Preparation and Evaluation of Florfenicol Nanocrystals for in Vivo Intestinal Absorption

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Abstract: Initiatives to avoid or reduce the use of antibiotics have a positive significance, and maximizing the retention of the effectiveness of existing antibiotics is one of the effective measures that can be taken in the field of food animal husbandry. A "top-down" method was used to prepare florfenicol nanocrystals, which appeared as irregular prismatic structures under electron microscopy. The particle size measured by laser diffraction was (291.9±2.9) nm, and the zeta potential value was - (23.63±1.64) mV. The absorption results in vivo showed that florfenicol was absorbed in the duodenum, jejunum, ileum, and colon, but primarily in the duodenum. The effective permeability coefficient (Peff) of florfenicol increased to 1.8 times that of the bulk drug group after being prepared as nanocrystals. The above research results indicate that nanocrystal technology has a promoting effect on the intestinal absorption of florfenicol.

Keywords: Florfenicol; Nanocrystals; In Vivo Evaluation; Intestinal Absorption

1. Introduction

In food-borne animal farming, low-dose and long-term administration of antibiotics can achieve the effects of growth promotion, disease prevention, and improved feed conversion ratio [1-3]. Long-term use of antibiotics produces drug-resistant bacteria, which can pose a fatal threat to the ecological environment and human health and safety, and it has become an international consensus to regulate the use of antibiotics [4,5]. The annual domestic consumption of antibiotics is 1/2 of the global total, and 52% of this is in agriculture and animal husbandry, where chronic or inappropriate use of antibiotics greatly contributes to the development of antibiotic tolerance[6,7]. Initiatives to avoid or reduce antibiotic usage have positive implications, and maximising the effectiveness of antibiotics is one of the effective means that can be adopted in the field of food-borne animal farming [8]. In this study, nanotechnology will be used to nanosize the difficult-to-solve drug florfenicol, thereby improving its in vivo absorption and prompting its application in livestock and poultry farming for feeding, which will provide support for "substitution of antibiotics and reduction of antibiotics" in China's animal husbandry industry [9-11].

2. Instruments Drugs and Laboratory Animals

JEOL JEM-1400 transmission electron microscope (Nippon Electron Co., Ltd.), Delsa Nano C nanoparticle size/zeta potential analyser (Beckman Coulter Scientific Instruments Co., Ltd., U.S.A.), IKARCT Basic Magnetic Stirrer (Eckhardt Instrument Co., Ltd., Germany), MEZ240E/02 Electronic Balance (METTLER TOLEDO (Shanghai) Co., Ltd.), Agilent 1200LC High Performance Liquid Chromatograph (Agilent Technologies (China) Co., Ltd.), HL-2B Peristaltic Pump (Gongyi IYUHUA Instrument Co., Ltd.), TGL-20B Centrifuge (Shanghai Anting Scientific Instrument Factory), 0.5mm zirconia beads (Hunan Ruichen Ceramics Co., Ltd.), vortex mixer (IKA, Germany), refrigerated high-speed centrifuge (Shanghai Anting Scientific Instrument Factory), medical silk braided wire (Shanghai Pudong Jinhuan Medical Supplies Co., Ltd.). WPDI608B, Shanghai Changwei Pharmaceutical Excipients Technology Co.), heparin sodium (Shanghai McLean Biochemical Technology Co., Ltd.), zirconia beads (diameter 0.2 mm, 0.5 mm, 1.0 mm, Hunan Ruichen Ceramics Co.)

Healthy male SD rats 300 g were purchased from Guangdong Provincial Medical Laboratory Animal Centre, and the relevant studies followed the basic principles of animal experiment ethics.

3. Methods and Results

3.1 Preparation and Evaluation of Florfenicol Nanocrystals

Add 300 mg of florfenicol API, 15 mg of hydroxypropylmethylcellulose and 15 mg of sodium dodecyl sulphate to 10 mL of deionised water, put them into a conical flask containing 0.5 mm zirconium oxide beads of 50 g, 4 cm magnetic stirrer and control the rotation speed of 700 r·min-1 stirring at room temperature for 8 h, so as to get the nanohybrid suspension. The nanosuspension was dried by spray drying to obtain florfenicol nanocrystals.

3.1.1 Appearance

The nanocrystals prepared by the above method are shown in Figure 1., the florfenicol nanocrystals were loose white powder, a small amount of the sample was taken and dissolved in distilled water, the nanocrystals could be dispersed rapidly and uniformly in a shorter period, and there was no precipitate present after shaking (Figure 2A), whereas the dissolution of the same concentration of florfenicol APIs was poorer, and most of them could not be dissolved to form a precipitate or floated on the surface of the water (Figure 2B). Under transmission electron microscopy, florfenicol nanocrystals were observed to have a lamellar structure (Figure 3.).

Figure 1. Appearance of Florfenicol Nanocrystals

3.1.2 Particle size, polydispersity coefficient and zeta potential

A small number of samples were taken and the particle size distribution and zeta-potential in florfenicol nanocrystals were determined using Delsa Nano C nanoparticle size/zeta-potential analyser. The average diameter of florfenicol nanocrystals was (291.9 ± 2.9) nm, and the particle size distribution range was concentrated. ζ-potential was -(23.63±1.64) mV, and the system was negatively charged, and the prepared system had good physical stability.

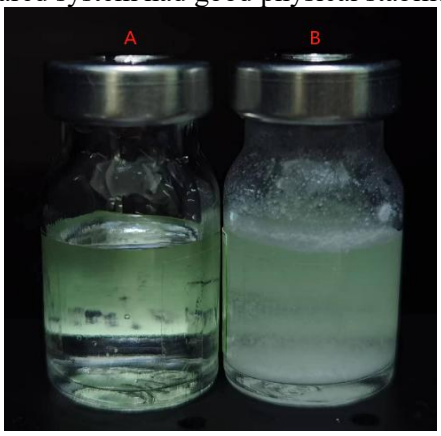


Figure 2. Dissolution of Florfenicol Nanocrystals

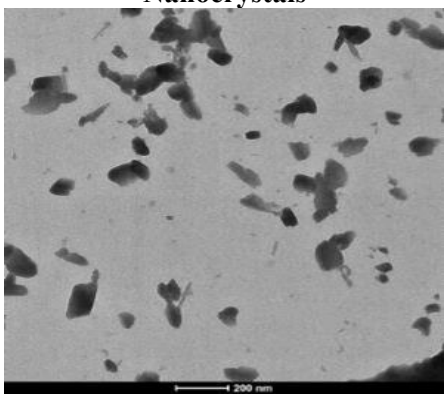


Figure 3. Transmission Electron Microscopy Morphology

3.2 Experimental Method for in Vivo Unidirectional Intestinal Perfusion in Rats

Saline and the perfusate to be tested (100 μg·mL-1 FF florfenicol API perfusate and 100 μg·mL-1 FF-NC florfenicol nanocrystals perfusate) were put into it to preheat. Take ten 50 mL dry and clean conical vials, divided into two groups of inlet and outlet and numbered (a, b, c, d, e for inlet vials and A, B, C, D, E for outlet vials), add the circulating drug solution to be tested (about 30 mL) into the inlet vials, record the weights of the two conical vials, and place them at the inlet and outlet ends of the circulating pipeline, respectively. Rats that had been fasted (without drinking water) for more than 12h were anaesthetised with an intraperitoneal

injection of 1% phenobarbital (at a dose of 60 mg·kg-1). The anaesthetised rats were fixed on a rat platform, and an incision of about 3 cm was made along the midline of the abdomen to locate and isolate the intestinal segments required for the experiments and incise the intestinal segments at the two ends, and the intestines were repeatedly rinsed with saline in a water bath of 40°C until the intestinal contents were drained away. Both ends of the intestine were intubated and fixed with surgical wires, the flow rate of the peristaltic pump was adjusted, and the whole circulatory line was rapidly filled with the drug-containing perfusate to be tested, and then the flow rate was adjusted to 0.5 mL·min-1, and after equilibrating for 10min, the inlet and outlet ends were replaced with a and A bottles respectively, and the formal collection of the perfusion was begun, and the conical bottles of the inlet and outlet ends were changed every 30min, and the collection was carried out for a total of 150min.The weight and density of the conical vials at the inlet and outlet ends were recorded, and a YMC-Pack ODS-A (250 mm × 4.6 mm, 5 μm) column was used, with the mobile phase: acetonitrile:1% glacial acetic acid in water=32:68 (v/v); the detection wavelength was 266 nm; the flow rate was 1 mL·min-1; and the sample volume was 10 μL.

The drug concentration in the conical vials at the inlet and outlet ends was calculated by HPLC external standard method, and the drug concentration in the vials was measured and then the drug content of the drug solution was calculated according to Equation 1.

At the end of the experiment, the experimental intestinal segment was cut off and its length and internal diameter were measured to calculate the perfusion volume of the segment, and then the Ka value and Peff value of FF in the perfusion cycle of each intestinal segment were calculated according to Equations 2 and 3.

$$X=\frac{C \bullet \Delta M}{\rho} \quad (1)$$

In the formula, C is the concentration of the drug solution to be tested, which was measured by the one-point method of the external standard of the control product; ΔM is the weight change before and after cycling of the conical flask at the inlet or outlet end; ρ is the density of the drug solution to be tested (take a clean vial of vials, weigh and peel off the skin, and pipette the gun to measure the drug solution to be tested by transferring 1mL to the vial, and the measured weight is equal to the density of the drug solution).

$$Ka=\left(1-\frac{Xout}{Xin}\right) \bullet \frac{Q}{V} \quad (2)$$

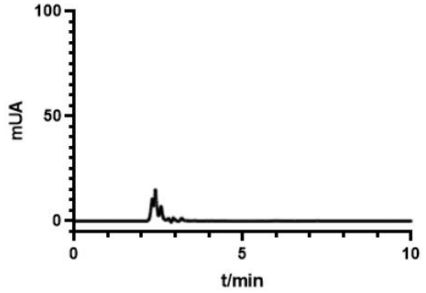
In the formula, Xout and Xin are the drug content of the conical vials at the inlet and outlet ends, respectively; Q is the flow rate of the peristaltic pump; and V is the perfusion volume of the experimental intestinal segment.

$$Peff=-Qln(\frac{Xout}{Xin})/(2RL\pi) \quad (3)$$

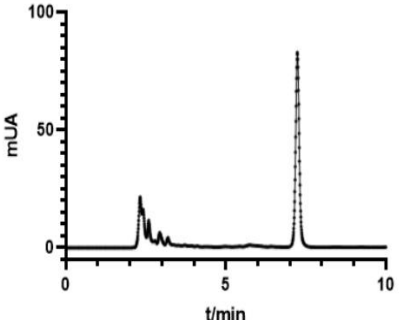
In the formula Q is the peristaltic pump flow rate during cycling, and R and L are the inner diameter and length of the experimental intestinal segment, respectively.

Blank enteric perfusion, florfenicol-containing enteric perfusion and a mixture of blank enteric perfusion and florfenicol API were taken, and the collected fluid was processed according to the experimental method of in vivo unidirectional perfusion in rats. Detect according to the above chromatographic conditions and record the results.

The endogenous intestinal material and the components in the blank perfusate did not interfere with the determination of florfenicol, as shown in Figure 4. , the peak of the florfenicol chromatogram had a good peak shape with a retention time of 7.4min.



(a) Intestinal Perfusate for the Blank Group



(b) Intestinal Infusion of Florfenicol

Figure 4. HPLC Chromatogram of Florfenicol

The drug solution of 100 μg·mL-1 of FF-NC was used as the perfusion solution to be tested, and four intestinal segments, including duodenum, jejunum, ileum and colon, were perfused respectively; The results showed that the main absorption intestinal segment of FF-NC was the duodenum, which was the same as that of FF API, followed by the jejunum, ileum, and the least absorption in the colon, in which the Ka value of the duodenum segment was 1.6 times of that of the jejunum segment, 1.5 times of that of the ileum segment, and 3.0 times of that of the colon, and the Peff value was 1.4 times of that of the jejunum segment, 1.5 times of that of the ileum segment, and 3.7 times of that of the colon, respectively. Meanwhile, the tendency of the Ka and Peff values of FF-NC in the duodenal segment to first increase and then decrease with time was most obvious in the four solid bowel segments.

The FF API and its nanocrystalline drug solution at a concentration of 100 μg·mL-1 were used as the perfusate to be tested, respectively, and the duodenum was selected as the experimental intestinal segment.The Ka and Peff values of two experimental FF-NC perfusion cycles in this intestinal segment were calculated. The Ka and Peff values of FF-NC group were higher than those of FF API group under the control of drug concentration, experimental intestinal section and irrigation time, and the Ka and Peff values of FF-NC group were 1.7 times and 1.8 times of those of FF API group respectively, and at the same time, the changes of Ka and Peff values over time were similar to those of FF API group, which had the tendency to increase firstly and then decrease later.It can be seen that making FF into nanoparticle formulation can improve its absorption rate constant (Ka) and effective permeability parameter (Peff) in the absorbing intestinal segment to a certain extent, but basically it will not change the law of its absorption with time.

4. Discussion

Florfenicol is a broad-spectrum antimicrobial agent that has been widely used in the field of veterinary medicine due to its excellent antimicrobial effects and good safety. Florfenicol is primarily used for the prevention and treatment of bacterial diseases in animals such as cattle, sheep, pigs, aquatic products, and poultry. With the advancement of technology and increased attention to animal welfare, the research and application of florfenicol nanocrystals have gradually become a hot topic in this field. The nanocrystallization of florfenicol can significantly improve the drug's solubility and bioavailability. This means that the drug can be absorbed by animals more quickly, thereby exerting therapeutic effects more rapidly. Due to the high efficiency of florfenicol nanocrystals, it may be possible to reduce the frequency of administration, thereby reducing breeding costs and workload, and also reducing the stress on animals caused by frequent administration. The aim of this paper is to evaluate the reduction and potentiation of florfenicol nanocrystalline formulations, prepare florfenicol nanocrystals by micro media milling method and perform a series of characterisation of their morphology and physicochemical properties. Finally, the florfenicol nanocrystals were obtained as a milky-white milky turbid liquid with particle size around 290 nm and uniform distribution. A high performance liquid chromatographic (HPLC) method was established for the determination of florfenicol nanocrystals content to obtain good specificity, high sensitivity, accurate measurement results, convenient operation, and suitable for the detection of florfenicol content in in vivo intestinal absorption. Through the study of florfenicol nanocrystals in the body intestinal absorption, the perfusion experimental study was carried out separately with the API, and the effect of making florfenicol into nanocrystals formulation on its absorption in the body intestines was investigated, and the results of the above studies showed that nanotechnology can effectively enhance the oral absorption of florfenicol, which in turn improves the bioavailability and reduces the amount of the drug administered.

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