Nimodipine (Fig. 1), a dihydropyridine calcium channel blocker, is significantly selective for cerebral arteries, mainly used in clinic cerebral vasospasm and cerebral ischemia [7]. Through preventing Ca^{2+} entering into cells, nimodipine promotes the cerebral blood glow to relieve ischemia [8,9]. Ginkgo biloba extraction (GBE), which contains various bioactive ingredients such as ginkgo flavonoids (Fig. 1) and terpene lactones (Fig. 1) [10–12], is commonly used in the traditional Chinese medicine. GBE is also widely used in the treatment of cardiovascular and cerebrovascular diseases through eliminating free radicals, regulating the activity of various antioxidant enzymes, reducing plasma viscosity by antiplatelet aggregation and increasing the blood flow velocity [13,14].

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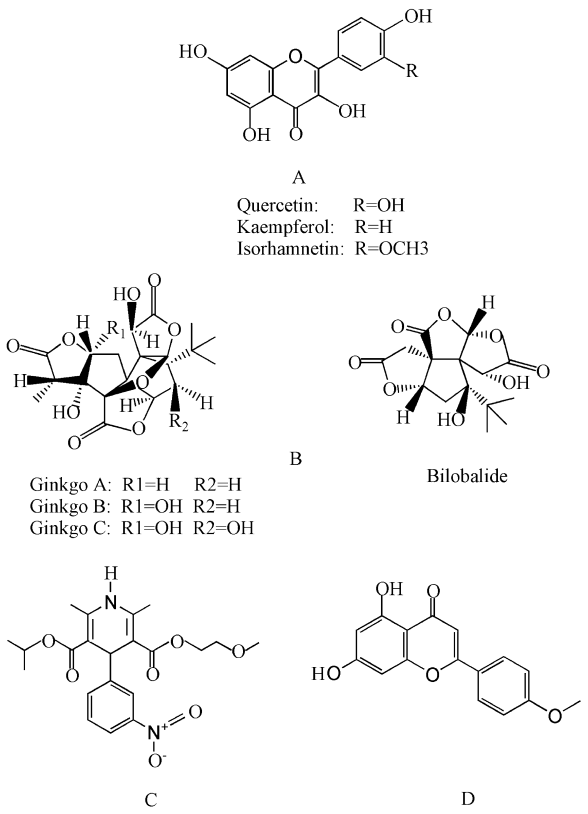

Fig. 1. Chemical structures of ginkgo flavonoids (A), terpene lactones (B), nimodipine (C) and Acacetin (internal standard, IS) (D).

Thus, the combination administration of nimodipine and GBE in clinic will have different mechanisms on different pathogenesis. Under the synergistic effect, the combination administration of the two agents has a more brilliant cure-effect on series of cerebral ischemia than either of the single administration, respectively. The synergistic effect has been studied fully in many studies [15,16], but the pharmacokinetic behaviors of the bioactive ingredients after combination administration are still unknown. As it can directly reflect the process of absorption, distribution, metabolism and excretion of the drug in the body and provide a scientific support for the clinical dosage at the same time, pharmacokinetic behavior after combination administration values great importance and worth further exploration. However, there were no efficient methods for simultaneous quantification of ginkgo flavonoids, terpene lactones and nimodipine. Therefore this study established a sensitive, reliable and accurate method, with two step liquid–liquid extraction tandem UHPLC–MS/MS, to analyze ginkgo flavonoids, terpene lactones and nimodipine simultaneously. It will be applied to reveal the differences of pharmacokinetic behaviors of the bioactive ingredients between combined and single administration groups.

2. Materials and methods

2.1. Chemical and reagents

Quercetin (purity 96.5%, Batch No. 100081-200907), Kaempferol (purity 93.2%, Batch No. 110861-201310), Ginkgolide A (purity 100%, Batch No. 110862-200608), Ginkgolide C (purity 97.7%, Batch No. 110864-200906), Bilobalide (purity 100%, Batch No. 110865-200605) and Nimodipine (purity 100%, Batch No. 10027-200002) were all purchased from the National Institute for Food and Drug Control (Shengyang, China); Isorhamnetin (purity ≥ 98%, Batch No. GZDD-0150-20109) and Ginkgolide B (purity ≥ 98%, Batch No. GZDD-0305-201107) were purchased from DiDa Biological Science Co., Inc. (Guizhou, China). Acacetin (internal standard, IS) (purity 99.43%, Batch No. MUST-15012704) (Fig. 1) was obtained from Must Bio-technology Co. Ltd. and Institute of Biology. CAS. (Chengdu, China).

Nimodipine tablets were supplied by the Central Pharmaceutical Co., Ltd. (Tianjin, China). Ginkgo biloba dispersible tablets were purchased from Chenpai Pharmaceutical Group Corp (Jiangsu, China) (each tablet has 19.8 mg flavonoids and 9.4 mg terpene lactones).

Vitamin C was purchased from BoDi Chemical Co., Ltd. (Tianjin, China). Methanol were purchased from Fisher Co., Inc. (USA) and formic acid was purchased from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China) (HPLC). The pure water was supplied by Wahaha Group Co., Ltd. (Hangzhou, China); Ethyl acetate was purchased from Huirui Chemical Technology Co., Inc. (Tianjin, China). Hexane and ether were provided by Shandong Yuwang Industrial Co., Ltd. (Yucheng, China). The chemicals except mentioned were all analytical grade.

2.2. Animals

Eighteen Sprague-Dawley male rats (230 ± 10 g) were obtained from Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The rats were acclimatized to the condition of the laboratory over one week before the experiments. Animal welfare and experimental procedures were strictly performed in accordance with the Animal Care and Committee of Shenyang Pharmaceutical University (Shenyang, China) and the protocol was approved by the Animal Ethics Committee of the Institution (SYPU-IACUC-2015-0722-206).

2.3. UHPLC–MS/MS condition

The UHPLC–MS/MS system was performed by using an XR LC-20 AD Prominence™ HPLC system equipped with a binary pump, a degasser, an autosampler, a thermostatted column compartment (Shimadzu, Japan) and a 4000 QTRAP™ MS/MS system equipped with a turbo ion spray source (AB Sciex, USA). All the operations and analysis of data were controlled by Analyst 1.6 (AB Sciex, USA).

Analytes and IS were separated on a ZORBAX SB-C_8 column (150 mm × 4.6 mm, 3.5 μm) (Shimadzu, Japan) at 30 °C with 0.1% formic acid in water (A) and methanol (B). The gradient elution was used as follows: 75% B → 95% B at 0.01–1.00 min; 95% B at 1.01–3.50 min; 95% B → 98% B at 3.51–5.00 min; 75% B at 5.01–9.00 min. Efficient and symmetrical peaks were obtained at a flow rate of 0.6 mL/min with a sample injection volume of 2 μL.

The negative ion mode (ESI^-) with select multiple reaction monitoring (MRM) was applied to detecting the analytes and IS with the ion spray voltage and a source temperature setting at −4500 V and 500 °C, respectively. The curtain gas, gas 1 and gas 2 (all gases: nitrogen) were set at 20, 50 and 40 psi. The quantitative parameters are listed in Table 1.

2.4. Preparation of standard solutions

The stock solutions of quercetin, kaempferol, isorhamnetin, ginkgolide A, B, C, bilobalide and nimodipine were prepared in methanol at the concentration of 1.0 mg/mL, respectively. They were further diluted with methanol to make a series of mixed working solutions at the concentration of 7.5–1500.0 ng/mL for quercetin, 5.0–1000.0 ng/L for kaempferol, 5.0–1000.0 ng/mL for isorhamnetin, 25.0–5000.0 ng/mL for ginkgolide A, 6.0–1200.0 ng/mL for ginkgolide B, 5.0–1000.0 ng/mL for ginkgolide C, 40.0–8000.0 ng/mL for bilobalide and 5.0–1000.0 ng/mL for