A New Ivermectin Formulation Topically Kills Permethrin-Resistant Human Head Lice (Anoplura: Pediculidae)

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ABSTRACT This study examines the effectiveness of a new ivermectin formulation for the topical treatment of the human head louse, *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae). Permethrin-resistant lice originally obtained from south Florida and maintained on an in vitro rearing system were 100% susceptible to ivermectin formulations by using a semiclinical hair tuft bioassay. The formulation was 100% effective at killing lice using 1, 0.5, and 0.25% ivermectin concentrations after 10-min exposures. As judged by the lethal time $(LT)_{50}$ and LT_{95} values, 0.5% formulated ivermectin was 3.8 and 3.2 times faster at killing lice, respectively, than 0.5% nonformulated ivermectin, indicating that the formulation may facilitate the penetration of ivermectin into the louse. The hair tuft-based bioassay in conjunction with the in vitro rearing system provides a standardized method to assess the comparative efficacy of pediculicide formulations in a reproducible format that mimics the exposure scenario that occurs on the human scalp.

KEY WORDS *Pediculus humanus capitis*, human head louse, in vitro rearing system, ivermectin formulation

The human head louse, *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae) is an ectoparasitic insect that causes prevalent infestations of humans in the United States and elsewhere (Gratz 1997). It is estimated that 2.6 million U.S. households are affected with 8% of all schoolchildren infested. Most people find head lice intolerable, and they often repeatedly and prophylactically apply costly pediculicides (insecticides) without realizing the potential harm and lethality if misused or overused. Overapplication impacts children, in particular, due to their small size and higher sensitivity to the toxic effects of these pediculicides (NRC 1993). Louse infestations are irritating, and they can lead to secondary infection. Moreover, social, mental, and economic consequences of recurring infestations are substantial (Chosidow et al. 1994; Meinking 1999; Meinking et al. 2001, 2002).

In the United States, over-the-counter (OTC) pediculicidal products are almost exclusively limited to those that contain the natural botanical insecticides, the pyrethrins, or to those that contain a pyrethroid, permethrin, as the active ingredient. There are only two U.S. Food and Drug Administration (FDA)-approved prescription medications available for the treatment of pediculosis; one medication contains 1% lindane and the other medication contains 0.5% malathion (e.g., Ovide). All insecticides are potentially toxic to the target group of largely pediatric patients who most commonly become infested with head lice, according to recent FDA guidelines (FDA 2003).

Over the past two decades, many investigations have established that resistance to currently used pediculicides, including permethrin, synergized pyrethrins, and malathion, has become a serious problem worldwide (Chosidow et al. 1994; Burgess et al. 1995; Mumcuoglu et al. 1995; Rupes et al. 1995; Picollo et al. 1998; Downs et al. 1999a, 1999b; Hemingway et al. 1999; Lee et al. 2000; Meinking et al. 2001, 2002; Vassena et al. 2003; Yoon et al. 2003; Yoon et al. 2004, 2006). There are two ways to combat pediculosis: proactive prevention or postinfestation treatment. Emphasis is increasingly on prevention (education) and physical removal (combing or shaving) because a crisis exists in the chemical management of pediculosis. The pediculicide arsenal is limited and shrinking, and health providers are spending an increasing and inordinate amount of time and resources dealing with infestations. Effective management information is limited and few, if any, alternatives exist when standard pediculicide treatments fail. Thus, there is a great demand for new pediculicidal formulations that possess novel chemistries, unique modes of action and are appropriately safe to treat children.

The avermectins are 16-membered macrocyclic lactones with four structurally related avermectins (avermectin A_1, A_2, B_1, and B_2) produced by the soil microorganism *Streptomyces avermitilis* (Fisher and Mrozik 1984). In general, the avermectin B_{1a}, B_{1b}, B_{2a}, or B_{2b} is biologically more active than avermectin A_{1a},

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A_{1b}, A_{2a}, or A_{2b}. Furthermore, the avermectins with B_1 components are commercially more important because those with B_2 are inactive in some insect pest species, such as nematode *Hemonchus contortus.*

Two avermectin mixtures that are highly similar have been commercially successful. Abamectin (MK-0936) is sold under the trade names Abba, Agri-Mek, Zephyr, Affirm, Avid, Dynamec, and Vertimec to treat mites, leaf miners, pear psylla, and other insect pests. Ivermectin (MK-0933) has been sold under the trade names Stromectol and Mectizan to treat *Onchocerca volvulus,* a skin dwelling microfilariae causing human onchocerciasis (river blindness), and *Dirofilaria immitis.* Both are mixtures of avermectins containing ≥80% avermectin B_{1a} and ≤20% avermectin B_{1b}. The chemical structure of abamectin differs from that of ivermectin by only a single double bond between C22 and C23 in abamectin.

Unique insecticidal and anthelmintic properties of the avermectins have been reported previously (Burg et al. 1979, Egerton et al. 1979, Ostlind et al. 1979, James et al. 1980, Putter et al. 1981), and avermectin B_{1a} was found to be highly toxic to several insect pests in the orders Coleoptera, Homoptera, Diptera, Orthoptera, Isoptera, Hymenoptera, and Lepidoptera. Yoon et al. (2004) reported that abamectin killed human head lice and that to date no resistance or cross-resistance had occurred. Campbell et al. (1983) reported high antiparasitic activity of ivermectin, and they suggested the use of ivermectin against *O. volvulus.* In clinical trials (phase I–III) for the treatment of onchocerciasis in Africa (1982–1987), a single annual dose of ivermectin (150 μg/kg) was determined to be effective, although ivermectin was not a permanent cure for onchocerciasis (Greene et al. 1989).

Several mechanisms of action for avermectin B_{1a} have been investigated. Initially, Fritz et al. (1979) reported that avermectin B_{1a} increased membrane permeability to chloride ions due to either an agonistic interaction with α-GABA binding sites or the regulation of presynaptic GABA release. Pong and Wang (1982) first identified a high-affinity binding site of avermectin B_{1a} in the mammalian cerebellum and reported that avermectin B_{1a} stimulated GABA binding. Huang and Casida (1997) investigated avermectin B_{1a} binding on GABA-gated Cl^- channel, and they found high- and low-affinity sites in cultured cerebellar granule neurons. An additional site of action for avermectin B_{1a} is at the glutamate-gated Cl^- channels, which are members of the inhibitory ligand-gated anion channel found in invertebrates (Cully et al. 1994, 1996; Rohrer and Arena 1995; Etter et al. 1996; Blackhall et al. 1998).

Although ivermectin contains polar functional groups, such as sugar moieties and hydroxyl groups, it has a limited water solubility of ≈5 μg/ml (0.00004%, wt:vol) at room temperature. Uniquely, ivermectin also has limited solubility (<0.1%, wt:vol) in apolar organic solvents, such as cyclohexane, *n*-hexane, and isooctane (Lo et al. 1985). Topical formulations of ivermectin have been solubilized in mixtures of propylene glycol and glycerol formal. Glycerol formal is the product of the condensation reaction between glycerol and formic acid. The solubility of ivermectin in glycerol formal is ≥20% (wt:vol). Topical treatment with ivermectin formulations have been used to treat ectoparasites, such as scabies and human body and head lice (Youssef et al. 1995). Aqueous formulations of ivermectin also have been made, but they are not effective as topical applications for treatment of ectoparasites because the waxy exoskeleton of the ectoparasite is impermeable to aqueous substances.

The worldwide problem of chemical resistance in human head lice to currently available pediculicide formulations containing pyrethrins, permethin, and malathion has led to research on a new head lice formulation containing ivermectin necessitating a chemical with a mode of action that lice have not developed a resistance mechanism. In this study, we tested formulated ivermectin at various concentrations and exposure intervals. Our results were compared with two commercially available pediculicidal products, Nix and Ovide. Using our semiclinical hair tuft bioassay in conjunction with our in vitro rearing system, we were able to directly compare the different products in a reproducible format that mimics the exposure scenario that occurs on the human scalp.

Materials and Methods

Human Head Lice. The SF-HL strain of permethrin-resistant *P. h. capitis* was collected from infested children in Plantation and Homestead, FL, and lice were maintained on an in vitro rearing system at the University of Massachusetts at Amherst, MA, as described by Yoon et al. (2006). Lice have been maintained without a human host on the in vitro rearing system for 24–36 generations. Permethrin-resistant SF-HL have been selected periodically using 1% permethrin-treated filter papers (Yoon et al. 2006). Filter papers (35 mm in diameter, Whatman no. 1, Whatman, Maidstone, United Kingdom) were immersed into 1% permethrin dissolved in acetone (wt:vol) for 10 s and air-dried in a dark fumehood for 20–30 min. Mixed developmental stages (first instars to adult) were placed on the treated filter paper and exposed for 5 h. Surviving lice were transferred back into the rearing system. The SF-HL strain has been determined previously to be susceptible to Ovide (0.5% malathion) but resistant to Nix (1% permethirn) and cross-resistant to DDT treatments (Yoon et al. 2003).

Chemicals. Ivermectin (MK-0933) formulations were prepared by Particle Science Inc. (Bethlehem, PA) and supplied through Topaz Pharmaceuticals LLC (Jenkintown, PA) in 1, 0.5, and 0.25% ivermectin concentrations in a formulation containing deionized water, olive oil USP, surfactants, shea butter, sorbitan tristearate, methylparaben, and propylparaben. Nix formulation (Pfizer, Morris Plains, NJ) containing 1% permethrin (vol:vol), Ovide formulation (Taro Inc., Hawthorne, NY) containing 0.5% malathion (vol:vol) and nonformulated ivermectin (Chem Service, West Chester, PA) were used as positive controls. Placebo formulation (ivermectin formulation sans ivermectin)

was used as a negative control and distilled, deionized water (ddH_2O) was used as a no treatment control.

Bioassays. Mortality bioassays were performed to determine lethal time to 50% mortality (LT_{50}) values of various ivermectin formulations for comparison to LT_{50} values of Nix and Ovide (Fig. 1). All lice used in experiments were newly hatched first instars (<24 h old), randomly taken from the in vitro rearing system, which had taken a bloodmeal overnight. Lice (30 lice per treatment) were placed on an individual hair tuft (≈300 strands, ≈4 cm in length) by using sterile forceps. All treatments were performed for three replicate experiments. The treatment was dispensed on a sterile glass plate and the hair tuft containing lice was gently rubbed into the treatment using several circular motions until saturation occurred. After a 10 min exposure period, the hair tuft with lice was washed sequentially in three separate water baths, containing ≈10 ml of ddH_2O for 5 s each. The washed hair tuft with lice was blotted onto stacks of filter paper and air-dried for 5 min. Any lice dislodged during treatment or washing were placed back onto the treated hair tuft. After drying, the treated hair tuft with lice was examined under a dissecting microscope, and the number of dead lice was recorded. A louse was considered dead if it could not right itself when inverted and when its legs had ceased all movements when probed. Treated hair tufts with lice were then placed onto the feeding membrane in the in vitro rearing system and maintained at 31°C and 75% humidity. The numbers of dead lice were reassessed at 10-min intervals until >90% mortality was achieved. The timing for mortality began immediately after the 10-min exposure period. Because of this, mortality was not assessed during the washing and drying intervals (≈5 min).

Log time versus logit percentage of mortality regression lines were generated to determine LT_{50} and LT_{95} values for all treatments and controls. Maximum log-likelihood ratio tests were performed on the regression lines to test the equality (slope and intercept) between treatments and controls. The null hypothesis of the maximum log-likelihood ratio test states that the regression lines being compared are equal. The null hypothesis was rejected at a P value <0.05 (Polo PC, LeOra Software 1987).

To determine whether ivermectin in formulation performed better than ivermectin alone, blood-fed lice were placed onto 0.5% (wt:vol) ivermectin formulation-treated hair tufts as described above. Blood-fed lice were also placed onto hair tufts that were treated with 0.5% ivermectin in acetone (acetone was allowed to volatilize in a fumehood for 1 h before transferring lice to the treated hair tuft), washed, and transferred to the in vitro rearing system as described above.

Results

Mortality Responses after 10-min Exposures. The mortality responses of SF-HL after a 10-min exposure to all three of the ivermectin formulations (1, 0.5, and 0.25%) were significantly different compared with lice exposed only to ddH_2O ($\chi^2 = 90.1$, df = 2, $P < 0.001$; $\chi^2 = 199.5$, df = 2, $P < 0.001$; and $\chi^2 = 213.5$, df = 2, $P < 0.001$, respectively) (Fig. 2). At the LT_{50} and LT_{95}, 1% ivermectin formulation was 426 and 491 times faster than ddH_2O treatments, respectively (Table 1). The 0.5% ivermectin formulation was 369 and 331 times faster and the 0.25% ivermectin formulation was 180 and 207 times faster than the ddH_2O treatments, respectively (Table 1). The mortality response to Nix was significantly different compared with ddH_2O ($\chi^2 = 74.7$, df = 2, $P = 0.001$). At the LT_{50} and LT_{95}, Nix was 3.5 and 2.0 (estimated) times faster than ddH_2O treatment, respectively (Table 1). The mortality response to the placebo (sans ivermectin) formulation was significantly different compared with ddH_2O ($\chi^2 = 53.3$, df = 2, $P < 0.001$) (Fig. 2). At the LT_{50} and LT_{95}, placebo formulation was 3.1 and 1.3 times faster than ddH_2O treatment, respectively (Table 1). The mortality response to the nonformulated 0.5% ivermectin treatment was significantly different compared with ddH_2O ($\chi^2 = 168.1$,

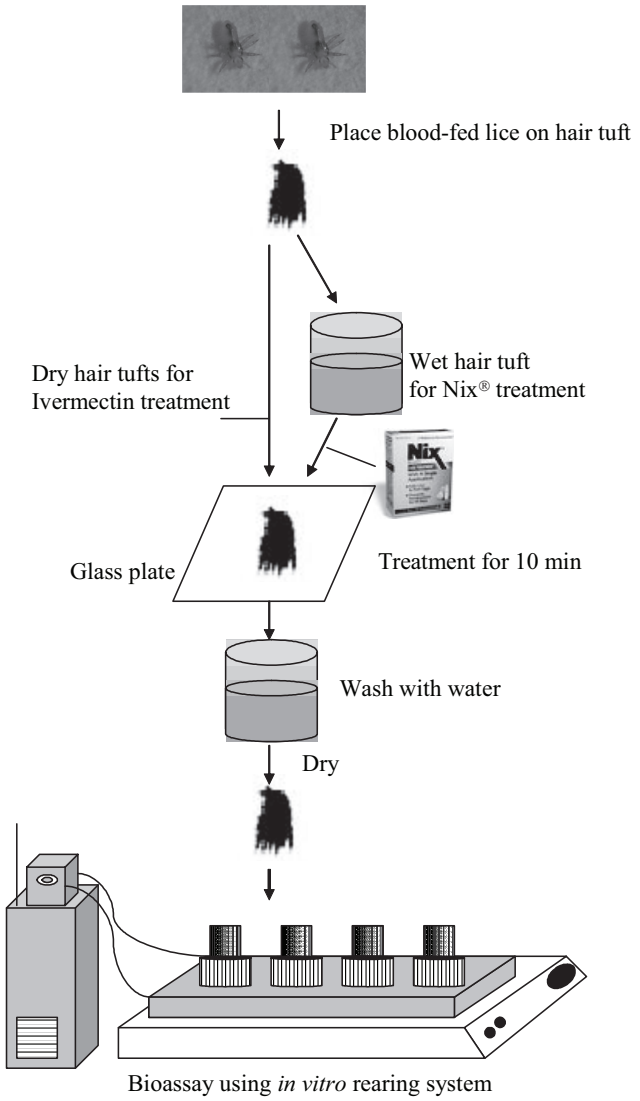

Fig. 1. Semiclinical bioassay procedure performed on the in vitro rearing system to determine mortality of head lice from south Florida (SF-HL) to ivermectin formulation.

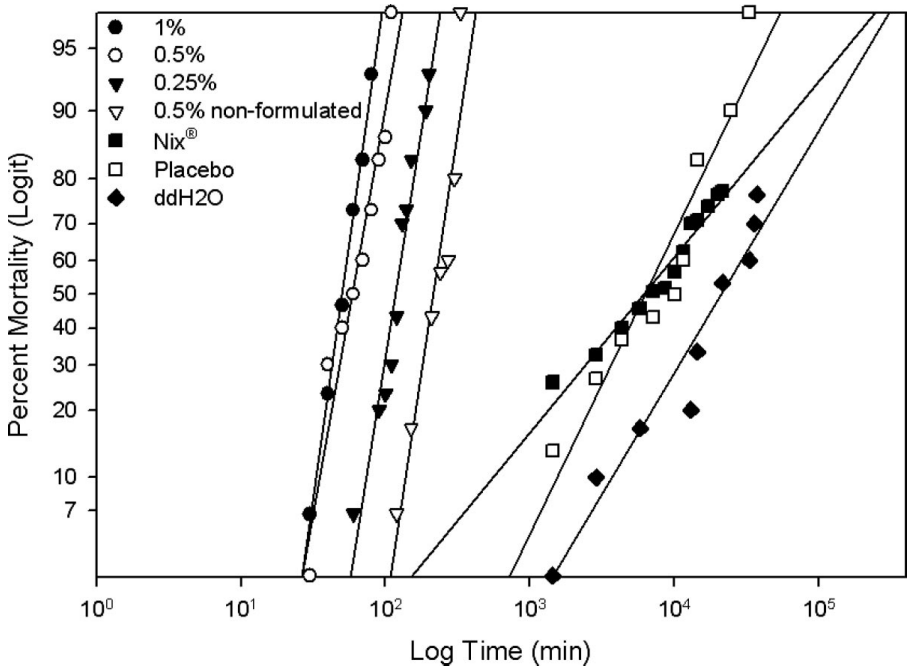

Fig. 2. Log time versus logit percentage of mortality of permethrin-resistant human head lice from south Florida (SF-HL) after a 10-min exposure to ivermectin and Nix formulations.

df = 2, $P < 0.001$) (Fig. 2). At the LT_{50} and LT_{95}, nonformulated 0.5% ivermectin was 96.7 and 103.0 times faster than ddH_2O treatment, respectively (Table 1).

The mortality responses to all three of the ivermectin formulations were significantly different compared with the placebo formulation ($\chi^2 = 157.5$, df = 2, $P < 0.001$; $\chi^2 = 178.8$, df = 2, $P < 0.001$; $\chi^2 = 190.0$, df = 2, $P < 0.001$, respectively). At the LT_{50} and LT_{95}, 1% ivermectin formulation was 137 and 393 times faster than placebo formulation, respectively (Table 1). The 0.5% ivermectin formulation was 119 and 265 times faster and the 0.25% ivermectin formulation was 58 and 165 times faster than the placebo formulation, respectively (Table 1). The mortality response to Nix was significantly different compared with the placebo formulation ($\chi^2 = 7.4$ df = 2, $P < 0.025$). At the LT_{50} and LT_{95}, Nix was 1.1 and 1.6 (estimated) times faster than placebo treatment, respectively (Table 1). The mortality response to nonformulated 0.5% ivermectin was significantly different compared with the placebo formulation ($\chi^2 = 143.8$ df = 2, $P < 0.001$). At the LT_{50} and LT_{95}, nonformulated 0.5% ivermectin was 31.2 and 82.4 times faster than placebo treatment, respectively (Table 1).

The mortality responses to all three of the ivermectin formulations were significantly different compared with the Nix ($\chi^2 = 211.8$, df = 2, $P < 0.001$; $\chi^2 = 244.0$, df = 2, $P < 0.001$; and $\chi^2 = 244.0$, df = 2, $P < 0.001$,

Table 1. Comparison of median lethal time (LT_{50} and LT_{95}, minutes) and slope values determined from log time versus logit mortality regression lines obtained using the hair tuft bioassay of the permethrin-resistant (SF-HL) and permethrin-susceptible (EC-HL) head louse populations treated with ivermectin, Nix, and Ovide formulations

Treatment	LT_{50} (CL)[a]	LT_{95} (CL)[a]	Slope
1%	50.4 (46.5–54.1)	88.0 (78.7–104.5)	12.1 ± 1.7
0.5%	58.1 (52.9–63.0)	130.4 (113.8–158.7)	8.4 ± 1.0
0.5% (5-min exposure)	93.7 (82.9–103.6)	196.7 (168.8–249.1)	8.5 ± 1.1
0.5% (3-min exposure)	204.7 (176.7–236.8)	426.4 (359.3–556.7)	
0.25%	119.3 (112.7–125.9)	208.9 (189.2–241.5)	12.1 ± 1.5
0.5% (nonformulated)	221.8 (204.5–238.5)	419.4 (363.9–532.5)	10.6 ± 1.5
Placebo (formulation sans ivermectin)	4.8 d (3.6–6.2)	24 d	3.3 ± 0.4
Nix[b]	4.3 d (3.9–4.8)	>15 d	2.1 ± 0.17
Nix (EC-HL)[c]	177.7 (126.1–223.3)	357.0 (282.7–628.6)	9.7 ± 1.8
ddH_2O	14.9 d (12.0–18.8)	30 d	3.1 ± 0.5
Ovide[d]	<5.0	<5.0	

[a] CL, 95% confidence interval limit.
[b] Nix LT_{50} values for SF-HL and EC-HL are from historical data previously determined using the same experimental bioassay (Yoon et al. 2006).
[c] EC-HL are permethrin-susceptible lice collected from Kuna Indians in Ecuador.
[d] LT_{50} and LT_{95} values following Ovide treatments are estimated times since log time versus logit mortality responses curves were not generated given the fast response times (see Materials and Methods).

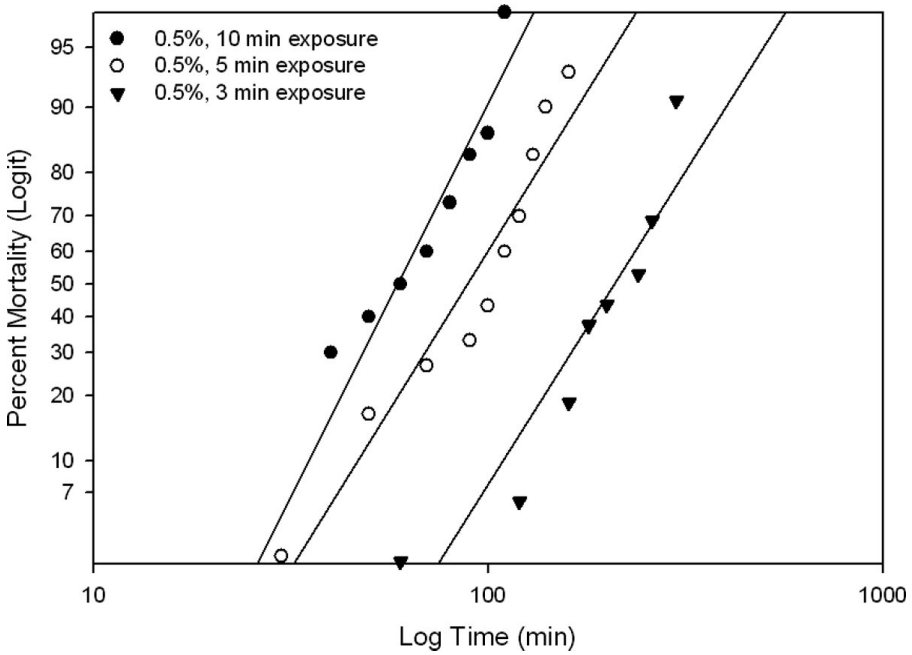

Fig. 3. Log time versus logit percentage of mortality of permethrin-resistant human head lice from south Florida (SF-HL) treated with 0.5% ivermectin formulations at different exposure periods.

respectively (Fig. 2). At the LT_{50} and LT_{95}, the 1% ivermectin formulation was 123 and 245 times faster than Nix, respectively (Table 1). The 0.5% ivermectin formulation was 107 and 166 times faster and the 0.25% ivermectin formulation was 52 and 103 times faster than Nix, respectively (Table 1). The mortality response to nonformulated 0.5% ivermectin was significantly different compared with Nix ($\chi^2 = 180.6$ df = 2, $P < 0.001$). At the LT_{50} and LT_{95}, nonformulated 0.5% ivermectin was 27.9 and 51.5 times faster than Nix, respectively (Table 1).

The mortality responses to all three of the ivermectin formulations were significantly different compared with nonformulated 0.5% ivermectin ($\chi^2 = 143.8$, df = 2, $P < 0.001$; $\chi^2 = 153.2$, df = 2, $P < 0.001$; and $\chi^2 = 100.3$, df = 2, $P < 0.001$, respectively (Fig. 2). At the LT_{50} and LT_{95}, the 1% ivermectin formulation was 4.4 and 4.8 times faster than nonformulated 0.5% ivermectin, respectively (Table 1). The 0.5% ivermectin formulation was 3.8 and 3.2 times faster and the 0.25% ivermectin formulation was 1.9 and 2.0 times faster than nonformulated 0.5% ivermectin, respectively (Table 1).

The mortality response for the 1% ivermectin formulation was significantly different from that produced by the 0.5% and the 0.25% formulations after 10-min exposures ($\chi^2 = 11.5$, df = 2, $P = 0.003$; and $\chi^2 = 134.8$, df = 2, $P = 0.001$, respectively) (Fig. 2). At the LT_{50} and LT_{95}, the 1% ivermectin formulation was 1.2 and 1.5 times faster than the 0.5% formulation, respectively (Table 1). At the LT_{50} and LT_{95}, the 1% ivermectin formulation was 2.4 and 2.4 times faster than the 0.25% formulation, respectively (Table 1). The mortality response of the 0.5% ivermectin formulation was significantly different from that produced by the 0.25% formulation after a 10-min exposure ($\chi^2 = 121.5$, df = 2, $P < 0.001$) (Fig. 2). At the LT_{50} and LT_{95}, the 0.5% ivermectin formulation was 2.0 and 1.6 times faster than the 0.25% formulation, respectively (Table 1).

Mortality Responses after 5- and 3-min Exposures. The mortality response of the 1% ivermectin formulation after a 10-min exposure was significantly different compared with the 0.5% formulation with a 5-min exposure and the 0.5% formulation with a 3-min exposure ($\chi^2 = 80.6$, df = 2, $P < 0.001$; and $\chi^2 = 163.3$, df = 2, $P < 0.001$, respectively). At the LT_{50} and LT_{95}, the 1.0% ivermectin formulation was 1.9 and 2.2 times faster than the 0.5% formulation with a 5-min exposure, respectively (Table 1). At the LT_{50} and LT_{95}, the 1.0% ivermectin formulation was 4.1 and 4.9 times faster than the 0.5% formulation with a 3-min exposure, respectively (Table 1). The mortality response of the 0.5% ivermectin formulation after a 10-min exposure was significantly different compared with the 0.5% formulation with a 5-min exposure and the 0.5% formulation with a 3-min exposure ($\chi^2 = 55.1$, df = 2, $P < 0.001$; and $\chi^2 = 175.2$, df = 2, $P < 0.001$, respectively). At the LT_{50} and LT_{95}, the 0.5% ivermectin formulation with a 10-min exposure was 1.6 and 1.5 times faster than the 0.5% formulation with a 5-min exposure, respectively (Table 1). At the LT_{50} and LT_{95}, the 0.5% ivermectin formulation was 3.5 and 3.3 times faster than the 0.5% formulation with a 3-min exposure, respectively (Table 1). The mortality response of the 0.25% ivermectin formulation after a 10-min exposure was significantly different from that produced by the 0.5% formulation with a 5-min exposure and the 0.5% formulation with a 3-min exposure ($\chi^2 = 25.6$, df = 2, $P < 0.001$; and $\chi^2 = 95.0$, df = 2, $P < 0.001$, respectively) (Fig. 3). At the LT_{50} and LT_{95}, the 0.25% ivermectin formulation after a 10-min exposure was 1.3 and 1.1 times slower than the 0.5% formulation with a 5-min exposure, respectively (Table 1). At the LT_{50} and LT_{95}, the 0.25% ivermectin formulation after

a 10-min exposure was 1.7 and 2.0 times faster than the 0.5% formulation with a 3-min exposure, respectively (Table 1). The mortality response of the 0.5% formulation with a 5-min exposure was significantly different compared with the 0.5% formulation with a 3 min exposure ($\chi^2 = 118.8$, df = 2, $P < 0.001$). At the LT_{50} and LT_{95}, the 0.5% formulation with a 5-min exposure was 2.2 and 2.2 times faster than the 0.5% formulation with a 3-min exposure, respectively (Table 1).

Discussion

Treatments with 1, 0.5, and 0.25% ivermectin formulation resulted in significantly faster mortality response than treatments with placebo or ddH_2O, indicating that ivermectin is pediculicidal on permethrin-resistant head lice. Ivermectin formulations had a faster mortality response than Nix treatments, indicating that ivermectin is a faster acting pediculicide. The 1% ivermectin formulation worked significantly faster than its 0.5% formulation and the 0.5% formulation worked significantly faster than its 0.25% formulation.

The 0.5% ivermectin formulation with a 10-min exposure worked significantly faster than the 0.5% formulation with a 5-min exposure and both were significantly faster than the 0.5% formulation with a 3-min exposure.

Nonformulated 0.5% ivermectin was 3.2–3.8 times slower at killing SF-HL than 0.5% formulated ivermectin. The exact reason for the superior killing power of ivermectin in formulation is currently unclear, but it may be due to the increased penetration or increased transfer of ivermectin residues to the louse cuticle.

Ovide was 100% effective at killing SF-HL, and it had the fastest mortality response time. The mortality response was estimated at <5 min because 100% mortality of SF-HL occurred within the period of time immediately after exposure but before the 5-min washing and drying period. Ovide formulation contains 78% isopropanol, which is flammable. The isopropanol in the Ovide formulation is actually more effective at killing head lice than the active ingredient 0.5% malathion (Yoon et al. 2003).

It has been proven that Nix is not 100% effective at killing treated SF-HL by using the hair tuft bioassay system (Yoon et al. 2006). All ivermectin formulations tested killed 100% of lice treated. Further investigation is necessary to determine whether this ivermectin formulation has effects on the ova or the developing embryos and larvae.

The hair tuft-based bioassay in conjunction with the in vitro rearing system provides a standardized method to assess the comparative efficacy of pediculicide formulations in a reproducible format that mimics clinical exposure trials. This bioassay setup standardizes louse exposure to treatment and the recording of mortality over developmental time after treatment, both of which are applicable to clinical application exposure scenarios for efficacy validations. Standardization of exposure includes the amount of treatment applied, the duration of exposure, the number and developmental stage of lice being treated, and the use of positive and negative treatment controls. Standardization in the recording of mortality after treatment includes daily visual inspection of lice under a dissecting microscope, control of environmental factors (i.e., temperature and humidity), and prevention of reinfestation during recording period.

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