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Pharmacokinetics of florfenicol after intravenous and intramuscular administration in New Zealand White rabbits

F. Koc [a,*], M. Ozturk [b], Y. Kadioglu [b], E. Dogan [c], L.E. Yanmaz [c], Z. Okumus [c]

[a] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Atatürk University, Erzurum 25400, Turkey
[b] Department of Analytical Chemistry, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey
[c] Department of Surgery, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

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ABSTRACT

The pharmacokinetic disposition and bioavailability of florfenicol (FF) were determined after single intravenous (i.v.) and intramuscular (i.m.) administrations of 25 mg/kg b.w. to ten healthy New Zealand White rabbits. Plasma FF concentrations were determined by high-performance liquid chromatography (HPLC). The plasma pharmacokinetic values for FF were best described by a one-compartment open model. The elimination half-life ($t_{1/2\beta}$) was different ($p < 0.05$) however, the area under curve (AUC) was similar ($p > 0.05$) after i.v. and i.m. administrations. FF was rapidly eliminated ($t_{1/2\beta}$ 1.49 ± 0.23 h), slowly absorbed and high (F, 88.75 ± 0.22%) after i.m. injection. In addition, FF was widely distributed to the body tissues (V_{ss} 0.98 ± 0.05 L/kg) after i.v. injection. In this study the time that plasma concentration exceeded the concentration of 2 μg/mL was approximately 6 h. For bacteria with MIC of 2 μg/mL, frequent administration at this dose would be needed to maintain the concentration above the MIC. However, it is possible that rabbit pathogens may have MIC values less than 2 μg/mL which would allow for less frequent administration. Further studies are necessary to identify the range of MIC values for rabbit pathogens and to identify the most appropriate PK-PD parameter needed to predict an effective dose.

1. Introduction

FF is a synthetic broad-spectrum antibiotic in veterinary treatment of infectious diseases. It is a novel antibiotic that belongs to the chloramphenicol (CP) family, but it is used only in animals (Papich and Riviere, 2001). The compounds act by inhibiting bacterial protein synthesis by binding to 50 S and 70 S subunits in the ribosomes (Cannon et al., 1990). Moreover, FF prevents bacterial enzymatic acetylation; consequently, this product has more antibacterial activity than CP and TP (Cannon et al., 1990). Because it is both activity against CP-resistant pathogens (Neu and Fu, 1980; Syriopoulou et al., 1981) and low adverse effects (Paape et al., 1990) it has been widely used in veterinary clinics to treat bacterial diseases (Booker et al., 1997; Jim et al., 1999; Angelos et al., 2000).

The pharmacokinetic disposition of FF has been extensively investigated in calves, horses, poultry, pigs, fish and sheep (Varma et al., 1986; Adams et al., 1987; Mestorino et al., 1993; Martinsen et al., 1993; El-Banna, 1998; Soback et al., 1995; Lobell et al., 1994; Afifi et al., 1997; Li et al., 2002; Jianzhong et al., 2004) but in rabbits has scarcely been documented (El-Aty et al., 2004; Park et al., 2007). In this study, both used analytical method and dose were different from previous reported two studies.

The aim of this study was to determine FF (25 mg/kg b.w.) kinetic disposition in plasma and bioavailability after i.m. and i.v. administration in healthy New Zealand White rabbits.

2. Materials and methods

2.1. Reagents and instruments

All solvents were HPLC grade and obtained from J.T. Baker (Deventer, Netherlands). FF (99.6% assay purity) and CP (99.7% assay purity) analytical standards and injectable formulation (Nuflor, 300 mg/mL, was commercial preparation of FF) were provided by the Schering-Plough (Segre, France).

HPLC was supplied from a Thermoseparations Spectra Series. The HPLC system consisted of a P 4000 gradient pump coupled with a Spectra System UV 6000 LP photodiode array detection system, Thermoseparations AS 3000 autosampler and software Chromoquest 30. The column was reverse phase nucleosil C_{18} (4.6 × 250 mm, 5 μm particle size) and it was supplied from Phenomenex (CA, USA).

* Corresponding author. Tel.: +90 442 631 4193x1007; fax: +90 442 631 4188.
E-mail address: feridekoc@yahoo.com (F. Koc).

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2.2. Animals and experimental design

Ten healthy rabbits (New Zealand White) approximately 6–12 months old were used in this study. Of ten rabbits, five animals were female (2.50 ± 0.41 kg), five male (2.78 ± 0.35 kg). Before use, they were housed for two weeks in Experimental Research Centrum of Ataturk University. These animals were fed pelleted feed (antibacterial-free) and water *ad libitum*. Animal experiments were performed in an ethically proper way by following guidelines as set by the Ethical Committee of Ataturk University (Report No.: 03/2007).

The rabbits were divided into two groups ($n = 5$, each group). Before i.v. and i.m. administrations, the restrain devices were placed in the marginal vein each animal. The blood (2 mL) samples were collected from the restrain devices for control (at 0 min). Following, in group One, florfenicol was administrated single dose i.m. injection of 25 mg/kg b.w. into semimembraneous muscle. Blood samples were taken from restrain devices of each rabbit and collected in tubes containing heparin as anticoagulant at 5, 10, 15, 30, 45, 60, 90 min, 2, 4, 6, 8, 12, 18 and 24 h after drug administration. In group Two, single dose (i.v.) FF (25 mg/kg b.w.) was injected into right the marginal vein. Blood samples were taken from the restrain devices of each rabbit (opposite ear) and collected in tubes with anticoagulant (heparin) at the same times. Samples were centrifuged within 1 h after collection and plasma samples were stored frozen (−20 °C) until analysis. All the samples were analysed within one week after the experiments.

2.3. Analytical method

The chromatographic method was performed as described by Kowalski et al. (2005). CP was used as an internal standard in the analytical method.

The mobile phase consisted of a mixture of acetonitrile–water at a ratio of 25:75 (v/v) and adjusted to pH of 2.7 with 85% orthophosphoric acid. The injection volume was 20 μL, monitoring wavelength was at 224 nm, and oven temperature was at 24−25 °C and the flow rate was 1.5 mL/min.

Calibration curve was prepared in the range of 0.1−25.0 μg/mL ($n = 6$). CP (internal standard) was added to spike samples 0.5 mL at 10.0 μg/mL concentration.

2.4. Sample preparation

The extraction procedure was also achieved as described by Kowalski et al. (2005). Briefly, the frozen plasma samples (0.5 mL) were thawed at a temperature of 25 °C (room temperature) and added 0.2 mL 1.0 M sodium hydroxide and 3 mL of ethyl acetate. The mixture was mechanically shacked and centrifuged for 15 min at 1650*g*. The organic layer was evaporated at 50 °C under nitrogen and supernatant was dissolved in 0.5 mL of mobile phase. Following again it was centrifuged at 11,200*g* during 10 min and 20 μL volume was injected into HPLC system.

2.5. Validation

The following parameters were determined for validation of method: linearity, precision (RSD), accuracy, limit of detection (LOD), limit of quantitation (LOQ), recovery, reproducibility. For linearity, calibration curve was calculated automatically using software (0.1−25.0 μg/mL). The recoveries were calculated as the percentage using extraction process after spiking from 0.1 to 25.0 μg/mL with eight different levels of florfenicol ($n = 6$). The inter-and intra-day reproducibility was determined at 1, 5 and 10 μg/mL concentrations. LOD's were calculated on the basis to be 3 of signal-to-noise ratio spiking at low concentrations the plasma samples. LOQ was calculated to be 10 of signal-to-noise ratio.

2.6. Pharmacokinetic and statistical analysis

Florfenicol plasma concentration vs. time plot of each animal was analysed using the computer program WinNonlin version 4.1 (Pharsight, Mountain View, CA, USA). The proper model was chosen by minimum Akaike's information criterion estimation (Yamaoka et al., 1978). Pharmacokinetic parameters for each animal were analysed using by one-compartmental open model. The absorption half-life ($t_{1/2abs}$), the elimination half-life ($t_{1/2\beta} = 0.693/_{\beta}$), the total body clearance (Cl_{tot}) and the apparent steady-state volume of distribution (V_{ss}) and bioavailability ($F\% = AUC_{i.m}/AUC_{i.v.}$) were calculated. Areas under the plasma concentration–time curves for both i.v. ($AUC_{i.v\cdot 0\rightarrow\infty}$) and i.m. ($AUC_{i.m\cdot 0\rightarrow\infty}$) studies were calculated by the method of trapezoids. Peak plasma concentrations (C_{max}) of drug and times to reach peak concentration (t_{max}) for the i.m. study were determined from the individual plasma concentration–time curves.

All results are presented as mean ± SD. Harmonic means were calculated for $t_{½\beta}$ and MRT. Mann–Whitney U test was used to test for significant difference in $t_{½\beta}$. Comparison of AUCs was made using the Independent Samples *t*-test. Statistical significance was assigned at $p < 0.05$.

3. Results

The HPLC analysis for the determination of FF was high degree of reproducibility. Calibration curves had an r^2 value of >0.99. Intra-assay variations were determined by measuring six replicates ($n = 6$) of three standard samples used for calibration curves. The intra-assay variation coefficient was 6.29 ± 2.58. Inter-assay precision was determined by assaying the three standard samples on three separate days. The inter-assay variation coefficient was 6.90 ± 3.53. The average of inter- and intra-day precision (RSD) was <7. The accuracy ranged from 95% to 108% and mean recovery was 85 ± 6.33%. LOD and LOQ were found to be 0.03 and 0.1 μg/mL, respectively.

After i.v. and i.m. administration to rabbits at a single dose (25 mg/kg b.w.), plasma concentration of FF time curves were shown (Fig. 1).

The pharmacokinetic parameters for FF were shown in Table 1.

After i.v. administration, its $t_{1/2\beta}$, MRT, AUC, V_{ss}, and Cl_{tot}, of FF were 1.21 ± 0.09 h, 1.75 ± 0.12 h, 44.59 ± 1.38 μg h/mL, 0.98 ± 0.05 L/kg, and 0.56 ± 0.02 L/kg/h, respectively.

Following i.m. injection, its $t_{1/2\beta}$, $t_{1/2abs}$, AUC, t_{max}, C_{max} and F were 1.49 ± 0.23 h, 0.83 ± 0.24 h, 39.10 ± 10.12 μg h/mL, 1.56 ± 0.13 h, 8.65 ± 2.19 μg/mL, and 88.75 ± 0.22%, respectively.

4. Discussion

In this study, all rabbits were clinically healthy and there were not observed any side effects after administration of FF by a single dose i.m. and i.v.

The elimination half-life ($t_{1/2\beta}$) was different ($p < 0.05$) however, the area under curve (AUC) was similar ($p > 0.05$), after i.v. and i.m. administrations.

Following i.v. injection, $t_{1/2\beta}$ of FF in rabbits was 1.21 ± 0.09 h in the present study. This value was similar to 1.54 ± 0.20 h (El-Aty et al., 2004) and 0.90 ± 0.20 h (Park et al., 2007) in rabbits. However, this finding was shorter than in calves (3.18 ± 1.01 h), cattle (159 min), turkeys (2.34 ± 0.44 h), broiler chickens (173.25 min), turtles (from 2 to 7.8 h), (Decraene et al., 1997; Lobell et al., 1994; Switała et al., 2007; Afifi et al., 1997; Stamper et al., 2003).