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Research Paper

Lack of dose dependent kinetics of methyl salicylate-2-O-β-D-lactoside in rhesus monkeys after oral administration

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ABSTRACT

Ethnopharmacological relevance: Methyl salicylate-2-O-β-D-lactoside (MSL) is one of the main active components isolated from *Gaultheria yunnanensis*, which is a traditional Chinese medicine used to treat arthritis and various aches and pains. Pharmacological researches showed that MSL had various effective activities in both *in vivo* and *in vitro* experiments. However, the pharmacokinetics features and oral bioavailability of MSL in primates were not studied up to now.

Aim: To study the pharmacokinetics of different doses of MSL in rhesus monkeys and investigate the absolute bioavailability of MSL after oral administration.

Materials and methods: Male and female rhesus monkeys were either orally administrated with MSL 200, 400 and 800 mg/kg or received an intravenous dose of 20 mg/kg randomly. The levels of MSL and salicylic acid (SA) in plasma were simultaneous measured by a simple, sensitive and reproducible high performance liquid chromatography method.

Results: Mean peak plasma concentration values for groups treated with 200, 400 and 800 mg/kg doses ranged from 48.79 to 171.83 μg/mL after single-dose oral administration of MSL, and mean area under the concentration–time curve values ranged from 195.16 to 1107.76 μg/mL h. Poor linearity of the kinetics of SA after oral administration of MSL was observed in the regression analysis of the C_{max}-dose plot ($r^2=0.812$), CL-dose plot ($r^2=0.225$) and $AUC_{(0-t)}$-dose plot ($r^2=0.938$). Absolute bioavailability of MSL was assessed to be 118.89 ± 57.50, 213.54 ± 58.98 and 168.72 ± 76.58%, respectively.

Conclusions: Bioavailability of MSL after oral administration in rhesus monkeys was measured for the first time. Pharmacokinetics parameters did not appear to be dose proportional among the three oral doses of treatments, and MSL showed an apparent absolute bioavailability in excess of 100% in rhesus monkeys based on the present study. In addition, a rapid, sensitive and reliable HPLC method was established and demonstrated for the research of traditional Chinese medicine in this study.

1. Introduction

Methyl salicylate-2-O-β-D-lactoside (MSL, Fig. 1A), a novel salicylic acid (SA, Fig. 1B) analog extracted from traditional Chinese herbal medicine *Gaultheria yunnanensis*, is one of the main components with best anti-inflammatory activities in the herbal (Zhang et al., 2011). In the southern regions of China, *Gaultheria yunnanensis*, as a folk medicine, has been widely used for treatment of swelling, arthritis, cephalalgia, and various painful inflammatory conditions (Ma et al., 2001; Wang et al., 2011). Our previous studies demonstrated that MSL showed anti-inflammatory activities in RAW264.7 macrophages and acted as an anti-inflammation agent on microglia and astrocytes (Lan et al., 2011; Zhang et al., 2012), and *in vivo* studies, MSL could suppress inflammation response on fibroblast-like

Abbreviations: MSL, methyl salicylate-2-O-β-D-lactoside; SA, salicylic acid; CIA, collagen-induced arthritis; HPLC, high performance liquid chromatography; UV, ultraviolet; CDE, Center for Drug Evaluation; SFDA, China's State Food and Drug Administration; IS, internal standard; QC, quality control; LLOQ, lower limit of quantification; AUC, area under the plasma concentration-time curve; RSD, relative standard deviation; C_{max}, mean maximum concentration; $T_{1/2}$, half-life elimination; T_{max}, time to reach C_{max}; MRT, mean residence time; CL, clearance rate; F_{app}, apparent absolute oral bioavailability

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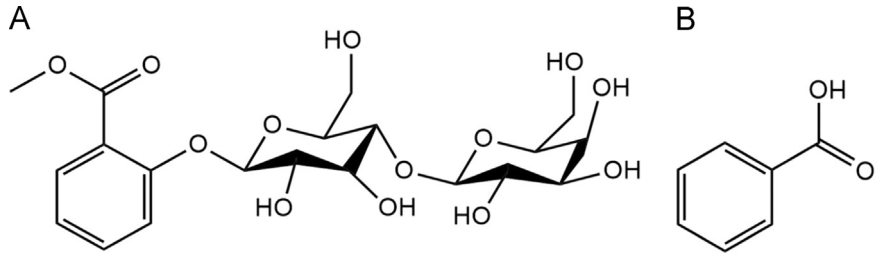

Fig. 1. Chemical structures of MSL (A) and SA (B).

synoviocytes and collagen-induced arthritis (CIA) by inhibiting NF-κB activation (Xin et al., 2014). Meanwhile, unlike other acidumsalicylicum, MSL remarkably prevented the progression of arthritis in CIA mice without gastric mucosa toxicity (Xin et al., 2014). In addition, as a novel natural product agent for prevention and/or treatment of rheumatoid arthritis, MSL had been confirmed to have a good absorption from gastrointestinal tract in rodents and Canidae (Zhang et al., 2013). However, metabolic characteristics of MSL in primates and whether the characteristics would be identical to that in non-primates have not been clear yet.

The present study, as a section of pre-clinical studies, was primarily for purposes of surveying the metabolic features of MSL in rhesus monkeys, and providing more valuable information for clinical application in future.

2. Materials and methods

2.1. Chemicals and reagents

MSL which had a high purity of 99%, was provided by Institute of Materia Medica, Chinese Academy of Medical Sciences (Beijing China). SA and benzoic acid were purchased from Sigma-Aldrich Co. (Shanghai, China), and both of them had a purity of more than 99%. Methanol (high performance liquid chromatography, HPLC grade) was purchased from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Phosphoric acid was purchased from Beijing Chemical Reagent Company (Beijing, China). Purified water was provided by Hangzhou Wahaha Group Co. (Beijing, China).

2.2. Instrumentation

An Agilent1200 chromatography system equipped with a quaternary pump, a vacuum degasser and an autosampler was purchased from Agilent Technologies (Santa Clara, California, USA). N-EVAP™III nitrogen evaporator was purchased from Organomation Associates, Inc. (Berlin, MA).

2.3. Chromatographic conditions

The analyses were performed on an Agilent Zorbax SB-C_{18} chromatographic column (5 μm, 4.6 × 250 mm) at 35 °C with an Agilent Zorbax SB-C_{18} pre-column before it. Mobile phase consisted of water containing 0.1% phosphoric acid and methanol. Gradient elution conditions are listed in Table 1. Flow rate was 1 mL/min and ultraviolet (UV) detection wavelength was 238 nm.

2.4. Animals

Sixteen healthy, adult rhesus monkeys (male and female, 5.2 ± 0.3 kg, Certificate no. SCXK (Beijing) 2010-0007, List no. 11805300-000014) were provided from Institute of Beijing Xieerxin Biology Resource (Beijing, China). The rhesus monkeys were maintained in an individually ventilated cage system with alternating 12 h light/dark cycles, a relative humidity of 50 ± 5% and a constant temperature of 20 °C. The rhesus monkeys were proved to be healthy based on physical examination both before initiation and after completion of the trial. All procedures involving care and use of the rhesus monkeys were reviewed and approved by Chinese Academy of Medical Sciences & Peking Union Medical College Biomedical Research Ethics Committee. In addition, the number of animals used in this study and designs for dose of administration followed relevant guidelines of Center for Drug Evaluation (CDE) of China's State Food and Drug Administration (SFDA).

Table 1
Gradient elution conditions for HPLC.

Time (min)	Mobile phase composition	
	Water with 0.1% phosphoric acid (%)	Methyl alcohol (%)
0	80	20
12	45	55
18	80	20
24	80	20

2.5. Preparation of stock solutions and standards

The stock solution of MSL and SA were prepared in methanol to be 10 mg/mL and diluted with methanol to obtain working standard solutions, respectively. Benzoic acid, as an internal standard (IS) in this HPLC method, was also stocked in methanol with a concentration of 1 mg/mL. All stock solutions were stored at −80 °C prior to use. Both quality control (QC) samples of MSL and SA contained 2.5, 25 and 250 μg/mL.

2.6. Plasma sample preparation and protraction of standard curves

To a 200 μL aliquot of rhesus monkey plasma samples, different concentrations of MSL and SA stock solution and 10 μL of the IS solution were added immediately. Then, the mixture was vortex-mixed with 1 mL methanol for 3 min at room temperature. After centrifugation at 13,400 r/min for 10 min, supernatant was transferred into a clean tube and evaporated to dryness using a nitrogen evaporator system at 37 °C. The residue was re-dissolved with 100 μL methanol vortex-mixing for another 3 min. After centrifugation for 5 min, 10 μL of the supernatant was detected by the HPLC system.

2.7. Method validation

The following method of validation in terms of selectivity, linearity, recovery, accuracy, precision and stability in rhesus monkey plasma was executed abiding by FDA's Guidance for Industry: Bioanalytical Method Validation, and Guiding Principle for Drug Non-clinical Pharmacokinetic Research in CDE of SFDA.