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Research Article

PHARMACOKINETICS OF FLORFENICOL VIA NANOSUSPERSION AFTER ORAL ADMINISTRATION TO RABBITS

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ABSTRACT
The purpose of this study was to evaluate the pharmacokinetic behavior of florfenicol spray-dried nanosuspersion after oral administration to rabbits and to compare its oral bioavailability characteristics to that of a known soluble powder. Florfenicol spray-dried nanosuspersion was produced by high pressure homogenization followed by spray drying. Plasma concentrations were determined by HPLC. When administered at equal doses, the area under the plasma concentration (AUC $_{0-\infty}$) of florfenicol following intragastric administration of the spray-dried nanosuspersion and soluble powder were 36.02±5.49 μg· h· mL^{-1} and 29.35±4.65 μg· h· mL^{-1}; the C $_{max}$ was 9.83±1.22 μg/mL and 9.00±1.61 μg/mL; the $T_{1/2}$ were 2.95± 0.81 h and 3.08± 0.83 h, respectively. We conclude that the oral bioavailability of spray-dried nanosuspersion was 1.26-fold higher than that of soluble powder. Based upon the results of this investigation, the florfenicol spray dried nanosuspersion is a viable potentially veterinary preparation for the oral administration.

Keywords: *Florfenicol, Nanosuspersion, High Pressure Homogenization Method, Absorption, Pharmacokinetics*

INTRODUCTION
Florfenicol is a fluorinated derivative of chloramphenicol and thiamphenicol. It is of great value in veterinary treatment of infectious diseases, and control of bacterial respiratory tract infections in rabbits, cattle and other domestic animal (Soback *et al.,* 1995; Voorspoels *et al.,* 1999; Ali *et al.,* 2003; Park *et al.,* 2007). In food animals, florfenicol has been shown to be effective against bacteria such as *Pasteurella spp.* (Cannon *et al.,* 1990; Kim and Aoki, 1996; Marshall *et al.,* 1996), *Actinobacillus pleuropneumoniae* (Ueda *et al.,* 1995), *Mycoplasma mycoides* (Ayling *et al.,* 2000), *Staphylococcus aureus* (Marshall *et al.,* 1996), *Salmonella typhimurium* (Afifi and Aboel-Sooud, 1997; Booker *et al.,* 1997) and *Escherichia coli* (Cannon *et al.,* 1990; Marshall *et al.,* 1996).
However, many orally administered drug compounds present formulation problems related to poor water solubility. In recent years, nanoparticle engineering process has been seen as a promising approach for enhancing drug solubility and therefore product dissolution rate (Patravale *et al.,* 2004; Wong *et al.,* 2006; Kesisoglou *et al.,* 2007).
The pharmaceutical benefits of nanocrystals include improvement in formulation performance, such as enhanced dissolution velocity and saturation solubility, reproducibility of oral absorption, improved dose-bioavailability, proportionality and increased patient compliance via reduction of number of oral units to be taken (Shegokar and Müller, 2010).
They had also been used for drug targeting (Chavhan *et al.,* 2011). Drug particle size reduction leaded to an increase in surface area, resulting in a high stickiness to gastrointestinal mucosa after oral administration, and causing a lower rate of elimination (Lenhardt *et al.,* 2008). Therefore, the nanosuspension can largely improve the bioavailability not only because of the increased saturation solubility, but also the high dispersion property and larger surface area. Moreover, the form of nanosuspensions was found to be a stable product. (Peters *et al.,* 2000) Nanosuspensions can be produced by high pressure homogenizers (Rabinow, 2004; Keck and Muller, 2006; Kocbek *et al.,* 2006; Kesisoglou *et al.,* 2007). This system can also be used to improve the bioavailability of poorly soluble compounds for enhancing the dissolution profiles of these compounds.

In our study, a new florfenicol powder formulation was prepared by high pressure homogenization method following by spray drying. This technology was applied to florfenicol in an effort to improve its oral bioavailability. The oral bioavailability of this product was evaluated relative to that of a marked medicated soluble powder in rabbits.

MATERIALS AND METHODS

Materials

Polyvinylpyrrolidone (PVP) K30 was provided by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Heparin was purchased from Shanghai Pharmaceutical Co. Ltd (Shanghai, China). Acetonitrile (HPLC grade) was obtained from Tianjin Kermel Chemical Reagent Co. Ltd (Tianjin, China). Shimadzu instrument consisted of a LC-20 A pump and SPD-20 A UV/VIS detector (Shimadzu, Kyoto, Japan) and the chromatographic column was a Kromasil C-18 (5 μm, 250× 4.6 mm). All other reagents were of analytical grade.

Preparation of Florfenicol Nanosuspension

Florfenicol nanosuspension was prepared by the high pressure homogenization method followed by spray drying. The amount of florfenicol was dissolved in N, N-dimethylformamide and then poured into 20 mL of 0.8% aqueous solution of tween 80 and stirred for 5 min.

The resulting dispersion was diluted with 50 ml of 1%PVP K30 solutions and passed through a high-pressure homogenizer to form florfenicol nanosuspension. It was subsequently was spray dried under the following conditions: inlet temperature, 150 °C; outlet temperature, 90 °C; aspiration, 80%; feeding rate of the suspension, 5 mL/min.

Characterization of Nanoparticles

Particle Size Analysis

Particle size distribution was analyzed by photon correlation spectroscopy (PCS) at 25 °C with a NICOMP particle sizing system at a fixed angle of 90° yielding the mean particle diameter of the suspension. The diameters were calculated using volume distribution.

Dissolution Study

In vitro dissolution behavior of the florfenicol nanosuspersion was studied by paddle method. The rotation speed of the paddles was set to 100 rpm and 900 mL of distilled water at 37± 0.5 °C was used as dissolution medium. Samples were withdrawn at predetermined time intervals and equivalent amounts of distilled water were added. Florfenicol in samples were analyzed by HPLC (Zhu and Zhao, 2000). The mobile phase consisted of a mixture of acetonitrile–distilled water at a ratio of 40:60 (v/v) using a C-18 reverse phase column. The injection volume was 20 μL; flow rate was set at 1.0 mL/min; the UV detector was operated at a wavelength of 223 nm.

Pharmacokinetic Studies in Rabbits

Experimental Animals

Pharmacokinetics was studied in Healthy male rabbits (weighing 2.46 ± 0.30 kg). Each animal was housed in stainless grid cages for two weeks. The cage set-up prevented coprophagia, which otherwise could have biases the PK study results. The room temperature and humidity were controlled 22 ± 2 °C and 60 ± 5%. Animal experiments were performed in an ethically proper way by following guidelines as set by the Ethical Committee of Hebei University.

Experimental Design

Florfenicol spray-dried nanosuspersion and florfenicol soluble powder were both dispersed with distilled water for oral pharmacokinetic study. The rabbits were randomly divided into two groups, and were orally administrated of florfenicol soluble powder or spray-dried nanosuspersion respectively (at a drug dose of 25 mg/kg body weight). The animals were fed with standard rabbit diet and water ad libitum. Blood samples were taken from preplaced intravascular catheters of each rabbit and collected in tubes containing heparin as anticoagulant at 0, 10, 20, 30, 45, 60, 90, 120, 240, 360, 480 and 720 min after drug administration. Samples were centrifuged after collection and plasma samples were stored at -20 °C until analysis.

Sample Preparation

Frozen plasma samples were thawed at room temperature. Chloramphenicol was added to a of plasma sample to provide an internal standard, after which 3 mL ethyl acetate and 0.5 mL phosphate buffer (pH 7.0) were added. After vortexing for 5 min, the mixture was centrifuged for 20 min. The ethyl acetate supernatants were evaporated to dryness at 40 °C under nitrogen and supernatant was dissolved in 0.2 mL of mobile phase. An aliquot of the clear supernatant (20 μL) was used for HPLC analysis. The mobile phase consisted of a mixture of acetonitrile–distilled water at a ratio of 40:60 (v/v) using a C-18 reverse phase column. The injection volume was 20 μL; flow rate was set at 1.0 mL/min; the UV detector was operated at a wavelength of 223 nm.

Linearity was calculated to base upon the freshly prepared spiked plasma samples within a concentration range of 0.2-20 μg/mL. Precision was tested by intra-day and inter-day repeatability, and assessed by multiple analyses of the plasma samples at three concentration levels (0.4, 2 and 20 μg/mL). The accuracy of florfenicol was determined by calculating the recovery at 0.4, 2 and 20 μg/mL. The specificity of the method was evaluated by analyzing blank plasma samples from rabbits.

Pharmacokinetic Analysis

The data analysis was performed by the 3P87 computing program (produced by the Committee of Mathematic Pharmacology of the Chinese Society of Pharmacology). K was estimated by the linear regression of Ln C/time profile (where Ln C is the log-normal transformed plasma concentration). The terminal elimination half-life ($T_{1/2}$) was calculated by $T_{1/2}$= 0.693/K. Peak observed concentrations (C_{max}) of drug and times to reach peak plasma concentration (T_{max}) were determined from the individual plasma concentration–time curves. The total area under the concentration–time curve (AUC) was calculated by the method of trapezoids. The area from time zero to infinity was calculated by $AUC_{0-\infty}$ = AUC_{0-t} + Ct /K. AUMC was the area under the first moment of the plasma concentration-time curve, calculated by $AUMC_{0-\infty}$ = $AUMC_{0-t}$ + Ct /K. Mean residence time was MRT, where MRT= AUMC/ AUC.

RESULTS AND DISCUSSION

Results

The Dissolution

The dissolution profiles of the spray-dried florfenicol nanosuspersion, in comparison with the drug soluble powder in water, were shown in Figure. 1. The dissolution rate was markedly enhanced in the nanosuspersion, as more than 90% of the drug dissolved in 15 min, as same to that of soluble powder.

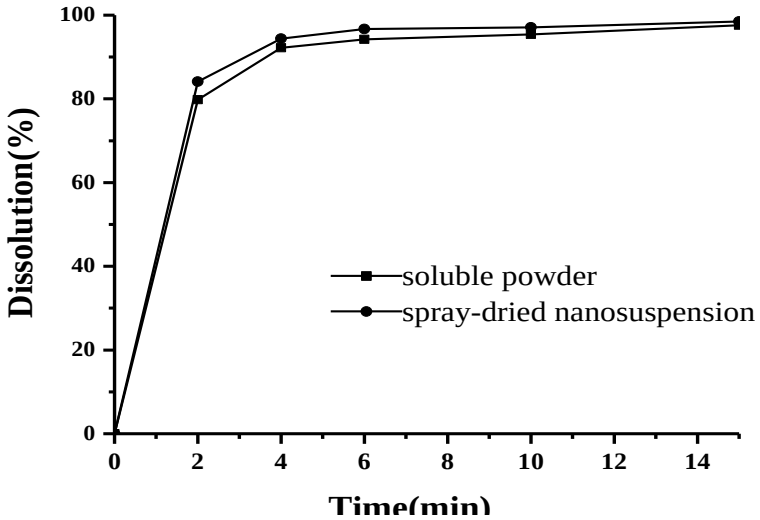

Figure 1: Dissolution profiles of florfenicol spray-dried nanosuspension and florfenicol soluble powder in distilled water (*n* = 6)

Pharmacokinetic Analysis

The plasma samples were stored at -20 °C for three days prior to analysis. Calibration curves were linear in the concentration ranges of 0.2-20 μg/mL, R^2=0.993. The mean recoveries for florfenicol were 92.3±5.7%, 95.4±6.3%, and 92.5±4.7% at the 8, 10, and 12 μg/ mL concentrations, respectively. The interday accuracy of the drug containing serum samples was more than 93.5% with a precision of 2.6–4.8%. The intraday accuracy was more than 92.3% with a precision of 3.9–6.0%. Results indicated that there was good reproducibility and accuracy for the determination of florfenicol for samples determined on the same or different days.

The pharmacokinetic parameters of the two formulations could both be described using non-compartmental analysis (Table 1) and the pharmacokinetic profiles were shown in (Figure 2). C_{max} value for florfenicol in spray-dried nanosuspension was found to be 9.83±1.22 μg/mL, whereas C_{max} value for soluble powder was found to be 9.00±1.61 μg/mL. The $T_{1/2}$ was 2.67±0.19 h for florfenicol in spray-dried nanosuspension, and 2.82±0.27 h for soluble powder. $AUC_{0-\infty}$ of florfenicol after oral administration for soluble powder was 29.35±4.65 μg· h· mL^{-1}. The AUC of florfenicol for spray-dried nanosuspension was 36.02±5.49 μg· h· mL^{-1}, which was 1.26 times greater than that of soluble powder.

Table 1 Pharmacokinetic parameters after oral administration of florfenicol to rabbits (a) spray-dried nanosuspension, (b) soluble powder (n = 6)

Parameter	Soluble powder	Spray-dried nanosuspension
Ke（h^{-1}）	0.25±0.02	0.26±0.02
$T_{1/2}$（h）	2.82±0.27	2.67±0.19
$AUC_{0-\infty}$（μg·h· mL^{-1}）	29.35±4.65	36.02±5.49
$AUMC_{0-\infty}$（μg·h^2· mL^{-1}）	116.31±19.35	136.96±18.43
Cmax(μg/ml)	9.00±1.61	9.83±1.22
MRT（h）	3.97±0.24	3.81±0.21
Fr		1.26±0.30

Ke, elimination constant; $T_{1/2}$, elimination half-life; $AUC_{0-\infty}$, the area under the plasma concentration time curve; $AUMC_{0-\infty}$, area under the moment curve; Cmax, maximum plasma concentration; MRT, mean residence time; Fr, relative bioavailability.

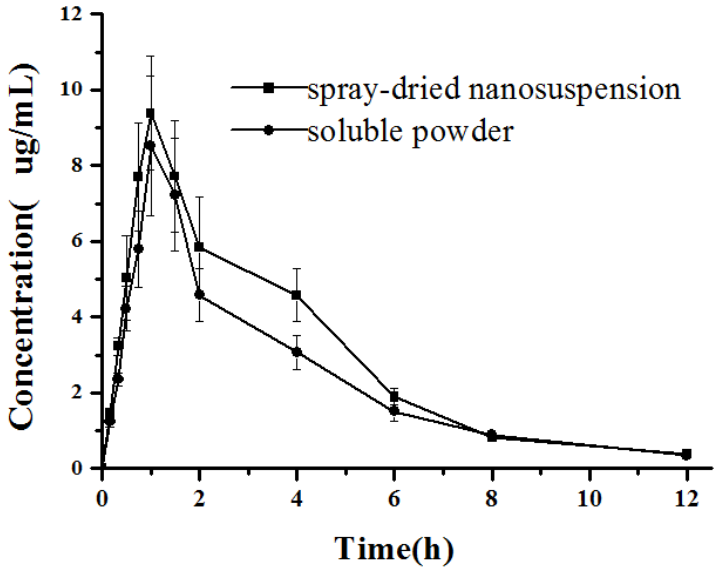

Figure 2: Plasma concentrations–time curves of florfenicol after oral administration to rabbits (a) spray-dried nanosuspension, (b) soluble powder (n = 6)

Discussion

In this study, all rabbits were clinically healthy and there were not observed any side effects after administration of florfenicol by a single dose oral administration. $T_{1/2}$ was similar ($p > 0.05$), which showed the spray-dried nanosuspersion had not changed the in vivo drug elimination behavior. However, the C_{max} was higher in spray-dried nanosuspersion than that obtained with soluble powder ($p < 0.05$), and spray-dried nanosuspersion also showed a much higher AUC. The relatively greater bioavailability suggested better absorption for spray-dried nanosuspersion than that of soluble powder. These differences in parameters may be due to difference of dosage forms, which nanosuspersion can enhance dissolution velocity and saturation solubility, reproducibility of oral absorption, improved dose-bioavailability.

Compared with the data reported by Abd El-Aty *et al.*, (2004) and Park *et al.*, (2007), the dose used was not the same.

There was 30 mg/kg and 20 mg/kg, while in our study was 25 mg/kg. AUC was 49.02±13.13 μg/mL· h^{-1} at the oral dose of 30 mg/kg, while AUC was 29.35±4.65 μg/mL· h^{-1} and 36.02±5.49 μg/mL· h^{-1} for soluble powder and nanosuspersion at the oral dose of 25 mg/kg in our study. Taking into account with different oral dose, we increased the dose to 30 mg/kg (1.2 times higher than 25 mg/kg), if the AUC and the dose was in linear relation, our AUC data maybe increase to 34.8μg/mL· h^{-1} and 43.2μg/mL· h^{-1}. It showed lower bioavailability as compared to this citation, but our AUC data was higher than that of 20 mg/ kg for oral route in rabbits compared as above, we reduced the dose to 20 mg/kg (0.8 times lower than 25 mg/kg), if the AUC increased linearly with dose, our AUC data maybe reduce to 28.2 μg/mL· h^{-1} which was higher than 23.78 μg/mL· h^{-1}. However, AUC and does actually were not linearly associated. In addition, there was individual variance in the rabbits.

Therefore, the formulation had not sub-optimal absorption properties. It was acceptable in pharmacokinetic study. Following oral administration, the $T_{1/2}$ of florfenicol in rabbits was 2.67±0.19 h in the present study. After 12 h oral administration of spray-dried nanosuspersion, the drug still reached 0.46±0.14 μg/ml. The higher drug concentration and maintenance time contributed to a good effect for the control of bacterial infections.

The solubility of florfenicol was very low resulting in a low and incomplete in vitro dissolution with a low bioavailability. Nanosuspensions were one of the most successful strategies to improve drug release of poorly soluble drugs. Consequently, in our study, florfenicol was prepared to nanosuspension and converted to powder by spray-drying. From these pharmacokinetic results, we can conclude that florfenicol absorption was enhanced by nanosuspension technology compared with a florfenicol soluble powder. Florfenicol spray-dried powder, by way of the attainment of high blood concentrations, could acquire a better therapy than that of florfenicol soluble powder. Nanosuspensions consequently represented a promising alternative to current delivery systems aiming to improve the pharmaceutical performance of drugs with low water solubility.

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