STUDIES ON POSSIBLE SULFADIMETHOXINE TOXICITY TO LIVER AND LIVER DRUG METABOLIZING ENZYME SYSTEM OF GOATS, QUAIL AND RATS

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SUMMARY

No significant increases in serum SDH, ALT and AST activities were observed in goats and rats receiving oral sulfadimethoxine at 5 times the therapeutic dose. The quail showed significantly higher activities of SDH and ALT when compared to control values. Moderate increases in liver microsomal cytochrome P-450 and aniline hydroxylase activity were observed in goats and quail but no appreciable change in benzphetamine N-demethylase activity was detected in any species. These results suggest a lack of hepatic toxicity of sulfadimethoxine to these species under the reported experimental conditions.

I. INTRODUCTION

Sulfadimethoxine, N'-(2,6-dimethoxy-4-pyrimidinyl) sulfanilamide, is one of the newer sulfa drugs, which has been shown to be a low-dose, rapidly absorbed, long-acting and therapeutically highly effective sulfonamide. Unlike some of the other sulfonamides, it is well-tolerated and has low toxicity probably due to its high solubility at the pH normally occurring in the kidney /1/. Sulfadimethoxine alone or in combination with other drugs has been found to be quite effective against coccidia and pathogenic bacteria in chickens, turkeys, ducks /2/, rabbits /3/ and other food producing species such as cattle /4-5/. Although pharmacokinetic studies on this important sulfonamide have been reported in a number of food producing species such as cattle /6/, pigs /7-8/, sheep /9/, Goats /10/, chickens /11/ and turkeys /12-13/, studies concerning the effect of this drug on the liver, the organ responsible for its metabolism and change in its antibacterial/anticoccidial activity and toxicity, are lacking both in major and minor food producing species. Furthermore, some sulfonamides have been reported to cause liver toxicity in some animal species /14-15/. Also, there is a species difference in sulfonamide metabolism in the liver. For example, sulfamethazine is hydroxylated in the iiver by sheep and cattle but not by swine /16/. Because of these reasons and to investigate whether sulfadimethoxine at a level of 5 x the recommended therapeutic dose for other food producing species may cause liver toxicity in goat, quail and rat (as a monogastric animal model), the following comparative studies were carried out.

II. MATERIALS AND METHODS

A total of 12 adult, male goats (mixed breed) were equally divided into two groups and used as control and treatment groups. The body weights of these animals ranged from approximately 25 to 45 kg. They were given hay and commercial feed, and water ad libitum. Sulfadimethoxine as sodium salt (Hoffmann-LaRoche, Nutley, NJ) at a dose of 137.5 mg/kg was dissolved in water (1 ml/kg) and administered by stomach tube daily for 15 days. The control group received tap water alone. Bobwhite adult, male quail were divided into two groups of 10 each and maintained on a commercial nonmedicated feed with access to water at all time. Sulfadimethoxine (0.25% concentration) was given in their drinking water for 15 days. Rats (10 per group) were also given sulfadimethoxine in their drinking water at 25% concentration for 15 days. The control quail and rats received tap water alone. At the end of the experimental period, blood samples were collected for the determination of the activity of sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Half of the animals from each group were stunned and exsanguinated to collect livers for isolation of liver microsomes to be used for the determination of cytochrome P-450, and the activity of benzphetamine N-demethylase and aniline hydroxylase.

The serum enzymes activity was determined using the assay kits purchased from Sigma Chemical Co. (St. Louis, MO). Cytochrome P-450 content of microsomes was determined by the method of Omura and Sato /17/ whereas benzphetamine N-demethylase and aniline hydroxylase activities were measured following the procedures published earlier /18/. Microsomal protein concentration was determined by the Biuret method.

The data were analyzed using Student's t-test. Significance of treatment mean differences was based on a P value of 0.05.

III. RESULTS

The results of the activity determination of serum SDH, ALT and AST in control and sufadimethoxine-treated animals (Table 1) show that there was no significant difference in the activities of these serum enzymes between the control and treated goats suggesting

TABLE 1

Effect of Sulfadimethoxine on the Serum Enzymes Profile of Goats, Quail and Rats

Species		SDH[1]	ALT[2]	AST[3]
Goat				
	Control	1306 ± 56	19 ± 2	104 ± 10
	Treated	1335 ± 94	18 ± 1	103 ± 3
Quail				
	Control	66 ± 2	57 ± 2	179 ± 14
	Treated	89 ± 4*	93 ± 7*	174 ± 8
Rat				
	Control	438 ± 12	59 ± 3	227 ± 1
	Treated	456 ± 14	67 ± 1	228 ± 1

Results are expressed as the means ± S.E.M.

1 Expressed as Sigma units/ml

2 Expressed as Karmen units/ml

* Significantly different from the corresponding control ($P < 0.05$)

sulfadimethoxine did not produce liver toxicity in goats. A similar trend is seen for rats. However, in quail serum SDH and ALT were significantly higher than their corresponding control values.

Since in toxicity study organ weights are used as one of the parameters to assess organ damage, body and liver weights of the control and treated animals were measured and expressed as relative liver weights. It is evident from the values of the relative liver weights (Table 2) that the sulfadimethoxine treatment did not produce appreciable change in the relative liver weights of any of the three species. Similarly, there was no effect of sulfadimethoxine on the liver microsomal protein content suggesting the drug did not inhibit microsomal protein synthesis in the liver. This is further supported by the fact that no loss of hepatic microsomal cytochrome P-450 was caused by sulfadimethoxine (Table 2). In fact, there was moderate but nonsignificant increase in the concentration of the haemoprotein from goats and quail while it was unchanged in rats. A

similar trend can be seen for other components of the enzyme system (Table 3). It can be seen from the results that there was no significant increase in the activity of benzphetamine N-demethylase caused by the drug in goats and quail. However, the increase in the activity of aniline hydroxylase in goats and quail treated with sulfadimethoxine is significantly higher than their corresponding controls.

TABLE 2

Species Difference in Relative Liver Weights
and Microsomal Protein and Cytochrome P-450 Concentration

Species		Relative Liver Weight (% b. wt)	Microsomal Protein (mg/g liver)	Cyt. P-450 (nmol/mg protein)
Goat				
	Control	1.55 ± 0.077	12.85 ± 0.485	0.425 ± 0.03
	Treated	1.47 ± 0.194	13.07 ± 1.440	0.510 ± 0.06
Quail				
	Control	2.80 ± 0.441	15.94 ± 0.080	0.165 ± 0.01
	Treated	2.38 ± 0.091	17.74 ± 0.660	0.236 ± 0.01
Rat				
	Control	4.89 ± 0.042	18.58 ± 1.655	0.433 ± 0.01
	Treated	4.11 ± 0.189	18.96 ± 0.910	0.446 ± 0.04

Results are expressed as the means ± S.E.M.

IV. DISCUSSION

The changes in the activity of serum SDH, ALT and AST have been widely used to assess the liver damage produced by chemicals, pathogens and parasites. From the results of the present study on the serum enzymes profile, it is evident that several days exposure of goats and rats to 5 x the recommended therapeutic dose of

TABLE 3

Effect of Sulfadimethoxine Treatment on the Activity of Benzphetamine N-Demethylase and Aniline Hydroxylase in Goats, Quail and Rats

Species		Benzphetamine N-demethylase[1]	Aniline hydroxylase[2]
Goat			
	Control	2.200 ± 0.219	0.244 ± 0.022
	Treated	2.378 ± 0.084	0.371 ± 0.045*
Quail			
	Control	1.447 ± 0.166	0.255 ± 0.038
	Treated	1.689 ± 0.144	0.556 ± 0.055
Rat			
	Control	2.643 ± 0.321	0.316 ± 0.007
	Treated	2.963 ± 1.333	0.380 ± 0.010

Results are expressed as the means ± S.E.M.

1 Expressed as nmoles of formaldehyde formed/min/mg protein

2 Expressed as nmoles of p-aminophenol formed/min/mg protein

* Significantly different from the corresponding control ($P < 0.05$)

sulfadimethoxine did not produce liver damage. Singh et al. /19/ administered sulfanilamide and sulfamezathine (100 mg/kg/day for 21 days) to buffaloes and found no change in serum GOT and GPT suggesting an absence of liver damage. The significant rise in the SDH and ALT levels in the treated quail may be indicative of sulfadimethoxine being relatively toxic to quail under the experimental conditions. Sulfonamides have been reported to cause haemorrhage in poultry in various tissues, notably the liver /20/, which may partially account for the observed higher activities of SDH and ALT in the treated quail. On the other hand, the modest but significant increase in the activity of serum SDH and ALT seen in the quail may be transient and may not be taken truly as an indicator of liver toxicity since other parameters of liver toxicity do not confirm this observation.

The data on relative liver weights and microsomal protein content are suggestive of sulfadimethoxine being not a hepatotoxic agent in

goats, quail and rats. Furthermore, results on the effect of sulfadimethoxine treatment on liver microsomal enzymes are particularly significant in that the drug does not inhibit the microsomal enzyme system. It has been suggested by Urbanek-Karlowski /21/ that inhibition of liver microsomal enzymes by chemicals can be taken as a more useful indicator of liver toxicity than elevation of serum enzymes. If this is true then sulfadimethoxine may be considered as nontoxic to liver. van Gogh and van Miert /10/ have also reported that sulfadimethoxine did not appear to have an inhibitory effect on the hepatic drug metabolizing enzymes in goats. The induction of microsomal aniline hydroxylase activity in goats and quail but not in rats may be construed as a species difference to drug metabolism /16/. Thus, sulfadimethoxine, a drug which is much less toxic than other sulfonamides, appears to produce no liver toxicity in goats, quail and rats at a dosage level which is five times higher than the recommended therapeutic dose for other food producing species.

V. ACKNOWLEDGEMENT

This work was supported by a grant No. FD-U-000064-03 from Food and Drug Administration.

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