

Chicken xenobiotic receptor upregulates the BCRP/ABCG2 transporter

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ABSTRACT The transporter breast cancer resistance protein (BCRP, encoded by ABCG2) influences the bioavailability and elimination of numerous substrate drugs during clinical therapy. The xenobiotic-sensing nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) reportedly regulate functional expression of BCRP in mammalian species. However, it is unknown whether chicken xenobiotic receptor (CXR) regulates the expression and activity of BCRP. This study aimed to investigate the role of CXR in regulation of BCRP in chicken using in vitro and in vivo models. CXR was expressed in the main drug-metabolizing tissues of chickens, and its expression correlated well with that of the prototypical target genes CYP2H1 and ABCG2. BCRP expression was upregulated, and transporter activity was increased, in chicken primary hepatocytes

exposed to the CXR agonist metyrapone. Using RNA interference and ectopic expression techniques to manipulate the cellular CXR status, we confirmed that ABCG2 gene regulation depended on CXR. In vivo experiments showed that metyrapone induced BCRP in the liver, kidney, duodenum, and jejunum of chickens. Coadministration of metyrapone significantly changed the pharmacokinetic behavior of orally administered florfenicol (substrate of chicken BCRP), with a lower C_{\max} (4.62 vs. 7.35 $\mu\text{g/mL}$, $P < 0.01$) and AUC_{0-t} (15.83 vs. 24.18 $\text{h}\cdot\text{mg/L}$, $P < 0.01$) as well as a higher T_{\max} (0.96 vs. 0.79 h, $P < 0.05$) and Cl/F (0.13 vs. 0.08 L/h/kg , $P < 0.05$). Together, our data suggest that CXR is involved in regulation of BCRP, and consequently, coadministration of a CXR agonist can affect the pharmacokinetic behavior of an orally administered BCRP substrate.

Key words: CXR, BCRP, induction, chicken

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INTRODUCTION

Breast cancer resistance protein (BCRP, encoded by ABCG2) is a member of the ATP-binding cassette (ABC) transporter family (Liu, 2019a). Using the energy garnered from hydrolysis of ATP, BCRP actively effluxes a broad range of substrates against their concentration gradients (Safar et al., 2019; Mehendale-Munj and Sawant, 2021). Our group previously demonstrated that commonly used antimicrobial agents licensed in veterinary medicine, including enrofloxacin, ciprofloxacin, tilmicosin, florfenicol, ampicillin, and clindamycin, are actively transported by chicken BCRP (Zhang et al., 2018). With its broad expression in pharmacologically important organs (e.g., intestine and kidney), BCRP may be a critical factor that influences the

pharmacokinetics and toxicity of substrate drugs (Hira and Terada, 2018; Liu, 2019b; Kawahara et al., 2020). Thus, the importance of researching the regulatory mechanisms of BCRP is becoming apparent.

A link between BCRP regulation and nuclear receptors has been established with the discovery that several orphan nuclear receptors play a role in induction of BCRP by xenobiotics (Lin et al., 2017; Gorczyca and Aleksunes, 2020). In particular, pregnane X receptor (PXR) and constitutive androstane receptor (CAR) play crucial roles in induction of BCRP in rodents and humans (Whyte-Allman et al., 2017; Wang et al., 2021). These nuclear receptors are characterized by the presence of a DNA-binding domain with 2 zinc finger motifs and a conserved ligand-binding domain (Yoshinari, 2019). PXR- and CAR-mediated regulation occurs in response to receptor ligands that are xenobiotics (e.g., therapeutic drugs and environmental toxicants) or endogenous metabolites (e.g., bile acids); therefore, both nuclear receptors are recognized as major xenobiotic sensors (Buchman et al., 2018; Daujat-Chavanieu and Gerbal-Chaloin, 2020). However, the regulatory mechanisms of BCRP in chicken are not completely understood.

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Chicken xenobiotic receptor (**CXR**) was first cloned by the Christoph group and belongs to the superfamily of nuclear receptors. Sequence comparisons and activation profiles indicate that CXR has a close relationship with PXR and CAR (Handschin et al., 2000). CXR is highly expressed in the main drug-metabolizing tissues and regulates the xenobiotic-metabolizing enzyme CYP2H1 (Baader et al., 2002). The same tissues also express chicken BCRP, which prompted us to study the regulatory effect of CXR on BCRP.

Here, we identified CXR as a positive regulator of chicken BCRP. In vitro experiments were performed using chicken primary hepatocytes, which have been used extensively as a model for studying the potential of transcription factors to modulate ABC transporter expression. Elucidation of this regulation provides an opportunity to improve pharmacotherapeutic outcomes by enhancing the efficacy and reducing the toxicity of drugs that are substrates of BCRP.

MATERIALS AND METHODS

Reagents and Chemicals

Metyrapone, ketoconazole, and mitoxantrone were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Ko143 was purchased from MedChemExpress (Monmouth Junction, NJ). Anti-ABCG2 (N338) and anti- β -tubulin polyclonal antibodies were purchased from Bioworld Technology (Louis Park, MN). DNA transfection reagents were purchased from Vazyme Biotech Co., Ltd (Nanjing, China). An RNA transfection reagent was purchased from Zeta Life (Menlo Park, CA). All other chemicals were of analytical grade and obtained from standard suppliers unless mentioned otherwise.

Isolation of Chicken Livers and Preparation of Primary Hepatocyte Cultures

Chicken primary hepatocytes were obtained from the livers of 14-day-old Arbor Acres broiler embryos according

to a previously described method (Zhang et al., 2020). They were cultured in M199 medium supplemented with 5 μ g/mL transferrin, 100 IU/mL penicillin, and 100 μ g/mL streptomycin. Cells were maintained at 37°C in 5% CO₂ and 95% humidity.

RNA Isolation and RT-PCR Analysis

Total RNA was extracted from chicken primary hepatocytes and chicken tissue samples using TRIzol reagent (Takara, Toyobo, Japan) according to the manufacturer's instructions. RNA concentration (absorbance at 260 nm) and purity (absorbance ratio 260/280 nm) were assessed using a NanoPhotometer-N60 spectrophotometer (Implen, Munich, Germany). cDNA was synthesized using a reverse transcription kit (Takara) according to the manufacturer's protocol. mRNA expression of chicken ABCG2, CYP2H1, and CXR was quantified using NovoStart SYBR qPCR SuperMix (Novoprotein, Shanghai, China) and a RT-PCR detection system (Bio-Rad Laboratories, Hercules, CA). The chicken β -actin housekeeping gene was used as an internal control, and the $2^{-\Delta\Delta C_t}$ method was used to calculate and analyze the data. All primers sequences were from previously reported studies (Handschin and Meyer, 2000; Xu et al., 2022) and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The primers sequences are listed in Table 1.

Western Blot Analyses

Total protein fractions were extracted using RIPA lysis buffer (Beyotime, Haimen, China) according to the manufacturer's instructions. The protein content of each cell lysate was quantified using the bicinchoninic acid assay (Beyotime). Proteins (50 μ g/lane) were separated on 5 to 12% SDS-polyacrylamide gels and transferred to PVDF membranes. Subsequently, the membranes were blocked with 5% skimmed milk powder for 1 h at room temperature and incubated with a primary antibody at the appropriate dilution (anti-BCRP,

Table 1. Oligonucleotide sequences of primers.

Name	Primer sequence (5'–3')
pcDNA3.1-CXR-F	Primers for plasmid construction CCCAAGCTTATGTCCAGTCCAGCCCCTCGGAC CTAGTCTAGATCAGCTGATGATTTTCGGAGAGCAGCGGAGTCATGC
pcDNA3.1-CXR-R	
siCXR-F	Primers for RNA interference UCAGGCGCUCCAUCCUUAATT UUAAGGAUGGAGCGCCUGATT UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT
siCXR-R	
NC-F	
NC-R	
Abcg2 (BCRP)-F	Primers for RT-PCR CCTACTTCTGGCCTTGATGT TCGGCCTGCTATAGCTTGAAATC TCCCTTCCGGCATCCCTGTC GGCGTTGGTCTCCTCGTTG TGCGTGACATCAAGGAGAAG TGCCAGGGTACATTGTGGTA ATCCCCATCATTGGAAATG TCGTAGCCATACAGCACCAC
Abcg2 (BCRP)-R	
CXR-F	
CXR-R	
β -actin-F	
β -actin-R	
CYP2H1-F	
CYP2H1-R	

1:500 and anti- β -tubulin, 1:5,000) overnight at 4°C. Thereafter, membranes were washed 3 times with PBS for 15 min and incubated with a secondary antibody (1:4,000; Bioss, Beijing, China) for 1 h at 37°C. Protein bands were visualized with enhanced chemiluminescence (Vazyme Biotech Co., Ltd) and scanned with a Tanon 5200 chemiluminescence imaging system. Densitometric analysis was performed using ImageJ software. β -Tubulin was used as a loading control.

Functional Detection of BCRP Activity

Transport activity of BCRP was assessed following intracellular accumulation of the BCRP fluorescent substrate mitoxantrone. In brief, chicken primary hepatocytes in 12-well plates were treated with the CXR ligand metyrapone (500 μ M) alone or in the presence of Ko143 (BCRP-specific inhibitor, 10 μ M) for 24 h. Nontreated cells were used as the negative control. Thereafter, all cells were incubated with 5 μ M mitoxantrone for 1 h, trypsinized, and washed 3 times with PBS. Intracellular fluorescence corresponding to mitoxantrone accumulation was recorded using a FACS Calibur instrument and CellQuest Pro software with an excitation wavelength of 633 nm.

Overexpression of CXR

The open reading frame of CXR was cloned from chicken liver and inserted into the pcDNA3.1(+) vector (Invitrogen, Carlsbad, CA) using the EcoRI and XbaI sites to generate the CXR expression vector pcDNA3.1-CXR. For the overexpression assay, chicken primary hepatocytes were seeded into 6-well plates, cultured until they reached 70% confluency, transfected with pcDNA3.1-CXR or pcDNA3.1-basic using ExFect 2000 (Vazyme Biotech Co., Ltd), treated with metyrapone (500 μ M) for 24 h, and harvested to analyze CYP2H1 and BCRP expression.

siRNA Studies

Chicken primary hepatocytes were seeded into 6-well plates, cultured until they reached 70% confluency, transfected with CXR-targeting siRNA (**siCXR**) or negative control scrambled siRNA (**NC siRNA**) using Advanced DNA RNA Transfection Reagent (Zeta Life) for 24 h according to the manufacturer's instructions, treated with metyrapone (500 μ M) for 24 h, and harvested to analyze CYP2H1 and BCRP expression. siRNAs were obtained from Sangon Biotech Co., Ltd., and the sequences are listed in [Table 1](#).

Experimental Animals and Sample Collection

HY-Line Brown commercial laying hens were purchased from a local commercial poultry farm (Nanjing,

Jiangsu, China). Chicken treatment procedures were approved by the Science and Technology Agency of Jiangsu Province (approval no. 2017-0007) and performed in accordance with the guidelines of the Science and Technology Agency of Jiangsu Province and Nanjing Agricultural University. Tissue samples were collected from chickens aged 2, 30, and 70 wk (6 chickens per group) for RNA extraction to analyze age-dependent mRNA expression of CXR, CYP2H1, and ABCG2. Another 12 third-wk-old chickens with a similar body weight were randomly divided into 2 groups equally. One group remained untreated and served as the negative control, while the other group was treated with metyrapone (single oral administration of 150 mg per chicken). All chickens were provided a basal diet and water ad libitum and managed under the recommended humidity and temperature. Chickens were slaughtered using a carbon dioxide asphyxiation machine at 24 h after metyrapone administration. Tissue samples were collected from all chickens, snap-frozen in liquid nitrogen, and stored at -70°C until further analysis.

Pharmacokinetic Studies of Florfenicol in Chicken

Twelve 30-wk-old chickens were randomly allocated to two groups. The first group received a single dose of 20 mg/kg florfenicol orally through crop tube gavage. The second group was orally dosed first with metyrapone (150 mg per bird) and then with florfenicol (20 mg/kg) 15 h later. Blood samples were taken from the wing vein of each chicken and collected in heparin-coated tubes at 0.083, 0.167, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, and 12 h after administration of florfenicol. Plasma was rapidly harvested by centrifugation at 5,000 g for 5 min and stored at -80°C until further analysis. The method to extract enrofloxacin from plasma and the HPLC method to detect florfenicol in plasma were described in our previous work ([Liu et al., 2018](#)). Pharmacokinetic parameters were calculated using noncompartmental analysis and a computer program (WinNonlin 6.1, Phoenix Software, Los Angeles, CA).

Statistical Analyses

Data are presented as mean \pm SD. Statistical differences between experimental groups were analyzed using SPSS software (version 20.0, SPSS Inc., Chicago, IL). One-way ANOVA was used to assess the statistical significance of differences for 3 or more groups of data. Independent-sample t test was used to assess the statistical significance of differences for only two groups of data. Repeated measure ANOVA was used for the statistical analysis of blood concentration data of florfenicol. All experiments were repeated at least 3 times. $P < 0.05$ was considered a significant difference, and $P < 0.01$ was considered an extremely significant difference.

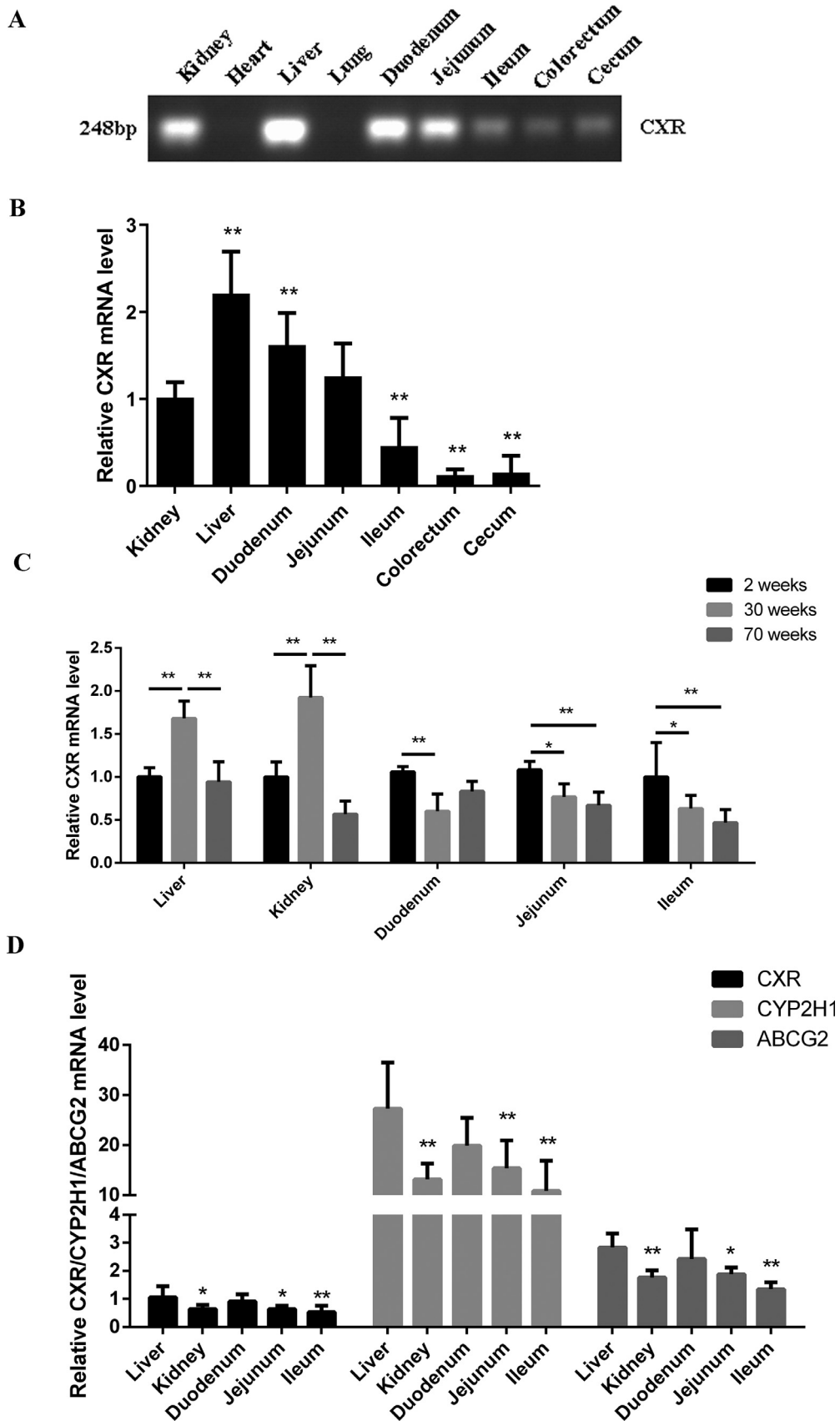


Figure 1. Expression pattern of CXR in HY-Line Brown commercial laying hens. (A) PCR analysis of CXR mRNA expression in different tissues of hens. (B) Comparison of CXR mRNA levels in different tissues of hens by RT-PCR. (C) Comparison of CXR mRNA levels in different tissues of differently aged hens. (D) Comparison of CXR, CYP2H1, and ABCG2 mRNA levels in different tissues of hens. Bars show means \pm SD of at least three independent experiments.

RESULTS

CXR mRNA Expression in the Main Drug-Metabolizing Tissues of Chicken

CXR mRNA expression was examined in various tissues of 30-wk-old chickens by PCR analysis. Expression of CXR mRNA was detected in the kidney, liver, duodenum, jejunum, ileum, colorectum, and cecum, but not in the heart or lung (Figure 1A). CXR mRNA was robustly expressed in the kidney, liver, duodenum, and jejunum according to real-time RT-PCR analysis, whereas very low basal expression of CXR mRNA was detected in the ileum, colorectum, and cecum (Figure 1B). We further investigated the ontogeny of CXR expression in chicken tissues (Figure 1C). mRNA expression of CXR was detected in HY-Line Brown commercial laying hens aged 2 wk (chick), 30 wk (adult chicken), and 70 wk (aged chicken), which correspond to the stages in the layer breeding industry. CXR mRNA levels in the liver and kidney were significantly higher in chickens aged 30 wk than in chickens aged 2 wk, but were significantly lower in chickens aged 70 wk than in chickens aged 30 wk. CXR mRNA levels in the small intestine decreased as age increased. Importantly, mRNA expression of CXR correlated well with that of CYP2H1 (the prototypical target gene of CXR) and ABCG2 (Figure 1D), which led us to study whether ABCG2 is a target gene of CXR.

Metyrapone Upregulates Expression of BCRP at the mRNA and Protein Levels

To investigate whether CXR is involved in regulation of BCRP expression, we first examined if BCRP expression is modulated in chicken primary hepatocytes exposed to the CXR agonist metyrapone. CYP2H1 is the prototypical target gene of CXR as previously described and therefore was used as a positive indicator of CXR activation. Results showed metyrapone with the concentration ranging from 50 to 500 μ M significantly up-regulated the mRNA expression level of CYP2H1 (3.8 to 12.0-fold compared with negative control group) and ABCG2 (1.3 to 3.8-fold compared with negative control group) in a concentration-dependent way in chicken primary hepatocytes at 24 h (Figures 2A and 2B). To determine whether the increases in mRNA expression result in changes in BCRP protein expression, western blot analysis was performed after metyrapone treatment for 24 h (Figure 2C). BCRP protein expression was significantly higher (up to 2.8-fold) in chicken primary hepatocytes treated with increasing concentrations of metyrapone than in negative control cells.

We further tested whether the observed induction of BCRP by metyrapone in chicken primary hepatocytes was primarily mediated by CXR using the CXR antagonist ketoconazole. Cells were exposed to ketoconazole in the presence of metyrapone for 24 h. As expected, ketoconazole dose-dependently prevented induction of CYP2H1 (0.7 to 0.6-fold) and BCRP (0.4 to 0.3-fold at

the mRNA level and 0.7 to 0.4-fold at the protein level) by metyrapone (Figure 3), further suggesting that CXR is involved in regulation of BCRP.

Agonist-Activated CXR Increases BCRP Function in Chicken Primary Hepatocytes

To determine whether induction of BCRP expression by metyrapone modulates the transport function of BCRP, a mitoxantrone (a selective BCRP substrate) accumulation assay was performed using chicken primary hepatocytes (Figure 4). Intracellular mitoxantrone fluorescence was 33% lower in cells pretreated with 500 μ M metyrapone for 24 h than in untreated cells ($P < 0.01$), indicating that BCRP-mediated efflux of mitoxantrone was increased. This metyrapone-induced efflux activity was reversed by cotreatment with the specific BCRP inhibitor Ko143. These results demonstrate that induction of BCRP expression by metyrapone is associated with an increase in the transport function of BCRP.

CXR-Dependence of BCRP Induction

We performed gain-of-function assays by overexpressing CXR and loss-of-function assays by knocking down CXR in chicken primary hepatocytes. Transfection of chicken primary hepatocytes with the CXR expression vector significantly enhanced induction of CYP2H1 (5.1-fold) and ABCG2 (3.1-fold at the mRNA level and 2.1-fold at the protein level) upon metyrapone treatment (Figures 5A–5C). By contrast, knockdown of CXR in chicken primary hepatocytes using siCXR (60% suppression of the CXR transcript level compared with NC siRNA treatment) attenuated induction of CYP2H1 (0.5-fold) and ABCG2 (0.7-fold at the mRNA level and 0.7-fold at the protein level) by metyrapone (Figures 5D–5F). These data suggest that activation of CXR is required for induction of chicken BCRP by metyrapone, indicating that CXR is directly involved in regulation of BCRP.

CXR-Mediated Induction of BCRP in Chicken

Metyrapone treatment significantly increased mRNA expression of CYP2H1 and ABCG2 in the liver (3.6-fold for CYP2H1 and 2.1-fold for ABCG2), kidney (8-fold for CYP2H1 and 2.2-fold for ABCG2), duodenum (5.6-fold for CYP2H1 and 1.8-fold for ABCG2), and jejunum (1.7-fold for CYP2H1 and 1.6-fold for ABCG2; Figures 6A and 6B). BCRP protein expression showed a similar trend as ABCG2 mRNA expression in chicken tissues (1.6-fold in the liver and kidney, 1.4-fold in the duodenum, and 1.3-fold in the jejunum; Figure 6C). However, metyrapone treatment did not influence expression of CYP2H1 or ABCG2 in chicken ileum.

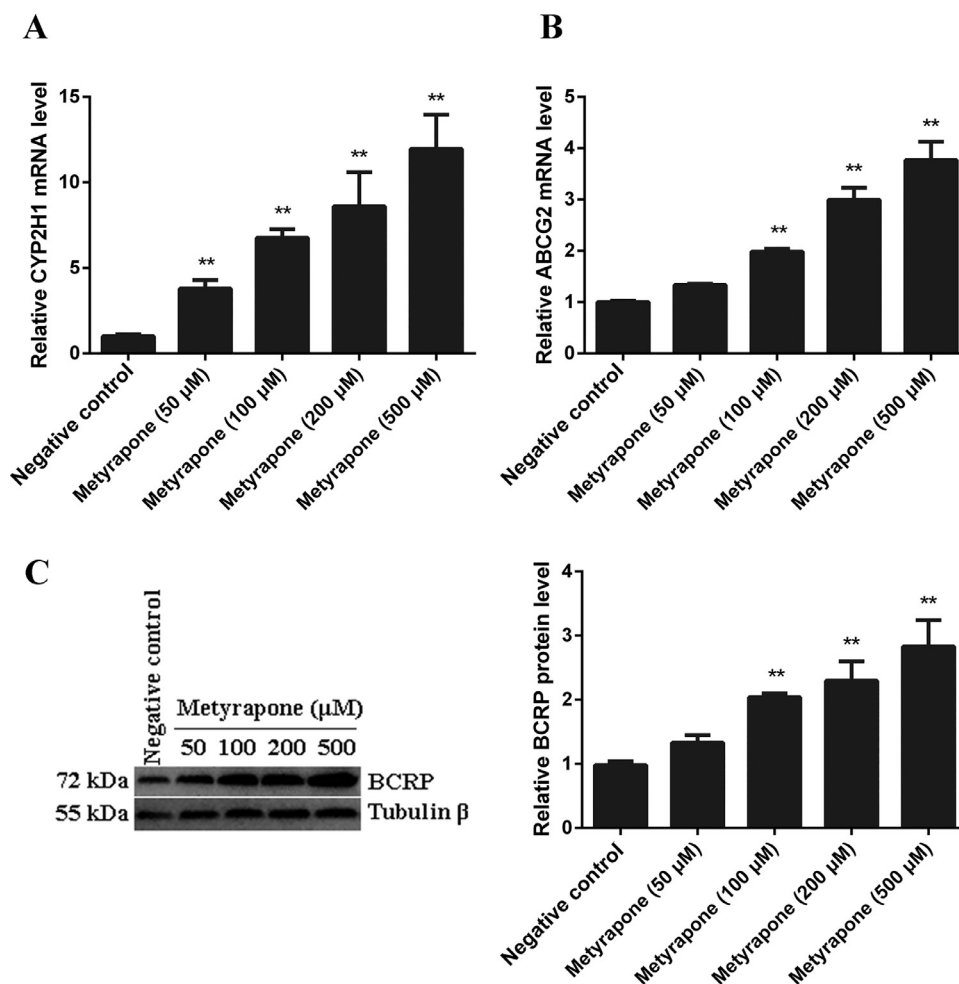


Figure 2. CXR-mediated upregulation of BCRP in primary hepatocyte cultures. Cells were exposed to various concentrations of the CXR agonist metyrapone for 24 h. RT-PCR analysis of CYP2H1 (A) and ABCG2 (B) mRNA levels. (C) Western blot analysis of relative chicken BCRP protein expression in cells. Bars show means \pm SD of at least three independent experiments. Abbreviations: BCRP, breast cancer resistance protein; CXR, chicken xenobiotic receptor.

Agonist-Activated CXR Affects the Pharmacokinetics of the Orally Administered BCRP Substrate Florfenicol in Chicken

To assess whether induction of BCRP by metyrapone is functionally relevant, pharmacokinetic analysis of the BCRP substrate florfenicol was performed in 30-wk-old HY-Line Brown commercial laying hens. The mean plasma concentration-time profiles of florfenicol orally administered alone or coadministered with metyrapone are shown in Figure 7, and the relevant pharmacokinetic parameters are listed in Table 2. The combination of florfenicol/metyrapone significantly changed the pharmacokinetic behavior of orally administered florfenicol in chicken, with a lower C_{max} (4.62 vs. 7.35 $\mu\text{g}/\text{mL}$, p) and AUC_{0-t} (15.83 vs. 24.18 h-mg/L) as well as a higher T_{max} (0.96 vs. 0.79 h) and Cl/F (0.13 vs. 0.08 L/h/kg).

DISCUSSION

CXR, a member of the nuclear receptor superfamily of transcription factors, was reported to regulate genes encoding xenobiotic-metabolizing enzymes, such as chicken CYP2H1 and CYP2C45. However, it remains

uncertain whether CXR is directly involved in transcriptional control of chicken BCRP. Here, we found that CXR is a direct transcriptional regulator of chicken BCRP. Our findings expand the roles of CXR to adaptive regulation of the BCRP transporter. This is the first report directly linking CXR to regulation of an important ABC efflux transporter in chicken.

CXR was highly expressed in the liver, kidney, and small intestine of chickens, whereas only a faint signal was detected in the large intestine. Tissues with high expression of CXR are pharmacologically important sites that affect the absorption, distribution, metabolism, and excretion of drugs (van den Anker et al., 2018; Akamine et al., 2019). We also studied the ontogeny of CXR, which is important for evaluation of therapeutic efficacy in juvenile and adult animals. To explore the mechanism regulating transporter expression, we also studied gene expression of CXR in relation to transporter expression levels. Importantly, expression of CXR correlated well with that of CYP2H1 (the prototypical target gene of CXR) and ABCG2.

BCRP has attracted growing research efforts directed at its involvement in toxicity and elimination of various drugs and xenobiotics (Chen et al., 2019; Ito et al., 2021;

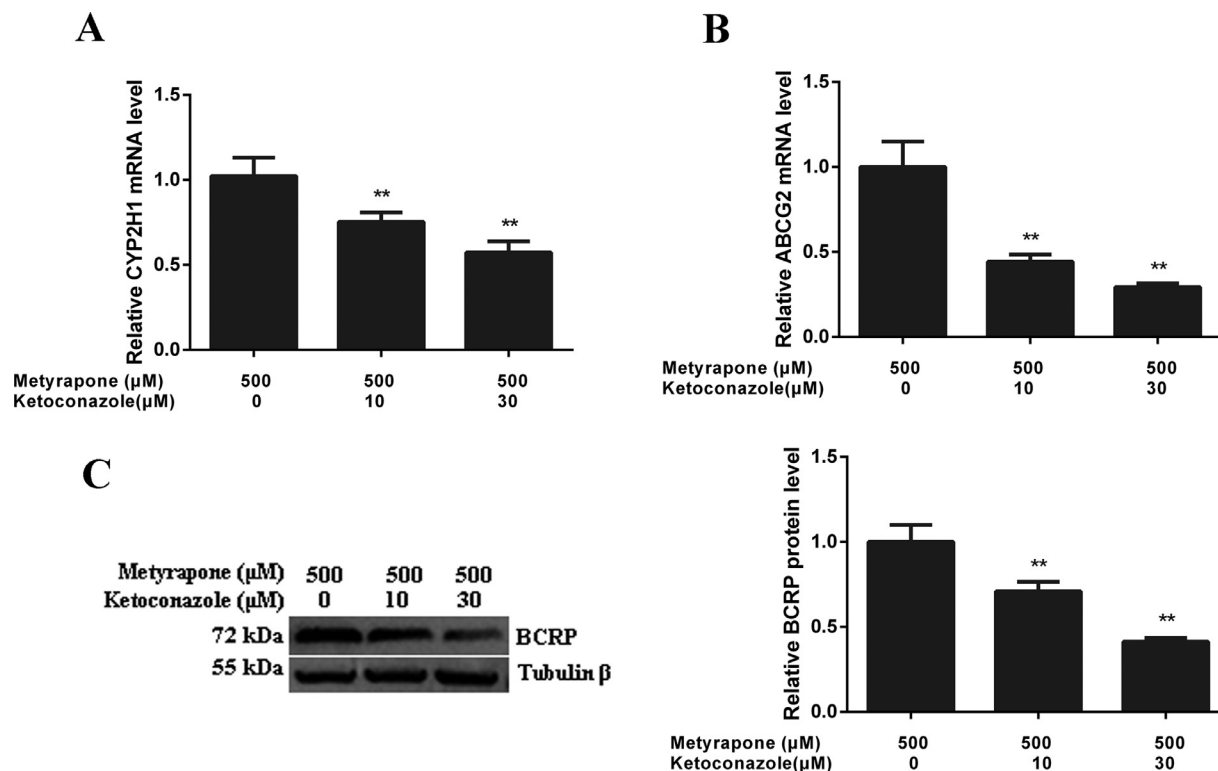


Figure 3. Effects of the CXR antagonist ketoconazole on BCRP expression in chicken primary hepatocytes. Cells were exposed to ketoconazole with or without the CXR agonist metyrapone for 24 h. RT-PCR analysis of CYP2H1 (A) and ABCG2 (B) mRNA levels. (C) Western blot analysis of relative chicken BCRP protein expression in cells. Bars show means \pm SD of at least three independent experiments. Abbreviations: BCRP, breast cancer resistance protein; CXR, chicken xenobiotic receptor.

Lee et al., 2021). An in vivo study with Arbor Acres broilers reported that florfenicol is more extensively absorbed upon coadministration of gefitinib (a BCRP inhibitor) (Liu et al., 2018). Clinical drug-drug interactions upon coadministration of BCRP substrates with inhibitors or inducers could be partly due to the presence of BCRP in pharmacologically important tissues (Pedersen et al., 2017; Schilling et al., 2021). Thus, it is of great clinical importance to elucidate the molecular mechanisms that regulate BCRP expression.

We first examined the involvement of CXR in regulation of BCRP using chicken primary hepatocytes, which are an in vitro model for elucidating the molecular mechanism underlying xenobiotic induction. CYP2H1, the prototypical target gene of CXR, was used as a positive indicator of CXR activation. The CXR agonist metyrapone significantly induced expression of CYP2H1 and ABCG2 in a concentration-dependent manner. The mitoxantrone (a selective BCRP substrate) accumulation assay showed that induction of BCRP expression

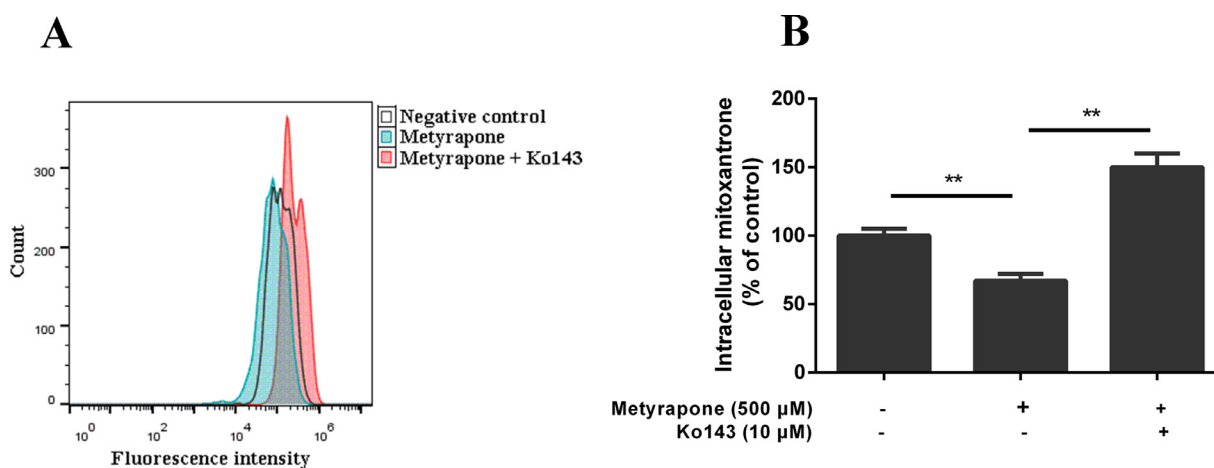


Figure 4. BCRP transport activity in chicken primary hepatocytes exposed to the CXR agonist metyrapone. Intracellular mitoxantrone fluorescence was assessed in untreated cells and cells treated with 500 μM metyrapone for 24 h with or without pre-exposure to the BCRP-specific inhibitor Ko143 (5 μM) for 1 h. (A, B) Representative results and summaries of mitoxantrone accumulation. The histogram shows fluorescence (x axis) representing mitoxantrone accumulation plotted as a function of the number of cells (y axis). Bars show means \pm SD of at least three independent experiments. Abbreviations: BCRP, breast cancer resistance protein; CXR, chicken xenobiotic receptor.

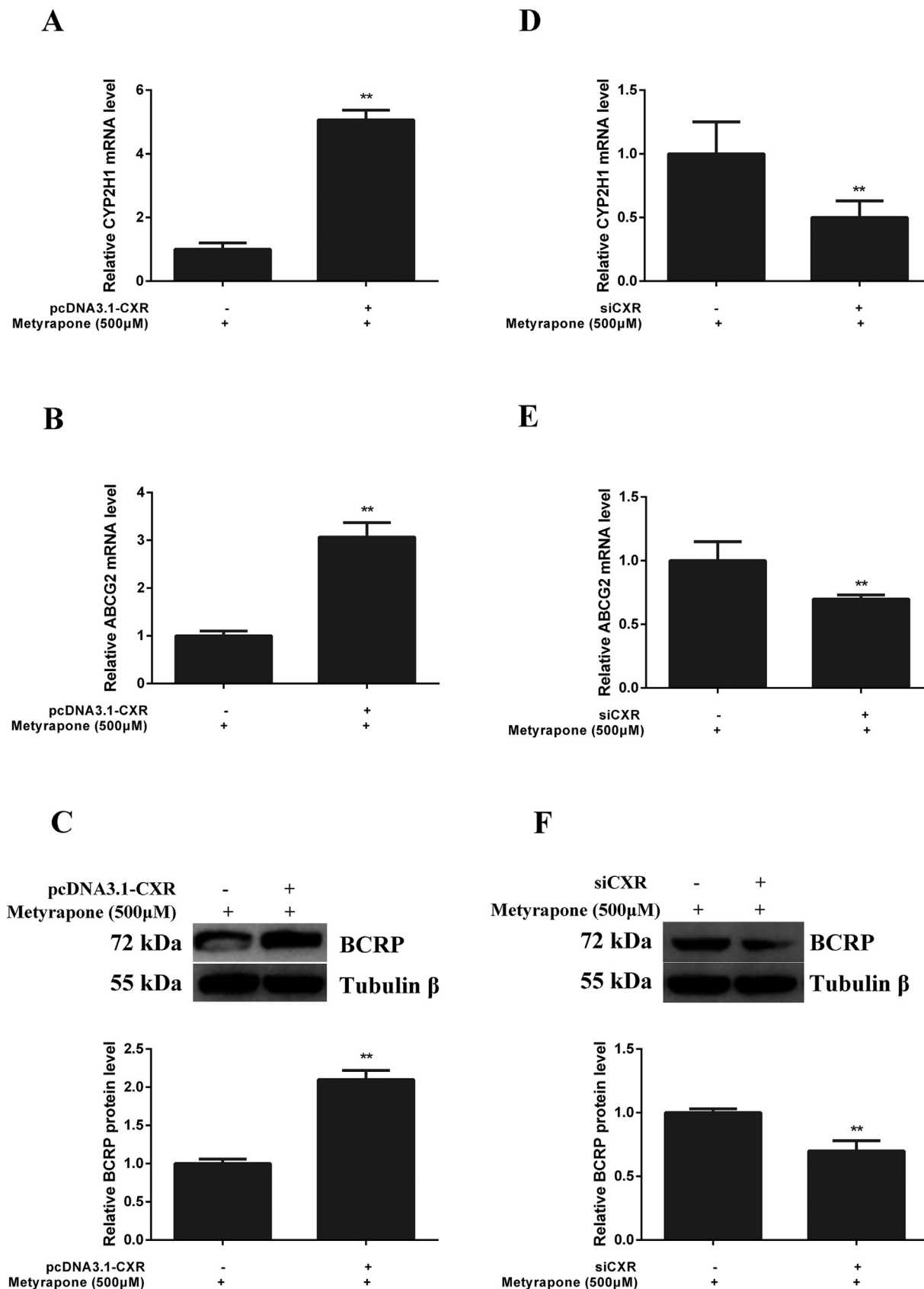
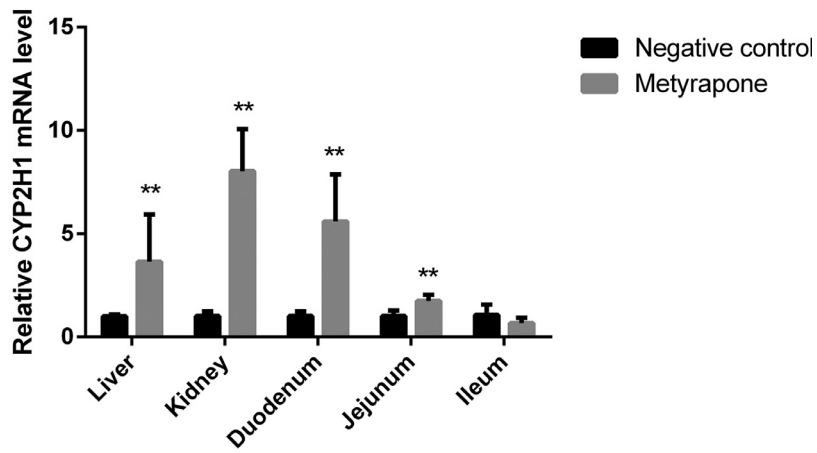
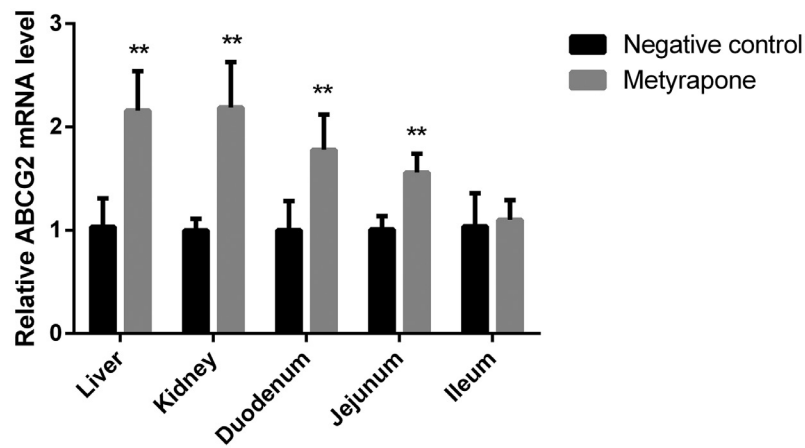


Figure 5. Dependence of BCRP induction on CXR. RT-PCR analysis of CYP2H1 (A) and ABCG2 (B) mRNA levels, as well as Western blot analysis of relative chicken BCRP protein expression (C) in chicken primary hepatocytes transfected with a CXR expression vector and then treated with 500 μ M metyrapone for 24 h. RT-PCR analysis of CYP2H1 (D) and ABCG2 (E) mRNA levels, as well as western blot analysis of relative chicken BCRP protein expression (F) in chicken primary hepatocytes treated with 500 μ M metyrapone after siRNA-mediated knockdown of CXR. Bars show means \pm SD of three independent experiments. Abbreviations: BCRP, breast cancer resistance protein; CXR, chicken xenobiotic receptor.

A



B



C

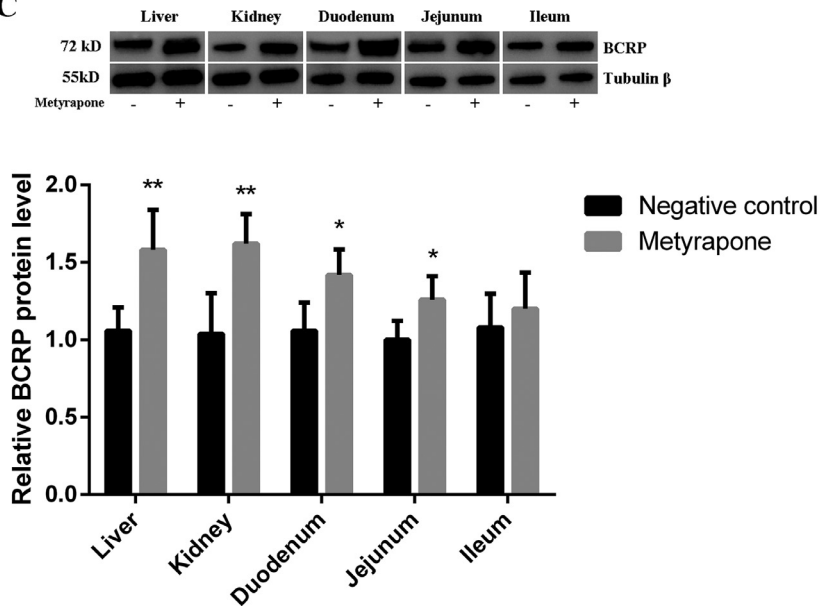


Figure 6. CXR-mediated induction of BCRP in chicken. RT-PCR analysis of CYP2H1 (A) and ABCG2 (B) mRNA levels, as well as western blot analysis of relative chicken BCRP protein expression (C) in the liver, kidney, duodenum, jejunum, and ileum of chickens treated with or without the CXR agonist metyrapone. Bars show means \pm SD of three independent experiments. Abbreviations: BCRP, breast cancer resistance protein; CXR, chicken xenobiotic receptor.

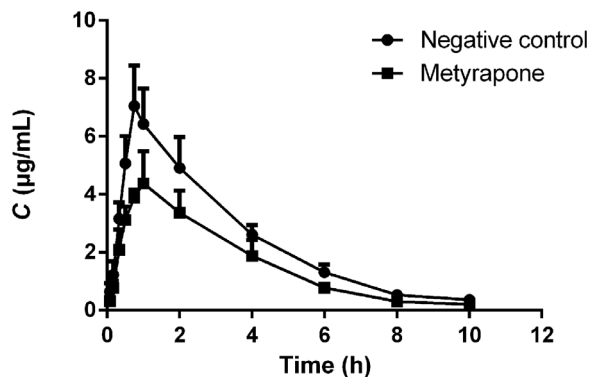


Figure 7. Plasma concentration-time profiles of florfenicol after single oral administration at a dose of 20 mg/kg body weight alone and coadministration with the CXR agonist metyrapone. Data represent means \pm SD ($n = 6$).

Table 2. Pharmacokinetic parameters of orally administered florfenicol in chicken.

Parameter	Florfenicol	Florfenicol+Metyrapone	<i>P</i> -value
C_{\max} ($\mu\text{g}/\text{mL}$)	7.35 ± 1.31	$4.62 \pm 0.71^{**}$	0.00
T_{\max} (h)	0.79 ± 0.10	$0.96 \pm 0.10^*$	0.02
$T_{1/2}$ (h)	2.13 ± 0.45	1.89 ± 0.21	0.27
V_z/F (L/kg)	0.24 ± 0.05	0.44 ± 0.24	0.10
Cl/F (L/h/kg)	0.08 ± 0.01	$0.13 \pm 0.03^{**}$	0.01
AUC_{0-t} (h·mg/L)	24.18 ± 2.45	$15.83 \pm 2.98^{**}$	0.00

Significant difference between parameters of florfenicol in the presence and absence of metyrapone in chickens. C_{\max} , the peak concentration; T_{\max} , time to reach peak concentration; $T_{1/2}$, the elimination half-life; V_z/F , apparent volume of distribution per fraction of the dose absorbed; Cl/F , plasma clearance per fraction of the dose absorbed; AUC_{0-t} , area under the curve up to the last measurable concentration.

* $P < 0.05$.

** $P < 0.01$.

by metyrapone modulated the transport function of BCRP. To exclude the possibility that other nuclear receptor pathways are involved in regulation of BCRP by metyrapone, RNA interference and ectopic expression techniques were used to manipulate the cellular CXR status. Induction of BCRP upon metyrapone treatment was significantly enhanced by overexpression of CXR and attenuated by silencing of CXR. These results confirm that metyrapone induces BCRP expression via a CXR-dependent mechanism, and that CXR directly regulates expression of BCRP.

We further investigated the role of CXR in regulating the expression and function of BCRP in chicken. Consistent with the *in vitro* results, metyrapone induced expression of BCRP at the mRNA and protein levels in the liver, kidney, duodenum, and jejunum of chickens. Our previous research showed that florfenicol is a substrate of chicken BCRP (Zhang et al., 2018). Modulation of the expression level and activity of BCRP may significantly change the pharmacokinetic profiles of florfenicol. Therefore, we further investigated the influence of metyrapone on the pharmacokinetics of florfenicol in chicken to examine potential interactions between them. Coadministration of metyrapone significantly decreased C_{\max} and AUC_{0-t} of orally administered florfenicol, but increased T_{\max} and Cl/F . Studies suggest that florfenicol can be absorbed from the small intestine and is primarily eliminated by renal

excretion as an unchanged compound (42%) and its metabolite florfenicol amine (25%) (Anadon et al., 2008). Accordingly, the decreased oral bioavailability of florfenicol upon coadministration of metyrapone could be mainly due to limitation of its intestinal absorption and enhancement of its elimination by the kidneys via BCRP.

In conclusion, both *in vitro* and *in vivo* results demonstrate that CXR upregulates the BCRP/ABCG2 transporter. Therefore, xenobiotics can alter the pharmacokinetic properties of BCRP substrate drugs through nuclear receptor-mediated pathways if they are CXR agonists or antagonists. This may have far-reaching significance regarding the usage and development of veterinary drugs.

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DISCLOSURES

The author(s) declare(s) no conflicts of interest.

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