Antidermatitic Perspective of Hydrocortisone as Chitosan Nanocarriers: An *Ex Vivo* and *In Vivo* Assessment Using an NC/Nga Mouse Model

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ABSTRACT: The aim of this study to administer hydrocortisone (HC) percutaneously in the form of polymeric nanoparticles (NPs) to alleviate its transcutaneous absorption, and to derive additional wound-healing benefits of chitosan. HC-loaded NPs had varied particle sizes, zeta potentials, and entrapment efficiencies, when drug-to-polymer mass ratios increased from 1:1 to 1:8. *Ex vivo* permeation analysis showed that the nanoparticulate formulation of HC significantly reduced corresponding flux [$\sim$24 μg/(cm^2 h)] and permeation coefficient ($\sim 4.8 \times 10^{-3}$ cm/h) of HC across the full thickness NC/Nga mouse skin. The nanoparticulate formulation also exhibited a higher epidermal (1610 $\pm$ 42 μg/g of skin) and dermal (910 $\pm$ 46 μg/g of skin) accumulation of HC than those associated with control groups. An *in vivo* assessment using an NC/Nga mouse model further revealed that mice treated with the nanoparticulate system efficiently controlled transepidermal water loss [15 $\pm$ 2 g/(m^2 h)], erythema intensity (232 $\pm$ 12), dermatitis index (mild), and thickness of skin (456 $\pm$ 27 μm). Taken together, histopathological examination predicted that the nanoparticulate system showed a proficient anti-inflammatory and antifibrotic activity against atopic dermatitic (AD) lesions. Our results strongly suggest that HC-loaded NPs have promising potential for topical/transdermal delivery of glucocorticoids in the treatment of AD. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1063–1075, 2013

Keywords: Corticosteroids; allergic contact dermatitis; percutaneous; biodegradable polymers; nanoparticles; *in vitro model*; *ex vivo* permeation; *in vivo* efficacy

INTRODUCTION

Glucocorticoids (GCs) are highly effective drugs that are widely used in dermatology for the management of various inflammatory complications. However, several local and systemic adverse effects often accompany their long-term use,[1,2] limiting the clinical significance, and excluding their applicability in chronic maintenance therapies. In recent times, hydrocortisone (HC), a mild-potency agent of the GC series, has been administered percutaneously to minimize the development of unwanted systemic effects. Compared with other high-potency GC compounds, HC possesses mild vasoconstrictive activity[3]; therefore, the incidence of adverse effects is significantly lower than that associated with the related anti-inflammatory compounds.[4] To further limit the adverse effects associated with GCs, special approaches have been attempted to mitigate the transcutaneous absorption of GCs and to increase therapeutic feasibility and patient compliance.

The development of successful topical/transdermal drug delivery systems has been limited[5] because of the significant penetration barrier provided by the stratum corneum (SC), the topmost skin layer. The SC is composed of a multilayered "brick- and mortar-" like structure, in which the bricks are composed of keratin-rich corneocytes, and the mortar is an intercellular matrix with a unique composition of lipids.[6] To overcome these penetration problems, various active and passive penetration-enhancing approaches, including chemical enhancers,[7] electroporation,[8] iontophoresis,[9] sonophoresis,[10] microneedles,[11] and laser ablation,[12] have been tested. However, each of these techniques has limitations in terms of toxicity to SC and therapeutic practicability.

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Nanotechnology is one of the more advanced and noninvasive techniques adopted for improving skin permeation of various drugs.[13] Among the various nanocarriers, micelles, liposomes, and polymeric nanoparticles (NPs) have been proposed to facilitate percutaneous delivery of therapeutic agents while mitigating damage to the skin barrier function of SC.[14,15] These colloidal nanocarrier systems could target GCs to the epidermis, where inflammatory reactions take place.[16] Among these colloidal nanocarriers, NPs have been reported to be more appropriate for topical/transdermal delivery because of their exceptional biopharmaceutical properties, such as high entrapment efficiency (EE), controlled release rates, and low enzymatic degradation.[17] Among the various biodegradable and biocompatible polymers used for preparing NPs, chitosan (CS) has generated enthusiasm because of its mucoadhesive and transepidermal penetrative properties, which are marked by regulation of intercellular tight junctions.[18]

Subsequent to optimization of colloidal characteristics (in terms of particle size, zeta potential, entrapment, and loading efficiencies) of unloaded and HC-loaded NPs, the study was aimed at mitigating the systemic absorption of HC and its accumulation in the epidermal and dermal layers. *Ex vivo* drug permeation across dermatomed NC/Nga mouse skin was investigated using Franz diffusion cells. To evaluate the *in vivo* clinical proficiency of the nanoparticulate system, transepidermal water loss (TEWL), erythema intensity, dermatitis index, and skin thickness were also assessed using the NC/Nga mouse model of atopic dermatitic (AD). To harmonize our results, histopathological examinations of NC/Nga mouse skin specimens were also performed by using hematoxylin–eosin and Masson's trichrome staining techniques.

MATERIALS AND METHODS

Materials

Eight-week-old NC/Nga mice were purchased from Experimental Animal Devision, RIKEN BioResource Center, Tsukuba Ibaraki, Japan. Isoflurane (inhalation anesthetic) was obtained from Pacific Pharmaceuticals (Lahore, Pakistan). CS (MW, 70 kDa; deacetylation degree, 85%), HC (base form), phosphate-buffered saline (PBS), hematoxylin–eosin, and Masson's trichrome stains were purchased from Sigma–Aldrich Chemicals Company Ltd. (Kuala Lumpur, Malaysia). Pentasodium tripolyphosphate (TPP) was sourced from Merck KGaA Company Ltd. (Dermstadt, Germany). High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and ethanol were obtained from Fisher Scientific Korea Ltd. (Seoul, Korea). QV cream (Ego Pharmaceuticals Pty. Ltd., Kuala Lumpur, Malaysia) was used as a vehicle base to formulate the nanoparticulate formulation.

Preparation of CS/TPP NPs

CS/TPP NPs were prepared via ionic gelation of CS with TPP.[19] CS solution (0.2%, w/v) was prepared in 1% (v/v) acetic acid at room temperature, and CS/TPP NPs were tested at various CS/TPP mass ratios (1:2 to 1:8) and different pH values of TPP solution (2, 3, 4, 5, 6, 7, and 8). CS/TPP NPs were spontaneously developed by adding 10 mL of TPP solution dropwise into 25 mL of CS solution with constant stirring at 700 rpm. Thereafter, CS/TPP NPs were centrifuged (72,000 × g) using Optima L-100 XP Ultracentrifuge (Beckman–Coulter, Brea, California, USA) with NV 70.1 Ti rotor (Beckman–Coulter) at 10°C for 30 min.

Preparation of HC-Loaded NPs

HC-loaded NPs were prepared by the incorporation method. CS solution (0.2%, w/v) was mixed and incubated with HC solution (1 mg/mL in a 30:70 ratio of ethanol and water solvent mixture) for 30 min at various drug-to-polymer mass ratios (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, and 1:8) before addition of TPP solution (0.1%, w/v). Thereafter, 10 mL of TPP solution was added dropwise to each reaction mixture with constant stirring (700 rpm). Finally, the HC-loaded NPs were harvested by centrifugation (72,000 x g) for 30 min and lyophilized at −40°C for 24 h.

Particle Size and Zeta Potential

To determine the mean particle size and zeta potential, the resulting pellets of unloaded and HC-loaded NPs obtained after centrifugation (72,000 × g) were resuspended in distilled water. Then, the mean particle size and zeta potential of NPs were measured using a ZS-90 Zetasizer® (Malvern Instruments, Worcestershire, UK) based on the photon correlation spectroscopy technique. All of the measurements were performed in triplicate at 25°C with a detection angle of 90°. Results were reported as mean ± standard deviation.

Entrapment Efficiency and Loading Capacity

To measure drug EE and loading capacity (LC) of HC, the standard corresponding calibration curve was made by analyzing various standard HC solutions (1–1000 μg/mL) by using reversed-phase HPLC (RP-HPLC) (Waters 600 controller, in-line degasser AF, 2707 Autosampler, and 2998 Photodiode Array Detector) with Waters Symmetry C_{18} column (250 × 4.5 mm; 5 μm) at 248 nm (λ_{max}). EE and LC of HC were calculated using Eqs. 1 and 2,