Viability of adult *Onchocerca volvulus* after six 2-weekly doses of ivermectin*

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Ivermectin is a safe, effective microfilaricide and microfilarial suppressant for *Onchocerca volvulus*; but in single doses of 100–200 μg/kg body weight it has no macrofilaricidal action. The present trial aimed to determine whether 6 doses of 100 μg/kg ivermectin, given at 2-week intervals, would kill the adult worms. Eighty-two nodules from 28 otherwise healthy adult male Liberian patients treated with this ivermectin schedule, and 102 nodules from a similar group of 25 control patients, were removed four months after the last dose of ivermectin. They were coded and assessed in a masked fashion either by routine histology or by examination of whole worms extracted from the nodules after collagenase digestion. The drug had no visible effect on adult male worms. More adult female worms were assessed as moribund or dead in the ivermectin-treated group than in the control group (for the collagenase digests P = 0.09; for the histological assessment P = 0.47). The data suggest that repeated dosage with ivermectin may lead to a slow attrition of some female worms and this possibility should be investigated in patients receiving regular doses every 3, 6 or 12 months as part of onchocerciasis control programmes.

Clinical and community-based trials in Liberia and elsewhere have shown ivermectin to be a safe, single-dose microfilaricide effective against *Onchocerca volvulus* (1, 2). The drug also prevents the escape of intra-uterine microfilariae from the female worm and leads to their degeneration in utero (3). However, when given in a single oral dose of 100–200 μg/kg body weight, ivermectin has shown no evidence of any macrofilaricidal action against *O. volvulus*.

The objective of the present trial was to find out whether a short, intense course of six doses of ivermectin at 100 μg/kg each (i.e., at the minimum effective single-dose microfilaricidal level), given at 2-week intervals, might kill the adult worms of *O. volvulus*.

**Materials and methods**

**Site of the trial, selection of patients and treatment**

The trial was carried out on the Liberian Agricultural Company’s (LAC) rubber plantation at Buchanan, in Grand Bassa County, Liberia, in the forest zone of West Africa. Sixty male plantation workers, aged 21–55 years and weighing 51–72 kg, volunteered to take part after having been made aware of the nature and purpose of the study. All had at least one palpable nodule, and usually several, but were otherwise in good health. Ethical clearance for the trial was obtained from the Liberian Ministry of Health, the World Health Organization, the Case Western Reserve University School of Medicine, and Merck Sharp and Dohme.

The 60 patients were divided at random into two groups of 30. The ivermectin group received six oral doses of ivermectin (scored Mectizan tablets (Merck) containing 6 mg of ivermectin), which were given at 2-week intervals over 10 weeks between 1 August and 10 October 1988. Those...
weighing 51–69 kg received a 6-mg dose and those of 70 kg and over received 7.5 mg, i.e., all treatments were in the range of 87–118 μg/kg.

Chlorpheniramine, a potent antihistamine with sedative properties, which is believed to have no effect on O. volvulus, was selected as a placebo. Patients in the control group received chlorpheniramine tablets, similar in appearance to the Mectizan tablets. A careful watch was kept for serious adverse reactions during and after treatment but none was encountered.

**History of previous treatment; removal of nodules**

All palpable nodules located before treatment and found again at the time of nodulectomy were removed surgically (under local anaesthesia with 2% lignocaine) four months after the last treatment, i.e., 5–20 February 1989. Six patients (five of whom were in the control group) did not report for nodulectomy. A few of the “nodules” removed (including the only one in one patient from the ivermectin group) turned out to be small lymph nodes or lipomata. Two, totally calcified “tombstone” nodules were also excluded from the analysis. In all, 184 O. volvulus nodules were examined from 53 patients.

In the ivermectin group, 28 patients (mean age 33 years; mean weight 59 kg) provided a total of 82 nodules (mean number per patient 2.9; range 1–13). In the control group, 25 patients (mean age 34 years; mean weight 60 kg) provided a total of 102 nodules (mean number per patient 4.1; range 1–12). As shown in Table 1, and as part of the community-based trial then being carried out on the LAC plantation (2), 19 patients in the ivermectin group and 21 of the controls had received single-dose treatment with ivermectin at 150 μg/kg in October 1987 (i.e., nine months before the present trial started and 15 months before the time of nodulectomy) and/or in October 1988.

| Table 1: Patients’ histories of previous ivermectin treatment; and numbers of nodules examined |
|-----------------------------------------------|-----------------|-----------------|
| **Ivermectin-treated**                       | **Controls**    |
| No. of patients                              |                 |
| At outset                                    | 30              | 30              |
| Attending for nodulectomy                    | 29              | 25              |
| Yielding O. volvulus nodules                 | 28              | 25              |
| Previous history of ivermectin treatment:    |                 |
| None                                         | 9               | 4               |
| Single dose (Oct '87)                        | 19              | 13              |
| Single dose (Oct '88)                        | 0               | 4               |
| Single doses (Oct '87 and '88)               | 0               | 4               |
| Total number of O. volvulus nodules examined | 82              | 102             |

* Six 2-weekly doses.

**Fixation and examination of nodules**

Of the 184 O. volvulus nodules removed, 81 were fixed whole in cold 10% buffered formalin (using 20 times the volume of the nodules) for 24 hours. The formalin was then changed and each nodule was bisected to allow better penetration of the fixative. The remaining 103 nodules were fixed whole for 24 hours in a mixture of ethanol 50%, glycerol 10% and water 40% (4) using 20 times their volume of fixative. After 24 hours the fixative was changed and the nodules were stored (without bisection) in the same fluid.

When the nodules arrived at the histological laboratory the patients’ numbers were coded, in order to mask the subsequent examination. The formalin-fixed nodules were embedded in paraffin wax, cut into sections at 6 μm, and stained with haematoxylin and eosin. Six sections per nodule were examined. The alcohol-fixed nodules were washed for 48 hours in water before being placed in a 0.3% solution of collagenase (made up in RPMI 1640 medium, with 2 mg/100 ml gentamycin added) and incubated at 37 °C for 8–24 hours. The contained worms were then extracted and cleaned using fine forceps and gentle squiring with RPMI 1640 from a Pasteur pipette, stained in Mayer’s haemalum and brought up, through ascending strengths of ethanol, into glycerol for unravelling. They were examined first as longitudinal mounts and later as transverse sections (5).

After all the worms had been examined and categorized, the code was broken and the results from the ivermectin and control groups were tabulated.

**Results**

**Classification of dead and moribund O. volvulus worms**

Table 2 gives, for each group of patients, the number of nodules examined, together with the
numbers of male and female worms and the numbers (and percentages) of dead and moribund worms classified according to the system devised by Duke et al. (6) and the results of other workers (7, 8).

Moribund or dead worms included (a) calcified worms; (b) degenerating worms which had lost their turgor and collapsed before being absorbed in a giant cell/fibrotic granuloma; (c) worms, usually old, with a thin body wall and empty degenerate genital tubes (or no genital organs at all), and which were often beginning to be attacked by giant cells; and (d) diseased worms, whose pseudocoelemic cavities were full of basophilic, polymorphic, space-occupying cellular material of uncertain, but probably neoplastic origin, which compressed and destroyed their internal organs. All these changes have been illustrated elsewhere as photomicrographs (6–9).

### Comparison of the histological and collagenase digestion methods for assessment of dead and moribund worms

For ivermectin nodules, histology gave 30/69 (43.8%) females dead or moribund as against 29/99 (29.3%) for collagenase digestion. For control nodules the corresponding figures were 34/93 (36.6%) and 19/105 (15.1%). The difference between the proportions of worms classed as dead or moribund by the two methods was significant ($P = 0.001$, using the Mantel-Haenzel $\chi^2$-test).

### Comparison of worms from the ivermectin and control groups

The mean numbers of female worms per nodule were closely similar in the ivermectin and control groups, whereas the mean numbers of male worms per nodule were slightly higher in the controls (Table 2). However, the difference between the numbers of males in individual nodules in the two groups was not significant ($P = 0.29$ for histology; $P = 0.59$ for collagenase digests, using the Wilcoxon 2-sample sum rank test—normal approximations). The ratios of male to female worms were higher in the controls than in the ivermectin groups, but again the differences were not significant ($P = 0.34$ for histology and $P = 0.47$ for collagenase digests, using the $\chi^2$-test).

Dead male worms were found only in the collagenase-treated nodules—4.5% in the ivermectin group and 3.5% in the control group. The difference was not significant ($P = 0.54$, using Fisher's exact test). By contrast, whichever method of assessment was used, the proportions of moribund and dead female worms in the ivermectin group (43.5% and 29.3%) were higher than in the controls (36.6% and 18.1%). The difference was not significant for the worms examined by routine histology but approached the significant level in those from collagenase-treated nodules ($P = 0.47$ and $P = 0.09$ respectively, using the $\chi^2$-test).

The proportion of all moribund or dead female worms which contained polymorphic basophilic cellular material was higher in the ivermectin groups (16.9%) than in the controls (11.3%) but the difference was not significant ($P = 0.56$, using the $\chi^2$-test). The proportion of moribund or dead females containing polymorphic basophilic cells among all the female worms examined was also higher in the ivermectin group (6.0%) than in the controls (3.0%) but again the difference was not significant ($P = 0.27$, using the $\chi^2$-test). We do not know whether the death of worms resulting from this possibly neoplastic process was in any way influenced or accelerated by multi-dose ivermectin treatment.

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### Table 2: Total numbers of male and female worms in nodules examined, and the numbers dead or moribund

<table>
<thead>
<tr>
<th></th>
<th>Histological examination</th>
<th>Collagenase-extracted worms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ivermectin-treated</td>
<td>Controls</td>
</tr>
<tr>
<td>No. of patients providing nodules</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>No. of nodules examined</td>
<td>34</td>
<td>47</td>
</tr>
<tr>
<td>No. of male worms per nodule (mean)</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>No. of female worms per nodule (mean)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>1:2.46</td>
<td>1:1.81</td>
</tr>
<tr>
<td>No. of male worms (total)</td>
<td>28</td>
<td>52</td>
</tr>
<tr>
<td>No. of males dead</td>
<td>0 (0.0)*</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>No. of female worms (total)</td>
<td>69</td>
<td>93</td>
</tr>
<tr>
<td>No. of females dead</td>
<td>14 (20.3)</td>
<td>18 (19.4)</td>
</tr>
<tr>
<td>No. of females moribund</td>
<td>16 (23.2)</td>
<td>16 (17.2)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are percentages.
Discussion

Comparison of the histological and collagenase-extraction methods

The two methods used, namely (a) histological sections of whole nodules and (b) examination of adult worms extracted by collagenase digestion, may be compared for their ability to assess the possible macrofilaricidal action of a drug, such as ivermectin, which also has effects on embryogenesis. Histological assessment suffers from the disadvantage that it is difficult in multi-worm nodules to be certain which of the cut coils belong to which worm; on the other hand it is relatively easy to make out where worms have died, even when their bodies have almost completely disappeared in a fibrotic granulomatous reaction. The collagenase digest method makes it possible to study each individual worm completely and to determine, sequentially and to an extent quantitatively, the type and condition of the ova or embryos along the whole length of the genital tracts (5, 6, 9). On the other hand, dead worms that are fragmented or have degenerated are sometimes difficult to see and some probably escape detection in the digests.

The proportions of moribund and dead females detected by the two methods were compared and the difference found to be statistically significant. Histological assessment apparently detected a higher proportion of worms which had been dead for a long time and whose remnants had almost disappeared. It also led to the classification of more worms as moribund owing to the easier detection of giant cell adherence—an important sign that could only rarely be assessed after collagenase digestion. Histology is probably the better method for detecting macrofilaricidal action per se, but it can very usefully be supplemented by examination of collagenase-extracted worms to assess accompanying changes in embryogenesis. Since alcohol-fixed worms give satisfactory results by histology even when the nodules have not been bisected, it may be advantageous to collect material in this way, when assessing the action of drugs on adult worms, for then either method of examination can subsequently be used.

Influence of previous ivermectin treatment

Because a community-based, large-scale treatment programme with ivermectin had been started on the LAC plantation in 1987, it was not possible exclusively to select control subjects who had never previously received ivermectin.

Although 19/28 patients (68%) in the group treated with multi-dose ivermectin had received a single dose of the drug (150 μg/kg) in October 1987, nine months before the present trial started (or 15 months before nodulectomy), this treatment would only be expected to have potentiated any macrofilaricidal action of the drug in the multi-dose trial. However, in the control group, an identical proportion (17/25 or 68%) had also received a similar dose of ivermectin at the same time interval before the present trial began. There was thus a balance between the two groups with regard to the 1987 dosage with ivermectin; and the single dose received by eight of the control patients in October 1988 could hardly be expected to have had any macrofilaricidal effect.

Effect of multi-dose ivermectin on the viability of adult female worms

The proportion of dead females in the collagenase digests from the controls (12/105 or 11.4%) was of the same order as the 12% found in other natural untreated forest populations of Liberian O. volvulus (10). This suggests that we were dealing with a worm population having an age distribution that was usual for the area, and that our assessments of death from old age were similar to those of previous workers.

No statistically significant difference was found by histological examination or by collagenase digestion between the combined proportions of moribund and dead females in the ivermectin and control groups. Nevertheless there were always relatively more moribund and dead females in the ivermectin groups than in the controls and this difference extended also to those worms dying from presumed neoplastic change.

Effects of multi-dose ivermectin on the adult male worms

Multi-dose ivermectin had no apparent macrofilaricidal effect on the male worms. However, as has been noticed in other trials (9, 11, 12), the mean number of male worms per nodule, and hence the ratio of male to female worms, were somewhat lower in the ivermectin groups than in the controls. Although the reduction in the number of males was not statistically significant, this is a parameter that should be watched in future ivermectin trials, for it could have important consequences, enhancing the anti-filarial effect of ivermectin (9).

Conclusion

Our results provide no statistically significant evidence of a macrofilaricidal effect on O. volvulus following six doses of ivermectin (100 μg/kg) given at intervals of two weeks. To this extent they agree with the negative macrofilaricidal results from multidose treatment of O. cervicalis infections in horses.
(13), but contrast with the significant increase in the numbers of dead and moribund *O. volvulus* worms of both sexes found after 12 monthly doses of ivermectin at 150 μg/kg in humans in Guatemala (9).

However, the tendency in the present trial was always for there to be more dead and moribund females in the ivermectin-treated group regardless of the method of assessment used. We consider that persistent retreatment with ivermectin, at optimum intervals yet to be determined, may cause a slow attrition of certain members of the female worm population which, for some reason, are more susceptible to the drug (9). This possibility merits further investigation in future trials when repeated doses of the drug are given at 3–12-month intervals in order to suppress macrofilariae in the skin and eye.

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**Résumé**

Viabilité des formes adultes d’*Onchocerca volvulus* après 6 doses d’ivermectine administrées à 2 semaines d’intervalle

L’ivermectine est un microfilaricide sûr et efficace et est également capable d’inhiber la ponte de nouvelles microfilaries d’*Onchocerca volvulus* mais, administrée en une dose unique de 100 à 200 μg/kg, elle n’a pas d’effet macrofilaricide. Le présent essai était destiné à déterminer si 6 doses de 100 μg/kg d’ivermectine, administrées à 2 semaines d’intervalle, tuerait les vers adultes.

Quatre mois après la dernière dose, 82 nodules ont été prélevés chez 28 Libériens adultes de sexe masculin par ailleurs en bonne santé et 102 nodules ont été prélevés chez un groupe analogue de 25 sujets témoins n’ayant pas reçu le traitement. Les nodules ont reçu un numéro de code et l’état des vers adultes qu’ils contenaient a été évalué en anomyne, par histologie classique ou par examen de coupes longitudinales et transversales de vers entiers extraits des nodules après digestion par la collagénase.

Le médicament n’avait pas d’effet visible sur les vers adultes mâles contenus dans les nodules, mais on a observé une chute du nombre relatif de vers mâles après le traitement, ce qui pourrait indiquer que certains vers mâles aient quitté les nodules sous l’effet du médicament.

Les vers femelles ont été classés comme vivants, moribonds ou morts au moment de la nodulectomie. Dans ces deux dernières catégories ont été inclus les vers calcifiés, les vers dégénérés avec perte de la turgescence, nécrose et coalescence des cellules géantes, les vers âgés à parois amincies et voies génitales dégénérées et vides, et enfin les vers malades présentant probablement des altérations néoplasiques.

Bien que les vers femelles morts et moribonds aient été plus nombreux dans le groupe traité par l’ivermectine que chez les témoins, les différences n’étaient pas statistiquement significatives (P = 0,47 pour l’évaluation histologique; P = 0,09 pour l’examen après digestion par la collagénase). Il semble néanmoins que l’administration de doses répétées d’ivermectine pourrait conduire à une élimination lente de certains vers particulièrement sensibles, possibilité qui devra faire l’objet d’une étude lors de programmes de traitement mensuel sur 3 à 12 mois.

**References**


