INTERACTION OF FLUOROQUINOLONES AND CERTAIN IONOPHORES IN BROILERS: EFFECT ON BLOOD LEVELS AND HEPATIC CYTOCHROME P450 MONOOXYGENASE ACTIVITY

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SUMMARY

The concomitant administration to broilers of ionophore coccidiostats and certain chemotherapeutic agents may cause deleterious interactions, with toxicosis and death as possible sequelae. In this study, co-administration of the ionophore monensin was not shown to alter blood levels of enrofloxacin or norfloxacin. In addition, exposure to lasalocid was not shown to change blood levels of enrofloxacin. However, norfloxacin + lasalocid co-administration induced aminopyrine *N*-demethylase (AD) activity by day 5 after the last administration of norfloxacin, and induced a rise of norfloxacin levels in the blood. This rise of blood norfloxacin levels after co-administration of norfloxacin + lasalocid implies that lower levels of norfloxacin could be administered in birds also receiving lasalocid.

KEY WORDS

enrofloxacin, norfloxacin, monensin, lasalocid, drug interactions, microsomal cytochrome P450 monooxygenases, blood level, broiler chickens

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INTRODUCTION

In broiler practice, ionophore coccidiostats, such as monensin, lasalocid, salinomycin and narasin, are very commonly administered in the feed for a significant part of their growth period. If illness is diagnosed, the flock may be prescribed treatment with antimicrobial or antibiotic drugs. Sometimes the concomitant usage of these drugs leads to toxic effects, for example, toxicoses due to monensin-tiamulin, salinomycin-tiamulin, and ionophore-sulfonamide combinations are well recognized /1-3/. The mechanisms of the toxic interaction have not been completely elucidated, but possible means may be elevated levels of free radical generation and lowering of antioxidant defenses, and also changes in microsomal cytochrome P450 monooxygenase activity /4-6/. Induction or inhibition of the latter system may change the velocity and direction of the metabolism of a drug (and also of endogenous substrates) and therefore may bring about undesirable side effects, such as lipid peroxidation and drug accumulation in the blood /5/.

Fluoroquinolone antimicrobials are also widely used for treatment of poultry diseases, due to a high antibacterial activity against Gram-negative bacteria and high efficacy at low dosages /7/. Incompatibility of some fluoroquinolones with penicillin, drug interactions of quinolones, such as prolongation of prothrombin time during warfarin therapy in combination with ofloxacin, norfloxacin and ciprofloxacin, and inhibition of the metabolic clearance of methylxanthines, have been recorded /8/. Data exist about the interaction between fluoroquinolones and some other drugs, particularly with theophylline, as the result of cytochrome P450 inhibition /9/.

In a previous study, we showed that the fluoroquinolone antimicrobials, norfloxacin and enrofloxacin, inhibit hepatic microsomal cytochrome P450 monooxygenases in the livers of broiler chickens /10/, and that the combinations, monensin + sulfadimethoxine or monensin + sulfadimidine, also modified cytochrome P450 monooxygenase activity /6,11/. The consequence of administration of the combination monensin + sulfadimidine is the induction of cytochrome P450 monooxygenases over a period of days, accompanied by a lowering of total antioxidant status /11/.

The present study is a continuation of previous research to investigate the role of hepatic microsomal cytochrome P450 mono-

oxygenases when the ionophores monensin and lasalocid are administered with the fluoroquinolones norfloxacin and enrofloxacin to broiler chickens, and the possible consequences of these modifications, as manifested by changes of fluoroquinolone levels in the blood.

MATERIALS AND METHODS

Chemicals

The drugs used were norfloxacin nicotinate (36%, Quinabic®, Teva-Abic, Israel), enrofloxacin (10%, Baytril®, Bayer, Germany), lasalocid and monensin (Bar Magen, Israel). Aminopyrine, aniline, nicotinamide adenine dinucleotide phosphate (reduced form), acetyl acetone, ammonium acetate and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Animals, treatments, microsomes and enzyme assays

One day-old male commercial broiler chickens were housed in electrically heated battery brooders. A broiler starter feed was supplied *ad libitum* throughout. When the birds were 25 days old, they were weighed, and birds in a weight range of 560-650 g were divided into two randomly constituted groups. Group 1 served as a control and received no drug treatment. Group 2 was administered monensin or lasalocid in the feed for 12 days (99 ppm), and from the 8^{th} to the 12^{th} day (a period of 5 days) concomitantly with one of the fluoroquinolones (norfloxacin, delivered *per os* at a dosage of 20 mg/kg body weight, or enrofloxacin, given *per os* at a dosage of 10 mg/kg body weight). At 1, 3, 5 days after the last fluoroquinolone dosage, and 24 h after the last feed was given, six birds in the appropriate group were killed for the determination of hepatic microsomal cytochrome P450 monooxygenases. The periods of 1, 3, 5 days were chosen for the determination of duration and direction of change in the activity of cytochrome P450 monooxygenases because it is known that in some cases after short-time inhibition the stable induction of this system may follow /12/. Blood samples were collected at 1 day after the last fluoroquinolone dosage for determination of serum levels of fluoroquinolones. This period is in accordance with the specificity of

the pharmacokinetics of fluoroquinolones in chickens after oral administration. The half-life of enrofloxacin, ciprofloxacin and norfloxacin is 6-10 h, and after 24 h, 0.2-0.4 mg/l of these drugs may be found in the plasma of chickens /13-15/.

Livers were immediately perfused with ice-cold 1.15% KCl solution from the cranial vena cava caudally, until the efferent perfusion fluid was blood free. Determination of aminopyrine *N*-demethylase (AD) activity /16/, aniline hydroxylase (AH) activity /17/ and protein /18/ was made in the 9,000 *g* supernatant liver fraction. Serum levels of norfloxacin and enrofloxacin were determined by liquid chromatography. The limit of quantification was 0.01 mg/l and the standard curve was linear within the range from 0.01 to 50 mg/l (coefficient of correlation: 0.996). Precision analysis showed that the interday coefficient of variation among six replicates was 9.0% at 0.01 mg/l, 4.0% at 0.5 mg/l, and 4.0% at 50 mg/l. The recovery from serum samples was more than 60% /19/.

Statistical analysis

Data were analyzed to test the difference between treatment and control groups using Student's t-test; $p < 0.05$ was taken as the significance level. All test and standard errors are based on interassay variation.

RESULTS

There were no statistically significant changes in AD or AH activity in liver microsomes when broilers were treated with the enrofloxacin + lasalocid combination (Fig. 1). The norfloxacin + lasalocid combination resulted in AD activity induction on the 5th day after the last treatment (Fig. 2), with no change in AH activity. The treatment of broilers with the enrofloxacin + monensin or enrofloxacin + lasalocid combinations did not show significant changes in blood enrofloxacin levels (Fig. 3). Similar results were seen with the metabolite of enrofloxacin, ciprofloxacin (which is also an active antibacterial agent in broilers) (Fig. 3). The lasalocid treatments showed a trend towards higher levels of blood fluoroquinolone levels (Fig. 3). The combination norfloxacin + lasalocid treatment resulted in a significant rise in blood norfloxacin levels compared with administ-

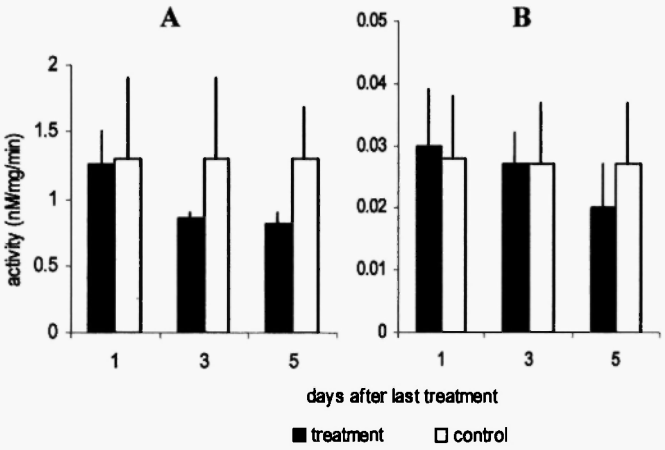

Fig. 1: Effect of enrofloxacin + lasalocid on (**A**) aminopyrine *N*-demethylase and (**B**) aniline hydroxylase activity in chicken liver microsomes.

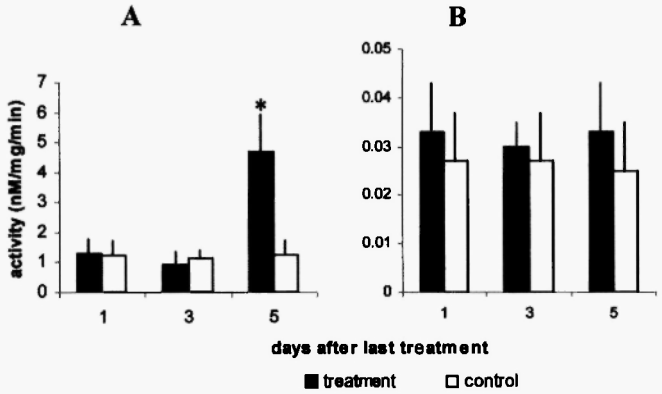

Fig. 2: Effect of norfloxain + lasalocid on (**A**) aminopyrine *N*-demethylase and (**B**) aniline hydroxylase activity in chicken liver microsomes.

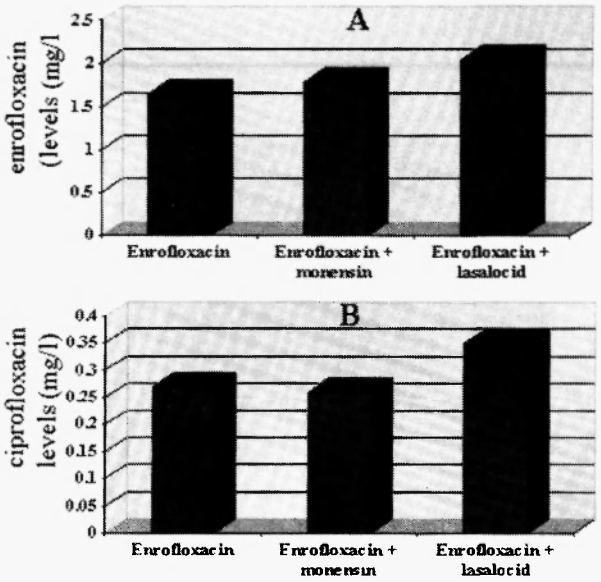

Fig. 3: Interaction of ionophores and enrofloxacin. Effect on blood levels of (**A**) enrofloxacin and (**B**) ciprofloxacin (n = 6 for each group). **A:** Enrofloxacin 1.65 ± 0.55; enrofloxacin + monensin 1.79 ± 0.42, enrofloxacin + lasalocid 2.05 ± 0.47 mg/l. **B:** Enrofloxacin 0.27 ± 0.02, enrofloxacin + monensin 0.25 ± 0.03, enrofloxacin + lasalocid 0.35 ± 0.08 mg/l.

ration of norfloxacin alone or with monensin (Fig. 4). There were no changes in relative liver weight on the 1st, 3rd and 5th days when birds were treated with the norfloxacin + lasalocid or enrofloxacin + lasalocid combinations (data not shown).

DISCUSSION

Incompabilities of monensin, salinomycin and narasin with erythromycin, oleandomycin, certain sulfonamides, tiamulin, furazolidone, and with certain antioxidants have been demonstrated /3,20-23/. Adverse reactions have been recorded when ionophores were used together with the dihydroquinoline compound duokvin and other related compounds /24,25/. The monensin-duokvin combination is

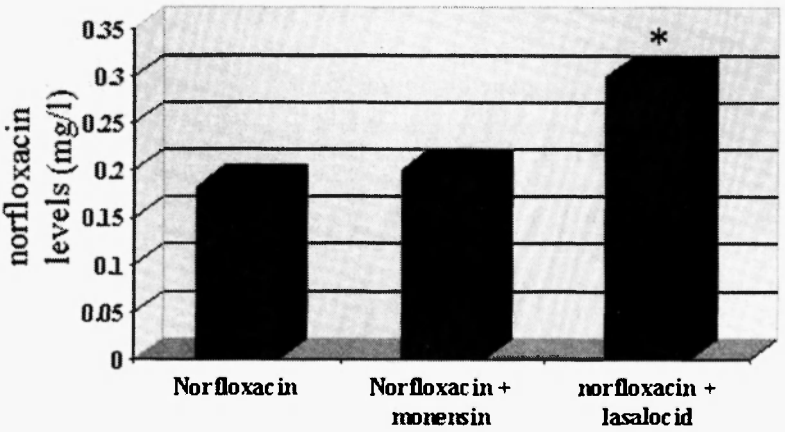

Fig. 4: Effect of ionophores on blood norfloxacin levels. Norfloxacin 0.18 ± 0.03, norfloxacin + monensin 0.20 ± 0.03, norfloxacin + lasalocid 0.3 ± 0.12 mg/l. *Significant change ($p < 0.05$) ($n = 6$ for each group).

compatible with erythromycin, sulphachlorpyrazine and sulphaquinoxaline when the ionophore dosages are lowered /26/. The mechanisms of these interactions are not fully understood, although the monensin-tiamulin incompatibility is considered to be due to the retardation of monensin metabolism because of cytochrome P450 monooxygenase activity inhibition by tiamulin /27/. The pharmacokinetics of antipyrine in chickens before and after prolonged tiamulin treatment showed that tiamulin inhibited the oxidative metabolism of antipyrine /27/. Antipyrine was used as an agent principally metabolized by the cytochrome P450 monooxygenase system and so as an index of hepatic drug metabolizing activity /28/. Lasalocid interacts with fewer drugs than monensin, but the lasalocid + chloramphenicol combination caused neuromuscular dysfunction and structural damage in broiler chicks /29/. Chloramphenicol is a strong inhibitor of cytochrome P450 monooxygenases and an effective "suicide substrate" for cytochrome P450 enzymes /30/.

In the present study, concomitant exposure to monensin was not shown to alter blood levels of enrofloxacin or norfloxacin. In addition, exposure to lasalocid was not shown to change blood levels of enrofloxacin. However, the norfloxacin + lasalocid combination

induced a rise of blood norfloxacin levels 1 day after the last fluoroquinolone administration (Fig. 4). It would be reasonable to expect that,in these periods, the inhibition of cytochrome P450 monooxygenase activity occurred, but our experiments did not reveal statistically significant changes in AD or AH activity. This phenomenon could be explained by the substrate specificity of P450 isoenzymes for different compounds. For example, in hepatic tissue from chicken embryos, treatment with phenobarbital-type inducers caused induction of ethoxycoumarin and aminopyrine dealkylase activities, but not of pentoxyresorufin and benzyloxyresorufin dealkylase, as was noted with other species /31/. The most specific test for chicken CYP1A2 activity is considered to be uroporphyrinogen oxidation /32/.

One of the substrates used in this study was aminopyrine, which, as with some other substrates, has been used with rodents and other species for measuring cytochrome P450b activity. In our previous experiments, AD activity seemed to be a sensitive reaction in broilers treated with enrofloxacin and norloxacin /10/. The other substrate used in this study was aniline, whose metabolism may be connected with some cytochrome P450 forms including cytochrome P450IIE. Birds have relatively high AH activity, which exceeds that of aldrin epoxidase or aminopyrine demethylase /33/. In our investigations, AH activity was also a sensitive indicator in broilers treated with enrofloxacin and norfloxacin /10/. However, when the norfloxacin + lasalocid combination was used, these indicators did not show statistically significant changes in the early periods, and only after 5 days was the rise of AD activity observed (perhaps in a compensatory manner). It may be that the reason for the elevated blood level of norfloxacin, when the norloxacin + lasalocid combination was used, was the modification of other isoenzymes, different from cytochrome P450b and P450IIE. This combination is preferable in comparison with norfloxacin alone /10/ which lowered the activity of both AD and AH at all times studied (1, 3 and 5 days after the last treatment). The rise of norfloxacin in blood, when the norfloxacin + lasalocid combination was used, may be considered as enhancing the antibacterial action of this combination, and not as negative effect, as lower levels could conceivably be administered in birds receiving lasalocid, without reducing the minimum inhibitory concentration (MIC).

The administration of enrofloxacin and norfloxacin in combination with ionophores seems to be preferable to their administration alone. Enrofloxacin and norfloxacin have been shown to be active inhibitors of cytochrome P450 monooxygenase systems, and this fact may be a reason for their cumulative action, which may result in a toxic effect.

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