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SHORT COMMUNICATION

Response of *Pediculus humanus humanus* (Pediculidae: Phthiraptera) to water or 70% ethanol immersion and determination of optimal times for measuring toxic effects

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Abstract Human pediculosis is caused by *Pediculus humanus humanus* (Linnaeus 1758) and *Pediculus humanus capitis* (De Geer 1767). We studied the response of body lice to immersion in water and ethanol 70% and determined the optimal times for measuring knockdown and mortality. After immersion in water, all lice remained alive from 5 min to 22 h for both times of exposure. A low proportion of lice were affected after 2 min of immersion in ethanol in the 10-min exposure test, but recovered completely after 5 min. Different proportions of lice were affected between 2 and 7 h after immersion in ethanol, depending on the immersion time. However, a high proportion of lice recovered after 22 h. The results suggest that the optimal times for measuring early knockdown effects of insecticides are the 5-min to 7-h interval for water and 5-min to 1-h interval for ethanol. On the other hand, the best time for measuring mortality is 22 h after immersion. These results should improve the interpretations of the effects of pediculicides in immersion bioassays.

Introduction

Human pediculosis is caused by *Pediculus humanus humanus* (Linnaeus 1758) and *Pediculus humanus capitis* (De Geer 1767). Chemical control of head lice, *P. humanus capitis*, has been performed using a wide variety of insecticides from DDT, dieldrin, and lindane to malathion, pyrethrum, and other synthetic pyrethroids such as permethrin and d-phenotrin. Unfortunately, the repetitive and inadequate application of pediculicidal products has resulted in the development of resistance to one or more of these products in several countries (Burgess 2004; Mougabure Cueto et al. 2008). Within this context, it is imperative to continue monitoring for resistance and evaluating the pediculicide activity of alternative molecules. For the latter, adequate standardized bioassays using insects to evaluate the biological effects of different molecules and formulations guarantee the validity and applicability of the results. Some of the proposed bioassays for evaluating pediculicidal activity include immersion and topical application as methods of insecticide exposure (Burkhart and Burkhart 2001; Meinking et al. 2001; Mougabure Cueto et al. 2002, 2008; González Audino et al. 2007; Heukelbach et al. 2008).

As head and body lice show similar toxicological phenotypes, a laboratory-reared *P. humanus humanus* is frequently used as a reference colony in insecticide resistance studies for head lice and as a test organism to evaluate the efficacy of potential pediculicides (Mumcuoglu et al. 1990; Downs et al. 2000; Kristensen et al. 2006; Priestley et al. 2006; Mougabure Cueto et al. 2008; Gallardo et al. 2009). Furthermore, a laboratory-reared colony allows carrying out bioassays under standardized physiological conditions.

The response of the insects to the control conditions of a bioassay (e.g., immersion in ethanol) allows establishing the optimal times for measuring effects such as mortality and knockdown and hence determines the pediculicidal activity of the evaluated compound. It has been shown that human lice have the capacity to physiologically shut down and go into stasis during immersion and then recover from an apparently dead state (Burkhart and Burkhart 2001; Heukelbach et al. 2008). Moreover, the insects can metabolize insecticides and recover from insecticide poisoning.

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Therefore, in insecticide assays, it is not easy to determine to what extent this shut down behavior is a physiological response to immersion itself or the result of intoxication with the compounds. Canyon and Speare (2007) reported that head lice become immobile during immersion in different aqueous solutions and recover from stasis 0–1 min after being rescued. Similar responses to immersion have been reported in body lice (Canyon and Speare 2007). The description of the symptoms of lice immersed in control conditions will clearly improve our interpretation on the effects of pediculicides.

Considering the above, the aims of this research were to describe the response of *P. humanus humanus* to immersion under the control conditions of a typical bioassay for evaluating pediculicides and to determine the optimal times for measuring toxic effects.

Materials and methods

Insects Body lice were obtained from a colony reared for 6 years at our laboratory and originated from a colony of the Department of Parasitology, University of Queensland, Brisbane, Australia. The colony was maintained at 28±1°C, 55±1% RH, and fed on rabbits six times a week for 20–30 min.

Chemicals Absolute ethanol was purchased from Merck (Buenos Aires, Argentina).

Bioassays Male lice were treated as a control group of an immersion bioassay for evaluating pediculicidal products (Mougabure Cueto et al. 2002). We evaluated two kinds of control conditions: immersion in an alcoholic formulation (ethanol 70%) and immersion in an aqueous formulation (water). Lice were submerged for 2 or 10 min in distilled water or ethanol 70%. Twenty-five lice were used for each of the four experimental groups. After the immersion period, the insects were placed on a wire mesh and washed with 100 ml of water. They were then individually placed on pieces of filter paper No. 1 (Whatman, Maidstone, UK), 5.5 cm in diameter, located in plastic Petri dish lids. Each louse was observed at short (2, 5, 10, and 30 min), intermediate (hourly from 1 to 7 h), and long intervals of time (after 22 h). The observation ended after 22 h due to a noticeable increase in lice mortality after 24 h due to physiological damage. At each observation time, the lice were categorized as alive (ability to walk on the filter paper) or affected (inability to walk). These categories were determined by inspecting each louse with an Olympus SZ4045 stereomicroscope. All bioassays were performed at the optimal temperature for comparative bioassays of head and body lice, 18°C and 97% RH (Gallardo et al. 2009).

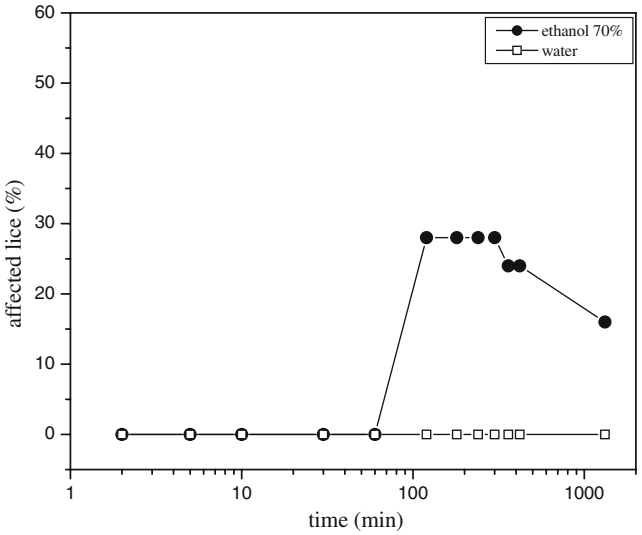

Fig. 1 Affected lice at different times after 2 min of immersion in ethanol or water

Results and discussion

After immersion in water, all the lice were alive at all the registered times of both treatments, except at 2 min in the 10-minute treatment where we found 2% of affected lice (Figs. 1 and 2).

The proportion of lice affected after immersion in ethanol for both exposure times are shown in Figs. 1 and 2. In general, all lice remained alive at 1 h after immersion. A low proportion of lice were affected at 2 min in the 10-min treatment, but then recovered completely at 5 min. Different proportions of lice became affected between 2 and 7 h after immersion, depending on the immersion time. However, a high proportion of lice recovered after 22 h.

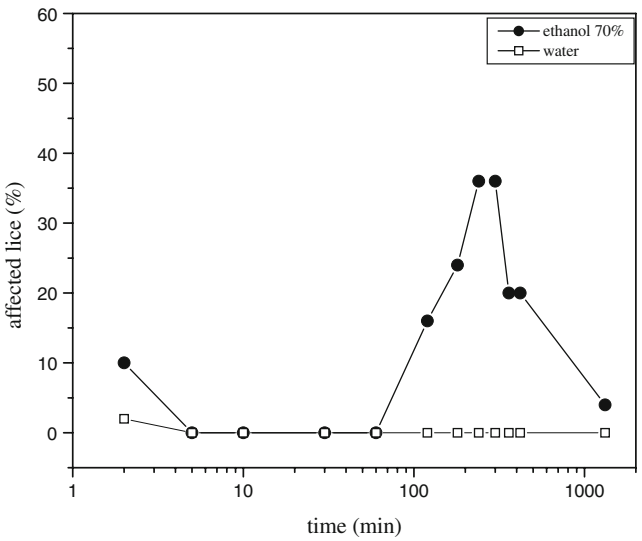

Fig. 2 Affected lice at different times after 10 min of immersion in ethanol or water

Canyon and Speare (2007) showed that lice went quickly into stasis during immersion, recovering shortly after being rescued (<1 min). The present study showed that in all the treatments most of the lice recovered from early stasis in less than 2 min. Only a low proportion of lice were found affected after 2 min in both the 10-min treatments, but fully recovered within the next 2 to 5 min.

All lice were alive between 5 min and 1 h after immersion in all the treatments; however, in both ethanol treatments, affected lice were observed again after this period. Percentages of affected lice higher than 20% were observed at each observation interval between 2 and 7 h after immersion in ethanol, which is the maximum admitted for mortality correction in toxicological bioassays (Abbott 1925). The highest percentage of affected lice observed at any time was 28% in the 2-min immersion treatment and 36% in the 10-min immersion treatment. The total amount of affected lice after 1 h was 36% and 44% for the 2- and 10-min immersion treatments, respectively. The proportion of affected lice at the end of the bioassay (22 h) did not exceed Abbott's threshold (20%) in any experimental group.

In conclusion, the results showed that there are two periods in which lice were affected and then recovered: an early period of transitory affection (before 5 min) in the 10-min treatment of both water and ethanol, and a later period of transitory affection (between 2 and 22 h) that was only observed for ethanol. Early affection seems to occur as a reaction to immersion independent of the evaluated solution (it could be a natural adaptation to immersion in lice). On the contrary, the later transitory affection depended on the solution used during immersion. This later affection was clearly a symptom of ethanol intoxication, a compound that the lice were capable of metabolizing and then recovering afterwards.

The above description opens the discussion regarding the time for measuring toxic effects (knockdown, mortality, etc.) in a bioassay. This time should be established at an interval where there are no affected control insects, so that any observed effects can be attributed to the evaluated molecule. For an immersion bioassay with human lice, this interval should be after the recovery time from early affection due to immersion. Therefore, we recommend the post-immersion interval from 5 min to 1 h, or 22 h after immersion, for measuring toxic effects in bioassays using ethanol in the controls (e.g., evaluation of alcoholic formulations), while any time after 5 min is recommended for bioassays using water (e.g., evaluation of aqueous formulations). The interval between 2 and 7 h is not recommended for assays with alcoholic formulations due to the high proportion of affected control insects.

Some insecticides (e.g., pyrethroids) produce knockdown effects from which the insects can recover after the early symptoms and survive or not recover and die, depending on the dose used. Recovery time from knockdown mainly depends on the insecticide molecule. Insecticide bioassays should be able to discriminate between the two toxic effects caused by insecticides, knockdown (with possible recovery) and mortality. Additionally, the time for measuring mortality should consider insect recovery from knockdown. The standardized WHO resistance test for human lice recommends that mortality records should be taken 24 h after exposure to the insecticide (WHO 1981). Burkhart and Burkhart (2001) recommend that mortality should be monitored within 24 h, as after this time the mortality of lice increases notably due to physiological damage. According to those authors, the optimal time for measuring mortality is close to 24 h (but lower) and with a mortality of control insects compatible with Abbott's correction (<20%). In this study, the mortality at 22 h is lower than 20% in all the treatments, and therefore, we recommended this time for measuring mortality. In consequence, any time during the 5-min to 7-h interval for water and 5-min to 1-h interval for ethanol is recommended for measuring insecticide knockdown effect.

The two immersion periods evaluated (2 and 10 min) allow measuring toxic effects at the recommended times, indicating that both periods can be used in an immersion bioassay. However, short times (e.g., 2 min) allow a better discrimination between the potencies of different molecules than long times (e.g., 10 min).

Although head and body lice show similar toxicological phenotypes, the results in body lice might be different from those obtained in head lice, which in general die earlier and require more restricted laboratory conditions of temperature and humidity for the survival than body lice (Gallardo et al. 2009).

In summary, we determined the optimal times for measuring toxic effects in human lice during an immersion bioassay by studying the response of lice to the control conditions of that kind of the test. These results will allow measuring and differentiating toxic effects efficiently, improving the subsequent interpretations on the pediculicidal activity of the evaluated compounds.

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