NOTE *Pharmacology*

Pharmacokinetics of a Florfenicol-Tylosin Combination after Intravenous and Intramuscular Administration to Beagle Dogs

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ABSTRACT. A pharmacokinetic study of a commercial florfenicol-tylosin (2:1) combination product was conducted in six beagle dogs after intravenous (IV) and intramuscular (IM) administration at doses of 10 mg/kg (florfenicol) and 5 mg/kg (tylosin). Serum drug concentrations were determined by a validated high performance liquid chromatography (HPLC) using UV detection. A rapid and nearly complete absorption of both drugs with a mean IM bioavailability of 103.9% (florfenicol) and 92.6% (tylosin), prolonged elimination half-life, and high tissue penetration with steady state volume of distribution of 2.63 *l*/kg (florfenicol) and 1.98 *l*/kg (tylosin) were observed. Additional studies, including pharmacodynamic and toxicological evaluation are required before recommendations can be made regarding the clinical application of the product in dogs.

KEY WORDS: canine, combination, florfenicol, pharmacokinetics, tylosin.

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Florfenicol is a broad-spectrum antibiotic belonging to the family of agents that includes thiamphenicol and chloramphenicol [22]. Its equal or even better efficacy, lower toxicity, and less development of resistance as compared with chloramphenicol made florfenicol an attractive antibiotic for use in various animals [4]. The pharmacokinetics of florfenicol has been reported in various animals, including camel, cattle, dog, primates, goat, pig, rabbit, sheep, and aquatic species [1, 4, 14–17, 19].

Tylosin, a macrolide antibiotic with bacteriostatic action against certain gram-positive and anaerobic bacteria, *Mycoplasma* spp. and some *Rickettsiae* [11], is registered exclusively for veterinary use in several countries. The pharmacokinetics of tylosin has been reported in many species, including pigs, buffaloes, cattle, sheep, goats, and dogs [2, 18, 20, 23, 26].

The rationale for a combination therapy with antimicrobial agents is the pharmacodynamic or pharmacokinetic interactions, leading to improved efficacy or safety profiles, compared with the single components [7]. Furthermore, combination therapy is considered to be a potentially effective means of minimizing the emergence rate of bacterial resistance. Among the frequently used combination products in veterinary area are amoxicillin/clavulinic acid, ampicillin/sulbactam, trimethoprim/sulfonamide, and ormetoprim/sulfadimethoxine combinations [9, 10]. Tylosin-sulfonamide combinations have also been used in small-animal practice to treat upper respiratory tract infections [11]. Florfenicol is combined with tylosin in a 2:1 ratio in a commercial preparation (FTD-inj, Shinilbiogen Co., Seoul, Korea). This ratio was selected because we found that it provided the best antibacterial effect against several pathogenic bacteria isolated from cattle, pigs and dogs (results not shown).

We have previously reported the intramuscular (IM) pharmacokinetics of the combination in pigs [13]. In the present study, we evaluated the disposition kinetics of florfenicol and tylosin after intravenous (IV) and IM injection of the combination product to dogs.

Pure standards of florfenicol and tylosin tartarate, and an injectable solution (FTD-inj, Shinilbiogen Co.) consisted of florfenicol 100 mg/m*l* and tylosin tartrate 50 mg/m*l* were used. All reagents used for extraction and analysis were analytical or high performance liquid chromatography (HPLC) grade. A cross-sectional study was conducted using six clinically healthy male beagle dogs aging 1 to 2 year and weighing between 8 and 10 kg. The study was approved by the bioethical committee of Kyungpook National University (Korea). Animals were kept in individual cages and fed commercial non-medicated feed. They were randomly divided into two groups. Three dogs received the product at the manufacturer-supplied dose level containing 10 mg/kg florfenicol and 5 mg/kg tylosin IV into the jugular vein, and the other three received the same dose IM into the inner thigh muscle. After fourteen days 'wash out' period, the route of administration was exchanged. This time was considered adequate because none of the two drugs could be detected in random serum samples of dogs collected one week after initial drug administration of both IV and IM routes. Furthermore, in our tissue residue study to be reported elsewhere (Korean Journal of Veterinary Research, Submitted) no detectable level of the two drugs was found in the serum or tissue samples of pigs slaughtered fourteen days after intramuscular injection of the combina-

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tion product. Blood samples were collected from the cephalic veins. By alternating the forelegs, a total of ten samples per animal were collected before and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hr after drug administration. Samples were stored immediately at room temperature for 20 min and then placed on ice to encourage clot retraction. Tubes were placed at 4°C for 12 hr. Serum was separated by centrifugation at 2,000 × *g* for 10 min and stored at –20°C until analysis by a validated HPLC. A Hewlett Packard 1100 system comprising an HPLC pump, HP octadecyl silica (ODS) column (200 × 4.6 mm; particle size, 5 μm), autoinjector and UV detector was used. The mobile phase for florfenicol detection comprised of 25% acetonitrile and 75% double-distilled water. No pH modifier was used. The flow rate was adjusted at 1 m*l*/min and was detected with a UV detector at a wavelength of 224 nm. Tylosin was eluted with a mobile phase of 37% acetonitrile and 63% phosphate buffer (KH_2PO_4, pH adjusted to 2.4 by adding hydrochloric acid). The flow rate was adjusted at 1 m*l*/min and the wavelength of the UV detector was set at 282 nm. The retention times of florfenicol and tylosin were approximately 6.2 and 7.3 min, respectively. No interfering peaks in all blank samples were noted in the elution position of either drug. A linear relationship existed in the calibration curves of peak area and peak height versus concentration of both florfenicol and tylosin at a range of concentration between 0.05 and 100 μg/m*l*. Calibration curves of peak area versus concentration were used to determine the serum concentration of each drug. Florfenicol recovery from serum ranged from 96 to 98%, and the inter-day precision (coefficient of variation, CV%) was 5–17.5%. Tylosin recovery from serum ranged from 94 to 98%, and the inter-day precision (CV%) was 1–10% for the tested range of concentrations. The limit of detection and limit of quantitation were 0.015 and 0.02 μg/m*l* for florfenicol, and 0.03 and 0.04 μg/m*l* for tylosin.

The pharmacokinetic analysis of serum concentration-time data for both florfenicol and tylosin was done using WinNonlin professional program (Version 5.2, Pharsight Corporation, Mountain View, CA, U.S.A.) using non-compartmental and compartmental methods. The most appropriate model was selected based on the Akaike information criterion (AIC) values. The weighting scheme $1/y^2$ was used. The serum concentration-time data for florfenicol were best described by a one-compartment open model following both routes of administration. For tylosin, a two-compartment open model was found to best fit the data following both routes of administration. Pharmacokinetic terms are defined in Table 1. A non-compartmental analysis based on trapezoidal method was used to determine model-independent parameters, such as the area under the concentration-time curve (AUC) and the area under the first moment curve (AUMC) with extrapolation to infinity. Mean residence time (MRT) was calculated as MRT=AUMC/AUC, mean absorption time (MAT) as $MAT=MRT_{IM} - MRT_{IV}$, the systemic clearance (Cl) as Cl=Dose/AUC, and the absolute bioavailability as AUC_{IM}/

Table 1. Pharmacokinetic parameters (mean ± SD) of florfenicol (10 mg/kg) and tylosin (5 mg/kg) after intravenous (IV) and intramuscular (IM) administration of a florfenicol-tylosin combination to beagle dogs (n=6)

Parameters	Florfenicol		Tylosin	
	IV	IM	IV	IM
A (μg/m*l*)			3.01 ± 0.58	
B (μg/m*l*)			1.11 ± 0.21	
C_0 (μg/m*l*)			4.12 ± 0.39	
AUC (μg.hr/m*l*)	20.25 ± 3.69	21.15 ± 5.25	23.71 ± 6.70	22.1 ± 4.60
T_{max} (hr)		2.49 ± 0.61		3.00 ± 1.00
C_{max} (μg/m*l*)		2.86 ± 0.59		2.28 ± 0.16
$t_{(1/2)}$abs (hr)		1.13 ± 0.89		1.54 ± 0.91
$t_{(1/2)}\alpha$ (hr)			1.56 ± 0.46	1.87 ± 0.77
$t_{(1/2)}$eli (hr)	4.92 ± 1.14	4.58 ± 1.22	8.52 ± 1.53	7.70 ± 1.43
K_{12} (*l*/hr)			0.89 ± 0.12	0.21 ± 0.11
K_{21} (*l*/hr)			0.57 ± 0.06	0.14 ± 0.01
K_{12}/K_{21}			1.57 ± 0.46	1.51 ± 0.13
AUMC (μg.hr^2/m*l*)	107.31 ± 30.50	138.60 ± 38.30	234.8 ± 54.10	260.60 ± 67.91
MRT (hr)	5.31 ± 0.22	6.59 ± 1.21	10.72 ± 0.18	11.80 ± 3.75
MAT (hr)		1.29 ± 0.82		1.72 ± 0.67
Cl (*l*/kg/hr)	0.49 ± 0.19		0.23 ± 0.08	
Vss (*l*/kg)	2.63 ± 0.12		1.98 ± 0.53	
F (%)		103.91 ± 11.60		92.63 ± 12.40

A, B, Y-axis intercept terms for distribution and elimination phases; C_0, serum concentration at zero time. AUC, area under the serum concentration versus time curve from time zero to infinity; T_{max}, time of maximum observed concentration; C_{max}, maximum observed concentration; $t_{(1/2)}$abs, absorption half-life $t_{(1/2)}\alpha$, distribution half-life; $t_{(1/2)}$eli, elimination half-life; K_{12}, rate constant for passage from central to first peripheral compartment; K_{21}, rate constant for passage from first peripheral to central compartment; AUMC, area under the first moment curve; MRT, mean residence time; MAT, mean absorption time; Cl, total body clearance; Vss, volume of distribution at steady-state; F, bioavailability.

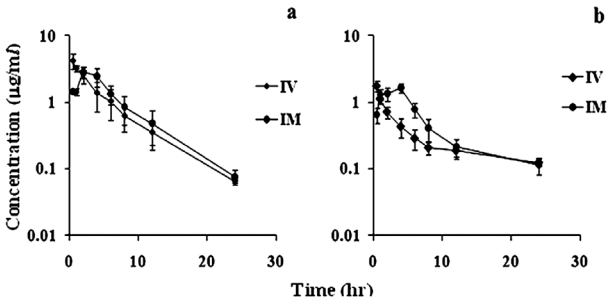

Fig. 1. Semi-logarithmic plot of serum drug concentration versus time for florfenicol (a) and tylosin (b) after intravenous (IV) or intramuscular (IM) administration of a 2:1 florfenicol-tylosin combination product. Values represent mean ± SD (n=6).

$AUC_{IV} \times 100\%$.

The pharmacokinetic parameters calculated for florfenicol and tylosin after IV and IM injection are presented in Table 1. Semi-logarithmic plots of the serum concentration versus time are shown in Fig. 1. After IV injection, the total body clearance and steady state volume of distribution were 0.49 *l*/kg/hr and 2.63 *l*/kg for florfenicol, and 0.23 *l*/kg/hr and 1.98 *l*/kg for tylosin. Mean peak serum concentrations of 2.86 μg/m*l* (florfenicol) and 2.28 μg/m*l* (tylosin) were reached at 2.49 and 3 hr, respectively after IM injection. The mean IM bioavailability was 103.9% for florfenicol and 92.6% for tylosin. The mean terminal half-lives obtained after IV and IM injection were 4.92 and 4.58 hr for florfenicol and 8.52 and 7.70 hr for tylosin, respectively.

After extravascular administration, florfenicol had a high bioavailability in various animals, including pigs, broiler chickens, dogs and aquatic species [12, 17, 19, 22]. Consistently, a rapid and nearly complete absorption of florfenicol was observed in our study after IM injection of the combination product to beagle dogs. However, the IM bioavailability of florfenicol in beagle dogs was higher than that of cattle, horses, camels, sheep, goats and rabbits [1, 14–16]. Florfenicol had a high volume of distribution in beagle dogs indicating its extensive distribution in well-perfused tissues after IV injection. The elimination half-life of florfenicol in our study (4.58 and 4.92 hr after IV and IM injection) was longer compared to a previous report in dogs (1.11 and 1.24 hr after IV and IM injection) [17]. This is consistent with the higher volume of distribution (2.63 versus 1.45 *l*/kg) and a slower clearance (0.49 versus 1.43 *l*/kg/hr) observed in our study. The differences may be attributed partly to the different commercial preparations and analytical methods used. Both florfenicol and tylosin bind to the same site of 50S subunit of ribosome in bacteria. Combinations of 50S subunit ribosomal inhibitors resulted in antagonism *in vitro* [7], although clinical reports demonstrating the *in vivo* relevance of these observations are scarce. The prolonged elimination half-life and mean residence time of florfenicol administered together with tylosin may have resulted from pharmacokinetic drug-interactions, in terms of altered protein binding or metabolism, or reduced renal clearance. However, this likely hypothesis is not supported by our present data and it should be validated in further *in vitro* and *in vivo* studies.

The efficacy and safety of florfenicol have been established in various animals [5, 8]. Although florfenicol is currently approved for use in cattle, pigs and some aquatic species like salmon and eel, it is not approved for use in dogs and no critical breakpoints have been defined for dogs [17]. However, its off-label use in a number of species and the issue of antibiotic resistance are common concerns that demand investigation to determine the value of this compound in other species [5, 8]. In this regard, the present study demonstrated a favorable pharmacokinetic profile of florfenicol in beagle dogs, in terms of rapid and complete absorption, extensive tissue distribution and prolonged elimination period.

We also investigated the disposition kinetics of tylosin after IV and IM injection of the combination product in dogs. The pharmacokinetics of tylosin was evaluated in dogs in an earlier study [24]. The high volume of distribution of tylosin obtained in our study (1.98 *l*/kg) is in agreement with previous reports in dogs, rats, cow, sheep and goats [3, 6, 23, 24], however, it is lower than that in pigs (14.6 *l*/kg) [18] and camels (11.9 *l*/kg) [26]. Tylosin is rapidly eliminated from blood after IM injection in different animal species, with the $t_{1/2}$ values ranging from 0.4 hr in rats [6] to 4.75 hr in sheep [23]. The elimination half life of tylosin in these dogs was longer than many species. In pigs, however, the tylosin elimination half-life exceeded 24 hr after IM injection of tylosin base at a dose of 10 mg/kg body weight [18]. The K_{12}/K_{21} ratio (>1) of tylosin administered with florfenicol also suggests that the drug moves from the central to the peripheral compartment at a faster rate than redistribution from the peripheral to the central compartment. The different formulation used here or possible pharmacokinetic interactions not addressed in this study may have contributed to the observed slow elimination of tylosin administered together with florfenicol to beagle dogs. The clinical application and safety of tylosin have also been established in dogs. For example, Scott *et al*. [21] reported a successful and safe treatment of staphylococcal pyoderma in dogs by administration of tylosin at 10 mg/kg body weight every 12 hr. In another study, treatment with tylosin eliminated diarrhea in dogs within 1–3 days [25]. Although it is rarely used parenterally in dogs and cats, the injectable form of tylosin is also approved in some countries, and oral tylosin is sometimes recommended for the treatment of chronic colitis in small animals. In the present study, good pharmacokinetic features for parental treatment of animals, including high bioavailability, good tissue penetration and prolonged duration of action of tylosin were observed in beagle dogs. However, this study has some potential limitations. First, we used here a single dose provided by the manufacturer, and the clinical relevance of the studied dose as well as possible pharmacokinetic interactions between the two drugs remain to be elucidated in further studies. Second, although we observed an additive antibacterial

activity of the combined drugs against some pathogens of dogs, including *Escherichia coli* and *Staphylococcus intermedius* (unpublished data), this should be validated in further *in vitro* and clinical studies. Future studies should also determine the presence and disposition kinetics of major metabolites of florfenicol or tylosin in dogs.

In conclusion, after IV and IM injection of the florfenicol-tylosin combination to beagle dogs, no overt adverse effects were observed. The pharmacokinetics of both drugs were characterized by a rapid and complete absorption, extensive tissue distribution and slow elimination. Additional studies, including pharmacodynamic and toxicological evaluation are required before recommendations can be made regarding the clinical application of the product in dogs.

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