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The Effect of Haemonchus Contortus Infection and Treatment with Ivermectin on Drug-Metabolizing Enzymes

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Abstract: This study was aimed at elucidating the interplay between the parasiticide ivermectin and haemonchiasis on the hepatic activities of some drug-metabolizing enzymes in Nubian goats. The in vitro experiments were confirmed *in vivo* by estimating the effect of ivermectin on hexobarbitone-induced sleeping time in rats. The in vivo experiments showed that there was no substantial influence of ivermectin on Hexobarbital Sleeping Time (HST) in rats, whether administered at its recommended therapeutic dose (200 ug kg^{-1} body weight) or two times the recommended dose. But there was significant decrease in HST 24 h post administration of the double dose, although all other values of HST from day 2 to day 15 were within the normal range. In in vitro experiments, the enzymes studied in goat liver were hexobarbitone oxidase, p-nitroreductase and UDP-glucuronyl transferase. Ivermectin given at a dose rate of 200 ug kg^{-1} body weight did not produce alterations in the activities of drug-metabolizing enzymes investigated, nor did it affect microsomal protein contents or liver weight. The activities of hexobarbitone oxidase, P-nitroreductase and UDP-glucuronyl transferase were significantly decreased three weeks post infection, also there was significant decrease in microsomal protein contents.Ivermectin when given at a dose rate of 200 μg kg^{-1} body weight subcutaneously resulted in a significant increase in the activities of hexobarbitone oxidase, P-nitroreductase and UDP-glyucuronyl transferase in infected animals given the drug three weeks post infection and killed one week later.

Key words: Parasiticide ivermectin, HST, hexobarbitone oxidase, glyucuronyl

INTRODUCTION

The main organ concerned with drug metabolism is the liver and many drugs are substrates for microsomal enzymes systems of hepatocytes. The kidneys, lungs, intestinal mucosa, plasma and nervous tissues also contain important drug-metabolizing enzymes (Williams, 1972).

Ivermectin is a derivative of a naturally occurring fermentation product, avermectin $B_{1,}$ which is one of a series of avermectins produced by the actinomycete, *Streptomyces avermitilis*. It is a highly potent drug and shows activity against a wide range of animal parasites, including internal and external parasites (Campbel and Benz, 1984; Barragy, 1987; Entrocasso *et al*., 1996). Ivermectin having these qualities was used in this study to monitor its effect on some drug-metabolizing enzymes in Nubian goats.

Haemonchiasis is one of the important parasitic diseases in farm animals of the Sudan. The disease is caused by *H. contortus* which dwells in the abomasum of sheep, goats and to a lesser extent cattle. Haemonchiasis is associated with clinical and pathological manifestations, comprising haemorrhagic anaemia, hypoproteinaemia and parasitic gastroenteritis (Ahmed and Ansari, 1989; Albers *et al*., 1990).

The decrease in sleeping time response to hexobarbital has been used as an indicator of increased hepatic microsomal metabolism activity (Lang *et al*., 1992; Mitoma, *et al*., 1967). It was stated that Hexobarbital Sleeping Time (HST) as a prescreening method before starting investigation in detail is of great importance (Lang *et al*., 1992).

MATERIALS AND METHODS

In vivo experiments

The effect of ivermectin on hexobarbitone-induced sleeping time in rats

Animals: Sexually mature male rats weighing 200-240gm were used in these experiments. The rats were kept in groups of ten in standard cages and were allowed food and water ad libitum.

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Administration of ivermectin: The rats were divided into three groups (A, B and C) of ten animals each. Group A rats were kept as control unchallenged with the drug. Rats of group B and C received ivermectin subcutaneously at a dose rates of 200 and 400 μg kg^{-1} body weight, respectively. This dose was given once before the beginning of the experimental work. The sleeping time was measured at intervals of 1, 2, 3, 5, 7, 10 and 15 days post administration of ivermectin.

Measurement of hexobarbitone-induced sleeping time: These experiments were carried out in a noise-free, normally-lit room. Hexobarbitone sodium in distilled water (150 mg mL^{-1}) was given as a single intraperitoneal injection at a dose of 150 mg kg^{-1} body weight. The period of time between the loss and regain of the righting reflex was taken as the hexobarbitone-induced sleeping time measured in minutes.

In vitro **experiment**

The effect of ivermectin and haemonchosis on the hepatic activities of drug-metabolizing enzymes in Nubian Goats

Animals: Twenty four male Nubian goats aged 1-2 years and weighing 10-20 kg were used. They were clinically healthy. Feed and water were available at ad libitum basis.

The goats were divided into four groups I. II, III and IV of six animals each. Goats in group I were kept as control neither challenged with the drug nor infected with the parasite. Group II goats were challenged with ivermectin subcutaneously at a dose of 200 μg kg^{-1} body weight and were killed 24 h following administration of the drug. Group III and IV goats were dosed orally with infective larvae of *Haemonchus contortus* of sheep origin at 500L3 kg^{-1} body weight. Goats in group III were killed 3 weeks post infection. Goats in group IV were treated with ivermectin subcutaneously at a dose of 200 μg kg^{-1} body weight 3 weeks post infection and were killed one week later.

Preparation of the Hepatic sub-cellualr fraction: The goats were killed by decapitation using a sharp knife, abdomen was opened and once liver had been removed, all steps of preparation were carried out on ice. The hepatic sub-cellular fractions was prepared as described by Mazel (1971). Livers were quickly removed washed with ice-cold 0.25M sucrose, blotted dry with filter paper and weighed. Liver weight was calculated as gm kg^{-1} body weight. Pieces of 5-7 grams of liver were removed, wrapped in aluminum foil and dipped in liquid nitrogen pending analysis within one week. It has been reported that the drug metabolizing enzymes may be stable for up to five months when tissues are frozen in liquid nitrogen (Wisniewski *et al*., 1987). The liver samples were thawed at 4°C. Pre-weighed specimens were minced with scissors and homogenized with 2 volumes of ice-cold sucrose in a potter S-homogenizer (Potter, B-Braum, Germany) for 1 min using 6-8 strokes.

The homogenate was centrifuged at 10,000xg for 20 min at 4°C in a refrigerated ultracentrifuge (Beckman model L7 U.S.A.) and the resulting supernatant was carefully decanted into plastic tubes immersed in ice. Aportion of this supernatant was used for the estimation of hexobarbitone oxidase, P-nitroreductase and supernatant protein. The remainder of the supernatant fraction was centrifuged at 105,000×g for 60 min at 4°C and the resulting microsomal fraction (having concentration equivalent to 500 mg fresh liver mL^{-1}) was used for determinations of UDP-glucuronyl transferase and microsomal protein.

Enzyme assays

Hexobarbitone oxidase activity: Hexobarbital was determined by the method of Cooper and Brodie (1955) with heptane as the extractant and using the wave-length 245 mμ for the final assay.

P-nitroreductase activity: The method involves the determination of the amount of P-aminobenzoic acid formed under standard conditions from the substrate P-nitrobenzoic acid (Fouts and Brodie, 1957). The amount of P-aminobenzoic acid formed was estimated spectrophotometrically using standard procedure of Bratton and Marshal (1939).

UDP-glucuronyl transferase: This enzyme catalyses glucuronide biosynthesis by the transfere of glucuronic acid from UDP-glucuronic acid to compounds containing reactive groups such as phenols, alcohol, carboxylic acids and amines. In this assay the amount of the substrate, P-nitrophenol, remaining at the end of the reaction is estimated (Pogell and Krisman, 1960 as modified by Hollman and Touster, 1962).

Protein determination: Protein estimations of the microsomal and 10,000xg supernatant fractions were carried out by the method of Lowry, *et al*. (1951), as modified by Miller (1959), using a Unicam 8625 uv/vis spectrophotometer and bovine serum albumin as a standard.

Parasitolgoical methods: During routine post-mortem examination of sheep at Omdurman slaughter house, the abomasal contents were poured into a large container and

preparation of larval faecal culture was done according to Anon (1977). The standard Baerman technique (Anon, 1977) was used to recover and to identify *H. contortus* larvae from the faecal culture.

Histopathological examination: At necropsy, small pieces of the liver and abomasum were fixed in 10% formal saline and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin (H and E). Show in Fig. 1.

Statistical analysis: The mean and Standard Deviation (SD) were obtained for all data within groups. Analysis of variance for sleeping time, liver weight, protein and enzyme assays were done using Complete Randomized Design (CRD) and significance was set at $p<0.05$.

RESULTS

Hexobarbitone-induced sleeping time HST in minutes: The results of this experiment are shown in Table 1. Group B rats showed no significant changes ($p<0.05$) in HST from day 1 to day 15 in comparison with the control group (group A) (39.74±6.23 mins). However within group B HST showed significant decrease in day 1, day 2 and day 3 compared with day 10 and day 15. Group C rats showed no significant changes in HST from day 2 to day 15 in comparison with the control group, but in day 1 there was significant decrease in HST in comparison with the control group, also within group C rats there was significant increase in HST in day 15 compared with day 1.

Drug-metabolizing enzymes and protein concentration: The results of drug-metabolizing enzymes, protein concentration and liver weight are shown in Table 2 and 3. Ivermectin given at the dose rate of 200 μg kg^{-1} body weight (group II goats) did not result in alterations in the activities of the drug-metabolizing enzymes investigated, also it did not affect microsomal protein contents and liver weight.

In this study all infected goats of group III developed a classical picture of haemonchiasis. This was assessed by the clinical signs of the disease and detection of the parasite eggs in the faeces. In group III goats, the activities of hexobarbitone oxidase, P-nitroreductase and UDP-glucuronyl transferase were significantly decreased three weeks post infection. A significant decrease in microsomal protein contents was also found in group III goats, but neither supernatant protein nor liver weight were affected.

Table 1: Summary of analysis of variance of hexobarbitone induced sleeping time of rats. Group A vs. group B and group A vs. group C.

	Group A vs. group B	Group A vs. Group C
Control	39.74± 6.23^{abc}	39.74± 6.23^{a}
Day 1	36.33 ±3.40^{c}	33.94± 6.07^{b}
Day 2	36.65 ±2.97^{c}	34.48± 5.99^{ab}
Day 3	36.77± 2.78^{c}	35.28± 6.53^{ab}
Day 5	37.2± 5.07^{bc}	36.24±5.68^{ab}
Day 7	39.39 ±4.69^{abc}	38.48±5.59^{ab}
Day 10	41.25± 5.50^{ab}	39.07±7.39^{ab}
Day 15	42.21 ±6.58^{a}	40.06±7.61^{a}
F-value	20.196	1.411
C.V%	12.53	17.43
LSD	4.323	5.763

-Values in the table, are mean ± SD. -Mean values having different superscript letters in each column differ significantly ($p<0.05$). -Ivermectin was given as a single subcutaneous dose at the dose rates of 200 and 400 μg kg^{-1} body wt. in groups B and C, respectively. -Animals in group A were kept as control unchallenged with the drug. -The sleeping time (in minutes) was measured at intervals of 1, 2, 3, 5, 7, 10 and 15 days post administration of ivermectin

Fig. 1: Abomasum of goat experimentally infected with *H. contortus* and was killed three weeks post infection . H and E x 10. (A) complete denudation of epithelial lining cells and villi. Glands elongate and affected area of mucosa is thickened. (B) Erosion of epithelial lining and cells. Mucous metaplasia and hyperplasia, thickening of the mucosa with moderate infiltration of lymphocytic cells in lamina propria between glands

Table 2: Effect of ivermectin and haemonchiasis on enzyme activities, protein contents and liver weight in Nubian goats

	Group I control	Group II	Group III	Group IV
Hexobarbitone Oxidase (HBO)	3.278±0.252	3.355±0.172	2.375±0.485	3.182±0.307
P-nitro-Reductase (PNR)	2.30±0.084	2.427±0.263	1.685±0.058	2.182±0.126
UDP-Glucuronyl transferase	1.030±0.111	1.062±0.118	0.698±0.032	0.998±0.076
Supernatant protein	111.169±5.828	113.192±7.981	93.667±5.212	105.25±5.261
Microsomal protein	25.767±1.591	27.633±1.892	20.883±2.153	24.000±1.7297
Liver weight	12.09±0.515	12.307±0.499	11.623±0.569	11.827±0.732

- Values in the table are means ± SD. Ivermectin was given as a single subcutaneous dose; 200 μg kg^{-1} body wt. in group II and IV. Each animal in group III and IV was dosed orally with infective larvae of *H. contortus* at 500 L3 kg^{-1} body weight. Animal representing group I were kept as control unchallenged with the drug. Enzymes activities are expressed as μmole gm^{-1} of fresh liver/hour. Protein concentration is expressed as mg/gm of fresh liver, Liver weight is expressed as gm/kg body wt.

Table 3: Summary of analysis of variance for enzyme activities, protein contents and liver weight

	Hexobarbitone oxidase	p-nitro-reductase	UDP-Glucuronyl transferase	Supernatant protein	Microsomal protein	Liver weight
Group I Control	3.278 ±0.25^{a}	2.300± 0.08^{ab}	1.030±0.11^{a}	111.169 ±5.83^{bc}	25.767± 1.59^{ab}	12.09 ±0.51^{a}
Group II Challenged with ivermectin	3.355 ±0.17^{a}	2.427±0.26^{a}	1.062±0.12^{a}	113.192± 7.98^{a}	27.633 ±1.89^{a}	12.307 ±0.50^{a}
Group III (infected unchallenged)	2.375± 0.49^{b}	1.685±0.06^{c}	0.698± 0.03^{b}	93.667 ±5.21^{c}	20.883 ±2.15^{c}	11.623 ±0.57^{a}
Group IV (infected and treated)	3.182± 0.31^{a}	2.182±0.13^{b}	0.998± 0.08^{a}	105.25 ±5.26^{b}	24.000± 1.73^{b}	11.827 ±0.73^{a}
F-value	11.689*	26.534*	20.505*	12.116*	14.401*	1.563^{ns}
C.V%	10.67	7.19	9.59	5.84	7.54	4.90
LSD	0.3921	0.1866	0.1077	7.436	2.232	0.7053

- Values in the table are means ±SD, - *Mean values having different superscript letters in each column differ significantly ($p<0.05$), - N.S. Not significant
- Complete Randomized Design (CRD) was used for analysis of variance

Fig. 2: Liver micrograph of goat experimentally infected with *H. contortus* and was killed three weeks post-infection. Note degeneration of some hepatic cells, in addition to presence of haemosiderosis. H and E×20

Group IV goats that were infected with the parasite and treated with ivermectin showed significant increase in the activities of phase I and phase II drug-metabolizing enzymes hexobarbitone oxidase, P-nitroreductase and UDP-glucuronyl transferase when compared with group III goats (infected untreated). Also there were significant increase in microsomal and supernatant protein contents of group IV goats when compared with group III, but there was no change in liver weight. Show in Fig. 2.

DISCUSSION

In the first experiment the influence of ivermectin increased with the dose only 24 h post administration, although all other values of HST from day 2 to day 15 were within the normal range (Table 1). These results show that there was no substantial influence of ivermectin on HST in this experiment whether administered at its recommended therapeutic dose (200 μg kg^{-1} body weight) or two times the recommended dose. The variations in HST within group B and within group C may be linked with the pharmacokinetics of the drug which showed slow absorption rate as the peak plasma concentration usually achieved in 2.7-3 days (Fink and Porras; 1989; Mariner *et al*., 1987).

In the second experiment, the present results with ivermectin in group 11 goats (Table 3) and the results in group B rats (Table 1) are in general agreement with those of Chiu and his collaborators (1987) who found that the *in vivo* metabolism of ivermectin is closely correlated with its *in vitro* liver microsomal metabolism. Relative to this work, Mattei and Rodrigues (1994) found that a 72 h time course following ivermectin exposure, the activities of glucose-6-phosphate dehydrogenase and glucose –6-phosphatase remained essentially unchanged.

Wallace *et al*. (1996); Dukkak (1988); Ahmed *et al*. (1990); Abott *et al*. (1985) found that infection with *H. contortus* influenced a number of erythrokinetic, metabolic and biochemical parameters in parasitized host. In the present study the decrease in enzymes activity in group III may be attributed to the biochemical and metabolic effects of the parasite on the host. Relative to this work Tekwani *et al*. (1988); Van Miert (1990) stated that generally infection and parasitic diseases exert their effects on the host's mixed function oxidase MFO system by the alteration of the microsomal drug metabolizing enzymes and electron transport carriers

such as cytochrome P450. It is universally accepted that all enzymes are proteins. Ahmed and Ansari (1989); Albers *et al*. (1990); Hunter and Mackenzie (1982) stated that the main effect of *H. contortus* infection in small ruminant is anaemia and hypoproteinaemia. Blood and Radostitis (1989) found that the presence of *H. contortus* in the abomasum seems to interfere with the digestibility and absorption of protein, calcium and phosphorus. Ahmed and Ansari (1989) recorded a decrease in RBC count Hb concentration and PCV values in sheep and goats naturally or experimentally infected with *H. contortus*. Tekwani *et al.* (1990) reported that experimental infection of hamster with the hook worm, *Ancylostoma ceylanicum*, caused impairment in hepatic mixed function oxidase MFO activities, which was confirmed by prolongation in HST.

Ivermectin treatment caused paralysis and death of the parasite by stimulating the release of the neurotransmitter Gamma- Aminobutyric Acid (GABA) (Turner and Shaeffer, 1989). The removal of the parasite by ivermectin chemotherapy would prevent the biochemical, metabolic and erythrokinetic disturbances that might have possibly affected the microsomal enzyme activities and protein contents. Also it is possible that the drug stimulates the immune systems as indicated by the increase of white blood cells as reported by Baraka (1994) and Entrocasso *et al*. (1996). Relative to this research, Zaha *et al*. (2000) found that a high incidence of liver dysfunction was observed with benzimidazole compound. However, ivermectin showed a strong anthelmintic effect with negligible liver dysfunction.

The effect of ivermectin and haemonchiasis on drug-metabolizing enzymes made use conclude that attention should be exercised when treating animals infected with haemonchiasis because the hepatic drug-metabolizing capacity is impaired and accordingly the choice of the drug and dosage should be given due consideration. Choice of dosage is important because Sudanese herders tend to increase the dose, particularly when the drug dose volume is small, such as in the case of ivermectin (0.2 mg kg^{-1} body weight), thinking that a larger dose might get rid of the parasite more efficiently.

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