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Pharmacokinetics of Dehydroevodiamine Following Intravenous Administration in Rats

Seong Yun Kim, Chan-Soo Moon, Yun-Sik Choi, and Sang Bok Lee

Department of Pharmacology, College of Medicine, The Catholic University of Korea, Seoul 137–701, Korea

Dehydroevodiamine (DHED) is one of the bioactive components of the Chinese herbal medicine *Wu-chu-yu-tang* that has been shown to produce various pharmacological effects. In the present study, we investigated the pharmacokinetics of DHED after intravenous administration of two doses (2.5 and 5 mg/kg) in anesthetized rats. The plasma concentration of DHED was measured by reverse-phase high-performance liquid chromatography with UV detection. The mean area under the curve of the time-concentration profile was 21.9 and 53.9 μg·min/ml after the 2.5- and 5-mg/kg doses, respectively, and the volume of distribution was 1584.9 and 1580.6 ml following 2.5- and 5-mg/kg doses, respectively. Plasma concentration profiles versus time were compatible with a two-compartment model and first-order kinetics. The terminal elimination half-life was 91.8±16.6 min and 78.7±11.9 min in the dose of 2.5 and 5 mg/kg, respectively. This is the first report to study the pharmacokinetics of DHED in animals.

Key Words: Dehydroevodiamine, Pharmacokinetics, *Wu-chu-yu*, HPLC, Intravenous injection, Rats

INTRODUCTION

Dehydroevodiamine (DHED) is an isoquinazolinocarboline alkaloid isolated from the Chinese herbal drug *Wu-chu-yu*, the dried unripe fruit of *Evodia rutaecarpa* (Chen et al, 1981; Chiou et al, 1996). *Wu-chu-yu-tang* (*Goshuyu-to* in Japanese Kampo medicine) is a traditional Chinese prescription for gastrointestinal disorders, abdominal pain, headache, postpartum hemorrhage, amenorrhea and vomiting accompanying a cold (Kiangsu Institute of Modern Medicine, 1977; Kano et al, 1991).

It has been reported that DHED has some discrete effects on cardiovascular function such as an increase of cerebral blood flow in cats (Haji et al, 1994). Recently, DHED was found to have antiacetylcholinesterase (Park et al, 1996) and antiamnesic activities (Park et al, 2000; Wang et al, 2001) and to reduce infarction volume induced by focal cerebral ischemia in rats (Park et al, 2000). These findings suggest that DHED has beneficial effects on both dementia and stroke.

While various pharmacological effects of DHED have been known, its pharmacokinetic studies have not yet been reported. In the present study, we investigated the pharmacokinetics of DHED after intravenous injection in rats. The plasma concentration of DHED was measured using high-performance liquid chromatography (HPLC) with UV detection.

METHODS

Fourteen Sprague-Dawley male rats (250~300 g) were housed in an animal care facility and were acclimated for a minimum of 7 days prior to experimentation. In rats anesthetized with pentobarbital sodium (60 mg/kg, i.p.), the femoral artery and vein were cannulated with polyethylene tube (PE-50). Twenty min after cannulation, DHED hydrochloride (Jeil Pharmaceutical Company, Korea), dissolved in boiling distilled water (Yang et al, 1990) and cooled to body temperature, was injected into the femoral vein (2.5 or 5 mg/kg). Arterial blood (less than 300 μl) was collected prior to administration of DHED and at 5, 15, 30, 45, 60, 90, 120, and 180 min thereafter. Rectal temperature was monitored and maintained at 37.5±0.5°C with a heating lamp.

Plasma was separated by centrifugation at 2,000 g for 5 min and stored at −70°C until analysis. To 0.1 ml of plasma, 2 ml of acetonitrile were added to precipitate the plasma protein. Following a second centrifugation at 2,000 g for 5 min, the supernatant was separated and blown dry by a centrifugal evaporator (SpeedVac, Savant, USA). The residue was finally redissolved in 100 μl of water, and 50-μl aliquots were injected into the HPLC apparatus for analysis.

Concentrations of DHED in plasma were determined by HPLC (Peng et al, 1993), with some modifications. The HPLC system consisted of a solvent-delivery system (Waters, M510, USA) delivering the mobile phase, which was water-acetonitrile-phosphoric acid [74.6 : 25 : 0.4 (v/v/v)] with the pH adjusted to 3.5 with tetrabutyl ammonium

Corresponding to: Sang Bok Lee, Department of Pharmacology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, Korea. (Tel) 590-1201, (Fax) 536-2485, (E-mail) sbl@catholic.ac.kr

ABBREVIATIONS: DHED, dehydroevodiamine; HPLC, high-performance liquid chromatography; Kel, terminal elimination constant; AUC, area under the concentration versus time curve; Cl, total body clearance; Vd, volume of distribution.

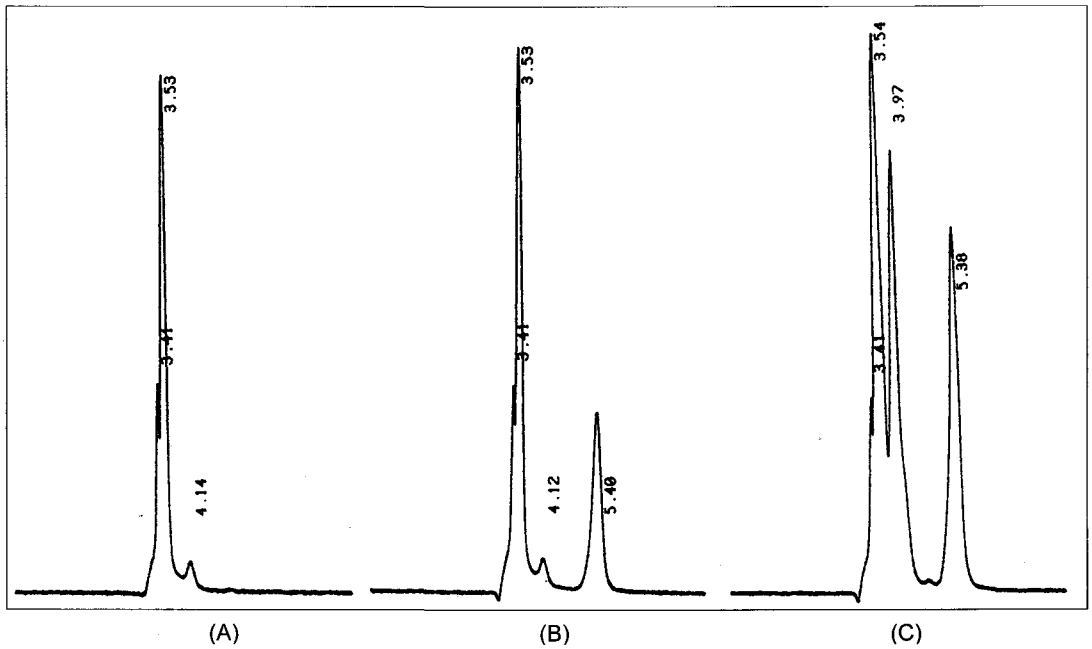

Fig. 1. HPLC chromatogram showing the detection of dehydroevodiamine (DHED) in rat plasma. (A) blank rat plasma; (B) standard rat plasma containing 0.8 μg/ml DHED (5.40); (C) rat plasma sample (DHED, 5.38) taken 5 min following intravenous administration of 5-mg/kg dose. The calculated DHED concentration was 1.5 μg/ml.

hydroxide solution and 25% ammonia solution. The mobile phase was delivered at a flow rate of 1.0 ml/min to an μBondapak C_{18} column (300×3.9 mm; particle size 10 μm, Waters) fitted with a guard column (μBondapak C_{18} Guard-Pak, Wters). Samples were injected with an autosampler (Triathlon Model 900, Spark Holland, The Netherlands). Detection was performed using an UV detector (M441, Waters) with the detection wavelength set at 365 nm. Data were processed by a chromatographic integrator (Spectra-physics, Chromjet, USA).

In order to construct a standard curve, various amounts of DHED were added to aliquots of the corresponding pooled blank plasma, which were then carried through the entire procedure. At the concentration range of 20 ng/ml $-1.5\,\mu$g/ml, a standard curve was constructed by plotting the amounts of spiked DHED against the response (peak area). The amount of DHED in a sample was calculated from the slope of the standard curve.

The terminal elimination constant (K_{el}) was calculated by linear least-squares regression analysis using the last five log-transformed plasma concentration-versus-time points. The area under the concentration versus time curve to infinite time (AUC) was calculated by the trapezoidal rule to the last quantifiable plasma concentration (C_{last}), and the area extrapolated beyond this point was calculated as C_{last}/K_{el}. Total body clearance (Cl) was calculated as dose (i.v.)/AUC. Volume of distribution (Vd) was determined as Cl/K_{el}. The terminal half-life ($t_{1/2}$) was calculated as $\ln 2/K_{el}$.

DHED was a kind gift from Jeil Pharmaceutical Company (Seoul, Korea). HPLC grade acetonitrile and phosphoric acid were purchased from Fisher (USA). Other chemicals and solvents (analytical reagent grade) for the mobile phase were purchased from commercial suppliers.

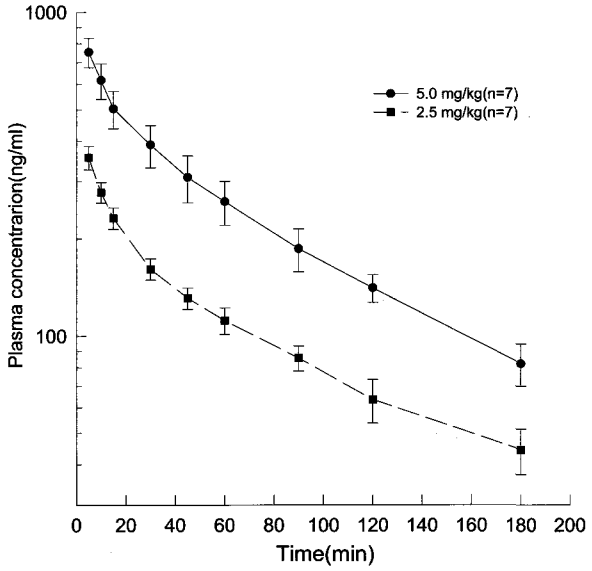

Fig. 2. Mean semi-logarithmic plots of plasma concentration-time data for dehydroevodiamine after intravenous injection. Data are expressed as mean±S.E.

RESULTS

No peak overlapping that of DHED was observed in rat plasma samples. DHED had retention time of about 5.4 min. Fig. 1 displayed representative chromatograms, showing the detection of DHED in rat plasma.

The mean plasma concentration data for DHED over time

Table 1. Pharmacokinetic parameters of dehydroevodiamine (DHED) after intravenous injection in anesthetized rats

Parameters	DHED (2.5 mg/kg)	DHED (5 mg/kg)
AUC (μg · min/ml)	21.9±1.7	53.9±6.1
Vd (ml)	1,584.9±151.6	1580.6±218.4
CL (ml/min)	31.3±2.3	27.3±4.7
$T_{1/2}$ (min)	91.8±16.6	78.7±11.9

Data are expressed as mean±S.E.
AUC: area under the curve, Vd: volume of distribution, CL: total body clearance, $T_{1/2}$: terminal elimination half-life.

after intravenous administration (2.5 and 5 mg/kg, 7 rats per each dose) in the rat are presented in Fig. 2. The calculated AUC of DHED increased in proportion to doses administered. AUC for DHED were 21.9±1.7 μg · min/ml and 53.9±6.1 μg · min/ml after 2.5-mg/kg and 5-mg/kg doses, respectively. Regardless of dose, both Vd and CL of DHED were fixed within a narrow range. The respective volumes of distribution for DHED were 1584.9±151.6 ml and 1580.6±218.4 ml, and the clearance were 31.3±2.3 ml/min and 27.3±4.7 ml/min, respectively. Pharmacokinetic parameters for DHED after intravenous injection in the rat are presented in Table 1.

DISCUSSION

DHED has been reported to have various pharmacological effects, such as analgesic (Park et al, 2003), hypotensive (Yang et al, 1990), bradycardic (Yang et al, 1990), ion channel depressant (Loh et al, 1992), thermoregulatory (Tsai et al, 1995) and cerebral blood flow enhancement (Haji et al, 1994) activities. In addition, DHED has recently been found to have antiacetylcholinese (Park et al, 1996) and antiamnestic activities (Park et al, 2000). Because DHED possesses these potential therapeutic properties, its pharmacokinetic studies are necessary for future applications. No previous attempt has been, however, made for characterization of the pharmacokinetics of DHED in animals.

In the present study, concentrations of DHED in the rat plasma were determined by HPLC, as described by Peng et al. (1993) with some modifications. For these modifications, the plasma volume required for sample processing was reduced to 0.1 μl from 0.35 μl for multiple blood samples. In addition, the change of mobile phase consisting of water-acetonitrile-phosphoric acid [from 64.4 : 35 : 0.6 to 74.6 : 25 : 0.4 (v/v/v)] shortened overall running time of 7 min (versus 20 min) with retention time of 5.4 min (versus 10.4 min) for DHED, as compared to the results reported by Peng et al. (1993). Our analytical method, consisting of reversed-phase HPLC coupled to UV detection, made it possible to analyse rat plasma DHED concentration, leading to its successful pharmacokinetic study in animals.

In the range of 2.5 and 5 mg/kg doses after i.v. injection in rats, the mean AUC increased in proportion to the dose administered. The volume of distribution and total body clearance of DHED were virtually identical for both two doses, indicating the validity of this study. Over the sampling times monitored, plasma concentration profiles versus time were compatible with a two-compartment model and first-order kinetics. Ueng et al. (2002) reported that *Wu-chu-yu-tang*-treatment in mice caused a significant increase of hepatic cytochrome b_5 which is involved in the metabolism of many endogenous and exogenous substances. The terminal elimination half-lives of DHED were 91.8±16.6 min and 78.7±11.9 min in the dose of 2.5 and 5 mg/kg, respectively. Indeed, although pharmacokinetics of its metabolites was not specifically studied in the present work, the short terminal half-life suggests a rather rapid metabolism. However, it can not be ruled out that the short terminal half-life is attributed to other factors such as a limited tissue accumulation.

In conclusion, this report represents for the first time valuable information about the pharmacokinetics of DHED in rats. Because this study was conducted in anesthetized rats, it is not possible to simply extrapolate plasma concentration profiles versus time obtained from this study to pharmacological responses in the conscious rat. Future studies are in need to explore this relationship.

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