# A pharmacokinetic study of Isatin in Beagles' bodies

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Received November 19, 2015; Accepted April 6, 2016

DOI: 10.3892/etm.2016.3232

**Abstract.** Isatin are marine active drugs that exert anti-cancer effects, have a cancer-prevention function, and possess many pharmacological activities. The study aimed to examine the pharmacokinetics of a single intravenous injection and oral medication of Isatin given to Beagles. Nine male and nine female Beagles were injected with 30 mg/kg of 2,3-indole quinones. The animals were divided into 3 groups (n=6 per group) and lavaged with a dose of 15, 30 and 60 mg/kg, respectively. Blood samples were collected prior to the medicine delivery (0 h) and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post-medicine delivery. The blood plasma samples were analyzed using the liquid chromatography-mass spectrometry (MS)/MS method following pretreatment for the protein precipitation. Pharmacokinetics software was applied to calculate relevant pharmacokinetic parameters through the atrioventricular model. The drug concentration in plasma decreased rapidly following the intravenous injection of Isatin. After 8 h, the prototype drugs could not be tested in the plasma and only trace amounts of drugs were tested in one dog, which was considered to be an endogenous drug. Indole quinone was absorbed following lavage into Beagles and peaked in <1 h, and the drug concentration in the plasma decreased rapidly. After 8 h, the prototype drugs could not be tested in the plasma. The elimination of the two drugs in the body had no evident gender differences. In conclusion, Isatin is rapidly absorbed in bodies of Beagles. Within the dose range of 15-60 mg/kg, no linear relationship was observed for the increase in Cmax and $AUC_{0-t}$ values with the increased dose.

## Introduction

Isatin is a type of marine active drug exerting anti-cancer effects, with a cancer-prevention function, and is an endogenous substance in human bodies, which possesses pharmacological activities (1-3), such as nerve protection, antibacterial and antivirus activities. Through the synthesis of a large number of materials, we aimed to identify a new drug and conducted relevant investigations (4,5), funded by the National Major Projects for New Drugs Innovation.

To design an improved drug dose and administration regimen, based on earlier studies of the lavage and dose of rats, pharmacokinetic studies were conducted following oral and intravenous injection of Isatin given to Beagles.

## Materials and methods

*Drugs and reagents*. Isatin was purchased from Shanghai Xin Sheng Yuan Biological Pharmaceutical Co., Ltd. (Shanghai, China) and served as a control. Internal standard, quetiapine, was purchased from Maddie Xipuya Medical Technology Co., Ltd. (Shanghai, China). Methanol of HPLC grade was purchased from Burdick and Jackson (Morristown, NJ, USA). Formic acid of HPLC grade and ultrapure water were purchased Acros Organics (Geel, Belgium).

*Instruments and equipment*. Liquid chromatography (LC) instrument (Agilent 1200) and mass spectrometer (6410B) were purchased from Agilent Technologies, Inc. (Santa Clara, CA, USA), and the electrospray ion source and tandem quadrupole mass analyzer were purchased from Zhejiang Haochuang Biotechnology Co. (Hangzhou, China). The data processing system was MassHunter software (Agilent Technologies, Inc.). The following instruments were also purchased: Vortex-Genie 2, a vortex generator (Scientific Industries, Inc., Bohemia, NY, USA); a small desktop high-speed refrigerated centrifuge (5417R; Eppendorf, Hamburg, Germany); a trace analytical balance [XP26; Mettler-Toledo Instrument (Shanghai) Co., Ltd., Shanghai, China]; and an ultrapure water machine (Millipore Corp., Billerica, MA, USA). The chromatographic column used was Venusil XBP PH, 5 $\mu$m, 100x2.0 mm.

*Experimental animals*. Nine male and nine female Beagles, weighing 7.80-9.60 kg were purchased from Beijing Thomas Biotechnology Co. Ltd. (Beijing, China), license no.: SCXK (Beijing) 2010-0003.

*Experimental methods*

*Solution preparation*. A suitable amount of Isatin (10 g) was weighed and the required concentration was compounded

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*Key words:* 2,3-indole quinine, Beagles, pharmacokinetics, intravenous injection, oral medication

Table I. The pharmacokinetic parameters after intravenous injection of Isatin to Beagles.

| Animals | $AUC_{0-t}$, μg*h/l | $AUC_{0-\infty}$, μg*h/l | MRT, h | $T_{1/2}$, h | CL, l/h/kg | Vss, l/kg |
|---|---|---|---|---|---|---|
| Male | 2391±669 | 2418±675 | 1.05±0.26 | 0.89±0.24 | 6.57±1.98 | 7.98±0.34 |
| Female | 2260±446 | 2293±438 | 1.82±0.38 | 1.13±0.47 | 6.72±1.41 | 11.49±7.19 |
| Average | 2326±514 | 2356±513 | 1.44±0.98 | 1.01±0.36 | 6.64±1.54 | 9.73±4.94 |

$AUC_{0-t}$, area under the perindopril concentration-time curve; $AUC_{0-\infty}$, AUC zero to infinity; CL, clearance; MRT, mean residence time.

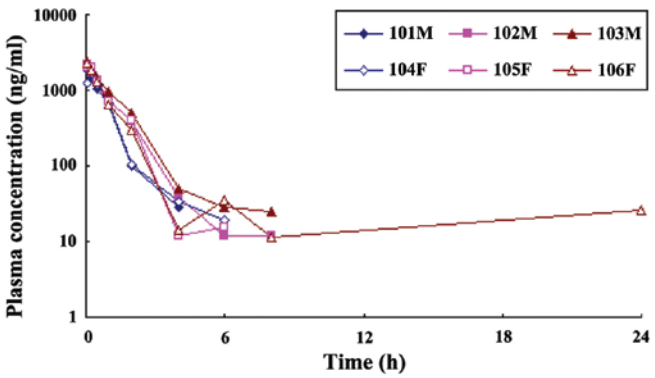


Figure 1. Blood Isatin concentration curve after injection of 15 mg/kg of drug was injected into Beagles' vein.

according to the dose and dose volume. West astragalus gum solution (1.25%) was compounded for the gavage, as well as 5% DMSO and 40% polyethylene glycol (both from Haian Petrochemical Co., Nantong, China), and 55% physiological saline (Shanghai Chemical Reagent Co., Shanghai, China) was compounded for intravenous injections.

*Drug delivery and sample collection*. Intravenous drug delivery was carried out in the Beagles. Briefly, 3 male and 3 female healthy beagles were injected with 15 mg/kg of Isatin through the saphenous vein at a dosing volume of 1.5 ml/kg. Blood (1 ml) was taken from the jugular vein prior to adminstration of test substances (0 h) and after 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h, in the K2EDTA tube and kept on ice. Blood samples were centrifuged at 1,900 x g for 10 min at 4˚C. Plasma was collected and stored at -80˚C until analysis.

Intragastric adminstration of Beagles was subsequently carried out. Briefly, 3 male and 3 female dogs in each dose group, were fasted for 14-18 h, albeit drinking water was provided *ad libitum* prior to drug delivery. The animals were lavaged with Isatin at doses of 15, 30 and 60 mg/kg, respectively at a dosing volume of 2 ml/kg. Blood (1 ml) was taken from the jugular vein prior to adminstration of test substances (0 h) and after 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h in the K2EDTA tube and kept on ice Blood samples were centrifuged at 1,900 x g for 10 min at 4˚C, and plasma was separated and stored at -80˚C prior to analysis.

*Measurement methods of plasma samples*. The LC-mass spectrometry (MS)/MS method was used to measure the concentration of Isatin in the plasma at different time points

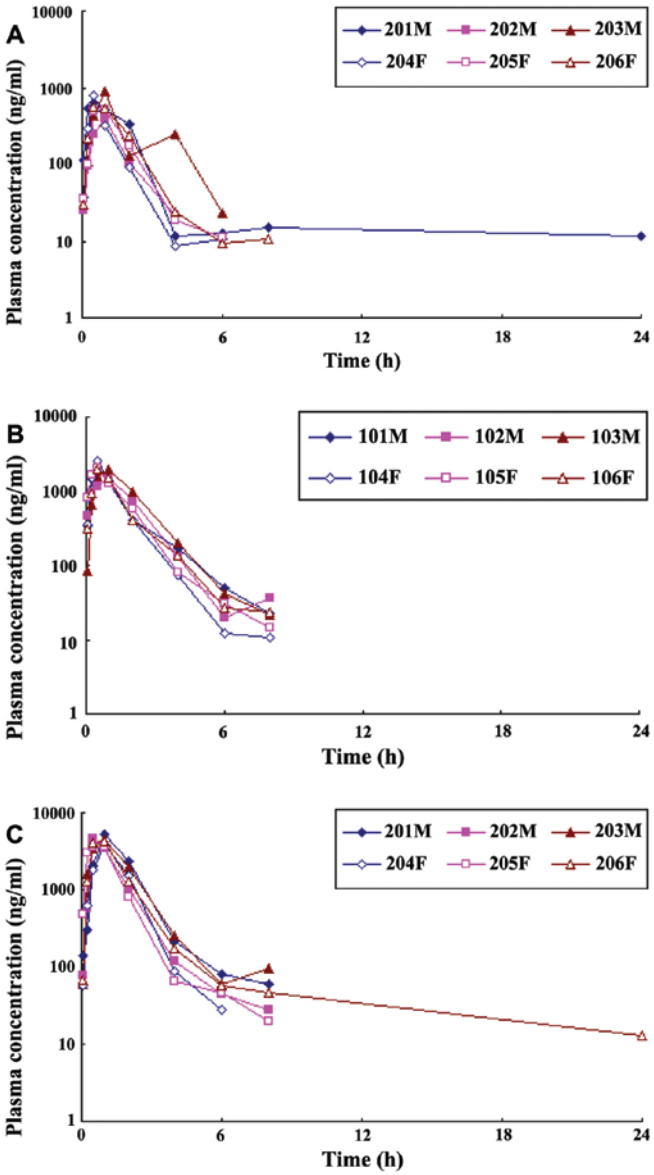


Figure 2. Blood concentration-time curve after Isatin was gavaged into Beagles at a dose of (A) 15, (B) 30 and (C) 60 mg/kg.

after drug injection. The required conditions for the measurement of Isatin in the plasma were similar to those reported in rats earlier (6), and were used in blank dog plasma to validate the methodology. The specificity, sensitivity, linearity, rate of extraction recovery, precision in the day or between days, stability, and the matrix effect of the analytical method was

Table II. The pharmacokinetic parameters after different dosages of Isatin are lavaged into Beagles.

| Pharmacokinetic parameters | Animals | Beagles' drug dosage of lavage (mg/kg) | | |
|---|---|---|---|---|
| | | 15 | 30 | 60 |
| $T_{max}$, h | Male | 0.67±0.29 | 0.83±0.29 | 0.83±0.29 |
| | Female | 0.67±0.29 | 0.50±0.00 | 1.00±0.00 |
| | Average | 0.67±0.26 | 0.67±0.26 | 0.92±0.20 |
| $C_{max}$, μg/l | Male | 634±253 | 1,902±357 | 4,812±412 |
| | Female | 619±152 | 2,213±347 | 3,891±284 |
| | Average | 631±187 | 2,057±358 | 4,352±596 |
| $AUC_{0-t}$, μg*h/l | Male | 1,012±466 | 3,578±553 | 8,071±1464 |
| | Female | 922±161 | 3,184±128 | 6,748±927 |
| | Average | 967±316 | 3,381±419 | 7,409±1313 |
| $AUC_{0-\infty}$, μg*h/l | Male | 1,031±459 | 3,624±541 | 8,164±1509 |
| | Female | 937±163 | 3,212±128 | 6,793±923 |
| | Average | 984±313 | 3,418±418 | 7,479±1348 |
| $T_{1/2}$, h | Male | 0.71±0.10 | 1.19±0.19 | 1.04±0.09 |
| | Female | 0.95±0.15 | 1.20±0.36 | 1.70±0.89 |
| | Average | 0.83±0.18 | 1.19±0.26 | 1.37±0.67 |
| MRT, h | Male | 2.06±1.23 | 1.69±0.07 | 1.58±0.2 |
| | Female | 1.40±0.23 | 1.37±0.17 | 1.61±0.47 |
| | Average | 1.73±0.87 | 1.53±0.21 | 1.60±0.32 |

$AUC_{0-t}$, area under the perindopril concentration-time curve; $AUC_{0-\infty}$, AUC zero to infinity; MRT, mean residence time.

confirmed to the relevant provisions of the biological sample analysis worldwide (7-9).

*Data analysis*. The pharmacokinetic parameters of Isatin were analyzed and processed by the atrioventricular model of WinNonlin5.2 software (Pharsight Corporation, Mountain View, CA, USA). The experimental data were presented as mean ± standard deviation).

## Results

*Blood concentration as well as concentration and time curve*. After 15 mg/kg Isatin (n=6) was injected into the Beagles' vein, the association between blood concentration of Isatin and time were measured (Fig. 1). The drug concentration in the plasma decreased rapidly after the intravenous injection of Isatin. After 8 h, the prototype drugs could not be tested in the plasma and only trace amounts of drugs were tested in one dog, which was considered an endogenous drug. Elimination of the drug in the body had no obvious gender difference. WinNonlin pharmacokinetic software was used to process the plasma concentration of Isatin using an atrioventricular model following drug administration in dogs and the pharmacokinetic parameters after fitting (Fig. 1).

The curve of blood Isatin concentration and time are shown in Fig. 2 after three different doses of Isatin were respectively lavaged into the Beagles. The blood Isatin concentration peaked within 1 h, and then decreased rapidly. After 8 h, the prototype drugs could not be tested in the plasma.

*Pharmacokinetic parameters*. An atrioventricular model was used to calculate the pharmacokinetic parameters (Table II). Indole quinone was rapidly absorbed following lavage into Beagles and peaked in <1 h, while the drug concentration in the plasma decreased rapidly. After 8 h, the prototype drugs could not be tested in the plasma. Elimination of the drugs in the body had no evident gender differences.

## Discussion

As an endogenous component, indole quinone exists widely in human and animal bodies (2). The current results showed that the plasma concentration of indole quinone in the majority of the Beagles was relatively high but extremely low in certain Beagles, and could not be tested. The plasma of the Beagles was initially tested, followed by methodology validation and pharmacokinetic examination to select the Beagles whose blood concentration was lower than the minimum quantitative limit 1/10. After 8 h of intravenous injection or intragastric administration, the prototype indole quinones could not be tested in the plasma of most of the Beagles, with the exception of some dogs, and this was considered an endogenous drug.

The clearance of Isatin in dogs was 6.64±1.54 l/h/kg, which is 6.64-fold that of the canine liver plasma flow (approximately 1.0 l/h/kg) (10). To calculate the average value of $AUC_{0-\infty}$, the absolute bioavailability of 15 mg/kg Isatin given to lavage Beagles was 41.77%, which was lower than the bioavailability (57.75%) of rats, and could therefore be used as oral medicine (6). However, additional experiments are required to

determine whether the drugs were likely to have renal excretion or liver metabolism.

**Acknowledgements**

This study was supported by the Natural Science Foundation of Shandong Province (no. ZR2013HM065) and the Science and Technology Bureau of Qingdao (no. 11-2-4-2-(4)-jch).

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