European Journal of Pharmaceutical Sciences 46 (2012) 468–474

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Pharmacokinetic behavior of argirein, derived from rhein, is characterized as slow release and prolonged $T_{1/2}$ of rhein in rats

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ARTICLE INFO

Article history:
Received 26 August 2011
Received in revised form 26 February 2012
Accepted 25 March 2012
Available online 10 April 2012

Keywords:
Argirein
Rhein
Pharmacokinetic
Bioavailability
HPLC
Rat

ABSTRACT

Aim: Rhein is an effective ingredient from *Rheum palmatum* L., *Polygonum cuspidatum* Sielb.et Zucc., *Polygonum multiflorum* Thunb. and has anti-inflammatory activity, however, plasma levels are too high and $T_{1/2}$ is not long enough following oral medication. Therefore, a modification of the rhein moiety was encouraged to improve the pharmacokinetic behavior. Argirein was produced by connecting rhein with L-arginine through hydrogen bond, which releases both rhein and L-arginine while getting into the body. The present study was to verify if the pharmacokinetic profile of argirein by measuring the released rhein is improved against those of untreated rhein administered alone.
Methods: A reversed-phase HPLC with a mobile phase of methanol mixed with acetate buffer was conducted. Rhein was monitored after arginine administration by i.g. and i.v. routes. Rhein alone was also administered and compared.
Results: The C_{max} and AUC_{0-48} of the released rhein following argirein medication were less than those following rhein administered. The bioavailability of argirein was 18.5–20.8% against 22.77–25.22% of rhein. A delayed T_{max}, a reduced C_{max} and AUC_{0-t} and an increased $T_{1/2}$ were significant in the argirein group as compared with those in the rhein group.
Conclusion: The pharmacokinetic behavior of oral argirein presents a slow release property against those following oral rhein in rats. The released rhein following oral argirein is suitable in suppressing chronic inflammatory reactions attributed to prolonged $T_{1/2}$ and delayed T_{max} due to its slow release pharmacokinetic characteristics.

1. Introduction

Rhein belongs to 1,8-dihydroxyanthraquinone derivatives (Fig. 1A) and is an active ingredient extensively found in plants of polygonaceae, including *Rheum palmatum* L., *Polygonum cuspidatum* Sielb.et Zucc., *Polygonum multiflorum* Thunb. (Zuo et al., 2008). It has bioactive activities in relieving liver fibrosis (Guo et al., 2002) and renal lesions in diabetic nephropathy (Gao et al., 2010; Zheng et al., 2008) due to its anti-inflammatory activities. Diacerein, a new compound formed by adding two acetyl groups to the moiety of rhein, has been launched for treating osteoarthritis in Europe (Nicolas et al., 1998). Actually, rhein as the active metabolite of diacerein is released and monitored after medication (Nicolas et al., 1998). In treating osteoarthritis diacerein modulates resorptive enzymes and osteoclastic differentiation/survival in the affected bones (Boileau et al., 2008).

Argirein contains two active molecules rhein and L-arginine connected each other by hydrogen bond. Like diacerein, the compound is to release rhein in the body after medication. As a consequence, pharmacological effects of argirein are relevant to the two active components released in the body. In treating diabetic nephropathy in streptozotocin injected rats, the beneficial effects of argirein have been proved due to suppressing ER (endoplasmic reticulum) stress and p66Shc via its anti-inflammatory activity (Hu et al., 2011). Argirein protects injuries of cardiac myocardium caused by over doses of isoproterenol through its anti-inflammatory activity (Zhang et al., 2011).

Following oral medication of untreated rhein, its plasma levels were high reaching 55 μg/ml at a dose of 70 mg/kg in rat (Zhang et al., 2010). It is unclear if high plasma concentrations of rhein are necessary for anti-inflammatory reactions. On the other hand, the $T_{1/2}$ of rhein is about 3 h, not long enough for sustained suppression on chronic inflammation. Therefore, it is interesting to improve the pharmacokinetic parameters of rhein through chemical modification by forming a new molecule which releases rhein, after oral medication, possibly keeping concentrations in plasma longer than those following oral rhein alone. Based on its

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http://dx.doi.org/10.1016/j.ejps.2012.03.014

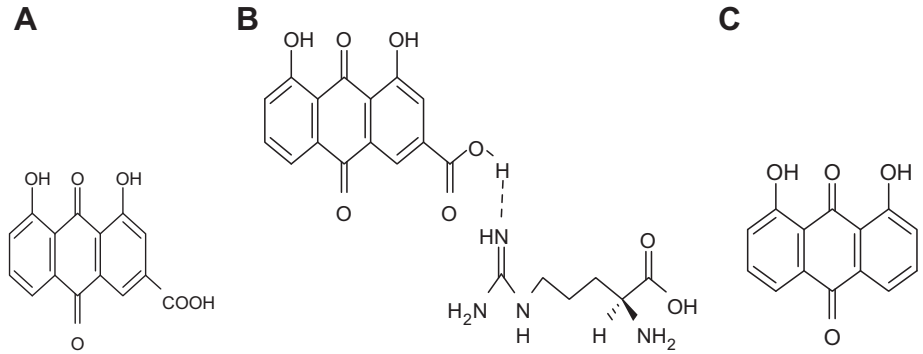

Fig. 1. Chemical structure of rhein (A), argirein (B) and 1,8-dihydroxyanthraquinone (C).

excellent anti-inflammatory activity, an attempt to produce derivatives of rhein for the purpose of novel drug discovery was encouraged. However, a modification of rhein moiety by esterification of carboxyl group did lead to unsatisfied pharmacokinetic properties with much reduced plasma levels of released rhein, therefore, making these unsuitable for new drug development (Peng et al., 2009).

L-Arginine is a precursor for biosynthesis of NO essential for normal vascular endothelial activity. An exogenous offer of L-arginine, a NO-donor, is beneficial for counteracting the adverse effects of high glucose on the vascular activity (Giugliano et al., 1997; Zhong et al., 2010). Therefore, we may suggest that L-arginine released from argirein could improve the vascular activity favorable to synergistic activity with anti-inflammatory activity of rhein (Fig. 1B). Furthermore, from the pharmacokinetic point of view, a combining L-arginine in argirein might cause an acceptable bioavailability and a relative high plasma concentration appropriate for suppressing inflammatory reactions in tissues.

Therefore, we hypothesized that argirein combining rhein with L-arginine through a hydrogen bond may exhibit a pharmacokinetic property of rhein slow-releasing presenting with a reduced C_{max} and a delayed T_{max} following oral administration, which appears to be beneficial for the pharmacological activities. The pharmacokinetic parameters and bioavailability of argirein were investigated by monitoring the released rhein in plasma by HPLC–UV assay following oral and i.v. administration in rats. The pharmacokinetic behavior of argirein and the untreated rhein was compared in rats.

2. Materials and methods

2.1. Animals

Adult Sprague–Dawley (SD) rats, either sex, weighing 220 ± 20 g, were applied, purchased from the Zhejiang Experimental Animal Center and were kept in a standard environment at a controlled temperature (20–25 °C). The handlings of animal were in accordance with the Guidelines of Animal Experiment set up by the Bureau of Sciences and Techniques of Jiangsu Province, China and were consistent with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

2.2. Reagents and chemicals

Argirein (purity >99%) and rhein (Fig. 1A) were offered from Zhejiang Chinese Medical University and 1,8-dihydroxyanthraquinone (Fig. 1C) as internal standard (IS) was purchased from the National Quality Control Institute for the Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was used (Jiangsu Hanbon Sci. & Tech. Co., Ltd.) and ultra-pure water was provided from Robust Company, Wuxi, China. Other solvents or reagents of analytical grade were purchased from the market.

2.3. Calibration curve

Standard stock solutions of rhein (100 μg/ml) and IS (10 μg/ml) were prepared in methanol and stored at 4 °C. The rhein stock solution was further diluted with methanol to make eight different concentrations of working standards in a range of 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25 and 50 μg/ml. Calibration standards of rhein were prepared by dissolving the evaporated working standards with blank rat plasma yielding final concentrations of 0.39, 0.78, 3.12, 12.5, 25, 50 and 100 μg/ml.

2.4. HPLC assay

The HPLC system was consisted of a LC-10AT *VP* liquid chromatograph (Shimadzu, Japan), a SPD-10A UV detector (Shimadzu, Japan), and a chromatography integrator (Zhejiang University N2000, China).

Rhein and 1,8-dihydroxyanthraquinone were separated on a reversed-phase C_{18} column (460 mm × 150 mm, 5 μm, Hanbon Sci. & Tech., China). A mixture of methanol–0.2% acetic acid (80:20, V/V) was used as the mobile phase at a flow rate of 1.0 ml min^{-1} with a column temperature of 30 °C and the detection wavelength at 254 nm. The total run time for sample each was 15 min. Under these conditions, the retention times were about 6.9 and 9.2 min for rhein and IS, respectively. Quality control (QC) samples were prepared at low (0.39 μg/ml), medium (6.25 μg/ml) and high (25 μg/ml) concentrations in the same way as the plasma samples for calibrations and were measured in the way of intra-day and between days.

2.5. Sample preparation

A compound of 1,8-dihydroxyanthraquinone was used as an IS mixed in plasma for HPLC assay, then, the mixture was acidified by hydrochloric acid (2 mol/l, 20 μl), followed by liquid–liquid extraction using 3 ml of diethyl ether, vortex-mixed for 3 min and centrifuged at 3500 r min^{-1} for 10 min. About 2.5 ml of upper organic layer was then transferred to a new tube and evaporated in a thermostatic bath at 50 °C (Zhang et al., 2005). The residue was reconstituted in 100 μl methanol, and an aliquot of 20 μl of each reconstituted sample was injected into the HPLC system to be analyzed.

Specificity was assessed by analysis of six different samples of blank matrix with and without rhein and IS. The calibration curves were performed with eight different concentrations. The regression equation was calculated in the form of $y = ax + b$, where y and x were the concentrations of rhein and peak area ratio of rhein to the IS, respectively.