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Effects of salvianolic acid B and tanshinone IIA on the pharmacokinetics of losartan in rats by regulating the activities and expression of CYP3A4 and CYP2C9

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ABSTRACT

Losartan (LST) is a common chemical drug used to treat high blood pressure and reduce the risk of stroke in certain people with heart disease. Danshen, prepared from the dried root and rhizome of *Salvia miltiorrhiza Bunge*, has been widely used for prevention and treatment of various cardiovascular and cerebrovascular diseases. There are more than 35 formulations containing Danshen indexed in the 2010 Chinese Pharmacopoeia, which are often combined with LST to treat cardiovascular and cerebrovascular diseases in the clinic. The effects of the two major components of Danshen, salvianolic acid B (SA-B) and tanshinone IIA (Tan IIA), on the pharmacokinetics of losartan and its metabolite, EXP3174, in rats were investigated by liquid chromatography coupled with mass spectrometry (LC–MS). Male Sprague-Dawley rats were randomly assigned to 3 groups: LST, LST+SA-B and LST+Tan IIA, and the main pharmacokinetic parameters were estimated after oral administration of LST, LST+SA-B and LST+Tan IIA. It was found that there are significant differences in the pharmacokinetic parameters among the three groups: C_{max}, $t_{1/2}$, *AUC*, *AUMC* in the LST+SA-B group was smaller than those in group LST, while larger in group LST+Tan IIA. Further, the effects of SA-B and Tan IIA on the metabolism of losartan was also investigated using rat liver microsomes *in vitro*. The results indicated that SA-B can induce the metabolism of LST, while Tan IIA can inhibit the metabolism of LST in rat liver microsomes *in vitro* by regulating activities of CYP450 enzymes. In addition, the effect of SA-B and Tan IIA on CYP3A4 and CYP2C9 expression was studied in Chang liver cells by western-blotting and Real-time PCR. It was concluded that the two components of Danshen, SA-B and Tan IIA have different influences on the metabolism of LST: SA-B can obviously speed up the metabolism of LST by inducing CYP3A4/CYP2C9 activities and expression, however, Tan IIA can slow down the metabolism of LST by inhibiting CYP3A4/CYP2C9 activities.

1. Introduction

During the past decade, the use of herbal medicines, which incorporate the therapeutic use of herbs and other natural products, continues to expand rapidly across the world (Wang and Yeung, 2012; Foster et al., 2005). The reason herbal medicines are so popular with people is that herbal medicines have many advantages over other synthetic drugs, including mild therapeutic efficacy, less toxic side effects and relatively low incidences of adverse reactions (Firenzuoli and Gori, 2007). However, contrary to common belief that "natural products are safe", herbal medicines can lead to drug interactions, significant toxic effects and even morbidity or mortality (Skalli and Soulaymani Bencheikh, 2012; Hu et al., 2005). Therefore, it is important to discover possible herbal–drugs interactions in order to prevent serious side effects. Danshen, prepared from the dried root and rhizome of *Salvia miltiorrhiza Bunge*, has been widely used in China for prevention and treatment of various cardiovascular and cerebrovascular diseases; it is also used in America and some European countries as a complementary medicine (Wang and Yeung, 2012). In recent years, the potential herb–drug interaction for Danshen has drawn more and more attention, due to its common application in combination with other drugs in the clinic.

Losartan (LST) is the first non-peptide, angiotensin-II receptor which is used to treat high blood pressure and reduce the risk of stroke in certain people with heart disease by selectively blocking the angiotensin-II type 1 (AT1) receptor within the renin–angiotensin–aldosterone system (Fujimori et al., 2011; Shiga et al., 2012). In China, patients usually were treated with LST 50 mg once

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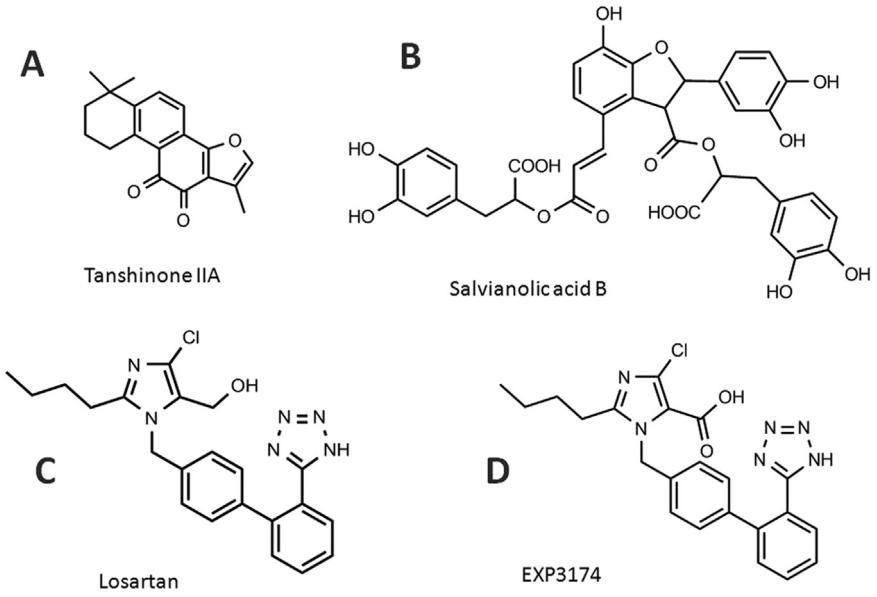

Fig. 1. Chemical structure. A: tanshinone IIA, B: salvianolic acid B, C: losartan, D: metabolite EXP3174.

daily and administrated 3–4 danshen tablets three times daily simultaneously to treat cardiovascular and cerebrovascular diseases. However, the current research of Danshen tablets and LST are mainly focused on the aspects of pharmacodynamics, and there have been few studies on the herb–drug interactions. The interactions may exist in the processes of absorption, distribution, metabolism and excretion. In our previous research, it was found that the compound Danshen tablet (CDST) could influence the metabolism of LST in rats, which indicated that there may be herb–drug interactions between LST and some components in CDST (Yuan et al., 2013). However, it is unclear which components in CDST influence the metabolism of LST and whether it acts through inducing CYP450 metabolizing enzymes or not. In this study, the two major components of CDST, tanshinone IIA (Tan IIA) and salvianolic acid B (SA-B) were chosen to investigate the potential influences on the metabolism of LST. The structure of Tan IIA is shown in Fig. 1A, and the structure of SA-B is shown in Fig. 1B.

Cytochrome P450 (CYP450) enzymes are the most important phase I drug-metabolizing enzyme systems and exit in almost all living organisms. They play important roles in the metabolism of a large number of xenobioties, including therapeutic drugs and some important endogenous substances (Fasinu et al., 2012; Sridhar et al., 2012; Kong et al., 2011). Currently, many clinically relevant drug-drug interactions may be the result of induction and/or inhibition of specific CYP450s (Wienkers, 2001; Pergolizzi et al., 2011; Hughes et al., 2010). Some studies found that active components of Danshen can influence the activities or expression of CYP450 enzymes (Li and Chen, 2014; Zhou et al., 2013; Zhou et al., 2012). The animal experiment showed that ethyl acetate extract, especially tanshinone IIA, were mouse CYP1A, CYP2C and CYP3A-inducing agents of Danshen (Kuo et al., 2006). Primary hepatocytes experiment showed that a formulated Danshen pill (containing mainly danshensu and salvianolic acid B and the tanshinones) up-regulated CYP1A2 protein expression and enzyme activity, but danshensu and salvianolic acid B, when used individually, did not affect CYP1A2 activity (Lee et al., 2012). In addition, tanshinone inhibited the activities of CYP450 enzymes in experiments with liver microsomes, although the aqueous extract had no effects on any of the activities tested (Wang et al., 2010; Qiu et al., 2008; He et al., 2007). These studies showed that Danshen and its active components have effects on the activities or expression of CYP450 enzymes. As we know, LST can be metabolized into EXP3174 by CYP3A4 and CYP2C9 enzymes (Yang et al., 2012). The structure of LST is shown in Fig. 1C, and the structure of EXP3174 is shown in Fig. 1D. However, it was unclear whether the main ingredients in Danshen, Tan IIA and SA-B, influence the pharmacokinetics of LST through regulating the activities and expression of CYP3A4 and CYP2C9.

Therefore, in order to discover the possible interactions between LST and CDST, this study was to investigate effects of SA-B and Tan IIA on the pharmacokinetics of LST and its metabolite, EXP3174, in rats by liquid chromatography coupled with mass spectrometry (LC–MS). Furthermore, the effects of SA-B and Tan IIA on the metabolism of LST were investigated using rat liver microsomes *in vitro*. In addition, the effects of SA-B and Tan IIA on CYP3A4 and CYP2C9 expressions in Chang liver cells were detected. This research could provide useful insight into the safe and effective use of Danshen preparations.

2. Materials and methods

2.1. Ethics statement

All animal experiments were approved by the Administrative Committee of Experimental Animal Care and Use of Second Military Medical University (SMMU, Licence No. 2011023), and conformed to the National Institute of Health guidelines on the ethical use of animals.

2.2. Chemical and reagents

Standards of LST (purity > 98%), irbesartan (purity > 98%) as an internal standard (IS), Tan IIA (purity > 98%) and SA-B (purity > 98%) were obtained from China's National Institute for the Control of Pharmaceutical and Biological Products (NICPB, Beijing, China). LST carboxylic acid (EXP3174) (purity > 98%) was purchased from Toronto research chemicals Inc., Canada. Methanol and formic acid of HPLC grade were purchased from Fisher Chemical Company (USA). Ultrapure water was prepared by Milli-Q System (Millipore, Bedford, MA, USA). Human Chang liver cells were purchased from American Type Culture Collection (USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was