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Simultaneous UPLC-MS/MS determination of four components of Socheongryong-tang tablet in human plasma: Application to pharmacokinetic study

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ABSTRACT

The purpose of this study was to develop a method for simultaneous analysis of schizandrin, ephedrine, paeoniflorin, and cinnamic acid as constituents of Socheongryong-tang tablet in human plasma using UPLC-MS/MS. These four components were separated using water containing 0.01% formic acid and methanol as a mobile phase by gradient elution at a flow rate of 0.3 mL/min with a HALO-C_{18} column (2.1 mm × 100 mm, 2.7 μm particle size). Quantitation was performed on a triple quadrupole mass spectrometer employing electrospray ionization technique operated in multiple reaction monitoring mode. Mass transitions were *m/z* 432.9 → 384.1 for schizandrin, 165.8 → 148.1 for ephedrine, 525.0 → 449.2 for paeoniflorin, 146.8 → 102.9 for cinnamic acid, and 340.0 → 324.0 for papaverine as internal standard. Liquid-liquid extraction and protein precipitation with ethyl acetate-methanol (1:2, v/v) were used to obtain these four components. Chromatograms showed high resolution, sensitivity, and selectivity without interference by plasma constituents. Calibration curves of schizandrin, ephedrine, paeoniflorin, and cinnamic acid in human plasma ranged from 0.02 to 8 ng/mL, 0.5 to 200 ng/mL, 0.2 to 80 ng/mL, and 1 to 400 ng/mL, respectively. Calibration curves of each analyte displayed excellent linearity, with correlation coefficients > 0.99. For all four components, both intra- and inter-day precisions (CV%) were < 5.99%. The accuracy was 99.35–103.30% for schizandrin, 98.48–104.38% for ephedrine, 97.06–103.34% for paeoniflorin, and 99.97–104.36% for cinnamic acid. This analytical method developed in this study satisfied the criteria of international guidance. It could be successfully applied to pharmacokinetic studies of schizandrin, ephedrine, paeoniflorin, and cinnamic acid after oral administration of Socheongryong-tang tablet to humans.

1. Introduction

Socheongryong-tang is called Xiaoqinglong-tang in China and Shoseiryu-to in Japan with a long history of usage [1]. Socheongryong-tang is a medicine that can improve the function of the respiratory system by warming the body, leading to sweating and improving water metabolism [2]. It is also often used for allergic rhinitis with runny nose or sneeze. In addition, it is prescribed for symptoms such as chills, fever, bronchitis, bronchial asthma, chronic bronchitis, allergic asthma, and acute bronchitis [3,4]. These Socheongryong-tang are made of eight herbal medicines: Ephedrae Herba, Cinnamomi Ramulus, Zingiberis Rhizoma, Asari Herba, Schizandra Fructus, Paeoniae Radix, Pinelliae Rhizoma and Glycyrrhizae Radix. Main components of each herbal medicine act in a complex manner, resulting in drug efficacy in the body [5]. Schizandrin is a major active ingredient of Schizandra fructus. Ephedrine is a major active ingredient of Ephedrae Herba. Paeoniflorin is a major active ingredient of Paeoniae Radix and cinnamic acid is a major active ingredient of Cinnamomi Ramulus. Fig. 1 shows structures of these four major active ingredients.

Schizandrin promotes liver protection and detoxification and prevents gastritis and ulceration by inhibiting gastric juice secretion and irritation of the respiratory center while facilitating phlegm removal and blood circulation [6]. Ephedrine has vasoconstriction effect. It can elevate blood pressure, leading to sweating. It is also diuretic. In

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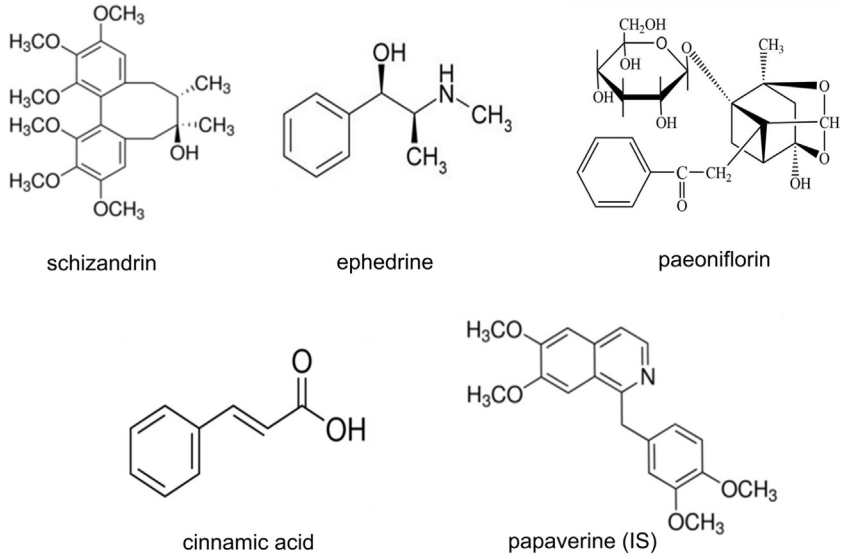

Fig. 1. Chemical structures of schizandrin, ephedrine, paeoniflorin, cinnamic acid, and papaverine (IS).

addition, it has sputum removal efficacy. Paeoniflorin has vasodilation, anti-oxidant, anti-cancer, and anti-inflammation effect [7–9]. Similarly, cinnamic acid has anti-bacterial, anti-cancer, and anti-oxidant properties [10,11]. The efficacy of these individual components of Socheongryong-tang is well known. However, pharmacokinetic (PK) studies or clinical trial data of Socheongryong-tang are unavailable. To the best of knowledge, there is no report of using LC-MS/MS to quantify pharmacologically active components or calculate their PK parameters in humans administered with Socheongryong-tang. Although Socheongryong-tang has been empirically used for a long time and listed in herbal medicine standard book, its safety or efficacy has not been verified properly yet [12]. Socheongryong-tang was registered as a herbal medicine covered by Korean health insurance in 2016. Therefore, its prescriptions are increasing in relation to cold diseases, and there is a growing interest in herbal medicines all over the world. However, these herbal medicines lack scientific evidence of safety and efficacy, and their PK research data are almost non-existent. Thus, it is necessary to perform PK investigation and safety evaluation for Socheongryong-tang. In addition, herbal medicines made up of various complex components such as Socheongryong-tang may result in PK patterns different from those obtained when a single component is administered into the body due to interactions among individual components. Therefore, clinical PK studies on these herbal medicines are needed. To do this, four components of Socheongryong-tang were selected for simultaneous analysis using UPLC-MS/MS method in human plasma taking Socheongryong-tang.

There are reports of analysis methods for Socheongryong-tang [13,14]. However, these analytical methods used dried aqueous extracts where its main constituents were mixed, different from a biological sample. In addition, reported lower limit of quantitation (LLOQ) values for the four components mentioned above were too high that these methods could only be applied to its content test. They could not be to study PKs in humans who take Socheongryong-tang. Thus, the aim of this study was to develop and validate an UPLC-MS/MS method for sensitive, accurate, and simultaneous determination of schizandrin, ephedrine, paeoniflorin, and cinnamic acid in human plasma obtained after administration of Socheongryong-tang tablet. To the best of authors' knowledge, this is the first report of simultaneous analysis for four major constituents of Socheongryong-tang and their PK analysis in humans.

2. Materials and methods

2.1. Chemicals and reagents

Socheongryong-tang tablets were obtained from Kyung-bang Pharmaceutical Company (Incheon, Republic of Korea). Contents of its four components per three tablets (test number: QPO-17011001) were as follows: 0.15 mg of schizandrin, 9.8 mg of ephedrine, 49.5 mg of paeoniflorin, and 0.25 mg of cinnamic acid. Cinnamic acid (purity ≥ 98%), schizandrin (purity ≥ 99%) and paeoniflorin (purity ≥ 98%) were obtained from Oriental Industry Promotion Agency (Jangheung, Republic of Korea). Ephedrine (purity ≥ 99%) and papaverine (purity ≥ 98%) as an internal standard (IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC-MS grade methanol, acetonitrile, water (18.2 mΩ), and HPLC grade ethyl acetate were purchased from Fisher Scientific (Hampton, NH, USA). LC-MS grade formic acid was supplied by Sigma-Aldrich (St Louis, MO, USA). All chemicals had the highest HPLC grade or quality available.

2.2. Instrumentation and chromatographic conditions

Simultaneous quantitative analysis for schizandrin, ephedrine, paeoniflorin, and cinnamic acid in human plasma by the newly developed UPLC-MS/MS method was performed using a Shimadzu Nexera X2 Series UPLC system (Shimadzu, Japan) coupled with a Shimadzu 8040 mass spectrometer. Various conditions were tested to obtain the best chromatography condition and excellent quantitation for materials. These included mobile phase [0.005–0.1% formic acid in water (pH 2.2–3.5), 0.01–0.1% acetic acid in water (pH 2.9–3.4), and 5–10 mM ammonium acetate buffer (pH 4.6–6.5)], column (HALO-C_{18}, Acquity UPLC® BEH C_{18}, Inertsil C_8-3, and Phenomenex KINETIC core-shell biphenyl column), and organic solvent [ethyl acetate, di-ethyl ether, methyl *tert*-butyl ether (MTBE), methylene chloride, acetonitrile, and methanol]. Optimized chromatographic separation of these four components was conducted with a HALO-C_{18} column (100 mm × 2.1 mm, 2.7 μm particle size; Advanced Materials Technology, Wilmington, DE, USA) at an oven temperature of 40 °C. The mobile phase was performed with 0.01% formic acid in water (mobile phase A) and 100% methanol (mobile phase B) with gradient elution at flow rate of 0.3 mL/min. The elution program consisted of 0–0.75 min (0% B), 0.75–1.0 min (0–65% B), 1.0–3.75 min (65% B), 3.75–3.76 min (65–0% B), and 3.76–5.0 min (0% B). Analytical procedures were evaluated with positive and negative electrospray ionization