The Journal of Veterinary Medical Science

FULL PAPER

Pharmacology

Effects of anemoside B4 on pharmacokinetics of florfenicol and mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in broilers

Sicong LI[1,2], Xuting LI[1,2]*, Rui YANG[1,2], Bin WANG[1,2], Jinliang LI[1,2], Liang CAO[3], Songyang XIAO[3] and Wei HUANG[3]

[1]Institute of Veterinary Pharmacology, Sichuan Animal Science Academy, 7 Niusha Road, Jinjiang district, Chengdu 610066, PR China
[2]Animal Breeding and Genetics Key Laboratory of Sichuan Province, 7 Niusha Road, Jinjiang district, Chengdu 610066, PR China
[3]Sichuan Dingjian Animal Pharmaceutical Co., Ltd., 19 7th East Road, Checheng, Longquanyi district, Chengdu 610100, PR China

ABSTRACT. *Pulsatillae radix*, a traditional Chinese medicine (TCM), is often used in combination with florfenicol for treatment of intestinal infection in Chinese veterinary clinics. Anemoside B4 (AB4) is the major effective saponin in *Pulsatillae radix*. This study aimed to investigate whether the pharmacokinetics of florfenicol in broilers was affected by the combination of AB4. In this study, broilers were given AB4 (50 mg/kg BW), or 0.9% sodium chloride solution by oral administration for 7 days. They were then fed florfenicol orally (30 mg/kg BW) on the eighth day. The results showed that the $AUC_{(0-\infty)}$, $MRT_{(0-\infty)}$, $t_{1/2z}$ and C_{max} of florfenicol were significantly decreased, and the Vz/F and CLz/F were significantly increased by AB4; the mRNA expression levels of CXR, CYP3A37 and MDR1 (except CXR and CYP3A37 in the liver) were up-regulated by AB4. In conclusion, AB4 altered the pharmacokinetics of florfenicol, resulting in lower plasma concentrations of florfenicol, this was probably related to the mRNA expression of CXR, CYP3A37 and MDR1 in the jejunum and liver (except CXR and CYP3A37) increased by AB4. The implications of these findings on the effect of traditional Chinese medicine containing AB4 on the effectiveness of florfenicol in veterinary practice deserve study.

KEY WORDS: anemoside B4, drug-metabolizing enzyme, efflux transporter, florfenicol, pharmacokinetics

J. Vet. Med. Sci.
81(12): 1804–1809, 2019
doi: 10.1292/jvms.19-0293

Received: 31 May 2019
Accepted: 2 October 2019
Advanced Epub:
14 October 2019

Use of veterinary herbal medicines is of interest because of the medicines' natural origin, lack of drug residue, and low frequency of side effects [20]. In veterinary practice, herbal medicines are widely used in combination with synthetic drugs to prevent and control diseases. However, herbs contain phytochemicals that may interact with co-administered synthetic drugs, altering pharmacokinetics of the latter and leading to interactions by inducing or inhibiting drug-metabolizing enzymes and/or efflux transporters [31]. Therefore, it is important to evaluate these interactions as they may affect clinical herb-drug interactions [35].

Florfenicol is a synthetic broad-spectrum antibiotic with activities like that of chloramphenicol; the drug is widely used to control bacterial infection in veterinary practice [27, 30, 32]. Florfenicol has low toxicity and better antibacterial activity than does chloramphenicol or thiamphenicol [3, 12, 25]. The pharmacokinetics of florfenicol has been documented in many animal species [1, 2, 6, 22, 29, 32]. A few studies have also reported on the possible metabolic pathways and mechanisms of florfenicol *in vivo*. Liu *et al.* (2012) have reported that P-gp and/or CYP3A are likely involved in the disposition of florfenicol in rabbits [19]. Wang *et al.* (2018) have suggested that CYP 3A plays a key role in the pharmacokinetics of florfenicol in chickens [33].

Pulsatillae radix, a traditional Chinese medicine (TCM), has been widely used for a long time. Anemoside B4 (AB4) (Fig. 1) is its major effective saponin, with diverse pharmaceutical effects, including antiviral activity, enhancing hypoglycemic, anti-tumor, neuroprotective and anti-angiogenic activity [11, 13]. In Chinese veterinary clinics, *pulsatillae radix* is commonly used for treatment of intestinal infection. In addition, the TCM preparations containing *pulsatillae radix* are also often used in combination with florfenicol, and however, the drug-drug interaction between *pulsatillae radix* and florfenicol is still unknown.

*Correspondence to: Li, X.: 676049640@qq.com

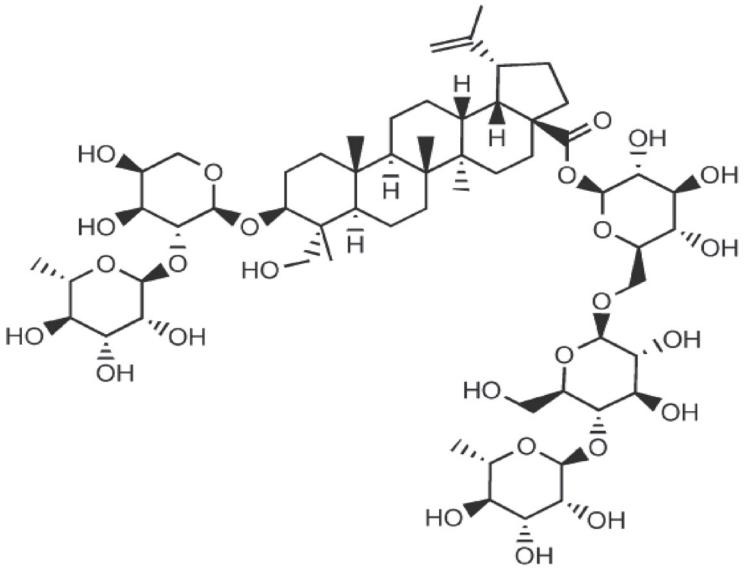

Fig. 1. Chemical structure of anemoside B4.

In order to avoid the potential adverse drug-drug interactions, the main purpose of this study was to investigate the effects of AB4 on pharmacokinetics of florfenicol in broilers. The *in vivo* pharmacokinetics of florfenicol in broilers with or without AB4 pretreatment were determined by ultra-high-performance liquid chromatography (UHPLC). In addition, the effects of AB4 on the mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in broilers in the liver and jejunum were analyzed with real-time PCR. We believe that the results may help predict the clinical effects of AB4-florfenicol interactions.

MATERIALS AND METHODS

Chemicals and reagents

AB4 was purchased from the National Institutes for Foods and Drug Control (Beijing, China). Florfenicol was provided by Sichuan Dingjian Animal Pharmaceutical Co., Ltd. (Chengdu, PR China), it was dissolved in polyethylene glycol 400 (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) to a concentration of 30 mg/m*l*. Florfenicol and chloramphenicol (internal standard) analytical standards were purchased from China Institute of Veterinary Drug Control (Beijing, PR China). HPLC-grade acetonitrile and methanol were obtained from Merck Chemicals Ltd. (Darmstadt, Germany). All other reagents were of analytical grade or better.

Animals

Qingjiaoma broilers were obtained from a commercial hatchery. The broilers were reared at the Laboratory Animal Research Center of Sichuan Animal Science Academy since they were one day old. The broilers had *ad libitum* access to water and a standard diet lacking drug supplements. Efforts were made to minimize animal suffering. All experimental procedures and protocols were reviewed and approved by the Animal Ethics Committee of Sichuan Animal Science Academy and were in accordance with the guide for the care and use of laboratory animals [24].

UHPLC Method for Detection of Florfenicol in Plasma

UHPLC was performed on UltiMate 3000 HPLC (Thermo Fisher Scientific Inc., Chelmsford, MA, U.S.A.) as previously reported [16]. Florfenicol and chloramphenicol were simultaneously determined with a Diamonsil C18 column (4.6 × 250 mm, 5 μm; Thermo Fisher Scientific Inc.) at constant 40°C. The mobile phase consisted of acetonitrile and water (27:73) at a flow rate of 1.0 m*l*/min and an injection volume of 20 μl. The detection wavelength was set at 223 nm and the overall run time of the analysis was 16 min.

The method validation was performed using the following parameters: limit of detection (LOD) and limit of quantification (LOQ), precision, extraction recovery, correlation coefficients of the calibration curves.

LOD and LOQ were detected based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. The precision was estimated by intra-day and inter-day precision for 3 days at 3 standard levels (0.1, 2.5, and 20 μg/m*l*). The extraction recovery was expressed as the ratio of the mean area of florfenicol in plasma samples to that of the analytes in neat standard samples at equivalent concentrations. The standard curve of florfenicol was derived from the ratios of the peak-areas of florfenicol and the internal standard chloramphenicol (S), and plotting them against the corresponding concentration of florfenicol in blank plasma (C). The standard samples of florfenicol were prepared at concentrations of 50, 20, 10, 5.0, 2.5, 0.5, 0.1, 0.05 μg/m*l* with each parallel processing 5 samples.

The LOQ and LOD of florfenicol were validated at 0.05 and 0.02 μg/m*l*, respectively. The intra-day and inter-day assay precisions at 3 standard levels were below 7.0%. The extraction recovery of 3 concentrations of florfenicol were all more than 84.0%. The correlation coefficient (R^2) for the calibration curves was 0.9998.

Effect of AB4 on pharmacokinetics of florfenicol in broilers

Study design, formulation and dosage regimen: The broilers (male; 1.50 ± 0.10 kg) were randomly assigned to 1 of 2 equal groups (6 broilers per group): control group (CG) and AB4 group (AG). According to the dose of pulsatillae radix in chicken and the content of AB4 in pulsatillae radix prescribed in the Veterinary Pharmacopoeia of the People's Republic of China (2015 edition) [5], the regimen of 50 mg/kg AB4 was administrated orally, directly into the crop by tube gavages in AG each morning for 7 consecutive days, and the same volume of double-distilled water was orally administrated by tube gavages in CG. During the whole trial period, there was no significant difference in feed intake, growth and health status between AG and CG.

Pharmacokinetic study: On the eighth day, after fasting for 12 hr, a suspension containing florfenicol (30 mg/kg BW), was administrated orally into the crop in each group. Blood samples from each broiler were collected at 0.167, 0.33, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12, and 24 hr after administration of florfenicol. Plasma samples were separated by centrifugation at 4,000 rpm for 5 min and stored at −80°C until use.

Sample preparation: Plasma sample preparation was performed as described with a slight modification [29]. Briefly, a 200 μl aliquot of thawed plasma in a 1.5 m*l* centrifuge tube was spiked with 5 μg of chloramphenicol (internal standard) in 10 μl methanol and added to 800 μl of ethylacetate. The tube was vortex mixed for 2 min, and the sample was centrifuged at 4,000 rpm for 10 min at room temperature. The subnatant was re-extracted with 800 μl ethylacetate solution to collect the extract again. The pooled supernatant was evaporated to dryness under a flow of nitrogen at 40°C. The residue was dissolved in 400 μl mobile phase and centrifuged at 12,000 rpm for 10 min at 4°C. Finally, 20 μl of the supernatant was injected into the UHPLC system for analysis.

Effect of AB4 on mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in the liver and jejunum

Drug administration and sample collection: Another 12 broilers (male; 1.50 ± 0.10 kg) were randomly assigned to 1 of 2 equal groups (6 broilers per group). The study design, formulation and dosage regimen are those in '*Effect of AB4 on pharmacokinetics of florfenicol in broilers.*': On the eighth day, after fasting for 12 hr, broilers were slaughtered by carbondioxide asphyxiation machine. Each liver and jejunum sample was removed quickly, perfused with ice-cold saline to remove blood residue, blotted dry, and stored at −80°C.

Total RNA isolation and synthesis of cDNA: Total RNA from each sample was isolated by use of TRIzol reagent (Invitrogen Corporation and Applied Biosystems, Inc., Calsbad, CA, U.S.A.) according to the manufacturer's protocol. Concentration, purity and integrity of the total RNA samples were measured according to our previous work [16]. RNA samples (800 *n*g) were reverse transcribed to cDNA with RevertAid Premium Reverse Transcriptase (Thermo Fisher Scientific Inc.) according to the manufacturer's protocol, using C100 PCR (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.; 2015). The products were stored at −80°C until assay.

Real-time RT-PCR analysis: Real-time RT-PCR was performed by using the ABI StepOne RT-PCR (Applied Biosystems, Foster City, CA, U.S.A.) for a 20 μl reaction mixture that contained 10 μl High RoxSybrGreen qPCR Master Mix (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, PR China), 2 μl cDNA, 0.4 μl of each oligonucleotide primer (10 μM), and 7.2 μl diethyl pyrocarbonate-treated autoclaved distilled water on the StepOne RT-PCR instrument. PCR was carried out using initial denaturation at 95°C for 3 min, followed by 45 cycles of denaturation at 95°C for 10 sec, annealing at 57°C for 15 sec, extension at 72°C for 20 sec, and final extension at 60°C for 1 min. β-actin was used as house-keeping gene. The fold change in the expression level of target gene in AB4 relative to the level in CG was calculated using the $2^{-(\Delta\Delta Ct)}$ method [17]. The data are represented as the fold change in gene expression relative to the control. The sequences, GenBank accession and PCR amplification efficiency of the forward and reverse primers used in this experiment are summarized in Table 1. The PCR amplification efficiency of 5 pairs of primers was between 100.792 and 109.793%.

Statistical analysis

The concentration-time profile of florfenicol was analyzed with Data Analysis System version 3.0 (Chinese Pharmacological Society, Beijing, PR China). All data were presented as mean ± SD. Statistical significant differences were evaluated between control and AB4 treatment groups by One-way ANOVA, using SPSS version 22.0 (SPSS Inc., Chicago, IL, U.S.A.; 2017). For all analyses, *P*-values less than 0.05 were considered significant.

Table 1. Sequences of the forward and reverse primers used for real-time RT-PCR

Isozymes	Accession number	PCR efficiency (%)	Forward	Reverse
CXR	NM_204702.1	100.792	5′-TCCCTTCGGCATCCCTGTC-3′	5′-GGCGTTGGTCTCCTCGTTG-3′
MDRl	XM_003641637.1	108.089	5′-GCTGTTGTATTTCCTGCTATGG-3′	5′-ACAAACAAGTGGGCTGCTG-3′
CYP3A37	NM_001001751.2	104.632	5′-CGAATCCCAGAAATCAGA-3′	5′-AGCCAGGTAACCAAGTGT-3′
UGT1E	XM_015289253.1	109.793	5′-CCCTGGTCCTTCCTTCTCATCC-3′	5′-CTCCCACCTACTGGCACTACC-3′
β-actin	NM_205518.1	104.719	5′-ATGTGGATCAGCAAGCAGGAGTA-3′	5′-TTTATGCGCATTTATGGGTTTTGT-3′

RESULTS

Effect of AB4 on pharmacokinetics of florfenicol

The effect of AB4 on pharmacokinetics of florfenicol in broilers is presented in Table 2 and Fig. 2. After oral administration of AB4 for 7 days, the area under the concentration-time curve from zero to infinity ($AUC_{(0-\infty)}$), mean residence time from zero to infinity ($MRT_{(0-\infty)}$), the elimination half-life ($t_{1/2z}$) and the peak concentration (C_{max}) of florfenicol in AG were significantly decreased (by 43.14, 23.58, 20.75 and 23.42%, respectively) compared with corresponding values in controls. The apparent volume of distribution fraction of the dose absorbed (Vz/F) and the plasma clearance fraction of the dose absorbed (CLz/F) were significantly increased (by 68.30% and 72.13%, respectively) compared with corresponding value in controls, whereas the time to reach peak concentration (T_{max}) was not significantly different in CG and AG.

Effect of AB4 on mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in the liver and jejunum

The effects of AB4 on the mRNA expression levels of CXR, MDR1, CYP3A37 and UGT1E in the liver are presented in Fig. 3. After oral administration of AB4 for 7 days, the mRNA expression levels of MDR1 in AG was significantly increased to 2.18-fold levels in CG. mRNA expression levels of CXR, CYP3A37 and UGT1E in the CG and AB4 treatment groups were not significantly different.

The effect of AB4 on the mRNA expression levels of CXR, MDR1, CYP3A37 and UGT1E in the jejunum are presented in Fig. 4. After oral administration of AB4 for 7 days, the mRNA expression levels of CXR, MDR1 and CYP3A37 were significantly increased, to 2.61-fold, 1.92-fold and 1.77-fold levels in CG, respectively. mRNA expression level of UGT1E in the CG and AB4 treatment groups were not significantly different.

Table 2. Pharmacokinetic characteristics of florfenicol in plasma of broilers after oral administration of florfenicol (30 mg/kg BW) with or without Anemoside B4 (AB4) (50 mg/kg BW for 7 days) pretreatment (n=6, Mean ± SD)

Characteristic	Control group (CG)	AB4 group
$AUC_{(0-\infty)}$ (mg/*l**hr)	28.30 ± 5.26	16.09 ± 4.97[a)]
$MRT_{(0-\infty)}$ (hr)	3.86 ± 0.47	2.95 ± 0.35[a)]
$t_{1/2z}$ (hr)	2.41 ± 0.29	1.91 ± 0.20[a)]
T_{max} (hr)	1.20 ± 0.47	1.39 ± 0.66
Vz/F (*l*/kg)	3.47 ± 1.32	5.84 ± 1.13[a)]
CLz/F (*l*/hr/kg)	1.22 ± 0.24	2.10 ± 0.37[a)]
C_{max} (mg/*l*)	5.38 ± 0.45	4.12 ± 0.39[a)]

a) Significantly different from CG, $P<0.05$.

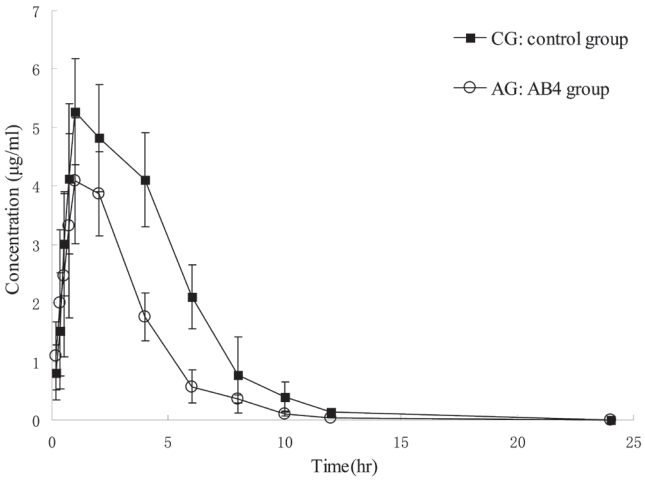

Fig. 2. Mean plasma concentration–time profiles of florfenicol in broilers after oral administration of florfenicol (30 mg/kg BW) with or without Anemoside B4 (AB4) (50 mg/kg BW for 7 days) pretreatment. Each symbol with a bar represents the mean ± SD of 6 broilers.

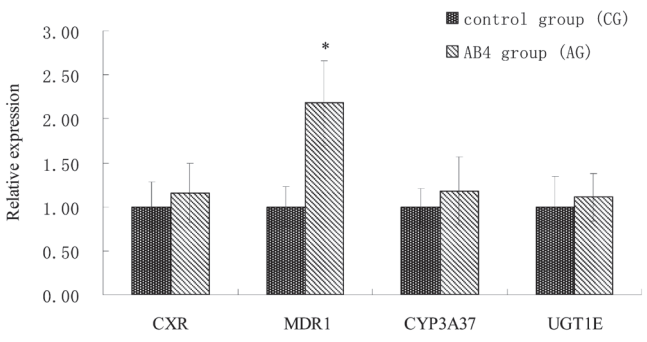

Fig. 3. Effect of Anemoside B4 (AB4) (50 mg/kg BW for 7 days) on mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in the liver (n=6). *Significantly different from control group (CG), $P<0.05$.

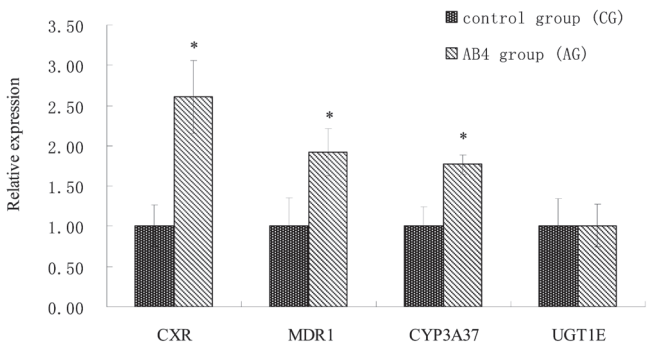

Fig. 4. Effect of Anemoside B4 (AB4) (50 mg/kg BW for 7 days) on mRNA expression of CXR, MDR1, CYP3A37 and UGT1E in the jejunum (n=6). *Significantly different from control group (CG), $P<0.05$.

DISCUSSION

In recent years, the negative effects of herb-drug interactions have gradually attracted attention. In human medicine, several studies have reported the severe negative effects of herb-drug interactions, but they often miss some important items. Most literature surveys showed that interactions of clinical importance were indeed certified by case reports, but mechanistic study in this field is still limited [7]. In veterinary research, reports about herb-drug interactions are also far from enough. In this study, we characterized the effects of AB4 on florfenicol pharmacokinetics and the mRNA expression levels of CXR, MDR1, CYP3A37 and UGT1E in broilers for the first time.

CYP3A is the phase I metabolic enzymes, in which CYP3A37 and CYP3A80 are present in birds. Wang *et al.* (2018) have suggested that CYP 3A plays a key role in the pharmacokinetics of florfenicol in chickens [33]. Watanabe *et al.* (2013) have reported that CYP3A37 is suggested to be more important in avian xenobiotic metabolism than CYP3A80 because of the higher expression level [34]. Based on this, we decided to study the effect of AB4 on the mRNA expression of CYP3A37. P-gp, an important transport protein, is the product of the multiple drug resistance (MDR) gene 1; it is expressed in the intestine along with CYP3A, forming a transport barrier to drug absorption [21, 26]. Liu *et al.* (2012) have reported that P-gp and/or CYP3A are likely involved in the disposition of florfenicol in rabbits [19]. In the liver, P-gp is present in bile canaliculi, where it transports endogenous and xenobiotic substrates. In our study, we found that the oral administration of AB4 decreased the $AUC_{(0-\infty)}$, $MRT_{(0-\infty)}$, $t_{1/2z}$ and C_{max} of florfenicol in AB4-treated broilers while it increased the corresponding Vz/F and CLz/F. We propose that the decreased $AUC_{(0-\infty)}$ and $MRT_{(0-\infty)}$ resulted from AB4 decreasing the intestinal absorption and increasing elimination of florfenicol. Meanwhile, the mRNA expression levels of jejunal CYP3A37 and MDR1 in AB4-treated broilers were significantly increased, which may have induced the activities of CYP3A and P-gp, leading to increased metabolism and efflux of florfenicol. Thus, the absorption of florfenicol was decreased. This inference is consistent with the altered pharmacokinetics of florfenicol that we observed. In addition, after treatment of broilers with AB4, the mRNA expression of MDR1 in the livers was significantly increased, which may induce the activities of hepatic P-gp, thus promoting efflux of florfenicol. Thus, the elimination of florfenicol was increased, which was also approximately consistent with the pharmacokinetic results. In this research, we have not carried out the determination of the activities of chicken CXR, CYP3A and P-gp yet, which makes it impossible to fully support the above inference. In addition, although the mRNA expression levels of CXR, CYP3A37 and MDR1 (except CXR and CYP3A37 in the liver) were significantly up-regulated by AB4, these changes are still small. Therefore, this inference needs to be further demonstrated.

CXR is the main xenobiotic-sensing nuclear receptor in chickens through its regulation of CYP450 expression [9, 10]. In this study, increased mRNA expression of CYP3A37 in the jejunum of AB4-treated animals was correlated with increased mRNA expression of CXR, which supports this relationship. The UGT superfamily consists of enzymes that are responsible for a major biotransformation phase II pathway: the glucuronidation process. Florfenicol is a chloramphenicol antibiotic, previous studies have reported that chloramphenicol can be glucuronidated primarily by UGTs *in vivo* [4]. However, in this study, AB4 had little effect on the mRNA expression of UGT1E.

The reported minimum inhibitory concentrations (MICs) of florfenicol for bacteria isolated from poultry, fish, swine and calves are approximately 0.4 to 8 μg/m*l* [8, 14, 15, 23, 28], and the bacterium is reported sensitive to antibiotics at average concentration in blood 2–4 times their MIC [18]. Therefore, 1–16 μg/m*l* may be the effective blood concentration of florfenicol against various pathogenic bacteria *in vivo*. The time above the MIC is a main criterion for the effectiveness of florfenicol [14, 30]. In this study, the time that the mean plasma concentration of florfenicol exceeded 1 μg/m*l* was about 7 hr in control broilers but about 5 hr after prior oral administration of AB4 (Fig. 3). This observation raises the possibility that feeding of AB4 reduces the therapeutic effectiveness of florfenicol. This possibility emphasizes the need for caution in co-administering TCM containing AB4 and florfenicol in broilers.

In conclusion, we suggested that AB4 affected the pharmacokinetics of florfenicol in broilers, this was probably related to the mRNA expression of CXR, CYP3A37 and MDR1 in the jejunum and liver (except CXR and CYP3A37) increased by AB4. Prior feeding of AB4 decreased the time that mean plasma concentrations of florfenicol were in the reported minimal inhibitory concentration for various pathogenic bacteria. These findings emphasize that when florfenicol is co-administered with AB4, there may be drug-drug interactions, which may cause therapy failure. Implications of these results for the use of TCM containing AB4 in combination with florfenicol in veterinary practice should be studied.

ACKNOWLEDGMENTS. This study was supported by the Program Sichuan Veterinary Medicine and Drug Innovation Group of China Agricultural Research System (CARS-SVDIP); and Finance Operation Special Project of Sichuan.

REFERENCES

1. Abd El-Aty, A. M., Goudah, A., Abo El-Sooud, K., El-Zorba, H. Y., Shimoda, M. and Zhou, H. H. 2004. Pharmacokinetics and bioavailability of florfenicol following intravenous, intramuscular and oral administrations in rabbits. *Vet. Res. Commun.* **28**: 515–524. [Medline] [CrossRef]
2. Balcomb, C. C., Angelos, J. A., Chigerwe, M., Byrne, B. A., Lane, V. M., Wetzlich, S. E., Sahin, O., Holler, L., Zhang, S. and Tell, L. A. 2018. Comparative pharmacokinetics of two florfenicol formulations following intramuscular and subcutaneous administration to sheep. *Am. J. Vet. Res.* **79**: 107–114. [Medline] [CrossRef]
3. Cannon, M., Harford, S. and Davies, J. 1990. A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated

derivatives. *J. Antimicrob. Chemother.* **26**: 307–317. [Medline] [CrossRef]
4. Chen, M., Howe, D., Leduc, B., Kerr, S. and Williams, D. A. 2007. Identification and characterization of two chloramphenicol glucuronides from the in vitro glucuronidation of chloramphenicol in human liver microsomes. *Xenobiotica* **37**: 954–971. [Medline] [CrossRef]
5. Chinese Veterinary Pharmacopoeia Committee. 2015. Veterinary pharmacopoeia of the People's Republic of China (2015 edition), China Agriculture Press, Beijing.
6. Feng, J. B., Huang, D. R., Zhong, M., Liu, P. and Dong, J. D. 2016. Pharmacokinetics of florfenicol and behaviour of its metabolite florfenicol amine in orange-spotted grouper (Epinephelus coioides) after oral administration. *J. Fish Dis.* **39**: 833–843. [Medline] [CrossRef]
7. Ge, B., Zhang, Z. and Zuo, Z. 2014. Updates on the clinical evidenced herb-warfarin interactions. *Evid. Based Complement. Alternat. Med.* **2014**: 957362. [Medline] [CrossRef]
8. Gharaibeh, S., Al Rifai, R. and Al-Majali, A. 2010. Molecular typing and antimicrobial susceptibility of *Clostridium perfringens* from broiler chickens. *Anaerobe* **16**: 586–589. [Medline] [CrossRef]
9. Handschin, C., Podvinec, M. and Meyer, U. A. 2000. CXR, a chicken xenobiotic-sensing orphan nuclear receptor, is related to both mammalian pregnane X receptor (PXR) and constitutive androstane receptor (CAR). *Proc. Natl. Acad. Sci. U.S.A.* **97**: 10769–10774. [Medline] [CrossRef]
10. Handschin, C., Podvinec, M., Stöckli, J., Hoffmann, K. and Meyer, U. A. 2001. Conservation of signaling pathways of xenobiotic-sensing orphan nuclear receptors, chicken xenobiotic receptor, constitutive androstane receptor, and pregnane X receptor, from birds to humans. *Mol. Endocrinol.* **15**: 1571–1585. [Medline] [CrossRef]
11. He, M., Ouyang, H., He, M., Tan, T., Li, J., Zhang, X., Jia, J., Feng, Y. and Yang, S. 2017. Application of a liquid chromatography-tandem mass spectrometry method to the pharmacokinetics, tissue distribution and excretion in the study of anemoside B4, a novel antiviral agent candidate, in rats. *Biomed. Chromatogr.* **31**: e3914. [Medline] [CrossRef]
12. Ho, S. P., Hsu, T. Y., Che, M. H. and Wang, W. S. 2000. Antibacterial effect of chloramphenicol, thiamphenicol and florfenicol against aquatic animal bacteria. *J. Vet. Med. Sci.* **62**: 479–485. [Medline] [CrossRef]
13. Hu, Y., He, K. and Wang, X. 2016. Role of Chinese herbal medicinal ingredients in secretion of cytokines by PCV2-induced endothelial cells. *J. Immunotoxicol.* **13**: 141–147. [Medline] [CrossRef]
14. Illambas, J., Potter, T., Sidhu, P., Rycroft, A. N., Cheng, Z. and Lees, P. 2013. Pharmacodynamics of florfenicol for calf pneumonia pathogens. *Vet. Rec.* **172**: 340. [Medline] [CrossRef]
15. Lei, Z., Liu, Q., Yang, S., Yang, B., Khaliq, H., Li, K., Ahmed, S., Sajid, A., Zhang, B., Chen, P., Qiu, Y., Cao, J. and He, Q. 2018. PK-PD integration modeling and cutoff value of florfenicol against *Streptococcus suis* in pigs. *Front. Pharmacol.* **9**: 2. [Medline] [CrossRef]
16. Li, S., Li, X., Yuan, D., Wang, B., Yang, R., Zhang, M., Li, J. and Zeng, F. 2017. Effects of paeoniflorin on the activities and mRNA expression of rat CYP1A2, CYP2C11 and CYP3A1 enzymes *in vivo*. *Xenobiotica* **2017**: 1–26. [Medline] [CrossRef]
17. Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25**: 402–408. [Medline] [CrossRef]
18. Liu, C., Wang, S. J., Zhang, Q. and Shao, Y. X. 2015. Influence of three coccidiostats on the pharmacokinetics of florfenicol in rabbits. *Exp. Anim.* **64**: 73–79. [Medline] [CrossRef]
19. Liu, N., Guo, M., Mo, F., Sun, Y. H., Yuan, Z., Cao, L. H. and Jiang, S. X. 2012. Involvement of P-glycoprotein and cytochrome P450 3A in the metabolism of florfenicol of rabbits. *J. Vet. Pharmacol. Ther.* **35**: 202–205. [Medline] [CrossRef]
20. Lloret, N. L. 2008. Traditional Chinese veterinary medicine. *Vet. Hist.* **14**: 97–103.
21. Lo, A. and Burckart, G. J. 1999. P-glycoprotein and drug therapy in organ transplantation. *J. Clin. Pharmacol.* **39**: 995–1005. [Medline] [CrossRef]
22. McKellar, Q. A. and Varma, K. J. 1996. Pharmacokinetics and tolerance of florfenicol in Equidae. *Equine Vet. J.* **28**: 209–213. [Medline] [CrossRef]
23. Michel, C., Kerouault, B. and Martin, C. 2003. Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. *J. Appl. Microbiol.* **95**: 1008–1015. [Medline] [CrossRef]
24. National Research Council (US) Institute for Laboratory Animal Research. 1996. Guide for the care and use of laboratory animals. **103**: 1072–1073.
25. Paape, M. J., Miller, R. H. and Ziv, G. 1990. Effects of florfenicol, chloramphenicol, and thiamphenicol on phagocytosis, chemiluminescence, and morphology of bovine polymorphonuclear neutrophil leukocytes. *J. Dairy Sci.* **73**: 1734–1744. [Medline] [CrossRef]
26. Pal, D. and Mitra, A. K. 2006. MDR- and CYP3A4-mediated drug-drug interactions. *J. Neuroimmune Pharmacol.* **1**: 323–339. [Medline] [CrossRef]
27. Pérez, R., Palma, C., Drápela, C., Sepulveda, M., Espinoza, A. and Peñailillo, A. K. 2015. Pharmacokinetics of florfenicol after intravenous administration in Escherichia coli lipopolysaccharide-induced endotoxaemic sheep. *J. Vet. Pharmacol. Ther.* **38**: 144–149. [Medline] [CrossRef]
28. Salmon, S. A. and Watts, J. L. 2000. Minimum inhibitory concentration determinations for various antimicrobial agents against 1570 bacterial isolates from turkey poults. *Avian Dis.* **44**: 85–98. [Medline] [CrossRef]
29. Shen, J., Hu, D., Wu, X. and Coats, J. R. 2003. Bioavailability and pharmacokinetics of florfenicol in broiler chickens. *J. Vet. Pharmacol. Ther.* **26**: 337–341. [Medline] [CrossRef]
30. Sidhu, P., Rassouli, A., Illambas, J., Potter, T., Pelligand, L., Rycroft, A. and Lees, P. 2014. Pharmacokinetic-pharmacodynamic integration and modelling of florfenicol in calves. *J. Vet. Pharmacol. Ther.* **37**: 231–242. [Medline] [CrossRef]
31. van Erp, N. P., Baker, S. D., Zhao, M., Rudek, M. A., Guchelaar, H. J., Nortier, J. W., Sparreboom, A. and Gelderblom, H. 2005. Effect of milk thistle (Silybum marianum) on the pharmacokinetics of irinotecan. *Clin. Cancer Res.* **11**: 7800–7806. [Medline] [CrossRef]
32. Wang, G. Y., Tu, P., Chen, X., Guo, Y. G. and Jiang, S. X. 2013. Effect of three polyether ionophores on pharmacokinetics of florfenicol in male broilers. *J. Vet. Pharmacol. Ther.* **36**: 494–501. [Medline] [CrossRef]
33. Wang, G. Y., Zheng, H. H., Zhang, K. Y., Yang, F., Kong, T., Zhou, B. and Jiang, S. X. 2018. The roles of cytochrome P450 and P-glycoprotein in the pharmacokinetics of florfenicol in chickens. *Majallah-i Tahqiqat-i Dampizishki-i Iran* **19**: 9–14. [Medline]
34. Watanabe, K. P., Kawai, Y. K., Ikenaka, Y., Kawata, M., Ikushiro, S., Sakaki, T. and Ishizuka, M. 2013. Avian cytochrome P450 (CYP) 1-3 family genes: isoforms, evolutionary relationships, and mRNA expression in chicken liver. *PLoS One* **8**: e75689. [Medline] [CrossRef]
35. Zhou, C. J., Qiao, L. M., Zhao, L. H. and Li, Z. Y. 2015. Evaluation of the impact of cantharidin on rat CYP enzymes by using a cocktail of probe drugs. *Fitoterapia* **107**: 49–53. [Medline] [CrossRef]