STUDY

Permethrin-Resistant Human Head Lice, *Pediculus capitis*, and Their Treatment

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Objective: To compare the pediculicidal activity of Ovide lotion and its active ingredient, 0.5% malathion, with Nix and its active ingredient, 1% permethrin, in permethrin-resistant head lice.

Design: In vitro pediculicidal product and active ingredient comparison. The presence of knockdown resistance–type mutations (*T929I* and *L932F*) was validated by DNA sequencing.

Setting: University of Massachusetts-Amherst; University of Miami School of Medicine, Miami, Fla; Plantation and Homestead, Fla; and Mathis, Tex.

Other Participants: Lice were collected in 3 geographical regions within the United States and in Yamburara, Ecuador, from healthy but infested individuals.

Intervention: Within 3 to 6 hours of collection, lice were given a blood meal, exposed to products or active ingredients, and observed at regular intervals.

Main Outcome Measures: Percent mortality of lice at regular intervals after exposure to products or active ingredients and presence of T929I and L932F mutations.

Results: South Florida lice exhibited a significantly slower mortality response to permethrin compared with susceptible Ecuadorian lice. Ovide and malathion killed permethrin-resistant lice faster than Nix or permethrin. The presence of T929I and L932F in permethrin-resistant south Florida lice was confirmed by DNA sequencing. The population of Texas lice from Mathis was slightly resistant to permethrin and included 13% with resistant genotypes.

Conclusions: The presence of the T929I and L932F mutations was confirmed by DNA sequencing in lice collected from children in south Florida that were resistant to the pediculicidal effects of permethrin and the leading permethrin-based head lice product, Nix. Malathion resistance was not observed in this study. The data also show that Ovide killed these same permethrin-resistant head lice approximately 10 times faster than permethrin or Nix.

Arch Dermatol. 2003;139:994-1000

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PEDICULOSIS, CAUSED by the human head louse (HL) *Pediculus capitis*, is the most prevalent parasitic infestation in children from the United States.[1] Infestations continue to be reported in the United States[1-7] and in other countries such as those of the United Kingdom (UK),[8-10] Israel,[11-12] the Czech Republic,[13] and Argentina[14] despite the many over-the-counter, self-treatment medications available today. In the United States, consumer (nonprescription) treatment products are almost exclusively limited to those that contain the natural botanical active ingredients, the pyrethrins (eg, RID [Pfizer Inc, New York, NY], A-200 shampoo [Hogil Pharmaceutical Corp, Purchase, NY], and Pronto [Del Laboratories, Farmingdale, NY]), or those that contain the synthetic pyrethroid permethrin (eg, Nix [Pfizer/Warner-Lambert, Morris Plains, NJ]), as the active ingredients. These products have been previously reported to be highly effective in the treatment of infestations.[8]

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It is well established in the published literature[1-16] that resistance to permethrin exists in certain locations. In the United States, resistance to permethrin has been found in lice from children in Brookline and Cambridge, Mass[15]; Chicopee and Holyoke, Mass[16]; Boise, Idaho[15]; and Plantation, Fla.[2] Although resistance to malathion has been reported in the UK,[10] none has been reported in the United States to date.[2,16]

Lee et al[16] reported that US lice were resistant to permethrin and also exhibited knockdown resistance (kdr) in behavioral assays. Knockdown resistance is associated with increased nerve insensitivity and is similar to the kdr noted for DDT, the pyrethrins, and the pyrethroids reported originally in the housefly, *Musca domestica*.[17-19] Two point mutations (T9291 and L932F) have been found to be associated with permethrin-resistant lice from Florida, Massachusetts, and Bristol, England, but not found in permethrin-susceptible lice from Panama.[16] The T9291 mutation functions as a kdr-type mutation in the diamondback moth, *Plutella xylostella*.[20] Recently, both mutations have been identified in lice throughout the UK and substantiate their importance in kdr.[21]

A recently reintroduced prescription treatment (1999), Ovide lotion (Medicis Pharmaceutical Corporation, Phoenix, Ariz), is a pediculicidal product that contains 0.5% pharmaceutical-grade malathion as the active ingredient in a special base. Meinking et al[2,3] recently reported that Ovide has superior pediculicidal and ovicidal activity on both susceptible and permethrin-resistant lice compared with the leading permethrin- and lindane-based products. These investigators concluded that Ovide killed permethrin-resistant lice and that these lice were not resistant to malathion.

The purpose of the present study was to confirm and extend the findings of Meinking et al.[2,3] Standard mortality bioassays determined comparative mortality profiles for both the Nix and Ovide products and their active ingredients. In addition, DNA sequencing determined whether the mutations associated with permethrin resistance were present and what influence they had on mortality.

METHODS

HUMAN HEAD LOUSE POPULATIONS

The Ecuador test population (EC-HL) was obtained from over 50 children in Yamburara, Ecuador (obtained by D.T.). They had never been exposed to pesticides, including permethrin and malathion, and were considered pediculicide susceptible. A mixed population of all stages and eggs were overnight expressed to the University of Massachusetts-Amherst and placed into a temporary colony fed on humans.[16] The south Florida population (SF-HL) was collected from over 30 infested children in Plantation and Homestead[2] and are permethrin resistant.[2,16] The Texas population (TX-HL) was obtained from a single heavily infested child (42 lice were obtained) from Mathis, Tex (obtained by L.B.). All collections and administration of informed consent forms were carried out using protocols previously approved by the institutional review board of the University of Massachusetts-Amherst.

IN VITRO MORTALITY BIOASSAYS

After collections, the EC-HL were transported to the Pesticide Toxicology Laboratory, Department of Entomology, University of Massachusetts-Amherst; the FL-HL to the Field Epidemiology Survey Team Laboratory; and the TX-HL to a temporary field laboratory in Mathis. All lice were given a blood meal prior to initiation of mortality bioassays by feeding on the investigator's hand.[16] Filter paper and contact bioassays were used to determine lethality of 1% permethrin, 0.5% malathion, Nix (ingredients: 1% permethrin, balsam Canada, cetyl alcohol, citric acid, FD&C yellow #6 fragrance, hydrolyzed animal protein, hydroxyethylcellulose, polyoxyethylene 10 cetyl ether, propylene glycol, stearalkonium chloride, water, isopropyl alcohol, methylparaben, propylparaben) and Ovide shampoo (ingredients: 0.5% malathion, terpineol, dipentene and pine needle oil in 78% isopropyl alcohol). Filter paper disks (Whatman No. 1) were dipped for 10 seconds into 1% (vol/vol [1 part 10% permethrin in acetone to 9 parts acetone]) permethrin in acetone, 0.5% (vol/vol) malathion in acetone, or Ovide and were subsequently air dried in a dark fume hood for 4 hours. Disks were likewise dipped into Nix for 1 minute and dried in a dark fume hood for 24 hours owing to the slow drying characteristics of this product. Disks were also dipped into neet acetone, dried, and used as nontreatment controls.

The bioassay procedure was conducted according to Lee et al[16] except that mixed developmental stages (first, second, and third instars and adults) were examined. Log time vs logit mortality regressions were performed (POLO PC; LeOra Software, Berkeley, Calif, 1987) to determine lethal time 50% (LT_{50}) values. To determine a susceptible or resistant phenotype following 1% permethrin exposure, the lethal time 95% (LT_{95}) value of the insecticide-susceptible EC-HL was used. Survival beyond the calculated 7.6 hour value of this susceptible strain was used to assess permethrin resistance on a phenotypic basis. The use of timed bioassays at a single dose rather than multiple doses at a single time point is becoming a standard procedure for the assessment of resistance.[22] We have chosen the same concentration that is used in the commercial products for both permethrin and malathion because this is how lice are actually exposed to the active ingredient (at a single concentration over a set time interval). The speed in which permethrin and malathion kills susceptible lice vs the resistant lice is therefore germane in the determination of resistance. Comparisons of the mortality responses due to different test materials were made using the maximum log-likelihood ratio test, which tests the hypothesis of equality of slopes and intercepts of the logit regressions ($P=.05$ [POLO PC]).

DETERMINATION OF THE T929I AND L932F MUTATIONS

To determine whether a correlation exists between increased survivorship (due to resistance) and an increasing frequency of kdr-type mutations (T929I and L932F) for permethrin resistance,[16] genomic DNA was extracted from individual SF-HL lice used in the mortality bioassays described previously, which were preserved in 95% ethanol. Extractions were performed using DNAzol (Molecular Research Center, Cincinnati, Ohio) with a slight modification (addition of 1% polyacryl carrier) to handle small samples according to the manufacturer's instruction. Genomic DNA was also obtained from a mixed population of permethrin-susceptible and -resistant lice from Mathis (TX-HL) and used as internal quality controls to assure that the DNA amplification and sequencing reactions were successful and capable of identifying lice with and without mutations. Two rounds of polymerase chain reaction (PCR) were performed with nested primers (BL5'Gn/3SP1 for first PCR and 5SP1L/3SP5N for the second PCR; **Table 1**) to amplify a DNA fragment (approximately 548 to 561 base pairs) containing the S4 to S6 regions of domain II of para-orthologous, voltage-sensitive sodium channel α-subunit gene of head lice. The presence or absence of kdr-type mutations (T929I or L932F) was determined by DNA sequencing (ABI 377XL; Applied Biosystems, Foster City, Calif) at the Automated DNA Sequencing Facility, University of Massachusetts-Amherst. Computer software, Gene Runner, Version 3.00 (Hastings Software, Bethesda, Md, 1994) was used to analyze and manage sequencing data.

RESULTS

IN VITRO MORTALITY BIOASSAYS

Treatment of the EC-HL with either 0.5% malathion or 1% permethrin resulted in significantly different and substantially reduced survival times compared with the acetone-treated EC-HL as judged by the maximum log-likelihood ratio test (χ^2_2=174.8 [P<.001] and χ^2_2=312.9 [P<.001], respectively) (**Figure 1**A). Malathion reduced the LT_{50} value 9.0-fold and permethrin reduced the LT_{50} value 5.2-fold compared with the acetone control (**Table 2**).

Treatment of the SF-HL with 0.5% malathion, likewise, resulted in significantly different and substantially reduced survival times compared with the acetone-treated SF-HL (χ^2_2=159.0 [P<.001]) (Figure 1B). Malathion reduced the LT_{50} value 5.9-fold compared with no treatment (Table 2). Although treatment of the SF-HL with 1% permethrin also resulted in a significantly different response (χ^2_2=59.9 [P<.001]) (Figure 1B), the LT_{50} value was not substantially reduced compared with the acetone treated SF-HL (1.3-fold, Table 2).

The SF-HL (Figure 1B) exhibited significantly slower mortality response to 1% permethrin compared with the EC-HL (Figure 1A) (χ^2_2=218.5 [P<.001]). The mortality resistance ratio (RR) based on the LT_{50} values of the SF-HL vs the EC-HL was 3.1 (Table 2) and confirmed that the SF-HL was resistant to permethrin.

Table 1. PCR Primers for the Amplification of a Genomic DNA Fragment of the Para-Orthologous Sodium Channel α-Subunit Gene From the Human Head Louse

Name	Sequence
BL5′Gn	5′-GAGTCTTCAAATTGGCCAAATCGTG-3′
3SP1	5′-CATTGTCAGCGGTGGGAGCAGA-3′
5SP1L	5′-CCACGTTAAATTTATTAATTTCAA-3′
3SP5N	5′-GATAAACTAGAGGAACCGAAATT-3′

Abbreviation: PCR, polymerase chain reaction.

The mortality response of the SF-HL (Figure 1B) to 0.5% malathion, however, was not statistically different from the EC-HL (Figure 1A) (χ^2_2=2.7 [P=.26]). Although the LT_{50} value of the SF-HL was slightly longer than for the EC-HL, the magnitude of difference was small (RR=1.2) and their confidence limits were overlapped (Table 2). These results confirm that the SF-HL and

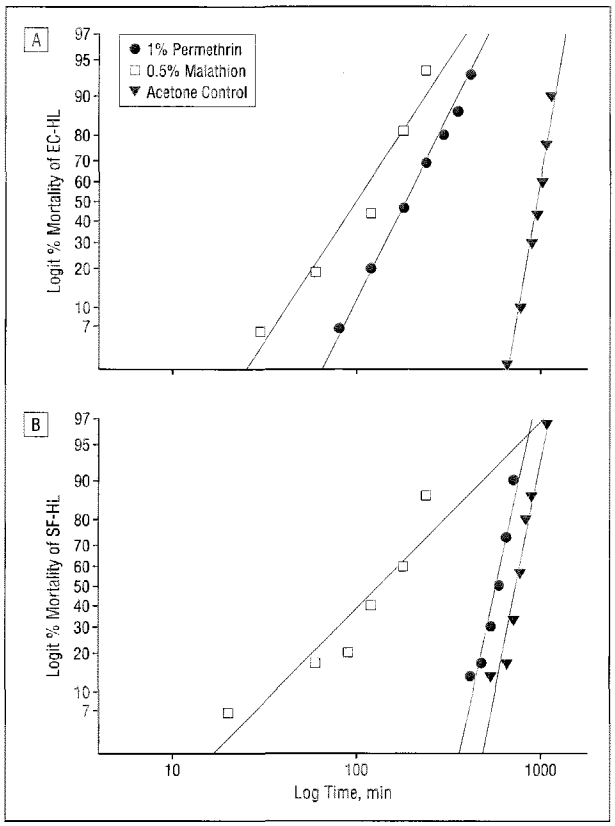

Figure 1. Log time vs logit percent mortality regressions of human head louse populations from Ecuador (EC-HL) (A) and south Florida (Plantation and Homestead [SF-HL]) (B) treated with 1% permethrin (EC-HL, n=45; SF-HL, n=30), 0.5% malathion (EC-HL, n=16; SF-HL, n=30), or acetone (EC-HL, n=30; SF-HL, n=30) as a solvent control.

Table 2. Lethal Time Values and Resistance Ratios From Human Head Louse Populations Treated With Various Pediculicidal Compounds and Products

	EC-HL		SF-HL		TX-HL			
Treatments	LT_{50} (95% CI), min	Louse Sample, No.	LT_{50} (95% CI), min	Louse Sample, No.	LT_{50} (95% CI), min	Louse Sample, No.	RR*	RR†
1% Permethrin	187 (169-204)	45	577‡ (538-616)	30	268‡ (178-339)	33	3.1	1.4
0.5% Malathion	107 (83-132)	16	129§ (79-197)	30	NA	NA	1.2	NA
Nix (Pfizer/Warner-Lambert, Morris Plains, NJ)	NA	NA	551‖ (516-587)	22	NA	NA	NA	NA
Ovide (Medicis Pharmaceutical Corporation, Phoenix, Ariz)	NA	NA	57 (35-79)	30	NA	NA	NA	NA
Ovide vehicle	NA	NA	38 (30-46)	15	NA	NA	NA	NA
Control	965 (934-993)	30	748 (702-790)	30	NA	NA	NA	NA

Abbreviations: CI, confidence interval; EC-HL, Ecuador test population; LT_{50}, lethal time 50%; NA, not applicable; RR, mortality resistance ratio; SF-HL, south Florida test population; TX-HL, Texas test population.

*RR = LT_{50} (SF-HL)/LT_{50} (EC-HL).

†RR = LT_{50} (TX-HL)/LT_{50} (EC-HL).

‡Significantly different from EC-HL (maximum log-likelihood test, P<.05).

§Significantly not different from EC-HL (maximum log-likelihood test, P>.05).

‖Significantly not different from 1% permethrin treatment in SF-HL (maximum log-likelihood test, P>.05).

EC-HL share a similar level of susceptibility to malathion and that the SF-HL lice were not resistant to this pediculicide.

Comparatively, the mortality responses of the SF-HL to 0.5% malathion (Figure 1B) and Ovide (**Figure 2**) were significantly faster than when treated with 1% permethrin (Figure 1B) (χ^2_2=141.9 [P<.001] and χ^2_2=173.0 [P<.001], respectively) or with Nix (Figure 2) (χ^2_2=105.6 [P<.001] and χ^2_2=140.6 [P<.001], respectively). These findings indicate that both 0.5% malathion and Ovide are more efficient in killing permethrin-resistant lice in the SF-HL than 1% permethrin or Nix. The data also show that Ovide kills permethrin-resistant lice from the SF-HL approximately 10 times faster than 1% permethrin and Nix (Table 2).

The mortality response of the SF-HL to Nix (Figure 2) is statistically the same as 1% permethrin (Figure 1B) (χ^2_2=1.8 [P<.4]). Similarly, the magnitude of the difference using the LT_{50} values was small (LT_{50} of 1% permethrin-treated SF-HL/LT_{50} of Nix-treated SF-HL=1.0) and their confidence limits were overlapped (Table 2).

In the SF-HL, Ovide (Figure 2) exhibited a somewhat faster mortality response (2.3-fold, Table 2) compared with 0.5% malathion (Figure 1B) (χ^2_2=61.1 [P<.001]). Additionally, Ovide vehicle (Ovide without malathion) also elicits lethality. These results suggest that other ingredients (eg, isopropanol and terpenes) in Ovide may have pediculicidal activity. These differences may or may not be clinically meaningful but deserve further investigation.

LINKAGE OF PERMETHRIN-RESISTANT PHENOTYPES AND KDR-TYPE MUTATIONS

Two populations were used to establish a linkage between increasing survivorship to 1% permethrin and increasing frequency of the 2 kdr-type mutations. The TX-HL was one of the most permethrin-susceptible US populations assayed to date as judged by its LT_{50} value compared with that of the SF-HL (2.2-fold more susceptible, Table 2) and by its calculated RR vs the EC-HL (RR=1.4; Table 2). Additionally, the overlap of its regression line with that of the permethrin-resistant SF-HL population only at the longer survival times would indicate a population that consists mostly of susceptible individuals with some highly resistant individuals (**Figure 3**). The SF-HL is resistant to permethrin (RR=3.1; Table 2) and its regression line is not substantially different from that of its acetone control line (Figure 1B), which indicates a uniformly high tolerance to permethrin.

Using the LT_{95} value of 7.6 hours from the EC-HL treated with 1% permethrin (Figure 1A) to assess the susceptible or resistant phenotype of individual lice (survival beyond 7.6 hours indicated resistance), it was determined that of the thirty-three 1%-permethrin–treated lice of the TX-HL, 15% (5) were resistant and 85% (28) were susceptible (**Table 3**). These same lice were genotyped using DNA sequence data, with 1 sample producing unreadable sequence (TPYE7). Only 13% (4/32) of these lice possessed the homozygous kdr-type mutations, and 87% (28/32) of the lice had no mutations or were heterozygous for these mutations (Table 3). It should be noted that kdr-type mutation has been widely determined to be inherited as a recessive factor and that heterozygous individuals are susceptible to DDT, the pyrethrins, and the pyrethroids in a variety of insect species.[23-28] Of the lice that possessed the homozygous kdr-type mutations, all died at times greater than 7.6 hours (11-20 hours). Only 1 sample (TPYE4) had a mismatch between its phenotype (resistant) and its genotype (heterozygous susceptible). Using these criteria, we found a 97% agreement (31/32) between the phenotypic and the genotypic determinations.

In contrast, it was phenotypically assessed that of the thirty 1%-permethrin–treated lice of the SF-HL (Figure 1B), 87% (26) were resistant and 13% (4) were susceptible (**Table 4**). Two samples, both of which were phenotypically resistant, failed to produced readable sequence (Per27 and 30). Genotypically, it was determined that in the SF-HL 96% (27/28) were resistant and 4% (1/28) were susceptible (Table 4). Using these criteria, we found an 86% (24/28) agreement between the phe-

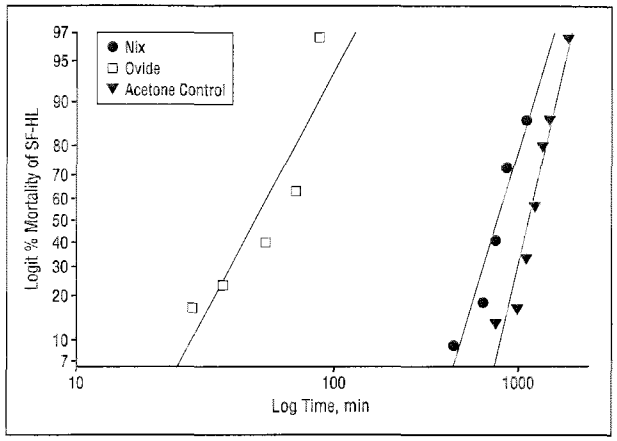

Figure 2. Log time vs logit percent mortality regressions of human head louse populations from south Florida (Plantation and Homestead [SF-HL]) treated with Nix (Pfizer/Warner-Lambert, Morris Plains, NJ) (n=22) or Ovide (Medicis Pharmecutical Corporation, Phoenix, Ariz) (n=30) compared with acetone control (n=30).

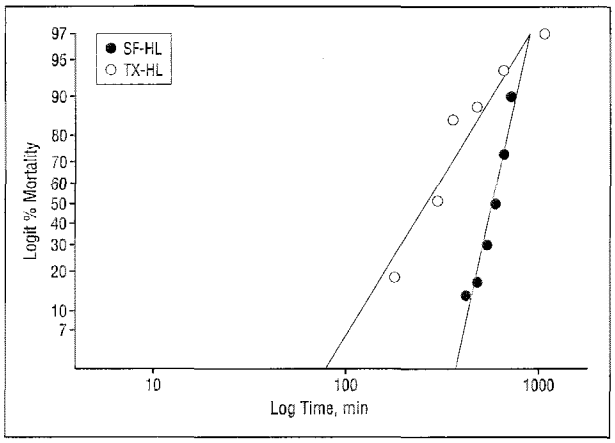

Figure 3. Log time vs logit percent mortality regressions of human head louse populations from south Florida (Plantation and Homestead [SF-HL], n=30) and Mathis, Tex (TX-HL, n=33) treated with 1% permethrin.

Table 3. Comparative Data of the Phenotype and Genotype of the Human Head Louse Population From Mathis, Tex, Following 1% Permethrin Mortality Bioassay (Phenotype) and DNA Sequencing Analysis (Genotype)

Pediculicide	Louse Sample (N = 33)	Time Died, h	Phenotype* (S or R)	Genotype†	
				T929I	L932F
1% Permethrin	TPYE1	18	R	R	R
	TPYE2-3	11	R	R	R
	TPYE4	8	R	H	H
	TPYE5	6	S	H	H
	TPYE6	3	S	H	H
	TPYE7	5	S	Unreadable‡	Unreadable‡
	TPYE8	5	S	H	H
	TPYE9	5	S	S	S
	TPYE10-11	5	S	H	H
	TPYE12-14	5	S	S	S
	TPYE15-17	5	S	H	H
	TPYE18	6	S	H	H
	TPYE19-20	6	S	S	S
	TPYE21-22	6	S	H	H
	TPYE23-27	6	S	S	S
	YOON1-3	3	S	S	S
	YOON4	3	S	H	H
	YOON5	3	S	S	S
	YOON6	20	R	R	R

Abbreviations: H, heterozygote allele; R, resistant allele; S, susceptible allele.
*Determined by lethal time 95% value (7.6 hours) of 1% permethrin bioassay using the Ecuador test population. A louse that died in 7.6 hours or less was susceptible. A louse that died in more than 7.6 hours was resistant.
†Genotype was assessed by actual DNA sequencing with T929 and L932 being susceptible and I929 and F932 being resistant genotypes.
‡The DNA sequence cannot be determined owing to the high background in chromatogram.

Table 4. Comparative Data of the Phenotype and Genotype of the Human Head Louse Population From South Florida Following 1% Permethrin and Nix Mortality Bioassay (Phenotype) and DNA Sequencing Analysis (Genotype)

Pediculicide	Louse Sample	Time Died, h	Phenotype* (S or R)	Genotype†	
				T929I	L932F
1% Permethrin (N = 30)	Per1	13	R	R	R
	Per2	13	R	S	S
	Per3-4	12	R	R	R
	Per5-6	11	R	R	R
	Per7-10	7	S	R	R
	Per11	8	R	R	R
	Per12-15	9	R	R	R
	Per16-21	10	R	R	R
	Per22-26	11	R	R	R
	Per27	12	R	Unreadable‡	Unreadable‡
	Per28-29	12	R	R	R
	Per30	13	R	NA	NA
Nix§ (N = 9)	Nix1-3	15	R	R	R
	Nix4	12	R	Unreadable	Unreadable
	Nix5	12	R	R	R
	Nix6-8	10	R	R	R
	Nix9	9	R	R	R

Abbreviations: NA, not available (sample lost following bioassay); R, resistant allele; S, susceptible allele.
*Determined by lethal time 95% value (7.6 hours) of 1% permethrin bioassay using the Ecuador population. A louse that died in 7.6 hours or less was susceptible. A louse that died in more than 7.6 hours was resistant.
†Genotype was assessed by actual DNA sequencing with T929 and L932 being susceptible and I929 and F932 being resistant genotypes.
‡The DNA sequence cannot be determined owing to the high background in chromatogram.
§Pfizer/Warner-Lambert, Morris Plains, NJ.

notypic and genotypic determinations. These results indicate a strong correlation between the presence of the T929I and L932F mutations and survivorship beyond 7.6 hours in the 1%-permethrin bioassay, which is indicative of kdr-type permethrin resistance.

DETERMINATION OF THE FREQUENCY OF KDR-TYPE MUTATIONS

Lice were randomly selected from the SF-HL used in the mortality bioassays for Nix (Figure 2), 0.5% malathion

(Figure 1B), and Ovide (Figure 2) and used for genotypic analysis. The LT_{50} values determined from the log time vs logit mortality regression lines for the 9 lice randomly selected from the SF-HL treated with Nix (Table 4, bottom), the 5 lice treated with 0.5% malathion (**Table 5**, top), and the 7 lice treated with Ovide (Table 5, bottom) were statistically not different from the LT_{50} values of their respective parent populations (Table 2) due to overlapping 95% confidence limits (552 [519-586] vs 642 [578-713], 129 [111-149] vs 227 [131-383], and 57 [51-64] vs 72 [55-92] minutes, respectively). Thus, the 3 randomly selected subpopulations used for sequencing were representative of their respective bioassayed parent populations.

Of the 9 randomly selected SF-HL lice treated with Nix, 100% (9/9) were phenotypically and 100% (8/8) were genotypically resistant, with 1 sample (Nix4) that was unreadable (Table 4). These results clearly establish the presence of kdr-type mutations and permethrin resistance in this subpopulation.

Of the 5 randomly selected SF-HL lice treated with 0.5% malathion and the 7 lice treated with Ovide, 100% were genotypically resistant to permethrin (5/5 and 7/7, respectively) (Table 5). However, all were assessed to be phenotypically susceptible to 0.5% malathion and Ovide as judged by the LT_{95} value of 0.5% malathion on the pediculicidal-susceptible EC-HL (5.1 hours, Figure 1A), and all died prior to the 7.6 hour value used to assess phenotypic resistance to permethrin, indicating that there is no cross-resistance to malathion in permethrin-resistant lice. Although based on limited numbers, these results show that, even in the presence of the kdr-type mutations and permethrin resistance, both 0.5% malathion and Ovide provided much faster kill times than 1% permethrin or Nix.

Table 5. Comparative Data of the Phenotype and Genotype of the Human Head Louse Population From South Florida Following 0.5% Malathion and Ovide Mortality Bioassay (Phenotype) and DNA Sequencing Analysis (Genotype)

Pediculicide	Louse Sample	Time Died, h	Phenotype* (S or R)	Genotype†	
				T929I	L932F
0.5% Malathion (N = 5)	Mal1-4	5	S	R	R
	Mal5	4	S	R	R
Ovide‡ (N = 7)	Ov1-3	1.7	S	R	R
	Ov4-6	1.3	S	R	R
	Ov7	1	S	R	R

Abbreviations: R, resistant allele; S, susceptible allele.

*Determined by lethal time 95% value (5.1 hours) of 0.5% malathion bioassay using the Ecuador test population. A louse that died in 5.1 hours or less was susceptible. A louse that died in more than 5.1 hours was resistant.

†Genotype was assessed by actual DNA sequencing with T929 and L932 being susceptible and I929 and F932 being resistant genotypes.

‡Medicis Pharmaceutical Corporation, Phoenix, Ariz.

COMMENT

We continue to confirm the presence of permethrin-resistant lice on individuals with pediculosis in the United States. Overall, the SF-HL is resistant to permethrin due to kdr-type mutations, but 0.5% malathion and Ovide kill these permethrin-resistant head lice in a manner not significantly different from that elicited by the insecticide-susceptible EC-HL. We were also able to demonstrate that a prescription-only product, Ovide, was able to kill the permethrin-resistant lice at a rate that was approximately 10 times faster than that observed for Nix.

Our mortality bioassay data are in agreement with findings from 3 other independent studies.[2,15,16] Although the overall conclusions are the same, the in vitro kill times observed in the present study are longer than those observed for both studies by Meinking et al[2,3] and another conducted by Pollack et al[15] owing to several differences between the in vitro mortality bioassay techniques used. In view of these differences, there is a clear need for standardizing the way in which pediculicidal effectiveness and resistance are evaluated in vitro. It must also be noted that the slower kill times for permethrin and Nix may not be clinically relevant as long as they also kill all the lice, albeit more slowly, in practice. This conundrum, however, can only be resolved by a clinical study that assesses the effectiveness of these products and the survivability of permethrin-resistant lice. The present work provides the means to carry out such a study.

The combination of mortality bioassays and DNA sequence analysis confirms the original findings of Lee and coworkers[16] that the presence of kdr-type point mutations T929I and L932F is highly associated with permethrin resistance in field-collected head lice. The data presented also confirm and extend the recent findings by Meinking et al[2] that Ovide is able to kill permethrin-resistant lice. This finding was expected because the 2 mutations associated with permethrin resistance in lice[16] do not confer cross-resistance to the differing pharmacological and pediculicidal action of malathion in Ovide. Additionally, Ovide is not available except as a prescription product and has only been reintroduced to the market since 1999, which has limited its impact on louse populations. Since Ovide was able to kill lice many times faster than Nix and other permethrin-based products,[2] its pediculicidal and ovicidal activity[3] make it an alternative treatment when permethrin resistance is a problem. Nevertheless, permethrin/malathion–dual resistant lice already exist in the UK and are a serious problem.[10] Thus, surveillance for malathion resistance should be implemented if Ovide becomes the treatment of choice. Finally, our in vitro bioassay and genotyping data clearly indicate that permethrin-resistant head lice are established in the United States and warrant the need for additional clinical studies to ensure the continued efficacy of the currently available over-the-counter formulations that contain permethrin.

Accepted for publication February 11, 2003.

This study was supported by grant PHS 1 R01 AI45062-01A1 from the National Institutes of Health National Institute of Allergy and Infectious Diseases, Bethesda, Md, and by a grant-in-aid from Medicis Pharmaceutical Corporation, Phoenix, Ariz.

We sincerely thank Lice Source Services, Inc, and Field Epidemiology Survey Team members for their assistance in louse collections and shipments.

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REFERENCES

1. Gratz NG. *Human Lice: Their Prevalence, Control and Resistance to Insecticides.* WHO/CTD/WHOPES/97.8. Geneva, Switzerland: World Health Organization. 1997:1-61.
2. Meinking TL, Serrano L, Hard B, et al. Comparative in-vitro pediculicidal efficacy of treatments in a resistant head lice population in the United States. *Arch Dermatol.* 2002;138:220-224.
3. Meinking TL, Entzel P, Villar ME, Vicaria M, Lemard G, Porcelain SL. Comparative efficacy of treatment for *Pediculus capitis* infestations: update 2000. *Arch Dermatol.* 2001;137:287-292.
4. Meinking TL. Infestations. *Curr Probl Dermatol.* 1999;11(3):73-120.
5. Chosidow O. Scabies and pediculosis. *Lancet.* 2000;355:819-826.
6. Poorbaugh JH. Head lice infestation—treatment failures with 1% lindane (Kwell). *Calif Morbidity.* April 17, 1987. No. 14.
7. Brandenburg K, Deinard AS, DiNapoli J, Englender SJ, Orthoefer J, Wagner D. 1% permethrin cream rinse vs 1% lindane shampoo in treating *Pediculosis capitis. Am J Dis Child.* 1986;140:894-896.
8. Burgess IF, Peock S, Brown CM, Kaufman J. Head lice resistant to pyrethroid insecticides in Britain [letter]. *BMJ.* 1995;311:752.
9. Downs AM, Stafford KA, Coles GC. Head Lice: prevalence in schoolchildren and insecticide resistance. *Parasitol Today.* 1999;15:1-4.
10. Downs AM, Stafford KA, Harvey I, Coles GC. Evidence for double resistance to permethrin and malathion in head lice. *Br J Dermatol.* 1999;141:508-511.
11. Mumcuoglu KY, Hemmingway J, Miller J, et al. Permethrin resistance in the head louse *Pediculus humanus capitis* from Israel. *Med Vet Entomol.* 1995;9:427-432.
12. Hemingway J, Miller J, Mumcuoglu KY. Pyrethroid resistance mechanisms in the head louse, *Pediculus capitis* from Israel: implications for control. *Med Vet Entomol.* 1999;13:89-96.
13. Rupes V, Moravec J, Chmela J, Ledvinka J, Zelenkova J. A resistance of head lice (*Pediculus capitis*) to permethrin in Czech Republic. *Cent Eur J Public Health.* 1995;3:30-32.
14. Picollo MI, Vanessa CV, Casidio AA, Massimo J, Zerba EN. Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (*Anoplura: Pediculae*). *J Med Entomol.* 1998;35:814-817.
15. Pollack R J, Kiszewski A, Armstrong P, et al. Differential permethrin susceptibility of head lice sampled in the United States and Borneo. *Arch Pediatr Adolesc Med.* 1999;153:969-973.
16. Lee SH, Yoon KS, Williamson MS, et al. Molecular analysis of kdr-like resistance in permethrin-resistant strains of head lice, *Pediculus capitis. Pesticide Biochem Physiol.* 2000;66:130-143.
17. Farnham AW. Genetics of resistance of house flies (*Musca domestica* L) to pyrethroids, I: knockdown resistance. *Pestic Sci.* 1977;8:631-636.
18. Schuler TH, Martinez-Torres D, Thompson AJ, et al. Toxicological, electrophysiological, and molecular characterization of knockdown resistance to pyrethroid insecticides in the diamondback moth, *Plutella xylostella* (L). *Pestic Biochem Physiol.* 1998;59:169-182.
19. Oppenoorth FJ. Biochemistry and genetics of insecticide resistance. In: Kerkut GA, Gilbert LI, eds. *Comprehensive Insect Physiology Biochemistry and Pharmacology.* Oxford, England: Pergamon; 1985:731-773.
20. Vais H, Williamson MS, Usherwood PN. The molecular interaction of pyrethroid insecticides with insect and mammalian sodium channels. *Pest Manag Sci.* 2001; 57:877-888.
21. Downs AMR, Williamson MS, Stafford KA, Coles GC. A common mechanism of resistance to pyrethroids in head lice. *Br J Dermatol.* 2002;114(S62):49.
22. Brogdon MG, McAllister JC. Insecticide resistance and vector control. *Emerg Infect Dis.* 1998;4:605-613.
23. Lee SH, Dunn JB, Clark JM, Soderlund DM. Molecular analysis of kdr-like resistance in a permethrin-resistant strain of Colorado potato beetle. *Pestic Biochem Physiol.* 1999;63:63-75.
24. Soderlund DM. Molecular mechanisms of insecticide resistance. In: Sjut V, ed. *Molecular Mechanisms of Resistance to Agrochemicals.* Berlin, Germany: Springer Verlag; 1977:21-56.
25. Knipple DC, Doyle KE, Marsella-Herrick PA, Soderlund DM. Tight genetic linkage between the kdr insecticide resistance trait and a voltage-sensitive sodium channel gene in the housefly. *Proc Natl Acad Sci U S A.* 1994;91:2483-2487.
26. Williamson MS, Denholm I, Bell CA, Devonshire AL. Knockdown resistance (kdr) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). *Mol Gen Genet.* 1993;240:17-22.
27. Taylor MFJ, Heckel DG, Brown TM, Krietman ME, Black B. Linkage of pyrethroid insecticide resistance to a sodium channel locus in the tobacco budworm. *Insect Biochem Mol Biol.* 1993;23:763-775.
28. Dong K, Scott JG. Linkage of kdr-type resistance and the para-homologous sodium channel gene in German cockroaches (*Blatella germanica*). *Insect Biochem Mol Biol.* 1994;24:647-654.

News and Notes

The 15th Ibero–Latin American Congress of Dermatology will take place from October 21 through October 25, 2003, in Buenos Aires, Argentina. For more information, contact the Secretariat at Av. Rivadavia 1977, 11th floor A, C1033AAJ, Buenos Aires, Argentina; fax: (5411) 4953-1505; e-mail: xvcongresso@xvcilad.org or xvcongreso@fibertel.com.ar.