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Pharmacokinetics of isochlorgenic acid C in rats by HPLC-MS: Absolute bioavailability and dose proportionality

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ABSTRACT

Ethnopharmacological relevance: Isochlorgenic acid C (IAC), one of the bioactive compounds of *Lonicera japonica*, exhibited diverse pharmacological effects. However, its pharmacokinetic properties and bioavailability remained unresolved.

Aim of the study: To determine the absolute bioavailability in rats and the dose proportionality on the pharmacokinetics of single oral dose of IAC.

Materials and methods: A validated HPLC-MS method was developed for the determination of IAC in rat plasma. Plasma concentration versus time data were generated following oral and intravenous dosing. The pharmacokinetic analysis was performed using *DAS 3.0* software analysis. Absolute bioavailability in rats was determined by comparing pharmacokinetic data after administration of single oral (5, 10 and 25 mg kg^{-1}) and intravenous (5 mg kg^{-1}) doses of IAC. The dose proportionality of $AUC_{(0-\infty)}$ and C_{max} were analyzed by linear regression.

Results: Experimental data showed that absolute oral bioavailability of IAC in rats across the doses ranged between 14.4% and 16.9%. The regression analysis of $AUC_{(0-\infty)}$ and C_{max} at the three doses (5, 10 and 25 mg kg^{-1}) indicated that the equations were $y=35.23x+117.20$ ($r=0.998$) and $y=121.03x+255.74$ ($r=0.995$), respectively.

Conclusions: A new HPLC-MS method was developed to determine the bioavailability and the dose proportionality of IAC. Bioavailability of IAC in rats was poor and both C_{max} and $AUC_{(0-\infty)}$ of IAC had a positive correlation with dose. Evaluation of the pharmacokinetics of IAC will be useful in assessing concentration-effect relationships for the potential therapeutic applications of IAC.

1. Introduction

Lonicera japonica, a well-known Chinese traditional medicine, listed in the Chinese Pharmacopeia, has been used to treat various diseases, especially in the treatment of various viral diseases, such as *SARS*, *H7N9* virus and infections (Guo et al., 2014). *Lonicera japonica* mainly contains chemical constituents including organic acids, flavonoids and volatile oil. Our laboratory has been dedicated to the study of pharmacokinetics of organic acids, such as chlorogenic acid (Wang et al., 2006), caffeic acid (Wang et al., 2014) and isochlorgenic acid A (Huang et al., 2015). Isochlorogenic acids, naturally a mixture of several isomers, are composed of unimolecular quinic acid and bimolecular caffeic acids (Guo et al., 2015). Isochlorgenic acid C (4, 5-Di-O-caffeoylquinic acid, IAC, PubChem CID: 6474309, Fig. 1) is one of the bioactive compounds of *Lonicera japonica*. The pharmacological properties of IAC beneficial to health are multifaceted. For example, IAC has been reported to significantly inhibit the reverse mutation induced by Trp-P-1 on *Salmonella typhimurium* TA 98 (Yoshimoto et al., 2002), to induce hepatocyte growth factor production in a dose-dependent manner (Kurisu et al., 2013) and to inhibit the formation of leukotriene B4 induced by calcium ionophore A23187 in human peripheral polymorphonuclear leukocytes (Kimura et al., 1987). IAC has also been found to have antioxidative (Chiang et al., 2004), anti-inflammatory (Peluso et al., 1995) and analgesic effects (Santos et al., 2005). Moreover, compared to other quinic acid derivatives, IAC has showed the highest protection against tetrahydropapaveroline-induced cell damage (Soh et al., 2003). However, the investigations have been limited in its pharmacological effects and plant extraction methods (Guo et al., 2015; He et al., 2012). Its analytical methods have only focused on the

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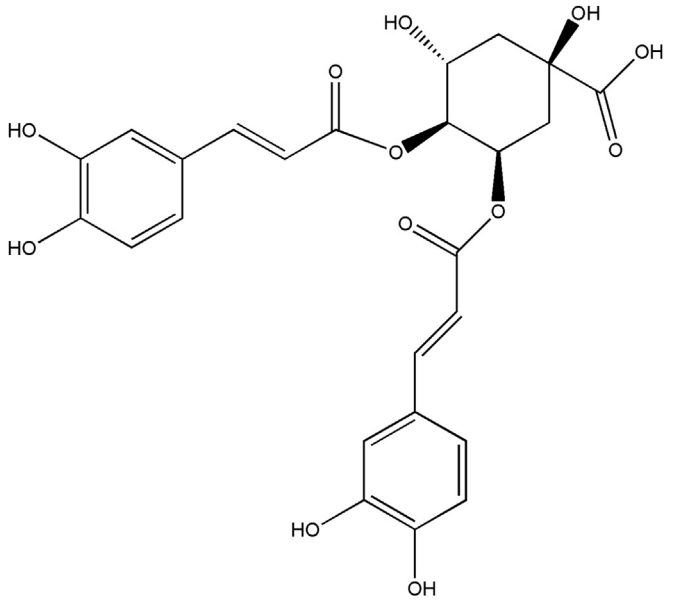

Fig. 1. Chemical structure of IAC.

simultaneous determination of muti-components extracted from *Lonicera japonica in intro* (He et al., 2012; Zheng et al., 2013). Up to now, the analytical method *in vivo* has not been reported. The pharmacokinetic properties and bioavailability of IAC remain unresolved. Pharmacokinetic study plays an important role in assessing the concentration-effect relationship for the potential therapeutic applications. In addition, bioavailability is one of the most crucial factors determining the therapeutic utility of drugs. Thus, the present study aims to develop a new analytical method (HPLC-MS) to investigate the pharmacokinetic properties, absolute bioavailability and dose proportionality of IAC in rats.

2. Materials and methods

2.1. Chemicals and reagents

IAC and protocatechuic acid (internal standard, IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity was above 99% which has been measured by HPLC-MS (Fig. 2 (A)). Purified water from a Milli-Q system (Millipore, Bedford, MA, U.S.A.) was used. Methanol (Fisher Company, U.S.A.) was of high-performance liquid chromatography grade. All other reagents and solvents were of analytical grade and were commercially available.

2.2. Experimental animals

Male Sprague-Dawley rats, weighing 180–220 g, were purchased from Laboratory Animal Center of Guangzhou University of Chinese Medicine (Guangzhou, China). The animals were acclimated for 7 days in an environmentally controlled room (12-h dark/light cycle, temperature: (25 ± 2) °C, humidity: (50 ± 5)%)) with free access to water and food. The rats were fasted for 12 hours before the experiments. The study was approved by the Animal Ethics Committee of Guangdong Pharmaceutical University (GDPUIACE No. 2011036).

2.3. HPLC-MS analytical conditions

Chromatography was performed on Agilent 1100 HPLC system (Agilent, USA). HPLC separations were performed on an Agilent

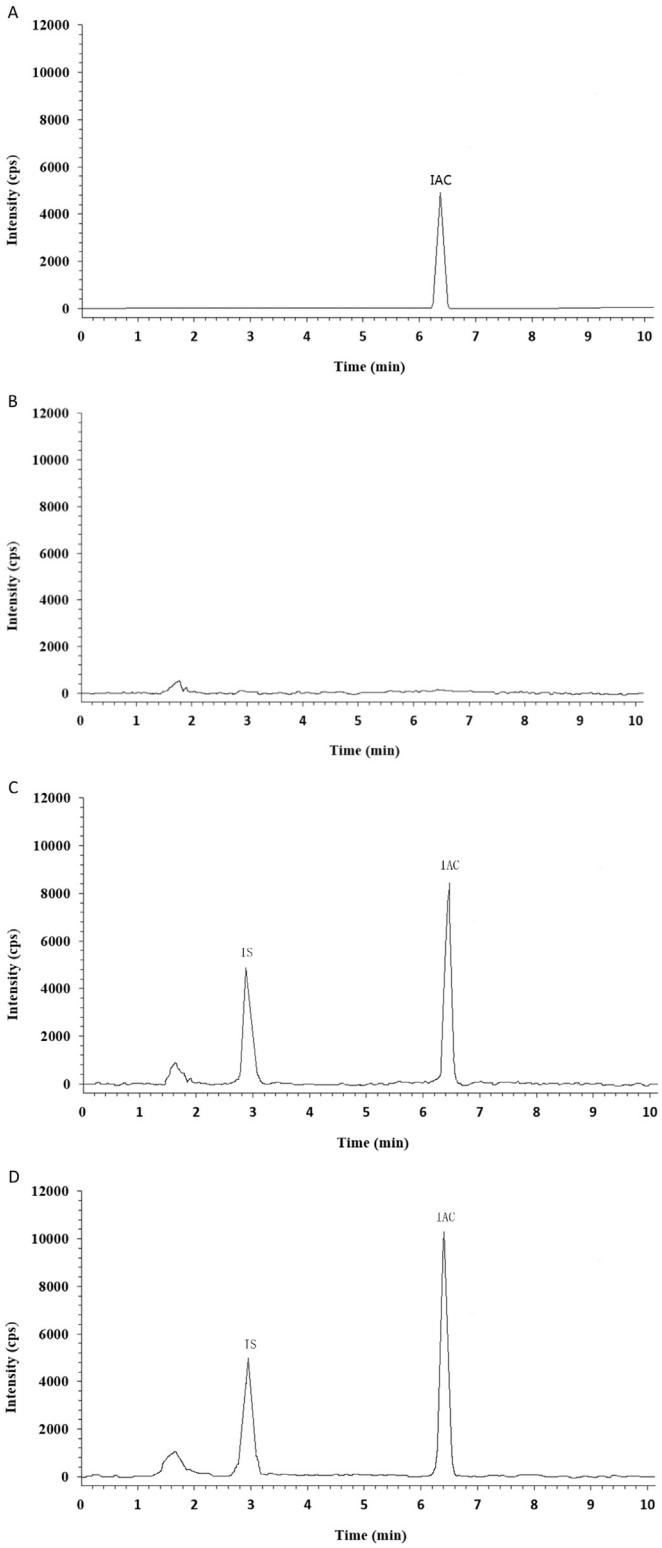

Fig. 2. Chromatogram of IAC ($50\ \mu g\ L^{-1}$) standard solution (A), Chromatogram after deproteinization for rat blank plasma (B), the rat plasma spiked with $100\ \mu g\ L^{-1}$ IAC and ($100\ \mu g\ L^{-1}$) IS (C), and rat plasma sample at 0.33 h after oral administration at a dose of $10\ mg\ kg^{-1}$ (D).

HC-C_{18} (4.6×250 mm, 5 μm) at 30 °C. Chromatographic conditions were as following: The mobile phase consisted of methanol-water containing 0.1% formic acid (20:80). The flow rate was set at $1\ mL\ min^{-1}$. Aliquots of 10 μL were injected into HPLC system for analysis.