Original Article

Pharmacokinetics of oxfendazole nanosuspension in sheep

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Abstract

The pharmacokinetics of our previous prepared oxfendazole nanosuspension was studied in sheep to understand its enhancement of bioavailability. Ten sheep were randomly divided into two groups, each of which received a single-dose (5mg/kg) oral oxfendazole nanosuspension and oxfendazole granules. After intragastric administration, oxfendazole rapidly reached peak concentrations of 11.25 μg/mL and 5.50 μg/mL at 5.6 h and 6.6 h in the nanosuspension group and granules group and the concentrations gradually reduced to below the detection limit at 96 h and 72h, respectively. The main pharmacokinetic parameters of oxfendazole after administration to the sheep: The T_{max}, C_{max}, AUC_{last}, MRT_{last}, $T_{1/2}$ and Ke of in nanosuspension were 5.6 h, 11.26 μg/mL, 179.22 μg*h/mL, 17.82 h, 87.22 h and 0.012 1/h, respectively, while the these main pharmacokinetic parameters in the OFZ granules group were 6.6 h, 5.50 μg/mL, 105.28 μg*h/mL, 16.55 h, 35.04 h, and 0.020 1/h, respectively. Compared with the granules group, the C_{max} and AUC_{last} of the nanosuspension group were increased by 2.05 and 1.70 times, respectively, and the C_{max} and AUC_{last} of the total active compound in the nanosuspension group were increased by 1.85 times and 1.71 times, respectively. These results suggest that the oxfendazole suspension might be an effective and promising formulation for use in sheep.

Keywords: Oxfendazole, Nanosuspension, HPLC, Pharmacokinetics

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Introduction

Oxfendazole (OFZ), whose chemical structure is Methyl 5-(phenylsulfinyl)-benzimidazol-2-carbamate, is a derivative of fenbendazole. It is a new broad-spectrum, high-efficiency and low-toxicity insect repellent drug. OFZ is metabolized to fenbendazole (FBZ) and fenbendazole sulfone (FBZSO) in ruminants but its main repellent effect is still derived from itself. OFZ, FBZ and FBZSO can be detected in serum after the use of OFZ, and OFZ is the main component (Lanusse *et al*., 1995). It has a good repellent effect on lung nematodes, abdominal worms and aphids (Barr *et al*., 1993; Blanton *et al*., 1998; Davies and Schwalbach, 2000; Gavidia *et al*., 2009, 2010). In addition, it has a good deworming egg effect (Duwel *et al*., 1975). Studies have reported that OFZ exhibits a more efficient deworming effect than other similar deworming drugs (Bauer, 1990; Ploeger and Everts, 2018). It is safe and well tolerated in animals and humans (Gonzales *et al*., 1996; Gonzalez *et al*., 1997; An *et al*., 2019) and it is gradually used to prevent and treat gastrointestinal parasitic diseases and various helminthiasis. However, it should be noted that OFZ is a biopharmaceutical classification system (BCS) class II drug (a drug with low solubility and high permeability) (An *et al*., 2019) with concentration-dependence; a higher oral absorption concentration is required to achieve good deworming effects *in vi*vo. However, the existing formulations have not solved the solubility problem of OFZ. Therefore, the challenge of solubility and oral absorption of OFZ should be urgently resolved.

In order to solve the problem of poor oral absorption, our group proposed a method for preparing OFZ nanosuspension by adding surfactant. The OFZ nanosuspension was prepared by acid-base neutralization combined with ultrasonic dispersion. 5 g of OFZ was dissolved in 20 mL saturated malic acid solution at 75℃ and stirred with a magnetic stirrer. A 2.5 mol/L NaOH of the same molar mass solution containing 1.5g polyoxyethylene ether hydrogenated castor oil (HEL-40) at 4℃ was quickly poured into an acidic aqueous solution containing OFZ under stirring at 500 r/min; after that the drug had complete precipitation. The recrystallized OFZ was sonicated for 6 mins using 6 mm microprobes with 91% amplitude (VCX 130 Vibra-CellTM, Sonics & Materials, Inc., Newtown, CT, USA) to obtain the uniform particle diameter. The preparation method was simple and could meet the requirements of clinical application. In previous experiments, the equilibrium solubility of nanocrystals in different solvents was significantly improved by 2.02-109.99-fold compared to OFZ powder. The *in vitro* release speed was improved significantly and the pharmacokinetics showed that the relative bioavailability of the OFZ nanosuspension was 265.61% compared to the OFZ granules in rats. This indicates that it is a very well worth developing the preparation.

In addition, there are no reports on the pharmacokinetics of OFZ nano preparations currently. The HPLC method was used to determine the concentration of OFZ and its metabolites in the plasma of sheep. This research can provide the basis for the clinical application of the preparation.

Materials and Methods

OFZ granule (Specifications 10%) was purchased from Jiangxi Tebang Biopharmaceutical Co., Ltd. (Jiangxi, China). OFZ nanosuspension (specification 7.5%) was obtained from our laboratory. Methanol, acetonitrile, and ammonium acetate were commercially available from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ultrapure water for all experiments was obtained with a Microporous Purification® Purified Water System (Milli-Q Ltd., France). All other chemicals and reagents used in this experiment were of analytical grade. The ten healthy small-tailed Han rams had an average weight of 38.6±1.2 kg. The experimental sheep were fed 3 times a day and drank freely at a room temperature of -3 to 10℃. The experiment was carried out according to the "Guidelines of the Care and Use of Laboratory Animals" of Huazhong Agricultural University and approved by the Ethics Committee of Huazhong Agricultural University.

Animal study: Ten sheep of uniform weight were selected and randomly divided into two groups; the first group was the OFZ nanosuspension group and the second group was the OFZ granules group. They were free to drink and eat. The feed was a full-price diet without antibacterial drugs. After clinical observation for 7 days, single-dose gastric administration at 5mg/kg and they could only drink freely within 12 hours before the test and within 4 hours after the test. After administration, 5 mL of intravenous blood was collected from sheep at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120 and 144 h. The collected blood samples were placed in a centrifuge tube containing sodium heparin, mixed, centrifuged at 8000 r/min for 10 minutes and the upper plasma was placed on standby at -20℃.

Plasma sample pretreatment: For the analysis of drug concentration in plasma, a 1 mL plasma sample was added to a 10 mL centrifuge tube and deproteinized with 1 mL acetonitrile. Furthermore, 2 mL of ethyl acetate and 0.1 mL of 0.1 mol/mL aqueous ammonia solution were added under vorticity for 3 mins to be completely mixed. The mixture was centrifuged at 8000 r/min at 4℃ for 10 minutes and the supernatant was transferred to another 10 mL centrifuge tube. 2 mL of ethyl acetate were added to the precipitate to repeat the extraction. Extracts was dried with nitrogen at 50℃ and resoluble with 0.2 mL of mobile phaseand analyzed by HPLC after filtration through a 0.22 μm cellulose membrane.

HPLC assay: The detection of OFZ, FBZ and FBZSO in all samples in this test was carried out according to the HPLC methods reported in our previous work(Chen *et al*., 2010). The chromatographic conditions were as follows: Agilent (1100 series) and Ecilpse XDB-C18 (4.6 ID×250 mm, Agilent, USA); detection wavelength, 292 nm; flow rate, 1 mL/min; column temperature, 25℃; and injection volume, 50 μL, A gradient elution mode of the mobile phase (A: 0.02 M ammonium acetate, B: acetylene) was set up to separate OFZ and its major

metabolites. The gradient was set as follows: 0 minutes, 85% A; 10 minutes, 50% A; 20 minutes, 50% A.

Statistical analysis: Non-compartmental analysis was accomplished using WinNonlin version 5.0 for pharmacokinetic analysis. The plasma concentration-time curve area (AUC), drug peak concentration (C_{max}), time reached the maximum concentration (T_{max}), elimination half-life ($T_{1/2}$) and average retention time (MRT) were calculated. The statistical significance of differences in pharmacokinetic parameters between the granules and the nanosuspension group was analysed by Anova Analysis using SPSS 17.0 (SPSS Co. USA). Significant differences and extremely significant differences were determined as p values of 0.05 and 0.01, respectively. The data was analysed in triplicate.

Results

HPLC method: The drug and its metabolites had a good linear relationship in the concentration range of 0.05-15 µg/mL, and the correlation coefficients were all above 0.999. The LOD and LOQ of the three compounds were 0.05 µg/mL and 0.5 µg/mL, respectively. The average recoveries of OFZ, FBZ and FBZSO in plasma were 89%-110% and the intra- and inter-assay coefficients of variation were less than 5% respectively. This established that the method was suitable for detecting OFZ and its active metabolites in plasma.

***Pharmacokinetics*:** All sheep tolerated the oral administration of OFZ granules and the OFZ nanosuspension well without any abnormal phenomena. The plasma concentration-time curves of OFZ granules and the OFZ nanosuspension are shown in figure 1-3. After oral administration, the concentrations of OFZ in blood reached maximum concentrations of 5.50 µg/mL and 11.26 µg/mL at 6.60 h and 5.60 h in granules group and nanosuspension group, respectively, and gradually decreased to the LOD at 72 h and 96 h. The metabolites FBZ reached maximum concentrations of 1.34 µg/mL and 1.45 µg/mL at 10.60 h and 3.69 h in granules group and nanosuspension group, respectively, and slowly fell to the LOD at 72 and 96 h. Another metabolite, FBZSO, reached maximum concentrations of 2.20µg/mL and 4.12 µg/mL at 17.60h and 18.40 h in granules group and nanosuspension group, respectively, and both fell to the LOD at 144 h.

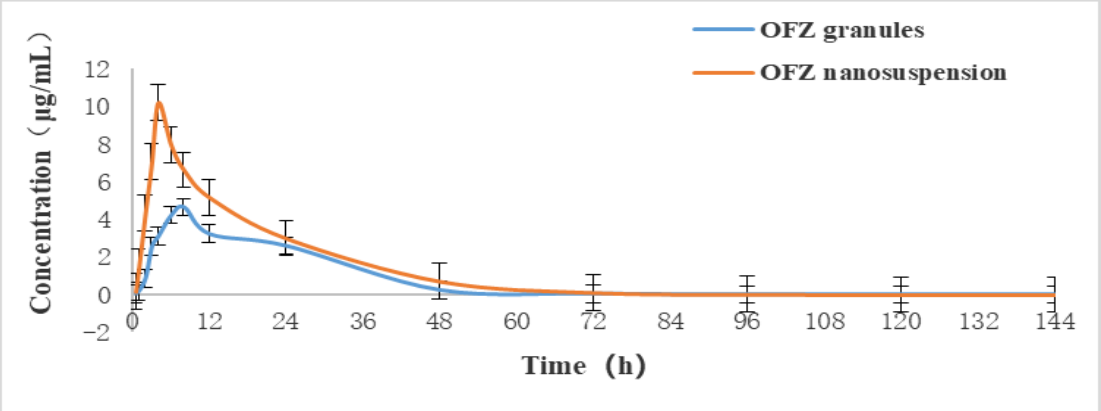

Figure 1 The plasma concentrations versus time curves of OFZ after oral administration at a dose rate of 5 mg/kg.B. W to sheep.

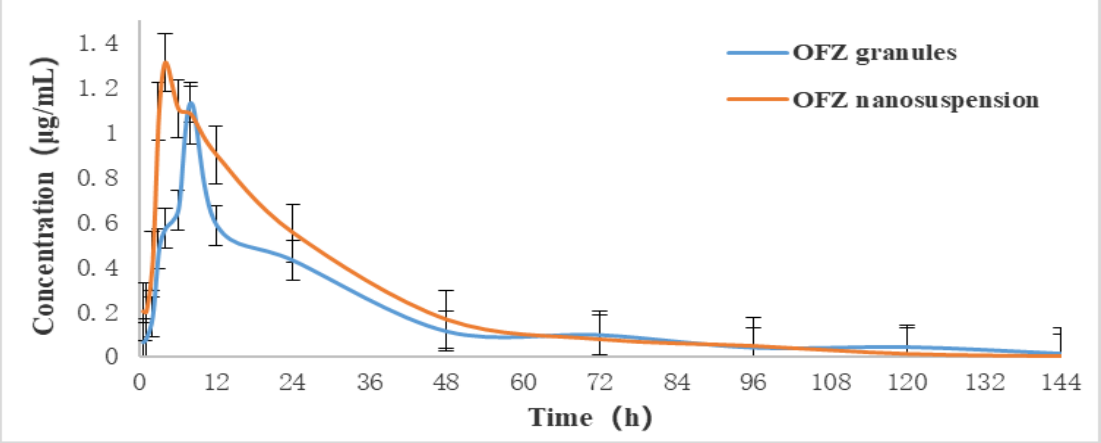

Figure 2 The plasma concentrations versus time curves of FBZ after oral administration at a dose rate of 5 mg/kg.B. W to sheep.

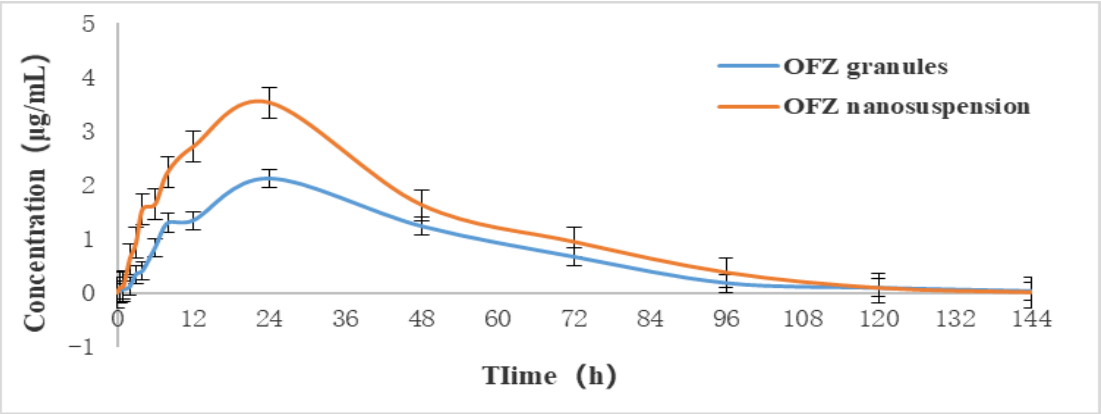

Figure 3 The plasma concentrations versus time curves of FBZSO after oral administration at a dose rate of 5 mg/kg.B. W to sheep.

The area under the concentration time curve of FBZSO (AUC_{last}) and the elimination half-life ($T_{1/2}$) in the OFZ nanosuspension groups were 170.84±12.76 h*ng/mL and 37.77±4.42 h, while these parameters for the OFZ granules were 107.35±15.50 h*ng/mL and 46.22±18.07 h, respectively. Compared to OFZ granules, the AUC_{last} was increased by 1.59 times, respectively. Comparison of the average MRT_{last} of the three active ingredients shows that the nanosuspension group was 17.82 h, 21.61 h, and 37.19 h, respectively. There was no significant difference between the two groups (Table1-3).

Table 1 Comparison of the pharmacokinetic parameters of OFZ after oral administration at a dose rate of 5 mg/kg.B. W to sheep.

Parameters	OFZ granules	OFZ nanosuspension	ANOVA test (p)
T_{max}(h)	6.60±2.19	5.60±2.19	0.398
C_{max}(μg/ml)	5.50±0.76	11.26±4.29	0.034*
AUC_{last} (h*μg/ml)	105.28±11.26	179.22±74.35	0.043*
MRT_{last} (h)	16.55±1.6	17.82±2.01	0.064
Ke	0.020±0.000	0.012±0.006	0.016*
$T_{1/2}$	32.00±20.62	41.20±23.94	0.533

Table 2 Comparison of the pharmacokinetic parameters of FBZ after oral administration at a dose rate of 5 mg/kg.B. W to sheep.

Parameters	OFZ granules	OFZ nanosuspension	ANOVA test (p)
T_{max}(h)	10.60±8.17	3.69±1.66	0.184
C_{max}(μg/ml)	1.45±0.98	1.34±0.82	0.299
AUC_{last} (h*μg/ml)	27.82±12.57	28.85±8.46	0.964
MRT_{last} (h)	27.70±8.06	21.61±8.10	0.914
Ke	0.011±0.003	0.009±0.000	0.779
$T_{1/2}$	30.25±12.48	57.38±29.01	0.091

Table 3 Comparison of the pharmacokinetic parameters of FBZSO after oral administration at a dose rate of 5 mg/kg.B. W to sheep.

Parameters	OFZ granules	OFZ nanosuspension	ANOVA test (p)
T_{max}(h)	17.60±8.76	18.40±7.80	0.762
C_{max}(μg/ml)	2.20±0.41	4.12±0.53	0.033*
AUC_{last} (h*μg/ml)	107.35±15.50	170.84±12.76	0.012*
MRT_{last} (h)	39.70±3.16	37.19±2.63	0.486
Ke	0.004±0.002	0.004±0.002	0.212
$T_{1/2}$	46.22±18.07	37.77±4.42	0.340

*Statistical significances compared with OFZ granules are $p<0.05$

Discussion

The HPLC analysis method of OFZ, FBZ and FBZSO in sheep plasma established in this study has certain specificity. The three drugs were completely separated by the gradient elution of acetonitrile and ammonium acetate. The retention times were 8.4mins, 16.9mins, and 14.1mins, respectively, and no

interference peaks appeared near the drug peak. After determining the analytical method of the mixed drug, the linearity, precision and stability were verified and the results met the requirements of the biological sample analysis method.

Compared with the pharmacokinetic parameters of OFZ granules in sheep, OFZ nanosuspension significantly improved bioavailability. The main reason for the increase in bioavailability may be that the particle size of the nanosuspension is in the nanometer range, and the surface area is greatly increased, which increases the solubility and enhances the adhesion to the gastrointestinal tract, making it possible to achieve higher drug locally concentrated, higher deworming effects may be possible for gastrointestinal nematodes. With time, the drug is slowly released from the interstitial space and the circulation time in the body is prolonged, so the residence time and absorption of the drug particles in the gastrointestinal tract are increased and it can prolong the deworming time in the body. Another reason may be that the stabilizers selected in the formulation have good compatibility with tissues and mucous membranes and the surface charge of the nanoparticles affects the permeability of small intestinal epithelial cells, thereby affecting plasma drug levels (Ban *et al.*, 2020).

In short, the results of this test show that OFZ nanosuspension is expected to become a more effective preparation for the treatment of gastrointestinal nematode disease in ruminants, with great further development value.

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References

An G, Murry DJ, Gajurel K, Bach T, Deye G, Stebounova LV, Codd EE, Horton J, Gonzalez AE, Garcia HH, Ince D, Hodgson-Zingman D, Nomicos EYH, Conrad T, Kennedy J, Jones W, Gilman RH, Winokur P. 2019. Pharmacokinetics, Safety, and Tolerability of oxfendazole in Healthy Volunteers: a Randomized, Placebo-Controlled First-in-Human Single-Dose Escalation Study. Antimicrob Agents Ch. 63.

Ban C, Jo M, Park YH, Kim JH, Han JY, Lee KW, Kweon DH, Choi YJ. 2020. Enhancing the oral bioavailability of curcumin using solid lipid nanoparticles. Food chem. 302, 125328.

Barr SC, Bowman DD, Heller RL, Erb HN. 1993. Efficacy of albendazole against giardiasis in dogs. Am J Vet Res. 54, 926-928.

Bauer C. 1990. Comparative efficacy of praziquantel, albendazole, febantel and oxfendazole against Moniezia expansa. Vet Rec. 127, 353-354.

Blanton RE, Wachira TM, Zeyhle EE, Njoroge EM, Magambo JK, Schantz PM. 1998. oxfendazole treatment for cystic hydatid disease in naturally infected animals. Antimicro Agents Ch. 42, 601-605.

Chen D, Tao Y, Liu Z, Liu Z, Huang L, Wang Y, Pan Y, Peng D, Dai M, Yuan Z. 2010. Development of a high-performance liquid chromatography method to monitor the residues of benzimidazoles in bovine milk. J Chromatogr. B. 878, 2928-2932.

Davies JA, Schwalbach LM. 2000. A study to evaluate the field efficacy of ivermectin, fenbendazole and pyrantel pamoate, with preliminary observations on the efficacy of doramectin, as anthelmintics in horses. J S Afr Vet Assoc. 71, 144-147.

Duwel D, Kirsch R, Reisenleiter R. 1975. The efficacy of fenbendazole in the control of trematodes and cestodes. Vet Rec. 97, 371.

Gavidia CM, Gonzalez AE, Lopera L, Jayashi C, Angelats R, Barron EA, Ninaquispe B, Villarreal L, Garcia HH, Verastegui MR, Gilman RH. 2010. Evaluation of oxfendazole, praziquantel and albendazole against cystic echinococcosis: a randomized clinical trial in naturally infected sheep. PLoS Neglect Trop D. 4, e616.

Gavidia CM, Gonzalez AE, Lopera L, Jayashi C, Angelats R, Barron EA, Ninaquispe B, Villarreal L, Garcia HH, Verastegui MR, Gilman RH. 1996. Effective, single-dose treatment or porcine cysticercosis with oxfendazole. Am J Trop Med Hyg. 54, 391-394.

Gonzalez AE, Falcon N, Gavidia C, Garcia HH, Tsang VC, Bernal T, Romero M, Gilman RH. 1997. Treatment of porcine cysticercosis with oxfendazole: a dose-response trial. Vet Rec. 141, 420-422.

Lanusse CE, Gascon LH, Prichard RK. 1995. Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfendazole and their metabolites in adult sheep. J Vet Pharmacol Ther. 18, 196-203.

Ploeger HW, Everts RR. 2018. Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands. Vet Parasitol. 262, 11-15.