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Effects of fluoroquinolones on CYP4501A and 3A in male broilers

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ABSTRACT

The inhibitory effects of fluoroquinolones on the enzyme activity, protein levels and mRNA expression of liver cytochrome P450 (CYP) 1A and 3A were investigated in male broiler chicks. Enrofloxacin (20 mg/kg), sarafloxacin (8 mg/kg) and marbofloxacin (5.5 mg/kg) were administrated in drinking water for 7 consecutive days. A cocktail of the probe drugs caffeine and dapsone was used to determine CYP1A and 3A activity. Western blot analysis and real-time PCR were used to determine the effects on protein levels of CYP1A and 3A, and on CYP1A4, 1A5, 3A37 mRNA levels. Enrofloxacin increased the half-life of elimination for both caffeine and dapsone, and decreased expression of CYP1A and 3A protein. Marbofloxacin decreased the metabolism of caffeine and expression of CYP1A protein. However, no change in mRNA expression was observed for any treatment group. This suggested that high doses of enrofloxacin and marbofloxacin, but not sarafloxacin, inhibit CYP in chick liver raising the possibility of drug–drug interaction when using these compounds.

1. Introduction

Cytochrome P450 (CYP) enzymes are known for their role in metabolism of non-polar compounds (Anzenbacher and Anzenbacherová, 2001). Within the complex group of cytochromes, the enzymes in CYP families 1–3 are primarily involved in the biotransformation of drugs, and are often referred to as drug-metabolising enzymes. The CYP1A subfamily enzymes 1A4 and 1A5 have been purified and characterized from chicken (Gilday et al., 1996). Phylogenetic analysis showed a tree topology consistent with orthology between the avian CYP1A5s and the mammalian CYP1A2s, and between the avian CYP1A4s and mammalian CYP1A1s (Kubota et al., 2006). The CYP2 family (2H1, 2H2) is inducible by phenobarbital, and the enzymes found in chicken liver are similar to human CYP2C (Ourlin et al., 2000; Hansen et al., 1990). The chicken CYP2C45 sequence is 56% identity with CYP2H1, and is highly related to a gene cluster of CYP2Cs in other species (Baader et al., 2002). Finally, when the CYP3A subfamily member 3A37 was cloned from phenobarbital-inducible chicken embryo livers, the expressed protein was recognized by polyclonal anti-rat CYP3A1 antiserum (Ourlin et al., 2000). Although studies have been published on the role of CYP isoforms in human drug metabolism, very little data are available on the effect of drugs on CYP enzymes in chickens.

The fluoroquinolones are important group of antibiotics that are used to control pulmonary, urinary and digestive bacterial infections in poultry and animals (Papich and Riviere, 2001), and are usually administrated to poultry in drinking water. They inhibit bacterial DNA gyrase, a bacterial topoisomerase II that is essential for DNA replication and transcription (Yorke and Froc, 2000). Fluoroquinolones have been shown to have an inhibitory effect on CYP4501A and 3A subtypes in human (Mclellan et al., 1996), rat (Mclellan et al., 1996), dogs (Regmi et al., 2005, 2007; Hirt et al., 2003), sheep (Rahal et al., 2008) and broiler chicks (Schlosberg et al., 1995; Sureshkumar et al., 2004). Mclellan et al. (1996) demonstrated that norfloxacin and ciprofloxacin, competitively inhibited ethroxyresorufin O-deethylation and erythromycin N-demethylation catalyzed by CYP1A and 3A of human and rat hepatic microsomes. Regmi et al. (2005, 2007) reported that enrofloxacin acted as a noncompetitive inhibitor, while ciprofloxacin was a mechanism-based inhibitor of the CYP1A subfamily, with no effect on CYP3A in dogs. When theophylline was co-administered intravenously (i.v.) once a day at 5 mg/kg with enrofloxacin, theophylline concentrations progressively and significantly increased over 5 days (Intorre et al., 1995). Hirt et al. (2003) demonstrated that marbofloxacin at 5 mg/kg body weight significantly decreased the total clearance of theophylline in dogs. In sheep, co-administration of enrofloxacin caused a more rapid absorption and significant reduction in elimination of diclofenac (Rahal et al., 2008), possibly because of direct inhibition of CYP isozymes by enrofloxacin. Schlosberg et al. (1995) reported that, in chickens, enrofloxacin at 10 mg/kg body weight, which is a dose roughly equal to the enrofloxacin concentration of 50 mg/L used in the

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work reported here, transiently inhibited the activity of aniline hydroxylase and aminopyrine N-demethylase. The reversible inhibition of aniline hydroxylase by enrofloxacin has also been demonstrated, and enrofloxacin coadministrated with monensin increased monensin toxicity, and heart and liver damage (Sureshkumar et al., 2004).

Inhibition of CYP is a principal mechanism for metabolism-based, drug–drug interactions, and may lead to an increase in residue from co-administered drugs. Enrofloxacin and sarafloxacin are commonly used in poultry, so understanding the effect of the extensive use of fluoroquinolones on CYP enzymes in broiler chicks is crucial. In this study, a cocktail of probes was used to evaluate enzyme activity, and Western blotting and real-time PCR were used to detect the protein and gene expression of CYP1A and 3A after fluoroquinolone treatment.

2. Materials and methods

2.1. Chemicals

A 10% enrofloxacin oral solution was from Bayer AG Corporation (Shanghai, China), water-soluble sarafloxacin powder was from Riedel-de Haen Corporation (Seelze, Germany) and marbofloxacin water-soluble powder was from Zhejiang Guobang Pharmaceutical Corporation (Zhejiang, China). Dapsone (97%, Gillingham, UK) and antipyrine (St. Louis, USA) were from Sigma–Aldrich Chemical Co. Ltd. Caffeine was from Axxora (Nottingham, UK). Acetonitrile and methanol were high-performance liquid chromatography (HPLC)-grade from Merck Corporation (Darmstadt, Germany). Trizol reagent was from Invitrogen Corporation (CA, USA). M-MLV reverse transcriptase was supplied by Takara Corporation (Shiga, Japan). SYBR Green mix enzyme was from Toyobo Corporation (Shiga, Japan). Rabbit polyclonal antibody anti-human CYP3A4/5 and anti-human/rat/mouse CYP1A1/2 were purchased from Bioworld Corporation (CA, USA). β-actin monoclonal antibody was from Abcam Corporation (Cambridge, MA, USA). Chemiluminescence Reagent Plus was from Beyotime Institute of Biotechnology (Jiangsu, China). All other reagents were from commercial companies.

2.2. Chickens

One-day-old Arbor Acres broiler chicks were from Hewei Agricultural Development Share Co. Ltd. (Anhui, China). Chicks were fed a diet recommended by the National Research Council (1994) and had free access to water. When 35 days old, male chicks were divided into four groups. Groups I, II and III were treated with enrofloxacin, sarafloxacin and marbofloxacin at 20, 8, and 5.5 mg/kg body weight (twice clinical dosage), respectively, for 7 days. Concentrations of drugs added to drinking water were based on daily water consumption rates and daily weights of the chicks. Control chicks (Group IV) received water without drugs or additives. No adverse effect was observed during administration of high dosage fluoroquinolones in any chicks. On day 8, each group was divided into two, with 10 chicks chosen for the drug probe cocktail trial, and 6 used to evaluate the protein and RNA levels of CYP1A and 3A.

2.3. Administration of probe drugs and blood sampling

Administration of the probe drugs and blood sampling from the wing vein were performed by technical staff with minimal delays. Dosages of caffeine at 10 mg/kg of body weight, and dapsone at 10 mg/kg of body weight generally corresponded to the use of probe drugs in rat (Tang et al., 2008), and were injected in 10 chicks per group. Serum samples were taken at 10, 30, 50, 70 and 90 min, and at 2, 3, 4, 6, 8, 10 and 12 h. Each sample was centrifuged at 900 *g* for 10 min and 400 μL of serum was removed. Aliquots of collected serum (100 μL) were supplemented with 20 μL antipyrine as an internal standard, and extracted with 1 mL chloroform: 2-propanol = 9:1 (vol/vol) with vortexing for 2 min, before centrifuging at 5000 rpm for 10 min. Organic supernatants were evaporated to dryness under nitrogen. Dry residues were dissolved in 200 μL HPLC mobile phase (see below) and 60 μL was injected through a Waters 717 autosampler (Milford, MA, USA) in a Waters 600 pump for HPLC analysis. The mobile phase was acetonitrile-20 mmol/L ammonium acetate (PH = 6.5) at a ratio of 30:70 (vol/vol) at a 1.0 mL/min flow rate. Chromatographic separation was performed on a Waters Sunfire C_{18} column (5 μm, 4.6 mm × 150 mm). The detection wavelength was 265 nm and column temperature was 28 °C.

2.4. Analytical methods

No adverse effects were observed during or following i.v. administration of caffeine or dapsone in any of the chicks. Chromatographic peaks of caffeine and dapsone in chick serum were sharp, and showed no matrix interference. The average retention times were 1.9 min for caffeine, 2.6 min for antipyine and 4.9 min for dapsone. The calibration curve was linear between 0.2 and 30 mg/L for caffeine with a determination coefficient of 0.9997, and between 0.05 and 10 mg/L for dapsone with a determination coefficient of 0.9993. Precision and accuracy were evaluated for fortified serum at three different concentrations: 1, 5 and 15 mg/L for caffeine and 0.25, 2 and 5 mg/L for dapsone. Recoveries ranged from 92.1% to 104.5%. For caffeine, intra-day coefficients of variation were 3.29%, 5.58% and 7.33%; and inter-day coefficients of variation were 8.12%, 9.13% and 11.05% (n = 5). For dapsone, intra-day coefficients of variation were 5.92%, 5.30% and 7.63%; and inter-day coefficients of variation were 6.32%, 8.24% and 10.91% (n = 5).

2.5. Pharmacokinetic analysis

A noncompartmental method was used to analyze the serum concentration–time data for caffeine and dapsone using a pharmacokinetic program (Chinese Pharmacological Association, Beijing, China) (Yang et al., 1988). The area under the concentration versus time curve (*AUC*) from time 0 to 12 h was calculated using the trapezoidal rule, and was extrapolated to infinity by dividing the last experimental concentration by the terminal elimination rate constant. Total body clearance (cl_B) was calculated as cl_B = dose/*AUC*. The mean residence time (*MRT*) was calculated as *MRT* = *AUMC*/*AUC*, where *AUMC* represented the area under the first moment versus time curve, computed in a similar fashion to the *AUC*. The half-life ($t_{1/2}$) was calculated as 0.693/terminal elimination rate constant. The steady-state volume of distribution (V_{SS}) was calculated as $V_{SS} = cl_B \times MRT$.

2.6. Preparation of microsomal fractions and cytochrome P450 content

Chicks were anaesthetized with pentobarbital (25 mg/kg body weight) and killed. Livers were removed immediately and differentially centrifuged to collect microsomes, as described by Khalil et al. (2001). The protein content was determined according to Bradford (1976) using bovine serum albumin as the standard, then total cytochrome P450 content and b_5 content were determined by the method of Omura and Sato (1964).

2.7. Western blotting

Microsome proteins (40 μg) were separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gels (SDS–PAGE), according to Laemmli (1970) and transferred to polyvinylidene

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difluoride (PVDF) membrane (Millipore Company, Bedford, MA, USA) using a Biorad Semi-Dry Electrophoretic Transfer Cell (Hercules, CA, USA). Membranes were blocked in Tris-buffered saline with 0.1% Tween 20 and 5% nonfat dry milk and incubated with polyclonal antibodies against anti-human CYP3A, anti-human/rat/mouse CYP1A, or β-actin monoclonal antibody. Immune complexes were visualized using peroxidase-labelled secondary antibody with a Western blot chemiluminescent reagent. The relative levels of each protein were quantified by densitometry using a Digital Science Imaging System (Version 2.0.1, Eastman Kodak Co., Rochester, NY, USA).

2.8. RNA extraction and real-time PCR

Total RNAs were extracted from liver samples using Trizol reagent and reverse transcribed into cDNA using M-MLV reverse transcriptase according to the manufacturer's procedure. mRNA was quantified on a Applied Biosystems 7300 Real-Time PCR System (Foster, CA, USA) using SYBR Green mix enzyme. PCR primers are in Table 1, and the β-actin housekeeping was used as a control. In general, 10 ng of total RNA was used per sample. PCR reactions were 95 °C for 1 min, then 45 cycles of 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 31 s. SYBR Green fluorescence data was collected at the end of each cycle.

2.9. Statistical analysis

Measures of cytochrome and b_5 and Western blots were done in duplicate. mRNA quantification was conducted in triplicate. The mean value of $t_{1/2}$ was calculated using a harmonic mean method (Lam et al., 1985), and the other data are presented as mean ± SEM. Where appropriate, data were analysed by one-way analysis-of-variance (ANOVA) followed by Fisher's least significant difference test using SPSS 16.0 software (SPSS Inc, Chicago, USA) with $p < 0.05$ denoting a significant difference.

3. Results

3.1. Effect on caffeine and dapsone pharmacokinetics

The intravenous pharmacokinetics of caffeine and dapsone were affected by oral treatment with high doses of enrofloxacin and marbofloxacin (Fig. 1). The pharmacokinetic parameters of the probe drugs are in Tables 2 and 3. Daily drug treatment for 7 days significantly increased the $t_{1/2}$ for elimination of caffeine from 4.23 h, to 5.02 h ($p < 0.05$) with enrofloxacin, and 5.63 h ($p < 0.01$) with marbofloxacin. The cl_B decreased from 0.15 L/kg/h with no antibiotic, to 0.13 L/kg/h ($p > 0.05$) with enrofloxacin, and 0.12 L/kg/h ($p < 0.05$) with marbofloxacin. The *AUC* of marbofloxacin-treated chicks was significant larger than the *AUC* of chicks in the untreated group (Table 2), suggesting that marbofloxacin and enrofloxacin inhibited the elimination of caffeine, which is a CYP1A substrate in mammals.

Treatment with enrofloxacin increased the *AUC* and $t_{1/2}$ of dapsone, and decreased the cl_B. The *AUC* increased by 2.28-fold compared to the control group, and the $t_{1/2}$ increased from 1.59 h to 5.99 h. Total body clearance decreased from 1.64 L/kg/h to 0.82 L/kg/h. All changes were significant ($p < 0.05$), suggesting that enrofloxacin decreased metabolism of dapsone, which is a CYP3A substrate in mammals. However, statistical analysis revealed that dapsone pharmacokinetics were not affect by marbofloxacin, and sarofloxacin affected neither caffeine nor dapsone metabolism (Table 3).

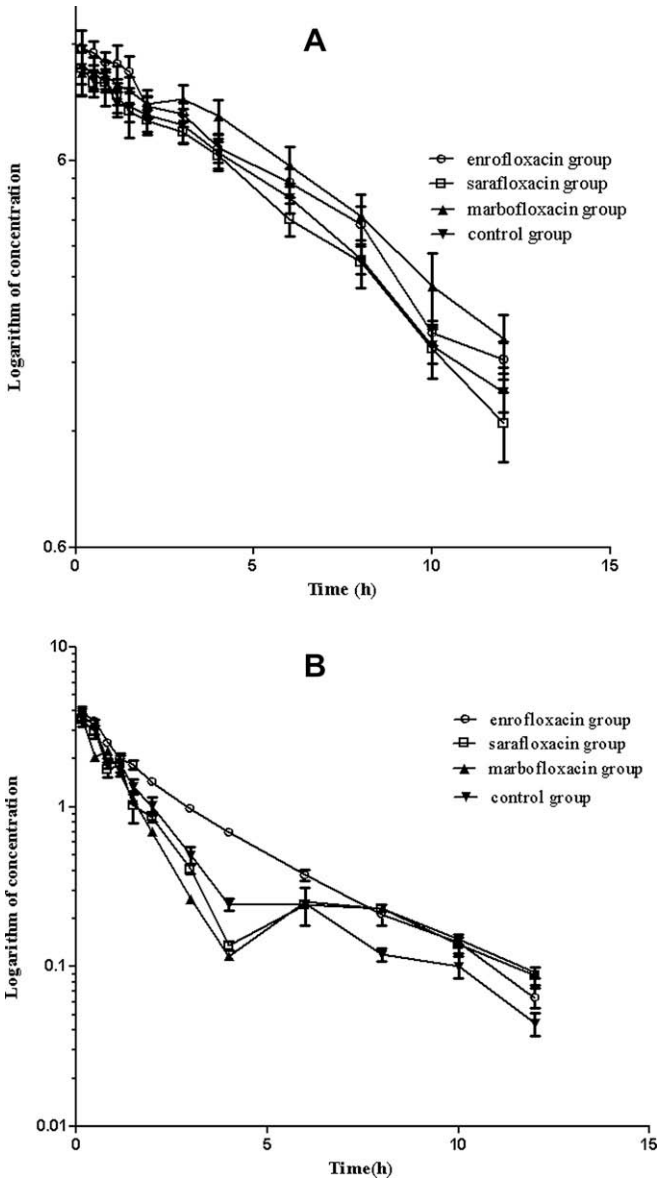

Fig. 1. Effect of fluoroquinolones on metabolism of probe drugs in male broiler chicks (semi-log graph of mean serum concentration versus time curves). (A) Caffeine and (B) Dapsone. Enrofloxacin, sarafloxacin and marbofloxacin at dosages of 20, 8 or 5.5 mg/kg daily for 7 days prior to caffeine (10 mg/kg) or dapsone (10 mg/kg) administration in the three fluoroquinolone-treated groups. Control group chicks received caffeine and dapsone on the 8th day. Each point with a bar represents the mean ± SEM of ten chicks.

Table 1
Primers for real-time PCR of CYP1A4, 1A5, 3A37 and chicken β-actin.

Gene	Sense primer (5′–3′)	Anti-sense primer
CYP3A37	CGAATCCCAGAAATCAGA	AGCCAGGTAACCAAGTGT
CYP1A4	AGGACGGAGGCTGACAAG	CAGGATGGTGGTGAGGAAGA
CYP1A5	TCACCATCCCGCACAGCA	AAGTCATCACCTTCTCCGCATC
β-actin	TGCGTGACATCAAGGAGAAG	TGCCAGGGTACATTGTGGTA

3.2. Effect on CYP450, b_5 content

Table 4 shows the effect of high doses of fluoroquinolone antibiotic treatment on the content of CYP in liver microsomes of broiler chicks. Both enrofloxacin and marbofloxacin strongly decreased the P450 content, which was reduced to 68% of the control value. Cytochrome b_5 content was not changed by fluoroquinolone treat-

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Table 2
Effects of fluoroquinolones on the pharmacokinetic parameters of caffeine.

Parameters	Enrofloxacin group	Sarafloxacin group	Marbofloxacin group	Control group
AUC (mg × h/L)	85.22 ± 7.90^{ab}	76.66 ± 5.41^{ab}	86.48 ± 4.79^{a}	72.22 ± 5.77^{b}
$t_{1/2}$ (h)	5.02 ± 0.26^{a}	4.39 ± 0.24^{bB}	5.63 ± 0.34^{aA}	4.23 ± 0.22^{bB}
CL (L/kg/h)	0.13 ± 0.02^{ab}	0.13 ± 0.03^{ab}	0.12 ± 0.02^{b}	0.15 ± 0.012^{a}
Vss (L/kg)	0.94 ± 0.08	0.83 ± 0.14	0.96 ± 0.05	0.91 ± 0.08

Values are represented as mean ± SEM (n = 10) except $t_{1/2}$ is the harmonic mean.
^{ab}Data within rows with different lowercase superscripts are significantly different ($p < 0.05$).
^{AB}Data within rows with different uppercase superscripts are highly significantly different ($p < 0.01$).

Table 3
Effects of fluoroquinolones on pharmacokinetic parameters of dapsone.

Parameters	Enrofloxacin group	Sarafloxacin group	Marbofloxacin group	Control group
AUC (mg × h/L)	15.72 ± 2.06^{A}	6.20 ± 0.45^{B}	6.58 ± 0.80^{B}	6.88 ± 0.77^{B}
$t_{1/2}$ (h)	5.99 ± 0.44^{A}	2.09 ± 0.29^{B}	1.60 ± 0.41^{B}	1.59 ± 0.46^{B}
CL (L/kg/h)	0.82 ± 0.20^{b}	1.81 ± 0.61^{a}	1.52 ± 0.27^{a}	1.64 ± 0.17^{a}
V_{SS} (L/kg)	7.96 ± 0.79	5.89 ± 0.57	4.21 ± 0.37	4.34 ± 0.93

Values are represented as mean ± SEM (n = 10) except $t_{1/2}$ is the harmonic mean.
^{ab}Data within rows with different lowercase superscripts are significantly different ($p < 0.05$).
^{AB}Data within rows with different upper superscripts are highly significantly different ($p < 0.01$).

Table 4
Effects of fluoroquinolones on weight gain, relative liver weight and CYP450 and b_5 content in male broiler chicks (n = 6).

Parameters	Enrofloxacin group	Sarafloxacin group	Marbofloxacin group	Control group
Weight gain (kg)	0.23 ± 0.04	0.25 ± 0.05	0.23 ± 0.02	0.29 ± 0.07
Relative liver weight^{A}	16.84 ± 0.42	15.90 ± 0.55	14.62 ± 0.65	16.79 ± 0.75
Cytochrome P450^{B}	0.23 ± 0.04^{b}	0.29 ± 0.05^{ab}	0.23 ± 0.01^{b}	0.34 ± 0.05^{a}
Cytochrome b_5^{B}	0.33 ± 0.02	0.38 ± 0.02	0.37 ± 0.12	0.44 ± 0.05

^{ab}Means data within rows with different superscripts are significantly different ($p < 0.05$); ^{A}g/kg; ^{B}nmol/mg protein.

ment. Weight gain and relative liver weight of chicks showed no significant difference among the four groups ($p > 0.05$).

3.3. Effect on CYP1A and 3A protein levels

The effect of fluoroquinolones on the expression of CYP1A and 3A in the liver of broilers was investigated. Changes were observed in the protein levels of hepatic CYP1A after pretreatment with enrofloxacin and marbofloxacin (Fig. 2), with both antibiotics causing a significant decrease in CYP1A. Enrofloxacin decreased the protein level of CYP3A by 39%, compared to untreated broilers, but marbofloxacin caused little decrease ($p > 0.05$). Sarafloxacin caused no significant differences in expression of CYP1A or 3A ($p > 0.05$) compared to untreated broilers (Fig. 3). The down-regulation of CYP3A by enrofloxacin, and CYP1A by marbofloxacin correlated well with delayed elimination of dapsone and caffeine, respectively.

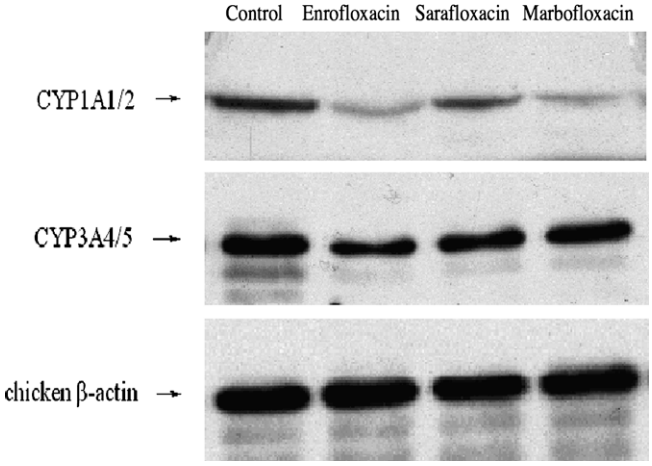

Fig. 2. Western blot of chicken liver microsomes with anti-human/rat/mouse CYP1A, anti-human CYP3A polyclonal antibody and β-actin monoclonal antibody. Immunoblotting was performed with 40 μg protein loaded on 10% polyacrylamide gels and transferred to PVDF membranes.

3.4. Effects on CYP 1A4, 1A5 and 3A37 mRNA expression

Real-time PCR was used to investigate the mRNA expression of broilers treated with high doses of enrofloxacin, sarafloxacin or marbofloxacin compared to untreated broilers. No significant differences were detected ($p > 0.05$) (Fig. 4), suggesting that fluoroquinolone inhibition of microsome activity was not through inhibition of expression of CYP1A4, 1A5 or 3A37 mRNA.

4. Discussion

The CYP1A family of the hepatic microsomal cytochrome p450 system is involved in the degradation of procarcinogens, and is highly induced by polycyclic aromatic hydrocarbons (PAHs). Drugs such as theophylline, caffeine, imipramine, paracetamol and phenacetin are known to be metabolized by CYP1A in humans (Zakia, 2008). In chickens, the CYP1A family consists of two enzymes, 1A4 and 1A5. CYP1A transcription expression is induced by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), and PAH through the aryl

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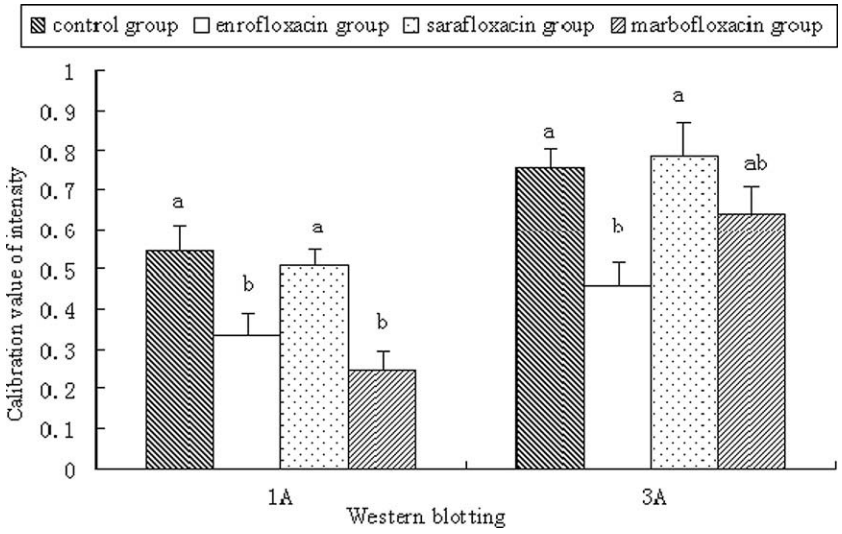

Fig. 3. Effects of fluoroquinolones on CYP1A and 3A protein expression, with different superscripts indicating significant differences ($p < 0.05$). Each bar shows the intensity ratio calibrated by β-actin. Values are the mean ± SEM ($n = 6$).

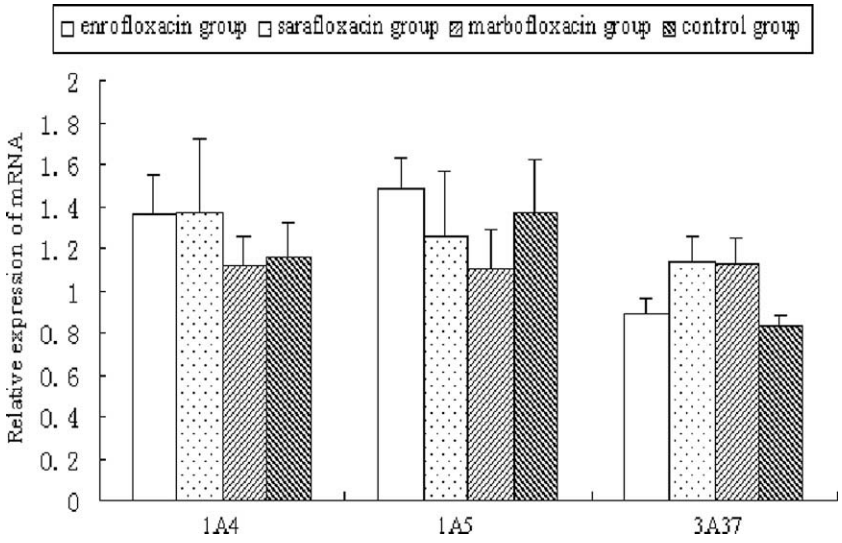

Fig. 4. Effects of fluoroquinolones on CYP 1A4, 1A5 and 3A37 mRNA expression compared to the control group. Total RNA preparation and real-time PCR quantification using second derivative calculations and the double correction method (Luu-The et al., 2005) with SYBR green fluorescence detection, as in Materials and Methods. Relative expression levels are calibrated with the housekeeping β-actin gene. Data are expressed as means ± SEM of triplicate measurements from six samples. No significant differences were found ($p > 0.05$).

hydrocarbon receptor (AHR) (Mahajan and Rifkind, 1999; Watanabe et al., 2005). Proteins that cross-react with antibodies against rat CYP1A have been found in liver microsomes of chickens (Coulet et al., 1996; Mahajan and Rifkind, 1999; Watanabe et al., 2005; Kubota et al., 2006), consistent with our results that only a single CYP1A cross-reactive band was detected with anti-human/rat/mouse CYP 1A1/2 polyclonal antibody in chicken liver microsomes.

CYP 3A isoforms play a primary role in the metabolism of 120 different human medications including sedatives, antidepressives, anti-arryhthmics, antihistamines, calcium channel antagonists, various antimicrobials and protease inhibitors (Zakia, 2008). The chicken CYP3A37 gene sequence is 60% homologous to human CYP3A4, and the steroid hydroxylase profile of CYP 3A37 exhibits a high degree of similarity to the mammalian 3A enzyme (Ourlin et al., 2000). Immunodetection of the chicken enzyme by polyclonal antibodies against rat CYP3A1 was reported by Coulet et al. (1996), and by Ourlin et al. (2000).

Through the administration of a cocktail of drugs, the activities of more than one enzyme at a time can be investigated, and the relative accuracy of the investigation can be increased. Probe drugs have been widely used for phenotyping individual cytochrome P450 activities, and caffeine and dapsone are considered the best for investigating the P450 isoforms CYP1A2 and 3A4 in rats (Fan et al., 2004; Tang et al., 2008) and in humans (Sharma et al., 2004). In addition, the phenotyping probes used in this study met other criteria, including safety at the doses administered, and availability in China.

Oral administration of enrofloxacin resulted in reduction of microsomal P450 activity, which has been reported in chicken (Schlosberg et al., 1995), rats (Vancutsem and Babish, 1996), sea bass (Vaccaro et al., 2003) and in the American alligator (Mayeaux and Winston, 1998). This study demonstrated that enrofloxacin inhibited the metabolism of caffeine and dapsone. Enrofloxacin is expected to inactivate the CYP1A system, but we also found that

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the 3A system was inhibited. In fish, enrofloxacin is reported to be a powerful, mechanism-based inhibitor primarily of the P450 3A isoform (Vaccaro et al., 2003). In chicks, enrofloxacin markedly inhibited the hydroxylation of aniline, which is an activity of CYP2E and 3A in mammals (Schlosberg et al., 1995). Thus, this antibiotic affects the CYP1A and 3A system in chicks, while marbofloxacin inactivates only the CYP1A system.

Orally administered marbofloxacin is not as commonly used in poultry as other fluoroquinolones, because of its lower bioavailability (Anadon et al., 2002). However, its inhibitory effects are important for two reasons. First, to our knowledge, this is the first report that the fluoroquinolone antibiotic marbofloxacin, has inhibitory effects on CYP1A. Second, comparing different fluoroquinolones in the same animal provides information about the inhibitory mechanism. We investigated whether enrofloxacin and marbofloxacin down-regulate CYP1A and 3A in the liver by Western blotting. The results showed that enrofloxacin significantly down-regulated the protein levels of both CYP1A and 3A, and marbofloxacin significantly down-regulated the level of CYP1A protein.

The effect of xenobiotics on CYP450 is generally measured at one of three levels: as mRNA that encodes for CYP450 protein (Courtenay et al., 1993; Williams et al., 1997), by cytochrome P450 subfamily protein concentration (Judith et al., 2000; Giorgi et al., 2000), or by catalytic activity of the enzyme (Martel et al., 1994; Munkittrick et al., 1992). In this study, enrofloxacin and marbofloxacin inhibited caffeine and dapsone metabolism, and correspondingly decreased CYP1A and 3A protein expression, but did not affect 1A4, 1A5 and 3A37 mRNA expression, which suggests that fluoquinolones does not affect transcription itself. Post-transcriptional level including the control of mRNA splicing, transcript stability, localization, translation, protein stability and modification is widespread in eukaryotes (Blencowe et al., 2009). Protein modification is catalyzed through biochemical reactions including enzyme acylation, alkylation, amidation, glycosylation, carboxylation, hydroxylation, oxidation and so on. Our finding that fluquinolones down-regulated P450 enzyme protein may due to the protein modification. The results presented here suggested inhibition of cytochrome P450 by fluoroquinolones through a post-transcriptional mechanism.

Enzyme inhibition is generally divided into reversible inhibition and mechanism-based inhibition. Reversible inhibitors, such as competitive and noncompetitive inhibitors are the most common. *In vitro*, enrofloxacin and norfloxacin are noncompetitive inhibitors of CYP1A activity (Regmi et al., 2005). However, ciprofloxacin, a metabolite of enrofloxacin in many species including broiler chicks (Mitchell, 2006; Garcia et al., 2001) is reported to show mechanism-based inhibition of ethoxyresorufin O-deethylation. Similar reports come from Vaccaro et al. (2003) both *in vitro* and *in vivo*, in sea bass. The mechanism elucidated by Vaccaro et al. (2003) is that the molecular structure of enrofloxacin and ciprofloxacin bind the oxidation site of p450 catalysis, and lead to reactive intermediates responsible for a suicide enzymatic reaction. In this study, a low dose of 10 mg/kg enrofloxacin and 2.75 mg/kg marbofloxacin were used, which did not affect the pharmacokinetics parameters of the cocktail probe drugs (data not shown), while a two fold dose decreased the total clearance of the cocktail of probe drugs. This dose-dependent inhibition of caffeine and dapsone metabolism indicated that enrofloxacin and marbofloxacin *in vivo* inhibited the enzymes in a irreversible inhibition manner. Thus it is possible that oxidized metabolite of fluoquinolones by particular P450 enzymes into a reactive intermediates, which tightly binds to the enzyme active site, leading to the suicide enzymatic reaction. In this case, the inactivation properties of CYP 1A and 3A enzymies may not react with corresponding antibodies and lead to the apparent down-regulate expression in Western blotting analysis. Since fluoroquinolones are administrated repeatedly, a drug–drug interaction may lead to fatal toxicity in chickens, because of changes in the depletion rate of co-administered drugs, which would also lead to an increase in drug residue in edible tissue.

In brief, this *in vivo* study is the first to report that fluoroquinolones modify the pharmacokinetics and metabolism of caffeine and dapsone in chicks. We found that enrofloxacin inhibited the activities of CYP 1A and 3A as well as protein levels, and the decrease in the metabolic clearance of dapsone was closely paralleled by a decrease in the protein level of CYP 3A. Marbofloxacin decreased the clearance of caffeine as well as altering the CYP1A protein level. The results suggested inhibition of cytochrome P450 by fluoroquinolones by post-transcriptional regulation of protein expression. Therefore, drugs co-administered with CYP substrates must be carefully monitored because continuous use of fluoroquinolones reversible inhibited the activity of CYP1A or 3A.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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