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Short communication

Pharmacokinetics of diclofenac sodium injection in swine

H.F. Yang[1,2], Y.J. Li[1,2], Y.Y. Li[1,2], C. Huang[1], L.X. Huang[1], S.J. Bu[2]

[1] The School of Animal Pharmacy, Jiangsu Agri-animal Husbandry Vocational College, No. 8, East Fenghuang Road, Taizhou 225300, China
[2] College of Veterinary Medicine, Yangzhou University, No. 12, East Wenhui Road, Yangzhou 225009, China

Abstract

The pharmacokinetics of a diclofenac sodium was investigated in swine. A single intravenous (i.v.) or intramuscular (i.m.) injection of 5% diclofenac sodium (concentration = 2.5 mg · kg^{-1}) was administered to 8 healthy pigs according to a two-period crossover design. The pharmacokinetic parameters were calculated by non-compartmental analysis with DAS2.1.1 software. After a single i.v. administration, the main pharmacokinetic parameters of diclofenac sodium injection in swine were as follows: the elimination half-time ($T_{1/2\beta}$) was 1.32±0.34 h; the area under the curve (AUC) was (55.50±5.50 μg · mL^{-1} h; the mean residence time (MRT) was 1.60±0.28 h; the apparent volume of distribution (V_d) was 0.50±0.05 L · kg^{-1}; and the body clearance (CL_B) was 0.26±0.04 L · $(h \cdot kg)^{-1}$. After the single i.m. administration, the pharmacokinetic parameters were as follows: peak time (T_{max}) was 1.19±0.26 h; and peak concentration (C_{max}) was 11.61±5.99 μg mL^{-1}. The diclofenac sodium has the following pharmacokinetic characteristics in swine: rapid absorption and elimination; high peak concentration; and bioavailability.

Key words: diclofenac sodium injection, pharmacokinetics, bioavailability, swine, HPLC

Introduction

Diclofenac sodium is an aryl acid non-steroidal anti-inflammatory drug (Yang et al. 2008) that suppresses the activity of cyclooxygenase isozymes, reduces prostaglandin synthesis, and shows clinical efficacy in curing patients. The pharmacokinetics of diclofenac sodium in humans, dogs, rats, and rabbits have been extensively investigated, while data on swine are lacking. In this work a single intravenous (i.v.) or intramuscular (i.m.) injection of 5% diclofenac sodium (concentration = 2.5 mg · kg^{-1}) was administered by cross-over experiment. The pharmacokinetics and bioavailability in the swine was evaluated, which may provide the experimental basis for new veterinary drug registration and reasonable clinical use.

Materials and Methods

Diclofenac sodium (Luye Animal Health Co. Ltd), diclofenac, indomethacin internal standard [IS] (99.9%;

Correspondence to: H.F. Yang, e-mail: yhf8142@sina.com, tel.: 0086-0523-86158187, fax: 0086-0523-86663808

National Institute for the Control of Pharmaceutical and Biological Products), acetonitrile, and methanol (chromatographic grade; TEDIA Company, Inc., USA) were used without further purification. Ether, hydrochloric acid, and glacial acetic acid were analytical grades (Sinopharm Chemical Reagent Limited Company). Specifically, eight (4 males and 4 females) healthy weaning York swine weighing 20-25 kg were used in this study. All the animals were fed by home-made breeding facilities for 2 weeks prior to the experiments, the rearing conditions are in compliance with national standards. The pigs had free access to drinking water and feed without any drugs. They were randomly divided into two groups. Each group comprised 4 swines (2 males and 2 females). In the first cycle group 1 was received a single i.v. injection of diclofenac sodium (concentration = 2.5 mg · kg^{-1}), while group 2 received a single i.m. injection (concentration = 2.5 mg · kg^{-1}).

The chromatographic separation of diclofenac and the IS was achieved with an Agilent1260 HPLC system (Agilent Technologie), which consisted of a high dual-gradient pump, an autosampler equipped with a 5 μm loop, a VWD detector, a vacuum degasser, and a Thermo Hypersil column (250×4.6 mm; Thermo, USA). The mobile phase consisted of 1.5% acetic acid (A) and methanol (B), and the flow rate was 1.0 mL · min^{-1}. The injection volume was 20 μL. The VWD detection wavelengths at maximum absorbance of diclofenac at 275 nm were chosen.

Blood samples (5 mL) were collected from the precaval vein at the following time intervals after i.v. injection: 0; 0.08; 0.25; 0.5; 1; 1.5; 2; 3; 4; 6; 9; 12; 24; and 36 hours. Similarly, blood samples were drawn at the following time intervals after the i.m. injection: 0.25; 0.5; 1; 1.5; 2; 3; 4; 6; 9; 12; 24; and 36 hours. After centrifugation, blood samples were frozen at -20°C for further analysis with HPLC. Briefly, 0.5 mL of plasma samples was initially placed into a 10 mL glass test tube to which 20 μL of IS working solution was added; the mixture was under eddying for approximately 1 min. Subsequently, 0.1 mL of 1 M hydrochloric acid was added and eddied for 30s, followed by the addition of 5 mL ether and eddying for 2 min. After free standing for 10 min, the ether layer was separated, 4 mL of which was transferred to a 10 mL glass test tube, followed by evaporation at 37°C under nitrogen gas. The residue was redissolved with 100 μL of the mobile phase, then transferred to 1.5 mL dactylethrae, followed by centrifugation at 13,000 r · $min^{-1}$for 10 min. The resultant sample was filtered through 0.22 μm Millipore filters.

Stock solutions of diclofenac (1000 μg · mL^{-1}) were prepared in methanol wrapped with aluminum plasma foil and stored at 4°C by wrapping with aluminum foil. The working solutions of diclofenac at concentrations of 0.625, 1.25, 6.25, 12.5, 25, 62.5, 125, 250, 500, and 1000 μg · mL^{-1} were prepared by serial dilutions with 70% methanol. The IS working solution (3 mg · mL) was also prepared by diluting IS stock solution (30 mg · mL) with 70% methanol.

The calibration standards were prepared fresh and assayed on the same day using 5 mL of diclofenac at concentrations of 0.025, 0.05, 0.25,0.5, 1, 2.5, 5, 10, 20, and 40 μg · mL^{-1} by spiking blank plasma. Diclofenac was identified and quantified by comparing the retention time and absorption spectra. This assay was repeated for five consecutive days with freshly prepared calibration standards.

The non-compartmental pharmacokinetic analysis of diclofenac concentrations versus time date was calculated using DAS 2.1.1 software. The data are expressed as the mean ± standard error (SD) and analyzed using Excel software. The doses for i.m. and i.v. diclofenac sodium injection were the same. The F (absolute bioavailability) was calculated as follows: $F = (AUC_{i.m.}/AUC_{i.v.})\times 100\%$.

Results and Discussion

The mean plasma concentration-time profiles of diclofenac are shown in Fig. 1 and Table 1. The pharmacokinetic parameters of diclofenac are presented in Table 2. The elimination half-lives ($T_{1/2\beta}$) for the i.v. and i.m. injections were 1.32±0.34 h and 1.87±0.70 h, respectively. The mean $AUC_{0-\infty}$ for the i.v. and i.m. injections were 55.50±5.50 μg · mL^{-1} · h and 43.17±7.77 (μg · mL^{-1}) · h, respectively. The absolute bioavailability of the 5% diclofenac sodium injection was calculated to be 78.29±14.81% according to the formula, $F = (AUC_{i.m.}/AUC_{i.v.})\times 100\%$.

Diclofenac was administered i.v. and the combined results shown in Table 2 indicate a rapid elimination process of diclofenac in swine. The main pharmacokinetic parameters of diclofenac sodium injection in swine after a single i.m. injection signify its high absolute bioavailability.

The short elimination half-life of diclofenac has been established previously, including 1.3 h for dogs (Tsuchiya et al. 1980), 2 h for rabbits (Said et al. 1981), 2.4 h for miniature swine (Oberle et al. 1994), 2.35 h for camels (Wasfi et al. 2003), and 1.1 h-1.8 h for humans (Willis et al. 1979), but 15 h for rats (Torres-Lopez et al. 1997). The differences were presumably due to species diversity. The apparent distribution volume (V_d) was 0.50±0.05 L · kg^{-1}, signifying a narrow diclofenac distribution in swine. This result was associated with the high binding ratio (95%-99%) of non-steroidal anti-

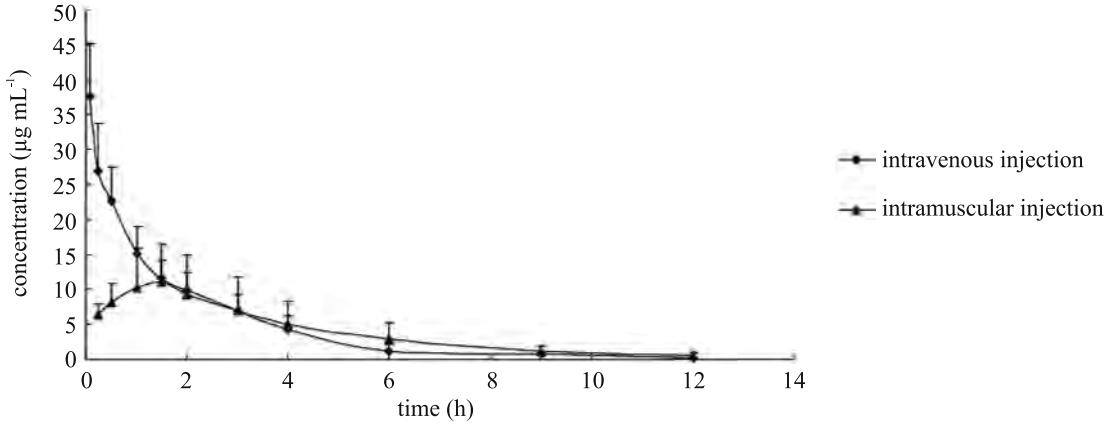

Fig. 1 Mean plasma concentration-time profiles of diclofenac via intravenous and intramuscular injections in swine.

Table 1. Plasma drug concentration of diclofenac sodium in swine after i.v. and i.m. administration.

Time (h)	Concentration in plasma	
	Intravenous administration μg · mL^{-1}	Intramuscular administration μg · mL^{-1}
0.08	37.66±7.53	–
0.25	26.90±6.86	6.43±1.46
0.5	22.61±4.99	8.06±2.79
1	15.19±3.80	10.28±5.59
1.5	11.57±2.49	10.98±5.47
2	9.98±2.52	9.35±5.65
3	6.98±2.35	6.99±4.81
4	4.33±1.88	5.13±3.24
6	1.21±0.90	2.89±2.34
9	0.68 (n=1)	1.20±0.72
12	0.10 (n=1)	0.63±0.41
24	ND	0.31±0.28
36	–	ND

ND – Not detected; – No sample

Table 2. Pharmacokinetics parameters of diclofenac sodium injection in swine after intravenous or intramuscular administration with a concentration of 2.5 mg · kg.

Parameters	Unit	Intravenous administration	Intramuscular administration
$T_{1/2\beta}$	h	1.32±0.34	1.87±0.70
T_{max}	h	–	1.19±0.26
C_{max}	μg · mL^{-1}	–	11.61±5.99
AUC	(μg · mL^{-1}) · h	55.50±5.50	43.17±7.77
MRT	h	1.60±0.28	2.864±0.64
CL_B	L · (h · kg)$^{-1}$	0.26±0.04	–
V_d	L · kg^{-1}	0.50±0.05	–

$T_{1/2\beta}$ – Elimination half-time, T_{max} – Peak time, C_{max} – Peak concentration, *AUC* – Area under the curve, *MRT* – Mean reaction time, CL_B – Body clearance, V_d – Apparent distribution volume, – No data

-inflammatory drug (NSAID) plasma proteins, which enabled the accumulation of drugs in inflammatory exudation sites. As inflammatory exudates originate from plasma and are rich in plasma proteins, the drug concentration in acute inflammatory sites was generally higher than in plasma after binding. By virtue of drug accumulation in exudates, a favorable anti-inflammatory effect can be retained even when the drug concentration of NSAIDS decreased to a level lower than the effective drug concentration.

Although the biological half-time of diclofenac sodium was short, the plasma drug concentration was high and prone to accumulate in inflammatory sites. A previous *in vivo* trial has revealed that the optimal inhibition time of prostaglandin synthesis would be later than the appearance of plasma peak concentration (Jim et al. 2012), thus indicating the duration of the pharmaceutical effect was longer than estimated from the plasma drug concentration. Therefore, the combined pharmacokinetic characteristics indicate that diclofenac sodium injection exerts powerful anti-inflammatory, analgesic, and anti-pyretic effects with a long duration time.

Conclusion

Based on the recommended dose derived from previous clinical trials, this work focused on an estimation of pharmacokinetic characteristics of diclofenac sodium injection depending on site in swine, and provide an experimental basis for new veterinary drug registration and reasonable clinical use. The study has shown that a diclofenac sodium injection gives rise to rapid absorption and elimination, a high peak concentration and bioavailability in swine, which is believed to be beneficial for adjuvant treatment of febrile, inflammatory pain in this species.

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