



HAL
open science

Effects of Fluoroquinolone Treatment on MDR1 and MRP2 mRNA Expression in *E. coli* Infected Chickens

Aneliya Milanova Haritova, Nikolina Rusenova, Anton Rusenov, Jan Schrickx,
Lubomir Lashev, Johanna Fink-Gremmels

► **To cite this version:**

Aneliya Milanova Haritova, Nikolina Rusenova, Anton Rusenov, Jan Schrickx, Lubomir Lashev, et al.. Effects of Fluoroquinolone Treatment on MDR1 and MRP2 mRNA Expression in *E. coli* Infected Chickens. *Avian Pathology*, 2008, 37 (05), pp.465-470. 10.1080/03079450802272945 . hal-00540125

HAL Id: hal-00540125

<https://hal.science/hal-00540125>

Submitted on 26 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Effects of Fluoroquinolone Treatment on MDR1 and MRP2 mRNA Expression in E. coli Infected Chickens

Journal:	<i>Avian Pathology</i>
Manuscript ID:	CAVP-2007-0130.R2
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	26-May-2008
Complete List of Authors:	Haritova, Aneliya; Trakia University, Pharmacology, Physiology of Animals and Physiological Chemistry Rusenova, Nikolina; Trakia University, Department of Microbiology, Infectious and Parasitic Diseases, Section of Microbiolog Rusenov, Anton; Trakia University, Department of Internal Diseases Schrickx, Jan; Utrecht University, Department of Veterinary Pharmacology, Pharmacy and Toxicology Lashev, Lubomir; Trakia University, Pharmacology, Physiology of Animals and Physiological Chemistry Fink-Gremmels, Johanna; Utrecht University, Department of Veterinary Pharmacology, Pharmacy and Toxicology
Keywords:	poultry, ABC transporters, E. coli infection, fluoroquinolones

SCHOLARONE™
Manuscripts

Cavp-2007-0130.R2

Effects of Fluoroquinolone Treatment on MDR1 and MRP2 mRNA
Expression in *E. coli* Infected Chickens

Aneliya Milanova Haritova², Nikolina Velizarova Rusenova³, Anton Georgiev
Rusenov⁴,
Jan Schrickx¹, Lubomir Dimitrov Lashev² and Johanna Fink-Gremmels^{1*}

¹Department of Veterinary Pharmacology, Pharmacy and Toxicology, Faculty of
Veterinary Medicine, Utrecht University, the Netherlands. ²Department of Pharmacology,
Veterinary Physiology and Physiological Chemistry, Faculty of Veterinary Medicine,
Trakia University, Bulgaria. ³Department of Microbiology, Infectious and Parasitic
Diseases, Section of Microbiology, Faculty of Veterinary Medicine, Trakia University,
Bulgaria. ⁴Department of Internal Diseases, Faculty of Veterinary Medicine,
Trakia University, Bulgaria

Short title: MDR1 and MRP2 mRNA Levels in *E. coli* Infected Chickens

*Corresponding author:

E-mail: J.Fink@uu.nl

Utrecht University

Faculty of Veterinary Medicine,

Department of Pharmacology, Pharmacy and Toxicology,

Yalelaan 104, De Uithof, P.O.Box 80152,

3508 TD Utrecht,

The Netherlands

Phone + 3130 2535453

Fax + 3130 2534125

Received: 27 August 2007

Cavp-2007-0130.R2

Effects of Fluoroquinolone Treatment on MDR1 and MRP2 mRNA Expression in *E. coli* Infected Chickens

Abstract

Current knowledge about the expression of ABC transport proteins suggests that their expression is regulated by a variety of factors, including pathological conditions, and in particular inflammatory reactions to infection. As ABC transporters are major determinants of absorption, distribution and excretion of many antimicrobials, modulation of their activity may result in increased or decreased tissue levels of drugs, affecting the efficacy of treatment. As fluoroquinolones have been identified as modulators and substrates of a number of drug transporters, we evaluated the effect of danofloxacin mesylate and enrofloxacin treatment on the levels of expression of MDR1 and MRP2 mRNAs in the intestines and livers of broilers with experimentally induced colibacillosis.

MDR1 mRNA expression was significantly decreased in infected animals and was partly restored over five days of treatment with orally administered danofloxacin mesylate or enrofloxacin. Changes in the level of expression of MRP2 mRNA were less prominent. This study suggests that the treatment of colibacillosis with fluoroquinolones, which resulted in a significant clinical improvement of the animals, also restored the expression of drug transporters. This is of clinical importance as these ABC transporters significantly contribute to the functionality of important biological barriers, protecting the bird and specific tissues from pathogens and bacterial toxins.

Introduction

Transmembrane efflux transporters of the ABC family are involved in the absorption, distribution and elimination of endogenous compounds, such as hormones and neurotransmitters, as well as of therapeutic agents and toxins (Dean & Annilo, 2005; Linton, 2007). Clinical experience and experimental work have indicated that exogenous substrates, including a number of drugs, modulate the expression and function of membrane transporters. In addition, metabolic disorders and infectious disease influence their activity (Yokooji *et al.*, 2006; Naud *et al.*, 2007).

Exposure to pathogens activates a cascade of defence mechanisms, generally described as the acute phase response to infection (Mireles *et al.*, 2005). Part of this response involves the expression of cytokines, in particular tumour necrosis factor alpha (TNF α), interleukin (IL) 1 β and IL-6, which are the major pro-inflammatory cytokines in all animal species, including chickens (Leshchinsky & Klasing, 2001). In mammals, the expression of pro-inflammatory cytokines is accompanied by a down-regulation of nuclear receptors, including FXR (farnesoid X receptor), PXR (pregnane X receptor) and CAR (constitutive androstane receptor), which are involved in the transcriptional regulation of a number of biotransformation enzymes, as well as ABC transporters (Beigneux *et al.*, 2002; Stienstra *et al.*, 2004). A number of investigations have further defined the role of the individual cytokines in the transcriptional and post-transcriptional regulation of expression of the ABC transporters MDR1 and MRP2 (McRae *et al.*, 2003; Siewert *et al.*, 2004). MDR1 mRNA and P-glycoprotein (P-gp, the product of the MDR1 gene) have been shown to be down-regulated by pro-inflammatory cytokines in a concentration-dependent manner.

IL-1 β appears to act post-transcriptionally, while IL-6 affects P-gp expression at the transcriptional level (Sukhai *et al.*, 2000, 2001).

Fluoroquinolones can be successfully used for treatment of colibacillosis in poultry (Charleston *et al.*, 1998; Chansiripornchai & Sasipreeyajan, 2002). They are substrates for ABC transporters, and are able to modulate their expression (Gollapudi *et al.*, 1995; Prime-Chapman *et al.*, 2005). However, the interactions between fluoroquinolones and ABC transporters have only been studied in cell lines or in healthy laboratory animals (Cormet-Boyaka *et al.*, 1998; Naruhashi *et al.*, 2001; Rodriguez-Ibanez *et al.*, 2003), and no studies have been conducted in birds. MDR1 and MRP2 are expressed in a variety of tissues, including the intestines. They may affect drug absorption (Giessmann *et al.*, 2004) at tissue barriers, prevent the entry of drugs into tissues such as the central nervous system (Fromm, 2004), and in excretory organs, including the liver and the kidneys, they also contribute to elimination (Lagas *et al.*, 2006; van de Water *et al.*, 2007).

In the study described here, we investigated the effect of the therapeutic application of two different fluoroquinolones, danofloxacin mesylate and enrofloxacin, on the levels of expression of MDR1 and MRP2 mRNAs in the intestines and the liver of broilers with experimentally induced colibacillosis.

Materials and Methods

Drugs. Danofloxacin mesylate (Danocin 180, Pfizer, 18% sterile solution) and enrofloxacin (Baytril 5%, Bayer, 5% sterile solution) were dissolved in water and used for oral treatment.

Animals. Mixed sex three-week-old broiler chickens (Plymouth Rock, line 99 x Cornish, line K) were obtained from a commercial poultry farm. The weights of the chickens at the start of the experiment were between 0.6 and 0.7 kg. All birds were kept at 19 to 20°C and had free access to standard commercial feed (without additives) and water. During the acclimatisation period the birds were examined for salmonellosis and colibacillosis. To this end, faecal samples from the birds were investigated for pathogenic serotypes of *E. coli* and *Salmonella spp.* using standard microbiological assays.

Experimental infection. *Escherichia coli* O78/K80 strain, isolated from a chicken with colibacillosis, was obtained from the National Scientific and Diagnostic Institute of Veterinary Medicine, Sofia, Bulgaria. The strain was stored on beads at -70°C prior to use. The day before infection, the *E. coli* were inoculated onto trypticase soy blood agar (TSA; Becton Dickinson) and incubated at 37°C for 24 h, and then eight colonies were suspended in 9 ml Mueller-Hinton broth (MHB; Becton Dickinson) and the broth was incubated at 37°C for 18 h. A 0.2 ml volume of the overnight culture, containing 2.6×10^8 colony forming units, was instilled into the trachea of each bird. The inoculum was also cultured on TSA for enumeration and confirmation of the identity and viability of the bacteria. Clinical signs of infection were seen in most animals within 24 h of inoculation. Three birds died during this period with lesions typical of colibacillosis. Tissue samples collected

from experimentally infected chickens at necropsy were cultured to confirm that the strain used to inoculate the birds caused the mortalities (Fernandez *et al.*, 1998). The MIC of danofloxacin and enrofloxacin for the *E. coli* strain was 0.25 µg/ml.

Study design and treatment. Infected birds were randomly allocated to one of three groups. The first group (10 birds) was infected, but remained untreated and served as a positive control group. A second group of infected birds was treated with danofloxacin mesylate (10 birds) and a third group of infected birds was treated with enrofloxacin (8 birds). A fourth group of 8 uninfected chickens served as uninfected controls. The birds from the first group were euthanized 24 h after infection and tissue samples (duodenum, jejunum, ileum, caeca, colon and liver) were taken. Tissue samples were also collected from four uninfected broilers.

Treatment was provided in the drinking water and commenced 24 h after inoculation and continued for a period of 5 days. The medicated water was offered *ad libitum*, and fresh batches of medicated water were prepared every 12 h, with water intake monitored every 12 h. The concentrations of the drugs in the drinking water were checked with a microbiological assay, using *E. coli* ATCC 25922, immediately after preparation and at the end of each 12 h interval. The treatments aimed for a daily dose of danofloxacin mesylate of 6 mg/kg, or a dose of enrofloxacin of 10 mg/kg, respectively. At the end of the experiment, water consumption and the concentration of fluoroquinolones in the water were used to calculate the mean dose ingested. The second group ingested 5 to 9.17 mg danofloxacin mesylate/kg, and the third group 10.43 to 13.95 mg enrofloxacin/kg. Twenty four hours after the last treatment birds were euthanized and tissue samples (duodenum,

jejunum, ileum, caeca, colon and liver) were collected. Tissue samples were also collected from the remaining four healthy animals, and served as controls. All samples were stored at -70°C until analysis.

RNA isolation and cDNA synthesis. Total RNA was isolated using Trizol Reagent (Invitrogen Life Technologies) according to the manufacturer's instructions. RNA concentrations were determined by ultraviolet absorbance at 260 and 280 nm, and stored for a short period at -70°C prior to cDNA synthesis.

Single-stranded cDNAs were synthesized from 1 µg total RNA using the iScript[™] cDNA Synthesis Kit (Bio-Rad Laboratories). To a master mixture containing 5 x iScript Reaction Mix and iScript reverse transcriptase, 1 µg of total RNA dissolved in sterile nuclease-free water was added. The reaction mixture (total volume 20 µl) was incubated for 5 min at 25°C, then for 45 min at 42°C, then the enzyme was heat inactivated at 85°C for 5 min and the reaction mixture rapidly cooled to 4°C. The cDNA was diluted 1:10 in sterile RNase-free water and stored at -20°C until analysis by PCR.

Real-time PCR analysis. Primer pairs specific for MDR1 and MRP2 (Table 1) and for the house-keeping genes for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and beta-actin were used for real-time PCR analysis. Primers were used at a concentration of 10 pmol/µl. Ten microlitres of diluted cDNA were mixed with 15 µl of iQTM SYBR Green Supermix (Bio Rad Laboratories), forward and reverse primers and sterile water according to the manufacturer's instructions. Real-time PCR was performed using an iCycler iQ system and MyiQ System software version 1.0.410 (Bio Rad Laboratories). The stability of

transcription of the housekeeping genes during experimental infection with *E. coli* O78/K80, treatment with danofloxacin mesylate and treatment with enrofloxacin was tested using the algorithm described by Radonic *et al.* (2004). Gene expression was assessed using the algorithm described by Vandesompele *et al.* (2002) and the geNorm software available at <http://medgen.ugent.be/~jvdesomp/genorm/>. Dilution series of target and reference nucleic acids were used to determine the fit coefficients of the relative standard curves (the efficiency of the PCRs). The standards were run at the same time as the samples. Each PCR reaction included a negative control that did not contain a cDNA template. All samples were run in duplicate.

Statistical analysis. All data were analysed using the Statistica 6.1 program (Statistica for Windows, StatSoft). Data were compared using the Mann-Whitney test with $P < 0.05$ considered significant. Comparative levels of transcription in different organs were assessed using the classification system of Vandesompele *et al.* (2002), with low levels of expression defined as being equal to or lower than the MDR1 mRNA levels in the caecum (0.2 relative units of expression), moderate levels defined as those lying between high and low levels of expression, and high levels defined as equal to or higher than MDR1 mRNA levels in the ileum (1 relative unit of expression).

Results

All birds developed clinical signs of disease, including depression and weakness, within 24 h of inoculation with *E. coli* O78/K80. Gross lesions included a two- to three-fold increase in the size of the spleen, pericarditis and dilation of the blood vessels in the gastrointestinal tract. Mild airsacculitis was observed in a few chickens. The most prominent changes were found in the small and large intestine, and included haemorrhagic enterocolitis with a large number of erosions, which could be seen in all infected animals, particularly in the untreated infected group (positive controls).

MDR1 mRNA was detected at high levels in all samples of the small intestine in healthy broilers. While the levels of mRNA in the caeca were low, those in the colon and the liver were moderate (Fig. 1). After infection with *E. coli* O78/K80, there was a significant decrease in MDR1 mRNA levels in the duodenum, jejunum, caeca and liver within 24 h (Fig. 2A). After treatment with danofloxacin, MDR1 mRNA levels increased, but were still significantly lower in the duodenum and liver than in healthy controls. After enrofloxacin treatment MDR1 mRNA levels remained significantly lower in the duodenum and caeca (Fig. 3A).

MRP2 mRNA was detected predominantly in the liver and small intestine, with high levels found in the duodenum, moderate levels in the jejunum and low levels in the ileum (Fig. 1). The levels in the caeca and colon were at the limit of detection (data not shown). MRP2 mRNA levels decreased significantly in the liver after infection (Fig. 2B). In the danofloxacin treated birds, levels of MRP2 mRNA in the liver remained significantly lower than in uninfected birds, but there was no significant difference between the levels in birds treated with enrofloxacin and uninfected birds (Fig. 3B).

The levels of house-keeping gene mRNAs did not differ between the four experimental groups.

Discussion

There have been few studies examining the expression and function of ABC transporters in birds, and none have examined mRNA levels in different organs of chickens. The distribution of MDR1 and MRP2 mRNAs in the intestines and livers of healthy broilers had a distribution comparable to that seen in mammalian species (Stephens *et al.*, 2002; Langmann *et al.*, 2003; Maher *et al.*, 2005; Thörn *et al.*, 2005). Our observations confirm those of the two previous reports on MDR1 mRNA and P-gp in poultry (Edelman *et al.*, 1999; Barns, 2001), but there have not been any published studies on the expression and function of MRP2 in birds.

MDR1 and MRP2 were examined in these studies because of their role as defence transporters. The product of MDR1, P-gp, is an important component of tissue barriers, including that in the gut (Borst & Elferink, 2002). The product of MRP2 plays a significant role in excretory organs, including the liver and the kidneys. In the liver, MRP2 (together with bile salt export pump, BSEP) is involved in the efflux of bile acids and has a protective role against intracellular accumulation of bile acids and their conjugates, including conjugates to many drugs and toxins (Elias & Mills, 2007). Several diseases, including obstructive cholestasis, are associated with a down-regulation of hepatic ABC transporters during the acute phase response to infection (Geier *et al.*, 2007). It is assumed

that pro-inflammatory cytokines mediate this reduced expression and activity via hepatic nuclear receptors. In mice and rats the expression of MRP2 mRNA is down-regulated in the liver and the intestines during the acute inflammatory response (Tang *et al.*, 2000; Hartmann *et al.*, 2002; Kalitsky-Szirtes *et al.*, 2004). In obstructive cholestasis in humans, no changes in MRP2 mRNA levels were detected, even though MRP2 protein levels were markedly reduced, suggesting post-transcriptional regulation (Geier *et al.*, 2005).

Injection of lipopolysaccharide has been shown to cause a decrease in the level of MRP2 and MDR1 in the livers of rats, but an increase in the level of other transporters belonging to MRP family such as MRP1 (Cherrington *et al.*, 2004). Dexamethasone was able to reverse these effects. On the basis of these results, the authors concluded that the adaptive response to LPS and to dexamethasone treatment is mediated through similar pathways, namely transcriptional regulation of hepatic transporters.

The work described in this paper found that infection of chickens with *E. coli* initially caused significant down-regulation of MDR1 mRNA in the liver and caeca, but the levels of this transporter in the small intestines also decreased, distinguishing the response in poultry from that seen in rodents to lipopolysaccharide. A decrease in MDR1 mRNA and a resultant decrease in P-gp expression in the intestines may increase permeability for toxins, including LPS, and accelerate the adverse effects of infection. MRP2 mRNA levels were significantly suppressed only in the liver after infection with *E. coli*. This decrease may affect excretory processes in the liver.

Following the five day treatment period with either of the fluoroquinolones, the levels of MDR1 mRNA in the small intestines returned towards those seen prior to infection, with the exception of those in the duodenum. This is most likely to have resulted from the

antimicrobial effect of the fluoroquinolones and the consequent reduction in inflammation, rather than a direct effect of these drugs on mRNA expression. Samples from the treated animals were obtained 6 days after infection, when the animals had improved clinically. Thus clinical improvement preceded the complete restoration of MDR1 mRNA levels.

The increase in the levels of MDR1 following therapy is likely to reflect a beneficial effect on the bird, as this up-regulation would improve the barrier function of the gastrointestinal tract (Panwala *et al.*, 1998; Schwab *et al.*, 2003; Leslie *et al.*, 2005). In the liver an increase in the levels of MRP2 is likely to be beneficial, as this transporter is essential for the efflux of glucuronides and other conjugates. Bilirubin can only be excreted as a glucuronide, and normal levels of MRP2 are required to protect hepatocytes from oxidative damage by free bilirubin. Thus, the persistence of down-regulation of MRP2 mRNA in the liver following the administration of danofloxacin mesylate requires further investigation.

In conclusion, our study provides evidence that the tissue distribution of these two ABC transporters in chickens is similar to that seen in mammals. The efflux transporters MRP2 and MDR1 are involved in a range of physiological functions, including the maintenance of the gut barrier, and the protection of hepatocytes. Infection with *E. coli* modulates the levels of mRNA from these genes in the liver and the intestines of chickens. Further investigation of the function of these ABC transporters is now needed to assess the clinical significance of these changes.

References

- Beigneux, A. P., Moser, A. H., Shigenaga, J. K., Grunfeld, C. & Feingold, K. R. (2002). Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. *Biochemical and Biophysical Research Communications*, 293, 145–149.
- Bertilsson, P.M., Olsson, P. & Magnusson, K.-E. (2001). Cytokines influence mRNA expression of cytochrome P450 3A4 and MDR1 in intestinal cells. *Journal of Pharmaceutical Sciences*, 90, 638–646.
- Borst, P. & Elferink, R. O. (2002). Mammalian ABC transporters in health and disease. *Annual Review of Biochemistry*, 71, 537–592.
- Chansiripornchai, N. & Sasipreeyajan, J. (2002). Efficacy of sarafloxacin in broilers after experimental infection with *Escherichia coli*. *Veterinary Research Communications*, 26, 255–262.
- Charleston, B., Gate, J.J., Aitken I.A., Stephan, B. & Froyman, R. (1998). Comparison of the efficacies of three fluoroquinolone antimicrobial agents, given as continuous or pulsed-water medication, against *Escherichia coli* infection in chickens. *Antimicrobial Agents and Chemotherapy*, 42, 83–87.
- Cherrington, N.J., Slitt, A.L., Li, N. & Klaassen, C. (2004). Lipopolysaccharide-mediated regulation of hepatic transporter mRNA levels in rats. *Drug Metabolism and Disposition*, 32, 734–741.
- Cormet-Boyaka, E., Huneau, J.-F., Morderelle, A., Boyaka, P.N., Carbon, C., Rubinstein, E. & Tome, D. (1998). Secretion of sparfloxacin from the human intestinal Caco-2 cell line is altered by P-Glycoprotein inhibitors. *Antimicrobial Agents and Chemotherapy*, 42, 2607–2611.
- Dean, M., & Annilo, T. (2005). Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annual Review of Genomics and Human Genetics*, 6, 123–142.
- Elias, E. & Mills, C.O. (2007). Coordinated defence and the liver. *Clinical Medicine*, 7, 180–184.
- Fernandez, A., Lara, C., Puyuelo, R., Gomez, J., Ramos, J. J., Loste, A., Marca, M. C. & Verde, M. T. (1998). Efficacy of phosphomycin in the control of *E. coli* infection of broiler chickens. *Research in Veterinary Science*, 65, 201–204.
- Fromm, F. M. (2004). Importance of Pglycoprotein at blood-tissue barriers. *Trends in Pharmacological Sciences*, 25, 423–429.
- Geier, A., Dietrich, C.G., Voigt, S., Ananthanarayanan, M., Lammert, F., Schmitz, A., Trauner, M., Wasmuth, H.E., Boraschi, D., Balasubramaniyan, N., Suchy, F.J., Matern, S. & Gartung, C. (2005). Cytokine-dependent regulation of hepatic organic anion transporter gene transactivators in mouse liver. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 289, G831–841.
- Geier, A., Wagner, M., Dietrich, C.G. & Trauner, M. (2007). Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. *Biochimica et Biophysica Acta*, 1773, 283–308.

- Giessmann, T., May, K., Modess, C., Wegner, D., Hecker, U., Zschiesche, M., Dazert, P., Grube, M., Schroeder, E., Warzok, R., Cascorbi, I., Kroemer, H.K. & Siegmund, W. (2004). Carbamazepine regulates intestinal P-glycoprotein and multidrug resistance protein MRP2 and influences disposition of talinolol in humans. *Clinical Pharmacology and Therapeutics*, 76, 192–200.
- Gollapudi, S., Thadepalli, F., Kim, C. H. & Gupta, S. (1995). Difloxacin reverses multidrug resistance in HL-60/AR cells that overexpress the multidrug resistance-related protein (MRP) gene. *Oncology Research*, 7, 213–225.
- Hartmann, G., Cheung, A.K.Y. & Piquette-Miller, M. (2002). Inflammatory cytokines, but not bile acids, regulate expression of murine hepatic anion transporters in endotoxemia. *The Journal of Pharmacology and Experimental Therapeutics*, 303, 273–281.
- Kalitsky-Szirtes, J., Shayeganpour, A., Brocks, D.R. & Piquette-Miller, M. (2004). Suppression of drug-metabolizing enzymes and efflux transporters in the intestine of endotoxin-treated rats. *Drug Metabolism and Disposition*, 32, 20–27.
- Lagas, J.S., Vlaming, M.L., van Tellingen, O., Wagenaar, E., Jansen, R.S., Rosing, H., Beijnen, J.H. & Schinkel, A.H. (2006). Multidrug resistance protein 2 is an important determinant of paclitaxel pharmacokinetics. *Clinical Cancer Research*, 12, 6125–6132.
- Langmann, T., Mauerer, R., Zahn, A., Moehle, C., Probst, M., Stremmel, W. & Schmitz, G. (2003). Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. *Clinical Chemistry*, 49, 230–238.
- Leshchinsky, T.V. & Klasing, K.C. (2001). Divergence of the inflammatory response in two types of chickens. *Developmental and Comparative Immunology*, 25, 629–638.
- Leslie, E.M., Deeley, R.G. & Cole, S.P.C. (2005). Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicology and Applied Pharmacology*, 204, 216–237.
- Linton, K.J. (2007). Structure and function of ABC transporters. *Physiology*, 22, 122–130.
- Maher, J.M., Slitt, A.L., Cherrington, N.J., Cheng, X. & Klaassen, C.D. (2005). Tissue distribution and hepatic and renal ontogeny of the multidrug resistance-associated protein (MRP) family in mice. *Drug Metabolism and Disposition*, 33, 947–955.
- McRae, M.P., Brouwer, K.L.R. & Kashuba, A.D.M. (2003). Cytokine regulation of P-glycoprotein. *Drug Metabolism Reviews*, 35, 19–33.
- Mireles, A. J., Kim, S. M. & Klasing, K. C. (2005). An acute inflammatory response alters bone homeostasis, body composition, and the humoral immune response of broiler chickens. *Poultry Science*, 84, 553–560.
- Naruhashi, K., Tamai, I., Inoue, N., Muraoka, H., Sai, Y., Suzuki, N. & Tsuji, A. (2001). Active intestinal secretion of new quinolone antimicrobials and the partial contribution of P-glycoprotein. *Journal of Pharmacy and Pharmacology*, 53, 699–709.
- Naud, J., Michaud, J., Boisvert, C., Desbiens, K., Leblond, F.A., Mitchell, A., Jones, C., Bonnardeaux, A. & Pichette, V. (2007). Down-regulation of intestinal drug transporters in chronic renal failure in rats. *The Journal of Pharmacology and Experimental Therapeutics*, 320, 978–985.

- Panwala, C.M., Jones, J.C. & Viney, J.L. (1998). A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis. *The Journal of Immunology*, *161*, 5733–5744.
- Prime-Chapman, H., Moore, V. & Hirst, B.H. (2005). Antibiotic exposure does not influence MRP2 functional expression in Caco-2 cells. *Journal of Drug Targeting*, *13*, 1–6.
- Radonic, A., Thulke, S., Mackay, I.M., Landt, O., Siegert, W. & Nitsche, A. (2004). Guideline to reference gene selection for quantitative real-time PCR. *Biochemical and Biophysical Research Communications*, *313*, 856–862.
- Rodriguez-Ibanez, M., Nalda-Molina, R., Montalar-Montero, M., Bermejo, M.V., Merino, V. & Garrigues, T.M. (2003). Transintestinal secretion of ciprofloxacin, grepafloxacin and sparfloxacin: in vitro and in situ inhibition studies. *European Journal of Pharmaceutics and Biopharmaceutics*, *55*, 241–246.
- Schwab, M., Schaeffler, E., Marx, C., Fromm, M.F., Kaskas, B., Metzler, J., Stange, E., Herfarth, H., Schoelmerich, J., Gregor, M., Walker, S., Cascorbi, I., Roots, I., Brinkmann, U., Zanger, U. & Eichelbaum, M. (2003). Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology*, *124*, 26–33.
- Siewert, E., Dietrich, C.G., Lammert, F., Heinrich, P.C., Matern, S., Gartung, C. & Geier, A. (2004). Interleukin-6 regulates hepatic transporters during acute-phase response. *Biochemical and Biophysical Research Communications*, *322*, 232–238.
- Stephens, R.H., Tanianis-Hughes, J., Higg, N.B., Humphrey, M. & Warhurst, G. (2002). Region-dependent modulation of intestinal permeability by drug efflux transporters: in vitro studies in *mdra* (-/-) mouse intestine. *The Journal of Pharmacology and Experimental Therapeutics*, *303*, 1095–10101.
- Stienstra, R., Lichtenauer-Kaligis, E. & Muller, M. (2004). Stress- (and diet-) related regulation of hepatic nuclear receptors and its relevance for ABC-transporter functions. *Drug Metabolism Reviews*, *36*, 391–406.
- Sukhai, M., Yong, A., Kalitsky, J. & Piquette-Miller, M. (2000). Inflammation and Interleukin-6 mediate reductions in the hepatic expression and transcription of the *mdr1a* and *mdr1b* genes. *Molecular Cell Biology Research Communications*, *4*, 248–256.
- Sukhai, M., Yong, A., Pak, A. & Piquette-Miller, M. (2001). Decreased expression of P-glycoprotein in interleukin-1b and interleukin-6 treated rat hepatocytes. *Inflammation Research*, *50*, 362–370.
- Tang, W., Yi, C., Kalitsky, J. & Piquette-Miller, M. (2000). Endotoxin downregulates hepatic expression of P-glycoprotein and MRP2 in 2-acetylaminofluorene-treated rats. *Molecular Cell Biology Research Communications*, *4*, 90–97.
- Thörn, M., Finnström, N., Lundgren, S., Rane, A. & Löf, L. (2005). Cytochromes P450 and MDR1 mRNA expression along the human gastrointestinal tract. *British Journal of Clinical Pharmacology*, *60*, 54–60.
- van de Water, F.M., Boleij, J.M., Peters, J.G., Russel, F.G. & Masereeuw, R. (2007). Characterization of P-glycoprotein and multidrug resistance proteins in rat kidney and intestinal cell lines. *European Journal of Pharmaceutical Sciences*, *30*, 36–44.
- Vandesompele, J., De Peter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data

by geometric averaging of multiple internal control genes. *Genome Biology*, 3, research0034.1–0034.11.

Yokooji, T., Murakami, T., Yumoto, R., Nagai, J. & Takano, M. (2006). Function of multidrug resistance-associated protein 2 in acute hepatic failure rats. *European Journal of Pharmacology*, 546, 152–160.

For Peer Review Only

Table 1. Nucleotide sequences of PCR primers used to assay gene expression by real-time PCR.

Gene	NCBI accession number	Forward primer 5' → 3'	Reverse primer 5' → 3'	Ta °C
MDR1	NM_204894	GCTGTTGTATTTCT GCTATGG	ACAAACAAGTGGGCT GCTG	58
MRP2	XM_421698	CTGCAGCAAATGA GAGGACAATG	CAGAAGCGCAGAGAA GAAGACCAC	63
GAPDH	NM_204305	GTGTGCCAACCC AATGTCTCT	GCAGCAGCCTTCACT ACCCTCT	65
Beta-actin	NM_205518	ATGTGGATCAGCAA GCAGGAGTA	TTTATGCGCATTTATG GGTTTTGT	61

Ta – optimal annealing temperature

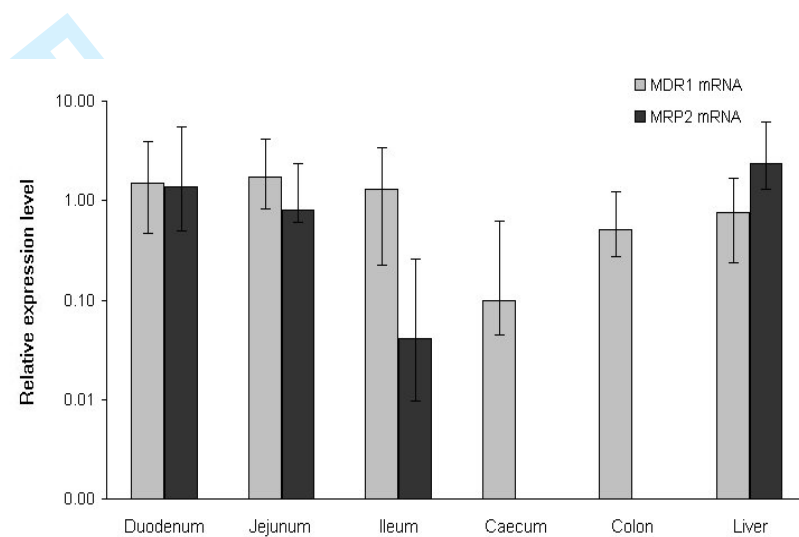


Figure 1. Levels of MDR1 and MRP2 mRNA relative to those of GAPDH and beta-actin mRNA in healthy broiler chickens ($n=8$). The data are presented as medians and ranges.

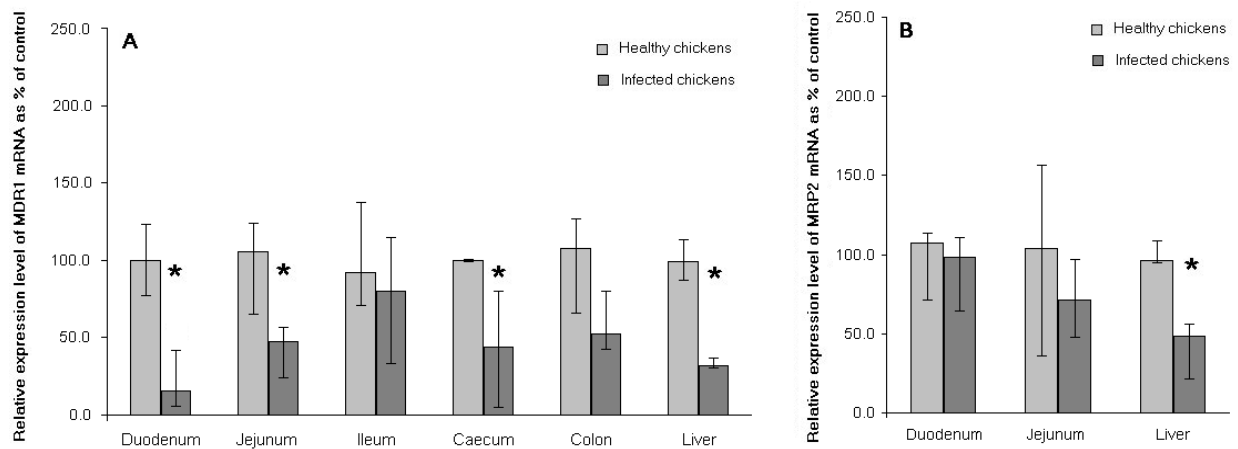


Figure 2. Levels of expression of MDR1 (A) and MRP2 (B) mRNA in chickens (n=8) experimentally infected with *E. coli* relative to those of uninfected animals (n=4). Tissue samples from both groups were obtained 24 h after inoculation of the experimentally infected birds. The data are presented as medians and ranges. * $P < 0.05$ compared to the uninfected controls.

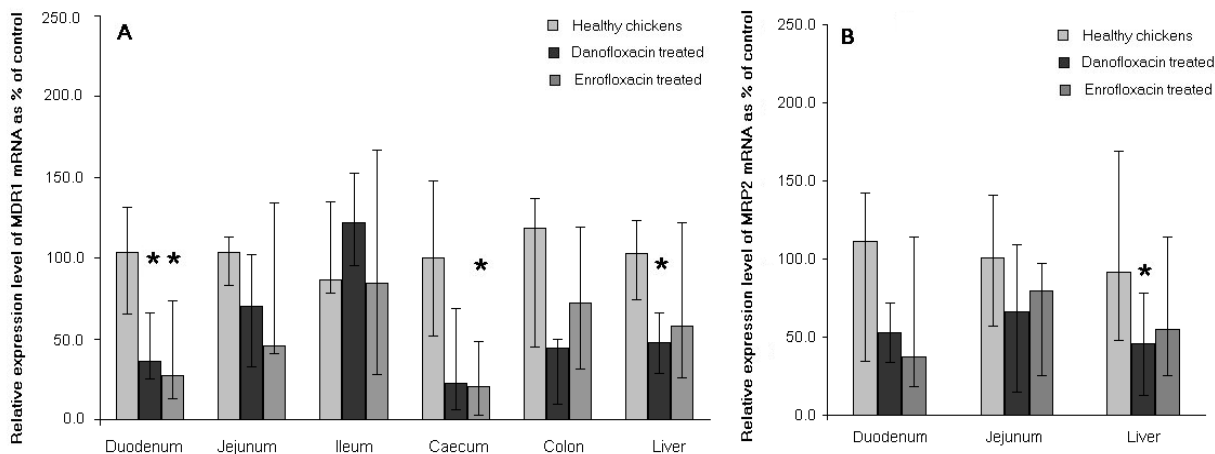


Figure 3. Levels of expression of MDR1 (A) and MRP2 (B) mRNA in control animals ($n=4$) and in infected chickens ($n= 8$ per group). The infected chickens were treated with either danofloxacin mesylate or enrofloxacin for 5 consecutive days, starting 24 h after inoculation with *E. coli*. Tissue samples from the uninfected controls and the treated chickens were taken on day 6 after inoculation. The data are presented as medians and ranges. * $P<0.05$ compared to the healthy controls.