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CHANGES IN PERIPHERAL LEUKOCYTE AND BODY FLUIDS OF ONCHOCERCIASIS PATIENTS TREATED WITH IVERMECTIN

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This study evaluated the peripheral leukocyte count and the presence of microfilariae in the body fluids of onchocerciasis patients treated with ivermectin. Fifty-three patients over the age 10 years were selected from Ipogun, an onchocerciasis endemic area in Ondo State, Nigeria. Before and after treatment, all patients received a parasitological and clinical examination that included physical examination, palpation of onchocercal nodules, assessment of microfilarial densities in iliac crest skin snips, diagnosis of concomitant parasitic infections in stool specimens and total leukocytes differential counts. Results indicated that ivermectin did not induce a decrease in the total number of peripheral leukocytes but there was a decrease in the number of eosinophils. Microfilariae were not found in increase frequency in the urine, blood and sputum, while the number of microfilariae per mg of skin snip decreased.

INTRODUCTION

Onchocerciasis is a major health problem in Nigeria. Epidemiological studies have shown that some communities are severely affected by the disease both in the Northern regions and in the Southern forested areas with foci of blinding disease found in the Northern parts of the country. Drug treatment and control of onchocerciasis have been unsatisfactory in the past. The available drug diethylcarbamazine (DEC) and suramin are too toxic for mass distribution.

The introduction of drug ivermectin has been responsible for the most recent dramatic advances both in disease control (1, 2) and in interruption of transmission (3, 4). Ivermectin has replaced DEC, and community based mass treatment campaigns against *Onchocerca volvulus* were initiated by the Onchocerciasis Control Program (OCP) in West Africa. Ivermectin is believed to paralyze susceptible nematodes by affecting neurotransmission mediated by gamma-amino-N-butyric acid (5), but total immobilization or killing of microfilariae *in vivo* has never been observed (6) and the exact mode of antifilarial action remain unclear.

Observations also indicate that ivermectin might not act on the filarial parasite directly, but rather, in synergism with the host immune response (7). Since ivermectin reduces microfilariae of *Onchocerca volvulus* and the drug acts in synergism with the host's immune response, it is expected that the drug would affect the clinical and systemic as well as immunological balance of the host.

The present study was designed to examine the eosinophilic leukocytic reactions to ivermectin provocation since filarial infections are usually followed by eosinophilia. It is also intended to correlate the signs and symptoms of the clinical response and the alterations in numbers of microfilariae in body fluids associated with a single oral dose of ivermectin in population of people with moderate to heavy infection with *Onchocerca volvulus*.

MATERIALS AND METHOD

Patient population and evaluation

The study was carried out in Ipogun, a town in Ifedore Local Government Area in Ondo State, Nigeria. Previous data from the study in the area showed that onchocerciasis is hyper endemic with a prevalence rate of 34% for leopard skin, and 16% nodular rate (8) which is representative of forest type disease. Ipogun is a rural community with a population of about 2000 residents.

Fifty three residents over the age of 10 years comprised the study patients. Children less than 10 years old, debilitated individuals, pregnant women and lactating mothers were excluded from the study. Patients were evaluated clinically and immunologically immediately before and after repeated 150 µg/kg doses of ivermectin given annually for 2 years. Samples (skin, blood, urine, and sputum) were collected before treatment, 2 days, 3 days, 3 months, 6 months, 12 months and 18 months after treatment. The patients co-operated all through in all aspects of the study except the blood sample at 48 hours and 72 hours. Clinical evaluation included physical examination, specific examination for the presence of onchocercal nodules, assessment of microfilarial densities in iliac crest skin snips and body fluids, total leukocyte and differential counts. All the subjects evaluated met the following criteria; positive history of exposure in endemic regions, clinical symptoms consistent with onchocerciasis and positive skin snips.

Skin snips were taken with corneo-scleral punch from both iliac crests and placed in wells of microtitre plates containing physiological saline. Emergent microfilariae was counted immediately and 24 hours after. The number of microfilariae were counted and scored quantitatively as reported by Crosskey and Crosskey (9).

Total and differential white blood cell count

Blood was anticoagulated with 1 mg/ml ethylene diamine tetra acetate (EDTA) and white blood cell count was done by conventional methods. Blood eosinophil count was done by the method of Discombe (10). Differential white blood cell count was done on smears stained with Leishmans stains.

Body fluid examination

The sediments of urine and blood specimens were treated according to previous methods (11, 12) and were checked for microfilaria of *Onchocerca volvulus*. Each sputum specimen was preserved with tincture of Merthiolate and examined similarly like the urine specimens.

Statistical analysis

Differences in means between time points were compared using student's test on logarithmically transformed data.

RESULTS

Pretreatment findings

Fifty three individuals aged 15-72 years (mean age 41.2 years) were evaluated before treatment. All were microfiladermic (geometric mean 22.72, 1-204/mg of skin) and 8 had detectable subcutaneous nodules. Thirty two of them were positive for leopard skin while ocular examination showed normal visual acuity except for 7 persons who complained of blurred vision (Table 1).

Complications during treatment

Fourteen patients (26.4%) had moderate side effects. Two patients experienced severe adverse reactions, of

which they received additional treatment during the follow up period of 3 days.

Skin microfilarial levels

Levels of microfilaraia, assessed by skin snips just before ivermectin administration, were measured at 6 months interval. There was a significant reduction ($p<0.05$) in the microfilarial level 6 months after the 1st dose of ivermectin (Table 2). Of the 53 patients, 22 (41.5%) had no skin microfilariae at the final sampling.

Total Leukocyte and Eosinophil counts

Ivermectin therapy had no effect on the total leukocyte counts. There was no significant difference at either the population or individual level between the pretreatment values and any of the subsequent time points (Table 2). In contrast, there was a rise in the absolute eosinophil counts two and three days after treatment. But by the third month after treatment, there was a considerable fall in the eosinophil count reaching statistical significance levels by 12 months. No correlation was seen between the reduction in eosinophil counts and the decrease in skin microfilarial levels, nor was there a correlation between eosinophil level and skin microfilarial density pretreatment.

Microfilariae levels in the body fluids

No onchocercal microfilaria was found in the urine, blood and sputum of patients before and after treatment. It is worthy to note that in 7 patients with concomitant *Schistosoma haematobium* infection, ivermectin was found not to have any effect *S. haematobium* ova was found in higher number in 2 cases of patients after treatment, while the number of *S. haematobium* ova remained the same in the other 5 patients before and after treatment.

Table 1: Baseline characteristics of patients treated with ivermectin

No of patients		53
Mean age (Range in kg)		41.2(15-72)
Mean weight (Range in Kg)		59(34-84)
No of patients with		
1.	Onchocerciasis	34
2.	Leopard skin	30
3.	Nodules	8
4.	Blurred vision	7

Table 2: Skin biopsy microfilaria counts and peripheral blood leukocyte levels in people with onchocerciasis over one year of therapy with ivermectin

Parameter	Before treatment	2days	3days	3mths	6mths	12mths	18mths
Mean no of MF/skin biopsy	22.72	21.5	4.5	17.7	5.5	9.6	6.8
Peripheral Blood Leukocytes	7662.4	8941.6	7936	7872.7	7522.8	7637.5	8428
Peripheral Blood Eosinophils	13.6	18.4	19.6	7.1	8.7	6.0	6.1

DISCUSSION

As expected from past studies (1, 13), levels of skin microfilariae decreased significantly during this period. Hematological assessment revealed no change in the leukocyte counts. Eosinophil numbers decreased significantly over the 18 months repeated observations. Such findings are particularly interesting since eosinophils have been known to be causally involved not only in the cytotoxicity to microfilariae after treatment (14) but also in the post treatment Mazzotti reactions (15) and the development of skin pathology (16).

Whether the decrease in blood eosinophil is the result of the hosts decreased antigen load (reduction in microfilariae) or whether if reflects some other possible treatment change in the host is unclear. By whatever mechanism, however, there was a reduction in eosinophil levels in these patients to less than half the pretreatment level. Within 2days after ivermectin was administrated, there was a rise in eosinophil and it has previously been demonstrated that there is a rise in eosinophil levels within the first month after treatment with either ivermectin or DEC (17). With ivermectin therapy the rise occurs more slowly than after DEC treatment, presumably because of a different mechanism of action (18) that results in decrease or clearance of the skin microfilariae.

The present finding indicate that after this initial early post treatment eosinophilia, there is a continual decline in eosinophil levels with repeated ivermectin treatment, suggesting that the patients' may be moving towards a normal (homoeostatic) state (19). The present study also demonstrated the effect of a single oral dose of ivermectin on migration of microfilariae in patients with a relatively heavy dose of infection on the skin. After administration of ivermectin the skin snips count tended to decrease with time while microfilariae were not found in the sputum, urine and blood as reported after DEC treatment by (20). This finding agrees with that of Richards *et al* (21) who also observed that the fall in microfilariae skip concentration after ivermectin treatment was not accompanied by any marked wave of microfilariae in the blood or urine.

The observations of Awadzi *et al* (17), Richards *et al* (21) and Basset *et al* (22) suggest that, after ivermectin administration although some microfilariae may enter the blood stream (presumably by way of the lymphatic system), their numbers are in no way comparable to those seen after DEC. Duke (23) also reported that ivermectin-affected microfilariae may be destroyed in

the lymph nodes and elsewhere more easily and with less reaction than those unmasked by DEC and fewer of them may survive the lymph node network to pass into the blood. They also observed that ivermectin caused microfilariae to move from the subepidermal layer into the deeper layers of the epidermis, subcutaneous fat, connective tissue and the regional lymph nodes. They concluded that the migration of microfilariae from superficial layers of the skin to the deeper connective tissues, fat lymph nodes, coupled with the mild cellular reactions that surround microfilariae dying from the effects on ivermectin are the main reasons why this effective microfilaricide for the control of onchocerciasis appears to be a promising chemotherapeutic strategy, particularly since it is not associated with severe side effects characteristic of DEC therapy.

Findings in this study demonstrated no increased symptoms that would preclude wide use of ivermectin to treat populations infected with generalized onchocerciasis.

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