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Utilization of the human louse genome to study insecticide resistance and innate immune response

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ABSTRACT

Since sequencing the human body louse genome, substantial advances have occurred in the utilization of the information gathered from louse genomes and transcriptomes. Comparatively, the body louse genome contains far fewer genes involved in environmental response, such as xenobiotic detoxification and innate immune response. Additionally, the body louse maintains a primary bacterial endosymbiont, *Candidatus* Riesia pediculicola, and a number of bacterial pathogens that it vectors, which have genomes that are also reduced in size. Thus, human louse genomes offer unique information and tools for use in advancing our understanding of coevolution among vectors, endosymbionts and pathogens.

In this review, we summarize the current literature on the extent of pediculicide resistance, the availability of new pediculicides and information establishing this organism as an efficient model to study how xenobiotic metabolism, which is involved in insecticide resistance, is induced and how insects modify their innate immune response upon bacterial challenge resulting in enhanced vector competence.

1. Pediculicides and resistance

The infestation of humans by lice is called pediculosis and the chemicals used to treat such infestations are called pediculicides. Before the availability of effective antibiotics, infestation by the human body louse, *Pediculus humanus humanus*, was common and the ability of the body louse to vector a number of bacterial diseases lead to the death of millions and in some cases changed the course of our history. Today, the infestation of the human scalp and hair by the human head louse, *Pediculus humanus capitis*, is more common. Although they are not vectors of disease, head louse infestations are a major economic and social concern worldwide because infestations are often associated with school-aged children, who often miss substantial number of school days and can suffer emotional distress not to mention the increased possibility of secondary infections due to self-inoculation upon intense itching [1]. Over the past 70 years, the control of pediculosis has been largely dependent upon the availability of natural and synthetic insecticides starting with DDT (1943), natural pyrethrins (1945), the organochlorine lindane (1960), organophosphorous insecticides (malathion, 1971), carbamates (carbaryl, 1977) and synthetic pyrethroids (permethrin, phenothrin, 1992) [2].

In the USA, the pyrethrins/pyrethroids have dominated the over-the-counter (OTC) market since their availability beginning in 1992, followed by the prescription only malathion-containing formulations, such as Ovide®, beginning in 1971. The pyrethrins/pyrethroids share a common target site in the nervous system, the voltage-sensitive sodium channel (VSSC), and act as agonistic neuroexcitants by increasing sodium current, leading to nerve depolarization and

Abbreviations: BR-HL, malathion- and DDT-resistant strain from Bristol, UK; DDT, dichlorodiphenyltrichloroethane; Duox, dual oxidase; EC-HL, insecticide-susceptible head louse strain from Ecuador; HLCbE3, head louse malathion carboxylesterase; Imd, immune deficiency pathway; *kdr*, knockdown resistance; KR-HL, insecticide-susceptible head louse strain form the Republic of Korea; MCE, malathion carboxylesterase; PGRP, peptidoglycan recognition protein; PPO, prophenoloxidase; qPCR, reverse transcriptase quantitative real-time polymerase chain reaction; RNAi, RNA interference; SF-HL, DDT- and permethrin-resistant head louse strain from south Florida; VSSC, voltage-sensitive sodium channel.

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hyperexcitation, followed by neuromuscular paralysis and death. Malathion is a phosphorodithioate-type organophosphorous insecticide, which is an indirect nerve toxin that acts as a competitive irreversible inhibitor of acetylcholinesterase associated with the cholinergic nervous system. When inhibited, acetylcholinesterase cannot efficiently hydrolyze the neurotransmitter, acetylcholine, allowing overstimulation of post-synaptic effector organs, including muscle, leading to paralysis and death.

Insecticide resistance to currently-used pediculicides, including permethrin, synergized pyrethrins and malathion, has occurred worldwide, is increasing [3–5] and is certainly contributing to increased incidences of pediculosis. Insecticide resistance threatens the success of all control programs but is particularly problematic in the control of human lice for several reasons: (1) they are obligate human blood feeders that are exposed to pediculicides at all stages; (2) they have short generational time and high fecundity; and (3) there are few pediculicidal products, the majority of which share common chemistry and elicit cross-resistance. Because of these issues, louse resistance to most commercial pediculicides has occurred and is increasing [2].

Both clinical and parasitological pyrethroid resistance to *d*-phenothrin was first reported in France in 1994 [6] with additional reports of clinical control failures following: permethrin (2001) in the USA [7], phenothrin (2005) in the UK [8], and permethrin (2005) in the UK [9]. Also, parasitological resistance has been reported in the Czech Republic [10], the UK [8], Denmark [11], Israel [12], the USA [13], Argentina [14], Japan [15] and Australia [16].

Malathion resistance was first reported in France in 1995 [17], followed by the UK in 1999 [18], Australia in 2003 [16], and Denmark in 2006 [11]. The lack of extensive resistance in the USA is likely due to the use of the Ovide® formulation, which also includes pediculicidal terpenes likely resulting in a mixture that has redundant killing action on multiple target sites [19].

Current control and resistance problems underscore the need to understand the molecular mechanisms of insecticide resistance in lice. The identification of resistance mechanisms and novel target sites may allow the development of resistance-breaking compounds and specific non-toxic synergists useful in novel control strategies. Recently, a number of new topical pediculicidal products have been introduced to the marketplace. They possess novel modes of action, show little cross-resistance to existing commercially-available pediculicides and appear safe and effective. The following section has been recently reviewed and the following is in large part from Clark et al. [1].

1.1. Dimeticone-based formulations

There has been a trend, primarily in Europe, for the development of physical means to control head lice because of increasing instances of resistance, particularly to the neurotoxic pediculicides, and the increased scrutiny of the use of such products on children. The dimeticone-based anti-louse products (silicone oils) are of interest due to their low mammalian toxicity, novel modes of action (not neurotoxic) and the possibility that they will have a low potential for the development of resistance. Dimeticones are linear polydimethylsiloxanes ($CH_3SiO[SiO(CH_3)_2]_nSi(CH_3)_2$), where *n* is the number of repeating monomers [$SiO(CH_3)_2$] of varying chain length. The chain length substantially influences the viscosity of the dimeticones and thus, they can vary considerably in spreading characteristics. Of the different dimeticone-based products available, two products are better characterized scientifically in terms of their effectiveness and probable modes of action.

Hedrin® 4% lotion (Thornton & Ross Ltd, Huddersfield, UK) is a 4% dimeticone lotion in 96% (w/w) decamethylcyclopentasiloxane (cyclomethicone D5). Head lice treated with this product are rapidly immobilized but small movements in their extremities over several hours indicate that death is delayed. Scanning electron microscopy coupled with X-ray microanalysis revealed that Hedrin® 4% lotion was found in the spiracles, in some cases blocking the opening completely, and penetrated into the outer aspects of the tracheae [20]. Asphyxia is unlikely as a mode of action given the slow onset of mortality. The inability of the louse to excrete the excess water acquired during blood feeding by transpiration via the spiracles has been suggested as a mode of action with death occurring by either prolonged immobilization or by the rupture of organs such as the gut [20].

The second dimeticone-based anti-louse product (NYDA®, G. Pohl-Boskamp GmbH& Co. Hohenlockstedt, Germany) contains a mixture of two dimeticones, one of low and the other of higher viscosity, at a final total concentration of dimeticones of 92% (w/w). Medium-chain length triglycerides, jojoba wax and two fragrances make up the remaining constituents. NYDA® rapidly enters the tracheal system, filling even the smallest branches, due to its superior spreading characteristics [21]. Within 1 min of treatment with NYDA®, lice do not show any major vital signs. This effect appears to be due to an interruption in the oxygen supply leading to suffocation. Additionally, NYDA® has been shown to be an effective ovicide [22].

1.2. Ivermectin-based formulations

Ivermectin is a macrocyclic lactone produced fermentatively by *Streptomyces avermitilis* and is a widely-used oral anthelmintic agent for both humans and companion animals. It has a unique mode of action by reducing motility and feeding in treated nematodes [23]. In addition to muscles used in motility, ivermectin also acts to paralyze the muscles associated with the pharyngeal pump, inhibiting the pumping action needed for feeding and attachment [24]. The concentration of ivermectin needed to cause paralysis of the pharyngeal pump is 10- to 100-fold lower than the concentration needed to cause mortality [25].

Ivermectin increases chloride ion permeability in insect [26] and nematode [27] neurons and muscle membranes through binding to glutamate-gated chloride ion channels. These channels are highly expressed in the neuromuscular system of the pharyngeal pump in the mouthparts of the free living nematode, *Caenorhabditis elegans*, which has been shown to be highly sensitive to ivermectin. It is believed that during de-worming, nematode parasites are killed by ivermectin acting on glutamate-gated chloride channels in the cells of the neuromuscular system. Ivermectin increases chloride ion influx, which hyperpolarizes the cells, leading to paralysis of the mouthparts. This action causes the worms to detach from the mammalian gut and be excreted. A similar mode of action in head lice, however, has not been directly characterized.

Recently, successive oral ivermectin treatments were used to treat hard-to-control head louse infestations [28]. The need for successive treatments in the oral ivermectin studies indicates that one treatment, while effective against feeding lice *in situ*, needs to be supplemented by a second systemic treatment to kill nymphs that emerge from eggs present at the time of the initial treatment. This implies an absence of an ovicidal effect of oral ivermectin.

Ivermectin also has been formulated as a less invasive topically-applied pediculicide that possesses the ability to kill permethrin-resistant head lice [29]. A 0.5% ivermectin topical cream formulation (Sklice®, Sanofi Pasteur Inc., Swiftwater, PA) killed permethrin-resistant head lice [29] but was not directly ovicidal to treated eggs, as hatchability was not decreased [30]. Nevertheless, the percent of hatched lice from treated eggs that took a blood meal significantly decreased (80–95%) compared with lice that hatched from untreated eggs and all treated lice died within 48 h of hatching, including those that fed. Dilutions of ivermectin formulation of 0.15 and 0.2 μg/ml, which were topically applied to 0–8 day old eggs, were not lethal to lice at 24 h post-eclosion. However, 9% and 16% less lice fed when hatched from these treated eggs, respectively. This observation led us to hypothesize that ivermectin may be acting on the glutamate-gated chloride channels in the neuromuscular system