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Neutrophil activation in ivermectin-treated onchocerciasis patients

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SUMMARY

Invermectin is a safe and effective drug for onchocerciasis treatment. In certain individuals, however, therapy is accompanied by adverse reactions. The mechanisms underlying these reactions are not yet known. The aim of the present study was to investigate whether neutrophils are involved in the development of these adverse reactions. Elastase and lactoferrin, two markers for the release of neutrophil azurophilic and specific granule contents respectively, were measured by radioimmunoassays in plasma of onchocerciasis patients with varying degrees of side effects, as well as in control subjects before and 1 and 2 days after ivermectin treatment. A considerable increase of elastase levels after treatment was observed, whereas lactoferrin levels did not change. The percentage of patients with elevated elastase levels was significantly correlated with the degree of side effects. These findings suggest that neutrophil activation may be involved in the development of adverse reactions in these patients.

Keywords ivermectin onchocerciasis adverse reactions elastase neutrophil activation

INTRODUCTION

Onchocerciasis is one of the most important causes of blindness in the world and is responsible for approximately 340 000 blind and one million partially sighted people in Africa and Latin America [1]. The disease is caused by the filarial nematode *Onchocerca volvulus* which is transmitted to man via blackflies belonging to the family *Simuliidae*. In addition to eye lesions, skin manifestations are also part of the disease. The death of microfilariae (mf), which are released in thousands each day by the adult female worms, and the resulting inflammatory reaction are thought to underlie the clinical symptoms [2,3].

Ivermectin has proved to be an acceptable, safe and effective drug to treat onchocerciasis. However, it can cause adverse reactions which may require additional medical care [3]. The mechanisms underlying these side effects are not yet known. *In vitro* studies have shown that granulocytes are able to kill mf from onchocerciasis patients [4]. However, there are no studies on the involvement of granulocytes *in vivo*, both with respect to efficacy of microfilarial killing as well as the development of side effects after ivermectin treatment. The present study is the first to demonstrate an *in vivo* activation of neutrophilic granulocytes after ivermectin treatment on onchocerciasis patients.

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PATIENTS AND METHODS

Study design

One hundred and fifty-six skin snip-positive onchocerciasis patients and 13 endemic control subjects were given a single oral dose of 150 μg/kg ivermectin. Before treatment, all persons received a general and an ophthalmological examination, and skin snips were taken [5]. The controls lived in the same villages in Sierra Leone (West Africa) as the patients, and although they were exposed to *O. volvulus* there was no clinical or parasitological (skin snip-negative) evidence of the disease.

Adverse reactions occurring during 3 days after ivermectin treatment were graded as described previously [5]. Briefly, pruritus, type of rash, extent of rash, lymph node enlargement, lymph node tenderness, arthralgia/synovitis, fever, extent of swollen extremity and headache were graded on a scale of 0–3. According to the highest score reached during these 3 days the patients were classified into four groups: no, mild, moderate and severe reactions. Severe side effects were an indication for additional treatment. However, patients were requested to withdraw from additional treatment as long as possible. Treatment comprised 500 mg acetylsalicylic acid and/or 25 mg promethazine depending on the nature of the side effects.

Sampling and assays

Plasma was obtained before and 1 and 2 days after ivermectin treatment, using siliconized vacutainer tubes (Terumo, Leuven,

Belgium) to which 10 mmol/l EDTA and 0·05% (w/v) Polybrene were added to prevent activation of the complement and contact coagulation systems [6]. Neutrophil elastase and lactoferrin were determined in plasma by radioimmunoassays as described previously [6]. Both radioimmunoassays were specific, sensitive and reproducible. Intra- and interassay coefficients of variation were less than 8%. Neutrophil elastase levels were measured by detecting complexes of elastase and its inhibitor α_1-antitrypsin. The normal values for elastase-α_1-antitrypsin (elastase-α_1AT) and lactoferrin were $\leqslant$100 mg/ml and $\leqslant$400 ng/ml, respectively. Before analysis we considered a rise of more than 20 ng/ml for elastase-α_1AT levels after ivermectin treatment to be significant. This limit corresponds with 2·5 times the intra- and interassay coefficient of variation of the highest normal value of elastase-α_1AT (i.e. 100 ng/ml). All determinations were performed blinded to the clinical side effects. Analysis of the data was done after completion of the determinations.

Statistical analysis

Statistical evaluation was performed using multivariance analysis, χ^2 trend test and Mann–Whitney *U*-test. A value of $P<0·05$ was considered significant.

RESULTS

Of the 156 treated patients enrolled in the study, one refused to fulfil the 3-day follow up. The remaining 155 patients were heavily infected: the overall geometric mean of microfilarial density was 43·1 mf/mg skin snip (range 0·63–627·38 mf/mg). Following ivermectin treatment, most patients had no ($n=31$, 20%) or mild ($n=91$, 58·7%) reactions. Twenty patients (12·9%) had moderate side effects. Thirteen patients (8·4%) experienced severe adverse reactions, of which seven received additional treatment during the follow-up period of 3 days. On day 1, three patients received acetylsalicylic acid and one obtained acetylsalicylic acid and promethazine. On day 2, five patients were given acetylsalicylic acid.

Plasma samples from seven patients could not be obtained. Thus the data presented here are those obtained from 148 skin snip-positive patients and 13 skin snip-negative endemic controls.

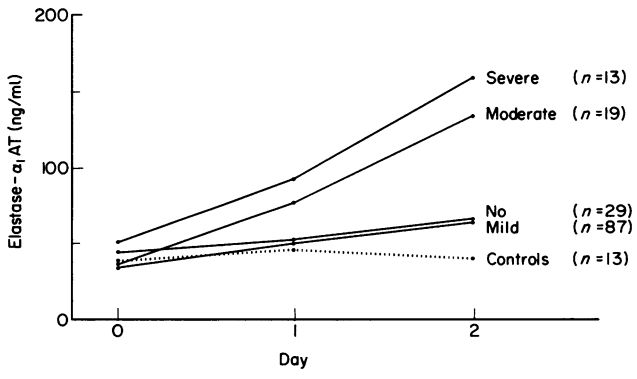

Fig. 1. The average elastase-α_1-antitrypsin (elastase-α_1AT) levels before, 1 and 2 days after ivermectin treatment in onchocerciasis patients with varying grades of side effects and skin snip-negative endemic controls.

Table 1. The percentage of patients showing a rise of more than 20 ng/ml of elastase-α_1-antitrypsin (elastase-α_1AT) after ivermectin treatment and the percentage of patients reaching values above the normal range at one or more occasions after treatment

Grade side effects	*n*	>20 ng/ml rise in elastase-α_1AT* *n* (%)	>100 ng/ml on one or more occasions* *n* (%)
No	29	8 (27·6)	4 (13·8)
Mild	87	49 (56·3)	15 (17·2)
Moderate	19	17 (89·5)	9 (47·4)
Severe	13	13 (100)	9 (69·2)
Controls	13	1 (7·7)	—

*$P<0·0001$, χ^2 trend test.

After treatment, a change of elastase-α_1AT levels was observed, which correlated with the severity of side effects (Fig. 1; $P<0·02$, multivariance analysis). The rise of elastase-α_1AT levels in onchocerciasis patients without adverse effects and in patients with mild reactions was comparable to that observed in the control group. In contrast, in patients with moderate and particularly with severe adverse reactions, elastase-α_1AT levels increased more markedly, exceeding the normal range of 100 ng/ml at day 2.

The percentage of patients showing a rise of more than 20 ng/ml of elastase-α_1AT at day 1 or 2 compared with day 0 increased significantly with the grade of adverse reactions (Table 1; $P<0·0001$, χ^2 trend test). Also, a significant correlation between the percentage of patients with elastase-α_1AT above the normal range and the grade of adverse reactions was found (Table 1; $P<0·0001$, χ^2 trend test). Although one of the controls showed a rise of more than 20 ng/ml elastase-α_1AT, all elastase-α_1AT values were within the normal range ($\leqslant$100 ng/ml), and comparable with those of healthy European controls (mean 66, range 33–96 ng/ml) measured at the same laboratory [6].

Futhermore, a correlation between skin snip microfilarial counts and a rise of more than 20 ng/ml of elastase-α_1AT was observed. Mean skin snip counts of patients with and without such a rise were 116·5 and 40·2 mf/mg respectively ($P<0·0001$, Mann–Whitney *U*-test).

Contrary to elastase-α_1AT levels, lactoferrin levels did not change significantly after ivermectin treatment in the patients and in the control group (data not shown). Only seven patients had lactoferrin levels above the normal range of 400 ng/ml: six of these had mild and one had moderate reactions.

DISCUSSION

The aim of the present study was to investigate whether neutrophils are involved in the development of side effects in onchocerciasis patients who have been treated with ivermectin. The results showed that neutrophils were activated in onchocerciasis patients after ivermectin treatment, as evidenced by increased plasma levels of elastase-α_1AT. Furthermore, it was demonstrated that the release of elastase from the neutrophilic azurophilic granules correlated with the degree of side effects.

It should be noted that in the present study a high incidence of adverse reactions was observed; only 20% of the patients did not experience side effects. The high degree of infection, as evidenced by the high overall geometric mean of 43·1 mf/mg skin snip may account for this finding [5]. The occurrence of side effects was related to skin snip counts and also to elastase-α_1AT levels, therefore we would expect elastase and skin snip counts to be related. Indeed, skin snip counts were significantly higher in patients with a rise >20 ng/ml elastase-α_1AT levels compared with patients without such a rise.

Seven of the 13 patients with severe side effects received additional treatment during the 3-day follow up, four of these on day 1, which may have interfered with the release of elastase. In particular, non-steroidal anti-inflammatory drugs at therapeutic levels may inhibit elastase release [7]. Consistently, at day 2 we observed a decrease in elastase-α_1AT levels; in one of the four patients this dropped to one third of the value on day 1. This patient received a single dose of 500 mg acetylsalicylic acid and twice 25 mg promethazine at day 1. However, in the other three patients, who received 500 mg acetylsalicylic acid once, elastase-α_1AT levels remained high. Thus, if these patients had not been treated with acetylsalicylic acid, the relation of elastase release to the severity of side effects might have been even more pronounced.

In contrast with elastase-α_1AT, lactoferrin which is released from specific granules did not increase above the normal values (i.e. 400 ng/ml), except in seven patients (six with mild and one with moderate reactions). All seven patients had raised elastase levels. This finding is consistent with earlier studies showing that neutrophils can mobilize the azurophilic and specific granules independently [6]. An alternative explanation is that lactoferrin is merely a less sensitive indicator of neutrophil activation than elastase.

The mechanisms of the development of adverse reactions in ivermectin-treated onchocerciasis patients are not yet known. It is thought, however, that side effects are associated with microfilarial destruction, which is in agreement with the observation that side effects are more pronounced in patients with high microfilarial loads [2,3,5]. How mf disintegrate following therapy is not known. Ivermectin's mode of action in fact still remains controversial [2].

Ivermectin is a γ-aminobutyric acid (GABA) agonist. It is thought that this property is related to inhibition of release of intra-uterine mf from the female adults, and paralysis of the mf [8]. Microfilariae obtained from skin snips as well as from aqueous humour show a reduced motility, and mf have been found to migrate into blood and urine as well as to deeper layers of the skin in ivermectin-treated individuals [2]. These latter processes, however, are not to the extent that they can be held responsible for the drastic reduction of microfilarial skin snip densities which is observed a few days after ivermectin treatment [2]. A direct microfilaricidal effect of ivermectin does not seem likely: ivermectin does not kill mf *in vitro*, except at concentrations of 1000 times higher than found in plasma [9]. A possible explanation for the elimination of mf from the skin is given by Darge *et al.* [10]. They observed that microfilarial densities in the lymph nodes were about a thousand-fold higher in ivermectin-treated patients compared with non-treated patients, and suggested that mf are degraded in the lymph nodes.

The present study is the first to report a role for neutrophilic granulocytes in ivermectin-induced adverse reactions in onchocerciasis patients. The factors causing neutrophils to degranulate, however, remain to be established. A role for ivermectin to act as a direct granulocyte stimulator has not been described, and does not seem probable in view of our observations that in skin snip-negative endemic control individuals no distinct increases of elastase or lactoferrin were found. Since we did not include ivermectin-treated non-exposed controls however (for instance healthy European individuals), we cannot rule out this possibility definitely.

Neutrophils can be activated by a large variety of agonists including C3a and C5a, factor XIIa and kallikrein of the contact system, cytokines (e.g. tumour necrosis factor, granulocyte-macrophage colony-stimulating factor, and IL-8), immune complexes, endotoxin and intact bacteria, and in particular by combinations thereof [6]. In a preliminary study we have determined levels of C3a, circulating immune complexes, tissue plasminogen activator and plasmin–anti-plasmin complexes in onchocerciasis patients with various grades of side effects. However, no clear changes of these parameters were found in these patients after ivermectin treatment (unpublished observations). Obviously, studies investigating stimuli that activate neutrophils in ivermectin-treated onchocerciasis patients, will provide more insight into ivermectin's mode of action and development of post-treatment adverse reactions.

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