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Differences of first-pass effect in the liver and intestine contribute to the stereoselective pharmacokinetics of rhynchophylline and isorhynchophylline epimers in rats

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Rhynchophylline (PubChem CID: 5281408)
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Diazepam (PubChem CID: 3016)
Verapamil (PubChem CID: 2520)

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ABSTRACT

Ethnopharmacological relevance: *Uncaria rhynchophylla* (Miq.) Miq. ex Havil., is a plant species used in traditional Chinese medicine to treat cardiovascular and central nervous system diseases. Rhynchophylline (RIN) and isorhynchophylline (IRN), a pair of epimers, are major alkaloids isolated from *U. rhynchophylla* and exhibit diverse pharmacological effects. Our previous study demonstrated that the pharmacokinetics of these epimers existed stereoselectivity after oral administration; however, the specific mechanism remains unknown and merits investigation.

Aim of the study: In the present study, the aim was to elucidate the mechanism underlying stereoselective pharmacokinetic characteristics of RIN and IRN in rats.

Materials and methods: The total (F), hepatic (F_h) and intestinal ($F_a \cdot F_g$) bioavailabilities of each epimer were measured using portal vein cannulated rats following different dosing routes (intravenous, intraportal and intraduodenal) to assess individual contributions of the liver and intestine in stereoselective pharmacokinetics. Then the differences of first-pass metabolism in the liver and intestine between two epimers were evaluated by in vitro incubation with rat liver microsomes, intestinal S9 and gastrointestinal (GI) content solutions, respectively. Meanwhile, the membrane permeability and efflux by P-glycoprotein (P-gp) were examined by in situ single-pass intestinal perfusion with and without P-gp inhibitor verapamil. The configurational interconversion at different pH values and the excretions via feces and urine were also examined.

Results: Pharmacokinetic data showed that the total bioavailability of RIN was 5.9 folds higher than that of IRN (23.4% vs. 4.0%). The hepatic availability of RIN was 4.6 folds higher than that of IRN (46.9% vs. 10.3%), whereas the intestinal availability of RIN (48.1%) was comparable to that of IRN (42.7%). In addition, intestinal perfusion showed that IRN possessed higher intestinal permeability than RIN and co-perfusion with verapamil could affect absorption process of RIN but not IRN. Conversely, the metabolism rate of IRN in rat liver microsomes was significantly faster than that of RIN, resulting in a lower systemic exposure of IRN after oral administration. The degradation in GI lumen and epimerization between two epimers also existed but had small contributions. Additionally, the excretions of both epimers via feces and urine were negligible.

Conclusions: Taken together, different first-pass metabolism in the liver was the major factor responsible for the stereoselective pharmacokinetics of RIN and IRN.

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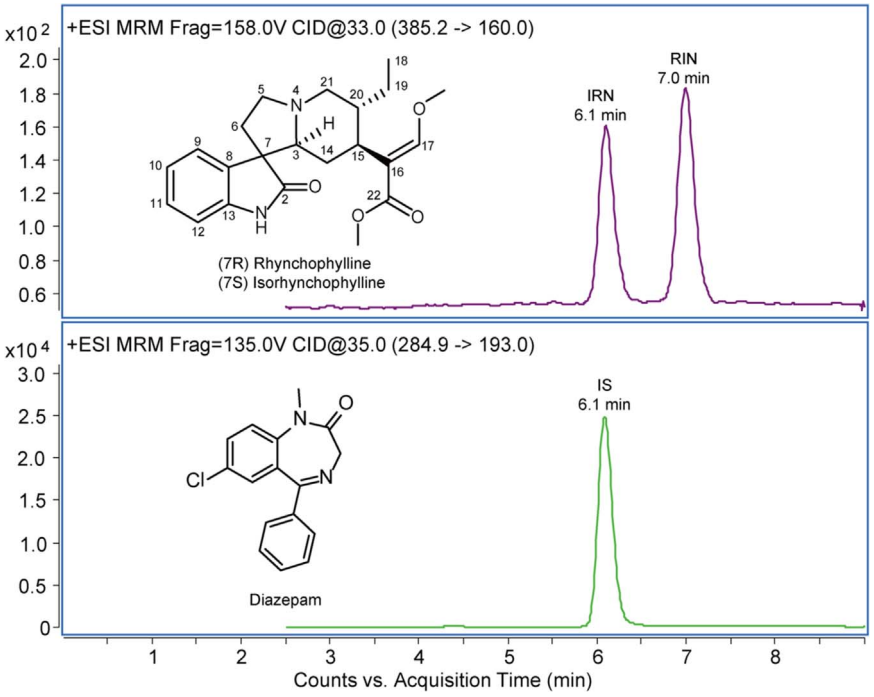

Fig. 1. Representative MRM chromatograms and chemical structures of rhynchophylline (RIN), isorhynchophylline (IRN) and diazepam (IS).

1. Introduction

Uncaria rhynchophylla (Miq.) Miq. ex Havil. (family *Rubiaceae*) is a well-known medicinal plant widely used in China to treat cardiovascular and central nervous system diseases (Heitzman et al., 2005). The fragments of dried stems or branches with hooks have been officially recorded in the Chinese Pharmacopoeia as “Gou Teng”, and in the European Pharmacopoeia 9th edition. Rhynchophylline (RIN) and isorhynchophylline (IRN) (Fig. 1) are the characteristic and active ingredients in *U. rhynchophylla* (Zhang et al., 2015), and have been reported to exhibit numerous pharmacological activities including antihypertension (Zhang et al., 2004), antimicrobial (Chen et al., 2010), anti-depressant (Kang et al., 2004), and anti-inflammatory (Yuan et al., 2008). Moreover, RIN and IRN are increasingly attracting research attention because of their neuroprotective actions (Ng et al., 2015). Investigations showed that they were beneficial to the treatment of Alzheimer's disease (AD) mediated via multiple mechanisms, such as ameliorating Aβ-induced neuronal toxicity (Xian et al., 2012) and blocking the receptor tyrosine kinase EphA4 to ameliorate the dysfunction of central nervous system (Fu et al., 2014). The basic structure of RIN and IRN is tetracyclic monoterpenoid oxindole alkaloid with four chiral centers at C-3, C-7, C15 and C-20 positions. Among them, only the spatial configurations at C-7 are different which contribute to these two epimers (Fig. 1).

Numerous researches revealed that epimeric compounds may behave differently in pharmacokinetics. For example, after oral administration at equal dose, the AUC of 22S-Budesonide was 6 folds higher than that of 22R-Budesonide in human (Lu et al., 2013) and for ginsenosides Rh2 and Rg3, plasma concentrations of S-epimers were much higher than that of R-epimers in rats (Bae et al., 2013). Therefore, it is important to investigate the in vivo disposition details of individual epimer. However, there is hardly any report about stereoselective pharmacokinetics of epimeric alkaloids in *U. rhynchophylla*. In our previous study, we found that following oral dosing, RIN displayed significantly higher plasma concentrations than IRN following oral dosing while no obvious difference was observed after intravenous administration, implying that dispositions of RIN and IRN may possess stereoselectivity during absorption (Wang et al., 2016b). It has been reported that various factors involved in absorption process would cause the different pharmacokinetic profiles between stereoisomers, such as membrane permeability (Gu et al., 2010), degradation in GI tract by microbial flora (Bae et al., 2002), transporters (Wang et al., 2014) and first-pass metabolism by hepatic or intestinal enzyme (Hanada et al., 2008). Particularly, the bidirectional epimerization between RIN and IRN in vivo complicated the issue. The relative contributions of above factors leading to the different dispositions of RIN and IRN are still unknown.

Therefore, in current study, our aim was to clarify the possible factors involved in the stereospecific absorption, with poor bioavailability, of RIN and IRN in rats. Portal vein cannulated (PVC) rats were used to determine the total (F), hepatic (F_h) and intestinal ($F_a \cdot F_g$) bioavailabilities to quantitatively investigate the contributions of liver and intestine in stereoselective first-pass metabolism of RIN and IRN. Intestinal perfusion study was conducted to further assess the permeability and efflux by P-glycoprotein (P-gp). Additionally, the epimerization and degradation of both epimers were entirely evaluated in vitro. The plasma protein binding, fecal and urinary excretions were also examined.

2. Materials and methods

2.1. Chemicals and reagents

Rhynchophylline (Lot. RH-151029) and isorhynchophylline (Lot. IH-160930) were obtained from Aktin Chemicals, Inc. (Chengdu, China). The purity determined by HPLC was above 98%. Diazepam and verapamil (purity ≥ 98%) were obtained from Shanghai Yuanye BioTechnology Co., Ltd. (Shanghai, China). β-Nicotinamide-adenine dinucleotide phosphate (NADPH) regenerating system was purchased from Roche Diagnostics (Mannheim, Germany). Bicinchoninic acid (BCA) kit for protein concentration detection was supplied by Beyotime Institute of Biotechnology (Haimen, China). Methanol and acetonitrile of HPLC grade were supplied by Tedia (Fairfield, USA). Deionized water was purified by a Milli-Q system (Millipore, USA) and all other reagents were of analytical grade.