UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO

**Isotretinoin-loaded *Delonix* polymeric nanoparticles prospects as a
delivery tool in the treatment of acne**

**Potencial de nanopartículas poliméricas de *Delonix* contendo
isotretinoína para o tratamento da acne**

ABAYOMI TOLULOPE OGUNJIMI

Ribeirão Preto
2018

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Doctoral thesis presented to the Graduate Program of School of Pharmaceutical Sciences of Ribeirão Preto/USP for the degree of Doctor in Sciences.

Concentration Area: Pharmaceutics and Cosmetics

Supervisor: Prof. Dra. Renata F.V. Lopez

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2018

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Abayomi Tolulope Ogunjimi

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DEDICATION

To everyone around the world displaced by bad leadership, religion and war

To everyone striving **genuinely** to make this world a better place

To my mother, Suzan Adunola Kehinde Ogunjimi

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Abstract

OGUNJIMI A.T. **Isotretinoin-loaded *Delonix* polymeric nanoparticles prospects as a delivery tool in the treatment of acne.** 2018. 163 p. Thesis (Doctorate). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018

Oral isotretinoin (IST) has been effective in acne treatment; however, its use is associated with side effects such as skin dryness, epistaxis, immune disorder and psychiatric concerns while its topical gel application's effectiveness is hampered by irritancy. Protecting IST from direct skin contact and targeting its release to the hair follicles where the acne develops can reduce irritation and increase the effectiveness of treatment. Delonix (DLX) polymer is a natural galactomannan derived from *Delonix regia* seed that may be engineered to yield nanoparticles for IST skin targeting. Therefore, the aim of this study was to develop IST-loaded DLX (IST-DLX) polymeric nanoparticles and assess its skin targeting prospects for acne treatment. DLX was collected, purified and characterized by monosaccharide composition and molecular weight (Mw). Blank DLX nanoparticles, fluorescent DLX nanoparticles (BODIPY-loaded (BOD-DLX) nanoparticles) and IST-DLX nanoparticles were prepared and characterized by size, polydispersibility index (PDI), zeta potential (zp) and encapsulation efficiency (EE). *In vitro* release study of IST-DLX nanoparticles was performed using diffusion cells and synthetic membrane, while skin targeting of BOD-DLX and IST-DLX nanoparticles were assessed by confocal microscopy and differential tape stripping technique in dermatomed pig ear skin respectively. IST-DLX polymeric nanoparticles' IL-6, IL-10 and TNF- α modulation was assessed *in vitro* using lipopolysaccharide (LPS) stimulated AMJ-2 macrophage cells and antimicrobial activity was assessed in *Propionibacterium acnes* culture medium. IST-DLX nanoparticles' skin penetration and irritation were evaluated *in vivo* in Wistar rats (ethics committee approval protocol CEUA-FCFRP n° 17.5.213.60.3). DLX polymer Mw was ~177 kDa, containing predominantly mannose and galactose in ratio 6.3:1. IST-DLX nanoparticles' size determined by light scattering was between 215 to 232 nm, PDI < 0.2, zp < -30 mV, EE > 25 %. IST-DLX nanoparticles sustained IST release, with about 37% IST being released in 48 h. Confocal images showed that BOD-DLX nanoparticles accumulated in the epidermis and hair follicles as compared to free BODIPY solution which permeated into the dermis. Significantly higher and uncontrolled IST penetration into all layers of pig ear skin with IST-DLX polymer solution was observed as compared to lower, controlled but optimum IST accumulation into the pig ear skin when IST-DLX nanoparticles were used. IST-DLX nanoparticles targeted the hair follicle (26 %) as compared to IST solution (6.5 %) which permeated through all skin layers. IST-DLX polymeric nanoparticles significantly reduced IL-6 production in LPS stimulated macrophage cells at 48 h as compared to free IST solution which significantly reduced IL-6 production only at 24 h. Both IST-DLX nanoparticles and free IST solution did not inhibit *P. acnes* growth. No sign of inflammatory erythema was observed in IST-DLX nanoparticles treated Wistar rats with or without UVA irradiation as compared to free IST treated ones. Epidermal thickness of rats treated with IST-DLX nanoparticles was significantly smaller than those treated with free IST with or without UVA irradiation. It can be concluded that IST-DLX nanoparticles can target, deliver and sustain IST release to the skin at optimum concentrations, modulate IL-6 inflammatory responses and may prevent IST related inflammatory erythema *in vivo*. DLX polymer could be promising polymer for drug delivery.

Keywords: *Acne vulgaris*, Isotretinoin, Delonix nanoparticles, follicular targeting, topical delivery

Resumo

OGUNJIMI A.T. Potencial de nanopartículas poliméricas de Delonix contendo isotretinoína para o tratamento da acne. 2018. 163 f. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018

A administração oral de isotretinoína (IST) é eficaz no tratamento da acne; no entanto, seu uso está associado a efeitos adversos, como ressecamento da pele, epistaxe, distúrbios imunológicos e psiquiátricos, enquanto a eficácia da aplicação tópica é prejudicada pela intensa irritação causada na pele. Proteger a IST do contato direto com a pele e direcionar sua liberação para os folículos pilosos, local onde a acne se desenvolve, pode reduzir a irritação e aumentar a eficácia do tratamento. O polímero Delonix (DLX), galactomanano natural derivado da semente de *Delonix regia*, pode ser manipulado para produzir nanopartículas para a liberação direcionada de IST na pele. Desta forma, o objetivo deste estudo foi desenvolver nanopartículas poliméricas de DLX (IST-DLX) contendo IST e avaliar seu potencial para o tratamento tópico da acne. O DLX foi coletado, purificado e caracterizado quanto a composição de monossacarídeos e massa molecular (MM). Nanopartículas DLX sem IST (branco), nanopartículas fluorescentes de DLX (nanopartículas contendo BODIPY (BOD-DLX)) e nanopartículas IST-DLX foram preparadas e caracterizadas por tamanho, índice de polidispersividade (PDI), potencial zeta (pz) e eficiência de encapsulação (EE). O estudo de liberação *in vitro* das nanopartículas IST-DLX foi realizado utilizando células de difusão e membrana sintética, enquanto a penetração cutânea das nanopartículas BOD-DLX e IST-DLX foi avaliada por microscopia confocal e *tape stripping* diferencial em pele de orelha de porco dermatomizada, respectivamente. A influência das nanopartículas IST-DLX na modulação de IL-6, IL-10 e TNF- α foi avaliada *in vitro* utilizando células de macrófagos AMJ-2 estimuladas com lipopolissacarídeos (LPS) e a atividade antimicrobiana foi avaliada em meio de cultura de *Propionibacterium acnes*. *In vivo*, as nanopartículas IST-DLX foram avaliadas quanto a penetração e irritação cutânea em ratos Wistar (protocolo de aprovação CEUA-FCFRP nº 17.5.213.60.3). A MM do polímero DLX foi de ~177 kDa, contendo predominantemente manose e galactose na proporção de 6,3:1. O tamanho das nanopartículas IST-DLX, determinado por dispersão de luz, foi entre 215 a 232 nm, PDI < 0,2, pz < -30 mV e EE > 25%. As nanopartículas IST-DLX sustentaram a liberação da IST, com cerca de 37% de IST sendo liberado em 48 h. As imagens de microscopia confocal mostraram que as nanopartículas BOD-DLX concentraram-se na epiderme e nos folículos pilosos em comparação com a solução de BODIPY livre, a qual permeou até a derme. Foi observada uma penetração de IST significativamente maior e descontrolada em todas as camadas da pele de orelha de porco quando a solução de polímero de IST-DLX foi usada em comparação com um acúmulo de IST menor e controlado quando as nanopartículas IST-DLX foram aplicadas. As nanopartículas IST-DLX direcionaram a IST para os folículos pilosos (26%) em comparação com a solução de IST (6,5%) que permeou por todas as camadas da pele. As nanopartículas IST-DLX reduziram significativamente a produção de IL-6 em células de macrófagos estimuladas por LPS, mesmo após 48 h, em comparação com a solução de IST livre, que reduziu significativamente a produção de IL-6 apenas até 24 h. Tanto as nanopartículas IST-DLX quanto a solução de IST livre não inibiram o crescimento de *P. acnes*. Não foi observado nenhum sinal de eritema nos ratos tratados com nanopartículas IST-DLX, com ou sem irradiação UVA, enquanto os tratados com solução de IST apresentaram eritema. A espessura da epiderme dos ratos tratados com as nanopartículas foi significativamente menor do que daqueles tratados com IST livre, com ou sem irradiação UVA. Conclui-se que as nanopartículas IST-DLX direcionam e sustentam a liberação de IST na pele em concentrações ótimas, modulam a resposta inflamatória da IL-6 e previnem o eritema inflamatório relacionado ao IST *in vivo*. Sendo assim, o polímero DLX pode ser considerado um polímero promissor para a liberação sustentada e direcionada de fármacos.

Palavras-chave: *Acne vulgaris*, isotretinoína, nanopartículas de Delonix, direcionamento folicular, liberação tópica

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LIST OF ABBREVIATIONS

AMJ-2	Macrophage (alveolar), infected with J2 virus
ARPE	Retina Pigment Epithelial cell
ATCC	American Type Culture Collection
DLX	Delonix
DMEM	Dulbecco's Modified Eagle Media
DMSO	Dimethyl sulfoxide
DoE	Design of Experiment
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
HEC	Human Epithelial Corneal cell
HEC gel	Hydroxy Ethyl Cellulose gel
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
IL-10	Interleukin 10
IL-6	Interleukin 6
IST	Isotretinoin
LoD	Limit of detection
LoQ	Limit of quantification
LPS	Lipopolysaccharide
MTT	Methyl thiazolyl diphenyl-tetrazolium bromide
NMR	Nuclear Magnetic Resonance spectroscopy
NRU	Neutral Red
PBS	Phosphate buffer solution
QbD	Quality by Design
SHEM	Supplemented Hormonal Epidermal Medium
TEM	Transmission Electron Microscopy
TNF- α	Tumor Necrosis Factor alpha

UFLC

Ultra-Fast Liquid Chromatography

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