

©2003, Acta Pharmacologica Sinica  
Chinese Pharmacological Society  
Shanghai Institute of Materia Medica  
Chinese Academy of Sciences  
<http://www.ChinaPhar.com>

# Pharmacokinetic-pharmacodynamic modeling of daurisoline and dauricine in beagle dogs<sup>1</sup>

SHI Shao-Jun<sup>2</sup>, CHEN Hui, GU Shi-Fen, ZENG Fan-Dian

*Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

**KEY WORDS** daurisoline; dauricine; pharmacokinetics; pharmacodynamics

## ABSTRACT

**AIM:** To study the combined pharmacokinetic-pharmacodynamic (PK-PD) model of daurisoline and dauricine, and compare their effects on cardiac electrophysiology, blood pressure, and hemodynamics in beagle dogs. **METHODS:** The plasma drug concentration was determined by the reversed-phase HPLC method and the effects on cardiac and hemodynamics were recorded by polygraph. The pharmacokinetic and PK-PD model parameters were calculated. **RESULTS:** The pharmacokinetics were best fitted to a two-compartment open model, and the relationship between effect and effect compartment concentration of both drugs could be represented by the sigmoid- $E_{\max}$  model. There were no significant differences in main pharmacokinetics and PK-PD parameters between the two drugs. **CONCLUSION:** No statistically different kinetic disposition characteristics and potencies of inhibitory effects on myocardial function of daurisoline and dauricine were found in beagle dogs.

## INTRODUCTION

The combined pharmacokinetic-pharmacodynamic (PK-PD) model builds the bridge between these two classical disciplines. It can provide a more rational basis for dosage individualization and may thus guide applied pharmacotherapy to a higher level of performance<sup>[1]</sup>.

Daurisoline (DS) and dauricine (Dau), the bisbenzyltetrahydroisoquinoline (BBI) alkaloids, were isolated from the root of *Menispermum dauricum* DC. Their anti-arrhythmic effects and mechanisms had been

demonstrated in our laboratory<sup>[2-4]</sup>. The previous studies have revealed that DS and Dau prolonged action potential duration (APD) in ventricular myocytes in a use-dependent manner<sup>[5-7]</sup>, which make them to be the very promising anti-arrhythmic agents. The present study was to construct the combined PK-PD model for both drugs, and compare their disposition characteristics and effects on cardiovascular system in beagle dogs.

## MATERIALS AND METHODS

**Reagents** DS and Dau with purity >99 % were provided by Institute of Clinical Pharmacology, Tongji Medical College. Acetonitrile, methanol, and dichloromethane were of HPLC grade. Other reagents were of analytical grade.

**Beagle dogs** (♀ and ♂,  $n=8$ , weighing 11.4 kg±1.2 kg, aged 8-10 months) were purchased from Shanghai Institute of Xingang Experimental Animal Center.

<sup>1</sup> Project supported by the Scientific Research Foundation of Committee of Science and Technology of Hubei Province. No 991P1609.

<sup>2</sup> Correspondence to Dr SHI Shao-Jun. *Department of Pharmacy, Xiehe Hospital Affiliated to Tongji Medical College, Wuhan 430022, China.*

E-mail sjshi1@163.com.

Received 2002-11-01

Accepted 2003-01-21

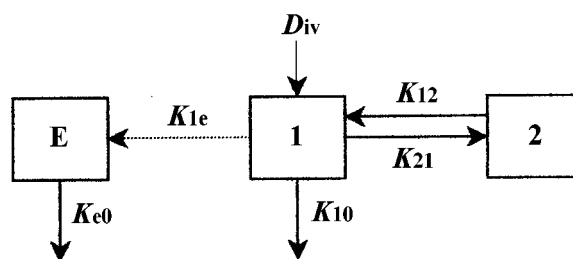
**Chromatography** The HPLC system consisted of a solvent pump (Merck-Hitachi L-6000, Japan), a UV-detector (Shimadzu, Japan) operated at 284 nm, a column (Shimadzu ODS, 5  $\mu$ m, 150 mm $\times$ 4 mm) were protected with a guard column packed with the same material. The column was kept at room temperature. The mobile phase was acetonitrile-water-triethylamine (18:82:0.28), plusing phosphoric acid to pH 3.0, at 1.0 mL/min. The limit of quantitation (LOQ) was 50  $\mu$ g/L of plasma, and the intra- and inter-assay coefficient of variation were less than 10 %. The absolute and relative recoveries were above 80 % and 85 %, respectively. The HPLC chromatograms of DS and Dau in plasma were shown in Fig 1.

**Sample preparation** IS 10  $\mu$ L, acetonitrile 0.5 mL, dichloromethane 4 mL, and plasma 1 mL was mixtured. The mixture was vortexed for 5 min, then centrifuged at 3000 r/min for 15 min. The organic phase was transferred to a centrifuge tube. This procedure was repeated after addition of 2 mL dichloromethane. The organic phase was evaporated to dryness at 40  $^{\circ}$ C under a nitrogen stream. The residue was reconstituted in 200  $\mu$ L of mobile phase, and then 20  $\mu$ L was analyzed by HPLC.

**Measurement of myocardial function** After the beagle dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) a canula was advanced into the left ventricle through the right common carotid artery. And the canula was connected to a pressure transducer which was connected to an amplifier and polygraph. The right femoral artery was canulated to measure the blood pres-

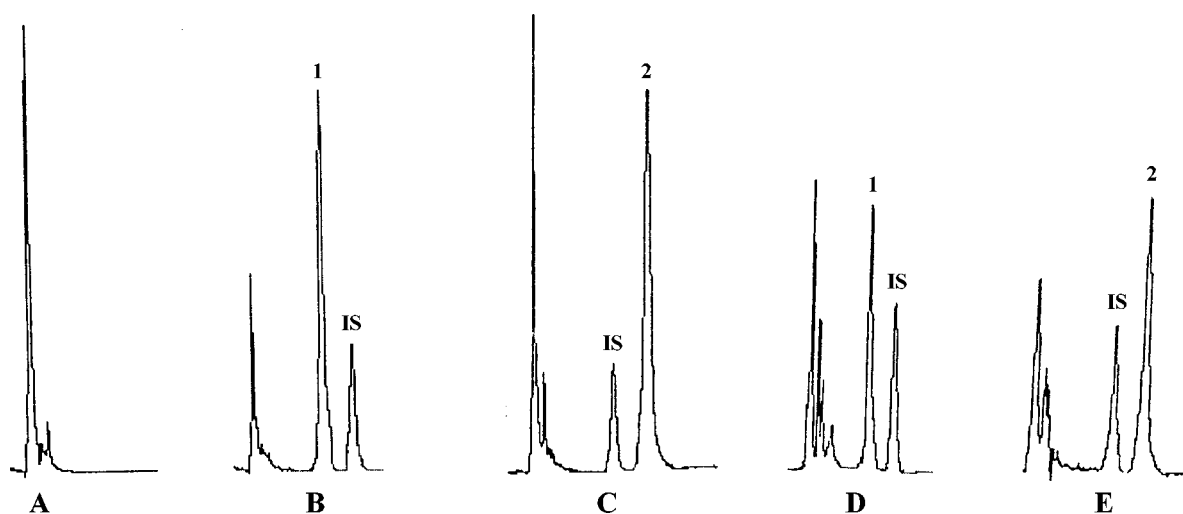
sure wave. ECG (lead II) was observed simultaneously. After iv injection of DS ( $n=4$ ) or Dau ( $n=4$ ) to beagle dogs, the ECG, BP, and LVP signals were recorded. Blood samples were taken before dosing and at 2, 5, 10, 15, 20, 30, 45 min, and 1, 1.5, 2, 3, 4, 6, 8 h after dosing.

**PK-PD model** The effect compartment originally proposed by Sheiner is modeled as a additional hypothetical compartment linked to central compartment by a first-order process<sup>[8]</sup>. But the compartment receives negligible actual mass of drug. Hence, the exponential term for the effect compartment does not enter into the pharmacokinetic solution for the mass of drug in the body (Fig 2).



**Fig 2.** Effect compartment model in association with a traditional two-compartment model after iv input. 1, 2, and E represent central, peripheral, and effect compartment respectively.

For two-compartment model with an iv input, the drug concentration in plasma ( $C_1$ ) and effect ( $C_e$ ) compartment can be expressed as follows:



**Fig 1.** Chromatogram of blank plasma (A), blank plasma spiked with DS and its IS (B), blank plasma spiked with Dau and its IS(C), plasma sample after iv DS (D), and plasma sample after iv Dau (E). 1: DS; 2: Dau; IS: internal standard.

$$C_I = \frac{X_0(\alpha - K_{21})}{V_c(\alpha - \beta)} \cdot e^{-\alpha t} + \frac{X_0(K_{21} - \beta)}{V_c(\alpha - \beta)} \cdot e^{-\beta t} \quad (1)$$

$$C_e = \frac{X_0 K_{e0}}{V_c} \left[ \frac{(K_{21} - \alpha)e^{-\alpha t}}{(\beta - \alpha)(K_{e0} - \alpha)} + \frac{(K_{21} - \beta)e^{-\beta t}}{(\alpha - \beta)(K_{e0} - \beta)} + \frac{(K_{21} - K_{e0})e^{-K_{e0}t}}{(\alpha - K_{e0})(\beta - K_{e0})} \right] \quad (2)$$

Where  $\alpha$  was distribution rate constant,  $\beta$  was elimination rate constant,  $K_{1e}$  was transfer rate constant from central compartment to effect compartment, and  $K_{e0}$  was the rate constant for drug removal from effect compartment.

The inhibitory effects on myocardial function were related to the effect compartment concentration. The relationship between the effects and effect compartment concentration of both drugs can be represented by the sigmoid- $E_{\max}$  model.

$$E = \frac{E_{\max} \cdot C_e^\gamma}{EC_{50}^\gamma + C_e^\gamma} \quad (3)$$

Where  $E_{\max}$  was the maximal effect,  $EC_{50}$  was the effect compartment concentration required to achieve 50 % of the maximal effect, and  $\gamma$  was the Hill parameter that allowed sigmoidicity of the concentration-effect relationship.  $C_e$  was the effect compartment concentrations.

**Statistical analysis** The pharmacokinetic parameters were calculated with 3P97 program. The PK-PD parameters were calculated by the PK-PD Parameters Estimate Program of Nanjing Military General Hospital. All data were expressed as mean $\pm$ SD. Statistical difference was determined by *t*-test.

## RESULTS

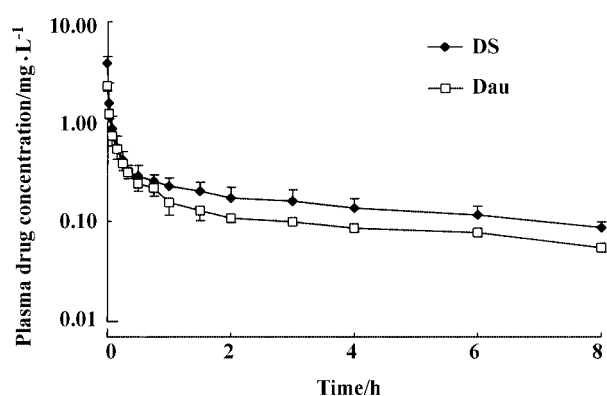
**PK** The results showed that plasma concentration existed a biexponential decline following iv administration of DS or Dau 6 mg/kg. The pharmacokinetics of both drugs were best fitted to a two-compartment open model.  $T_{1/2\alpha}$  for DS and Dau were (0.026 $\pm$ 0.014) and (0.049 $\pm$ 0.016) h and  $K_{12}$  for both test drugs were larger than  $K_{21}$ . So these two test drugs were distributed quickly. And both test drugs were eliminated quickly from central compartment, the mean  $T_{1/2\beta}$  were (3.1 $\pm$ 0.9) and (2.7 $\pm$ 0.6) h, respectively. The  $V_d$  were (12 $\pm$ 3)

and (16 $\pm$ 3) L/kg, which were much larger than the actual body fluid volume of dog.

Pharmacokinetic parameters  $T_{1/2\alpha}$  and  $T_{1/2\beta}$  showed no significant differences between two test drugs. It revealed that the distribution and elimination processes of DS were similar to that of Dau. But  $C_0$  and AUC of DS were significantly higher than the corresponding values of Dau ( $P < 0.05$ ). (Tab 1, Fig 3)

**Tab 1. Pharmacokinetic parameters of dauricine (Dau) and daurisolone (DS) after iv 6 mg/kg in beagle dogs.  $n=4$ . Mean $\pm$ SD. <sup>a</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs DS.**

Parameters	DS	Dau
$\alpha/h^{-1}$	32 $\pm$ 13	16 $\pm$ 6 <sup>a</sup>
$\beta/h^{-1}$	0.24 $\pm$ 0.07	0.27 $\pm$ 0.07 <sup>a</sup>
$T_{1/2\alpha}/h$	0.026 $\pm$ 0.014	0.049 $\pm$ 0.016 <sup>a</sup>
$T_{1/2\beta}/h$	3.1 $\pm$ 0.9	2.7 $\pm$ 0.6 <sup>a</sup>
$K_{21}/h^{-1}$	3.3 $\pm$ 1.5	2.1 $\pm$ 0.9 <sup>a</sup>
$K_{10}/h^{-1}$	2.3 $\pm$ 0.4	2.0 $\pm$ 0.5 <sup>a</sup>
$K_{12}/h^{-1}$	27 $\pm$ 12	12 $\pm$ 5 <sup>a</sup>
MRT/h	8.0 $\pm$ 1.9	7.5 $\pm$ 1.0 <sup>a</sup>
$V_d/L \cdot kg^{-1}$	12 $\pm$ 3	16 $\pm$ 3 <sup>a</sup>
AUC/mg $\cdot$ h $\cdot$ L <sup>-1</sup>	2.17 $\pm$ 0.17	1.48 $\pm$ 0.17 <sup>c</sup>
$C_0/mg \cdot L^{-1}$	3.8 $\pm$ 0.7	2.26 $\pm$ 0.23 <sup>b</sup>



**Fig 3. Mean plasma concentration-time curves of DS and Dau after iv 6 mg/kg in beagle dogs.  $n=4$ . Mean $\pm$ SD.**

**PD** After iv DS and Dau 6 mg/kg in beagle dogs, HR, LVSP,  $dp/dt_{\max}$ , and SBP were decreased. But the maximum pharmacological effects of both drugs peaked at 10-15 min later than the maximum plasma concentration was observed. There was no direct correlation between pharmacological effects ( $E$ ) and plasma concentrations ( $C_p$ ), and a counterclockwise hysteresis loop

existed between them. By means of effect compartment theory, the measured pharmacological effects were parallel to the effect compartment concentrations ( $C_e$ ). The relationships between the effects and  $C_e$  were successfully characterized by the sigmoid- $E_{max}$  model (Fig

4). It was in good agreement between the predicted effects and experimental effects.

No significant differences of  $K_{e0}$ ,  $EC_{50}$ ,  $E_{max}$ , and  $\gamma$  on HR, BP, LVSP, and  $dp/dt_{max}$  between DS and Dau at the same dosage were found ( $P>0.05$ , Tab 2).

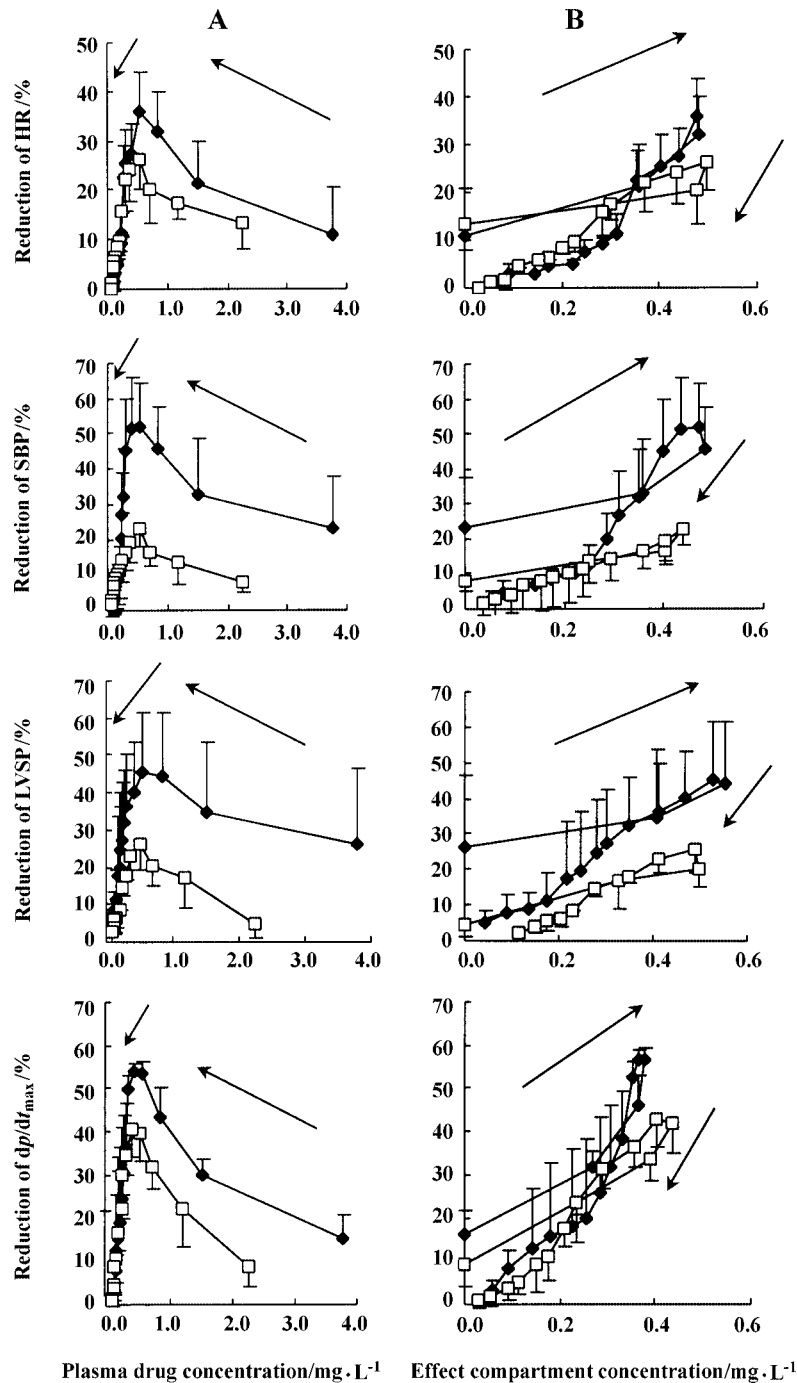


Fig 4. Relationships between inhibitory effects on HR, SBP, LVSP, + $dp/dt_{max}$  and plasma drug concentration (A) or effect compartment concentration (B) after iv daurisoline (◆) and dauricine (□) 6 mg/kg in beagle dogs. Open arrows denote the counterclockwise hysteresis loop between  $E$  and  $C_p$  (A) and the parallel relationship between  $E$  and  $C_e$  (B).

**Tab 2. Pharmacodynamic parameters after iv DS and Dau 6 mg/kg in beagle dogs.  $n=4$ . Mean $\pm$ SD.**

	$K_{e0}/h^{-1}$		$E_{max}/\%$		$EC_{50}/mg\cdot L^{-1}$		$\gamma$	
	DS	Dau	DS	Dau	DS	Dau	DS	Dau
HR ( $min^{-1}$ )	5.4 $\pm$ 2.2	6.0 $\pm$ 0.8	59 $\pm$ 30	34 $\pm$ 12	0.42 $\pm$ 0.15	0.29 $\pm$ 0.04	4.2 $\pm$ 1.5	2.5 $\pm$ 0.4
SBP (kPa)	5.1 $\pm$ 1.6	4.9 $\pm$ 1.2	75 $\pm$ 24	32 $\pm$ 6	0.40 $\pm$ 0.12	0.33 $\pm$ 0.15	3.7 $\pm$ 2.1	1.9 $\pm$ 0.9
LVSP (kPa)	5.5 $\pm$ 2.4	7 $\pm$ 3	72 $\pm$ 44	33 $\pm$ 9	0.34 $\pm$ 0.07	0.32 $\pm$ 0.04	3.5 $\pm$ 2.6	3.6 $\pm$ 2.0
$dp/dt_{max}$ ( $kPa\cdot s^{-1}$ )	4.0 $\pm$ 1.8	4.6 $\pm$ 1.3	66 $\pm$ 8	59 $\pm$ 23	0.28 $\pm$ 0.06	0.29 $\pm$ 0.06	3.4 $\pm$ 1.9	3.8 $\pm$ 2.5

## DISCUSSION

In the study, the hysteresis between effects and plasma drug concentrations indicated that there was equilibration processes between plasma and effect compartment. Hence, we successfully collapsed the hysteresis loops when using the effect compartment model.

The pharmacokinetic parameters revealed that both drugs were distributed quickly from central compartment to tissue and had a wide distribution in beagle dogs, which was due to their high affinity to tissues and organs. The main pharmacokinetic parameters of DS were similar to that of Dau, suggesting both drugs had the same process of pharmacokinetics.

$K_{e0}$  precisely characterized the temporal aspects of equilibration between  $C_p$  and effects, which governed the degree of lag. The smaller the value of  $K_{e0}$ , the greater the drug hysteresis loop<sup>[9,10]</sup>. The results revealed that the equilibration process between plasma and effect compartment of DS were similar to that of Dau.  $E_{max}$  reflected the efficacy of drugs and  $EC_{50}$  characterized the potency of drugs<sup>[11]</sup>. Hence, their efficacy and receptor affinity were not statistically different.

In conclusion, no statistically different kinetic disposition characteristics and potencies of the inhibitory effects on myocardial function of DS and Dau were found in beagle dogs.

## REFERENCES

- Derendorf H, Lesko LJ, Chaikin P, Colburn WA, Lee P, Miller R, *et al*. Pharmacokinetic/pharmacodynamic modeling in drug research and development. *J Clin Pharmacol* 2000; 40: 1399-418.

- Du ZH, Zeng WC, Gong PL, Zeng FD, Hu CJ. Antiarrhythmia effects of daurisoline. *Pharmacol Clin Prac TCM* 1996; (4): 21-3.
- Zhu JQ, Zeng FD, Hu CJ. Effect of dauricine and lidocaine alone and combined on electrophysiological properties of canine Purkinje fibers. *Acta Pharmacol Sin* 1990; 11: 220-5.
- Yao WX, Jiang MX. Effects of tetrandrine on cardiovascular electrophysiologic properties. *Acta Pharmacol Sin* 2002; 23: 1069-74.
- Guo DL, Zeng FD, Hu CJ. Effects of dauricine, quinidine, and sotalol on action potential duration of papillary muscles *in vitro*. *Acta Pharmacol Sin* 1997; 18: 348-50.
- Xia JS, Guo DL, Zhang Y, Zhou ZN, Zeng FD, Hu CJ. Inhibitory effects of daurisoline on potassium currents in guinea pig ventricular myocytes. *Acta Pharmacol Sin* 2000; 21: 60-4.
- Qian JQ. Cardiovascular pharmacological effects of bisbenzylisoquinoline alkaloid derivatives. *Acta Pharmacol Sin* 2002; 23: 1086-92.
- Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to *D*-tubocurarine. *Clin Pharmacol Ther* 1979; 25: 358-71.
- Donati F, Varin F, Ducharme J, Gill SS, Theoret FY, Bevan DR. Pharmacokinetics and pharmacodynamics of atracurium obtained with arterial and venous blood samples. *Clin Pharmacol Ther* 1991; 49: 515-22.
- Fuseau E, Sheiner LB. Commentary: simultaneous modeling of pharmacokinetics and pharmacodynamics with a nonparametric pharmacodynamic model. *Clin Pharmacol Ther* 1984; 35: 733-41.
- Meibohm B, Derendorf H. Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modeling. *Int J Clin Pharmacol Ther* 1997; 35: 401-13.