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Bioavailabilty and pharmacokinetics of four active alkaloids of traditional Chinese medicine Yanhuanglian in rats following intravenous and oral administration

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Abstract

Corydalis saxicola Bunting (Yanhuanglian) is an important component in various prescriptions in traditional Chinese medicine. Yanhuanglian has been demonstrated to possess many pharmacological activities, including antibacterial, antiviral and anticancer activities. The active fractions are dehydrocavidine, coptisine, dehydroapocavidine and tetradehydroscoulerine. The purpose of the present study was to examine in vivo pharmacokinetics and tissue distribution in rats by using high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry. Systemic clearance of the four active alkaloids in plasma was over 93% of hepatic blood flow, indicating they may be quickly eliminated via hepatic clearance. Less than 10% drugs was excreted via urine following intravenous and oral administration, suggesting that these four alkaloids may undergo significant metabolism in the body or the drug may be excreted via other routes other than urine. There was significantly lower excretion of these four alkaloids following oral than intravenous administration, suggesting a significant first pass effect after oral administration. There appeared to be wide distribution of those four alkaloids in rats as demonstrated by the higher apparent volume of distribution. Our results have also demonstrated that the four alkaloids can be absorbed following oral administration although there were less than 15% of drugs absorbed into systemic circulation. In summary, the favorable oral bioavailability properties of those four active alkaloids in rats make Yanhuanglian extract worth further investigation for improving oral bioavailability.

Keywords: *Corydalis saxicola* Bunting (Yanhuanglian); Pharmacokinetics; HPLC/MS/MS

1. Introduction

Hepatitis B virus (HBV) is known to cause acute hepatitis, chronic hepatitis, fulminant hepatitis, and has been linked to hepatocelluar carcinoma (HCC). Treatment of chronic hepatitis B with interferon, antiviral agents and immunomodulatory drugs has been employed, either alone or in combination. However, there is still an urgent need to search for even more effective drugs.

In China, and other Asian countries, the use of medicinal plants is commonplace for the treatment of hepatic disease such as hepatitis. Among these plants, *Corydalis saxicola* Bunting (Yanhuanglian) is used. Yanhuanglian grows in south China and is an important component in various prescriptions in traditional Chinese medicine. Yanhuanglian has been demonstrated to possess many pharmacological activities, including antibacterial, antiviral and anticancer activities. Clinically, Yanhuanglian has been reported to protect hepatic tissues from hepatitis B virus and hepatitis A viral damage. Moreover, Yanhuanglian can also be used for alleviating fever, detoxification and as a painkiller [1–5].

The isolation and purification of the methanol extract afforded mainly four alkaloids of quaternary ammonium protoberberine type: dehydrocavidine, coptisine, dehydroapocavidine and tetradehydroscoulerine, which are named as YHL-I, YHL-II, YHL-III and YHL-IV (for chemical structures see Fig. 1),

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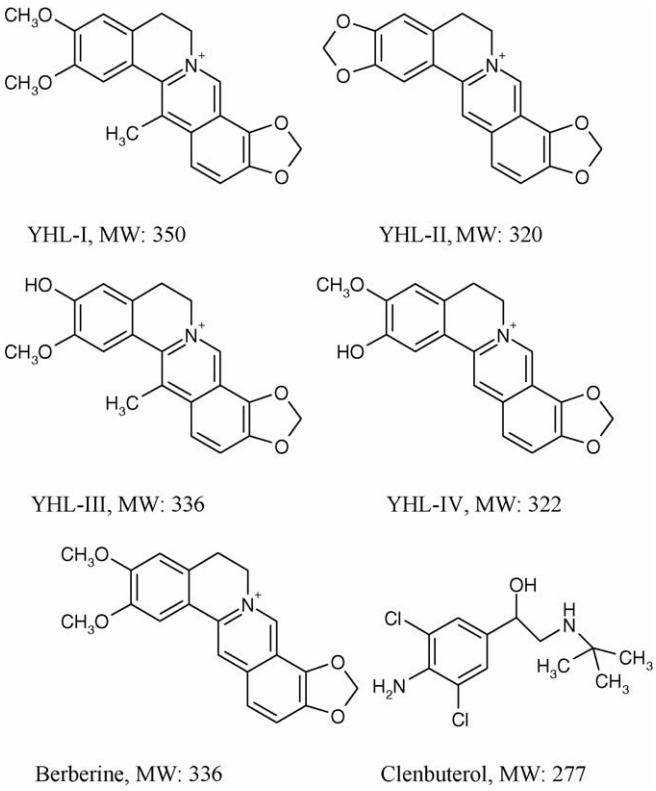

Fig. 1. Chemical structures of dehydrocavidine (YHL-I), coptisine (YHL-II), dehydroapocavidine (YHL-III), and tetradehydroscoulerine (YHL-IV) extracted from the Chinese medical herb Yanhuanglian (*Corydalis thalictrifolia* Franch). Berberine was the internal standard for plasma analysis; clenbuterol was the internal standard for urine analysis.

respectively. In addition, further studies showed that the active fraction contained 40% YHL-I, 15% YHL-II, 40% YHL-III and 5% YHL-IV by HPLC-UV analysis.

There is merit in characterizing the pharmacokinetics of four active alkaloids from Yanhuanglian extract in animals, since to our knowledge there was no detailed pharmacokinetic profiles for simultaneous characterization of these alkaloids. Recently, we have developed an HPLC coupled with tandem mass spectrometry method for simultaneously quantitating the four active alkaloids in plasma and urine [6]. By using this method, we evaluated in detail the in vivo pharmacokinetics, bioavailability and excretion via urine in rats following intravenous and oral administration.

2. Materials and methods

2.1. Plant material

The herb of *C. saxicola* was collected in Jinchengjiang, Guangxi Province, PR China, in July of 2003, and authenticated by Prof. Hanchen Zheng, Department of Pharmacognosy, Second Military Medical University. The voucher specimens (collection No. 188) are deposited at Herbarium of School of Pharmacy, Second Military Medical University, Shanghai, China.

2.2. Preparation of the total alkaloids

The dried and powdered herb of *C. saxicola* (1 kg) was extracted with 20 L ethanol by infiltration. The solvent was evaporated under vacuum to afford 90 g crude extract, which was suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate and water-saturated *n*-butanol successively. The *n*-butanol partition (15 g) was dissolved in hot water and filtered through a syringe filter (0.45 μm). The filtrate afforded a yellow powder after concentration, which was purified by recrystallization in ethanol to give the total alkaloids (8 g).

2.3. Chemicals and drug

Methanol and acetonitrile (HPLC grade) were obtained from TEDIA Company (Tedia Fairfield, OH, USA). Formic acid, 99%, was purchased from ACROS Organics (New Jersey, USA). All other reagents were of analytical purity.

Dehydrocavidine (YHL-I), coptisine (YHL-II), dehydroapocavidine (YHL-III), and tetradehydroscoulerine (YHL-IV) (lot No. 050201, 050202, 050203, and 050205, respectively) were isolated and purified from the total alkaloids by silica gel column chromatography eluted with chloroform/methanol (5:1) as eluent. The purified compounds were kept at 4 °C. Their purity was determined to be 98.9%, 99.2%, 99.0%, 99.4% and 99.3%, respectively by HPLC analysis. The chemical structures of the four alkaloids were identified based on spectral analysis (^{1}H NMR, ^{13}C NMR, 2D NMR, MS, UV and IR) and by comparison their spectral data with those reported previously in the literatures [1,7,8,9]. Internal standard berberine (lot No. 0015-9706) and clenbuterol (lot No. 0015-9706) were purchased from Shanghai Institute of Drug Control (Shanghai, China). Distilled de-ionized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

2.4. LC/MS/MS quantitation of four alkaloids in plasma and urine

The quantitation of the four alkaloids used the method was published in our previous paper [6]. The method was validated in the concentration range 1–1000 ng/ml in plasma and 10–1000 ng/ml in urine for the four test compounds, and the calibration curves were linear with coefficients of correlation >0.999. The lowest limits of quantitation for four substances were 1 ng/ml in 0.1 ml rat plasma and 10 ng/ml in 0.1 ml urine. The intra-assay accuracy and precision in plasma for YHL-I, YHL-II, YHL-III and YHL-IV ranged from 88.1% to 115.7% and 2.3% to 10.8%, respectively, while inter-assay accuracy and precision for YHL-I, YHL-II, YHL-III and YHL-IV ranged from 96.5% to 113.3% and 0.4% to 16.9%, respectively. The intra-assay accuracy and precision for YHL-I, YHL-II, YHL-III and YHL-IV in rat urine ranged from 96.1% to 112.9% and 1.2% to 8.3%, respectively, while inter-assay accuracy and precision in urine for YHL-I, YHL-II, YHL-III and YHL-IV ranged from 95.0% to 106.8% and 2.2% to 10.3%, respectively.

The HPLC system (Agilent 1100, Böblingen, Germany) consisted of a quaternary pump, an autosampler, a degasser, an

automatic thermostatic column compartment and a computer with a Chemstation software (Analyst 1.4, Applied Biosystems Inc., USA). The analytical column used was a Luna 5μ C18 (2) 100A reversed-phase column (5 μm, 100 mm × 2 mm, Phenomenex Inc., Torrance, CA, USA) and an Agilent Zorbax SB-C18 guard column (5 μm, 20 mm × 4 mm). The mobile phase was a mixture of methanol and water containing 0.1% formic acid (75:25 v/v). The mobile phase was degassed automatically using the electronic degasser system. The column was equilibrated and eluted under isocratic conditions utilizing a flow rate of 0.3 ml/min at 25 °C.

Mass detection was carried out using a triple quadruple mass spectrometer with TurboIonSpray (MDS Sciex Inc., Toronto, Canada), which is connected to the liquid chromatography system. High-purity nitrogen was provided by a liquid nitrogen tank.

2.5. Drug administration and sampling

Sixteen male Sprague–Dawley rats (180–220 g) were provided by Shanghai SLAC Lab Animal Co., Ltd. (Shanghai, China) and housed four to a cage with unlimited access to food and water except for 12 h before and during the experiment. The animals were maintained on a 12 h light–dark cycle (light on from 8:00 to 20:00 h) at ambient temperature (22–24 °C) and at 60% relative humidity. Animal studies were approved by the Second Military Medical University Animal Ethics Committee (Shanghai, China).

In order to determine the pharmacokinetics of the four compounds in rat after intravenous or oral administration, the animals were deprived of food but free access to water for 12 h before and during the experiment. Total Yanhuanglian extract was dissolved in 0.9% saline immediately before oral or intravenous administration and the injected volume was adjusted to 0.5 ml/100 g for rats. Both intravenous and oral bolus dose was 10 mg/kg. The plasma samples (0.3 ml) were withdrawn from carotid vein using cannulation at 0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 360, 480 min after intravenous administration and at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240 min after oral administration. The plasma samples were placed in heparinized tubes and were separated following centrifugation at 3000 g for 5 min and stored at −20 °C until analysis. Another eight rats were used to collect the urine samples: The urine samples were collected during the following time range 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–48, 48–96 h after intravenous administration and after oral administration. The actual volume of each urine sample was recorded and the samples were stored 4 °C until analysis.

2.6. Pharmacokinetic and statistical analysis

Data fitting and pharmacokinetic parameter calculations were carried out using the DAS pharmacokinetic program (Chinese Pharmacology Society). An appropriate pharmacokinetic model was chosen on the lowest Akaike's information criterion (AIC) value, lowest weighted squared residuals, lowest standard errors of the fitting parameters, and dispersion of the residual under equal weight scheme [10–14]. The area under the curve (AUC) was calculated by the trapezoidal rule between first (0 h) and last sampling time plus C_n/λ_n, where C_n is the concentration of last sampling, and λ_n is the elimination rate constant. $AUC_{0\to t} = \Sigma(C_i + C_{i-1}) \times (t_i - t_{i-1})/2$; $AUC_{0\to\infty} = AUC_{0\to t} + C_n/\lambda_n$. Bioavailability was calculated according to the equation:

$$\text{bioavailability} = AUC_{0\to\infty}(\text{PO})/AUC_{0\to\infty}(\text{IV})$$

3. Results and discussion

3.1. Pharmacokinetics of YHL-I, II, III, IV in rats following intravenous administration

Following intravenous administration, plasma drug concentration–time profiles can be best described as a two compartmental model (Fig. 2A). The pharmacokinetic parameter values are summarized in Table 1. YHL-I, II, III, IV were quickly eliminated with systemic clearance of 0.10 ± 0.02, 0.08 ± 0.03, 0.06 ± 0.01, and 0.05 ± 0.01 l/min/kg, which is about 180%, 147%, 111% and 93% of hepatic blood flow

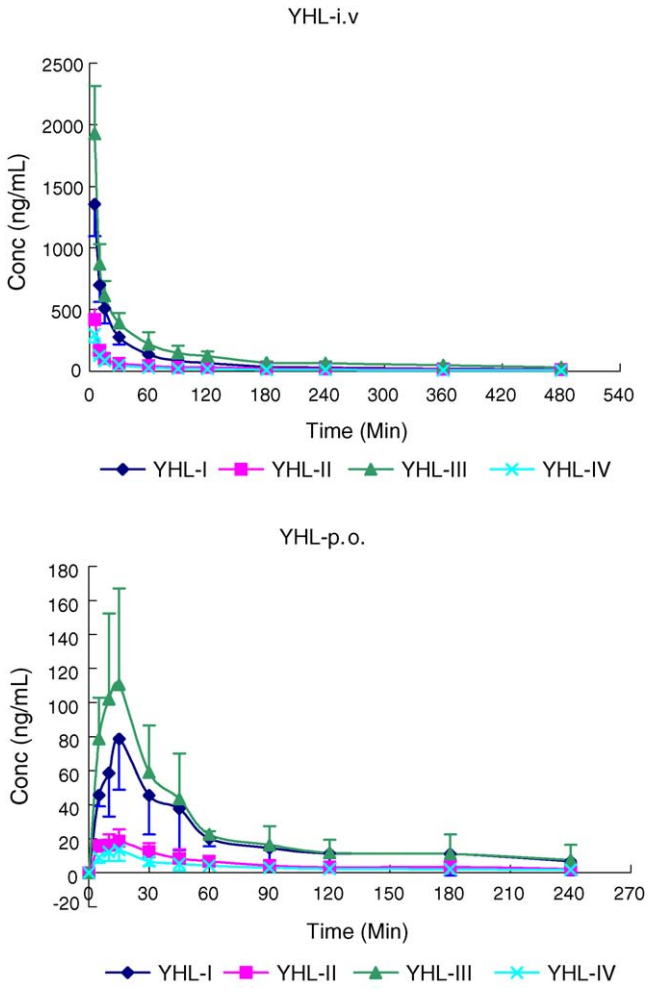

Fig. 2. Plasma concentration–time profiles of YHL-I, YHL-II, YHL-III and YHL-IV following intravenous bolus injection (A) and oral administration (B) in rats at doses of 10 mg/kg.

Table 1
The pharmacokinetic parameters of the four alkaloids in rats following intravenous administration at dose of 10 mg/kg ($n = 4$)

	$t^{1/2\alpha}$ (min)	$t^{1/2\beta}$ (min)	Vd (l/kg)	V1 (l/kg)	CL (l/min/kg)	AUC_{0-480} (mg/l min)	$AUC_{0-\infty}$ (mg/l min)
YHL-I	16.9 ± 9.36	207 ± 27.6	27.8 ± 3.78	3.80 ± 1.33	0.10 ± 0.02	38.5 ± 8.38	42.1 ± 9.41
YHL-II	4.44 ± 1.14	288 ± 112	30.1 ± 9.38	2.11 ± 0.99	0.08 ± 0.03	14.6 ± 3.49	20.4 ± 6.54
YHL-III	8.38 ± 4.82	214 ± 104	16.9 ± 5.99	1.83 ± 0.92	0.06 ± 0.01	59.4 ± 11.8	68.0 ± 16.0
YHL-IV	7.93 ± 4.72	253 ± 170	15.6 ± 6.81	1.56 ± 0.88	0.05 ± 0.01	8.68 ± 1.60	10.1 ± 1.93

Table 2
The pharmacokinetic parameters of the four alkaloids in rats following oral administration at dose of 10 mg/kg ($n = 4$)

	$t^{1/2\alpha}$ (min)	$t^{1/2\beta}$ (min)	AUC_{0-240} (mg/l min)	$AUC_{0-\infty}$ (mg/l min)	Bioavailability (%)	C^{max} (ng/ml)	T^{max} (min)
YHL-I	14.2 ± 5.32	154 ± 94.51	4.61 ± 3.21	5.57 ± 4.57	13.2 ± 10.9	88.4 ± 29.8	15.0 ± 0
YHL-II	14.8 ± 8.09	309 ± 157.2	1.23 ± 0.790	1.47 ± 1.03	7.21 ± 5.06	19.0 ± 6.52	13.8 ± 2.5
YHL-III	10.3 ± 2.24	146 ± 101.88	6.06 ± 3.82	6.72 ± 4.29	9.88 ± 6.3	115 ± 52.2	13.8 ± 2.5
YHL-IV	10.1 ± 4.95	312 ± 278.71	0.849 ± 0.390	1.06 ± 0.549	10.5 ± 5.42	13.8 ± 5.72	13.8 ± 2.5

(0.055 l/min/kg in rats) [15], suggesting that those four active alkaloids were quickly cleared via hepatic clearance. The elimination half-life of YHL-I, II, III, IV was 206.56 ± 27.65, 287.55 ± 112.85, 213.74 ± 104.76, and 253.22 ± 170.24 min. The volume of distribution of YHL-I, YHL-II, YHL-III and YHL-IV at terminal phase was 27.84 ± 3.78, 30.07 ± 9.38, 16.89 ± 5.99, and 15.58 ± 6.81 l/kg, which were greater than total body water at 0.67 l/kg, suggesting that these four alkaloids may be widely distributed into extravascular systems (Table 1).

3.2. Pharmacokinetics of YHL-I, II, III, IV in rats following oral administration

The plasma concentrations for those four active alkaloids in plasma vs. time can be best described as two-compartmental model (Fig. 2B). The pharmacokinetic parameter values are summarized in Table 2. The terminal elimination half-life for YHL-I, YHL-II, YHL-III and YHL-IV was 154.86 ± 94.51, 307.80 ± 157.20, 146.05 ± 101.88, and 311.81 ± 278.71 min, respectively. The oral bioavailability for YHL-I, YHL-II, YHL-III and YHL-IV was 13.24 ± 10.86%, 7.21 ± 5.06%, 9.88 ± 6.3%, 10.47 ± 5.42%, respectively. The time to reach the maximum plasma drug concentration was 15.00 ± 0.00 min for YHL-I, 13.75 ± 2.5 min for YHL-II, 13.75 ± 2.5 min for YHL-III and 13.75 ± 2.5 min for YHL-IV.

3.3. Urine excretion in rats following intravenous and oral administration

The accumulation of the four alkaloids excreted in the urine after intravenous and oral administration of Yanhuanglian extract is presented in Fig. 3A. The percent of drugs excreted in the urine over the dose administered following intravenous and oral administration is presented in Table 3. The percent of drugs excreted in the urine over the dose administered was 8.30% for YHL-I, 5.16% for YHL-II, 8.90% for YHL-III and 9.76% for YHL-IV following intravenous administration. There were lower percentages of drugs excreted in the urine following oral administration than following intravenous administration; the

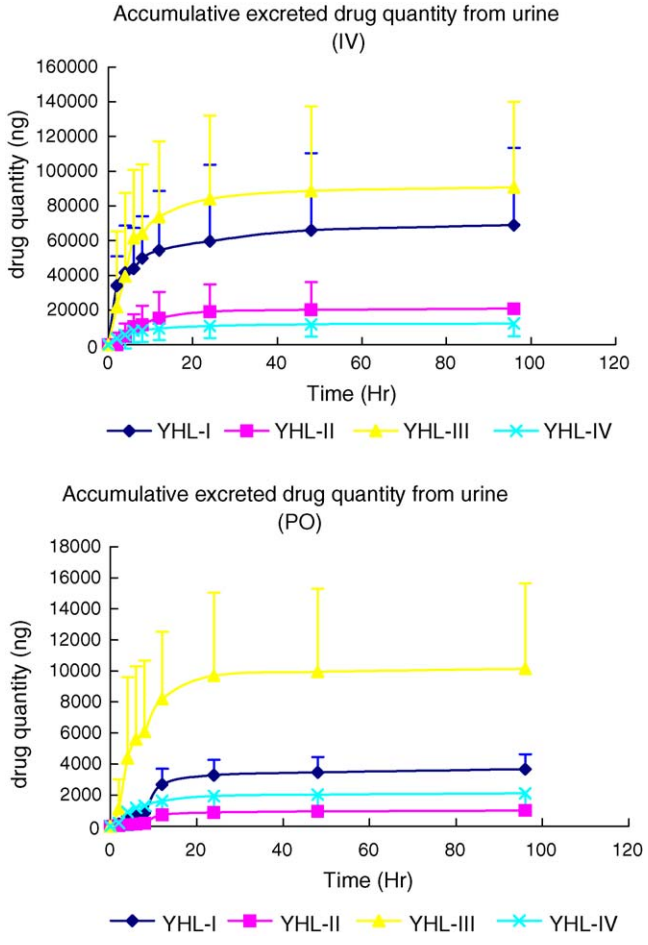

Fig. 3. The accumulation of the four alkaloids excreted in the urine after intravenous (A) and oral (B) administration of Yanhuanglian extract at dose 10 mg/kg.

Table 3
The percent of excreted drug from urine over the dose following intravenous and oral administration ($n = 4$)

	YHL-I (%)	YHL-II (%)	YHL-III (%)	YHL-IV (%)
IV	8.30 ± 3.00	5.16 ± 3.14	8.90 ± 4.53	9.76 ± 6.20
PO	0.35 ± 0.35	0.26 ± 0.24	1.06 ± 0.67	0.66 ± 0.46

percentage of drugs excreted in the urine over the dose administered was 0.35% for YHL-I, 0.26% for YHL-II, 1.06% for YHL-III and 0.66% for YHL-IV. The results show that these four alkaloids may undergo the first pass effects.

4. Conclusions

Systemic clearance of the four active alkaloids in plasma was over 93% of hepatic blood flow, indicating that they may be quickly eliminated via hepatic clearance. Less than 10% of the drugs was excreted via urine following intravenous and oral administration. This result demonstrated that these four alkaloids may undergo significant metabolism in the body or the drug may be excreted via routes other than urine. The results were supported by higher hepatic clearance for these four alkaloids. There was significantly lower excretion of the four alkaloids following oral than intravenous administration, suggesting a significant first pass effect after oral administration. The four alkaloids appeared to be widely distributed in rats as demonstrated by the higher apparent volume of distribution.

Our results have also demonstrated that four alkaloids can be absorbed following oral administration although there were less than 15% of drugs absorbed into systemic circulation. Our results further support efficacy results following oral administration. To our knowledge, this is the first study demonstrating that four alkaloids extracted from Yanhuanglian are orally available.

In summary, the favorable oral bioavailability properties of these four active alkaloids in rats make Yanhuanglian extract worth further investigation for improving oral bioavailability.

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