Parasitol Res
DOI 10.1007/s00436-017-5510-2

ORIGINAL PAPER

Ivermectin lipid-based nanocarriers as novel formulations against head lice

Gabriela Ullio-Gamboa[1,2] · **Santiago Palma**[1,2] · **Jean Pierre Benoit**[3,4] · **Daniel Allemandi**[1,2] · **María Inés Picollo**[5] · **Ariel Ceferino Toloza**[5]

Received: 18 March 2017 / Accepted: 8 May 2017

Abstract The use of pyrethroids to control the human head louse, *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae), has suffered considerable loss of efficacy due to the evolution of resistance. Thus, the development of efficiently insecticide delivery systems is imperative for the control of head lice. We studied the insecticidal activity of ivermectin-loaded lipid nanocapsules (IVM-LNC) against permethrin-resistant head lice from Argentina. The LNC, prepared by a phase inversion procedure, were characterized in terms of size, surface potential, and physical stability. These nanoparticles were nearly spherical with mean diameters of 55 nm and narrow size distribution (PI ≤ 0.2). The KT_{50} mortality values of head lice after exposure to two IVM-LNC formulations (0.11 and 0.28%) were significantly smaller (5 and 3 h, respectively) compared to those exposed only to LNC control group (8 h). This investigation showed the effectiveness in the encapsulation of ivermectin (IVM) into stable LNC dispersion with a potential clinical activity against head lice.

✉ Ariel Ceferino Toloza
atoloza@conicet.gov.ar

[1] Unidad de Investigación y Desarrollo en Tecnología Farmacéutica, UNITEFA-CONICET, Ciudad Universitaria, 5000HUA Córdoba, Argentina

[2] Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina

[3] LUNAM Université - Micro et Nanomédecines Biomimétiques, F-49933 Angers, France

[4] INSERM U1066, IBS-CHU, 4 Rue Larrey, F-49933, 9 Angers Cedex, France

[5] Centro de Investigaciones de Plagas e Insecticidas (CONICET-UNIDEF), Juan Bautista de La Salle 4397, B1603ALO Villa Martelli, Buenos Aires, Argentina

Keywords Lipid nanoparticles · *Pediculus humanus capitis* · Nanotechnology · Ivermectin · Pediculosis

Introduction

The human head louse *Pediculus humanus capitis* De Geer is an important cosmopolitan pest mainly affecting school-aged children. Louse infestation is annoying and may cause itching, loss of sleep, and social sanctioning (Burgess 2004). Transmission of head lice occurs mainly by direct host-to-host contact and by inanimate objects, called fomites. Although head lice have not been incriminated in the transmission of deleterious pathogens, it has been suggested that they could be potential transmitters of *Rickettsia prowazekii* Da Rocha-Lima and *Bartonella quintana* Brenner, a causative agent of epidemic typhus and trench fever, respectively (Mumcuoglu et al. 2009). However, the presence of these diseases occurs in exceptional situations like war, famine, and deprivation.

There are several treatment options for the control of head lice, which include over-the-counter (OTC) and prescriptions drugs, topical and oral treatments, natural chemical, and mechanical treatment (wet combing) (Burgess 2004). Nevertheless, the infestation with head lice is widespread throughout the world and has been increasing since the beginning of the 1990s due to the lack of effectiveness of pediculicides (Burgess 2009). This lack of efficacy is mainly due to the incorrect use of pediculicides and the resistance developed by lice to insecticides such as DDT, lindane, malathion, carbaryl, permethrin, and *d*-phenotrin (Mumcuoglu et al. 2009).

Pediculosis is very common in elementary schools of Argentina. The overall prevalence of active infestation (nits and living lice) in several cities of Argentina varied from 30 to

Published online: 18 May 2017

60% (Toloza et al. 2009), which is considerably higher than the 5% infestation value considered of epidemic importance (Clore 1988). In Argentina, the first line of pediculicide treatments is based on the application of pyrethroids (permethrin and *d*-phenothrin). In the last 5 years, the market share related to the sale of commercial pediculicides containing pyrethroids as their main active varied from 52 to 40% in Argentina. Thus, the selective pressure of pyrethroid-based pediculicides is still high in human head louse populations. This might be explained since the overall frequency of knockdown resistance mutations (*Kdr*) conferring target site insensitive of pyrethroids was 88%, suggesting that this mechanism is well established and closed to fixation in the studied populations of Argentina (Toloza et al. 2014). This widespread use of pyrethroids has precipitated their current ineffectiveness in the control of head louse infestations. Hence, it is important to treat persistent lice infestations with products with different modes of action.

Ivermectin (IVM) is a broad-spectrum anthelmintic agent that had been used since 1987. It binds to glutamate-gated chloride ion channels resulting in hyperpolarization with secondary paralysis and death. This compound also paralyzes the muscles associated with the pharyngeal pump, thus inhibiting the capacity of the animals to attach and feed (Brownlee et al. 1997). In addition, numerous studies showed that the concentration of IVM required to paralyze or block the pharyngeal pump is 10- to 100-fold lower than the concentration needed to produce irreversible knockdown following mortality (Gill et al. 1995; Strycharz et al. 2011). This mechanism is distinctly different from those of neurotoxic insecticides, such as pyrethroids and organophosphorus employed to control lice infestations. Several reports indicated that both orally and topically administered IVM were effective in controlling active head lice infestations (Strycharz et al. 2008; Chosidow et al. 2010; Currie et al. 2010; Meinking et al. 2013). The first treatment consists of two single doses of 200 μg/kg spaced 7 to 10 days apart to address re-infestations from eggs that hatch subsequent to treatment (Currie et al. 2010). Topical ivermectin 0.5% is now available in several countries and has proved to be safe and effective after a single 10-min application. This formulation found that 94.8% of treated individuals were louse-free after 2 days (Meinking et al. 2013). Similarly, these very high cure rates have been also achieved with a 92% concentration dimeticone product. After 9 days, 97% of treated patients were free of lice, as compared to 68% for those treated with 1% permethrin (Heukelbach 2010).

Over the past two decades, the development in nanotechnology has allowed the incorporation of multiple therapeutic, sensing, and targeting agents into nanoparticles, for detection, prevention, and treatment of many diseases. Recently, it was proposed as a strategy to decrease the irritation and degradation of active substances after application to the skin, by improving their stability and avoiding direct contact between the substance and the skin (Dianzani et al. 2014). The main benefits of nanomaterial-based formulations could be attributed to the improvement of the active ingredient efficacy due to higher surface area and lower toxicity by the elimination of organic solvents that are mostly used in conventional pesticide formulations. Concerning this, lipid nanocapsules (LNC) are synthetic lipoproteins, prepared according to a phase-inversion temperature method with size ranges between 20 and 100 nm that offer a versatile approach to insecticidal delivery (Huynh et al. 2009). LNC are made of a medium-chain triglyceride core with a surrounding polymer layer composed of lecithins and a hydrophilic surfactant derived from free polyethyleneglycol (PEG) and hydroxystearate PEG. Moreover, these nanocapsules offer to the active ingredient a protective core from the surrounding medium by reducing the fragile sites of the molecule that are vulnerable to chemical processes (i.e. oxidation, reduction, and hydrolysis). This improved the effectiveness of the formulation by achieving therapeutic or effective drug concentration at the target site (Lacoeuille et al. 2007). The purpose of this current study is to determine the insecticidal activity of IVM lipid nanocapsules (IVM-LNC) against permethrin-resistant head lice of infested children from Argentina.

Materials and methods

Chemicals

Labrafac® WL 1349 (caprylic-capric acid triglycerides) was provided by Gattefosse S.A. (Saint-Priest, France). Lipoid® S75–3 (soybean lecithin at 69% of phosphatidylcholine) and Kolliphor® HS-15 (Polyethylene glycol (15)-hydroxystearate) were gifts from Lipoid Gmbh (Ludwigshafen, Germany) and BASF (Ludwigshafen, Germany), respectively. Pure Ivermectin ($C_{48}H_{74}O_{14}$) (96%) was purchased from Parafarm (Buenos Aires, Argentina), and sodium chloride (NaCl) was obtained from Cicarelli laboratories (Santa Fe, Argentina). All other chemical reagents were from Sigma (Steinheim, Germany) and Fisher Scientific (Elancourt, France).

LNC formulation

The formulation of LNC was based on the phase inversion process described previously (Heurtault et al. 2002). Briefly, 20.6% of Labrafac® (*w*/*w*), 1.5% of Lipoid® S75–3 (*w*/*w*), 16.9% of Kolliphor® HS-15 (*w*/*w*), 59.2% of water (*w*/*w*), and 1.8% of NaCl (*w*/*w*) were mixed together under magnetic stirring. Three temperature cycles (between 60 and 90 °C) were applied to reach the phase inversion from oil-in-water to a water-in-oil emulsion. Thereafter, the mixture underwent a fast cooling dilution process with water at 0 °C, leading to the formation of nanocapsules. In order to obtain IVM-loaded

LNC, 5, 20, and 40 mg of IVM were first solubilized in Labrafac® and then all the components were added to obtain a final concentration of 0.028, 0.11, and 2.28% (*w*/*v*) respectively. Finally, the formulation was filtered with a 0.22 μm Ministar® high flow filter (Sartorius, Aubagne, France) to remove the non-encapsulated active ingredient.

LNC characterization and ivermectin payload

The size distribution and surface charge of LNC were determined by triplicate at 25 °C using a Malvern Zetasizer NanoSerie DTS 1060 (Malvern Instruments S.A., Worcestershire, UK). Sample suspensions were diluted in deionized water to ensure convenient scatter intensity on the detector. To calculate IVM-encapsulation ratio, three samples of an exact quantity of filtered LNC were dissolved in acetonitrile (ACN) and filtrated on a Minisart® 0.2-μm filter to eliminate the remaining excipients of LNC. A 10 μl aliquot of each filtrate was injected into a ZORBAX Eclipse XDB C18 Rapid Resolution column (4.6 × 150 mm, 3.5 μm) and analyzed with a Waters® 717 plus autosampler, Waters 600 controller, and Waters 2487 Dual Absorbance Spectrometer (Waters SA, Saint-Quentin-en Yvelynes, France) equipped with an UV detector set at 245 nm. The mobile phase contained ACN/methanol/water mixture (64:30:6 *v*/*v*/*v*) and the flow rate was 1 ml/min. The assays were performed in triplicate, and data were analyzed by the Empower Pro® software. The mean ± SD of IVM loading was calculated as milligrams of IVM per gram of LNC dispersion.

DSC and SEM

The size and the shape of LNC were also evaluated by using a scanning electron microscope (SEM) LEO Model EVO 40XVP (Zeiss, Oberkochen, Germany). The nanoparticle suspensions were diluted (1:400) in distilled water and fixed on a brass stub using a double-sided aluminum tape. In order to improve the conductivity, samples were gold-coated under vacuum employing a sputter coater PELCO Model 3 (Schneider Electric, Nevada, USA). DSC curves were recorded on a Pyris 1-PerkinElmer calorimeter (MA, USA), and the study was performed on the particles after lyophilization in presence of a cryoprotectant (trehalose). Then, samples (8 mg) were heated in sealed aluminum pans from 10 to 260 °C at a scanning rate of 10 °C/min under nitrogen purge with an empty aluminum pan as reference.

In vitro stability studies

The stability of IVM-LNC was evaluated under storage conditions for 2 months at 4 and 20 °C. Three sets of parameters were assessed at different time points: (i) macroscopic aspect (presence of aggregated, cream formation, changes in color); (ii) particle size, polydispersity, and zeta potential; and (iii) IVM concentration in the preparation and encapsulation efficiency. All these characteristics were determined as described above.

Head lice

Head lice were collected from infested children 6–13 years old, using a fine toothed antilouse metallic comb. A total of 396 lice were obtained from an elementary school located in Buenos Aires with a resistance degree to permethrin of 53 (LD_{50} = 125 ng/insect) (Toloza 2010). The studied schools were Argentinean Government owned and non-fee-paying. Only pupils whose parents had given informed consent for participation were examined. The freedom to refuse to participate in the research was clearly and amply established in each case. The entire head was examined carefully although special attention was paid to the nape of the head and behind the ears. Once collected, head lice were transported to our laboratory. The protocol for lice collection was approved by the ad hoc committee of the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-UNIDEF, Buenos Aires, Argentina) and archived in our laboratory (# BA20061995ARG, June 1995) (Picollo et al. 1998).

Toxicity bioassay

Immersion test

The adulticidal and third-stage nymph activity was assessed using an ex vivo immersion test (Gallardo et al. 2012). Briefly, batches of at least 10–15 adult and third instar nymphs were immersed for 5 min in 2 ml of each tested concentration of IVM-LNC (0.028, 0.11, and 0.28% *w*/*v*). Once the exposure period finished, the insects were placed onto a wire mesh and washed with 100 ml of water. Then, they were transferred onto a Whatman No. 1 filter paper (7.0 cm in diameter) moistened with 0.5 ml of water that was placed in the bottom of a plastic Petri dish (9.0 cm in diameter). During the studied period, Petri dishes containing head lice were placed in plastic containers (27 × 16 × 15 cm) at 18 °C, where 550 ml of distilled water (97 ± 2% RH) was added (with no contact with the insects). Then, these containers were kept inside an environmental chamber (Ambi-Hi-Low Lab-line, Iowa, USA) set at 18 ± 0.5 °C and 70–80 ± 1% relative humidity (RH) in the dark (Gallardo et al. 2009). Three controls were performed: (a) an insect control that consisted of healthy living lice (n = 10–15 per replicate per treatment) placed in the lid of a Petri dish and immersed into distilled water following the same procedures as the experimental group, (b) a formulation LNC control where insects were exposed to the base of the formulation without the addition of ivermectin, and (c) a commercial OTC

product containing 1% permethrin. Exposed lice were examined at room temperature (19–24 °C) for sign of any altered symptoms at 0.5, 1, 2, 3, 4, 5, 6, and 7 h post-treatment. The time for measurement of affected lice in a bioassay should consider the recovering of insects after the exposure treatment (Combescot-Lang et al. 2015). These authors suggested that physical conditions observed after 3 h post-treatment are fully reliable to detect formulation effectiveness. At this experimental time, it is possible to detect lice recovering after the intoxication phase. However, external factors like dehydration and starvation could interfere with the formulation effectiveness and give false positive results. Thus, we considered 7 h after treatment as the observational reference period reliable to estimate formulation effectiveness. However, after this period, it is still possible to detect lice recovering. Thus, lice were also observed at 18 h since it was estimated as the optimal period for comparative bioassays of human lice (Gallardo et al. 2009). Criterion of affected insects was according to Combescot-Lang et al. (2015). Briefly, a louse was considered alive if it showed no symptoms (normal moving) or some abnormal movements and having difficulties turning over. On the other hand, it was considered dead if it remained on its back showing no external or internal movements, except for slight contraction of the digestive tube. Each experiment was replicated at least three times. Once used in a given assay, the insects were discarded and not used in another experiment.

Statistical analysis

Data for treated head lice were subjected to probit analysis. Log time vs logit percentage of affected regression lines were computed to determined time (in hours) to knockdown 50 and 90% of exposed insects of each experimental treatment (KT_{50} and KT_{90}, respectively), by using POLO Plus Software (LeOra software 2002). Maximum log-likelihood ratio test was performed on the regression lines to test the equality (slope and intercept) among treatments and control. The null hypothesis of the maximum log-likelihood ratio test assumes that regression lines being compared are equal. The null hypothesis was rejected at $P < 0.05$. Differences in median mortality were assessed using analysis of variance (ANOVA) for repeated measures with percentage of mortality as the dependent variable, and formulation and time as main factors. Normal distribution was assessed by the Shapiro-Wilks test, but the Levene test for the homogeneity of variance was statistically significant at $P < 0.05$. Therefore, the hypothesis of homogeneous variances was rejected and a generalized linear mixed model (GLMM) using the R interface version 3.0.3 was performed (R Core team 2013). We assumed a binomial distribution for the response variable. Formulation and exposure time were the fixed effects, whereas the group of individuals was the random effect.

Results

Formulation and physical-chemical characterization

The lipid environment inside the LNC might solubilize molecules that are rather soluble in water and has a high octanol-water partition coefficient (5 ppm in saturated solution, K_{od} = 1651 of ivermectin). In order to confirm this, several studies utilizing differential scanning calorimetric were performed (Fig. 1). When the solid substance was assayed, the presence of an exothermic peak corresponding to the melting point of IVM was evident. However, this signal disappeared when samples containing IVM-LNC (0.028% *w*/*v*) were evaluated. This suggested that IVM would be totally dissolved in the lipid core of LNC. SEM images also show the homogeneity of LNC although few particles of higher size were observed (Fig. 2).

Since the ex vivo studies were performed at room temperature (19–24 °C) in order to maintain the homeostasis of the lice, we evaluated the stability of our formulations at this temperature over 8 weeks and compared them with previous results obtained at 4 °C. As shown in Table 1, the values of mean particle size (MPS), polydispersity index (PI), and zeta potential (ZP) remained practically unchanged during 60 days in samples stored either at 4 or at 20 °C. In addition, the loading and encapsulation efficiency (% EE) of IVM was higher than 90%, and no alteration was detected over the evaluated time, suggesting the physical stability of the systems at these temperatures (Fig. 3).

Toxicity bioassay

The immersion test produced lethality against permethrin-resistant head lice after 5-min exposure to the IVM-LNC formulations (0.11 and 0.28% *w*/*v*). They were significantly

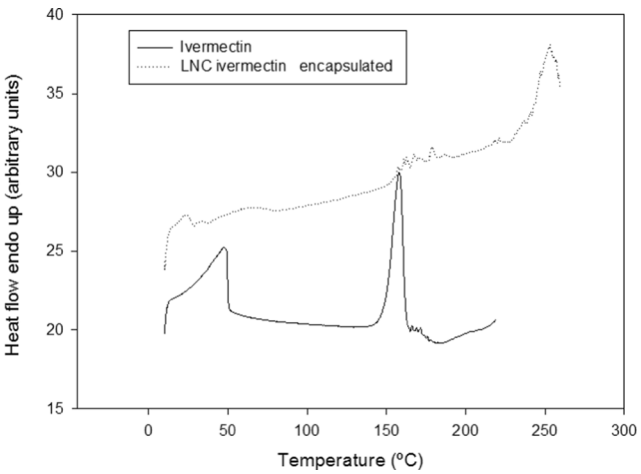

Fig. 1 DSC analysis (second heating curves) of IVM and LNC-IVM (0.028% *w*/*v*). The sample was heated and cooled twice applying a heating rate of 10 °C/min. Bulk IVM was used as reference. Analysis was performed 1 day after production

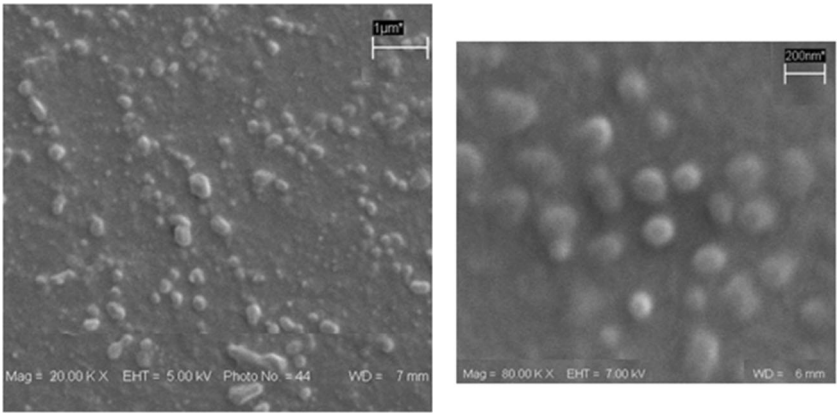

Fig. 2 SEM photographs of IVM-LNC (0.028% *w*/*v*). Note the presence of 50 nm round shape objects with the dark triglyceride rich oil core surrounded by a weak electron-dense external shell

different in comparison to lice only exposed to the control LNC formulation (Table 2), with the knockdown time affecting 50% (KT_{50}) of the individuals varying from 2.9 to 7 h. Moreover, the KT_{50} value of the IVM-LNC (0.28%) formulation was 2.7 times faster than the LNC formulation control treatment. Finally, IVM-LNC formulations were 20-fold faster than 1% permethrin OTC formulation (Table 2).

The generalized linear mixed model showed that there were significant differences in either the fixed effects of time or formulation ($\chi^2 = 3930.03$, $df = 7$, $P < 0.0001$; $\chi^2 = 21.17$, $df = 4$, $P = 0.0003$), respectively. There were also significant differences in the interaction of both fixed factors: time and formulation ($\chi^2 = 208.7$, $df = 28$, $P < 0.0001$). Due to these differences, we performed a contrast test (DGC) comparison of the interaction to analyze statistical differences among the treated groups (Fig. 4). The mortality response of the LNC formulation control was significantly higher in comparison with the control (insects without any exposure) after 5 h (Fig. 4). Similarly, after 5 h, the mortality response of IVM formulations showed some differences between them (Fig. 4). For instance, IVM-LNC (0.28%) formulation showed 80% of affected lice, whereas IVM-LNC (0.11%) formulation killed around 50%. Finally, IVM-LNC (0.028%) formulation affected after 5 h post-exposure around 25% of the exposed lice ($P < 0.05$). At 7 h, the only formulation that killed 100% of the lice was IVM-LNC (0.28%). There was no significant difference between the mortality values of the tested formulations at either 7 or 18 h.

Discussion

The present work revealed, for the first time, the effectiveness of lipid nanocapsules as carriers of ivermectin against permethrin-resistant head lice from Argentina. Treatments with 0.11 and 0.28% IVM-LNC formulation resulted in a significantly faster mortality response than treatment with a commercial 1% permethrin pediculicide. Similarly, Strycharz et al. (2008) tested the efficacy of several IVM concentrations (0.25, 0.5, and 1%) against head lice at several exposure times (3, 5, and 10 min). Despite the different employed methodology, they found that these formulations had a faster killing effect than the OTC 1% permethrin lotion Nix®. These values are in accordance with the effects of IVM-LNC against head lice reported here. These effects could be achieved through the combination of two main features of our system: (1) the efficient encapsulation of the insecticide in the lipid core coupled to the stability of the final formulation protecting IVM from degradation and (2) the enhancement of the specific surface contact due to the nanometric scale of the carrier that increases the bioavaibility and penetration of IVM through the lice cuticle.

Firstly, we previously reported the development of a novel nanocarrier for the delivery of IVM and its potential application in antiparasitic control. The IVM-LNC formulations had submicron size (50–57 nm) and were relatively monodispersed (Ullio-Gamboa et al. 2016). In this work, we confirmed that the presence of IVM at different concentrations

Table 1 Physicochemical characterization of IVM-LNC

Formulation	MPS (nm)	PI	ZP (mV)	pH
LNC Formulation control	57.13 ± 2.83	0.063	−10.9 ± 0.9	6.3 ± 0.05
IVM-LNC 0 days[a]	55.58 ± 0.62	0.048	−13.3 ± 3	6.4 ± 0.05
IVM-LNC 60 days 4 °C	56.49 ± 1.51	0.066	−13.4 ± 2.74	6.45 ± 0.05
IVM-LNC 60 days 20 °C	55.11 ± 0.96	0.081	−12.1 ± 0.9	6.5 ± 0.05

Mean particle size (MPS), polydispersity index (PI), zeta potential (ZP), and pH after storage for 60 days at 4 and 20 °C ($n = 3$; data are shown as mean ± SD)

[a] IVM theoretical concentration in the final suspension: 0.28 mg/ml (0.028% *w*/*v*)

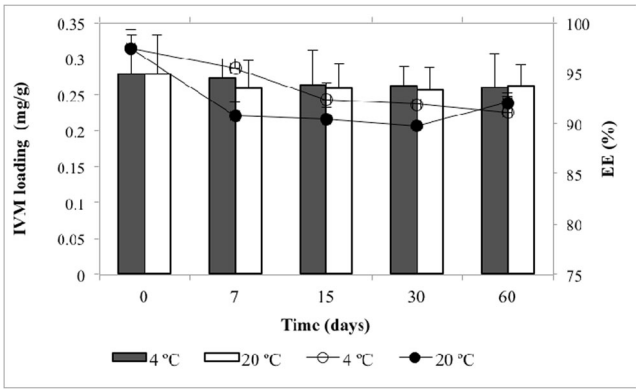

Fig. 3 Stability data of IVM-loaded LNC stored at 4 and 20 °C. Loading (mg/g) and efficiency encapsulation (%EE) evolution of nanocapsules after storage at 4 and 20 °C for 60 days. (n = 3, data are shown as mean ± SD)

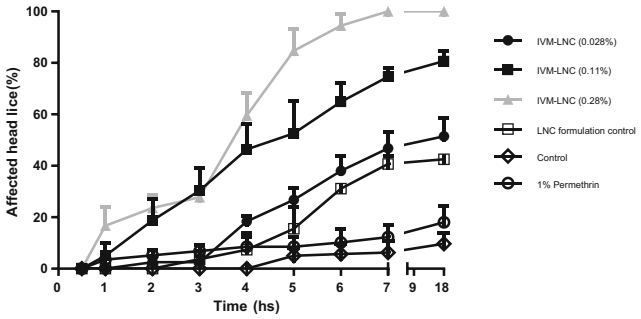

Fig. 4 Percentage of mortality of head lice after exposure to ivermectin lipid nanocapsules (IVM-LNC)

in the lipid core (0.028, 0.11, and 0.28%) affected neither the efficiency of the production method nor the physico-chemical parameters of the final suspension. The stability studies of IVM-LNC at 20 °C revealed no statistically significant differences in the results compared to LNC formulation control and IVM-LNC stored at 4 °C. Moreover, the final pH of these formulations was over 6, which accounts for a possible topical application since the skin surface pH is on average between 5.0 and 6.0.

Secondly, the effectiveness of local treatment might be due to the increased penetration or transfer of IVM residues to the cuticle of lice (Strycharz et al. 2008).

The ideal head lice treatment has been defined as being safe, effective, easy to use, inexpensive, and available without a prescription as a single dose (Mumcuoglu et al. 2009). Since the effectiveness of a pediculicide relates to the effective concentrations (for an appropriate period) at the site of action, the vehicle in the final formulation plays an important role in their local action. Regarding the prescription of pediculicides containing IVM in Argentina, it should be pointed out that there is a 0.5% ivermectin topical formulation (Evanix®) whose main vehicle compounds are 60% ethanol and 30% isopropyl alcohol. Previous studies indicated that products containing >60% of alcohol resulted in mortality values within a range of 80–100% (Mougabure Cueto et al. 2002; González-Audino et al. 2011). Although water-based pediculicides have a better market acceptance, few OTC products prove to be effective against human head lice (Heukelbach 2010).

In our study, the nanocapsules were obtained without organic solvent and with pharmaceutically acceptable excipients (general regarded as safe: GRAS, FDA 2016). The core of the particles was made of medium-chain triglycerides (Labrafac® Lipophile WL 1349) used in the manufacture of topical spray, emulsions, ointments, and lotions in the pharmaceutical industry. Moreover, the epidermal lipids are found in great homology with the synthetic lipids used in the LNC that accounts for its biocompatibility and possess low risk of toxic dermal effects. The lowest effective studied dose of 0.11% is expected to have little or no dermal effect on humans because percutaneous absorption of IVM is poor (in rats: acute dermal LD_{50} after 24 h > 660 mg/kg) and only the less-sensitive GABA-gated chloride channels are present in humans (Campbell 1989).

Finally, LNC complies with the pharmaceutical industry regulations and could be employed following established manufacturing practice (GMP) conditions on an industrial scale (Pardeike et al. 2009). Even if IVM-LNC formulations showed to be effective in ex vivo laboratory tests against head lice, two important points should be taken into account before their potential incorporation into commercial formulations as pediculicides: a battery of tests for acute and chronic toxicity, and in vivo clinical trials.

Table 2 Effectiveness of IVM-LNC against head lice

	Knockdown activity			
Formulation (%)[a]	KT_{50} (h) (95%CL[b])	KT_{90} (h) (95%CL[b])	Slope ± SE	χ^2
LNC formulation control	7.87 (6.93–10.82) a	14.30 (10.52–52.49) a	0.66 ± 0.87	0.49
IVM-LNC (0.028%)	7.0 (6.30–8.55) a	14.25 (11.26–23.85) a	0.24 ± 0.33	1.96
IVM- LNC (0.11%)	4.7 (4.02–6.13) b	15.19 (10.94–34.51) a	0.49 ± 0.72	0.81
IVM- LNC (0.28%)	2.96 (2.24–4.12) b	7.18 (5.28–18.29) a	0.65 ± 0.10	15.73
Permethrin 1% *w/w*	104.32 (31.35–5450.6) c	2104 (223.83–37,360) c	0.99 ± 0.32	1.17

Means in the KT_{50} and KT_{90} activity columns followed by different letters are significantly different at $P < 0.05$

[a] For each formulation and for each treatment, the number of head lice was 45

[b] 95% Confidence limit

Acknowledgements The authors want to thank all the authorities of the elementary school where head lice material was collected. We would like to thank Georgina "G" Fronza and Agustín Alvarez-Costa for statistical assistance. This investigation received financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina).

Compliance with ethical standards The authors declare no competing financial interests with any of the evaluated products. The experiments in this work comply with the current laws of Argentina.

References

Brownlee DJ, Holde-Dye L, Walker RJ (1997) Actions of the anthelminic ivermectin on the pharyngeal muscle of the parasitic nematode, *Ascaris suum*. Parasitology 115:553–561

Burgess IF (2004) Human lice and their control. Annu Rev Entomol 49: 457–481

Burgess IF (2009) Current treatments for *pediculosis capitis*. Curr Opin Infect Dis 22:131–136

Campbell W (1989) Ivermectin and abamectin. Springer-Verlag, New York

Chosidow O, Gireadeau B, Cottrell J, Izri A, Hofmann R, Mann SG, Burgess I (2010) Oral ivermectin versus malathion lotion for difficult-to-treat head lice. N Engl J Med 262:896–905

Clore ER (1988) Nursing management of pediculosis. Pediatr Nur 3:4

Combescot-Lang C, Vander Stichele RH, Toubate B, Veirron E, Mumcuoglu KY (2015) *Ex vivo* effectiveness of French over-the counter products against head lice (*Pediculus humanus capitis* de Geer, 1778). Parasitol Res 114:1779–1792

Core Team R (2013) R : a language and environment for statistical computing. R Foundation for statistical Computing, Vienna

Currie MJ, Reynolds GJ, Glasgow NJ, Bowden FJ (2010) A pilot study of the use of oral ivermectin to treat head lice in primary school students in Australia. Pediatr Dermatol 27:595–599

Dianzani C, Zara GP, Maina G et al (2014) Drug delivery nanoparticles in skin cancers. BioMed res: article ID:895986

Food Drug Administration (FDA) (2016) 81 FR54959, http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/. [Accessed 1 October 2016]

Gallardo A, Mougabure-Cueto G, Picollo MI (2009) *Pediculus humanus capitis* (head lice) and *Pediculus humanus humanus* (body lice): response to laboratory temperature and humidity and susceptibility to monoterpenoids. Parasitol Res 105:163–167

Gallardo A, Mougabure-Cueto G, Vassena C, Picollo MI, Toloza AC (2012) Comparative efficacy of new comercial pediculicides against adults and eggs of *Pediculus humanus capitis* (head lice). Parasitol Res 110:1601–1606

Gill JH, Redwin J, Van Wyk JA, Lacey E (1995) Avermectin inhibition of larval development in *Haomonchus contortus* effects ivermectin resistance. Int J Parasitol 25:463–470

González-Audino P, Picollo MI, Gallardo A, Toloza A, Vassena C, Mougabure-Cueto G (2011) Comparative toxicity of oxygenated monoterpenoids in experimental hydroalcoholic lotions to permethrin-resistant head lice. Arch Dermatol Res 303:361–366

Heukelbach J (2010) Management and control of head lice infestations. Uni-Med Verlag AG, Bremen

Heurtault B, Saulnier P, Pech B, Proust JE, Benoit JP (2002) A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharm Res 18:875–880

Huynh NT, Passirani C, Saulnier P, Benoit JP (2009) Lipid nanocapsules: a new platform for nanomedicine. Int J Pharm 379:201–209

Lacoeuille F, Garcion E, Benoit JP, Lamprecht A (2007) Lipid nanocapsules for intracellular drug delivery of anticancer drugs. J Nanosci Nanotechnol 7:4612–4617

Meinking TTL, Mertz-Rivera K, Villar ME, Bell M (2013) Assessment of the safety and efficacy of three concentrations of topical ivermectin as treatment for head lice infestations. Int J Dermatol 52:106–112

Mougabure Cueto GA, Gonzalez Audino P, Vassena C, Picollo MI, Zerba E (2002) Toxic effect of aliphatic alcohols against susceptible and permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). J Med Entomol 39:457–460

Mumcuoglu K, Gilead L, Ingber A (2009) New insights in pediculosis and scabies. Expert Review Dermatol 4:285–302

Pardeike JA, Hommoss RH, Müller R (2009) Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharma 366:170–184

Picollo MI, Vassena C, Casadio A, Massimo J, Zerba E (1998) Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura: Pediculidae). J Med Entomol 35:814–817

Software LO (2002) POLO-PC: a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA

Strycharz JP, Yoon KS, Clark J (2008) A new ivermectin formulation topically kills permethrin-resistant human head lice (Anoplura: Pediculidae). J Med Entomol 45:75–81

Strycharz JP, Berge N, Alves A, Clark J (2011) Ivermectin acts as a posteclosion nymphicide by reducing blood feeding of human head lice (Anoplura: Pediculidae) that hatched from treated eggs. J Med Entomol 48:1174–1182

Toloza AC (2010) Bioactividad y toxicidad de componentes de aceites esenciales vegetales, en *Pediculus humanus capitis* (Phthiraptera: Pediculidae) resistentes a insecticidas piretroides. PhD Thesis. University of Buenos Aires

Toloza A, Vassena C, Gallardo A, González-Audino P, Picollo MI (2009) Epidemiology of *pediculosis capitis* in elementary schools of Buenos Aires, Argentina. Parasitol Res 104:1295–1298

Toloza AC, Ascunce M, Reed D, Picollo MI (2014) Geographical distribution of pyrethroid resistant allele frequency in head lice (Phthiraptera: Pediculidae) from Argentina. J Med Entomol 51: 139–144

Ullio-Gamboa GV, Palma SD, Lifschitz A, Ballent M, Lanusse C, Passirani C, Benoit JP, Allemandi DA (2016) Ivermectin-loaded lipid nanocapsules: toward the development of a new antiparasitic delivery system for veterinary applications. Parasitol Res 115:1945–1953