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Metabolism and pharmacokinetics of alantolactone and isoalantolactone in rats: Thiol conjugation as a potential metabolic pathway

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ABSTRACT

Alantolactone (AL) and isoalantolactone (IAL), two major active sesquiterpene lactones isolated from Radix Inulae extract, have a wide range of pharmacological activities. The predominant metabolic pathway of AL and IAL observed was glutathione (GSH) conjugation *in vitro*, which could occur in the absence of metabolic enzymes. Non-enzymatic conjugation with cysteine (Cys) couldalso be observed. Four metabolites (AL-GSH, AL-Cys, IAL-GSH, IAL-Cys) were subsequently isolated and confirmed by nuclear magnetic resonance (NMR). The results indicated that the thiol of GSH or Cys can be reacted with the exomethylene carbon atoms of α, β-unsaturated carbonyl of AL and IAL. After intravenous administration in rats, AL and IAL were extensively metabolized, and the exposure, as measured by area under the concentration-time curve (AUC), for AL-GSH, AL-Cys, IAL-GSH, and IAL-Cys was approximately 1.54-, 0.96-, 1.50-, and 0.91-fold that of the parent drug, respectively. The AUC ratio of metabolites to parent compounds of oral administration was 3.66-, 9.19-, 12.97-, and 9.92-fold that of the parent drug for the above metabolites, respectively. The bioavailability of AL-total (AL, AL-GSH, AL-Cys) and IAL-total (IAL, IAL-GSH, IAL-Cys) was, respectively, 8.39% and 13.07%, which was 3.62- and 6.95- fold that of AL (2.32%) and IAL (1.88%), respectively. The oral exposure will be underestimated if the parent drugs are tested alone. These findings provide useful information for preclinical safety evaluation, and for predicting AL and IAL metabolism in humans.

1. Introduction

Radix Inulae, the root of *Inula helenium* L. or *I. racemosa Hook. f.*, which is known as Tu-Mu-Xiang or Zang-Mu-Xiang, is a popular traditional Chinese medicine (TCM) that has been officially documented in China Pharmacopoeia [1]. In TCM therapy, Radix Inulae has been used against a variety of ailments, including asthma, cough, bronchitis, lung disorders, tuberculosis, indigestion, chronic enterogastritis, infectious and helminthic diseases [2]. Recent studies have shown that the extract of *I. helenium* has remarkable pharmacological activities, such as anti-tumor, anti-bacterial and insecticidal activities [3–5]. In addition, our previous study indicated that Radix Inulae extract may be used to treat rheumatoid arthritis (RA) [6], and it was reported elsewhere to be useful in the treatment of irritable bowel syndrome (IBS) [7]. Alantolactone (AL) and isoalantolactone (IAL) (Fig. 1) are two major active sesquiterpene lactones in Radix Inulae [8] that have been reported to exhibit a wide range of biological effects, including antifungal, anthelmintic, antimicrobial, anti-inflammatory, and anti-trypanosomal activities as well

Abbreviations: AL, alantolactone; AL-GSH, glutathione conjugate of alantolactone; AL-Cys, cysteine conjugate of alantolactone; AUC, area under the concentration-time curve; Cys, cysteine; CYP450, cytochrome P450; DAD, diode array detector; GSH, glutathione; GST, glutathione-*S*-transferase; HPLC, high-pressure liquid chromatography; IAL, isoalantolactone; IAL-GSH, glutathione conjugate of isoalantolactone; IAL-Cys, cysteine conjugate of isoalantolactone; LC–MS, liquid chromatography with mass spectrometry; LC–MS/MS, liquid chromatography with tandem mass spectrometry; NMR, nuclear magnetic resonance; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase; UDPGA, UDP-α-D-glucuronic acid; UGT, UDP-glucuronosyltransferase; UV, ultraviolet

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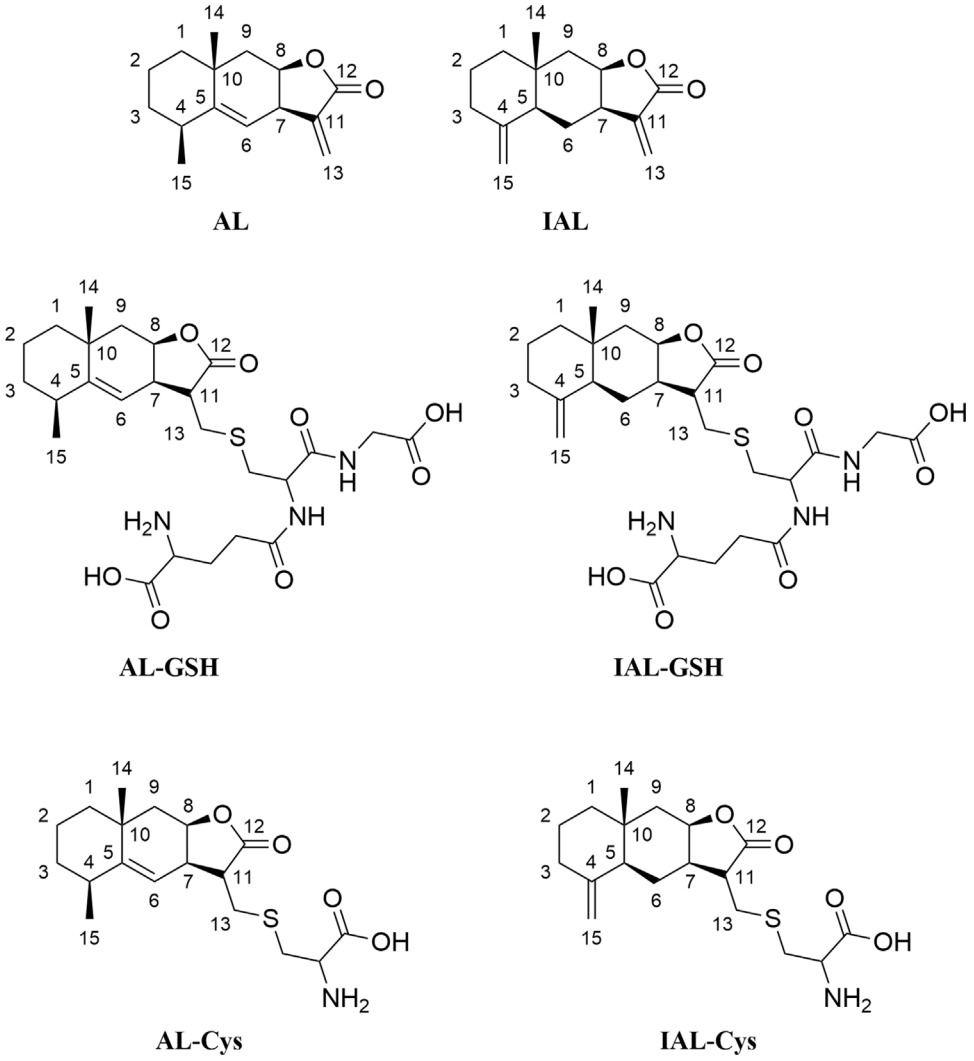

Fig 1. Chemical structures of AL, IAL from Radix Inulae and their four biothiol conjugates AL-GSH, IAL-GSH, AL-Cys, and IAL-Cys.

as anti-proliferative effects on several cancer cell lines, such as colon, ovary, prostate, and lung cancers as well as melanoma and leukemia [9].

Due to the various biological activities of Radix Inulae and its two most abundant lactones, some studies concerning the pharmacokinetics of IAL and AL in rats have been published. Interestingly, the pharmacokinetic parameters of IAL and AL after intravenous and oral administration suggest that these two compounds are poorly absorbed and can be eliminated rapidly in rat plasma [1,7]. Previous studies have also inferred that metabolism likely plays an important role in the elimination of these two sesquiterpene lactones. Drug-metabolism studies have played key roles in medicinal chemistry for lead optimization, detection of potentially toxic metabolites, and identification of the route and rate of drug clearance from the body [10]. To date, no studies have been published to investigate the metabolism of IAL and AL.

In this work, the metabolic stabilities of AL and IAL in both human/rat liver microsomes and S9 fractions were examined, and glutathione (GSH) conjugation was observed as their predominant metabolic pathway. Non-enzymatic conjugation with GSH and cysteine (Cys) were detected and isolated, and the structures of the conjugates were elucidated using two-dimensional nuclear magnetic resonance (NMR). The pharmacokinetics of IAL and AL and their metabolites after a single oral and intravenous Radix Inulae extract dose in rats were also studied, which suggested that AL and IAL were largely metabolized by conjugation with GSH and Cys. These results could provide useful information for preclinical safety evaluation of Radix Inulae extract, and better understanding its mechanism of action.

2. Materials and methods

2.1. Materials

Isoalantolactone, alantolactone and psoralen (all purity > 98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). UDP-α-D-glucuronic acid (UDPGA), 3′-phosphoadenosine 5′-phosphosulfate (PAPS), formic acid, glucose-6-phosphate dehydrogenase, nicotinamide-adenine dinucleotide phosphate ($NADP^+$), and D-glucose 6-phosphate were purchased from Sigma-Aldrich (Sigma, St. Louis, MO). Human liver microsomes (HLMs) and S9 fractions and rat liver microsomes (RLMs) and S9 fractions used in this study were purchased from the Research Institute for Liver Disease Co., Ltd (Shanghai, China). MS-grade methanol and acetonitrile were purchased from Merck (Merck Company, Germany), and ultra pure water was generated from the Milli-Q system (Millipore Corp., USA).Acetic acid, phosphoric acid and petroleum ether were all of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). SPE cartridges (300-mg Oasis HLB) were from Waters (Milford, MA).

2.2. Animals

Male SD rats (180–220 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The rats were housed in air-conditioned animal quarters at 22 ± 2 °C and a relative humidity