INTERACTION OF MONENSIN AND SULFADIMETHOXINE IN BROILERS, AS MEDIATED BY HEPATIC MICROSOMAL CYTOCHROME P-450 MONOOXYGENASES

E. Ershov, M. Bellaiche, V. Hanji, S. Soback, M. Gips, Y. Weisman and A. Shlosberg

Kimron Veterinary Institute, P.O.Box 12, 50250 Bet Dagan, Israel

SUMMARY

The influence of monensin + sulfadimethoxine on cytochrome P-450 monooxygenase activity in broilers, and the possible consequences of modification of this system, including changes in blood levels of sulfadimethoxine, influence on the duration of xylazine-ketamine anesthesia, total antioxidant status and superoxide dismutase activity were studied.

The results indicate that the combination of monensin + sulfadimethoxine gave a short-term inhibition of microsomal cytochrome P-450 monooxygenase activity but apparently did not influence the metabolism of other (exogenic) substances (ketamine, xylazine), and did not change the state of antioxidant systems or the relative liver weight. There was a rise in blood sulfadimethoxine levels.

KEY WORDS

monensin, sulfadimethoxine, drug interactions, microsomal cytochrome P-450 monooxygenases, antioxidant status, broiler chickens

INTRODUCTION

Broiler chickens are raised under intensive conditions with the emphasis on utilizing every day of their life span to realize the potential of maximum weight gain. If bacterial disease is diagnosed, the whole flock may be given feed or water medicated with an antibacterial drug or a combination of drugs. The efficacy of these drugs will depend on several factors, mainly the susceptibility of the pathogenic organism to that drug, and the pharmacodynamics and pharmacokinetics of the drug in the broiler. The last factor largely involves Phase 1 metabolism in the liver mediated by enzymes, many of which are microsomal cytochrome P-450 monooxygenases. Changes in cytochrome P-450 monooxygenase activity could cause unwanted complications, including lipid peroxidation resulting in damage to cell membranes, drug accumulation in blood with consequent toxic manifestations and possible undesirable tissue residues, or lowered blood levels with consequent poor therapeutic efficacy. Side reactions caused by the change of direction and velocity of drug biotransformation may also be observed when a combination of substances is used. It has been shown that tiamulin is capable of inhibiting the oxidative metabolism of antipyrine /1/, and chloramphenicol inhibited microsomal cytochrome P-450 monooxygenase, both leading to an accumulation of an ionophore in the blood, and consequently clinical toxicity, although all dosages were at therapeutic levels /2,3/.

Our literature search revealed few relevant publications on this topic in broilers, although considerable data exist on laboratory rodent cytochrome P-450 monooxygenases. Species differences preclude a valid extrapolation to broilers and there clearly exists a knowledge deficit on this topic.

Research trials have been conducted by this team on interactions between drugs that are of much importance to the broiler industry, including the ionophore coccidiostat monensin and the antibacterial agent sulfadimethoxine (SM). The present study was carried out to reveal the potential ability of these two drugs and their combination to modify cytochrome P-450 monooxygenase activity in the broiler, to assess possible harmful consequences of these modifications, including changes in blood levels of SM, the influence of the drugs on the

duration of xylazine-ketamine induced anesthesia, and changes in total antioxidant status and superoxide dismutase activity.

MATERIALS AND METHODS

Chemicals

Commercial preparations of monensin (Bar Magen Ltd., Israel) and sulfadimethoxine (Teva-Abic Ltd., Netanya, Israel) used for broilers were utilized. Aminopyrine, aniline, nicotinamide adenine dinucleotide phosphate (reduced form), acetyl acetone, ammonium acetate and bovine serum albumin were obtained from the 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 three randomly constituted groups. Group 1 served as a control and received no drug treatment. Group 2 was administered SM, given orally to each bird at a dosage of 20 mg/kg body weight daily for a 5-day period. Group 3 received monensin in the feed for 12 days at a concentration of 99 mg/kg, and from the 8^{th} to the 12^{th} day (a period of 5 days) concomitantly with SM. At 1, 3, 5 and 7 days after the last administration of SM, and 24 h after the last feed was given, six birds in the appropriate group were killed for the determination of hepatic microsomal cytochrome P-450 monooxygenases. Livers were immediately perfused with ice-cold 1.15% KCl solution injected caudally into the cranial vena cava, until the efferent perfusion fluid was blood-free. Determination of aminopyrine N-demethylase (AD) activity /4/, aniline hydroxylase (AH) activity /5/ and protein /6/ was made in the 9000 *g* and 100,000 *g* supernatant liver fractions /7/. SM concentration in serum from blood taken immediately before slaughter was determined by HPLC /8/. The total antioxidant level in the plasma was determined using a kit (Randox Laboratories Ltd., Grumlin, Co. Antrim, UK) /9/. Superoxide dismutase (SOD) activity in red blood cells was determined using a specific kit from the same firm.

The duration of sleeping time as induced by xylazine + ketamine /10/ was measured as the time from loss of the righting reflex to the time the animal regained the reflex and returned to sternal recumbency.

Statistical analysis

Data were analyzed to test the difference between treatment and control groups using Student's t-test with $P<0.05$ taken as the significance level. All tests and standard errors are based on inter-assay variation.

RESULTS

The influence of monensin and SD individually on microsomal monooxygenase activity and antioxidant status differed from the action of the combination of the two drugs. Monensin administration caused a significant rise in AH activity on the seventh day after the last treatment (Fig. 1) and led to a significant lowering of the total antioxidant status with a significant rise of SOD activity (Fig. 2). SM significantly inhibited both cytochrome P-450 monooxygenases on the first day (Fig. 3), but did not affect total antioxidant status or SOD activity (Fig. 2). Administration of the monensin + sulfadimethoxine combination significantly lowered AD and AH activity on the first day after the last treatment; on the 3rd and 7th day there were no differences in enzyme activity (Fig. 4); there were no changes in the antioxidant defense indices (Fig. 2).

The treatment of broilers with the combination of monensin + sulfadimethoxine resulted in a rise in the level of SD in serum. No significant effect was seen on the duration of ketamine-xylazine anesthesia: the duration of sleeping time 1 day after the last dosage was 52.6±21.7 min (control 63.5±25.5 min), and at 7 days after the last dosage was 70.0±9.7 min (control 67.5±8.8 min). There were no significant changes in relative liver weight when monensin, sulfadimethoxine or the combination was given (Table 1).

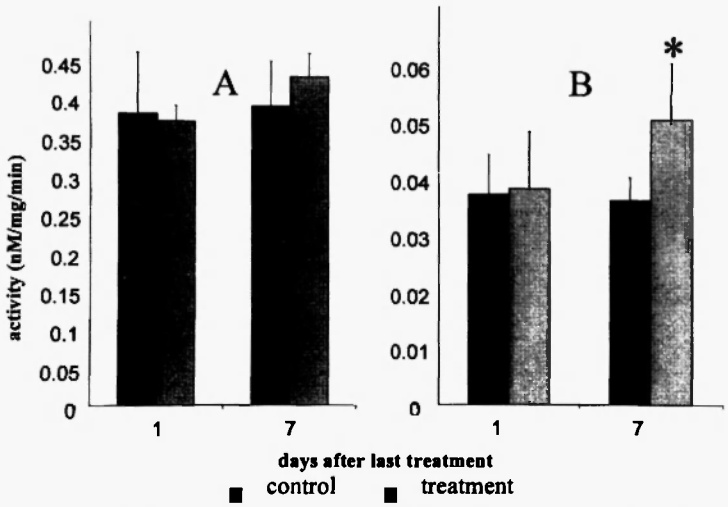

Fig. 1: Effect of monensin on (**A**) aminopyrine N-demethylase and (**B**) aniline hydroxylase activity in broiler chickens.
*Significant change ($p<0.05$). n=6 for each group.

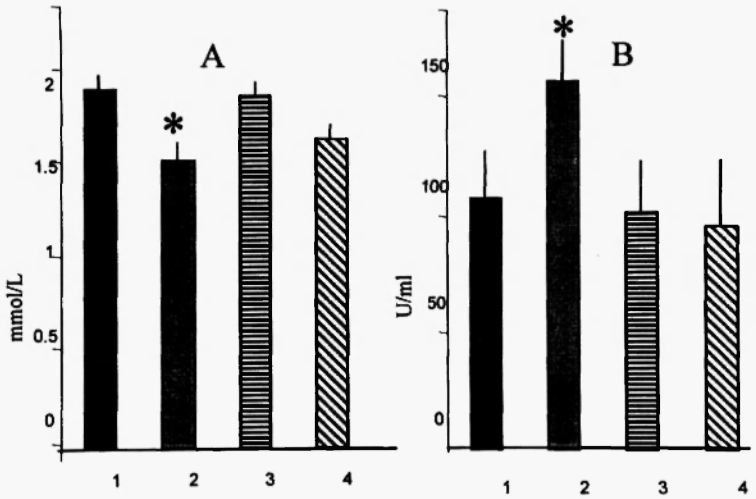

Fig. 2: Effect of monensin, sulfadimethoxine and the combination of monensin + sulfadimethoxine on (**A**) total antioxidant status in plasma and (**B**) superoxide dismutase activity in red blood cells in broiler chickens. 1 = control; 2 = monensin; 3 = sulfadimethoxine; 4 = monensin + sulfadimethoxine.
*Significant change ($p<0.05$). n=6 for each group.

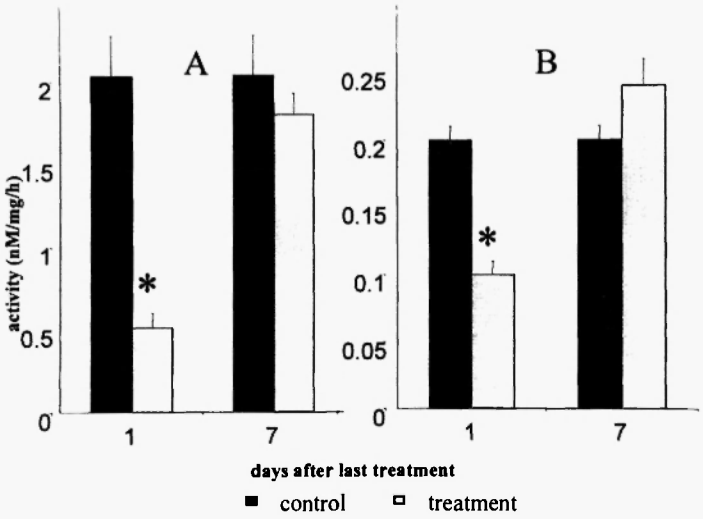

Fig. 3: Effect of sulfadimethoxine on (**A**) aminopyrine N-demethylase and (**B**) aniline hydroxylase activity in broiler chickens.
*Significant change ($p<0.05$). n=6 for each group.

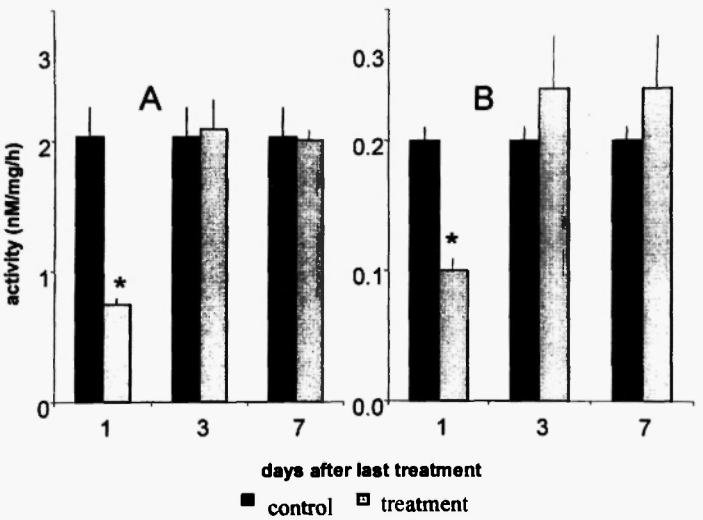

Fig. 4: Effect of monensin +sulfadimethoxine on (**A**) aminopyrine N-demethylase and (**B**) aniline hydroxylase activity in broiler chickens.
*Significant change ($p<0.05$). n=6 for each group.

TABLE 1

Effect of monensin, sulfadimethoxine and their combination on relative liver weight in broiler chickens

Days after last treatment	Monensin		Sulfadimethoxine		Monensin + Sulfadimethoxine	
	control	treatment	control	treatment	control	treatment
1	3.24±0.14	3.05±0.16	2.99±0.05	3.23±0.21	2.99±0.05	3.14±0.07
3	2.95±0.05	2.64±0.16	–	–	2.95±0.05	3.32±0.1
7	2.99±0.05	2.92±0.11	2.99±0.05	2.47±0.07	2.99±0.05	2.87±0.14

Means ± SE of relative liver weight (% body weight), n=6 for each group

DISCUSSION

Interaction and incompatibility of ionophore coccidiostats with other drugs, such as chloramphenicol /3,11/, tiamulin /12/ and various sulfonamides /13/, have been defined and quantified mainly on the level of gauging subsequent changes in apparent health, feed and water intake, and weight gain. In some investigations, biochemical, electrophysiological and morphological characteristics were examined, and changes in heart and nervous system function, degenerative changes of the muscle fibers, and disturbances in calcium ion exchange were found /14-16/.

Some investigations referred to the mechanism of action on a molecular level, with estimation of the activity of hepatic cytochrome P-450 monooxygenases /15,17,18/. The results of these studies have been inconsistent, probably due to varying experimental conditions, diverse dosages and different species of bird investigated. It was shown that monensin given for 2 wk at 110 ppm induced hepatic cytochrome P-450 (in quail) /18/, whereas at 330 ppm for 6 wk, it inhibited the same enzymes. In broilers, when a dosage of 10 mg/kg b.wt. was given for 3 days, monensin inhibited cytochrome P-450 activity, particularly that of AH /15/. The effect of monensin on cytochrome P-450 activity in broilers when used in the diet at 120 and 160 ppm for 3-4 wk was not examined /17/. Combined administration of monensin and tiamulin caused, in contrast to previous findings /19/, a marked induction of representative hepatic cytochrome P-450 /15/.

The cytochrome P-450 monooxygenases of birds show some differences in comparison with this system in mammals /20,21/. Birds have lower levels of cytochrome P-450, and total liver microsomal enzyme activity is about 80% lower than that of rats /22/. Birds are more reactive to inducers of the 3-methylcholanthrene type /23/; the classic inducer phenobarbital induces fewer cytochrome P-450 isoenzymes in birds (2 isoenzymes) than in rats (4 isoenzymes) and in rabbits (6 isoenzymes) /24/. There are also interspecies differences in hepatic cytochrome P-450 activity in avians /25/. There has been much interest in the monooxygenase system of poultry as they are the preferred model for research on delayed neurotoxicity, and this has led to the necessity for identification, purification and detailed examination of some cytochrome P-450 isoforms /26-28/. The results obtained give a better understanding of the pharmacokinetics and

specificity of biotransformation of drugs (and their combination) in hens.

It is often possible, on the basis of knowledge of the metabolism of particular drugs, to predict the character of their combined action, but there are exceptions to this. Such cases may be explained by the possibility of superoxide oxygen radical generation when xenobiotic metabolism occurs. Induction may cause drug biotransformation to be accelerated, with the formation of biotransformation products of reduced toxicity. However, there is no real lowering of toxicity because simultaneously the generation of oxygen active forms takes place giving hydrogen peroxide. Peroxide oxidation of lipids is enhanced, and the permeability of biological membranes may be partly damaged /29/. The yield of toxic products such as superoxide oxygen anion, singlet oxygen, H_2O_2 and lipid peroxides during xenobiotic biotransformation with cytochrome P-450 participation may be so high that this process may predetermine the result of combined xenobiotic action. In order to prevent and eliminate the toxic effects of free radicals, biochemical systems exist to ensure homeostasis /29/. It is known that one of the reasons for toxicosis in cases of monensin and salinomycin usage is free radical generation and lowering of antioxidant defenses /30-32/. Analogous changes have been revealed in cases of incompatibility between ionophores and tiamulin /30/. Oxidation of lipids by peroxides may be a consequence of cytochrome P-450 activity modification /33/.

The MFO activity inhibition when broilers are treated with the monensin + SM combination may lead to delay of SM metabolism and may be the reason for its accumulation in the blood. The rise of SM levels in blood possibly enhances its antibacterial action, but, on the other hand, could give a subtoxic or even toxic effect. However, when the monensin + SM combination is assessed, it should be taken into consideration that the resultant reduction of cytochrome P-450 activity is rather short term (only 1 day after the last dosage). There were no changes in indices characterizing the influence of monensin + SM on the metabolism of exogenic substances (ketamine, xylazine), or antioxidant system status. The components of the monensin + SM combination have different effects on the cytochrome P-450 and the antioxidant systems. Monensin elevates AH activity, lowers the total antioxidant status, and raises SOD activity (evidently, as compensation). SM acts as a cytochrome P-450 inhibitor, but did not affect

the antioxidant system. It is possible to compare the combination of monensin + SM with another drug combination used in broilers, monensin + sulfadimidine /34/. Broilers treated with monensin + sulfadimidine showed short-term (1 day) inhibition of cytochrome P-450, followed by a steady (up to 7 days) induction of the enzyme system, accompanied by a lowering of total antioxidant status. The negative effects of the influence of monensin + sulfadimethoxine were less manifested, and usage of this combination is therefore preferable. The rise of blood sulfadimethoxine levels, with the absence of other changes, may be considered as the factor that raises the efficacy of the antibacterial action of the combination of monensin + sulfadimethoxine.

REFERENCES

1. Anadon A, Martinez-Larranaga M, Diaz M, Bringas P. Effect of tiamulin on antipyrine kinetics in chickens. J Vet Pharmacol Ther 1989; 12: 94-98.
2. Hapke H, Abel J, Ghosch H, Tachampa S, Youssef S. Chloramphenicol-Wirkungen auf Enzymsysteme innerhalb des Azrneimittelabbaus. Zbl Vet Med 1977; 4: 701-714.
3. Broz J, Frigg M. Incompatibility between lasalocid and chloramphenicol in broiler chicks after a long-term simultaneous administration. Vet Res Comm 1987; 11: 159-172.
4. Cochlin J, Axelrod J. Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. J Pharmacol Exp Ther 1959; 125: 105-108.
5. La Du B, Mandel G, Way E, eds. Fundamentals of Drug Metabolism and Drug Disposition. Baltimore, MD: Williams and Wilkins, 1971; 569-572.
6. Lowry O, Rosenbrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-270.
7. Shlosberg A, Ershov E, Bellaiche M, Hanji V, Weisman Y, Soback S. The inhibitory effects of the fluoroquinolone antimicrobials norfloxacin and endofloxacin on hepatic microsomal cytochrome P-450 monooxygenases in broiler chickens. Drug Metab Drug Interact 1997; 14: 109-122.
8. Roos R. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides. J Assoc Anal Chem 1981; 64: 851-854.
9. Miller N, Rice-Evans C, Davis M, Copinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci 1993; 84: 407-412.
10. Roder J, Akkaya R, Amouzadeh H, Sangiah S, Burrous G, Qualls C. Effect of hepatic P-450 enzyme inhibitors and inducers on the duration of xylazine-

ketamine anaesthesia in broiler chickens and mice. Vet Hum Toxicol 1993; 35: 116-118.
11. Weisman Y, Shkap I, Egyed M, Shlosberg A. Chloramphenicol induced monensin toxicity in turkeys. Refuah Vet 1984; 41: 3-6.
12. Weisman Y, Shlosberg A, Egyed M. Acute poisoning in turkeys caused incompatibility of monensin and tiamulin. Vet Sci Com 1980; 4: 231-235.
13. Frigg M, Broz J, Weber G. Compatibility studies of ionophore anticoccidials with various antibiotics and chemotherapeutics in broiler chicks. Arch Geflügelk 1983; 47: 213-220.
14. Langston V, Galey F, Lovell R, Buck W. Toxicity and therapeutics of monensin: a review. Vet Med 1985; 80: 75-84.
15. Laczay P, Dobos-Kovacs M, Lehel J, Mora L. Some biochemical, electrophysiologic and morphologic characteristics of the monensin-tiamulin interaction in broiler chicks. Acta Vet Scand 1991; 87: 280-281.
16. Laczay P, Simon F, Lehel J. Einfluss der gleichzeitigen Applikation von Monensin und Tiamulin auf die periphere Nerventätigkeit und das Elektrokardiogramm bei Broiler. Dtsch Tierärztl Wschr 1991; 98: 306-310.
17. Brenes A, Beyer R, Cervantes H, Jensen S. Dietary and monensin effects on activity of hepatic microsomal mixed function oxidase system in chickens. Poultry Sci 1990; 69: 1285-1291.
18. Savant S, Terse P, Dalvi R. Toxicity of dietary monensin in quail. Avian Dis 1990; 34: 571-574.
19. Meingassner J, Schmook F, Czok R, Mieth H. Enhancement of the anticoccidial activity of polyether antibiotics in chickens by tiamulin. Poultry Sci 1979; 58: 308-313.
20. Short C, Flory W, Hsieh L, Aranas T, Ou S-P, Weissinger J. Comparison of hepatic drug metabolizing enzyme activities in several agricultural species. Comp Biochem Physiol 1988; 91C: 419-424.
21. Gay L, Ehrich M. A comparative study of drug metabolizing enzymes in adrenal glands and livers of rats and chickens. Int J Biochem 1990; 22: 15-18.
22. Banton M, Winston G, Flory W. Liver microsomal alkoxyphenoxazone O-dealkylases of white Leghorn chickens: response to mixed function oxidase inducers. Comp Biochem Physiol 1992; 102C: 455-458.
23. Ronis M, Walker C. Microsomal monooxygenases of birds. Rev Biochem Toxicol (Hodgson E, Bend J, Philpot R, eds). Elsevier Science Publishing Co., Inc. 1989; 10: 303-384.
24. Nebert D, Gonzales F. P-450 genes: structure, evolution and regulation. Ann Rev Biochem 1987; 56: 945-993.
25. Dalvi R, Nunn V, Juskevich J. Studies on comparative drug metabolism by hepatic cytochrome P-450 containing microsomal enzymes in quail, ducks, geese, chickens, turkeys and rats. Comp Biochem Physiol 1987; 87C: 421-424.
26. Hokama Y, Koga N, Yoshimura H. Purification and characterization of two forms of chicken liver cytochrome P-450 induced by 3,4,5,3′,4′-pentachlorobiphenyl. J Biochem 1988; 104: 355-361.

28. Gupta R, Lapadula D, Abou-Donia M. Purification and characterization of cytochrome P-450 isozymes from phenobarbital-induced adult hen liver. Comp Biochem Physiol 1990; 96C: 163-176.
29. Sevanian A, Hochstein P. Mechanism and consequences of lipid peroxidation in biological systems. Ann Rev Nutr 1985; 5: 365-390.
30. Mezes M, Surai P, Salyi G, Speake B, Gaal T, Maldjian A. Nutritional metabolic diseases of poultry and disorders of the biological antioxidant defence system. Acta Vet Hung 1997; 45: 349-360.
31. Laczay P, Simon F, Lehel J. Untersuchungen über den Einfluss von Monensin, Tiamulin bzw. der gleichzeitigen Applikation der beiden Substanzen auf die mikrosomalen mischfunktionellen Oxygenasen und auf die Peroxidbildung bei Broilern. Dtsch Tierärztl Wschr 1990; 97: 354-357.
32. Mezes M, Salyi G, Banhidi G, Szeberenyi S. Effect of acute salinomycin-tiamulin toxicity on the lipid peroxide and antioxidant status of broiler chicken. Acta Vet Hung 1992; 40: 251-257.
33. Kappus H, Sies M. Toxic drug effect associated with oxygen metabolism redox cycling and lipid peroxydation. Experientia 1981; 37: 1233-1241.
34. Ershov E, Shlosberg A, Bellaiche M, Hanji V, Weisman M, Gips M, Soback S. The effect of drugs, mediated by the hepatic microsomal cytochrome P-450 monooxygenases, on the efficacy of therapeutics in broilers. Eur J Pharm Sci 1999; 8: 87.