Long-Term Toxicity Evaluation of Sulfadimethoxine-Trimethoprim in Broilers: A Pharmacological and Toxicological Analysis

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ABSTRACT

Although potentiated sulfonamides as effective and broad-spectrum antimicrobials are frequently used in broiler farms, prescribing these agents may be associated with concerns about their toxicity especially regarding the fact that their toxic and therapeutic doses are usually close. This study evaluates toxicity of sulfadimethoxine-trimethoprim due to administration of higher doses or for longer period than what is recommended in broilers with regard to clinical signs, gross pathological examination, histopathological changes of liver and kidney as well as selected serum parameters. We found that sulfadimethoxine-trimethoprim use is relatively safe in broilers although high doses may be more damaging than long term use especially with regard to kidney function. The outcome of this study is useful in reducing the concerns related to severe toxic effects in chickens when unintentional over administration has been occurred but does not affect the need for use of recommended dosages as indicated in drug label.

Keywords: sulfadimethoxine-trimethoprim, toxicity, liver, kidney, broilers

INTRODUCTION

Sulfonamides especially those combined with diaminopyrimidine derivatives (generally known as potentiated sulfonamides) are broad-spectrum antimicrobials that are vastly used in different veterinary species including broiler chickens as chemo preventive and/or therapeutic agents. Although this group of agents is very efficient and helpful in treating microbial diseases, their use is usually associated with some concerns about detrimental side effects especially with regard to this fact that their therapeutic and toxic levels are relatively close[1]. Moreover, they are difficult to be mixed evenly in feed and have low solubility in acidic water which may lead to high intake of drug by some birds even when the dose has been accurately estimated; particularly in the case of increased feed or water consumption of birds due to different reasons (for example high water intake in hot climates or improperly ventilated broiler houses)[2]. Moreover, deliberate and/or unintentional over doses can happen and unfortunately some farmers may arbitrary tend to use these agents for longer periods than what is indicated in drug label in order to gain better therapeutic or preventive results. All these situations can lead to an increase in the possibility of toxicosis and raise the risk of deleterious effects due to sulfonamide over doses.

About half a century ago, it was clearly established that sulfadimethoxine is an effective sulfonamide against all

after oral administration in normal chickens and keeps its serum therapeutic level for relatively long time (24 hours)[4]. Although sulfadimethoxine-trimethoprim is frequently used for bacterial and protozoal diseases in poultry, there is not much evidence available about its toxicity in broilers especially when administered in higher doses or longer periods than what is indicated in drug label. This motivated us to evaluate its high dose and long term use toxicity in broilers with regard to effects on liver and kidney as major organs that are involved in its metabolism and excretion.

MATERIALS AND METHODS

Birds and Experimental Design

Seventy five 7day-old broiler chickens (Cobb 500) from both sexes were randomly allocated into 7 groups (n=10 each except for control group with n=15 in 2 and 3 replicates, respectively). Birds had free access to broiler commercial feed and tap water. The rearing conditions were the same for all birds and were chosen as suggested by Cobb 500 broiler management handbook. Bio security conditions were considered in order to minimize the risk of infectious diseases. The birds were treated as follows: control birds (group C) that received no drug; drug control birds (group DC) that received sulfadimethoxinetrimethoprim (TS® oral solution, each ml contained 200 mg sulfadimethoxine and 40 mg trimethoprim, Arshia Darou, Tabriz, Iran) at manufacturer's recommended dosage regimen (500mL/1000L in drinking water, for 5 days); T1 and T2 groups treated with sulfadimethoxinetrimethoprim at 500 mL/1000L for 10 and 15 days (2 and 3 times the recommended period, respectively); T3, T4 and T5 groups received the drug for 5 days but at the dosages of 1250mL/ 1000 L, 2500 mL/ 1000 L and 5000 mL/ 1000 L (2.5, 5 and 10 time the recommended dosage, respectively).

Birds were daily evaluated for clinical signs. At the end of the experiment (day 6 for groups DC, T3, T4 and T5 plus 5 birds from group C; day 11 for T1 plus 5 birds from group C and day 16 for T2 plus 5 birds from group C), over night fasting blood samples were collected for determination of biochemical parameters. After blood collection, all birds were sacrificed by cervical dislocation and the carcasses were evaluated for gross pathological signs. Kidney and liver samples were removed immediately for histopathological evaluation. Procedures used in the

present study are in accordance with institutional ethical guidelines for use of animals in experiments which are compatible with European convention for the protection of vertebrate animals used for experimental and other scientific purposes. *Determination of Serum Biochemical Parameters*

Collected blood samples were centrifuged for 10 min at 2000 rpm and harvested sera were preserved at -20°C until use. All biochemical parameters were determined by using commercial kits (Pars Azmun, Tehran, Iran) and spectrophotometric methods. The biochemical parameters were included activities of aspartate aminotransferase (AST), creatine kinase (CK), gamma glutamyl transferase (GGT), and concentration of uric acid, urea, albumin, total protein and potassium.

Histopathological Evaluation of Liver and Kidney At the end of the experiment, samples from liver and kidney were removed immediately and immersed in 10% buffered formalin. After fixation and routine histological laboratory methods, 5µm-thick transverse cross-sections were made and stained with haematoxylin and eosin (H&E). Kidney slides were evaluated for hyperemia and hemorrhage, interstitial nephritis, tubular necrosis, tubular distention, cast formation and glomerular changes and other possible lesions. Presence of hyperemia, hemorrhage, inflammatory cell infiltration, hepatocyte necrosis or lesions related to biliary system were evaluated in liver samples under light microscope.

Statistical procedure

Data were presented as mean±SD. One-way ANOVA method followed by Tukey's multiple comparison test was used for data comparisons (SPSS software, 11.5) with $p<0.05$ as the significant level.

RESULTS

Clinical signs and gross pathological examination All birds remained clinically normal until the end of the experiment except for birds in T5 group which were treated with 10 times the recommended dosages of sulfadimethoxine-trimethoprim for 5 days. These birds showed lethargy, depression and huddling and severe anorexia from day 3 post commencing the treatment which lasted until the end of the experiment. One death was recorded in this group which showed enlarged kidneys in gross pathology. A few other birds in group T5 also showed enlarged kidneys with urate deposition. Other birds were normal in gross pathological examination. *Serum biochemical parameters*

All assayed biochemical parameters remained statistically the same in group C in 3 sampling occasions; moreover, administration of drug with recommended dosage regimen (group DC) had no significant effect on any of the assayed parameters with respect to group C ($p>0.05$). Although administration of sulfadimethoxinetrimethoprim did not significantly increase AST level as compared with DC group; this parameter slightly increased in birds of T3, T4 and T5 groups. On the other hand activity of GGT was significantly increased in birds of T1, T2, T3 and T4 groups compared to DC group. Moreover, serum uric acid and potassium levels of birds in groups T4 and T5 were appreciably higher than DC group. Other parameters remained statistically the same among different groups. Data are summarized in table 1.

Histopathological evaluation

Except for little hyperemia and very mild hemorrhage which were observed in both liver and kidney samples of few birds in different groups no other appreciable changes were observed in samples.

DISCUSSION

Side effects and detrimental reactions to drugs pose a risk whenever a drug is administered; moreover, slips in proper dose calculation or intentional administration of drugs at higher dosages or for longer period that what is recommended, in a false effort to increase their efficiency increases the propensity for drug toxicity which is observed especially when collective treatment is used for example in broiler industry. Sulfonamides are the oldest group of antimicrobials and owing to their relatively low cost and high efficacy in some common bacterial and protozoal diseases; they still have kept their niche among the most widely used antimicrobials in veterinary medicine. The discovery of synergistic action of sulfonamides with DAP derivatives and introduction of potentiated sulfonamides has made these drugs even much more demanded[5]. However, they are notorious for a long list of adverse effects and drug interactions which could get worse by extra label use of these drugs at higher doses or for longer period than what that has been approved.

Sulfadimethoxine is mainly metabolized by hydroxylation and acetylation in chickens; hydroxylated metabolites still possess antibacterial effects while

Table 1: Serum biochemical parameters (mean±SD) in different groups.

	AST(U/L)	CK (U/L)	GGT (U/L)	Urea (mg/dL)	Uric acid (mg/dL)	Albumin (g/dL)	Total protein C(da (g/dL)	Potassium (mmol/L)
	63.0±36.0[a]	729±15	3.57±0.48	4.81±1.29[a]	6.33±0.132[a,]	2.07±0.49	3.06±0.574	4.66±1.19[a,b,] y 5) 8[a] 5[a d] 9[a a,b,c c,d]
C(da y 10)	85.6±7.08[a, c]	523±106[a]	4.16±0.999[a]	3.53±0.234[a]	5.84±1.97[a,d]	1.93±0.523[a,b]	2.92±0.592[a,b,c]	3.10±0.60[a]
C(da y 15)	130±1.37[a,b]	491±77[a]	5.78±1.15[a ,c]	4.70±0.969[a]	8.13±0.519[a,c ,d]	1.75±0.593[a,b]	3.06±0.619[a,b,c]	3.67±0.42[a,b, c]
DC	102±11.29[a ,b]	610±158[a]	6.88±1.07[a ,c]	6.97±1.25[a]	6.83±1.72[a,d]	1.60±0.295[a,b]	2.85±0.243[a,b,c]	3.85±0.67[a,b]
T1	101±26.6[a,b]	676±190[a]	13.8±2.90[b]	8.90±3.12[a]	5.73±1.25[a]	1.56±0.140[a,b]	3.06±0.699[b]	3.35±0.60[a]
T2	96.0±23.8[a]	506±101[a]	15.9±3.31[b]	8.12±3.04[a]	6.02±1.28[a]	1.32±0.182[b]	2.15±0.506[c]	3.95±0.46[a,b]
T3	135±34.5[b]	610±144[a]	13.7±2.56[b]	6.22±3.82[a]	9.28±2.02[d,g]	1.54±0.323[a,b]	2.82±0.703[a,b,c]	5.09±1.06[b]
T4	128±30.2[b,c]	721±152[a]	13.5±2.77[b]	5.49±1.53[a]	11.3±1.33[b,c, g]	1.61±0.297[a,b]	3.10±0.424[a,b]	5.25±1.36[c]
T5	126±27.6[b,c]	713±127[a]	8.87±1.87[d ,c]	5.39±3.50[a]	14.0±2.16[b]	1.60±0.264[a,b]	3.36±0.701[a,b]	5.82±1.01[d]

Different superscript letters are used to demonstrate significant difference in a column ($p<0.05$).

acetylated metabolite has no antibacterial activity with a lower solubility, which may lead to renal toxicity by precipitation in kidney; moreover, it has higher plasma protein binding than the parent drug, which can prolong the excretion phase[6]. As far as we know, individual information about the harmful effects of over administration (at higher doses and/or for longer periods than what indicated in drug label) of potentiated sulfonamides is relatively scarce. The only experimental study that has evaluated toxicity of sulfadimethoxine and a DAP derivative (2,4-diamino-5-(4,5-dimethoxy-2methylbenzyl) pyrimidine) in poultry is made by MARUSICH et al., in 1969 [3]. These authors reported that 4 times the prophylactic use level of this combination is not associated with adverse effects in broiler chickens with regard to mortality rate, gross examination and some performance parameters; moreover, continuous feeding of this dose for 20 weeks in layers does not affect subsequent egg production, fertility, hatchability and the quality of eggs produced. Although this is a relatively illustrative study, as it may be concluded, the authors have not used biochemical or histopathological approaches. Moreover effect of administration of different higher doses or longer periods in broilers has not been investigated, and the potentiator is not the routinely used trimethoprim (5-(3,4,5-Trimethoxybenzyl) pyrimidine-2,4-diamine). Our results with regard to mortality, gross examination and clinical signs of treated birds were relatively in accordance with what reported by MARUSICH et al[3]. except for birds in T5 group which were treated with 10 times the recommended dosages. In contrast to generally good health status of most birds in the present study and relatively normal features in histopathological evaluation of liver and kidney samples, some appreciable changes were observed in biochemical parameters in comparison with DC group including a rise in GGT levels of groups T1 to T4. Although reference intervals for GGT activity have not been established in birds, GGT values of 0-10 U/L are considered normal and significant increases in GGT are due to obstruction of or damage to the biliary tree[7]. We did not observe an appreciable change in AST activity levels therefore hepatocellular damage may not be present, moreover in gross and histopathological examination of birds no appreciable damages to liver parenchyma or bile ducts were observed. Therefore, the plausible liver damage in these groups may be very mild. Consistent with this, in 1988, DALVI and TRIVEDI[8] evaluated toxicity of oral sulfadimethoxine at 5 times the therapeutic dose in different species including goats, quails and rats. The authors reported no significant increases in serum sorbitol dehydrogenase (SDH), ALT and AST activities in goats and rats; however, the quails showed significantly higher activities of SDH and ALT when compared to control values (no drug administration). Moderate increases in liver cytochrome P-450 and aniline hydroxylase activity were observed in goats and quails without a change in benzphetamine N-demethylase activity in any species.

These authors suggested a lack of hepatic toxicity of sulfadimethoxine in these species under the reported experimental conditions.

In our study, uric acid and potassium were other significantly changed parameters that were increased in T4 and T5 groups as compared to DC group.

Uric acid is the major end product of nitrogen metabolism in birds and constitutes about 60 to 80 percent of total excreted nitrogen in avian urine[9]. Renal function disorders can result in hyperuricemia[10]. In a study by LIANG et al., 2015, administration of aflatoxin B1 in broiler diet was associated with renal toxicity and an appreciable increase in serum uric acid[11]. As previously stated in our study, a significant elevation in uric acid level was observed in birds of groups T4 and more profoundly T5 which can be a sign of renal toxicity especially by considering the fact that a significant hyperkalemia was also present in birds of these two groups accompanied by enlarged kidneys in some (but not all) birds in T5 group in gross pathology. It seems that this toxicity was mild since it was not associated with a rise in urea or appreciable detrimental changes in histopathological samples. It should be mentioned that hyperkalemia may also be present in severe muscular damages, since CK activity was not significantly changed in the present study, muscular damage can be ruled out. Statistically the same concentrations of albumin and total protein among different groups also potentiate the idea that kidney and liver were not severely injured.

In conclusion, it seems that long term or high dose administration of sulfadimethoxine-trimethoprim in broilers is not associated with severe toxicity although high doses may be more damaging than long term use especially with regard to kidney function. It should be emphasized that the outcome of this study does not affect the need for use of recommended dosages as indicated in drug label and only is useful in reducing the concerns related to toxic effects in chickens when unintentional over administration has been occurred.

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