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RESEARCH NOTES

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Ivermectin Inhibits Molting of *Wuchereria bancrofti* Third Stage Larvae In Vitro

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ABSTRACT: The effect of ivermectin or diethylcarbamazine (DEC) on *Wuchereria bancrofti* molting from the third to the fourth larval stage (L_3 to L_4) was evaluated in vitro. L_3 larvae were harvested from laboratory-reared *Aedes togoi* 2 wk after feeding upon a microfilaremic human volunteer. The larvae were kept in an artificial medium (Franke's NI medium) with 10% human serum under an atmosphere of 5% CO_2 for 20 days. Experimental tubes also contained ivermectin (0.1–1,000 ng/ml) or DEC (0.1–10,000 ng/ml). An estimated concentration of 50 ng/ml ivermectin inhibited molting in 50% of the larvae expected to molt. For DEC, this value was roughly 1,000 ng/ml. In this in vitro culture system, ivermectin inhibited the L_3 to L_4 molt of *W. bancrofti* and was roughly 20-fold more potent in this activity than DEC.

Humans suffering infection of the dermis and conjunctiva by microfilariae of *Onchocerca volvulus* have enjoyed marked therapeutic advantages with ivermectin versus diethylcarbamazine (DEC). Although ivermectin and DEC both greatly diminish numbers of viable microfilariae, ivermectin appears much less likely to elicit the potentially maiming and life-threatening Mazzotti reaction that occasionally marks therapy with DEC. Ivermectin shares with DEC chemoprophylactic activity against *Dirofilaria immitis* in dogs (McCall et al., 1984) and against *Onchocerca lienalis* in vitro, i.e., inhibition of L_3 molting (Court et al., 1985; Lok et al., 1987). However, Taylor et al. (1988) found that ivermectin failed to prevent infection of chimpanzees by *O. volvulus*. In view of a possible chemoprophylactic role for ivermectin against bancroftian filariasis in humans, we evaluated the effects of ivermectin on the molting of L_3 larvae of *Wuchereria bancrofti* in vitro.

Third stage larvae of *W. bancrofti* were harvested from laboratory-reared *Aedes togoi* 14 days after feeding upon a microfilaremic human volunteer living in Jakarta, Indonesia. The larvae were harvested and cultured as described by Franke et al. (1987), except that ivermectin or

DEC was added to the culture medium (Frank's NI medium with 10% human serum). The drugs were present in the culture medium through the duration of the experiments. Negative controls consisted of at least 2 tubes of 20–25 larvae per tube cultured in medium lacking drugs. These controls were run with each experiment in which effects of drugs were evaluated. Tubes for evaluation of effects of drugs also contained 20–25 larvae, and for each determination the duplicate sets of tubes were run on at least 2 occasions. Experiments were terminated after 20 days, soon after most control larvae had molted. The percent molt among drug-treated larvae was calculated using the following equation, where T denotes test condition (i.e., drug present) and C denotes negative control: $\% \text{ molt} = \{L_4T \div [(L_4C \div (L_4C + L_3C))(L_3T + L_4T)]\} \cdot 100$.

Inclusion of the proportion of negative control larvae molting in the denominator of the % molt calculation eliminated effects of drug-independent inhibition of molt. In all experiments, this proportion ranged between 0.75 and 1.0, i.e., the proportion of larvae expected to molt in the absence of drug effects. Most L_3 larvae in either test or control tubes were living when the experiment was terminated. Mortality specifically attributable to drugs was not documented.

Figure 1 illustrates the dose-dependent inhibition of molting by ivermectin or DEC. Neither drug caused appreciable inhibition below 10 ng/ml. The estimated dose for inhibiting molt among half the larvae was 50 ng/ml for ivermectin, and 1,000 ng/ml for DEC. As much as 500 ng/ml ivermectin was required to inhibit molt completely, and this occurred only at the highest concentration of DEC (10,000 ng/ml).

Just what the results in Figure 1 mean in terms of how the drugs may act in humans is uncertain. There are several distinct concerns in this regard, foremost among them are questions regarding

FIGURE 1. Molting of the third stage larva (L3) of *Wuchereria bancrofti* in vitro in the presence of increasing concentrations of ivermectin or diethylcarbamazine (DEC). Each point is the mean of at least 2 replicates measured on 2 occasions. The line drawn for each drug is a best-fit curve.

activity of metabolites of ivermectin versus parent. Fink and Porras (1989) found that appreciable proportions of ivermectin were metabolized rapidly (<5 hr) and that after a single therapeutic dose, metabolites persisted at about 50 ng/ml human plasma for at least 3 days whereas less than 5 ng parent ivermectin remained per ml plasma after just 24 hr. From the pharmacokinetic point of view, hope for chemoprophylactic activity of ivermectin lies in its longer lived metabolites. Another concern is the possibility of sequestration of ivermectin in glass and plastics as reported by Rew et al. (1986). Unless the concentration of drug in the final incubation medium actually is measured, it is not possible to know the concentration available to act upon the parasites.

In summary, we have shown that ivermectin inhibits a molt of *W. bancrofti* that occurs in human tissues as part of the development of this parasite. Ivermectin was roughly 20-fold more effective than DEC in this activity. Studies evaluating the effects of the human metabolic derivatives of ivermectin are required to evaluate the

potential of this drug as a chemoprophylactic agent against bancroftian filariasis.

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