Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Inhibition of Re Du Ning Injection on Enzyme Activities of Rat Liver Microsomes Using Cocktail Method

Xiao-qian Xu[1, 2, 3], Ting Geng[2, 3, 4], She-bing Zhang[1], Dan-yu Kang[1, 4], Yan-jing Li[1, 2, 3], Gang Ding[1, 2, 3], Wen-zhe Huang[1, 2, 3], Zhen-zhong Wang[2, 3], Wei Xiao[2, 3*]

1. Jiangsu Kanion Modern Chinese Medicine Institute, Nanjing 210017, China

2. State Key Laboratory of Pharmaceutical New-tech for Chinese Medicine, Jiangsu Kanion Pharmaceutical Co., Ltd., Lianyungang 222001, China

3. National Enterprise Technology Center, National Post-doctoral Research Workstation, Jiangsu Enterprise Academician Workstation, Lianyungang 222001, China

4. China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history

Received: November 4, 2015

Revised: January 4, 2016

Accepted: March 17, 2016

Available online:

July 11, 2016

DOI:

10.1016/S1674-6384(16)60045-X

ABSTRACT

Objective Re Du Ning Injection (RDN), a Chinese materia medica injection, is made from the extracts of *Lonicerae Japonicae Flos*, *Gardeniae Fructus*, and *Artemisiae Annuae Herba.* Since last decade, RDN has been widely used in China for the treatment of viral infection, fever, and inflammation. To assess the potential interacting of RDN with co-administered drugs, the inhibitory effects of RDN on the enzyme activities (CYP1A1, CYP1A2, CYP2C11, CYP2D1, and CYP3A1/2) of rat liver microsomes were investigated by a cocktail method. **Methods** A sensitive and specific LC-MS method capable of simultaneous quantification of five metabolites in rat liver microsomes was developed and validated. Then RDN (0.625%–1.0%) was incubated with rat liver microsomes and specific substrates. The enzyme activities were expressed as the formation rate of the specific metabolites of the substrates (pmol·mg·protein^{-1}·min^{-1}). **Results** RDN competitively inhibited the activities of CYP1A2 and CYP2C11, with inhibition constant (K_i) values determined to be 0.18% and 0.63%, respectively. RDN exhibited the mixed inhibition on the activity of CYP2D1, with a K_i value of 0.15%. The activities of CYP1A1 and CYP3A1/2 were not markedly inhibited even by 1.0% RDN. **Conclusion** RDN could inhibit the rat enzyme activities of CYP1A2, 2C11, and 2D1 *in vitro* with different inhibition modes, which is worthy of promoting safety and efficacy of RDN.

Key words

cocktail; cytochrome P450; inhibition; rat liver microsomes; Reduning Injection

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*Corresponding author: **Xiao W** Tel: +86-25-8658 7935 E-mail: kanionxw2010@126.com

Ding G E-mail: dingg2000@126.com

Funds: National Science and Technology Major Project "Creation of Major New Drugs" from China (No. 2013ZX09402203); Natural Science Foundation of Jiangsu Province (No. BK2013403).

1. Introduction

With the prevalence of chronic diseases in the world, Chinese materia medica (CMM) preparation is becoming increasingly popular as alternative therapies due to the mild and broad therapeutic efficacy, and the relatively low adverse reactions comparing with Western medicine. Re Du Ning Injection (RDN), a pure CMM preparation, developed by Jiangsu Kanion Pharmaceutical Co., Ltd. (Lianyungang, China), contains a combination of extracts from *Lonicerae Japonicae Flos* [bud and flower of *Lonicera japonica* Thunb. (Caprifoliaceae)], *Gardeniae Fructus* [fruit of *Gardenia jasminoides* Ellis (Rubiaceae)], and *Artemisiae Annuae Herba* [aerial part of *Artemisia annua* L. (Asteraceae)] in a special ratio (Wang et al, 2013; 2010; Xu et al, 2009). It has been demonstrated to feature a variety of pharmacological activities in clinical practice including fever relief in viral infection diseases, such as influenza, hand-foot-mouth disease, and herpes angina efficacy (Chang et al, 2015). Moreover, it exhibited anti-inflammatory and anti-pneumonia activities (Feng et al, 2007a; 2007b; 2007c; Huang et al, 2006; Lan et al, 2011; Sun et al, 2014; Zhang et al, 2013). In our previous research, the chemical constituents, quantitative analysis, and fingerprint of RDN have been systematically studied by HPLC-DAD, LC-MS, DART-MS, and so on (Li et al, 2012; 2013; Zhang et al, 2015). The HPLC characteristic fingerprint of RDN was established and the main peaks were identified, which accounted for 78.8% of the total peak areas. Phenolic acids and iridoid glycosides, which were about 40% of the total solid contents, have been reported to be the principal active components of RDN (Li, 2013). In the manufacturing process, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, gardenoside, secoxyloganin, and isochlorogenic acid A/B/C were usually chosen as the main quality control indexes to evaluate the quality of intermediates and RDN (Chang et al, 2013; Geng et al, 2015). Recently, with the growing significance of a potential beneficial role of RDN in clinic and in order to have a better therapeutic effect, it was necessary to operate a combination therapy with other drugs. However, several herbal medicines from China have been reported to have the potential to inhibit or induce cytochrome P450 (CYP450) enzymes activities in recent years. Consequently they affected the combined drug metabolism and resulted in drug-drug interactions (DDIs), which should receive our greater attention.

CYPs are responsible for the metabolism of a variety of drugs, xenobiotics, and endogenous substances (Chen et al, 2012; Han et al, 2012). Over 90% of the clinical drugs are metabolized by human CYPs. Among many CYPs isoforms, human CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 are involved in approximately 80% of the CYP-mediated drug metabolism (Badal et al, 2011; Ingelman-Sundberg 2004). Clinically, induction or inhibition of the CYP activities has been recognized as the most important contributor to the unexpected or even serious clinical DDI (Allis et al, 1994). So it is important to study the *in vitro* inhibition and induction of CYPs with the purpose of predicting the potential DDI. At this stage human liver microsomes are the most popular *in vitro* testing systems for predicting potential DDI via CYP inhibition (Pandit et al, 2011). As for rat liver microsomes, it has been demonstrated that human CYP1A2 and CYP2E1 are homologous to rat CYP1A2 and CYP2E1, respectively (Bogaards et al, 2000). In addition, human CYP2C9, CYP2D6, and CYP3A4 are homologous to rat CYP2C11, CYP2D1, and CYP3A1/2, respectively (Ando et al, 2002; Lee et al, 2007b; 2006; Martignoni et al, 2006; Venhorst et al, 2003). Hence, rat liver microsomes are also useful *in vitro* testing system for providing a basis of potential clinical DDI of RDN.

In a conventional DDI study, the CYP activities were evaluated by an individual method, which required extensive time, animal and/or human liver microsomes or hepatocytes (Pillai et al, 2013). To increase throughput, the cocktail method was proposed as a screening tool for potential *in vitro* DDIs. In this method, the preferred and acceptable probe substrates for individual CYP isoform will be mixed together as a cocktail and then incubate with suitable *in vitro* system for the simultaneous measurement of different CYP enzymes activities. The results will provide more inhibition or induction information in a single experiment and greatly improve efficiency. Our previous studies have found that organic acids and iridoid glycosides were the major active ingredients of RDN and showed high exposure levels in rat plasma (Bi et al, 2010; Zheng et al, 2014). It was reported that chlorogenic acid was substrate of CYP3A4, CYP2C9, and CYP1A2 (Zhou et al, 2011). It did not affect the activity of CYP3A4 in human intestinal Caco-2 cells (Melillo de Magalhaes et al, 2012), but inhibited the recombinant human CYP3A4 (Xu et al, 2012). Moreover, some induction effects of chlorogenic acid on the activities of CYP1A1 and CYP3A1 were discovered (Jia et al, 2009; Tang et al, 2013). Teel and Huynh (1998) found that chlorogenic acid, caffeic acid, and ferulic acid had the selective inhibitory effects on isoforms of CYP450. However, to our knowledge, no systematic study has been reported emphasizing the influence of RDN preparation on the activities of clinically important CYP enzymes. Therefore, the purpose of current study was to evaluate the inhibitory effects of RDN preparation on enzyme activities of CYP1A1, CYP1A2, CYP2C11, CYP2D1, and CYP3A1/2 in rat liver microsomes using cocktail method, which provided a basis for the further study about potential DDI of RDN.

2. Materials and methods

2.1 Materials and reagents

Tinidazole (internal standard, IS), paracetamol, omeprazole, and dextromethorphan were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Tolbutamide, testosterone, phenacetins, and tris (hydroxymethyl) aminomethane were purchased from Aladdin reagent Co., Ltd. (Shanghai, China). Reduning Injection (RDN) was manufactured by Jiangsu Kanion Pharmaceutical Co., Ltd. (Lianyungang, China). Rat liver microsomes were obtained from Research Institute for

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