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Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

Study on pharmacokinetics and tissue distribution of single dose oral tryptanthrin in Kunming mice by validated reversed-phase high-performance liquid chromatography with ultraviolet detection

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ARTICLE INFO

Article history:
Received 24 March 2017
Received in revised form 7 May 2017
Accepted 10 May 2017
Available online 25 May 2017

Keywords:
mouse
natural products
pharmacokinetics
tissue distribution
tryptanthrin

ABSTRACT

Background: Tryptanthrin is a major active constituent of several Chinese herbal plants, such as *Isatidis radix*. Tryptanthrin had been demonstrated to have several beneficial pharmacological effects *in vitro* for human diseases, including antitumor, anti-inflammatory and antibacteria activities. In contrast to the extensive *in vitro* investigations, the *in vivo* disposition process of tryptanthrin was explored limitedly.

Methods: In this study, the pharmacokinetics (PK) and tissue distribution of tryptanthrin in Kunming mice following a single oral dose of 80 mg/kg tryptanthrin were investigated for the first time. Mouse plasma, liver, heart, spleen, lung, kidney and brain were collected and analyzed using a validated reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC–UV) method after biological sample preparation by a simple liquid–liquid extraction.

Results: The chromatographic analysis was performed on a Diamonsil C_{18} column (5 μm, 250 mm × 4.6 mm) and ultraviolet detection was set at a wavelength of 251 nm. The analysis was achieved with a mobile phase of methanol (A) and water (B) (60:40, v/v) at a flow rate of 1.0 mL/min. The method was linear over the concentration range of 4.0–400.0 μg/mL with a lower limit of quantification of 0.10–0.30 μg/mL. Inter- and intraday precisions (relative standard deviations %) were all within 2.93%. Recoveries of tryptanthrin were more than 86.44%. Maximal tryptanthrin concentrations in plasma and tissues of mice were reached within 2.5 hours. The actual highest concentration (C_{max}) in mouse plasma was 3.13 μg/mL, the area under the curve (AUC_{0-t}) was 9.38 h μg/mL, and the terminal half-life was 2.27 hours. The volume of distribution

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http://dx.doi.org/10.1016/j.imr.2017.05.001

was 343.89 mL, the clearance rate was 204.58 mL/h, and the PK of tryptanthrin in mice after oral administration was fit to 2 compartment 1 st Order. After oral dosing of tryptanthrin to Kunming mice, the analyte was well distributed to the plasma and main tissues. C_{max} was found in the liver with a mean value of 3.54 μg/g, followed by that in the kidney, lung, spleen, heart, and brain.

Conclusion: In this study, a validated RP-HPLC–UV method was developed and successfully applied to PK and tissue distribution of oral tryptanthrin in mice. We confirmed that tryptanthrin was closely related and targeted to plasma, liver, kidney, and lung. These results indicate that tryptanthrin will have a good clinical application in the liver, kidney, or lung. The clinical use of tryptanthrin should focus on its pharmacodynamics and safety study in these tissues.

1. Introduction

Tryptanthrin as a natural indole quinazoline alkaloid, 6,12-dihydro-6,12-dioxoindolo-[2,1-*b*]-quinazoline, originally isolated from Chinese herbal medicines *Isatidis radix*, *Baphicacanthis Cusiae Rhizoma et Radix*, reportedly has a wide range of pharmacological effects and diverse pharmacological mechanistic pathways without potential cytotoxicity,[1–3] such as antitumor,[4–8] anti-inflammatory[9–11] and antibacterial[12–14] effects in modern pharmacological studies. Its antileukemic activity is considered its most important clinical application. Tryptanthrin has proliferation-attenuating and apoptosis-inducing effects on human chronic myeloid leukemia cells, exerting its antitumor effect on the murine myelomonocytic leukemia cells by causing cell cycle arrest and by triggering cell differentiation.[8] Tryptanthrin as a highly selective cyclooxygenase-2 inhibitor showed inhibitory activity on prostaglandin and leukotriene synthesis, and reduced leukotriene B4 levels with a potency comparable to that of the clinically used 5-lipoxygenase inhibitor[10,11] and so on. Because of its very low content in plants, less than one ten-thousandth (mass/crude drug mass),[2,15] and the fact that it is difficult, laborious, time-consuming, and inefficient in separation and purification of such complex natural samples, targeted synthetic methods were preferred to obtain this potential drug through microbial fermentation,[16] biomimetic synthesis[17] and chemical synthetic methods.[18–20] The pharmaceutical analysis method, an important tool for tryptanthrin drug development and clinical drug evaluation, can timely reflect the problems arising in the drug production process. Using validated analysis methods, drug pharmacokinetics (PK) and tissue distribution can be carried out in the preclinical experimental research stage; these were an effective tool to reduce the failure rate of drug development. Although there were researchers who reported the use of these methods for tryptanthrin, such as a LC-UV method with cinnamaldehyde as internal standard (IS) to determine tryptanthrin in rats,[21,22] or 2-hydroxy acetophenone as IS in rats,[23] no relevant studies of tryptanthrin have been reported in mice. Moreover, the disposition process of tryptanthrin in mice was unknown until now. In this study, a new sensitive and simple reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC–UV) method using 4(3*H*)-quinazolinone as IS, which is most similar to the structure of the analyte, was first established to determine tryptanthrin in plasma and main tissues of Kunming (KM) mice following oral administration. These results are projected to guide subsequent clinical trials of tryptanthrin and its new drug development work.

2. Methods

2.1. Instruments

The LC-100 chromatographic system (WuFeng HPLC, Shanghai, China) consisted of two LC-P100plus pumps, an LC-UV100 plus UV detector and an LC-CO100 column oven. Peak areas were integrated automatically using the WuFeng LC-WS100 chromatographic workstation. Other apparatuses included an SB-5200D ultrasonic device (Scientz, Ningbo, China), a TGL-16G high-speed centrifuge (Anting Scientific, Shanghai, China), an AY-120 electronic balance (Shimadzu, Tokyo, Japan), an SK-1 vortex mixer (JinTan, Jiangsu, China) and a YAZD-5L double-distilled water preparation system (HengXing, Shangyu, China).

2.2. Chemicals and reagents

Tryptanthrin oral solution (made in our laboratory, lot. no. 20150712), tryptanthrin reference substance (>98%, RN 13220-57-0, provided by the College of Life Sciences, Northwest University, Xi'an, China); 4(3*H*)-quinazolinone (98%; RN 491-36-1, provided by JiuDing, Shanghai, China), heparin sodium (lot. no. 425C0215; Solarbio, Beijing, China), 0.9% sodium chloride injection (lot. no. D15031506, Guizhou KeLun Pharmaceutical Industry Ltd Co., Guizhou Sheng, China), methanol (HPLC-pure; TEDIA Co. Inc. Shanghai, China). The other reagents were analytical grade, double distilled in water.

2.3. Animals

Male♂ KM mice, weighing (18–25) g, were obtained from the Experimental Animal Center of Guizhou Medical University (Guiyang City, Guizhou Province, China), Permit No. SYXK