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Enhanced cutaneous bioavailability of dehydroepiandrosterone mediated by nano-encapsulation

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ABSTRACT

Polymeric nanocarriers, especially nanospheres (NSs) and nanocapsules (NCs), can promote the penetration of their cargo through the skin barrier, towards improved cutaneous bioavailability. Dehydroepiandrosterone (DHEA), an endogenous hormone exhibiting poor aqueous solubility, was shown to be effective in modulating skin-aging processes following topical application. In this study, we designed adequate DHEA preparations, in an attempt to enable local delivery of the active ingredient to the viable skin layers. In addition, the potential efficiency of DHEA NCs on dermal collagen synthesis was evaluated. Cryo-TEM observations and thermal analysis indicated that DHEA was successfully incorporated within a stable NC-based delivery system. Moreover, higher [^{3}H]-DHEA levels were recorded in the viable skin layers following different incubation periods of NCs on excised pig skin specimens as compared to DHEA oil solution (free molecule). Furthermore, significantly higher (4-fold) skin flux values were observed for the DHEA NCs as compared to the values elicited by the oil control solution. Finally, collagen synthesis in human skin organ culture, assessed by the incorporation of [^{3}H]-proline, was up to 42% higher for DHEA NCs 48 h post-topical application than for the untreated specimens. Overall, these results suggest that poly lactic-*co*-glycolic acid (PLGA)-based NCs have promising potential to be used topically for various skin disorders.

1. Introduction

The skin distribution profile of an active agent topically applied will mainly depend on its physico-chemical features. Therefore, it is most likely that a small, slightly lipophilic molecule will more easily penetrate the horny layer of the skin toward its viable compartments, while the passive diffusion path, if any, of a large hydrophilic molecule would be limited to the stratum corneum (SC) outermost layers [1,2]. The passive penetration of poorly absorbed active ingredients can be improved by adding specific enhancers to the formulation, or by using adequate nanodelivery systems. These novel drug delivery systems are able not only to enhance the permeation of active principal to the systemic circulation but also to improve therapeutic benefits in the treatment of various skin pathologies: inflammation, fungal infection, acne, autoimmune disorders, hair disorders, vaccination and cancer [3–5]. Numerous particulate drug delivery systems facilitating skin permeation of stroids have been widely explored, including liposomes containing triamcinolone [6], which are believed to be the first drug nanocarriers investigated for this purpose. Ainbinder *et al.* [7] reported that a nonpatch ethosomal formulation enhanced the transdermal delivery of testosterone. When steroids were incorporated into lipid-based nanoparticles (NPs), it appeared that skin penetration was improved and elevated concentrations of the drugs were observed in the epidermis [8]. Other authors have shown that hydrogel-loaded dexamethasone nanocapsules (NCs) elicited a controlled release profile while using an *in vitro* diffusion experiment setting [9]. In addition, few studies have pointed out that polymeric (poly lactic acid (PLA), poly lactic-co-glycolic acid (PLGA), polystyrene and poly methyl methacrylate) fluorescent NPs did not penetrate the intact SC but accumulated in the upper skin layer and exhibited size-dependent affinity towards hair shafts [10–13]. Depending on the formulation, NPs can remain on the skin as demonstrated by the limited skin permeation of octyl methoxycinnamate when encapsulated within poly(ε-caprolactone) NPs [14]. Alternatively, NPs can facilitate dermal delivery of their cargo following transfollicular permeation and subsequent uptake, by antigen presenting cells, of solid fluorescent 40-nm- or 200-nm-sized polystyrene NPs [15]. Although several investigations involving polymeric nanocarriers were conducted, their skin biofate is still not completely elucidated. Controversies prevail mainly due to the significant variation in the experimental settings described in the literature [16,17]. NPs of PLGA exhibit an intrinsic advantage, since they are biocompatible, safe and biodegradable in physiological and biological environments. However, because of their sensitivity in aqueous medium, it is very difficult to maintain long-term stability within aqueous-based topical formulations. Therefore, they need to be stored as freeze-dried powders following lyophilization, and their aqueous solution should be

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reconstituted prior to topical application or incorporated within water-free topical formulation. Consequently, NPs of PLGA appear to be promising nanocarriers for enhanced topical delivery of steroidal agents. The endogenous steroidal hormone dehydroepiandrosterone (DHEA) is known to decrease with aging [18]. Interestingly, an improvement of the skin status was observed, particularly in women, in terms of hydration, epidermal thickness, sebum production and skin pigmentation, when DHEA was administered orally at a dose of 50 mg/day over 1 year [19]. Moreover, topical administration of DHEA of various concentrations in different treatment regimens not only demonstrated beneficial effects on skin characteristics, where other treatments did not [20], but also modulated the expression of several dermal genes, especially those involved in collagen synthesis [21,22]. Furthermore, elevated androgen receptor levels were correlated with the increased expression of procollagen mRNA in the dermis [23]. In addition, heat shock protein 47, a molecule believed to have a chaperone-like function in collagen biosynthesis, was found to increase in the dermis following a 13-week treatment regimen of local DHEA in a group of post-menopausal women [23]. Based on the overall reported results, it can be anticipated that topically administered DHEA can promote several anti skin-aging activities. However, DHEA exhibits complex solubility limitations in common cosmetic and pharmaceutical solvents such as water, polar oils and vegetable oils. DHEA is practically insoluble in water (0.02 mg/mL) and precipitates rapidly within regular topical formulations even at concentrations lower than 0.5%, yielding several polymorphic crystal forms which exhibit a very slow dissolution rate and consequently poor skin penetration [24]. To the best of our knowledge, no nano-encapsulation of DHEA has yet been reported. Ceschel *et al.* [25] exhibited the permeation properties of DHEA and cyclodextrin complexes formed in different vehicles. Although a significant increase in solubility was reported when DHEA was incorporated in cyclodextrin complex compared to the pure drug, the calculated membrane flux values for most of the formulations appear to be quite similar, irrespective of the vehicle, with the exception of DHEA in cyclodextrin complex dispersed in microemulsion. It is the objective of the present study to design a PLGA-based nanocarrier, free of DHEA crystals and safe to use, in an attempt to enhance the skin penetration of this steroidal hormone, while providing adequate dermato-biodistribution (DBD) profiles in the various skin layers. Additional objectives of this study are to examine the effect of different formulation variables on the skin penetration profile and to evaluate the potential efficiency of DHEA preparations on dermal collagen synthesis.

2. Materials and methods

2.1. Materials

DHEA, polysorbate 80 (Tween® 80), octanoic acid and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Rehovot, Israel). [^{3}H]-DHEA, [^{3}H]-proline, Ultima-Gold® liquid scintillation cocktail and Solvable® were obtained from Perkin-Elmer (Boston, MA, USA). PLGA 4K (molecular weight (MW): 4000 Da) and PLGA 100K (MW: 100000 Da) were acquired from SurModics Pharmaceuticals (Birmingham, AL, USA). Macrogol 15 hydroxystearate (Solutol HS 15) was purchased from BASF (Ludwigshafen, Germany). Oleic acid was acquired from Fisher Chemicals. Middle chain triglyceride (MCT) was kindly provided by Societé des Oleagineux (Bougival, France). Collagenase was purchased from Worthington Biochemical Corporation (Lakewood, NJ, USA). Dimethyl sulfoxide (DMSO) was purchased from Fluka (Steinheim, Switzerland). DiD′ oil; DiIC18(5) oil (1,1′-dioctadecyl-3,3,3′,3′-Tetramethylindodicarbocyanine Perchlorate) was purchased from Invitrogen (Carlsbad, CA, USA). Semi-solid silicone elastomer blend Dow Corning 9040, Dimethicone PMX-200 (Dow Corning 350) and Cyclohexasiloxane PMX-246 (Dow Corning 246) were from Dow Corning (Midland, MI, USA). Dimethicone/Vinyl Dimethicone Crosspolymer KSG-16 was from Shin Etsu (Tokyo, Japan). Lauroyl Lysine (Amihope LL) was from Ajinomoto (Tokyo, Japan). Polymethylsilsesquioxane (Gransil PSQ) was from Grant Industries (Elmwood Park, NJ, USA). Allantoin was from Ziv Chemicals (Holon, Israel). All organic solvents were HPLC grade and purchased from J.T. Baker (Deventer, Holland). All tissue culture media were from Biological Industries Ltd (Beit Ha Emek, Israel).

2.2. Human skin organ culture

Human skin was obtained from patients undergoing elective cosmetic surgery (abdominoplasty), approved by the Hadassah University Hospital Ethics Committee. Full-thickness human skin organ culture was performed as described previously [26]. The skin was kept at 37 °C in an atmosphere containing 5% CO_2 in serum-free DMEM containing 2 mM L-glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin.

2.3. Porcine tissue treatment

About 750 μm thick, trimmed, porcine ear skin was purchased from Lahav Animal Research Institute (Kibbutz Lahav, Israel). The skin was cleaned carefully, and the dermatomed skin was either treated or stored frozen at −20 °C for up to a maximum of 1 month before use. Skin integrity was measured by transepidermal water loss (TEWL) [27] using a VapoMeter device (Delfin technologies, Finland). Skin samples with TEWL values ≤ 15 g h^{-1} m^{-2} were used in the experiments [28].

2.4. Preparation of empty or drug-loaded NPs

The various PLGA nanocarriers were prepared according to the well-established solvent displacement method [29]. Briefly, the polymers PLGA 4K and 100K, at 50:50 blend of LA:GA, were dissolved acetone containing 0.2% w/v Tween® 80, at a concentration of 0.6% w/v. DHEA was added at various concentrations into the organic phase. The organic phase was added to the aqueous phase containing 0.1% w/v Solutol HS 15 to the formation of nanospheres (NSs). The suspension was stirred at 900 rpm over 15 min and then concentrated by reduced pressure evaporation. Alternatively, when appropriate oils were added to the organic phase, NCs were formed. For NC preparation, the solubility of DHEA in different oils was determined by UV spectroscopy (UVIKON, SECOMAM, France). When radiolabeled NPs were prepared, 7.2 μCi of [^{3}H]-DHEA was mixed with 0.02% w/v of DHEA acetone solution before adding to the aqueous phase. If fluorescent NPs were prepared, an aliquot of acetone DiD′ oil solution at a concentration of 1 mg/ml was also added to the organic phase resulting in a final concentration of 1.5 μg/ml.

2.5. Physicochemical characterization of NSs and NCs

The mean diameter and the zeta potential of the various NPs were characterized using Malvern's Zetasizer (Nano series, Nanos-ZS, UK) at 25 °C and using water as diluent. Morphological evaluation was performed using transmission electron microscopy (TEM, Philips Technai F20 100 KV) following negative staining with phosphotungstic acid and by cryo-transmission electron microscopy (Cryo-TEM). In the Cryo-TEM method, a drop of the solution is placed on a carbon-coated holey polymer film supported on a 300-mesh Cu grid (Ted Pella Ltd, Redding, CA, USA), and the specimen is automatically vitrified using Vitrobot (FEI) by means of a fast quench in liquid ethane to −170 °C. The samples were studied using an FEI Tecnai 12 G2 TEM, at 120 kV with a Gatan cryo-holder maintained at −180 °C, and images were recorded on a slow scan cooled charge-coupled device camera. Thermal analysis was performed using a DSC 1 STARe system differential scanning calorimeter (Mettler-Toledo, Switzerland), calibrated with In standard under nitrogen atmosphere. Samples of about 1–20 mg were