Short Report

# *In vitro* effects of ivermectin on *Onchocerca volvulus* microfilariae assessed by observation and by inoculation into *Simulium damnosum sensu lato*

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Ivermectin has proved to be an effective microfilaricide against *Onchocerca volvulus*. A single oral dose of 150 μg/kg results in a peak plasma concentration of 40–50 ng/ml 4 h after treatment and will reduce numbers of microfilariae (mf) in the skin to almost zero (AWADZIE *et al.*, 1985; EDWARDS *et al.*, 1988). To determine the direct effect of ivermectin on mf *in vitro* the following experiments were carried out.

**To determine the concentrations of ivermectin needed to kill microfilariae *in vitro***

Microfilariae were collected after emergence from skin taken during nodulectomy and incubated for 1–2 h at room temperature in a solution of 199 medium plus 20% foetal calf serum, antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) and an anti-fungal agent (0·25 μg/ml amphotericin). After resuspension in clean solution, between 20 and 40 mf were introduced into microtitre wells containing different concentrations of ivermectin made up from a 1 mg/ml solution of ivermectin powder in propylene glycol, diluted in the same 199 medium. The mf were incubated in these solutions for 24 h or longer at 37°C. At about 6 h intervals, the numbers of dead (immobile) mf were recorded. Each ivermectin concentration was tested in duplicate or triplicate. Observations were compared with mf incubated in control wells containing the same medium without ivermectin. In the first series of trials 5 concentrations between 10 and 50 ng/ml (in the range of concentrations found in plasma) were used (Table 1). It soon became apparent that much higher concentrations were needed to kill mf *in vitro* under these conditions (Table 2).

**Table 1. Effect of low concentrations of ivermectin on *Onchocerca volvulus* microfilariae *in vitro* at 37°C**

| Ivermectin (ng/ml) | No. of microfilariae | Mortality (%) 24 h | 36 h | 48 h |
|---|---|---|---|---|
| 10 | 27 | 0 | 15 | 37 |
| 20 | 28 | 0 | 29 | 46 |
| 30 | 30 | 0 | 10 | 37 |
| 40 | 28 | 0 | 11 | 61 |
| 50 | 31 | 0 | 16 | 52 |
| Control | 30 | 0 | 7 | 33 |

**Table 2. Effect of higher concentrations of ivermectin on *Onchocerca volvulus* microfilariae *in vitro* (24 h at 37°C)**

| Ivermectin (ng/ml) | No. of microfilariae | Corrected mortality (%)[a] |
|---|---|---|
| 5000 | 111 | 23·8 |
| 8000 | 76 | 38·7 |
| 10000 | 74 | 53·5 |
| 12000 | 110 | 73·2 |
| 15000 | 96 | 78·3 |
| 17000 | 95 | 79·0 |
| 20000 | 82 | 86·5 |
| 25000 | 110 | 91·3 |
| 30000 | 65 | 100·0 |

[a]Using Abbott's correction.

**To determine the concentration of ivermectin needed to impair the infectivity of microfilariae when injected into *Simulium damnosum s.l.***

In a series of experiments, mf were introduced into different concentrations of ivermectin as in the first series of experiments, before inoculation into wild-caught *S. damnosum* from a site where the predominant species was *S. soubrense* 'B'. In each experiment a control was included in which the mf were incubated in medium without ivermectin. The incubation was carried out at 4°C for half an hour because it had been found during preliminary experiments that nearly all mf incubated in control medium at 37°C for 1 h or longer failed to develop after inoculation into the flies. The lower temperature and reduced incubation time greatly improved the proportion of larvae that developed after inoculation. After incubation the mf were resuspended in fresh medium without ivermectin and centrifuged. This step was vital because the high concentrations of ivermectin used in some experiments meant that sufficient ivermectin could be carried over in the inoculum to be lethal to *S. damnosum*.

Mf were injected into the thorax of *S. damnosum* in quantities of 5, 10 or 15 mf per fly using the technique described by HAM & BIANCO (1983). The flies were then maintained for 4 d at room temperature, after which they were preserved in 80% ethanol, stained with Mayer's haemalum and later dissected in 50% acetic acid. The number of developing larvae in the thoracic muscles was counted and compared with the number developing in flies injected with control mf. Eight trials of 4 concentrations were tested (Table 3).

**Conclusions**

The concentrations of ivermectin needed to kill all mf *in vitro* were about 1000 times higher than those found in the plasma. This may indicate that the lethal effect *in vivo* of ivermectin is not due to the direct action of the drug on mf, or that the effect is not apparent over the 24–48 h observation time used (longer incubation led to high control mortalities).

Concentrations of ivermectin that have no effect *in vitro* on mf mortality over 24 h at 37°C inhibit mf development in *S. damnosum s.l.* even after incubation for only 30 min at 4°C. This suggests that infectivity

**Table 3. Numbers of *Onchocerca volvulus* $L_1$ and $L_2$ larvae recovered 4 days after inoculation of microfilariae into *Simulium damnosum* following incubation in various concentrations of ivermectin for 30 min at 4°C**

| Trial | Ivermectin (ng/ml) | Microfilariae inoculated per fly | Flies dissected | Larvae found | Percentage developed |
|---|---|---|---|---|---|
| 1 | 5000 | 5 | 17 | 0 | 0·0 |
| | Control | 5 | 19 | 15 | 15·8 |
| 2 | 5000 | 10 | 10 | 0 | 0·0 |
| | Control | 10 | 10 | 4 | 4·0 |
| 3 | 5000 | 10 | 4 | 0 | 0·0 |
| | Control | 10 | 11 | 3 | 2·7 |
| 4 | 1000 | 5 | 14 | 0 | 0·0 |
| | Control | 5 | 10 | 5 | 10·0 |
| 5 | 1000 | 10 | 21 | 0 | 0·0 |
| | Control | 10 | 16 | 56 | 35·0 |
| 6 | 100 | 15 | 21 | 2 | 0·6 |
| | Control | 15 | 16 | 130 | 54·2 |
| 7 | 100 | 5 | 18 | 1 | 0·1 |
| | Control | 5 | 13 | 18 | 27·7 |
| 8 | 50 | 5 | 17 | 20 | 23·5 |
| | Control | 5 | 26 | 63 | 48·5 |

is a more sensitive bioassay technique than measuring mortality *in vitro*. Clearly this technique should be investigated further.

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