Journal of Pharmaceutical Sciences xxx (2017) 1-9

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

In Vitro, *Ex Vivo*, and *In Vivo* Evaluation of a Dual pH/Redox Responsive Nanoliposomal Sludge for Transdermal Drug Delivery

Simphiwe Mavuso, Thashree Marimuthu, Pradeep Kumar, Pierre P.D. Kondiah, Lisa C. du Toit, Yahya E. Choonara, Viness Pillay*

Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 7 York Road, Parktown 2193, South Africa

ARTICLE INFO

Article history:
Received 2 October 2017
Revised 6 November 2017
Accepted 16 November 2017

Keywords:
copper-glyglycine-prednisolone succinate complex [Cu(glygly)(PS)]
nanoliposome
pH/redox stimuli responsiveness
transdermal drug delivery

ABSTRACT

A dual pH/redox responsive copper-glyglycine-prednisolone succinate–loaded nanoliposomal (NL) sludge was successfully synthesized and optimized using a Box-Behnken design of experiments. Preformulation design variables indicated that relative ratios of phospholipids, considerably influences NL size, thus altering the degree of drug loading in the formulation. *In vitro* evaluation further confirmed optimum release kinetics of the NL sludge, corresponding closely to *ex vivo* permeation studies, demonstrating effective transdermal delivery of prednisone succinate (PS) through a pig skin model, which closely resembles human skin anatomy. The pH/redox stimuli responsiveness of the NL sludge further demonstrated superior properties *in vivo* using a Sprague-Dawley rat model. The NL sludge displayed the greatest release of PS within 24 h of evaluation, falling within the acceptable therapeutic range of PS dose efficiency. *In vivo* results further displayed the greatest absorption of PS under inflammatory induced conditions, thus confirming the unique pH/redox responsive properties of the NL sludge. It was thus confirmed that the copper-glyglycine-prednisolone succinate–loaded NL sludge has significant potential for application in chronic inflammatory conditions such as tumor necrosis factor receptor–associated periodic syndrome (TRAPS), designed to release an effective dose of corticosteroid, as a transdermal drug delivery formulation, for effective therapeutic efficacy.

Introduction

Management of tumor necrosis factor receptor–associated periodic syndrome (TRAPS) using corticosteroids is mainly limited by long-term side effects from oral drug delivery,[1] mainly due to high systemic drug exposure. This may result in poor bioavailability of drugs at the site of action, which may be circumvented by using an intelligent metal-drug complex–loaded nanocarrier design, for transdermal drug delivery. Although this system has many advantages over other drug administration routes, the stratum corneum forms a strong barrier to most topically applied formulations.[2] However, by combining the advantages of both a liposomal nanocarrier and a gel-based vehicle, a liposomal nanogel with superior properties can be formulated. A liposomal nanogel may be described as lipid coated, drug-loaded nanoparticle system, dispersed in a gel matrix,[3] of which the gel matrix further enhances the overall drug delivery outcome of the design.

In the design and development of a nanoliposomal (NL) sludge, hydroxypropyl methylcellulose (HPMC) was used due to its gel-forming, hydration, and skin-permeating properties.[4,5] Poly(*N*-vinylpyrrolydone) (PVP) was also added to the formulation, to promote the bioadhesiveness[4] and stability[6] of the NL sludge system. The topical NL sludge delivery system was designed with consideration to elicit superior viscoelastic, bioadhesion, and pH/redox responsive properties. These physicomechanical advantages for application of the NL sludge in topical inflammatory conditions, has the ability to respond directly at the site of injury (inflammation), due to its unique pH/redox responsive nature, further producing a slow release of loaded drug from the nanoliposomes (NLs), due to a highly viscous and bioadhesive nature of the sludge. These properties of the sludge, further promote patient compliance, resulting in optimum clinical efficacy of the sludge formulation. Other novel properties designed as part of the sludge formulation included sheer thinning properties as stress was applied to the

Conflicts of Interest: The authors declare that there are no conflicts of interest.

* *Correspondence to:* Viness Pillay (Telephone: +27-11-717-2274; Fax: +27-11-642-4355).
E-mail address: viness.pillay@wits.ac.za (V. Pillay).

https://doi.org/10.1016/j.xphs.2017.11.011
0022-3549/

sludge; gradual release of loaded drug once stimuli responsive conditions of the sludge were initiated; and further demonstration of cationic zeta potential (>21 mV) for potential stability of the colloidal system once applied *in situ*. Researchers in the field previously reported a gel matrix based on a blend of HPMC and PVP, designed for enhanced transdermal drug delivery.[7,8] The outcome of the study presented potential success in treating TRAPS, by combining the chemical bonds between these 2 polymers, resulting in an effective transdermal nanogel drug delivery system.

In the present study, a dual pH/redox responsive cationic polymer, Eudragit E100-cystamine (EuE100-cyst) and phospholipids were conjugated to produce a pH/redox responsive NL formulation. This research is a trajectory from our previous study, following preformulation studies employing drug-loaded transdermal formulations.[9] Corticosteroids, which are known to be effective anti-inflammatory agents, were administered for the treatment of skin inflammation conditions and regularly prescribed for patients with TRAPS. Oral formulations are characterized by erratic absorption kinetics, high dosages, and associated with nontrivial side effects. Moreover, corticosteroids are relatively hydrophilic with low skin permeability that inhibits effective cutaneous absorption. Chemical permeation enhancers are often applied to aid transdermal delivery; however, these bring with them additional side effects and do not increase the potency of the drug. Cutaneous absorption can be potentially improved by coordination of corticosteroids to a metal center, with an appropriate nontoxic ancillary ligand to enable more consistent and efficient transdermal absorption. In this study, prednisolone 21-hemisuccinate was employed as this derivate enabled coordinative to the copper metal center through the available "O" donor on the succinate moiety. Glycylglycine a conventional amino acid was employed as the nontoxic ancillary ligand due to its ability to form stable complexes with copper, resulting in a further improved solubility of the resulting complex. Copper was identified as the metal of choice based on the following concepts; (a) copper is a *d* block transition metal which possess inherently low toxicity at specified thresholds, (b) Cu (II) has the ability to form mixed ligand complexes that facilitates coordination of ancillary ligands, and (c) copper complexes incorporating anti-inflammatory bioactives have been reported to be more effective than the parent drugs.[9]

Nanoliposomes have previously been investigated as promising drug delivery systems for various applications in transdermal delivery.[10-12] In this study, NLs were loaded with the previously reported (copper-glyglycine-prednisolone succinate [Cu(glygly)(PS)]) complex, and optimized using the design of experiments, employing a 3-factor, 3-level Box-Behnken (BB) statistical design. Independent variables in the study consisted of varying ratios of lipids, cholesterol constituent, and sonication time. Dependent variables in the study were selected as average particle size, polydispersity index (PDI), zeta potential, percentage entrapment efficiency (%EE), and percentage drug loading (%DL). Data obtained produced response surface plots, statistical validity of the polynomial according to mathematical sequence, and an optimized formulation according to response parameters of data input.

Further characterization of the optimized formulation was undertaken to elicit its physicochemical and physicomechanical properties. The transdermal delivery system was thereafter evaluated for its *in vitro* drug release properties and *ex vivo* drug permeation evaluation using an animal skin model. On successful results of previously mentioned experiments, *in vivo* evaluation was undertaken using a Sprague-Dawley rat model to determine the efficacy of drug absorption from the delivery system. Ultra-performance liquid chromatography (UPLC) was employed to determine the concentration of drug in plasma sampling during various time intervals in the study.

Materials and Methods

Materials

Eudragit E100 and phospholipids including L-α-phosphatidylcholine (PC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphatidyl-ethanolamine (DSPE) were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate-buffered saline (PBS) tablets, prednisone succinate (PS) salt, and copper (II) nitrate were also purchased from Sigma-Aldrich). Sodium hydroxide (NaOH) pellets and dimethylformamide, ammonium sulphate, sodium acetate, and glacial acetate acid were purchased from Merck (Wadeville, South Africa). Dimethylsulfoxide was purchased from Saarchem (Pty) Ltd. (Brakpan, South Africa), and liquid nitrogen was obtained from Afrox (Pty) Ltd. (Industria West, Germiston, South Africa). Sprague-Dawley rats used in this study were obtained according to ethical clearance from the Central Animal Services of the University of the Witwatersrand (animal ethics number: 2015/08/32/B). All solvents used for UPLC-UV detection were of UPLC grade. Remaining reagents were of analytical grade and used as received.

Preparation and Optimization of the Dual pH/Redox Responsive NL Sludge

A 3-factor, 3-level Box-Behnken design was implemented for the optimization of the dual pH/redox (copper-glyglycine-prednisolone succinate [Cu(glygly)(PS)])—loaded NLs, with independent and dependent variables listed in Table 1. Box-Behnken experimental design studies were generated using Minitab®V15 statistical software (Minitab® Inc., State College, PA). Synthesis of the formulation was undertaken as described in our previous reported study.[9] The concentration of PC, cholesterol, DSPE, and sonication time was used to prepare each of the 27 formulations, which were selected from preformulation studies, as seen in Table 2. Sonication time was selected due to the impact on response variables, such as particle size and % drug entrapment, thus promoting optimization of the NL sludge system.

NL Sludge Sterilization and Freeze-Drying

The NL suspension (10 mg/mL) was diluted to a concentration of 10 mg/5 mL with distilled water and sterilized by filtration through sterile disposable syringe filters (0.20-μm Millipore filter) into receiving 5 mL glass vials. All glassware was sterilized by autoclaving and performed under laminar air flow. The NLs were then lyophilized in the presence of a cryoprotectant (1% sucrose), and samples thereafter were stored at −20°C. The gel was then sterilized under UV light overnight, and the sterility of the gel was validated by incubating the sludge in an agar plate at 37°C for 24 h.

Table 1
Box-Behnken Design Employing Independent and Dependent Variables

Variable	Low	Middle	High
Independent variables			
X1: PC	50	87.5	125
X2: cholesterol (CHOL)	0	37.5	75
X3: DSPE	0	25	50
X4: sonication time	5	17.5	30
Dependent variables			
Y1: Z-average size (nm) (minimize)			
Y2: PDI (minimize)			
Y3: zeta potential (Mv) (in range)			
Y4: %DL (maximize)			
Y5: %EE (maximize)			