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Research paper

In vivo anti-melanogenesis activity and *in vitro* skin permeability of niacinamide-loaded flexible liposomes (Bounsphere™)

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ABSTRACT

This study was conducted to evaluate preparation and characteristics of flexible liposomes (Bounsphere™) consisting of a biocompatible lipid membrane with dipotassium glycyrrhizate (DPG) as an edge activator and containing niacinamide (NA). We evaluated particle size and zeta potential of the prepared liposomes by dynamic light scattering (DLS), and investigated their deformability, safety, skin permeability, and anti-melanogenesis activity in Bounsphere™ that remained stable for more than 6 months. The average size of Bounsphere™ was about 200 nm, and the change in liposome size after 6 months was not greater than that of conventional liposomes. The zeta potential of Bounsphere™ was about 32 mV with a positive charge, indicating relatively good stability. The deformability of Bounsphere™ was higher than that of conventional liposomes. In addition, the skin permeability of Bounsphere™ was higher than that of conventional liposomes or the phosphate buffer solution. These results indicated that Bounsphere™ with DPG as an edge activator has advantages over conventional liposomes as NA carriers. Furthermore, we found that 2% NA-loaded Bounsphere™ enhanced skin whitening. Taken together, our results demonstrate that Bounsphere™ can be used as a delivery system to enhance transdermal permeation and skin whitening agent effectiveness.

1. Introduction

Niacinamide (NA) is a natural compound that is found in many plants and is commercially available as a skin-whitening agent in cosmetic products. NA reduces melanogenesis in the skin by inhibiting melanosome transfer from melanocytes to surrounding keratinocytes. Moreover, NA protects the skin from reactive oxygen species and functions as a primary barrier with the skin, preventing the loss of moisture by enhancing production of ceramides and fatty acids. NA is very stable under exposure to UV radiation, heat, oxygen, acids, and bases (other than other vitamins) [1,4,8,17,21].

The outer layers of the skin, which act as the main barrier against harmful environmental materials, because corneocytes and hydrophobic intercellular lipid lamellae make external materials difficult to penetrate to our skin [7,13,20]. Especially intercellular route is main channel of skin permeation, passing the intercellular lipid. These lipids are arranged as multi-layer form and have unique liquid crystal structure, consisting of ceramide (40%), cholesterol (25%), cholesteryl sulfate (10%) and free fatty acid (25%). Therefore, hydrophilic compounds such as NA is almost difficult to penetrate through the skin and lipophilic compounds is also not easy to permeate. Thus, NA, which is water-soluble compound, does not easily penetrate into the stratum corneum in spite of low molecular weight [6,11]. Furthermore, NA is an irritant due to nicotinic acid in NA. Therefore, we have focused on ways to improve NA skin permeability and prevent its irritating effects.

Liposomes are highly studied drug delivery systems due to their good bio-affinity and ability to contain both hydrophilic and hydrophobic drugs. In comparison with conventional liposomes, Bounsphere™, which include a surfactant called an edge activator

Abbreviations: Dipotassium glycyrrhizate, DPG; Niacinamide, NA; Dynamic light scattering, DLS; Hydrogenated lecithin, HPC; Cholesterol, CHOL; Ceramide 3, CER; Triethanolamine, TEA.

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along with phospholipids, are known as deformable liposomes, elastic liposomes, or transfersomes. Edge activators in flexible liposomes are mostly single chain surfactants, such as Tween-80, Span 80 and sodium deoxycholate. Edge activators can increase the fluidity of bilayers in the stratum corneum by lowering the interfacial tension of the bilayer, thus enhancing the permeability of encapsulated drug into the skin [2,3,5,14,15].

In order to enhance the skin permeability and anti-melanogenesis activity of NA, we developed flexible liposomes incorporating the edge activator dipotassium glycyrrhizate (DPG) in a masksheet formula. Flexible liposome, which was comprised of optimal formula, was named Bounsphere™ by mixing term 'bounce' and 'sphere' in order to highlight the characteristics. In addition, we evaluated the chemico-physical properties and the efficacy of NA-loaded Bounsphere™ *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Hydrogenated lecithin (from soybean, ≥75% phosphatidylcholine, HPC), cholesterol (≥99%, CHOL), ceramide 3 (99≥ %, CER), dipotassium glycyrrhizate (99≥ %, DPG), triethanolamine (99≥ %, TEA), glycerin, and niacinamide (≥99%, NA) were used without further purification for the preparation of the liposomes. All other chemicals were of reagent grade and used as received, and water was freshly distilled.

2.2. Preparation of liposomes with and without NA

Bounsphere™ were prepared by a modified high-pressure homogenization method [18]. To prepare coarse liposome suspensions, 1.5 g of an HPC/CHOL/CER/DPG mixture (molar ratio of 5:1:0.1:0.1) was dispersed in 10 mL of glycerin at >80 °C. Using an agitator, 20 g of NA and 1 g of preservatives were dissolved in 60 mL of water at 50–60 °C (Eurostar 20, IKA, Wilmington, NC, USA), and the oil phase was dispersed into the water phase. The pH of coarse liposomes was adjusted to 6.5–7.5 using TEA and passed through a 100-mesh filter. Fine suspensions were produced using a microfluidizer (Panda 2K, GEA Niro Soavi, Parma, Italy) at >900 bar (2 cycles). Unloaded liposomes were prepared using the same method without NA. Conventional liposomes were prepared as a control sample by the above method, with the same composition as Bounsphere™, with the exception of DPG.

2.3. Stability test of liposomes

To investigate the stability of liposomes, particle size, polydispersity index (PDI) and zeta potential were evaluated by dynamic light scattering (DLS) and electrophoresis light scattering (ELS) using a particle size analyzer (Nanotracwave, Microtrac, Montgomeryville, PA, USA). Size, distribution, PDI and zeta potential were assessed for 90 s, with 3 repeated measurements, at a fixed scattering angle of 90°, and at room temperature. Liposome dispersion was used as it is, because sample had range of concentration and viscosity that did not require dilution.

Table 2
Skin irritancy-scoring system for the human patch test.

Score	Description
0 (−)	No signs of inflammation, normal skin
0.5 (±)	Doubtful or slight reaction
1 (+)	Slight erythema
2 (++)	Moderate erythema with or without partial edema or papules
3 (+++)	Moderate erythema with diffuse edema
4 (++++)	Intense erythema with diffuse edema with vesicles

2.4. Deformability of liposomes

The deformability of liposomes was determined using the method reported by Maitani et al., with slight modification [9]. The deformability of liposomes was calculated using the following Eq. (1):

$$Deformability = J_{flux}\, X\, (r_v/r_p)^2 \quad (1)$$

Where J_{flux} was the rate of penetration through the membrane r_v was the size of liposomes after extrusion, and r_p was the pore size of membrane.

Conventional liposome and Bounsphere™ were extruded through a micro-porous polycarbonate membrane (Whatman® Nuclepore, GE Healthcare, Little Chalfont, UK) with a pore diameter of 50 nm (r_p) using a mini-extruder with a syringe pump (Avanti® Polar Lipids, Alabaster, AL, USA) at a flux of 0.2 mL/min (Fusion 100 touch, Chemyx, Stafford, TX, USA). After 1 min of extrusion, the extrudate was weighed (J), and the average liposome diameter after extrusion (r_v) was measured by DLS. The experiment was performed in triplicate.

2.5. In vitro studies of skin permeation and deposition

A skin permeation study was performed using a 9 mm Franz diffusion cell with a 5 mL receptor volume (FDC-6T, Logan Instruments, Somerset, NJ, USA). The control sample (2% NA) was dissolved in distilled water, whereas the experimental samples were prepared with conventional liposome and Bounsphere™ containing 2% NA.

Skin sample was removed from back of human cadaver (age of 67, women) by Ohio Valley Tissue & Skin Center (USA), which has solid know-how and system for human cadaver process. And we obtained the skin samples from HANS Biomed (Korea) with its tissue bank with the FDA and the KDFA approvals. Skin that could not be transplanted to human was processed for research. The skin sample (area of 1.5 cm × 1.5 cm, depth of 1044 ± 231 μm) was fixed between the donor and the receptor phase of stratum corneum side, facing upward into the donor compartment. Phosphate-buffered saline (pH 7.4) was used as the receptor phase in the

Table 1
HPLC condition for niacinamide.

Condition of HPLC analysis	
Column	Waters SunFire™ C18 (250 mm × 4.6 mm, 5 μm), 35 °C
Detector	YL 9160 PDA detector (YL 9100 HPLC system)
Detection wavelength	263 nm
Flow rate	1 ml/min
Injection volume	20 μl
Mobile phase	0.05 M KH_2PO_4 buffer (pH 7.0):MeOH = 75:25