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Effect of aspirin on the pharmacokinetics and absorption of *panax notoginseng* saponins

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ABSTRACT

Background: *Panax notoginseng* saponins, a traditional Chinese medicine extraction, and aspirin are both widely used to treat cerebral infarction in China. Good results in clinical practice have been achieved, when *Panax notoginseng* saponins was taken together with aspirin.

Methods: To investigate the interaction of the two drugs in vivo, the concentration of notoginsenoside R_1, ginsenoside Rg_1, Rb_1, Re and Rd. in blood were simultaneously measured by UPLC/MS/MS. Sample preparation was carried out by the protein precipitation technique with an internal standard saikosaponin A standard. The separation of six components was achieved by using an ACQUITY UPLC ®BEH C18 column (1.7 μm 2.1 × 100 mm) by gradient elution using water (containing 0.2% formic acid) and acetonitrile (containing 0.2% formic acid) as the mobile phase at a flow rate of 0.2 mL/min. The pharmacokinetic parameters were determined using non-compartmental analysis. The transport of notoginsenoside R_1, ginsenoside Rg_1, Rb_1, Re and Rd. in MDCK -MDR1 cell monolayer was also used to verify the conclusion of pharmacokinetic drug-drug interaction and study the mechanism of drug interaction.

Results: The concentrations of the five components increased in a certain extent when the two drugs administered together in rats. The values of apparent permeability coefficients were significantly increased when the two drugs were used together. Aspirin and salicylic acid could destroy the tight junction protein and open the intercellular space to increase the absorption of *Panax notoginseng* saponins.

Conclusion: Pharmacokinetic drug-drug interaction in vivo existed between *Panax notoginseng* saponins and aspirin. The drug-drug interaction mainly occurred in the process of absorption.

1. Introduction

Panax notoginseng Saponins (PNS) are extracted from *Panax notoginseng (Burk.) F.H. Chen (Sanqi)* and also extensively used in cardiovascular disease [1,2,3]. It has been widely used to treat thrombotic diseases in China [4]. Aspirin (ASA), as the drug of antiplatelet, has been widely used in the primary or secondary prevention and treatment of atherosclerotic cardiovascular diseases. However, the adverse reactions of ASA cannot be ignored, such as ASA resistance and bleed [5]. At present, in order to achieve a better clinical efficacy and minimize side effects, ASA combined with PNS were used to treat cerebral infarction in China [6]. Although many clinical data suggested a drug–drug interaction between PNS and ASA in vivo, clinical researches only focus on the observation of pharmacodynamics at present in China [7,8,9,10]. We also found that PNS could increase absorption of salicylic acid (SA) [11]. This trial sought to assess the effect of ASA on the in vivo process of PNS.

MDCK cells (Madin-Darby canine kidney cell line) are widely used to study the drug transport [12,13]. MDCK cells have differentiated into a columnal epithelium to form tight junctions, and showed highly functionalized epithelial barrier with remarkable morphological and biochemical similarity when cultured on semi permeable membrane. The growth rate of MDCK cells was significantly faster than that of Caco-2, which could significantly shorten experimental period [14,15,16]. Besides, compared with Caco-2, MDCK cells showed higher reproducibility [17,18]. In a word, MDCK cells were selected to study the transport of PNS.

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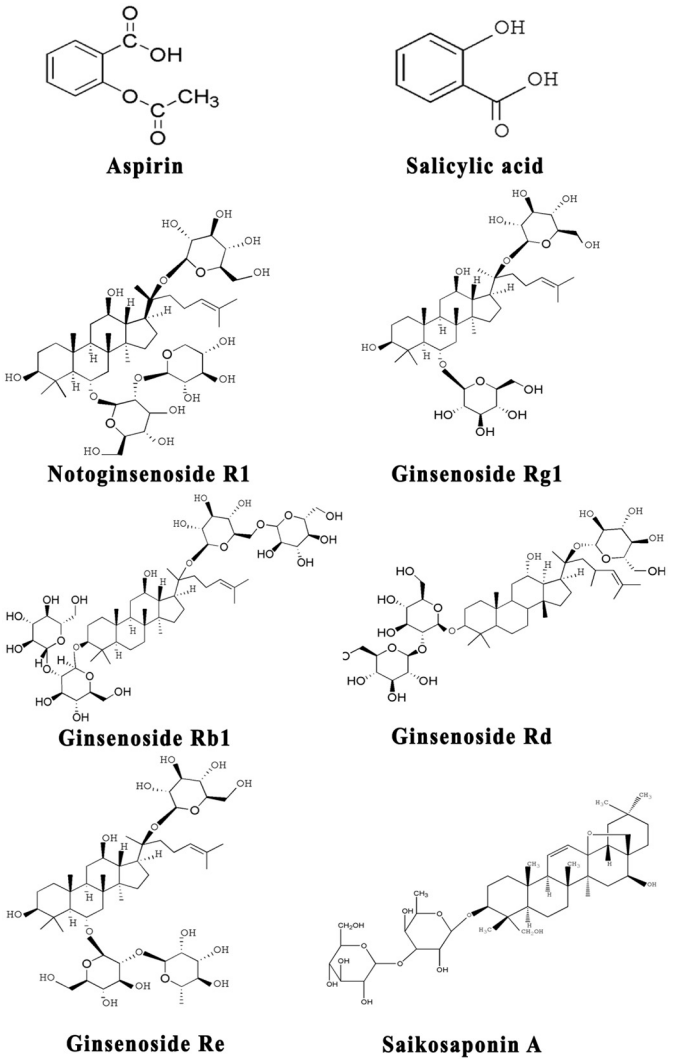

Fig. 1. Chemical structures of ASA, SA, Notoginsenoside R_1, ginsenoside Rg_1, Rb_1, Re, Rd. and saikosaponin A. (Notoginsenoside R_1 and ginsenoside Rg_1, Rb_1, Re, Rd. were active components in *Panax notoginseng* saponins.)
Abbreviations: ASA, aspirin; SA, salicylic acid.

2. Materials and methods

2.1. Chemicals

Notoginsenoside R_1 (NR_1), ginsenoside Rg_1 (GRg_1), Rb1 (GRb_1), Re (GRe), Rd. (GRd), and saikosaponin A were got from the National Institute for Food and Drug Control (Beijing, China). *Panax notoginseng* saponin extracts were got from Yunnan Baiyao Group Limited by Share Ltd. PNS (purity of 81%, NR_1, 6.9%; GRg_1, 28.0%; GRb_1, 29.7%; GRe, 3.8%; GRd, 7.3%). Raw material medicine of SA, ASA were purchased from Xi'an Yue Lai Medicine Technology Co., Ltd. The structure of ASA, SA, Notoginsenoside R1, ginsenoside Rg1, Rb1, Re, Rd. and saikosaponin A were shown in Fig. 1. Culture flasks (25 cm^2 growth area), polyester (PET) cell culture inserts (12 mm diameter, 0.4 μm pore size), 96-well plates, costar 12-well plates and transwell were got from Corning Costar Corporation (MA, USA) and untreated white opaque multi well plates were got from NUNC Denmark;NBD-C6-HPC was got from Life Technologies (USA). Acti-stain488 (green) fluorescent phalloidin was bought from Cytoskeleton Inc. (Denver, CO, USA). Acetonitrile (HPLC grade) was got from Fisher Scientific. HPLC-quality water was obtained using a Cascada™ IX-water. All other chemicals used in this experiment were analytical grade.

2.2. Animals and cell experiment

Male Sprague-Dawley (SD) rats (240–260 g) were got from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Rats were housed in the Beijing University of Chinese Medicine Laboratory for 7 days before the start of experiments in a controlled environment with consistent temperature and humidity. All the animal studies were performed under the guidelines for the care and use of laboratory animals and the experimental protocols was approved by the institutional animal experimentation committee of Beijing University of Chinese Medicine.

MDCK-MDR1 cells were cultured in DMEM (10% heat-inactivated fetal bovine serum as well as 100 U/mL penicillin and 100 μg/mL streptomycin). For transport experiments, MDCK-MDR1 cells (passage numbers: 77–83) were seeded at a density of 2.5×10^5 cells/mL on PET inserts at 37 °C with 5% CO_2 for 5–7 days to reach confluence. Fresh media were changed every other day.

2.3. Instruments and conditions

The UPLC system was consisted of Nexera X2 LC-30AD (a solvent delivery unit; Shimadzu (China) Co., Beijing), LC solution (an operating system software; Shimadzu) and 4500 AB QTRAP-LC/MS/MS (Mass spectrometric detection, Palo Alto, CA, USA) equipped with an electrospray ionization source (ESI). Chromatographic separation of all samples was peformed on an ACQUITY UPLC ®BEH C18 (1.7 μm 2.1 × 100 mm Column). The auto-sampler was maintained at 16 °C, and the UPLC mobile phase consisted of solvent A (0.2% formic acid acetonitrile solution) and solvent B (0.2% formic acid aqueous solution). The gradient condition was as follows: 20% A at 0–3 min, 20–50% A at 3–5 min, 50–100% A at 5–7 min, 100–100% A at 7–8 min, 100–20% A at 8–8.01 min, 20–20% A at 8.01–12 min at 30 °C·The flow rate was set at 0.2 mL/min, and the injection volume was 10 μL. Mass spectrometer was set in positive ionization mode with the capillary voltage setting at 3500 V scanning in multiple reactions monitoring (MRM) mode. The parameters in the source were set as the following: source temperature 350 °C; desolvation gas flow 10 L/min; nebulizer gas (N2) pressure 40 psi, and the parent and daughters *m/z* were listed as follows: NR_1, GRg_1, GRb_1, GRd, GRe, and IS: *m/z* 931.5 → 637.2 for NR_1, *m/z* 845.4 → 637.3 for GRg_1, *m/z* 1107.5 → 621.2 for GRb_1, *m/z* 945.5 → 637.2 for GRe, *m/z* 945.3 → 783.4 for GRd, *m/z* 779.4 → 617.3 for IS. The optimized collision energies were − 54 eV, − 43 eV, − 75 eV, − 51 eV, − 56 eV and − 55 eV respectively.

2.4. Sample preparation

2.4.1. Pharmacokinetic samples

Acetonitrile were used to extracted protein precipitation. Plasma samples were treated with acetonitrile (1:3, Plasma/acetonitrile, v/v) containing the IS (saikosaponin A), mixing and centrifuging at 14,000 rpm for 10 min at 4 °C. The supernatant was transferred and evaporated to dryness at 30 °C. The residue was reconstituted in 200 μL of ethyl acetate/water (2:8, v/v), vortexed for 3 min and then centrifuged at 14,000 rpm for 10 min at 4 °C. After centrifugation for twice, the supernatants were transferred to vials, and 10 μL of each was injected into the 4500 AB QTRAP-LC/MS/MS system for analysis at 16 °C.

2.4.2. Cell samples

The transfer solution was evaporated to dryness at 30 °C with concentrator. The residue was reconstituted in 200 μL of methanol, vortexed and centrifuged at 14,000 rpm for 10 min. The supernatant fluid was transferred to vials, and 10 μL of each was injected into the UPLC/MS/MS system for analysis.