Comparative Efficacy of Treatments for Pediculosis Capitis Infestations

Terri Lynn Meinking; David Taplin; Debra Chester Kalter, MD; Mark W. Eberle, PhD

Pediculosis capitis has become a widespread problem in the United States, reaching epidemic proportions in some areas. In 1973 and 1976, it was estimated that there were 6 million cases in this country annually. Rasmussen has observed an increase in new cases of head lice since then, with a substantial rise in the last five years. Most cases of pediculosis capitis are unreported. Market sales of over-the-counter pediculicides do not indicate whether the product was purchased for pediculosis capitis, corporis, or pubis. The incidence of head lice in the United States, therefore, is not truly known.

Parents, school health officials, and physicians face a dilemma in the choice of therapy, and advertising claims by manufacturers of pediculicides make the evaluation of relative efficacy difficult. Although the preferred method of testing therapeutic products involves adequately controlled clinical trials conducted by experienced, unbiased investigators, these studies are costly, time consuming, and would require several hundred patients.

In 1981, we initiated studies to develop an in vitro method that would be standardized and reproducible and that would reflect the results to be expected in a full-scale clinical trial with respect to pediculicidal and ovicidal activity. Our previous experience with the most currently used in vitro method did not reflect the results we obtained in clinical trials. Our in vitro method takes into account not only the active ingredients but also the activity of the vehicle that is often an important component of the finished product's activity.

To our knowledge, all attempts to establish laboratory colonies of Pediculus humanus var capitis, the human–head louse (Fig 1), have failed. Those colonies that do exist are those of the body louse or possibly hybrids of body lice and head lice. They have been maintained through hundreds or thousands of generations, are fed on rabbits, and have become attenuated to an artificial laboratory existence. For these reasons, they differ in feeding habits, life-style, and appearance (Fig 2) from body lice obtained from infested humans and bear even less resemblance to head lice.

We therefore based our model on recently captured head lice from infested children. Ovicidal activity was also evaluated for freshly collected viable eggs on hairs clipped from the same subjects.

The test results reported herein are from a study requested by the National Pediculosis Association, Newton, Mass. The study was conducted in Central America between April and August of 1984 in a population with endemic pediculosis capitis. The study site was an isolated mainland village with no prior history of pediculicide or pesticide usage, so that previous exposure or tolerance to any of the products tested was not a factor. Informed consent was obtained from the parents of children from whom lice were collected. These subjects and other family contacts were offered treatment with approved pediculicides.

Early work showed that careful attention to techniques and environmental variables is vital to standardization and reproducibility. The methodology is therefore reported in detail.
Fig 1.—Pediculus humanus var capitis, the human-head louse (adult male), recently obtained from scalp of infested child. Note tough chitinous exoskeleton and dark bands of pigmentation (X16).

Fig 2.—Laboratory strain evolved from body louse, Pediculus humanus var corporis. After thousands of inbred generations, normally rigid chitinous exoskeleton is thin and flexible, resulting from protection from trauma under artificial conditions. Note engorgement following recent blood meal (X8).

Fig 3.—Four head lice eggs laid on single human hair. From left to right, first two eggs have already hatched, and operculums are absent. Third egg is viable, with intact operculum and visible eye spot. Fourth nit, which has eyespot and intact operculum, is nonviable, since it contains air pocket (X16).

Fig 4.—Scanning electron micrograph of stillborn head louse nymph. Egg was treated with 1% lindane shampoo, which did not kill nymph in situ. Note that adult female louse produces cementlike coating on egg and hair which is unaffected by pediculicide (X57).

Fig 5.—In vitro method of evaluating pediculicides using head lice. Five minutes after initial contact with 0.5% malathion lotion, all lice were dead (X8).

Fig 6.—Scanning electron micrograph of viable head louse egg. Note pores or spiriclelike structures of operculum through which, we believe, ovicides penetrate to kill nymph in situ (X90).
Materials and supplies were protected at all times from pesticide or other chemical contamination. Routine spraying of storage and laboratory areas was prohibited. Three bottles of each product tested were obtained from different pharmacies in the United States and protected from heat and sunlight. Expiration date (which were all within one year), lot number, and place of purchase were recorded for each bottle.

All water used in these experiments was unchlorinated and filtered through 0.45-μm-dense charcoal to remove any bacteria or chemical contaminants (Pressure Pure, Seagull IV filter system).

Cotton-polyester kitchen towels were boiled with a small quantity of nonmedicated anionic shampoo, thoroughly rinsed in several changes of boiling water, and air dried. Five-centimeter diameter disks were cut from these prepared towels and sterilized by autoclaving.

At the time of testing, one prepared cloth disk was placed in the bottom of a sterile plastic 60 X 15-mm Petri dish.

For each experiment, at least 70 adult male and female head lice and nymphs were collected from the heads of six or more infested children. These lice were pooled in a Petri dish containing a cloth disk dampened with filtered water.

Specimens were tested within three hours of collection and were protected from sunlight and excess heat. Prior to testing, all lice were allowed to feed on the alcohol-cleansed forearms of one of us (T.L.M.). This circumvented the differences in susceptibility we had noted in pilot studies between starved and freshly fed lice.

One milliliter of the test material was evenly distributed over the cloth disk and allowed to thoroughly impregnate. This amount of product produced a wet surface with which the lice remained in intimate contact. One milliliter of water was used in control dishes. The lice grasped the threads of this cloth as if they were human hairs. This open system simulated an in vivo treatment but allowed the lice to remain in contact with the product until death.

Ten lice, including adults of both sexes and nymphs, were gently transferred to the test dishes using single strands of human hair to avoid physical damage to the lice.

They were observed with a ×10 hand-held lens until the death of the last louse. Time of death was recorded when all movement and peristalsis of the gut had ceased.

Tests were conducted under artificial lighting at ambient temperatures (27 to 30 °C) and humidity levels of 70% to 90% relative humidity. Sets of test materials and controls were evaluated at least ten times on different days. The mean killing time for ten or more experiments was estimated, and the SD was calculated. On each test day, ten or more lice were used as controls.

Ovicidal Activity

Three- to 4-centimeter lengths of single hairs, each with a viable egg attached, were snipped from infested children and placed in a clean polyethylene container. Viable eggs were plump, shiny, and tan to coffee colored, with an intact operculum and, frequently, a pigmented eye spot. Nits that were empty, shriveled, misshapen, or indented and those that contained air pockets were discarded (Fig 3).

Ten hairs with viable eggs were attached to small adhesive labels, allowing a 2-cm-long strand of hair to protrude, with the eggs aligned at the distal end. When clamped with hemostat forceps, the label formed a convenient holder for transferring ten eggs simultaneously to the test solutions and rinses.

Immediately prior to testing, medications were dispensed into clean 8-mL glass vials, which were discarded after the test. Each set of ten eggs was immersed in the product for exactly ten minutes. The eggs were then agitated in several changes of filtered water and air dried at an ambient temperature.

Each set of ten hairs with eggs were transferred to clean sterile glass vials (15 X 45 mm), which were capped and incubated at 30 to 34 °C for two weeks in the dark. Each experiment was replicated ten or more times. On each day experiments were conducted, ten eggs were dipped in filtered water for ten minutes and air dried as controls.

Ovicidal activity was expressed as the percentage of eggs hatched after 14 days, using the following formula:

\[
\text{Percent Hatched} = \frac{\text{Number Hatched}}{\text{Number Tested}} \times 100
\]

Eggs from which the newborn nymph was able to lift the operculum but did not fully emerge were termed *stillbirths* (Fig 4). These were not counted as hatched.

Results

Killing Time

There was considerable variation in the killing time between products (Table 1); 0.5% malathion lotion (Prioderm Lotion) demonstrated the quickest knock down and kill. No louse survived five minutes after initial contact (Fig 5). The synergized natural pyrethrin products were the next quickest killers.
We were concerned with the value of this in vitro model in reflecting the results of actual clinical use. In all clinical trials, the products were used in accordance with the manufacturer's directions. During 1984, in a nearby community not previously exposed to pesticides, our team personally treated more than 300 patients with 1% lindane shampoo (Kwell Shampoo). Seventy-nine of these subjects were closely followed up on a daily basis for two weeks. We found live lice on all subjects 15 minutes after treatment; in several patients, live lice were found six hours later. Many patients complained that they could feel lice "dancing" on their scalps for several hours after treatment. We have consistently observed hyperactivity and twitching of lice after exposure to lindane. Thus, the slow-killing effect of lindane shampoo was confirmed by clinical experience.

In clinical trials conducted in two similar villages in this area, none of 280 subjects treated with Prioderm Lotion and none of 98 subjects treated with RID complained of "dancing" lice, and no live lice were found 15 minutes after application of the products. The relatively quick-killing effect of RID and Prioderm Lotion was therefore confirmed in clinical use. Pediculidal activity of the other products tested has not been validated in clinical trials by us.

Ovicidal activity in clinical trials was also precisely measured by collecting eggs from each patient before and after treatment and determining hatching rates. Table 3 shows the comparison of hatch rates obtained by our in vitro method compared with results obtained in clinical trials with Kwell Shampoo and Prioderm Lotion.

The low hatch rates for malathion lotion and the considerably higher rates following treatment with lindane shampoo are therefore evident in both the in vitro method and clinical use.

Other products have not been evaluated for ovicidal activity in clinical trials by our team.

**COMMENT**

We consider the conditions of this test procedure to represent a maximum exposure test, since the lice were in close contact with the undiluted product until death. Similarly, the eggs were totally immersed in the formulations for ten minutes and protected in vials until hatching. We believe that the results indicate the maximum effect that could be achieved in actual clinical use. The times chosen for the test were standardized to allow direct comparison of activity and, for all products except Prioderm Lotion, the time of exposure of lice and eggs equaled or exceeded the manufacturers' instructions.

The results suggest several guidelines for those faced with the management of pediculosis. First, 1% lindane shampoo, which is available only by prescription, offers no advantage in pediculidal or ovicidal activity compared with several over-the-counter products. It is cosmetically elegant and requires less than ten minutes for treatment. For

---

Table 2.—Ovicidal Activity as Determined by In Vitro Method

<table>
<thead>
<tr>
<th>Pediculicide</th>
<th>No. of Eggs Hatched</th>
<th>% of Eggs (Stillborn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prioderm Lotion</td>
<td>5/123</td>
<td>5/100</td>
</tr>
<tr>
<td>A-200 Pyrinate Shampoo</td>
<td>23/98</td>
<td>23/23</td>
</tr>
<tr>
<td>R&amp;C Shampoo</td>
<td>29/117</td>
<td>25/18</td>
</tr>
<tr>
<td>RID</td>
<td>26/99</td>
<td>26/34</td>
</tr>
<tr>
<td>Kwell Shampoo</td>
<td>42/141</td>
<td>30/18</td>
</tr>
<tr>
<td>A-200 Pyrinate Liquid</td>
<td>32/100</td>
<td>32/23</td>
</tr>
<tr>
<td>Control (water)</td>
<td>91/98</td>
<td>93/0</td>
</tr>
</tbody>
</table>

Table 3.—Comparison of Ovicidal Activity in In Vitro Method and Clinical Trials

<table>
<thead>
<tr>
<th>Pediculicide</th>
<th>Clinical Trials</th>
<th>In Vitro Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prioderm Lotion (0.5% malathion lotion)</td>
<td>26/560 (3)</td>
<td>5/103 (5)</td>
</tr>
<tr>
<td>Kwell (1% lindane shampoo)</td>
<td>288/560 (51)</td>
<td>42/141 (30)</td>
</tr>
<tr>
<td>Controls</td>
<td>1,338/1,480 (90)</td>
<td>91/98 (93)</td>
</tr>
</tbody>
</table>

*All clinical trials were conducted in accordance with manufacturers' instructions (Prioderm Lotion, eight- to 12-hour application; Kwell Shampoo four-minute application). Ovicidal activity in clinical trials was determined by taking ten viable nits from patients before treatment and ten viable nits from patients after treatment. Our in vitro method consisted of a ten-minute exposure.

with killing times from 10.5 (± 3.4) minutes for RID to 22.5 (±9.0) minutes for A-200 Pyrinate Liquid. Finally, 1% lindane shampoo (Kwell Shampoo) was consistently the slowest-killing product (190.2 [± 46.5] minutes). In none of the ten experiments did this product kill all lice in less than two hours.

All products tested killed lice within four hours. All control lice were alive and active at four hours, with none surviving longer than 15 hours. Six to 15 hours is the normal longevity of head lice removed from the host and given no opportunity to feed.

**Ovicidal Activity**

Our results are shown in Table 2. Prioderm Lotion was the most effective ovicide, killing 95% of the eggs. It was the only alcoholic lotion tested and the only one that penetrated the eggs to kill the nymphs in situ. All other products yielded significant numbers of stillbirths after treatment. The synergized pyrethrin products were not completely ovicidal. The percentage of eggs that produced viable nymphs after treatment with these products ranged from 23% (A-200 Pyrinate Shampoo) to 32% (A-200 Pyrinate Liquid); 1% lindane shampoo (Kwell Shampoo) showed ovicidal activity in the same range as the synergized pyrethrin products (30% hatch rate).
practical purposes, the four synergized pyrethrin preparations ranked approximately equal in effectiveness as pediculicides and ovicides. All were considered cosmetically elegant and easy to use, requiring only ten minutes’ application.

Second, 0.5% malathion lotion is a highly effective, rapid-acting pediculicide and was the only product tested that showed excellent ovicidal activity. Many patients found the odor unpleasant and objected to the eight- to 12-hour application time; the high alcohol content necessitates precautions to avoid open flames and hair dryers. The particular product (Pridiern Lotion) used in our study is no longer available in the United States. The results of the pediculicidal and ovicidal activity of 0.5% malathion lotion have been included as a positive control. All pediculicides require careful application to avoid potential irritation of eyes and mucous membranes.

We stress that head lice exposed to all of the tested products died within four hours. Thus, they may all be considered effective pediculicides. In our experience, however, patients prefer a product that kills lice quickly. The appearance of crawling or twitching lice or the sensation of crawling lice on the scalp is often a source of emotional stress.

The relatively poor ovicidal activity of the synergized pyrethrin preparations after a single treatment leaves something to be desired. However, current recommendations instruct the user to repeat the treatment seven to ten days later. Our in vitro studies suggest that this should prove effective in killing nymphs that have hatched from eggs still viable after the first treatment, but we have not had the opportunity to test this hypothesis in clinical trials.

The high rate of stillbirths (18% to 34%) in eggs treated with all formulations except Pridiern Lotion raises questions concerning the mechanism of ovicidal activity. It suggests that the product did not penetrate the egg to kill the nymph in situ. No trials.

We believe that the system reported herein represents an advance in technology. We used marketed products and freshly collected, recently fed head lice. The end point of time required to kill all lice more accurately reflects the desired clinical results as an alternative to the laboratory variable of LD50. Similarly, we believe that the percent of eggs hatching into viable nymphs following treatment is more pertinent to the clinical situation.

In attempting to control the current epidemic of head lice, we should be concerned with the lice and eggs that survive treatment. Our results suggest that a single-treatment, fast-acting, completely ovicidal, and cosmetically elegant pediculicide has yet to be developed.

The authors gratefully acknowledge the support of the National Pediculosis Association (NPA) and the encouragement of the NPA Scientific Advisory Board; the medical assistance of R. Sanchez, MD, J. A. Chen Lee, MD, and P. Castillero, MD; the field assistance of Isidela Perez; the photography of Fig 2 by Jack Clark; and the secretarial assistance of Gloria Hernandez.

References

7. "World Health Organization, WHO/VBC/81.888, Instructions for determining the susceptibility or resistance of body lice and head lice to insecticides."