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Tacrolimus and curcumin co-loaded liposphere gel: Synergistic combination towards management of psoriasis

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ABSTRACT

Psoriasis is an autoimmune skin disorder characterized by hyper proliferation and poor differentiation of keratinocytes. It significantly affects patient's quality of life. This study reports the anti-psoriatic efficacy of tacrolimus and curcumin loaded liposphere gel formulation. Poor solubility, poor skin penetration and erratic absorption are some problems associated with the topical delivery of these drugs. To overcome these problems, lipospheres containing combination of tacrolimus and curcumin was prepared with a particle size of nearly 50 nm and incorporated into a gel for topical application. Liposphere gel showed slow release of both the drugs and shear thinning behaviour that is desirable property of topical formulation. Further, dermal distribution study using dye loaded formulation suggested penetration of dye into skin layers. The therapeutic efficacy of tacrolimus and curcumin loaded liposphere gel was assessed on imiquimod induced psoriatic plaque model, and the level of expression of psoriatic biochemical markers was evaluated using enzyme-linked immunosorbent assay. Results indicated improvement in the phenotypic and histopathological features of psoriatic skin treated with tacrolimus and curcumin loaded liposphere gel. There was reduction in the level of TNF-α, IL-17 and IL-22 compared to imiquimod group. These results corroborate the premise that liposphere gel containing combination of tacrolimus and curcumin can be an effective strategy for the treatment of psoriasis.

1. Introduction

Psoriasis is a chronic immune-mediated skin disease characterized by hyperproliferation and poor differentiation of epidermal keratinocytes coupled with prominently increased vascularisation of the skin, fibroblast activation and leukocyte infiltration [1]. The disease affects 2–3% of the world population with a substantial negative impact on the patient's quality of life [2,3]. Aberrant response of keratinocytes is mediated by activation of the cellular immune system, T-cells, dendritic cells and various immune-related cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-17, IL-22, IL-23 etc. [4]. The multi-factorial etiology of psoriasis and the myriad of antigenic trigger of disease development provide a wide range of treatment options such as topical therapy [5], phototherapy [6], immunomodulators [7], vitamin D derivatives [8], and systemic therapy [9]. Topical administration, however, is always preferred over systemic therapy due to the reduced systemic burden of the drug, thus minimizing side effects [6]. The first line anti-psoriatic therapy comprises the topical administration of corticosteroids [10]. However, the risk of cutaneous atrophy and the rebound of psoriasis associated with topical steroids show a need for the development of another effective topical treatment system for psoriasis [11].

Tacrolimus (TAC) is an immunosuppressant that acts by the inhibition of calcineurin, a calcium-binding cytoplasmic protein involved in T-cell activation and proliferation (Fig. 1A). The initial binding of TAC with FK506 binding protein forms a complex that binds to calcineurin, thus inhibiting the calcineurin mediated dephosphorylation of the nuclear factor of activated T-cells [12]. This essentially involves the cascade of cytokine gene transcription, such as IL-2, IL-4, interferon-ϒ (IFN-ϒ), and TNF-α [12,13]. Therefore, tacrolimus has been widely explored in the treatment of psoriasis [14–16] and immunological disorders [17–19]. It is commercially available as a topical ointment, but the ointment has been reported with low and highly variable absorption and doesn't ensure adequate topical delivery of the drug into deeper skin layers [20–22].

Curcumin (CUR) is known from ancient time for its beneficial effect on skin related complications. It acts through multiple molecular targets

Abbreviations: TAC, Tacrolimus; CUR, Curcumin; TAC liposphere gel, Tacrolimus loaded liposphere gel; TAC-CUR liposphere gel, Tacrolimus and curcumin co-loaded liposphere gel; TAC-CUR plain gel, Tacrolimus and curcumin co-loaded plain gel; FITC, Fluorescein isothiocyanate; IMQ, Imiquimod; CLSM, Confocal laser scanning microscopy; H&E, Hematoxylin and eosin; PASI, Psoriatic area severity index; IL, Interleukin; PDI, Polydispersity index; SBWR, Spleen weight to body weight ratio; TNF-α, Tumor necrosis factor alpha; IFN-ϒ, interferon-ϒ; TP, Tripalmitin; TM, Trimyristin; TL, Trilaurin; TA, Triacetin; TC, Tricaprin; NMP, *N*-methylpyrrolidone; PG, Propylene glycol; IPA, Isopropyl alcohol; EtOH, Ethanol; EtAC, Ethyl acetate; HPMC, Hydroxyl propyl methyl cellulose E-15; EtLC, Ethyl lactate.

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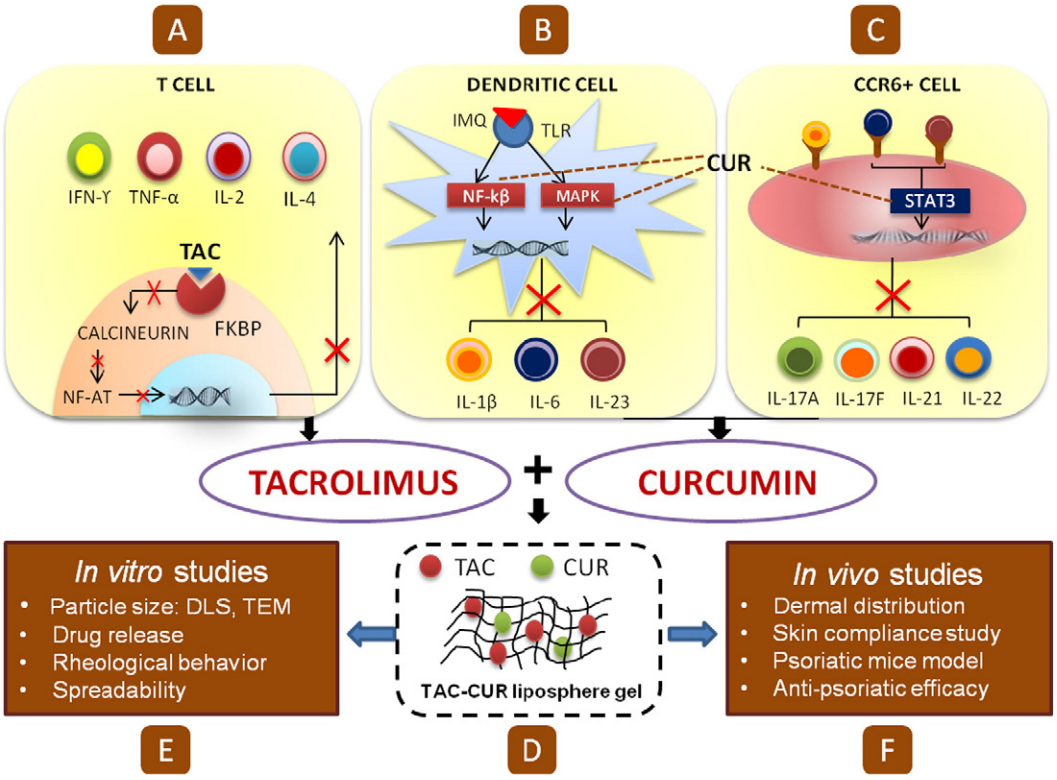

Fig. 1. Schematic presentation of mechanism of tacrolimus, curcumin and characterization of TAC-CUR liposphere gel. (A) Molecular mechanism of tacrolimus: tacrolimus acts on T-cells by inhibition of calcineurin thus inhibit the expression of IL-2, IL-4, IFN-ϒ, and TNF-α; (B) and (C) molecular mechanism of curcumin: curcumin shows inhibitory effect on NF-Kß and MAPK pathways and also suppresses STAT3 thus inhibits the production of different interleukins involved in the pathogenesis of psoriasis; (D) TAC-CUR liposphere gel: tacrolimus and curcumin co-loaded liposphere gel; (E) in vitro characterization and (F) in vivo studies. (Dotted lines and cross mark indicates inhibitory effects.)

(Fig. 1B and C) and mechanisms of action [23,24]. Sun et al., reported the therapeutic potential of CUR gel in psoriasis using imiquimod (IMQ) induced psoriatic model [25]. It inhibits expression of IL-1β, IL-6, TNF-α and cyclin E through its action on NF-kB and MAPK pathways [26]. Suppression of IL-1β/IL-6 down-regulates IL-17A/IL-22 production [25]. This down regulation shows impact on IL-23/IL-17 cytokine axis which is associated with pathogenesis of psoriasis [27,28]. However, poor solubility and poor skin penetration are the main problems associated with its topical delivery [29].

Combination therapy may be regarded as one of the most powerful strategies to obviate the compensatory mechanisms and dose related undesired off-target effects. Synergistic combinations of two or more therapeutically relevant molecules, acting through different mechanisms, maximize therapeutic effect by providing a multi-target treatment approach [30]. Various polymer and lipid nano-carrier systems have been used to co-deliver multiple drug molecules to their action site. Lipid based systems are preferred for topical application due to their better penetration through skin layers [31]. Lipospheres are lipid based nano-carrier containing a solid hydrophobic lipid core stabilized by a layer of phospholipid molecules (coat lipid) embedded on their surface. Lipospheres have several advantages over other lipid based systems such as stability, low cost of reagents, ease of manufacture, high dispersibility in an aqueous medium, and a release rate for entrapped substances that is controlled by phospholipid coating and carrier [32–35]. Lipospheres were discovered and patented in 1993 by our group [36,37]. In our previous work, a lipospheres based formulation was developed for oral delivery and improving bioavailability of cyclosporine. This formulation is now in clinical use (Deximune® soft-gelatin capsules, Dexcel® Pharma Ltd.) [38,39].

The main objective of the present work is to evaluate the potential of TAC and CUR co-loaded liposphere gel (TAC-CUR liposphere gel) in the management of psoriasis (Fig. 1D). TAC induced calcineurin inhibition and the action of CUR on NF-kB and MAPK pathways can provide a multi-target treatment approach to maximize therapeutic effect. TAC and CUR are co-loaded within the lipospheres to assist effective penetration of both drugs in skin layers. These lipospheres are incorporated in gel to provide ease of application, spreading and retention on skin for longer period of time. In vitro characterization and in vivo studies were performed for optimized formulations and the anti-psoriatic efficacy has been evaluated using IMQ induced psoriatic plaque model (Fig. 1E and F). This study reports for the first time, the novel combination of TAC and CUR loaded in liposphere gel in the management of psoriasis.

2. Materials and methods

2.1. Materials

Tacrolimus was a gift sample from Vivimed Labs (Hyderabad, India). Tripalmitin (TP), Trimyristin (TM), Trilaurin (TL), *N*-methylpyrrolidone (NMP) and Hydroxyl propyl methyl cellulose E-15 (HPMC) were purchased from HiMedia Laboratories (Mumbai, India). Ethyl lactate (EtLC), Egg lecithin, Curcumin and Fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (Missouri, USA). Triacetin (TA) and Tricaprin (TC) were purchased from SDFCL (Mumbai, India) and Tokyo Chemical Industry Co. Ltd. (Japan), respectively. Ethyl acetate (EtAC) was purchased from Avantor Performance Materials Ltd. (Gurgaon, India). Tween 80 was purchased from Sisco Research Laboratories Pvt. Ltd. (Hyderabad, India). ELISA kits for IL-17, IL-22, IL-23 and TNF-α were purchased from R&D Systems (Minneapolis, Canada). Acetonitrile (MeCN) and Ethanol (EtOH) were purchased from Merck Specialities Pvt. Ltd. (Mumbai, India). All other ingredients and reagents were of analytical grade.