Eosinophil Sequestration and Activation Are Associated with the Onset and Severity of Systemic Adverse Reactions following the Treatment of Onchocerciasis with Ivermectin

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To investigate the role of eosinophil activation and sequestration in the development and severity of adverse reactions after the treatment of *Onchocerca volvulus* infection, 40 *O. volvulus*–infected Ghanaians were randomized to receive placebo or standard- or high-dose ivermectin. Subjects were examined for typical physiologic and clinical events before and up to 48 h after treatment. Plasma samples were tested for interleukin (IL)-5 and eosinophil degranulation products (e.g., eosinophil-derived neurotoxin, EDN). After treatment, peripheral eosinophil counts declined in ivermectin-treated groups ($P < .001$), whereas circulating levels of IL-5 ($P < .01$) and EDN ($P < .05$) increased. Cumulative levels of IL-5 and EDN correlated with reaction scores ($P < .01$). High-dose ivermectin was associated with more-severe reactions, more-profound eosinopenia, and higher circulating levels of IL-5 and EDN, compared with the standard dose. These results suggest that eosinophil sequestration and activation/degranulation are associated with the initiation and severity of ivermectin-associated adverse reactions.

Ivermectin is a safe and highly effective microfilaricidal drug that now forms the mainstay of onchocerciasis control in most *Onchocerca volvulus*–endemic areas. Ivermectin treatment is associated with adverse reactions that affect up to 30% of patients receiving the first dose [1].

The pathophysiology of these adverse reactions is poorly understood but is thought to be related to microfilarial killing. The severity of the reaction has been shown to correlate with microfilarial density [2]. In addition, the close relationship between the appearance and degranulation of eosinophils in the peripheral tissues at the sites of microfilarial killing has suggested a central role for eosinophils in this reaction [3–5].

The eosinophil active cytokine, interleukin (IL)-5, is thought to be important in the differentiation and expansion of eosinophil reserves from the bone marrow and mobilization to the peripheral circulation [6]. IL-5 also activates eosinophils and enhances adhesion, tissue sequestration, and prolonged survival of eosinophils [6]. In fact, circulating IL-5 may be essential for infiltration of eosinophils into the tissues [7]. Activated eosinophils at the sites of allergic inflammation are induced to release toxic granule products such as major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). These cationic proteins are toxic for helminth larvae; raised circulating levels have been described after the treatment of onchocerciasis with diethylcarbamazine [4] and can be used as systemic markers of eosinophil activation [8, 9].

A recent double-blind placebo-controlled study of the safety and efficacy of high-dose ivermectin in the treatment of onchocerciasis [1] provided the opportunity to study the relationship between eosinophil recruitment and activation and the development of posttreatment reactions and to examine whether drug dose might affect this relationship.

Patients and Methods

Patients and drug administration. Forty healthy men with moderate to heavy infections from *O. volvulus*–endemic communities in Southeastern Ghana were admitted to the Onchocerciasis Chemotherapy Research Centre. The study design was double-blind and placebo-controlled. The design and conduct of this study is described in detail elsewhere [1]. Patients were allocated to one of the following 4 treatment groups: placebo (group A, $n = 6$); 150 μg/kg ivermectin (group B, $n = 18$); 400 μg/kg ivermectin (group C, $n = 8$); and 600 μg/kg ivermectin (group D, $n = 8$).

Sample collection and analysis. Blood samples (for plasma and total and differential blood cell counts) were taken before treatment on two occasions and then at 2, 4, 6, 8, 12, 18, 24, 30, 36, 44, and 48 h after the first dose of ivermectin or placebo. Levels of IL-5, ECP, and EDN were measured by specific sandwich ELISA using assays described previously [4, 10]. Skin snips, taken using standard

Received 19 August 1998; revised 3 November 1998.

Informed consent was obtained from all subjects, and the study was done with protocols approved by the World Health Organization Secretariat Committee for Research Involving Human Subjects (SCRIHS).

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The Journal of Infectious Diseases 1999; 179:738–42

0022-1899/99/7903-0033$02.00

methods, were obtained before treatment and at 48 h after treatment.

Grading of adverse reactions. The clinical assessment of each patient during the study period has been described in detail elsewhere [1]. Briefly, each patient received a thorough physical examination before treatment, and baseline values of the features used to quantify the posttreatment reaction were recorded at 4-h intervals up to 48 h after treatment. Cumulative reaction scores were calculated as the sum of scores for each (overall individual reaction) and all features (overall total reaction), respectively, over the observation period for each patient.

Statistical analysis. Skin microfilarial intensities are expressed as geometric mean number of microfilariae per milligram of skin. Cytokine levels are expressed as geometric means of the percentage change from the mean of the two baseline measurements. The association between two continuous variables was calculated using Spearman's rank correlation coefficients. Parametric statistical analyses were performed using $\log_e$-transformed data. The comparison of two means was calculated using Student's *t* test for independent groups and a paired *t* test for paired data. Comparison of more than two means was calculated using one-way analysis of variance (ANOVA) for independent groups and ANOVA for repeated measurements for paired data. Cumulative or total levels of eosinophils, cytokine levels, and eosinophil degranulation product levels were calculated as the area under the respective curves (for the 48-h observation period) using the trapezium rule.

Results

Clinical reactions and parasitologic findings. The age distribution and baseline microfilarial counts for the treatment groups are shown in table 1. Pretreatment microfilarial levels were similar in all groups and, by 48 h after treatment, had fallen dramatically to comparable levels in all ivermectin-treated groups.

The clinical reactions seen in these patients have been described previously [1]. As there were no significant differences in individual or total reaction scores between the groups receiving the highest doses (C and D), these 2 groups have been combined as a single group, group C/D, for subsequent analyses (table 1). Objective clinical reactions increased from negligible levels in the placebo group in a dose-dependent manner by ANOVA (e.g., comparisons of groups A, B, and C/D) (table 1). There were no severe or life-threatening reactions.

Changes in peripheral blood leucocyte counts. There were no significant differences between placebo and treatment groups in baseline total white cell counts and lymphocyte counts over the observation time. Neutrophil counts increased in both treatment groups after ivermectin treatment and remained elevated at 48 h after treatment (data not shown).

Eosinophil counts did not differ between the study groups before treatment. After treatment, eosinophil counts did not change significantly compared with baseline counts in the placebo group (figure 1A); however, eosinophil counts declined significantly in both treatment groups ($P < .001$). The overall decline in eosinophil levels or cumulative eosinophil counts was significantly reduced in both treatment groups compared with the placebo group ($P < .01$); a dose-dependent effect was seen, as the high-dose treatment group (C/D) had reduced total counts compared with the standard treatment group (B) between 18 and 24 h after treatment ($P < .05$).

Changes in circulating levels of IL-5 and eosinophil degranulation products. Changes in IL-5 levels over the study period in the treatment groups are shown in figure 1B. Baseline levels of IL-5 were negligible (e.g., <7.8 pg/mL) in all 3 groups at baseline. After treatment, IL-5 levels did not change significantly over the observation period in the placebo group (A). In contrast, significant increases were seen ($P < .001$) in both treatment groups (B and C/D) and continued to increase up to the 48-h observation time. IL-5 levels at 44 and 48 h were significantly greater ($P < .01$) in both ivermectin-treatment groups compared with the placebo group, and levels increased earlier in the high-dose (C/D) than in the standard-dose (B) regimens. Cumulative levels of IL-5 over the 48-h observation period were significantly greater in the 2 treatment groups than in the placebo group ($P < .05$).

Likewise, circulating levels of both eosinophil degranulation products measured (EDN and ECP) increased in both iver-

Table 1. Parasitologic and objective clinical reaction findings in the study group.

Treatment group	Age, median, years (range)	Geometric mean (range)		Adverse reactions[a]						
		Mf-S 0	Mf-S 48	Fever	Rash	BPL	BPS	PRL	PRS	Total
A (n = 6)	26 (22–36)	219 (64–318)	258 (162–432)	0.9	0	0	0	0	1.2	8.6
B (n = 18)	31 (24–50)	173 (52–367)	13 (0–79)	13.7	1.7	0	1.6	3.6	9.2	126.1
C (n = 8)	26 (20–45)	193 (70–342)	7 (1–27)	20.0	2.9	0	7.5	25.4	49.6	258.3
D (n = 8)	35 (19–50)	200 (53–520)	10 (2–24)	27.7[b]	4.9	0.4	13.0[c]	12.4[d]	44.3[d]	227.9[d]
C/D (n = 16)	32 (19–50)	197 (53–520)	8 (1–27)	23.5[b]	3.8[b]	0.2	9.9[d]	17.8[d]	46.8[d]	242.2[d]

NOTE. Treatment groups are placebo (A), 150 μg/kg ivermectin (B), 400 μg/kg ivermectin (C), 600 μg/kg ivermectin (D), and groups C and D combined (C/D). Skin microfilarial infection densities are shown before treatment (Mf-S 0) and 48 h after treatment (Mf-S 48). Reaction score indices are geometric means of cumulative individual reaction scores and total reaction scores for subjects in each group. Comparisons between groups (A, B, C, D, or A, B, C/D) were calculated by analysis of variance; shown are significant intergroup differences.

[a] Adverse reaction parameters are lying blood pressure (BPL), lying pulse rate (PRL), standing blood pressure (BPS), and standing pulse rate (PRS).

[b] $P < .05$.

[c] $P < .01$.

[d] $P < .001$.

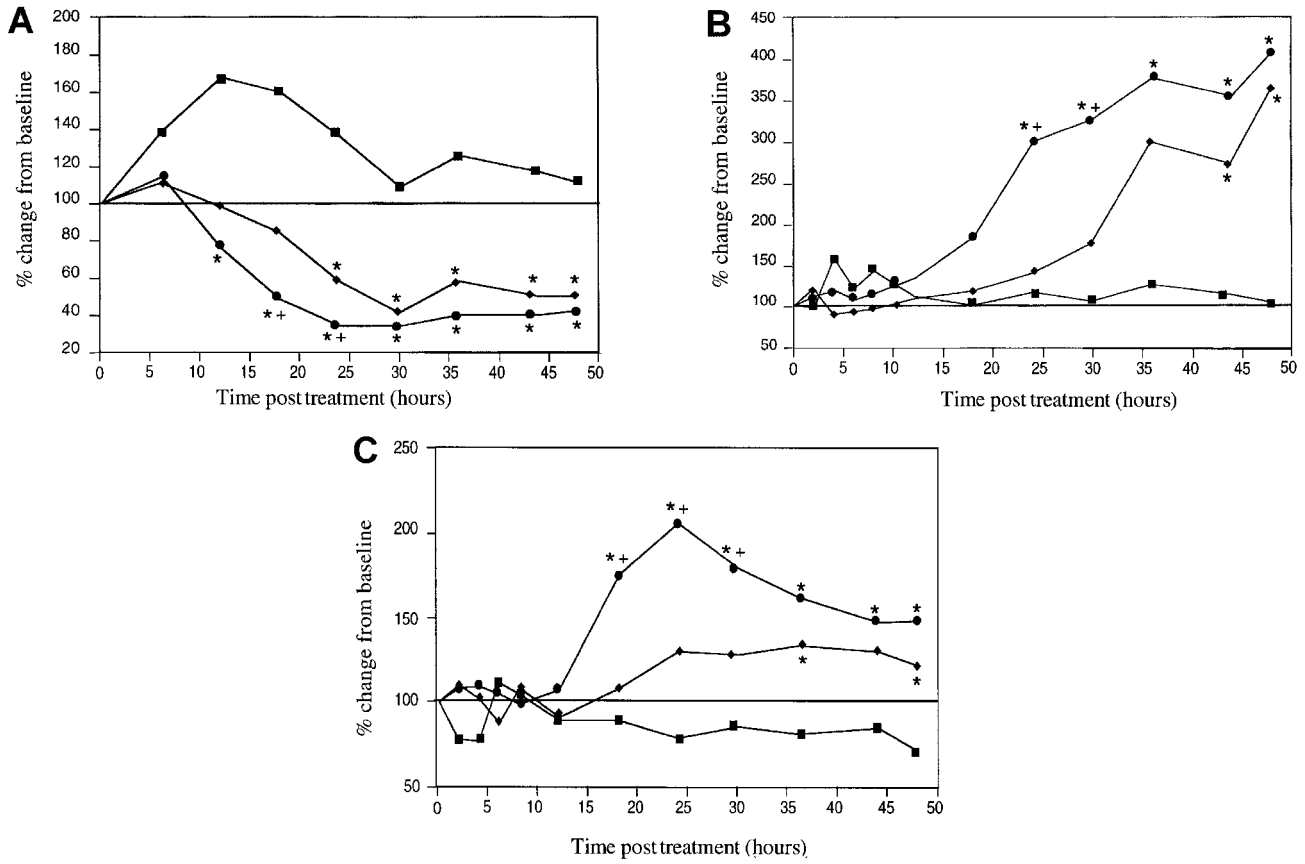

Figure 1. Changes in circulating eosinophil counts *(A)* and in plasma levels of IL-5 *(B)* and eosinophil-derived neurotoxin *(C)* after treatment with ivermectin. Shown are geometric mean % changes vs. baseline (horizontal line) for group A (placebo, ■), group B (150 μg/kg ivermectin, ♦), and group C/D (400–600 μg/kg ivermectin, ●). Shown are time points at which there were statistically significant differences ($P < .05$) between group A and group B or C/D (*) and between group B and group C/D (+).

mectin-treated groups after treatment, but the changes were statistically significant only for plasma EDN (group B, $P < .05$; group C/D, $P < .001$). After ivermectin treatment, EDN levels started to increase at 18 h in both groups, reaching a peak at 24 h in group C/D and a plateau in group B at the same time (figure 1C). Cumulative levels of EDN were significantly greater in both treated groups than in the placebo group ($P < .05$). EDN levels were also greater in the high-dose (C/D) than standard-dose (B) regimen between 18 and 30 h after treatment ($P < .05$).

Relationships between reaction severity and changes in circulating levels of eosinophils, cytokines, and eosinophil degranulation products. Strong positive correlations were seen between total reaction scores and cumulative levels of IL-5 and EDN ($P < .01$), whereas a negative correlation was seen between total reaction scores and cumulative eosinophil levels ($P < .05$). Individual reaction score parameters were also correlated with cumulative levels of IL-5, EDN, ECP, and eosinophils: IL-5 correlated with fever ($P < .01$) and rash ($P < .05$); EDN correlated with fever ($P < .01$), standing blood pressure ($P < .05$), and standing pulse rate ($P < .01$); ECP correlated with fever ($P < .05$); and eosinophil levels correlated negatively with rash ($P < .01$), standing pulse rate ($P < .05$), and standing blood pressure ($P < .01$).

Discussion

Ivermectin is a potent microfilaricidal drug that, when administered at a community level with broad coverage, reduces community microfilarial levels and may lead to interruption of transmission [11]. Ivermectin is associated, however, with significant adverse reactions that may affect compliance with the necessary repeated treatments. Little is known of the pathogenesis of these reactions, although eosinophils are thought to have a central role [3, 4]. The present study, with a double-blind placebo-controlled design, examined the role of eosinophils in the generation of this reaction early after ivermectin treatment, using systemic markers of eosinophil recruitment and activation (IL-5 and EDN, respectively) to determine the impact of drug dose on these parameters.

Eosinophils are able to kill filarial larvae, including *O. volvulus,* in vitro [12], and this killing is thought to involve the generation of reactive oxygen intermediates [13] and the deposition of cationic granule proteins (e.g., MBP, ECP, and EDN) [5, 14] on the larval surface. In vivo, after administration of ivermectin, dead and degenerating microfilariae become surrounded by closely apposed and degranulating eosinophils [4, 5, 14]. In the current study, as shown previously [3, 4], circulating levels of eosinophils declined after treatment, and this was followed by a rise in circulating IL-5. These changes were seen only in the ivermectin-treated groups, not in the placebo group. At the same time, circulating plasma levels of the eosinophil degranulation product EDN also started to rise.

Levels of IL-5 and eosinophils correlated negatively in both ivermectin treatment groups (data not shown), suggesting that eosinophil sequestration at the sites of inflammation in the tissues is resulting in high tissue expression of this cytokine. Raised circulating levels of IL-5 probably represent spillover from the sites of microfilarial killing; a number of cell types are capable of producing IL-5, including Th2 $CD4^+$ lymphocytes, eosinophils, and mast cells, all of which are involved in this tissue reaction [4, 5, 14]. Further, most objective reaction score parameters (e.g., fever, rash, and standing blood pressure and pulse rates) as well as total reaction scores correlated negatively with circulating eosinophil counts and positively with plasma IL-5 and EDN levels.

These findings suggest that eosinophil recruitment (as suggested by IL-5) and widespread eosinophil activation and degranulation (as suggested by plasma EDN) in the tissues (principally the lymph nodes and skin [5]) occur after ivermectin treatment. The disappearance of eosinophils from the circulation and their tissue localization at the sites of microfilarial killing preceded the onset of clinically apparent adverse reactions, which started after 24 h and peaked between 32 and 36 h [1]. Previous studies have shown that eosinophils begin to appear around microfilariae in the regional lymph nodes at 24 h, and numbers are maximal at 40–48 h [5]. These observations suggest a central role for eosinophils in microfilarial killing and the resultant inflammatory reactions.

The original and larger study from which this subject population was derived did not show evidence of a statistically significant effect of ivermectin dose on reaction severity [1]; however, in this study, there was evidence of a dose-dependent effect of ivermectin on the severity of adverse reactions. Two possible reasons for this follow: (1) The shorter observation period (i.e., 48 h) during which all reactions occur and start to disappear allowed true differences to be derived without the "dilutional" effects of longer periods (e.g., 30 days in the original study) obscuring such differences, and (2) the reaction parameters included in this study were restricted to those that could be assessed objectively (e.g., blood pressure, rash).

Eosinophil numbers started to decline earlier in the high-dose ivermectin group, and plasma levels of IL-5 and EDN started to rise earlier and reached higher peaks in this group. This suggests that ivermectin may be having a dose-dependent effect on the rate of microfilarial killing. There is evidence that microfilariae are killed more rapidly with high-dose ivermectin than with the standard regimen [1]. Ivermectin may act directly on eosinophils and is known to enhance the generation of active oxygen intermediates by eosinophils in a dose-dependent manner [13].

In summary, posttreatment reactions that were seen after the treatment of onchocerciasis with ivermectin were associated with an increase in eosinophil sequestration and the activation/degranulation that occurs, most likely, at the sites of microfilarial killing in the lymph nodes and skin. A dose-response effect was seen, in which subjects treated with higher doses of ivermectin experienced more-severe adverse reactions and a greater level of eosinophil sequestration and activation than did those receiving a standard dose regimen. We therefore provide further evidence for an important role for the eosinophil in the initiation of the posttreatment adverse reactions in onchocerciasis.

Acknowledgment

We thank Brenda Rae Marshall for her careful editorial assistance.

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