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EFFECT OF *ONCHOCERCA GUTTUROSA* ON MINERAL CONCENTRATION IN THE KIDNEY AND PLASMA OF MICE

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ABSTRACT

The effect of Onchocerca gutturosa on concentrations of some minerals in the plasma and kidney of mice was investigated. Thirty mice weighing 15-25g were divided into six groups. Groups 1, 2, and 5 were infected with O. gutturosa microfilaria (2000-3000mf); groups 2 and 3 were administered 150µg/kg body weight of ivermectin, 2days post infection. Groups 5 and 6 were administered 0.1% tween 80 (placebo) and served as control (uninfected and untreated). The concentrations of zinc (Fe), copper (Cu), iron (Fe) and manganese (Mn) were determined in the plasma and kidney on days 1 and 14 post-treatment.

On day 1, the uninfected and treated animals (group3) had higher plasma zinc and copper concentrations (103.29±0.33 and 253.00±0.97µg/L respectively) compared to the uninfected and treated group (Group 4) (101.65±0.66 and 241.92±1.93µg/L respectively). The concentration of Mn and Fe were however lower in group 3 compared to group 4. The infected and untreated group (group 1) had lower Zn, Cu, and Fe concentration (37.05±0.33µg/L and 91.32±0.23µg/L respectively) compared to their uninfected counterparts (group 4). Similar patterns of change were observed in the kidney. The study indicated that in the uninfected animals, ivermectin caused a reduction in plasma and kidney concentrations of Mn and Fe. In infected animals however, only Mn was reduced. Infection with *O. gutturosa* on the other hand caused a reduction in both plasma and kidney concentrations of Zn, Cu and Fe. The concentration of Mn was increased.

Key Words: Onchocerciasis, Onchocerca gutturosa, Micronutrients, Animal model, Plasma minerals, Kidney minerals

INTRODUCTION

Onchocerciasis is one of the world's most distressing disease of helminth origin, often resulting in blindness. About 19million people are reportedly infected and about 1million being blind. In humans, the disease is caused by *Onchocerca volvulus* and is transmitted by the black fly, *Simullium damnosum.* Occasionally, other species of Onchocerca from domestic animals have been recorded in man (Nelson, 1991). Of the thirty known species of Onchocerca, only four have been used in the laboratory- *O. gutturosa, O. lienalis, O. gibsoni* and *O.cervicalis.* In an attempt to maintain microfilaria (mf) in the laboratory, Nelson et al (1996) used rodents as proxy host for the study of *O. gutturosa* mf. Rabalais (1974) reported the distribution of *O. cervicalis* mf in jirds. Aoki et al (1980), also infected mice with *O. volvulus* mf. These suggested that rodents could be used as proxy hosts for Onchocerca.

Onchocerca gutturosa (wander worm) is a parasite of cattle found free in connective tissues including that which attaches the spleen to the lumen. The worms are threadlike with males having lengths of 2.9-3.9cm, and females 60cm or more (Mellor, 1973). The adult parasite causes nodular lesions in the ligamentum nuchae, especially at its insertion into the spinous process of the thoracic vertebrae. The mf causes dermatitis in cattle with lesions in the head, withers, neck and shoulders, thickening of the skin and loss of hair (Mellor, 1973).

Prescription drugs may interfere with nutrient absorption, metabolism, utilization or excretion. Similarly, nutritional status and diet can affect the action of drugs by altering their metabolism and function. Drugs may alter nutritional

status which in turn can result in anorexia and weight loss (Reid, 1985). High doses of laxatives decreases gastrointestinal transit time and reduce the absorption of glucose, calcium, protein, sodium and potassium (Frier and Scott, 1977). The anti inflammatory agent, colchicine, used to treat gout may also alter intestinal transport mechanism leading to potassium, sodium, lipid and nitrogen fecal loses (Race, 1970). Circulating zinc and release of zinc from tissues have also been reported to be reduced among users of oral contraceptives, but there is no evidence that such changes alter the dietary requirement for this mineral (King, 1987). Most drug-nutrient interactions would rarely manifest clinically; they can only be demonstrated in the laboratory as most drugs with recognized, frequently serious adverse effect would have been abandoned before marketing (Anon, 1986).

At present, there is no information on the effect of onchocerciasis on micronutrient status. Preliminary studies (unpublished) showed that ivermectin increased plasma zinc, copper, calcium and potassium concentrations, but reduced magnesium concentration in mice. This study was therefore intended to investigate the effect of *O. gutturosa* infection and ivermectin administration on the concentration of some minerals in the plasma and kidney of mice.

MATERIALS AND METHODS

Animals

Albino mice (16-20g) were obtained from the Animal Centre of the Nigerian Institute of Medical Research, Yaba. The animals were allowed to acclimatize after which they were divided into six groups labeled as follows:Group1- Infected, not treated; Group2- infected, treated; Group3- not infected, treated; Group4- not infected, not treated; Group5- infected, placebo; Group6- not infected, placebo. All the animals were kept in Perspex cages and allowed free access to food and water.

Parasite Extraction and Infection

Onchocerca gutturosa mf was obtained from the ligament of naturally infected and freshly slaughtered cattle at the Lagos State Abattoir, Agege. Adult worms were extracted from the ligaments with a needle into Petri dishes containing physiological saline and kept for 30min to allow the mf emerge. The solution was centrifuged at 1000rpm for 5 min and the supernatant was discarded. The residue was washed with saline and centrifuged thrice. Microfilaria load was determined by microscopic examination and 2000-5000mf was syringe- inoculated subcutaneously, under aseptic condition, into the scalp of the animals in the "infected" groups.

Drug Preparation and Administration

Ivermectin was obtained as a commercial preparation, Mectizan (MSD, France), from Franca Olamiju, MITOSATH, Jos. Tween 80 was a gift from Dr Bola Oyefolu, Lagos State University, Ojo. A tablet of Mectizan, containing 3mg of ivermectin, was dissolved in 20ml of 0.1% tween 80. This served as the stock solution. Two days after infection, Animals in the "treated" groups were administered 150µg/kg body weight of ivermectin orally. The "placebo" groups were administered 0.1% tween 80 (Tagboto and Townson, 1996)

Sample Collection

Animals were anaesthesized with chloroform and blood was collected by cardiac puncture and transferred immediately into heparinized bottles. The samples were centrifuged at 1000rpm for 5min and the plasma was collected into plain bottles for mineral assay. Kidneys were excised from the animals and placed on filter paper to drain the blood, after which they were kept in labeled plastic containers at -16°C until analyzed. Two animals from each group were sacrificed on days 1 and 14 post treatment.

Mineral Determination

Five hundred microgram of kidney was weighed into platinum crucible and wet-ashed with 2ml of HNO_3-$HClO_4$ (4:1 v/v) mixture at 80°C for 2hr and later at 130°C under pressure for 1hr. A 500µl of plasma and the residue from the wet- ashed kidney samples were each added to 5M HCl and digested for 30min. The samples were filtered through a Whatman No 44 filter paper, washed with HCl solution and made up to 100mml with all glass distilled water. The concentrations of zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) were determined with a Phillip PU3100X atomic absorption spectrophotometer.

RESULTS

On day 1(Table 1), animals in group3 (not infected, treated) had the highest Zn, Cu and Fe concentration in the kidney (42.96±0. 66, 77.11±0.97 and 251.45±2.15µg/kg, respectively) and group 1 (infected, not treated) had the least concentration for all the minerals (4.76±0.17, 24.10±0.49, 74.65±1.08 and 1.65±0.09µg/kg respectively). Group 2 (infected, treated) had the highest Mn concentration (5.92±0.18µg/kg). On day 14 (Table 2), a similar pattern occurred. Plasma zinc and copper concentrations were also highest in the group 3 mice on day 1 (Table 3) and Mn and Fe were highest in groups 1 and 4 respectively. Group 1 had the least Zn, Cu and Fe concentrations while group 2 had the least concentration of Mn. On day 14 (Table 4), plasma Zn and Cu were also highest in group 3, Fe was highest in group 4 (12.87±0.18µg/L and Mn in group 5 (567.15±0.75µg/L). Zinc and Cu and Fe were the least in group 521.48±0.82, 49.88±0.73, and 153.95±1.05µg/L respectively), and Mn in group 4 (2.44±0.18µg/L).

Table1. Mineral Concentration in Kidney of Mice (Day1)*

Animal Group	Mineral concentration (µ g/kg) Zinc	Copper	Manganese	Iron
1	4.76±0.17	24.10±0.49	1.65±0.09	74.65±1.08
2	19.68±0.66	48.43±0.72	5.92±0.18	150.00±1.42
3	42.96±0.66	77.11±0.97	1.47±0.99	251.45±2.15
4	36.56±0.82	86.98±0.24	0.78±0.09	227.16±1.44

*Values are mean ±SD of two animals per group; Group1-Not infected, treated; Group2- Infected, treated; Group3- Infected, not treated; Group4- Not infected, not treated; Group5- Infected, placebo; Group6- Not infected, placebo.

Table2. Mineral Concentration in Kidney of Mice (Day 14)

Animal Group	Mineral concentration (µ g/kg) Zinc	Copper	Manganese	Iron
1	3.77±0.16	17.35±0.97	13.74±0.70	105.35±1.06
2	40.01±0.66	80.92±0.00	1.13±0.09	233.56±2.16
4	33.94±0.17	80.48±0.49	1.31±0.90	241.43±0.73

*Values are mean ±SD of two animals per group; Group1-Not infected, treated; Group2- Infected, treated; Group3- Infected, not treated; Group4- Not infected, not treated; Group5- Infected, placebo; Group6- Not infected, placebo.

Table3. Mineral Concentration in Plasma of Mice (Day1)

Animal Group	Mineral concentration (µ g/kg) Zinc	Copper	Manganese	Iron
1	37.05±0.33	91.32±0.73	7.39±0.26	241.79±1.07
2	79.19±0.49	187.22±1.21	3.22±0.09	429.94±0.08
3	103.29±0.33	253.00±0.97	3.91±0.08	544.30±1.43
4	101.65±0.66	241.92±1.93	6.00±0.24	555.75±2.15
5	43.94±6.53	109.64±0.73	4.87±0.18	309.29±2.14
6	45.43±0.65	134.21±1.20	6.70±0.09	325.23±1.08

*Values are mean ±SD of two animals per group; Group1-Not infected, treated; Group2- Infected, treated; Group3- Infected, not treated; Group4- Not infected, not treated; Group5- Infected, placebo; Group6- Not infected, placebo.

Table4. Mineral Concentration in Plasma of Mice (Day 14)

Animal Group	Mineral concentration (μ g/kg) Zinc	Copper	Manganese	Iron
3	114.77±0.66	266.74±1.20	3.04±1.10	562.50±1.10
4	106.73±1.15	263.84±0.72	2.44±0.18	567.15±0.75
5	21.48±0.82	49.88±0.73	12.87±0.18	153.95±1.05
6	30.33±0.16	80.00±0.97	11.47±0.18	209.30±0.70

*Values are mean ±SD of two animals per group; Group1-Not infected, treated; Group2- Infected, treated; Group3- Infected, not treated; Group4- Not infected, not treated; Group5- Infected, placebo; Group6- Not infected, placebo.

DISCUSSION

Minerals play important roles and the different minerals studied perform different functions in the body system. Various effects of the deficiency or alteration in the normal levels of these minerals in man and animals have been reported (Scrimshaw et al, 1968; Fenwick et al, 1990). The vicious cycle of the interaction between nutritional status and immunity to infections was a major factor that influenced this study. Zinc and copper deficiencies have been reported to affect the susceptibility of man and animals to various bacterial and parasitic infections (Hansen et al, 1982; Jones and Suttle, 1983, Prohaska and Lukasewycz, 1989). Infections have also been reported to affect micronutrient status in populations with marginal nutritional status (Hucker and Yong, 1986).

In this study, *O. gutturosa* infection caused a drastic reduction in both kidney and plasma mineral concentration, notably those of Zn, Cu and Fe. This is shown by the very low values recorded for the infected mice groups (1, 2 and 5) compared to the uninfected groups (3, 4 and 6). Treatment of the infected animals (group 2) with ivermectin, which is the current drug of choice in the treatment of onchocerciasis and other parasitic infections, greatly increased the mineral concentration, but these were still lower than in the uninfected groups. The reduced mineral concentration in both plasma and kidney, suggests the possibility of impairment of both absorption and sequestration of these minerals.

Some drugs have been reported to increase or decrease plasma minerals (Janice, 2004). In this study, ivermectin only had marginal effects on mineral concentrations in uninfected mice (comparing groups 3 and 4). There was a slight reduction in Zn concentration in both plasma and kidney. In the infected animals (groups 1 and 2) however, ivermectin administration greatly increased Zn, Cu and Fe concentrations except Mn which was reduced by ivermectin.

The significance of this study is underscored by the fact that onchocerciasis is a disease which affects mainly people in the rural areas and such populations are known to be at risk of micronutrient deficiencies (WHO, 2003; UNICEF, 2004).

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