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Quantitative analysis of ligand effects on bioefficacy of nanoemulsion encapsulating depigmenting active

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ABSTRACT

Efficient skin delivery of active molecules is the main challenge to overcome in order to achieve significant therapeutic efficiency of cosmetics or dermo-pharmaceutical products. Nanocarriers such as nanoemulsions have been envisaged to overcome main challenges of active solubilization, protection and transport to their site of biological action. Nonetheless, their skin permeation is still limited and a new approach is required to significantly improve bioavailability. We here explored the possibility of increasing the whitening activity of a model active, licorice, by implementing a targeting approach of nanoemulsions to melanocyte cells. Targeting requires particle surface modification with specific molecules favoring nanoemulsion/cells contact through ligand–receptor interactions. The uniqueness of our strategy is that unlike classical covalent chemical grafting, we propose a self-assembled strategy based on a selection of amphiphilic ligands able to localize at nanoemulsion droplets interface. Four ligand candidates were thus assayed in terms of formulation and in vitro biological evaluation: a palmitoyl-peptide (palmitoyl-GQPR), a lipidized hyaluronic acid (caproyl-HA) and two amphiphilic actives (polydatin and isopilosine). A functional analysis based on a cellular assay of melanin inhibition was realized. The intrinsic properties of ligand candidates were first evaluated. Then, nanoemulsions encapsulating a drug model, licorice, and targeted with the different ligand candidates were assayed. The use of caproyl-HA significantly improved bioefficacy of the encapsulated licorice, suggesting a better interaction with the cells. The improved value observed was not attributed to a synergetic action as caproyl-HA did not evidence intrinsic melanogenesis modulation activity. In this study, we demonstrated the feasibility of targeting nanoemulsion droplets without chemical covalent modification of nanoemulsion droplets to increase bioefficacy of encapsulated drugs in vitro.

1. Introduction

Drug delivery implies that the administered active compound is properly dispersed in the aqueous environment, distributed through the whole body and readily available onto the site of action. While hydrophilic substances can be directly administered, lipophilic substance formulation is a prerequisite for good bioavailability. Numerous colloidal systems have been developed from oil-in-water emulsions, nanoemulsions and microemulsions to nanoparticles systems such as liposomes, solid lipid nanoparticles, etc. All those forms allow dispersing actives into an aqueous continuous phase for a better distribution of the drug. Moreover, these colloids may protect drugs from degradations and in some cases control drug release. Ultimately, colloid surfaces can be functionalized in order to make them stealth to immune system and to address particles to their biological target (i.e. specific cell or tissue). For review, see [1]. As a consequence, colloids can concentrate to their site of action, requiring less drug quantity and, in turn, reducing side effects. Such strategy is particularly interesting for tumor treatment where anticancer drugs produce significant side effects [2] or for tumor *in vivo* imaging [3]. However, addressing these particles to specific cells generally requires surface modification through chemical binding of ligands. Surface modification is

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usually achieved through bioconjugation after particle production, a chemical strategy aiming to form stable covalent link between the ligand molecules and the preformed particle. This approach has been proved to be effective at laboratory levels, but dramatically complexify the process and consequently limits its industrial application for cost and regulatory perspectives. Indeed, *in situ* chemical modification subsequently requires efficient purification steps to eliminate potential by-products that may cause immunogenicity or toxicity and may also affect the inherent activity of the covalently modified ligand [4]. In order to overcome these problems, we developed a nanoemulsion system targeting specific cells through direct ligand incorporation during the process [5]. Ligands are selected based on their physicochemical properties in order to favor their interface localization onto nanoemulsion droplets. Nanoemulsions present numerous advantages for drug or active delivery in dermatology as well as in medicine. Nanoemulsion characteristics can be finely tuned such as size, surface charge, prolonged blood circulation, specific targeting and binding ability, controlled release and imaging capability [6]. The physicochemistry of our developed nanoemulsions is well characterized [7] and the addition upon formation of substances with potential ligand effect depicts targeting abilities [5]. Selected molecules with ligand potential should combine two characteristics: (i) amphiphilic properties in order to be located at the surface of the droplets and (ii) targeting potential onto cell receptors. Among registered cosmetic ingredients presenting such characteristics, we selected one palmitoyl-peptide, a lipidized form of rigin derivative which has immunomodulating abilities and was suggested to target liposomes toward lymphocytes [8]. An additional selected ligand tested is caproyl-hyaluronic acid; hyaluronic acid is an anionic, nonsulfated glycosaminoglycan of the extracellular matrix interacting with cells and associated with moisture retention into the skin. It has been proposed as a potential ligand for targeting cancer cells [9,10]. Finally, two amphiphilic actives were selected as ligands for their amphiphilic properties: (i) polydatin glucoside which inhibits melanogenesis through inhibition of melanogenesis enzyme transcription and translation [11] and (ii) isopilosine which is an alkaloid extracted from *Pilocarpus microphyllus* with pilocarpine and pilosine. No proper action onto skin cells is actually known.

In the work presented here, a functional analysis of self-assembled ligands addressed nanoemulsions is realized. We aim to demonstrate that without chemical binding we are able to target nanoemulsions to specific cells and increase the efficacy of encapsulated actives. As a biological model, we used melanocytes and assayed the whitening efficacy of licorice depending on encapsulation and targeting ligands.

Skin pigmentation disorders, particularly benign solar lentigines, are of special interest in cosmetic and dermo-pharmacy. These brown spots appear to the skin following sun exposure or during ageing and are associated with melanocyte accumulation to the basement membrane between epidermis and dermis. Melanocytes are responsible for skin pigmentation. They are normally associated with 35–40 keratinocytes, main cells of the epidermis, and produce melanin pigments which are transferred to keratinocytes. This pigmentation protects cell's nucleus from ultraviolet radiations. Lentigines and other skin pigmentation disorder are usually treated by inhibiting melanin production. However, treatments often require long-term application (weeks to month) and strict observance for good results. Actives used for inhibiting melanin synthesis (e.g. alpha hydroxy acids, vitamin C, retinoids, azelaic acid) are sensitive to oxidation or enzymatic degradation. Using specific self-assembled ligand-addressed nanoemulsions, we demonstrated that active encapsulation and effective melanocyte targeting can be achieved to improve bioavailability and efficacy.

Table 1
Dispersed phase composition and physicochemical characteristics of blank nanoemulsion and ligand-targeted nanoemulsion droplets.

Composition (%)	NE	NE PDA	NE GQPR	NE HA	NE ISO
Wax	22.7	23	23	23	23
Oil	22.7	23	23	23	23
Ligand	–	0.4	0.4	0.4	0.4
Lipophilic surfactant	8.7	9	9	9	9
Hydrophilic surfactant	46.0	44.6	44.6	44.6	44.6
					Physicochemicalmeasurement
Size (nm)	53.5	58.3	50.3	61.8	46.5
Polydispersity (a.u.)	0.144	0.175	0.191	0.165	0.178

2. Experimental

2.1. Nanoemulsion formulation

All formulations prepared here were produced according to previously published work [7,5]. Nanoemulsions can be easily obtained through dispersion of a lipid phase containing wax (semi-synthetic glycerides, suppocire NC, Gatefosse, St Priest, France), oil (soybean oil, Sigma-Aldrich, St Quentin Fallavier, France) and lipophilic surfactant (soybean phospholipids, Phospholipon 75, Lipoid, Germany) into an aqueous phase made of phosphate-buffered saline (PBS) solution (pH 7.4) and hydrophilic surfactant (polyethoxylated fatty acid, Myrj S40, Croda, France). Both phases are crudely mixed using a vortex and finely emulsified through high-energy process ultrasonication with a tip sonicator (Bioblock Scientific VibraCell. TM. 75042) at 25% amplitude for 10 min with a 10 s on/30 s off cycle. Lipophilic/hydrophilic surfactants ratio of 0.2 was conserved through the different formulations to achieve a define size of 50 nm of the blank formulation. Actives were encapsulated according to their lipophilicity. The more lipophilic actives can replace part or the totality of the wax/oil core up to 46% (w/w) of the dispersed phase, whereas the more amphiphilic one can only replace part of the surfactants. Licorice (Glycyrrhiza glabra root extract, Bioland, Chungnam, Korea) with more than 40% glabridin, was used as an active model with an estimated log *P* (*X* log P3) of 3.9 suggesting a core/interface location. Licorice was loaded to 1% (w/w) of the dispersed phase. Selected compounds with targeting potential and amphiphilic properties were added to 0.4% of the final formulation. These compounds were palmitoyl-glycine-glutamine-proline-arginine (GQPR) from Creative Peptides (Shirley, NY, USA), caproyl-hyaluronic acid (HA) from Tenneliderm (Dolní Dobrouč, Czech Republic), polydatin glucoside (PDA) from Induchem (Neuilly Sur Seine, France) and isopilosine (ISO) from Sourcetech quimica (Pindamonhangaba, Brazil). Table 1 resumes the compositions in mass percent of the different formulations. All formulations were diluted to a 10% (w/v) dispersed phase ratio for long-term storage.

2.2. Zeta potential measurement

Zeta potential of nanoemulsion droplets was determined by electrophoretic light scattering (NanoZS, Malvern, UK). Data are reported as the means and standard deviations of two independent measurements performed on suspensions diluted 10-folds with 1× PBS buffer.

2.3. Physicochemical stability

Sizes of nanoemulsion droplets were measured by light-scattering measurement (NanoZS, Malvern, UK). All samples were previously diluted in 0.1× PBS buffer at a dispersed phase weight fraction of 0.03% to avoid multiple scattering effects. Measurements were realized at a fix angle of 173° using a 633 nm laser with each