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Determination and pharmacokinetic study of indirubin in rat plasma by high-performance liquid chromatography

X.Y. Deng, S.N. Zheng, G.H. Gao, G. Fan, F. Li*

School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China

Abstract

A specific, simple and sensitive HPLC method with UV detection was developed and validated for the pharmacokinetic studies of indirubin in rat plasma for the first time. Indirubin, with osthole as the internal standard, was extracted from plasma samples by liquid–liquid extraction. Chromatographic separation was conducted on a reverse-phase ODS column (200 mm × 4.6 mm, i.d., 5 μm), using a mixture of methanol–water (75:25, v/v) as the mobile phase at a flow rate of 1.0 ml/min with UV detection at 289 nm. The calibration curve of indirubin was linear over the range of 6.5–1950 ng/ml in rat plasma. The lower limit of quantification (LLOQ) was found to be 6.5 ng/ml. The present method was successfully applied for estimating the pharmacokinetic parameters of indirubin following intravenous and intraperitoneal administration of indirubin to rats.

Keywords: Indirubin; Pharmacokinetics; High-performance liquid chromatography; Rat plasma

Introduction

Indirubin, a 3,2′-bisindole isomer of indigo, exists in several medicinal plants such as *Baphicacanthus cusia* (Nees) Bremek., *Polygonum tinctorium* Ait. and *Isatis indigotica* Fort. Since this red-colored crystal was discovered as a compound with antileukemic properties by Chinese researchers in the Traditional Chinese Medicine Danggui Longhui Wan, which was effective against chronic granulocytic leukemia (Bradbury, 2005). Elucidation of its pharmacological efficacy and mechanism has kept drawing researcher's attention. It has been shown that indirubin inhibits cell growth and induces differentiation and apoptosis of leukemic cells (Wu and Fang, 1980; Suzuki et al., 2005; Kasahara, 2005; Sethi et al., 2006). Besides the antiproliferative effect, indirubin possesses also anti-inflammatory effects by inhibiting the production of interferon-gamma (Kunikata et al., 2000). It has been proved that indirubin and its analogs can bind to and inhibit glycogen synthase kinase, rabbit muscle glycogen phosphorylase b and aryl hydrocarbon receptor (Suzuki et al., 2001; Kosmopoulou et al., 2004; Adachi et al., 2001). Cyclin-dependent kinases (CDKs) are key regulators of the cell-division cycle, and their frequent deregulation in human tumors makes them attractive targets for the identification of new antineoplastic agents (Buolamwini, 2000). Indirubin was identified to represent a novel structure with potent inhibitory property towards CDKs due to its high affinity with the kinase's ATP-binding site (Hoessel et al., 1999; Jautelat et al., 2005). Since indirubin and its analogs hold strong promise for clinical anticancer activity and also might be used against other diseases, the *in vivo* pharmacokinetic study of indirubin is of great significance.

Several methods including TLC, HPLC/UV and HPLC/MS have been described for the identification

*Corresponding author. Tel./fax: +86 24 2398 6289.
E-mail address: lifamei@syphu.edu.cn (F. Li).

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and determination of indirubin in herbal extract and Chinese medicinal compound preparations (Zhao, 1981; Dai et al., 1986; Zou and Koh, 2007; Liau et al., 2007). However, to our knowledge, in contrast with the in-depth pharmacological mechanism study of indirubin and its analogs, there is no report on HPLC (high-performance liquid chromatography) method for the determination of indirubin in plasma. The only one report on the pharmacokinetics of indirubin involved the determination of specific radioactivity of tritium-labeled indirubin in mouse plasma (Qi et al., 1981). Due to lack of specificity of the method, the results cannot reflect the real process of parent molecule of indirubin *in vivo* since both indirubin and its metabolites contain radioactive isotope tritium, which limits the comprehension of systemic indirubin exposure and its activity. It is therefore necessary to develop an analytical method to evaluate systemic indirubin exposure. In this paper, we described an HPLC method for the determination of indirubin in rat plasma and applied it successfully to pharmacokinetic study in rat after intravenous and intraperitoneal administration of indirubin for the first time.

Materials and methods

Chemicals and reagents

The internal standard (IS), osthole, was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of indirubin and osthole are given in Fig. 1. Methanol of HPLC grade was obtained from Jiangsu Hanbon Sci. & Tech. Co., Ltd. (Jiangsu, China). All other chemicals were of analytical reagent grade purchased from Concord Technology Co. (Tianjin, China). Double distilled water was used for all the preparations.

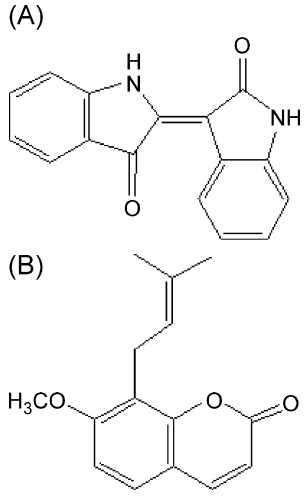

Fig. 1. Chemical structures of indirubin (A) and osthole (B).

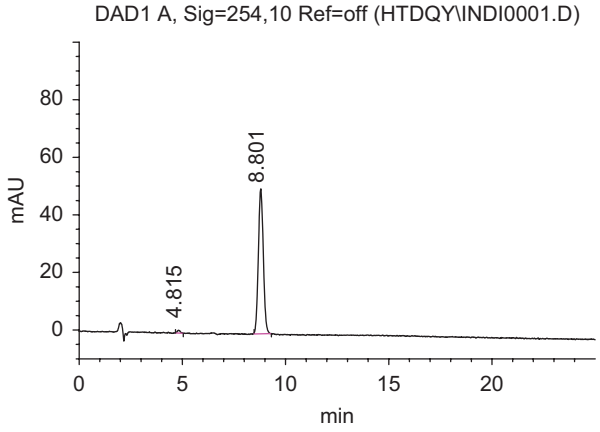

Fig. 2. HPLC chromatogram of indirubin.

Indirubin was isolated from the leaves of *I. indigotica* Fort. in our laboratory. The structure of indirubin (Fig. 1A) was fully characterized by ^{1}H NMR (300 MHz, DMSO) δ: 11.02 (1H, s, NH), 10.89 (1H, s, NH), 8.77 (1H, d, $J = 7.5$ Hz, H-4′), 7.66 (1H, d, $J = 7.5$Hz, H-4), 7.57 (1H, t, $J = 7.5$, 8.0 Hz, H-6′), 7.42 (1H, d, $J = 8.0$ Hz, H-7′), 7.25 (1H, t, $J = 7.5$, 7.8 Hz, H-6), 7.02 (2H, t, $J = 7.5$ Hz, H-5,5′), 6.91 (1H, d, $J = 7.6$ Hz, H-7) and ESI-MS m/z 261 $[M-H]^-$ compared with the data in literature (Ruan et al., 2005). The purity of indirubin determined by HPLC (Fig. 2) was 98.8%.

Chromatographic conditions

The analysis was performed on a Shimadzu HPLC system (Kyoto, Japan), consisting of an LC-10AT pump and an SPD-10A VP UV–vis spectrophotometric detector. Chromatographic data were recorded and processed using a Shimadzu Chromato-Solution Light workstation (Kyoto, Japan). The analytes were separated on a Zirchrom Kromasil ODS column (200 mm × 4.6 mm, i.d., 5 μm, Techcomp Ltd., Shanghai, China) protected by a guard column (Phenomenex SecurityGuard^{TM}, ODS, 4 mm × 3.0 mm, Cheshire, UK). The mobile phase was composed of a mixture of methanol–water (75:25, v/v). The analysis was carried out at a flow rate of 1 ml/min with the detection wavelength set at 289 nm at room temperature.

Preparation of standard and quality control samples

Stock solutions of indirubin (26 μg/ml) and IS (40 μg/ml) were prepared in methanol and stored at 4 °C. Stock solution of indirubin was diluted quantitatively with methanol to give working standards. The IS solution was diluted with methanol to prepare the working IS solution containing 4 μg/ml of IS. Indirubin calibration standards were prepared at concentrations of 6.5, 32.5,