Pharmacokinetics of isoforskolin after administration via different routes in guinea pigs

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Abstract
1. The objective of this study was to characterize the pharmacokinetics of isoforskolin after oral, intraperitoneal and intravenous administration, as well as to compare bioavailability.
2. Isoforskolin was administered to guinea pigs at a dose of 2 mg/kg. Plasma concentrations were determined by high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) method. The pharmacokinetic parameters were calculated by a noncompartmental method. A compartment model was also adopted to describe the pharmacokinetic profiles.
3. The pharmacokinetic behavior of intravenously administered isoforskolin was characterized by rapid and extensive distribution ($V_z = 16.82 ± 8.42$ L/kg) followed by rapid elimination from the body ($Cl = 9.63 ± 4.21$ L/kg/h). After intraperitoneal administration, isoforskolin was absorbed rapidly ($T_{max} = 0.12 ± 0.05$ h). The pharmacokinetic profiles of isoforskolin were similar after intraperitoneal and intravenous administration, except for the concentrations at the initial sampling times. Isoforskolin was also absorbed rapidly following oral dosing; however, the concentration–time data were best fit to a one-compartment model, which was different from that observed after intravenous and intraperitoneal administration. Following intraperitoneal and oral administration, the absolute bioavailability of isoforskolin was 64.12% and 49.25%, respectively.
4. Isoforskolin is a good candidate for oral administration because of its good oral bioavailability.

Keywords
Bioavailability, guinea pigs, HPLC–ESI–MS/MS, isoforskolin, pharmacokinetics

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Introduction
Isoforskolin (Figure 1) is a labdane diterpene isolated from Coleus forskohlii Briq., a perennial herb distributed over the tropical and subtropical regions including India, Pakistan, Brazil and China (Alasbahi et al., 2010a; Wang et al., 2009). Isoforskolin is an analog of forskolin, which is the most extensively studied constituent of C. forskohlii (Kavitha et al., 2010). Forskolin and isoforskolin activate adenylate cyclase and increase intracellular cAMP levels, thus producing various biological effects, including lowering blood pressure, inhibiting platelet aggregation and blocking bronchospasm (Alasbahi et al., 2010b). Because the content of forskolin is extremely low in C. forskohlii originating in China (Yin et al., 2013), isoforskolin is generally considered to be the principal active ingredient obtained from such plants. Oral solutions and capsules containing medicines, including isoforskolin extract, have been used to treat asthma in China since the 1990s.

Assessment of the pharmacokinetic properties of a given drug is an important step in the process of designing rational dosage regimens, because such properties influence formulation, administration route, drug dose, dosing interval and treatment duration. Unfortunately, current knowledge of the pharmacokinetics of isoforskolin is very limited. In our previous work, we developed a high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) method to determine the plasma concentrations of isoforskolin and applied it to a pharmacokinetic study of isoforskolin in beagle dogs following intravenous bolus injection of the drug (Tian et al., 2011). Our 2011 study is the only report on the pharmacokinetics of isoforskolin; however, our results are insufficient to allow clinicians to formulate guidelines for the appropriate use of isoforskolin, because the drug is administered orally in clinical practice.

The objective of this study was to characterize the pharmacokinetics of isoforskolin after oral, intraperitoneal and intravenous bolus administration, as well as to compare...
the bioavailability of isoforskolin after administration via different routes.

Materials and methods

Chemicals and materials

Isoforskolin (purity ≥ 98.0%) was isolated in our laboratory. Eplerenone (purity ≥ 99.0%, Figure 1) was obtained from Changzhou Siyao Pharm (Jiangsu, China) and used as the internal standard (IS). HPLC-grade formic acid and methanol were purchased from Tedia Company Inc. (Fairfield, OH). HPLC-grade methyl tert-butyl ether (MTBE) was purchased from J.T. Baker (Denventer, the Netherlands). Other reagents and chemicals of analytical grade were purchased from Sino Pharm Chemical Reagent Co. Ltd. (Shanghai, China). Deionized water was prepared using a Milli-Q water purifying system from Millipore Corp. (Bedford, MA).

Animals

Guinea pigs weighing between 350 and 400 g were purchased from Shanghai SIPPR/BK Laboratory Animals Ltd. (Shanghai, China) and acclimatized to laboratory conditions (temperature, 22–26 °C; relative humidity, 45–60%; and 12-h/12-h light/dark cycle) for 1 week prior to the experiments.

Drug administration and sampling

The animal experimental protocol was approved by the Ethics Committee of the School of Pharmacy of Fudan University. All animal studies were carried out according to the Guide for the Care and Use of Laboratory Animals.

Fifteen male guinea pigs were randomly divided into three groups with five animals in each group. All subjects were fasted overnight (12–15 h) before dosing and for another 4 h after dosing. Water was freely available at all times. Each animal was anesthetized with urethane (1 g/kg i.p.) and placed on a warming pad to maintain its body temperature. The jugular vein was isolated and cannulated for blood collection. The dosing solution (400 μg/mL) was prepared fresh daily by dissolving isoforskolin in a mixed solvent consisting of 10% ethanol (v/v), 10% polyethylene glycol (PEG) 400 (v/v) and 80% sterile saline (v/v). The isoforskolin dosing solution was administered at a dose of 2 mg/kg by gastric gavage, intravenous bolus administration via the marginal ear vein and intraperitoneal injection. Following isoforskolin administration, blood samples (approximately 0.25 mL) were collected into heparin-wetted tubes (0.08, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6 and 8 h for the gavage group and 0.08, 0.17, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 5 h for the intraperitoneal and intravenous administration groups). All blood samples were centrifuged at 7000 g for 5 min at 4 °C to separate the plasma. The obtained plasma was stored at −20 °C.

Sample analysis

Plasma concentrations of isoforskolin were determined by a validated HPLC–ESI–MS/MS method as previously described (Tian et al., 2011) with a slight adjustment. Briefly, a 100 μL aliquot of each plasma sample was spiked with 10 μL of IS solution (1.0 μg/mL) and extracted with 1.0 mL of MTBE by vortex mixing for 1 min. The organic layer was separated and evaporated to dryness at 45 °C under a stream of nitrogen. The residue was reconstituted in 200 μL of a solution of methanol and water (50:50, v/v), after which the sample was centrifuged at 5000 g for 5 min at 4 °C and 10 μL of the supernatant was injected into the LC–MS/MS system for analysis.

An Agilent (Agilent, Waldbronn, Germany) 1100 series LC system equipped with a quaternary pump (G1312A), a vacuum degasser (G1379A), a column oven with a thermostat (G1316A) and an HTS PAL autosampler (CTC Analytics, Switzerland) was used for HPLC. The chromatographic separation was performed on an Agela Venusil XBP Phenyl column (100 mm × 2.1 mm, 5 μm; Agela Technologies Inc., Wilmington, DE) coupled with a phenomenon C18 guard column (4.0 mm × 3.0 mm, 5 μm). The mobile phase was a mixture of methanol, 2 mM ammonium acetate and formic acid (62:38:0.1, v/v/v) delivered at 0.35 mL/min. Mass spectrometric detection was performed on an API 3000 triple quadrupole instrument (Applied Biosystems, Toronto, Canada) in multiple reaction monitoring (MRM) mode. A TurboIonSpray ionization (electrospray ionization)
interface in positive ionization mode was used. The selected precursor/product ion transitions were m/z 433.4/373.3 for isoforskolin and m/z 415.3/163.5 for IS.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by noncompartmental analysis using Kinetica® PK/PD version 5.1 SP1 (Adept Scientific, Luton, United Kingdom). The maximum plasma concentration (C_{\text{max}}) and the time to maximum plasma concentration (T_{\text{max}}) were obtained from experimental observation. The area under the plasma concentration–time curve (AUC_{0–\infty}) and the area under the first moment curve (AUMC_{0–\infty}) were calculated using the trapezoidal rule. The total area under the curve (AUC_{0–\infty}) was calculated as AUC_{0–\infty} = AUC_{0–t} + C_{\text{last}}/\lambda_{z}, where C_{\text{last}} is the last quantifiable concentration and \lambda_{z} is the elimination rate constant determined as the slope of the terminal portion of the natural log-transformed concentration–time plot. The total area under the first moment curve (AUMC_{0–\infty}) was calculated as AUMC_{0–\infty} = AUMC_{0–t} + C_{\text{last}}t_{\text{last}}/\lambda_{z} + C_{\text{last}}/\lambda_{z}, where t_{\text{last}} is the time at which C_{\text{last}} was measured. The mean residence time (MRT) was calculated as MRT = AUMC_{0–\infty}/AUC_{0–\infty}. The absolute bioavailability (F\%) of oral and intraperitoneal administration was calculated by the formula F\% = AUC_{0–\infty}(oral, intraperitoneal)/AUC_{0–\infty}(intravenous). The apparent clearance (Cl/F) was calculated as Cl/F = X_{0}/AUC_{0–\infty}, where X_{0} is the dose administered. The apparent volume of distribution during the terminal phase (V_{d}/F) was calculated as V_{d}/F = X_{0}/(AUC_{0–\infty}\lambda_{z}). The terminal elimination half-life (t_{0.5}) was calculated as t_{0.5} = 0.693/\lambda_{z}. The classical compartment model was also adopted to describe the obtained pharmacokinetic profiles. The Akaike information criterion (AIC) was utilized to compare different models, and the best model selection was determined by the smallest AIC value found.

Statistical analysis

Statistical analysis was performed using Excel 2010 (Microsoft) and IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY). Results are expressed as means ± standard deviation (SD). An unpaired Student’s t-test was used to compare means of two groups and a Duncan’s multiple range test was applied to compare means of three or more groups. A p < 0.05 was deemed statistically significant.

Results

Analytical method validation

Specificity

The degree of interference by endogenous plasma constituents with isoforskolin and the IS was assessed by inspection of chromatograms derived from the processed blank plasma sample. The representative MRM chromatograms of (A) a blank plasma sample, (B) a blank plasma sample spiked with isoforskolin (1 ng/mL) and the IS (100 ng/mL) and (C) a plasma sample after intraperitoneal administration of 2 mg/kg isoforskolin are illustrated in Figure 2. Isoforskolin and the IS were eluted at about 2.6 and 2.1 min, respectively. No interfering peaks were eluted at the retention times of interest. In addition, “cross-talk” was not observed between isoforskolin and the IS.

Linearity and sensitivity

Linearity was obtained for isoforskolin determinations over the concentration range of 1–1000 ng/mL (Y = 0.0443C + 0.0056, r = 0.994) using 1/x^2 weighted linear least squares regression (where Y is the peak area ratio of isoforskolin to IS and C is the nominal concentration of isoforskolin). The lower limit of quantification (LLOQ, signal-to-noise ratio (S/N) >10), defined as the lowest concentration of the standard curve at which isoforskolin was quantified with accuracy of 80–120% and precision of ≤20%, was 1 ng/mL. The limit of detection (LOD, S/N ≥3) was 0.03 ng/mL.

Accuracy and precision

The intra- and interday accuracy and precision of the method were evaluated by determining the quality control (QC) samples at concentrations of 1, 100 and 500 ng/mL (Table 1). The extraction recovery and matrix effect

The extraction recovery rates for isoforskolin using the selected method were 88.91–106.19% and 88.31–110.54%, respectively. The intra-day precision was 2.78–10.72%, whereas the inter-day precision was 4.15–10.83%. The accuracy and precision of the method met the criteria for acceptability.

Extraction recovery and matrix effect

The extraction recovery rates for isoforskolin were 68.42%, 77.58% and 71.66% for the QC plasma samples at concentrations of 1, 100 and 500 ng/mL (n = 3), respectively. The mean recovery rate of isoforskolin was 72.5% (n = 9). The RSDs of the extraction recovery rates were less than 5.66% at each concentration.

The average matrix effect values were 108.39, 112.40 and 106.31% at concentrations of 1, 100 and 500 ng/mL, respectively, indicating that the extracts had little or no detectable coeluting compounds with the potential to influence isoforskolin ionization.

Stability

In plasma, isoforskolin was stable at the chosen stability testing conditions, which included short-term storage for 8 h at room temperature, long-term storage for 10 days at –20°C and three freeze-thaw cycles (–20°C to 37°C) (Table 2).

Pharmacokinetics and bioavailability in guinea pigs

The average plasma concentration–time profiles of isoforskolin following oral, intraperitoneal and intravenous administration in guinea pigs are shown in Figure 3, whereas the pharmacokinetic parameters are summarized in Table 3. The concentrations of isoforskolin at the last sampling point were all less than 10% of their maximal plasma concentrations (C_{\text{max}}), while the AUC_{0–\infty} covered at least 90% of the AUC_{0–\infty}, indicating that the duration of blood sampling was long...
enough to provide a reliable estimate of the extent of exposure.

Following intravenous administration, the concentration–time data were best fit to a two-compartment model with a rapid initial distribution phase and terminal elimination phase. Isoforskolin was extensively distributed throughout the body with a $V_z$ of 16.82 ± 8.42 L/kg. Elimination of isoforskolin from the body was also rapid ($t_{0.5} = 1.20 ± 0.39$ h; $Cl = 9.63 ± 4.21$ L/kg/h).

As shown in Figure 3, the plasma concentration–time profile of isoforskolin following intraperitoneal administration was similar to that observed after intravenous

| Nominal concentration (ng/mL) | Intraday ($n=5$) | Interday ($n=3$) |
|-----------------------------|-----------------|-----------------|
|                             | Accuracy (%)    | RSD (%)         | Accuracy (%)    | RSD (%)       |
| 1                           | 106.19          | 9.12            | 106.91          | 7.99          |
| 100                         | 102.33          | 10.72           | 110.54          | 10.83         |
| 500                         | 88.91           | 2.78            | 88.31           | 4.15          |

Figure 2. Representative MRM chromatograms of isoforskolin and the internal standard (IS) in guinea pig plasma: (A) blank plasma sample; (B) blank plasma sample spiked with isoforskolin at the LLOQ of 1 ng/mL and the IS (100 ng/mL); (C) plasma sample from a guinea pig 4 h after a single intraperitoneal administration of 2.0 mg/kg isoforskolin. The retention times of isoforskolin and the IS were 2.6 and 2.1 min, respectively.
administration, except for the concentrations at the initial sampling times. Isoforskolin was absorbed rapidly into the bloodstream ($T_{max}$ from 5 to 10 min). The absolute bioavailability of intraperitoneally administered isoforskolin was 64.12%.

Following oral administration of isoforskolin, the concentration–time data were best fit to a one-compartment model, which was different from that observed after intravenous and intraperitoneal administration. Isoforskolin was absorbed rapidly following oral administration ($T_{max} = 0.30 \pm 0.11$ h). In addition, the $C_{max}$ of isoforskolin following oral administration was much lower than that measured following intraperitoneal administration. After peak concentrations were reached, isoforskolin plasma levels declined gradually. However, $t_{0.5}$ of terminal phase did not show a significant difference ($p > 0.05$) among the three tested administration routes. The absolute bioavailability of orally administered isoforskolin was 49.25%.

### Discussion

An appropriate experimental design is crucial for any pharmacokinetics study. The design of a preclinical pharmacokinetic experiment involves selection of many factors, including the animal species, anesthesia method, doses and administration route. Usually, the animal species used in pharmacokinetics studies should be those used in pharmacological and toxicological investigations. The guinea pig (Cavia porcellus) is the preferred animal model for asthma in the evaluation of antiasthmatic drugs and has been used in our preliminary in vitro (Wang et al., 2013) and in vivo antiasthmatic studies of isoforskolin. Therefore, we selected the guinea pig as a model to study the pharmacokinetics of isoforskolin.

In the present study, guinea pigs were anesthetized by urethane, one of the most commonly used anesthetics in pharmacological studies, to allow convenient blood sampling. Some recent studies showed that urethane could be employed in preclinical pharmacokinetics investigations for animal anesthetization (Azeredo et al., 2015; Bertera et al., 2009). The result of our preliminary study showed that urethane anesthesia had a negligible influence on the pharmacokinetics of isoforskolin in the guinea pig (Supplementary material, Figure S1).

Pharmacokinetic studies are generally performed using proposed therapeutic doses for a given drug. Unfortunately, the in vivo effective doses of isoforskolin that prevent asthma have not been reported. In our preliminary in vivo antiasthmatic study, isoforskolin showed a significant bronchodilating effect in guinea pigs at an intraperitoneal dose of 2 mg/kg. Therefore, a dose of 2 mg/kg was chosen as the dose for the study of the pharmacokinetics of isoforskolin. A mixed solvent consisting of 10% ethanol, 10% PEG 400 and 80% sterile saline was adopted to ensure complete dissolution of isoforskolin in the dosing solution. No precipitation was observed at any dilution with 0.1 M HCl, phosphate buffer (pH 6.8) and saline. The amounts of ethanol and PEG 400 used in the dosing solution were within published acceptable ranges (Neervannan, 2006).

Following intravenous administration, the pharmacokinetic behavior of isoforskolin was characterized by rapid and extensive distribution, followed by rapid elimination from the body. Isoforskolin was found to be preferentially distributed in peripheral tissues, because the obtained $V_z$ was much greater than the reported blood volume of the guinea pig (approximately 0.072 L/kg) (Ancill, 1956). The mean clearance of isoforskolin was $9.63 \pm 4.21$ L/kg/h, which was approximately equal to 70% of the maximal possible blood clearance calculated according to Toutain’s method (Toutain, 2004). This result demonstrates that isoforskolin is rapidly cleared from the blood in a process that may involve organs other than the kidney and liver (Toutain, 2004).

Intraperitoneal administration is a commonly used parenteral route of administration in animal studies. The pharmacokinetic profiles of isoforskolin were similar after intraperitoneal and intravenous administration. The rapid absorption of isoforskolin observed following intraperitoneal administration may have been due to the large surface area of the abdominal cavity and its abundant blood supply. Following intraperitoneal administration, absorbed
isoforskolin will enter the mesenteric vessels, drain into the portal vein and pass through the liver (Turner et al., 2011). Therefore, isoforskolin administered intraperitoneally undergoes hepatic metabolism that decreases systemic exposure. The absolute bioavailability of intraperitoneally administered isoforskolin was 64.12%.

The oral route is the most convenient route of drug administration. Marketed medicines containing isoforskolin are taken orally. Following oral administration to guinea pigs, isoforskolin was absorbed rapidly, with a $C_{\text{max}}$ much lower than that observed following intraperitoneal injection. Probable explanations for this observation were as follows: the plasma concentration data for intraperitoneally administered isoforskolin best fit a two-compartment model. Most of the absorbed isoforskolin was in the central compartment shortly after the injection, resulting in a high initial plasma concentration. However, the concentration data following oral administration best fit a one-compartment model, indicating that the absorbed isoforskolin distributed and equilibrated instantaneously and rapidly throughout the body. Because of the large apparent volume of distribution of isoforskolin, low peak concentrations with high absorption rates were observed following oral administration. The exact causes of the differences in the pharmacokinetic models that best describe the behavior of isoforskolin following oral and intraperitoneal administration are unknown. The absolute bioavailability of oral isoforskolin was lower than that of intraperitoneally administered isoforskolin, suggesting that isoforskolin may be degraded in the gastrointestinal tract, because the clearance rates measured following oral administration by each route were equal.

The absolute oral bioavailability of isoforskolin in guinea pigs is 49.25%, while the oral bioavailability of forskolin, an analog of isoforskolin, in rats is lower than 1% (Guo et al., 2012). The pharmacokinetics and bioavailability of isoforskolin in guinea pigs following intravenous, intraperitoneal and oral administration at a dose of 2 mg/kg (mean ± SD, n = 5).

### Table 3. Pharmacokinetic parameters of isoforskolin in guinea pigs following intravenous, intraperitoneal and oral administration

| Pharmacokinetic parameters | Intravenous administration | Intraperitoneal administration | Oral administration |
|----------------------------|---------------------------|--------------------------------|---------------------|
| $C_{\text{max}}$ (ng/mL)   | NA                        | 246.66 ± 96.20                 | 48.44 ± 12.66       |
| $T_{\text{max}}$ (h)       | NA                        | 0.12 ± 0.05                    | 0.30 ± 0.11         |
| AUC$_{0-t}$ (ng·h/mL)      | 225.70 ± 85.52            | 147.41 ± 44.93                 | 108.17 ± 29.52      |
| AUC$_{0-\infty}$ (ng·h/mL)| 239.23 ± 91.44            | 153.38 ± 45.33                 | 117.81 ± 32.62      |
| $F\%$                      | NA                        | 64.12%                         | 49.25%              |
| $\lambda_z$ (h$^{-1}$)     | 0.63 ± 0.19               | 0.78 ± 0.31                    | 0.45 ± 0.13         |
| $t_{0.5}$ (h)              | 1.20 ± 0.39               | 1.06 ± 0.59                    | 1.65 ± 0.46         |
| MRT (h)                    | 0.82 ± 0.21               | 0.97 ± 0.34                    | 2.75 ± 0.44         |
| CI/F (L/kg/h)              | 9.63 ± 4.21               | 14.04 ± 4.25                   | 18.10 ± 5.31        |
| V/F (L/kg)                 | 16.82 ± 8.42              | 20.37 ± 9.19                   | 41.40 ± 11.29       |

$aC_{\text{max}}$, maximum plasma concentration; $T_{\text{max}}$, time to maximum plasma concentration; AUC$_{0-t}$, area under the plasma concentration–time curve; AUC$_{0-\infty}$, total area under the curve; $F\%$, absolute bioavailability; $\lambda_z$, the elimination rate constant; $t_{0.5}$, terminal elimination half-life; MRT, mean residence time; CI/F, apparent clearance; V/F, apparent volume of distribution during the terminal phase.

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Supplementary material available online.