Physically acting products for head lice – the end of the beginning

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Abstract

Treatment of head louse infestation has evolved from widespread use of neurotoxic insecticides that have been extensively affected by resistance since the mid-1990s into the use of so-called physically acting treatments. It is widely believed that physically acting products are effectively “resistance proofed” because they do not act to inhibit any particular physiological mechanism and most have some kind of occlusive effect on the target organism. Over the past 20 years various new active materials have been utilized ranging from natural oils, synthetic oils, through to surfactants both as excipients and active substances. Relatively few of these products have been adequately tested clinically and, of those that have, there is now some indication that they are less effective than when first introduced. The question therefore arises whether lice can become resistant to these physically acting products. Only adequate testing both in the laboratory and in clinical trials can determine their real effectiveness and claiming efficacy based on the presence of a named chemical rather than demonstrated activity may result in acquired resistance to these types of product also.

Introduction

Head lice with acquired resistance to pyrethroid insecticides were identified more or less concurrently by several investigators from different countries in the early 1990s (Chosidow et al., 1994; Mumcuoglu et al., 1995; Rupeš et al., 1995; Burgess et al., 1995). Subsequently resistance and resistance mechanisms were identified in widely distributed territories (Picollo et al., 1998; Pollack et al., 1999; Hunter & Barker, 2003; Kasai et al., 2009) and were also found to affect other insecticides in addition to the pyrethroids (Downs et al., 1999, 2002; Kristensen et al., 2006).

Despite repeated reports of treatment failure, and even attempts at litigation through class action by frustrated consumers (Williams, McIntyre & Pilitere, 2001), most pediculicide manufacturers failed to take positive action to address the problems, relying in some cases on historical data to support efficacy (Vander Stichele, Dezeure & Bogaert, 1995); professional assessments that suggested method or thoroughness of application technique influenced treatment outcome more than physiological tolerance (Aston, Duggal & Simpson, 1998); or specific formulations that showed better efficacy in some geographic regions (Bialek, Zelck & Fölster-Holst, 2011; Bouvresse et al., 2012).

During the late 1990s, as resistance to various insecticides increased in intensity, many consumer groups began to show a renewed interest in the use of natural materials for louse control, although this was minimally reflected in scientific investigation (Mumcuoglu et al., 1996; Veal, 1996), and only more recently have researchers developed this interest further, with a plethora of studies of the pediculicidal activity of plant extracts, essential oils, and other materials of plant origin. In most cases, the underlying thought process has been that plant extracts of any kind are sufficiently complex that any successful treatment would be less likely to be affected by resistance; that naturally derived materials are potentially safer than synthetic neurotoxic insecticides; and that they are environmentally more acceptable because they are biodegradable with minimal if any environmental accumulation. In reality most of these factors have not been tested fully, either experimentally or clinically, and reviews of the naturally derived treatment prospects show a diverse and extensive range of preliminary investigations but few actual products that have been properly evaluated and brought to market (Heukelbach, Speare & Canyon, 2007; Heukelbach, Canyon & Speare, 2007). In many cases claims are made that plant derived products act against insects by physical rather than physiological mechanisms. While that concept can feasibly apply to fixed plant oils, such as unrefined coconut oil or pressed olive oil, the majority of plant extracts operate through physiological mechanisms. For example, Tea tree oil, a popular essential oil distillate from *Melaleuca alternifolia* used against lice (Barker & Altman, 2010), like most mixtures of monoterpenes and terpene esters, can exert a solvent effect on insect lipids but the major active components, terpinen-4-ol and 1,8-cineol the latter of which is also the main constituent of eucalyptus oil, are both neurotoxins acting as acetylcholinesterase inhibitors in a similar manner to the mode of action of organophosphorus insecticides (Ryan &

Byrne, 1988; Mills et al., 2004; Miyazawa & Yamafuji, 2005; Jankowska et al., 2018). Therefore, contrary to the perceived ideas about these natural materials that they are not affected by resistance, it is quite possible that a cross-resistance between essential oil components and synthetic insecticides could result that not only renders the natural materials ineffective but also enhances levels of resistance by selection of more tolerant insects capable of detoxifying both groups of chemicals.

Consequently, only truly physically acting treatments could be considered not to be affected by the resistance mechanisms to conventional insecticides. The first breakthrough alternative therapeutic agents not relying on physiological mechanisms were actually based on technologies that had been investigated, but never used, from the early 1980s. These products with a siloxane (silicone) base proved to be highly effective in clinical studies in areas where resistance to insecticides was prevalent and overall were significantly more effective than conventional insecticide-based products (Burgess, Brown & Lee, 2005; Burgess, Lee & Matlock, 2007; Burgess, Lee & Brown, 2008; Heukelbach et al., 2008), showing that these products were not affected by the physiological resistance mechanisms affecting insecticides. Further studies showed that these products exerted a physical mode of action by coating the insects and smothering them by "asphyxiation" (Richling & Böckeler, 2008), prevention of water excretion (Burgess, 2009), or else by disrupting cuticle lipid integrity resulting in dehydration (Barnett et al., 2012). Each of these modes of action has been considered "resistance proof", i.e. no known mechanism of resistance was considered likely to affect the effectiveness of these types of product and it was anticipated that there could be no pathway that would permit lice to develop resistance to these types of technologies.

Although the two formulations first to market in this group of products based on siloxanes were patent protected (Ansell, 2001; Campbell, Palma & Paulsen, 2003), this did not prevent the development of a series of other alternative treatments based on siloxanes, mineral oils, and synthetic oils, because the intellectual property was limited in its scope by prior art (Lover et al., 1977) and newer or novel chemical entities were used in some cases (Boscamp, Hilscher & Vater, 2007; Rossel, 2008; Panin, 2010). However, despite the fact that there are now probably as many as 100 siloxane and synthetic oil-based products available worldwide, only a few have ever been subjected to clinical evaluation and even fewer of these studies have been published in peer reviewed journals (Burgess, Brown & Lee, 2005; Burgess, Lee & Matlock, 2007; Burgess, Lee & Brown, 2008; Heukelbach et al., 2008; Kurt et al., 2009; Izri et al., 2010; Burgess & Burgess, 2011; Burgess, Brunton & Burgess, 2013; Burgess, Burgess & Brunton, 2013; Wolf et al., 2016).

In addition to synthetic oil-based materials, designed to coat lice and "smother" them, a number of other materials have been investigated for activity that are believed to not directly affect physiological processes. In some cases these include plant-derived fixed oils or derivatives of these fixed oils, especially from coconut and neem. Neem oil is a complex mixture of compounds, the principal components of which make it essentially similar to extra virgin olive oil, but also including around 80 triterpenoids and similar compounds that putatively have some pharmacologic function. None of these terpenoids is present at any great concentration and the activity, if any, of the vast majority is unknown. Only azadirachtin, a highly oxidized tetranortriterpenoid that has been demonstrated by those interested in plant protection to be an antifeedant for caterpillars and other leaf eating insects, can be considered active. However, how a large and complex molecule that primarily acts upon the function of the gut after ingestion could act upon the biology of a blood feeding insect that draws its nutrition directly from its host's circulatory system is difficult to reconcile. Nevertheless claims have been made for its activity in an unspecified shampoo basis against head lice (Abdel-Ghaffar & Semmler, 2007; Abdel-Ghaffar et al., 2012; Semmler et al., 2017). The product used has only been clinically evaluated in rural communities in Egypt, where hair care products may be at a premium for the population concerned, so it is possible that any modern surfactant based shampoo would have exerted a similar effect to kill lice. Evidence from the UK suggests that neem itself has a low activity against lice (Brown & Burgess, 2017) with a cure rate of just 6/24 (25.0%) following four applications and a study performed in Thailand required not only a high concentration of neem extract (6%), but also 16% eucalyptus oil in an alcoholic basis applied twice, in order to achieve elimination of lice in 40/45 (88.9%) of those treated (Thawornchaisit et al., 2012).

In contrast to neem, coconut oil is rarely used in its original form but generally it is used as the primary feedstock for a wide range of chemical entities used in the toiletry and cosmetic industries, the majority of which are surfactants, wetting and spreading agents, or emollients. Unfortunately this has resulted in considerable confusion over what if any activity coconut may have against lice but, when it is considered that people in the tropical developing world have used coconut oil as a hair conditioner treatment for centuries yet still have lice in their communities, it makes it clear that coconut oil has little if any effect on lice. In contrast, derivative surfactants may or do have some activity. This is quite variable depending upon the chemical constituents of toiletry hair treatments used in any particular community and, just as insecticides and essential oils used in low doses can select for lice capable of tolerating them, lice exposed to low doses of surfactants and similar compounds can be selected for tolerance. A good example was shown in a study of cocamide diethanolamine (cocamide DEA) lotion in which *in vitro* screening using lice that had never been exposed to toiletry shampoos suggested 100% efficacy but when the product was used in community-based randomized clinical studies it failed to achieve a cure rate better than 33.9% (19/56) irrespective of the dosing used (Burgess, Brunton & Brown, 2015). Similarly, a study of a cocamide DEA-based shampoo, including other coconut derived surfactants, performed in a different locality also produced a low treatment success rate of 22/41 (53.7%) (Connolly et al., 2008). Theoretically this surfactant should be effective to cause damage to the lipid waterproofing layer on the surface of lice, and this was shown in the *in vitro* studies (Burgess, Brunton & Brown, 2015). However, since cocamide DEA has been so widely used in toiletry shampoos and conditioners, many lice would have experienced low dose exposures, which may have been increased after the popular rise of the wet-combing with conditioner approach to treatment in which hair toiletry products are applied to facilitate combing for quite long periods (around 30 minutes or so) on several occasions over a two week period (Ibarra, 1996).

Other surfactant products have been shown to kill lice in a similar way by damage to the waterproofing lipid coating of the lice (Burgess et al, 2014), but this activity was also influenced by the formulation vehicle and less irritant, aqueous-based, preparations were less effective than alcohol-based mixtures (Burgess et al, 2012), which suggests that surfactants alone have only a limited capacity for disrupting cuticle lipids and require additional solvent action to facilitate the activity. A similar lipid disruption effect is observable with a mixture of the fatty acid ester isopropyl myristate and cyclomethicone (Barnett et al., 2012), which proved acceptably effective (77.0%) during initial clinical studies (Burgess, Lee & Brown, 2008).

In North America there were no new products based on essentially similar physical modes of action until 2017. Instead, the majority of new preparations have employed alternative neurotoxins such as ivermectin (Pariser et al., 2012) and spinosad (Stough et al., 2009). One product type sold in North America and Australia, based on the named active substance, 5% benzyl alcohol, does claim to have a physical mode of action (Barker & Altman, 2010; Meinking et al., 2010). However, the claim that the benzyl alcohol component of the product "*effectively asphyxiates lice by ''stunning'' the spiracles open, allowing the vehicle, comprising mineral oil and other inactive ingredients, to infiltrate the ''honeycomb'' respiratory apparatus and kill lice*" (Meinking et al., 2010) is illogical and contrary to prior studies of louse anatomy. Webb (1946) showed histologically that the spiracle is a highly sclerotized and immovable structure and that the musculature controlling the opening of the neck of the trachea needs to contract, rather than relax, in order to open the respiratory pathway. It is not possible to "stun" a muscle into contraction. External stimuli cannot trigger contraction of the muscle unless a pharmacological event occurs e.g. by inducing a tonic-clonic spasm or some similar neuro-stimulatory effect. Additionally, the formulation of the 5% benzyl alcohol product (Ulesfia® Lotion, Shionogi Inc., Florham Park, New Jersey, USA), is such that even if benzyl alcohol was capable of having the claimed effect, the mineral oil is so effectively emulsified that it either could not separate out and block the respiratory structures or would be washed away when the product was rinsed off with water after just 10 minutes.

Interestingly, benzyl alcohol is listed as an excipient in some other products investigated for use on the North American market. The currently available 0.9% spinosad product (Natroba™, ParaPRO LLC, Carmel, Indiana, USA) contains 10% benzyl alcohol, making it difficult to work out how it could possibly be considered an "inactive" component of the formulation. Similarly a benzyl alcohol component has been reported as an excipient in an experimental treatment containing 0.74% abametapir, which to date has not received its marketing approval from the FDA but which proved effective in several clinical studies (Bowles et al., 2018).

Current Trends

After approximately 12 years of use, since the introduction of siloxanes and other lipids and lipid emulsifying chemical products, what is the current level of effectiveness of these products in clinical use? If the hypothesis were to hold true that the physical mode of action operates in such a way that resistance is unlikely, or even not possible, then all of the products should remain as efficacious as they were when first introduced. However, to eliminate entirely the possibility that lice could acquire a tolerance for physically acting treatments is at best naïve and at worst risky. Naïve in that it eliminates from thought the possibility that insects may either be selected by these products for different physical and/or physiological characteristics that could help them adapt to a the effects of the treatments and allow them to tolerate or avoid the active effects. Risky in that, as was seen with continued reliance on neurotoxic insecticides long past the point when they became ineffective, there could be a widespread failure to control the parasites.

Evidence for resistance

We have already considered the risks of cross-resistance between essential oils and neurotoxic insecticides, or perhaps different mechanisms developing in parallel that can affect essential oils as well as insecticides. In 1970 a product was formulated of 0.5% malathion in an alcoholic basis that also contained approximately 12% of the essential oil terpenoids *d*-limonene and α-terpineol. This product (Prioderm lotion, Napp Laboratories Ltd, Cambridge, UK), and a similar competitor preparation (Suleo-M, Charwell Pharmaceuticals Ltd, Alton, UK), was sold in the UK until 1988 when the original Prioderm was reformulated to remove the terpenoids to improve the odor. Within a few weeks, complaints of failure were reported to health professionals and it was later confirmed that the new preparation was less effective than the original because much of the activity of the original was conferred by the terpenoids (Burgess, 1991). However, the Suleo product as well as the Prioderm versions available in mainland Europe and the American branded version (Ovide, Taro Pharmaceuticals USA, Inc., Hawthorne, NY, USA) retained the terpenoids and the associated activity for considerably longer.

In 1999 the Suleo-M lotion product was used for a clinical study conducted in and around Cambridge, UK (Burgess, Brunton & Brown, 2015) as a rescue treatment. Although lice in the area were malathion resistant it was assumed the terpenoid content of the product would be sufficient to effect a cure. It was subsequently found after several failed “rescue” attempts that in some lice from failed treatments could be immersed in the fluid and survive. As a follow up to investigate this further, and to confirm that insecticide resistant lice were susceptible to silicone treatment, a group of head lice were collected families with long-term infestations and then treated using two different malathion preparations in the laboratory. These treatments gave similar results to those observed previously (Table 1) but all lice that survived the malathion exposure were silicone susceptible. For the aqueous emulsion, the resistance was to malathion alone but failure of the alcoholic product with terpenoids indicated a parallel acquired resistance to the terpenoids.

Table 1. Survival of resistant head lice treated using different malathion preparations, one a simple aqueous emulsion and the other an alcoholic solution containing 12% terpenoids.

Malathion product	Number of lice			Mortality %
	Total	Alive	Killed	
Aqueous	89	47	42	47.2
Alcohol/terpenoid	94	63	31	33.0
Control (water)	42	40	2	4.8

Although this observation confirmed that the lice were not affected by the neurotoxic activity of the terpenoids it also indicated that they were not affected by any solvent activity of the terpenoids on cuticle lipids. If that were the case it also raises a question as to whether other solvents or emulsifiers used to disrupt the protective lipid coating of the louse cuticle could be similarly affected by acquired tolerance.

Some similar effect appears to be happening with physically acting materials, for example, apparent loss of sensitivity appears to have developed to the isopropyl myristate/cyclomethicone (IPM/C) mixture, indicated by the differences between studies conducted a few years apart (Burgess, Lee & Brown, 2008; Burgess et al., 2017). In the first UK clinical investigations of the isopropyl myristate/cyclomethicone 50:50 mixture in 2005/6, it showed a consistent 77% cure rate (no lice found after the second application of treatment) in two related trials with 27/35 and 57/74 participants cured (Burgess, Lee & Brown, 2008). Around 18 months later a different comparative trial found a non-significantly ($p = 0.25$) lower rate of 68% with 36/53 participants louse free after two treatments (Burgess et al., 2017). However, since then achieving successful treatments with this and other lipid-based products appears to be getting more difficult, with repeated individual cases reporting failure to eliminate infestation, irrespective of the formulations used, and recently an IPM-based preparation showed a clinical success rate of only 41% (unpublished observations).

Was this difference between studies part of a continuing process of loss of effectiveness or the type of random observational variation that could occur in comparing any two clinical studies? If it was a loss of activity would it be confined to just that product or active material or would it affect other and different formulations and dosage forms in the same way as tolerance of pesticides? Few products use isopropyl myristate (IPM) as a named active substance for use against lice, although it is much more widely used in toiletry products at low levels. One product that does name IPM as the named active substance in Europe is a pressurized isopropanol-based mousse (Vamousse™, Tyratech Inc., Morrisville, NC, USA). However, according to the USA documentation for the same product, IPM is an "inactive" ingredient and the product is sold as an unregistered homeopathic with the named active ingredient as "natrium muricatum 2x HPUS" (sodium chloride 1%). There is also a shampoo with the same ingredients plus 1% (2x HPUS) *Eucalyptus globulus* (presumably the oil). It only takes a few seconds thought to realize that 1% saline cannot eliminate lice; otherwise every child who swam in the sea (approximately 3.5% salts) would be louse free. Given the apparent "weasel words" surrounding these claimed active substances, how effective is the product? To date the evidence for these products has been limited to a white paper produced for the manufacturer (Krader, 2017) that only cites evidence from treating six (6) head lice and a larger number of insecticide susceptible laboratory reared body lice; and consumer reviews online suggest there is considerable geographic variability in success (Amazon, 2018). It is possible to see how IPM in an alcohol-based mousse might work against lice because these compounds are skin penetration enhancers that are just as likely effective on insect cuticle as on skin (Lane 2013). However, *ex vivo* tests following the pack instructions performed on freshly collected UK head lice (Table 2) suggested the bioavailability of the active material, whatever it is, is insufficient to kill head lice even 18 hours after treatment.

Table 2. Effect of Vamousse™ products on head lice recorded 18 hours after treatment.

Treatment	Number of lice				Mortality
	Total	Alive	Moribund	Immobile	
Mousse	15	10	3	2	33.3
Shampoo	20	20	0	0	0.0
Control	21	19	1	1	9.5

If physically acting products with differences of formulation, but containing the same named materials, demonstrate considerably different effectiveness, in much the same way as was observed with pesticides (Burgess, 1991), how many other products may not be as effective as claimed? Many of these products, like the neem shampoo and two of the silicone-based products referred to earlier (Abdel-Ghaffar & Semmler, 2007; Abdel-Ghaffar et al., 2012; Semmler et al., 2017; Heukelbach et Al., 2009; Izri et al., 2010), have only been evaluated in developing countries where lice may never have encountered many of the product excipients that are supposedly inactive. These excipient chemicals possibly have no effect on lice in countries with developed economies because they are included in so many other hair care preparations at low concentrations, so the lice have acquired a tolerance for them, but, when applied to chemically naïve lice, they can exert just as much killing activity as the named "active" substance.

Fixed and synthetic oils

Non-volatile oils that consist of molecules with no likely pharmacological activity, primarily glycerides of saturated and non-saturated fatty acids, as well as a range of synthetic and naturally derived mineral oils, are all considered to be capable of coating lice and inducing some kind of occlusive effect. The level of interaction with the physical structures of the insects and the ability of the oils to create and maintain a thorough coating over the body surface depend upon the viscosity, surface tension, and wetting angle of the fluid. Since lice are coated with a lipid layer the wetting capacity of these oils is relatively improved compared with some types of product but the ability of the oily fluid to remain in contact with the louse depends upon the flow characteristics of the fluid and the van der Waals forces applying to the interaction between the oil and the substrate, in this case the louse cuticle (Perez, Schäffer & Steiner, 2001).

It has been claimed that silicone-based oils are capable of entering the tracheae of lice, filling up the tracheal tracts completely, and blocking off all possibility of gaseous transfer within approximately 1-3.5 minutes (Richling & Böckeler, 2008), although other authors claiming similar observations state this process is slower (Candy, et al., 2018). How any fluid, irrespective of its surface tension or viscosity, could fill a closed-ended narrow capillary tube like an insect trachea that is already filled with air and, at the terminal portions in the tissues, is also filled with water (Wigglesworth, 1930) is impossible to conceive unless the tracheae and tracheoles are evacuated by means of a vacuum (Wigglesworth 1950). Consequently, attempts to repeat these experiments as described by Richling & Böckeler (2008) were not successful (Burgess, 2009). The evidence from gallium ion beam milling scanning electron microscopy (Burgess, 2009) suggested that penetration of silicone fluids actually progressed no further than the constricted part of the trachea proximal to the spiracle described by Webb (1946).

There is no controversy about the fact that these oily materials block the openings of the louse respiratory system. However, recent work has shown that the effect of blocking access to air does not result in asphyxiation (Candy, et al., 2018) so disruption of water excretion appears to be a primary effect (Burgess, 2009), with the addition of some damage to the insect's cuticle lipids resulting from the solvent action of the applied oily materials dissolving them so they are emulsified as the treatment product is washed off (Barnett et al., 2012). Consequently, it is widely believed, and promoted by the pharmaceutical manufacturers of these products, that siloxanes, synthetic oils, and fatty acid ester-based products are effectively "resistance proofed", primarily because there is no easily predictable mechanism whereby lice would be able to overcome the process of fluid flowing into the spiracles and blocking them.

As with many treatments, a certain "failure to cure" rate occurred in all but one of the clinical studies of these types of head louse treatment that have so far been published (Burgess, Brown &

Lee, 2005; Burgess, Lee, & Matlock, 2007; Burgess, Lee & Brown, 2008; Heukelbach et al, 2008; Kurt et al., 2009; Izri et al., 2010; Burgess & Burgess 2011; Burgess, Brunton & Burgess, 2013; Burgess, Burgess & Brunton, 2013; Ihde et al., 2015; Wolf et al., 2016; Semmler et al, 2017; Burgess et al., 2017). Interpretation of failure has varied from study to study but in all cases there were some participants who had lice that unequivocally failed to respond to treatment. If failure to apply the product correctly, in sufficient quantity to cover the head/hair, and other compliance issues are eliminated, the question arises as to how lice could have or develop a tolerance of these treatments.

If dissolution of cuticular lipid is a primary aspect of mode of action of these types of product (Barnett et al., 2012), as well as that of some surface active compounds like 1,2-octanediol (Burgess et al., 2012, 2014), a selection process for lice that have a different range or proportion of lipids, with different solubility characteristics from those affected by the current treatments, would prove less susceptible. In the study of IPM/C four hydrocarbon compounds were identified as constituting the main components of the cuticle lipid that were most dissolved by the product but their exact chemical nature was not identified. In the 1,2-octanediol study three major hydrocarbons: C25, C27, and C29, plus various low concentration analytes, were found to be affected by the treatment. Both of these studies were conducted on more or less naïve populations of lice on which the chemical had not been previously used. Over time, incomplete treatment regimens would select for lice less affected by the solvent action in much the same way as lice were selected by physiologically active insecticides in the past. That some lice have been able to adapt to tolerate materials capable of dissolving cuticle lipids, such as those in commercial "stripping" shampoos used by hairdressers to remove conditioning lipids from hair, was shown by the trials of cocamide diethanolamine (cocamide DEA) preparations referred to earlier (Burgess, Brunton & Brown, 2015; Connolly et al., 2008).

Is it possible to test for resistance to physically acting materials?

Testing insects for susceptibility to physiologically active chemical substances is relatively straightforward with numerous published protocols and guidelines from the World Health Organization and others. Even testing of formulated products is reasonably straightforward because most preparations investigated only contain one potentially active substance with known activity. In contrast, physically acting materials, by their very nature described above, rely on occlusive effects of some kind and *in vitro* it is almost impossible to mimic the relatively low level of contact with lice that occurs as these, often low viscosity, fluids are dispersed over and along hair shafts. In the laboratory, testing usually involves complete immersion of the insects or their eggs in the fluid for a relatively prolonged period of time without draining off excess fluid. Such a prolonged contact ensures that any surface interaction to disrupt the integrity of the lipid coat of the insects, or flow of the fluid into the openings of the spiracles, can proceed as completely as possible. In contrast, when applied to the hairs on the head, there is a momentary immersion of the insects followed by a "draining" effect as the fluid spreads out over the surface of the scalp and hair so that, unless the preparation is relatively viscous, the surface of the insect may only retain a thin film of fluid, if any at all, depending upon the wetting characteristics of the product.

In view of the difficulty in assessing the true effect of some of the oil-based preparations that coat the insect cuticle, an assessment of whether they are effective can only be made clinically. How that should be done is also open to some question. Historically most of these products have been assessed in efficacy studies, i.e. trials in which the treatments were applied by members of the investigation team (Burgess, Brown & Lee, 2005; Burgess, Lee & Matlock, 2007; Burgess, Lee & Brown, 2008; Heukelbach et al., 2008; Kurt et al., 2009; Izri et al., 2010; Burgess & Burgess, 2011; Burgess, Brunton & Burgess, 2013; Burgess, Burgess & Brunton, 2013; Wolf et al., 2016; Burgess et al, 2017). This should ensure a thorough dosage and coverage so that the outcome is a potentially "true" reflection of the likely best outcome effect. In this category of products, only one published study has so far been conducted as a pragmatic or effectiveness study, in which the treatment was given to the care giver and applied by them rather than by an investigator (Ihde et al., 2015) but it suffered from a high rate of exclusion from analysis 39/97 (40.2%) and also required extensive nit combing along with application of a viscous silicone, so it is difficult to identify true effectiveness of the applied product. Of all these investigations, only one has so far shown an efficacy outcome of 100% (Burgess & Burgess, 2011) and all other studies with that product and other products have resulted in some level of failure to cure. Whether that failure was due to reinfestation from contacts in the community or simply because the product was not able to kill all insects and their eggs varied between products and investigations. However, very few of these investigations gave a cure rate close to the ideal proposed by Vander Stichele and colleagues (1995), "*Moreover, inspection of the figure* [not shown here] *leads us to recommend that only products with an expected cure rate of over 90% should be tested and that this should be done in trials with sufficient power to establish cure rates with a lower confidence limit above 90%*." How such a high cure rate could be predicted is impossible to determine because, as outlined above, *in vitro/ex vivo* tests are no useful guideline in many cases.

Discussion

Recent clinical observations and consumer reports both suggest that at least some of the physically acting preparations are losing effectiveness. Just as with the neurotoxic insecticides in the 1980s and 90s (Aston, Duggal & Simpson, 1998), this phenomenon is occurring slowly with as yet no substantiation and can easily be written off as failure by care givers to adequately apply the treatment. Certainly, this is a factor contributing to the effect, partly because some people have become blasé about the efficacy of products, partly because they are trying to economize when faced with repeated need to treat, and partly through lack of skill. However, those same factors contributed to the development of acquired resistance to commonly used insecticides like permethrin and malathion and, when the warning signs of consumer dissatisfaction 25-30 years ago were not heeded, it resulted in complete loss of usefulness of the insecticides in most territories and regions within a little over a decade (Chosidow et al., 1994; Mumcuoglu et al., 1995; Rupeš et al., 1995; Burgess et al., 1995; Picollo et al., 1998; Pollack et al., 1999; Downs et al., 1999, 2002; Hunter & Barker, 2003; Kristensen et al., 2006; Kasai et al., 2009).

Irrespective of how well products might perform when applied by investigators, how well would they work when applied by consumers? We have seen an interesting metamorphosis of reporting since the introduction of these products in about 2005, from complete satisfaction and more or less every time cure through to repeated treatment failures. Of course, some of the products that are reported as failing either have never been subjected to clinical investigations or else such investigations have never been published but even those products that may have given acceptable results in clinical investigations have been reported as failing repeatedly by caregivers. In some cases this is definitely due to inadequate application of the treatment but some appear to be due to survival of either lice or their eggs after having been thoroughly saturated.

Identifying a mechanism whereby lice could now survive a treatment to which their ancestors were wholly susceptible is really quite difficult, especially if the perceived mode of action is one of occlusion of some anatomical feature like blocking of spiracle openings. As shown by histology (Webb, 1946), the spiracles of lice are sclerotized and rigid objects inhibiting the ingress of fluids into the respiratory tract. Such structures do not change easily in response of selection pressures and, in this sense, there is little or no true selection pressure as might be found in relation to physiologically acting insecticides that stimulate production of degradative enzymes even in susceptible insects. Inevitably some lice may encounter incorrectly applied treatments, and some of those may survive, but there would be no physiological stimulus and no "mutational" effect to change the spiracle structure. However, what may occur is the survival of lice that happen to have spiracles that are physically smaller or that, within individual variation, are structured slightly differently so that it is more difficult for the fluid to enter or persist therein. These features could be formed by heritable traits so their offspring could be more successful in surviving a more intense exposure to the same treatment.

Such an interpretation might be considered fanciful or wholly speculative but observations of recently collected lice in the laboratory suggest otherwise. Two observations of louse behaviour support this possibility.

The first was of lice immersed in water. It has long been reported that lice in water become inactive and that when immersed in water the louse "..*holds its breath, and continues to do so until unconscious*" (Maunder, 1983), and more recently it was demonstrated that lice immersed for extended periods did not take water into their tracheae (Candy et al., 2018). However, recently we have observed lice in water moving around for several minutes, unlike previously, and climbing out from the water if a suitable substrate was available.

The second observation relates to lice exposed to silicone–based and other oily preparations that coat the lice over the whole body surface. If the preparation was insufficiently viscous to result in a superficial build-up on the louse cuticle the insects were not immobilised but continued to walk around with no apparent ill effects with the result that the treatment failed and the lice continued to feed and reproduce normally. Lice surviving in this way were observed to carry a film of the treatment fluid on their surfaces and they were only killed by complete immersion in the fluid, something that could not physically occur on a patient's head.

If head lice are being selected for greater tolerance of being soaked in oily fluids, action needs to be taken now by pediculicide manufacturers, clinical investigators, and regulators to establish which types of formulations and which "active" materials are losing their effect. The same vigilance needs to be set in place in parallel to ensure that the alternative types of product, such as the newer insecticide products used in the U.S.A., do not show signs of loss of activity also. Resistance to ivermectin has been identified in Senegal in Africa (Diatta et al., 2016; Amanzougaghene et al., 2018) and this could spread outside of the region through travel and migration. Also, since several of the topical formulations of these newer insecticides also contain what are effectively therapeutic levels of benzyl alcohol any loss of sensitivity to that material could also result in loss of activity of products not listing it as an active substance.

We have already discovered that some surfactants like cocamide diethanolamine have lost much of their effect (Burgess, Brunton & Brown, 2015), and it is likely that others such as 1,2-octanediol could suffer the same fate because they are slow acting and this may give lice the opportunity to avoid lipid dissolution effects. Selection pressures may result in selecting lice that produce a different ratio or range of lipids that protect their cuticle from those that were previously shown to be removed by the surfactants (Burgess et al., 2012). The same problem applies to lipid dissolution effects from volatile and fluidly mobile silicones and synthetic oils (Barnett et al., 2012), which may account for the diminishing effectiveness of those products that contain isopropyl myristate (Burgess et al., 2017; and unpublished observations). If the synthetic oils lose effect it will create a major problem in those territories where they are widely used.

Viscosity of the final preparation appears to be important to ensure a thorough and complete covering of the louse. This not only helps to immobilize it physically, in much the same way as other viscous materials such as reported for hair conditioners (Ibarra, 1996) but also ensures that all vulnerable surfaces, such as spiracles and cuticle lipids, are coated and occluded. Consequently, a gel-like product appears to offer the best covering effect for delivery of whatever active principle is in the preparation. However, in the light of some recent observations, further investigation of the real activity of many physically acting preparations is necessary through properly constructed clinical trials. As with insecticide products in the past, it is not satisfactory to perform a write across desktop exercise based on one or two chemical entities that happen to be included in each preparation. Increasingly specific physical and chemical aspects of each formulation appear to be critical in determining the effectiveness of the products in the elimination of infestation, meaning that unless the products are adequately evaluated before marketing commences not only may they fail to perform as claimed but may also create longer term problems by initiating the development of acquired resistance through selection of altered physical characteristics of the target insects.

Conclusions

Physically acting treatments for head lice have made a considerable positive impact on control and management of insecticide resistant populations of lice. So much so, that they have virtually ousted insecticide-based products from the market in several European and other countries. However, the interpretation of the term "physically acting" is somewhat loose in some regulatory jurisdictions so that some of the products making this claim may be just a sensitive to the risks of acquired resistance as the insecticides that preceded them because there is alternative evidence that the chemicals in questions have a physiological activity in addition to any physical effect.

Irrespective of the physical nature of the activity of a preparation, the idea that lice cannot become resistant to it is a false concept. Insects have demonstrated an ability to develop resistance to a wide range of killing measures over the past 100 years and there is no reason to justify a belief that synthetic oils and other physically acting chemicals are exempt from this risk. As with claims about fixed vegetable oils like coconut, which may kill lice in urban communities in developed countries, these are only good for use against lice that have never encountered them. Regular use of these oils result in sub-lethal encounters that can select for lice able to tolerate the exposure because they have some difference of physical characteristics or physiology, and the same may apply to synthetic oils just as much. Consequently, in order to avoid problems in the future for the currently successful products, a greater degree of care and thoroughness is required in their pre-marketing evaluation and consumer use.

Literature search

Databases searched include PubMed, Science Direct, Scopus, Cochrane, Google Scholar, https://worldwide.espacenet.com, phthiraptera.info, as well as hand searching online, https://clinicaltrials.gov, the ISRCTN registry, and my own collection of more than three thousand electronic reprints and references, using terms including: head lice, pediculosis, treatment, clinical trials, and more specific target terms such as "physically acting treatment" or, "non-insecticide treatment".

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