EFFECTS OF IVERMECTIN AND DIETHYLCARBAMAZINE ON MICROFILARIAE AND OVERALL MICROFILARIA PRODUCTION IN BANCOFTIAN FILARIASIS

WILMA A. STOLK,* GERRIT J. VAN OORTMARSSSEN, S. P. PANI, SAKIE J. DE VLAS, S. SUBRAMANIAN, P. K. DAS, AND J. DIK F. HABBEBA

Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; Vector Control Research Centre (Indian Council of Medical Research), Pondicherry, India

Abstract. Ivermectin and diethylcarbamazine (DEC) are used in mass treatment programs for the elimination of lymphatic filariasis because of their strong effects on microfilariaemia. However, the effects of treatment on adult worms and the degree of individual variation in efficacy are unclear. We analyzed series of microfilaria (Mf) counts from individuals treated with a single dose of 400 μg/kg ivermectin or 6 mg/kg DEC (N = 23 in each group; 1 year follow-up). For each individual, we estimated the microfilaricidal effect and the reduction in overall Mf production (e.g., caused by death or sterilization of worms, or inhibited Mf release from the female worm uterus). Ivermectin on average killed 96% of Mf and reduced Mf production by 82%. DEC killed 57% of Mf and reduced Mf production by 67%, with some individuals responding very poorly. The strong reduction in overall Mf production is good news for control of lymphatic filariasis, but the prospects of elimination will be diminished if part of the population systematically responds poorly to treatment.

INTRODUCTION

Programs are being initiated worldwide to eliminate lymphatic filariasis by yearly mass treatment with ivermectin or diethylcarbamazine (DEC), given alone or in combination with albendazole. It is unclear, though, how many treatment rounds will be required to achieve the goal of elimination. A major problem is our incomplete understanding of the effects of treatment on the adult worm. Control needs to be continued for many years if overall microfilaria (Mf) production by adult worms is largely unaffected by treatment. Antigen tests have been used to demonstrate macrofilaricidal effects,1,2 but it is unclear how the reduction in antigen level relates to the proportion of worms killed. Ultrasound has been used to assess the macrofilaricidal effects of treatment directly3,4; however, its application is limited to the scrotal area and superficial lymphatics. Neither of these tools can assess an effect on fecundity.

Most commonly, the effects of treatment have been assessed by measuring the change in Mf density over time. Many clinical trials and community-based interventions showed that treatment with ivermectin or DEC, given alone or in combination with albendazole, leads to a strong and sustained reduction in Mf density (reviewed in Refs. 5–8). Mathematical models that describe the development of parasites in the human body can be used to analyze such trends in Mf density for indirect quantification of the effects of treatment. In this way, it was estimated from published data that a single dose treatment with 200 or 400 μg/kg ivermectin not only results in immediate killing of all Mf but also in a reduction in the overall Mf production in the follow-up period of respectively 35% or 65% at least.9 The reduction in Mf production indicates that adult worms are affected, but the nature of this effect (e.g., death or sterilization of worms, reduced Mf release from female worm uterus) cannot be determined. However, there was no indication that the reduction in Mf production was only temporary. Similar estimates for the efficacy of a single dose of DEC are not available yet.

Another aspect of interest is the variation in treatment efficacy that occurs between individuals. This has received little attention in literature. However, the impact of mass treatment may be undermined when there is a number of individuals who respond poorly to treatment and who continue to transmit infection in the population.10

Here, we present the results of a double-blind, randomized, hospital-based trial that was carried out to investigate the efficacy of a single dose of ivermectin (400 μg/kg body weight) or DEC (6 mg/kg body weight) for treatment of bancroftian filariasis.11 We analyzed the one-year follow-up trends in Mf density at the individual level to quantify the effects of treatment and the individual variation in these effects.

MATERIALS AND METHODS

Data. A double-blind, randomized, hospital-based trial was carried out in Pondicherry, India, to compare the safety and efficacy of a single dose of ivermectin (400 μg/kg body weight) or DEC (6 mg/kg body weight) for treatment of bancroftian filariasis.11 In each treatment group, 30 Mf carriers with pretreatment Mf counts ≥ 100 Mf/mL were included. Mf density in the blood was determined by membrane filtration of 1 mL venous night blood, and all blood samples were taken between 8:30 pm and 9:30 pm (not always on the exact same time for an individual). Mf counts were taken with monthly intervals during the first year after treatment. Available observations for part of the individuals made 24 months after treatment were not included in our analysis. This was because these observations are not only determined by the effects of treatment, but to a large extent also by trends in transmission intensity or other external factors that are not accounted for in our model. One-year follow-up is long enough to measure the effects of treatment, but distortion of the trends due to reinfection will be minimal because of the long immature period of the worms. Only individuals with complete follow-up were included in the analysis (23 individuals in each group).
The two treatment groups were comparable with respect to age and gender: the mean age was 20 years in the ivermectin group and 22 years in the DEC group, and the male:female ratio was 14:9 and 12:11, respectively. The mean pretreatment Mf load was higher in the ivermectin group than in the DEC group (538 Mf/mL versus 338 Mf/mL), but this difference was not significant (t test on log-transformed values, \( P = 0.118 \)).

**Statistical analysis.** We used a mathematical model, which describes the course of *Wuchereria bancrofti* infection in individuals over time and the impact of treatment on the different parasite stages (see Appendix). We assumed that the pretreatment Mf density represents an equilibrium situation where the acquisition of worms and Mf is balanced by the loss. This equilibrium is disturbed by two immediate and irreversible effects of treatment: a fraction of Mf is killed (resulting in an immediate drop in Mf density) and the overall Mf production is reduced by a certain fraction (resulting in a lower rate of Mf recurrence in the blood than expected if Mf production had not been affected). The cause of the reduced Mf production (e.g., death or sterilization of adult worms or any other mechanism that inhibits the release of Mf from the female worm uterus) cannot be determined from the data on Mf density.

The rate of recurrence of Mf after treatment (relative to an individual’s pretreatment level) depends not only on the effects of treatment, but also on assumptions on the duration of the immature period of worms and the adult worm and Mf life span. Based on literature, we assumed these durations to be, respectively, 8 months, 12 years, and 12 months on average. As argued above, new infections acquired during the first year after treatment will have little impact on trends in Mf density and were ignored in this analysis. Under these assumptions, Mf density 1 year after treatment is 61% of the pretreatment level if treatment kills all Mf but has no effect on adult worms. The behavior of the model is further explained elsewhere.

Individual trends in Mf density are described by the pretreatment force-of-infection (\( \beta \)), the fraction Mf killed due to treatment (\( \delta \)), and the effect of treatment on overall Mf production (\( \lambda \)). The values of these parameters are estimated by fitting the model to the individual data using nonlinear regression and assuming extra-Poisson variation. A more detailed description of the model and the estimation procedure is given in the Appendix.

In a sensitivity analysis, we assessed how the estimates of the efficacy parameters depend on assumptions on the immature period and the worm and Mf life span by halving and doubling their values. We also checked how the results change if we take account of new infections acquired during follow-up with the rate of acquisition being equal to the pretreatment rate. Spearman’s rank correlation was used to test for correlations between efficacy estimates (\( \delta \) or \( \lambda \)) and the predicted pretreatment Mf intensities (reflected by \( \beta \)).

**RESULTS**

The results of the analysis are summarized in Figure 1 and Table 1. On average, the efficacy of ivermectin was higher than that of DEC. The fraction of Mf killed (\( \delta \)) was high in all ivermectin-treated individuals; in 87% of the individuals, even more than 90% of the Mf was killed. Usually there was also a strong reduction in overall Mf production (\( \lambda \)). The effects of DEC treatment were somewhat lower on average and varied strongly between individuals. In both groups, there was no significant correlation between the individual estimates of \( \delta \) or \( \lambda \) and the pretreatment Mf intensity \( \beta \), indicating that the effects of treatment do not depend on the pretreatment level of infection.

Figure 2 shows the average trend in observed and predicted Mf intensities. Figure 3 gives some typical examples of individual trends in Mf density after treatment. Ivermectin led to
TABLE 1

<table>
<thead>
<tr>
<th>Impact on Mf</th>
<th>Ivermectin (N = 23)</th>
<th>DEC (N = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of Mf killed (δ)</td>
<td>0.96 (0.05)</td>
<td>0.57 (0.39)</td>
</tr>
<tr>
<td>Median (25th to 75th percentile)</td>
<td>0.98 (0.95-1.00)</td>
<td>0.77 (0.00-0.87)</td>
</tr>
<tr>
<td>Number (%) of individuals with all Mf killed (δ &gt; 0.999)</td>
<td>7 (30%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Number (%) of individuals with no Mf killed (δ &lt; 0.001)</td>
<td>0 (0%)</td>
<td>6 (26%)</td>
</tr>
<tr>
<td>Reduction in overall Mf production (λ)</td>
<td>0.82 (0.27)</td>
<td>0.67 (0.36)</td>
</tr>
<tr>
<td>Median (25th and 75th percentiles)</td>
<td>0.96 (0.78-1.00)</td>
<td>0.87 (0.38-0.96)</td>
</tr>
<tr>
<td>Number (%) of individuals with complete cessation of Mf production (λ &gt; 0.999)</td>
<td>7 (30%)</td>
<td>5 (22%)</td>
</tr>
<tr>
<td>Number (%) of individuals with no change in Mf production (λ &lt; 0.001)</td>
<td>1 (4%)</td>
<td>2 (9%)</td>
</tr>
</tbody>
</table>

Discussion

Our analysis of individual-level trends in Mf density after treatment showed that a single dose of ivermectin (400 µg/kg) in all treated individuals resulted in death of a large fraction of Mf and in most instances also in a strong reduction in overall Mf production. The effects of DEC were somewhat lower on average and more variable. In some individuals treated with DEC, almost all Mf were killed and Mf production was nearly completely interrupted; in others, the drug had little effect. The data provide no information about the cause of the reduction in overall Mf production. For DEC it is probably explained by a macrofilaricidal effect. Ivermectin probably does not kill adult worms. Possibly, ivermectin causes damage to the reproductive system of female worms, so that embryogenesis, maturation, or release of Mf from the uterus is inhibited.

Based on ultrasound examination of the male scrotum, it was previously estimated that a single dose of DEC kills about half of the adult worms. The estimated reduction in overall Mf production in our study was only slightly higher. Care is required in this comparison: the reduction in Mf production may be higher than the proportion of adult worms affected, because unmated worms may have survived and retained their ability to produce Mf. Any effect of ivermectin on the fertility of adult worms cannot directly be measured. However, the current estimates are in agreement with the results of a previous model-based analysis. This analysis of 2-year follow-up data provided no indication that the effect on Mf production was only temporal, but studies with longer follow-up are required to be more certain on this aspect. Analysis of combined data on Mf and antigen density and on the presence of motile worms may enhance our qualitative and quantitative understanding of the effects of treatment on adult worms.
The validity of our efficacy estimates depends on the validity of the model that was used to describe the average trends. We do not know exactly how the filarial worm develops in the human body. However, assumptions about the immature period or worm life span proved to have little impact on our efficacy estimates and did not change the main conclusions. The results were more sensitive to assumptions about the Mf life span. The effect of changing the assumed Mf life span depends on the observed trend. Assuming a shorter Mf life span results in higher estimates of the reduction in overall Mf production, if a strong initial decline in Mf density is followed by a gradual increase. However, it results in lower estimates, if a gradual decline in Mf density is observed over time. Assuming a longer Mf life span results in changes in the other direction. Although individual estimates were influenced by assumptions on the Mf life span, the impact on the average efficacy estimate was rather limited and strong variability remained.

Assumptions on the acquisition of new infections during follow-up had little impact on the outcomes. Because of the long immature period of the worm (8 months), the contribution of newly acquired infection on the Mf density 1 year after treatment is very limited. Indeed, when we allowed for the acquisition of new infections, assuming that transmission in the post-treatment continues at the same rate as before treatment, we found only slightly higher estimates for the reduction in overall Mf production, and the estimated fraction Mf killed hardly changed.

To assess the generalizability of our efficacy estimates, we compared our data with that from other trials. Higher effec-
tiveness of ivermectin (400 mg/kg) compared with DEC (6 mg/kg) was reported in several studies, but other studies revealed only small differences between both treatment regimens, and one study found that DEC was even more effective than ivermectin. For DEC, the geometric mean Mf density 1 year after treatment varied widely in published studies from 4.5% to 33.4% (average 12%) of the pretreatment level. In our data, it was reduced to about 17% of the pretreatment level, which is within the range of other studies. For ivermectin, too, trends in Mf density varied between studies. Analysis of data from other studies may therefore yield somewhat different efficacy estimates.

For part of the individuals in our study one additional observation made 2 years after treatment was available, but these observations were not used. These observations were usually low relative to the observed trend during the first year after treatment. Explorative attempts to fit the model to all data (including the 2-year follow-up data) resulted in somewhat higher estimates of the reduction in overall Mf production, but a poorer fit. This suggests that these observations were probably influenced by (external) factors that are not accounted for by our model.

A problem in the analysis of individual level data is the large variability in Mf counts, so that sometimes trends were difficult to interpret. The pretreatment Mf density was based on only one measurement. In some individuals, the pretreatment Mf count by chance will have been lower than the true density. This was probably seen in some DEC-treated individuals, who had higher Mf counts during follow-up than before treatment (e.g., Figures 3E and 3F). In other individuals, the observed Mf count by chance have been higher than the true Mf density. With our approach, however, we cannot identify when this occurs. This might have led to a small overestimation of the average effects of treatment. The selection of Mf positives for our study population may have added to the overestimation. In the whole population, therefore, the average efficacy may be somewhat lower than we estimated.

Our study provides important information for the ongoing elimination programs for lymphatic filariasis, which are based on mass treatment with DEC and ivermectin in combination with albendazole. The average effects of DEC and ivermectin treatment are high, which triggers optimism about the potential impact of mass treatment. However, ivermectin is usually given in lower dosages (150–200 µg/kg instead of the 400 µg/kg given in this study), which is less effective in reducing the overall Mf production. ivermectin is unknown to what extent the impact of treatment is improved by giving the drugs in combination with albendazole.

Especially in the DEC group, there was much variation in treatment efficacy, and in several individuals the effects were poor. A remaining question is whether the observed variation is random or systematic. More information is needed about the impact of a second treatment in individuals who had a poor response. The presence of systematic nonresponders in a population will considerably reduce the probability of elimination, or at least necessitate a longer duration of treatment programs (until most adult worms have died naturally). It would be interesting to study whether the average efficacy of treatment increases and whether the number of people with poor response to treatment is reduced when ivermectin or DEC are given in combination with albendazole, as is recommended for the ongoing elimination programs.

Received March 17, 2005. Accepted for publication May 31, 2005.

Acknowledgments: The authors thank Paul Simonsen (DBL), Dan Meyrowitsch (DBL), and Anton Plaisier for their contribution to this work in earlier stages of the project. The authors thank Theo Stijnen for his statistical advice.

Financial support: This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Authors’ addresses: Wilma A. Stolk, Gerrit J. van Oortmarssen, Sake de Vlas, and J. Dik F. Habbema, Department of Public Health, Erasmus MC, University Medical Center Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands, Telephone: +31 10 4087714, Fax: +31 10 4089449, S. P. Pan, S. Subramanian, and P. K. Das, Vector Control Research Centre, Indian Council of Medical Research, Indira Nagar, Medical Complex, Pondicherry 605 006, India, Telephone: +91 413 2272396/2272397, Fax: +91 413 2272041.

Reprint requests: Wilma A. Stolk, Department of Public Health, Erasmus MC, University Medical Center Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands, Telephone: +31 10 4087714, Fax: +31 10 4089449, E-mail: w.stolk@erasmusmc.nl.

REFERENCES


APPENDIX

MATHEMATICAL DESCRIPTION OF THE MODEL

The structure of the model is schematically presented in Figure A1. The dynamics of parasite development and Mf production are described by the following set of differential equations:

\[
\frac{dL(t)}{dt} = \beta_1 - (\gamma + \mu_1) L(t)
\]

\[
\frac{dW(t)}{dt} = \gamma L(t) - \mu_2 W(t)
\]

\[
\frac{dM(t)}{dt} = \rho W(t) - \mu_3 M(t)
\]

The rate of acquisition of new worms depends on the force-of-infection \( \beta_1 \), which is defined as the average number of successfully inoculated new parasites per year. The rate of maturation, \( \gamma \), is defined by the duration of the immature period (immature period = 1/\( \gamma \)). Similarly, the death rate of larvae and worms, \( \mu_1 \), is defined by the average life span of the parasites (parasite life span = 1/\( \mu_1 \)). Mature adult worms start producing Mf (M) at a constant per capita rate \( \rho \). Parameter \( \rho \) is defined as the rate of Mf production per mature worm per unit of blood taken for diagnosis. The death rate of larvae and worms, \( \mu_2 \), is defined by the average Mf life span (Mf life span = 1/\( \mu_2 \)).

We assume that the force-of-infection has been constant over time, so that the worm and Mf density are in equilibrium prior to treatment, meaning that death of worms is balanced.

\[
\beta_1 \quad \gamma \quad W \quad \mu_1 \\
L \quad \mu_2 \quad M
\]

Figure A1. Flow-chart of the model, showing the dynamics of immature worms (L), adult worms (W), and microfilariae (M) in the human host.
by new infections. The force-of-infection varies between individuals, reflected in different pretreatment counts. Because of the long immature period of worms, new infections acquired during the first year after treatment will have little impact on trends in Mf density, and we ignore these in our analysis. In other words, \( \beta_i = 0 \) in the post-treatment period for all individuals.

At the moment of treatment \( (t = 0) \), a fraction \( \delta_i \) of the Mf \( (M) \) is being killed instantaneously and a fraction \( \lambda_i \) of all worms present in the body (\( L \) and \( W \)) stop producing Mf or, in the case of immature worms, lose their ability to produce Mf.

**SOLUTION OF THE DIFFERENTIAL EQUATIONS**

For estimating the effects of treatment, we are interested in the relationship between the Mf density \( M \) and time \( t \). By solving the set of differential equations \( A1 \) for \( dL(t)/dt = dW(t)/dt = dM(t)/dt = 0 \), we derived the following relationship for the equilibrium Mf density pretreatment \( M^* \):

\[
M^* = \frac{\beta \gamma}{\mu_1 \mu_2 (\gamma + \mu_1)}
\]  
(A2)

From the moment of treatment onwards, the relationship is given by a nonlinear function:

\[
M(t) = \frac{\beta \gamma}{(\gamma + \mu_1)(\mu_2 - \mu_1)} \left[ \gamma(1 - \delta_i)e^{-\mu_2t} - \frac{\mu_2(\gamma + \mu_1)}{\mu_2 - \mu_1}(\lambda_i - 1)e^{-\mu_1t} \right]
\]  
(A3)

with \( \beta_i \) reflecting the pre-treatment individual force-of-infection. For \( t = 0 \) (i.e., directly after treatment), this becomes:

\[
M(0) = \frac{\beta \gamma (1 - \delta_i)}{\mu_1 \mu_2 (\gamma + \mu_1)}
\]  
(A4)

**ESTIMATION OF MODEL PARAMETERS**

Equations (A2) and (A3) were fitted to the data. Because we have no sound knowledge of the worm load of a person or the Mf production per worm, and because mathematically one of the parameters \( \beta_i \) and \( \rho \) is redundant, we put \( \rho = 1 \) and only estimated \( \beta_i \). Further, we estimated the individual values of \( \delta_i \) and \( \lambda_i \). These parameters were estimated by nonlinear regression, using SAS (v. 8.2). In doing so, we assumed that Mf counts follow a Poisson distribution with overdispersion (i.e., extra-Poisson variation, the variance being a factor \( \theta \) larger than the mean Mf density). The value of \( \theta \) was estimated at 30.9, indicating a high variation in Mf counts. Assuming a negative binomial distribution of Mf counts resulted in a worse fit of the data.

Explorative analyses showed that the individual level parameters did not follow a normal distribution and that efficacy estimates were frequently on the boundaries of the possible range of values (implying full or no effect on Mf or Mf production). Including these parameters as random effects in the model was not useful, and we therefore estimated all parameters as fixed effects.