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Polymeric nanoparticles modified with fatty acids encapsulating betamethasone for anti-inflammatory treatment

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ABSTRACT

Topical glucocorticosteroids were incorporated into nanocarrier-based formulations, to overcome side effects of conventional formulations and to achieve maximum skin deposition. Nanoparticulate carriers have the potential to prolong the anti-inflammatory effect and provide higher local concentration of drugs, offering a better solution for treating dermatological conditions and improving patient compliance.

Nanoparticles were formulated with poly-ε-caprolactone as the polymeric core along with stearic acid as the fatty acid, for incorporation of betamethasone-21-acetate. Oleic acid was applied as the coating fatty acid. Improvement of the drug efficacy, and reduction in drug degradation with time in the encapsulated form was examined, while administering it locally through controlled release. Nanoparticles were spherical with mean size of 300 nm and negatively charged surface. Encapsulation efficiency was 90%. Physicochemical stability in aqueous media of the empty and loaded nanoparticles was evaluated for six months. Drug degradation was reduced compared to free drug, after encapsulation into nanoparticles, avoiding the potency decline and promoting a controlled drug release over one month. Fourier transform infrared spectroscopy and thermal analysis confirmed drug entrapment, while cytotoxicity studies performed *in vitro* on human keratinocytes, *Saccharomyces cerevisiae* models and *Artemia salina,* showed a dose–response relationship for nanoparticles and free drug. In all models, drug loaded nanoparticles had a greater inhibitory effect. Nanoparticles increased drug permeation into lipid membranes *in vitro*. Preliminary safety and permeation studies conducted on rats, showed betamethasone-21-acetate in serum after 48 h application of a gel containing nanoparticles. No skin reactions were observed.

In conclusion, the developed nanoparticles may be applied as topical treatment, after encapsulation of betamethasone-21-acetate, as nanoparticles promote prolonged drug release, increase drug stability in aqueous media, reducing drug degradation, and increase drug permeability through lipid membranes.

Abbreviations: BTMA, Betamethasone-21-acetate; DMSO, Dimethyl sulfoxide; DMEM, Dulbecco's Modified Eagle's Medium; DSC, Differential scanning calorimetry; EE, Encapsulation efficiency; FTIR, Fourier transform infrared spectroscopy; GCs, Glucocorticosteroids; HaCaT, Human adult low-calcium high-temperature keratinocytes; HPLC, High performance liquid chromatography; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NP, Nanoparticles; NSAID, Non steroid anti-inflammatory drugs; OA, Oleic acid; PCL, Poly-ε-caprolactone; RC, Refrigeration conditions; RH, Relative humidity; RT, Room temperature; ROS, Reactive oxygen species; SA, Stearic acid; TLC, Thin layer chromatography; YPD, Yeast–peptone–dextrose.

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1. Introduction

Treatment of inflammatory diseases benefit from a localized therapy accomplished with polymeric-based nanoparticles (Zhang et al., 2013). Recently, we have developed innovative nanoparticles (NP) for treatment of skin diseases, through application of drugs including glucocorticosteroids (GC) (Rosado et al., 2012), non-steroid anti-inflammatory drugs (NSAID) (Pinto Reis et al., 2011), antimicrobial agents (Gomes et al., 2013; Pinto Reis et al., 2013; Rijo et al., 2014) and anticancer drugs for melanoma (unpublished).

Glucocorticosteroids (GC) cover a broad spectrum of therapeutic actions such as anti-inflammatory, immunosuppressive, anti-proliferative and vasoconstrictive, having also apoptotic and anti-angiogenic effects (Banciu et al., 2006; Lebwohl et al., 2013). The absence of natural GC produced by skin cells is mainly visible in inflammatory diseases, such as atopic dermatitis and psoriasis (Slominski et al., 2013).

However, when treating common skin diseases like atopic dermatitis or psoriasis, chronic application of topical corticosteroids generally leads to local adverse effects such as skin atrophy, rosacea, *striae* and skin infections. When highly absorbed, systemic side effects (e.g., hypothalamic-pituitary-adrenal suppression, glaucoma, hyperglycemia and hypertension) appear, compromising the therapeutic effectiveness and patient adherence (Ference and Last, 2009). For this study, we chose betamethasone-21-acetate (BTMA) with the structure illustrated in Fig. 1, since it is a high-potency synthetic derivative of betamethasone and agonist to the GC-receptors. Betamethasone offers a 10-fold higher potency than hydrocortisone (Arica and Lamprecht, 2005), and has been applied through nanosystems for topical and percutaneous permeation, reducing associated side effects (Abdel-Mottaleb et al., 2012; Zhang and Smith, 2010). Previously, Abdel-Mottaleb et al. (2012) demonstrated that non-coated polymeric NP work as drug reservoirs, penetrating to 25 μm of skin depth (Abdel-Mottaleb et al., 2012), due to limited interaction with skin lipids. In contrast, some reported lipid NP (Zhang and Smith, 2010) interact with skin lipids but show many stability problems (e.g., drug leakage and chemical modifications during storage).

In this study, we provide evidence that the association of both fatty acids and polymers for development of hybrid nanoparticles may counteract individual disadvantages of these materials. In addition, our nanoparticles may also improve local drug delivery to specific inflammatory sites in the skin, by reducing hydrolysis and degradation of BTMA and controlling its release from the nanoparticles over a prolonged period. The concept of hybrid lipid-polymeric structures was first described for formation of bi-layered membranes (Shen et al., 2000). In the present study, the goal was to develop a stable platform for drug delivery, based on the association of a biodegradable polymer, poly-ε-caprolactone (PCL) and stearic and oleic acids as long chain fatty acids. The potential of these carriers is to increase skin permeation. Poly-ε-caprolactone (PCL) was used to control drug release, reduce the drug percutaneous penetration and protect the drug from potential photochemical degradation (Pohlmann et al., 2013). In addition, PLC was selected as the core polymer as previous work showed promise as an ideal depot system for prolonged drug release, with appropriate NP size and spherical shape when used for skin applications (Rosado et al., 2012). However, to overcome previous problems, such as the low encapsulation efficiency (62%), stearic acid (SA) was added to the core, to improve drug entrapment within the NP structure, also reduce the possibility of burst release (Chen et al., 2001; Lee et al., 2003). Since the penetration of polymeric NP across the skin is hindered by the *stratum corneum* mechanical barrier properties (Abdel-Mottaleb et al., 2012), oleic acid (OA) was incorporated as the coating lipid, since it has been previously shown to be a skin permeation enhancer and membrane fluidizing agent (Al Abood et al., 2013). OA is also reported to reduce nanoparticle aggregation (Bennet et al., 2012). SA is a saturated fatty acid unlike OA, but both are C_{18} fatty acids, and are presently approved for skin and food applications (Inoue et al., 2004). In addition, OA and SA are also present in many essential oils, providing higher skin permeation allied with lower toxicity, and have been reported as accepted for cosmetic and alimentary applications and documented by several organizations, such as the International Flavor and Fragrance Association (Herman and Herman, 2015).

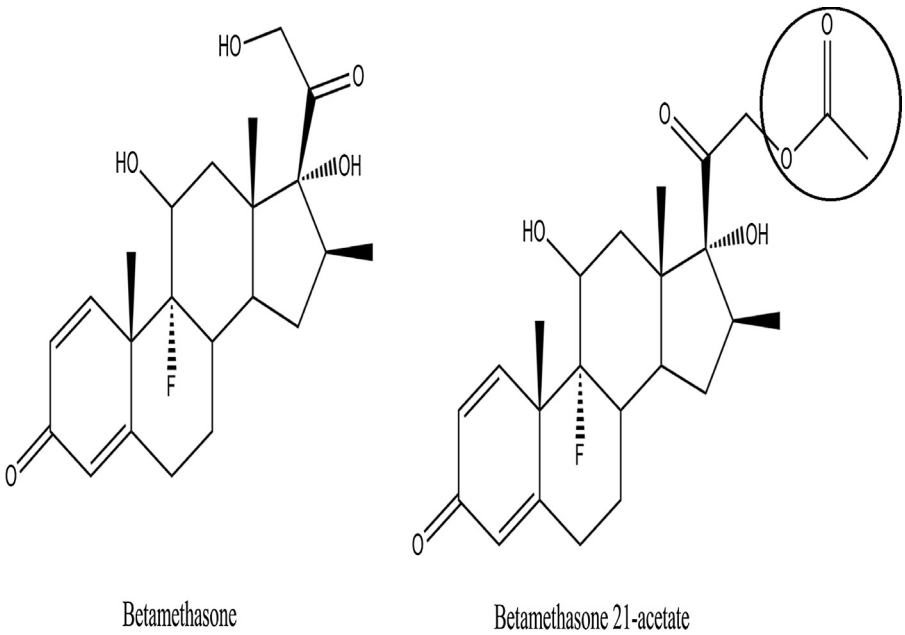

Fig. 1. Chemical structures of betamethasone and betamethasone-21-acetate.The modification of ester group in C_{21} of betamethasone-21-acetate, responsible for the molecule's high-potency action, is highlighted.