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Effect of Chemical and Physical Enhancers on the Skin Permeation of Cromolyn Sodium

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Effect of Chemical and Physical Enhancers on the Skin Permeation of Cromolyn Sodium

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ABSTRACT

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Cromolyn sodium (CS) has clinically shown to be an effective topical remedy for atopic dermatitis, however its physiochemical properties prevent efficient passive drug delivery beyond the outermost skin layer. This project aimed to optimize CS gel formulations and applications to improve drug delivery to the dermis of skin by examining various topical enhancement strategies. Oleic acid, salcaprozate sodium, and microneedles were investigated as enhancers for their effect on skin permeation of CS. *In vitro* permeation studies across dermatomed porcine ear skin tested CS gels to determine 24-hour drug permeation profiles and skin layer distribution of drug. Further, extraction method efficiency, the enhancement mechanism of salcaprozate sodium, and gel stability were investigated. It was concluded that microneedle pretreatment delivered the greatest amount of CS to the dermis using a 4% CS gel without chemical enhancement. These results provide a promising option for a commercially available topical treatment of atopic dermatitis.

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CHAPTER 1. INTRODUCTION

Atopic Dermatitis

Atopic dermatitis, also known as atopic eczema, is a chronic and inflammatory noncommunicable skin disease indicated by persistent erythema (redness) and pruritis (itching) accompanied by skin lesions. Atopic dermatitis presents variably in affected individuals based on age or race/ethnicity, making it difficult to make correct diagnoses. To compensate, diagnostic criteria for each country assess major and minor symptoms clinically observed in patients which include, but are not limited to, xeroderma (dry skin), morphology and location of skin lesions, patient history, serum levels of immunoglobulin E, and propensity toward skin infections.[1]

Although difficult to accurately diagnose, it is the most common skin disease to affect children and, of those diagnosed with atopic dermatitis in the United States, 60% experience symptoms before the age of 1 and 90% experience symptoms by the age of 5.[2] Nearly 15-20% of children worldwide suffer from atopic dermatitis, and on average 10-30% of children diagnosed with the disease experience persistence of symptoms into adulthood, with disease severity playing a contributing role.[2–4] Continued development of skin lesions, skin infections, and itching has been shown to severely deprive children of sleep, correlating to greater probability of developing attention-deficit/hyperactivity disorder and decreased overall health.[2] Furthermore, adolescents and adults with atopic dermatitis experience embarrassment and decreased self-esteem, negatively affecting their social lives and increasing comorbidity for anxiety and depression.[2,5] Among the physical and mental detriments, many patients and their family members struggle with the financial weight associated with atopic dermatitis. The cost of

hospital care, prescription and over-the-counter medications, and emergency visits place a heavy strain on patients and families, while indirect costs such as decreased productivity and missed work or school due to flare-ups or primary care of a loved one can also impact financial stability.[2,6] Additionally, a 2018 publication found that in the United States, a higher likelihood of atopic dermatitis was associated with income levels less than $50,000,[5] contributing to the financial burden experienced by patients and families.

Pathogenesis of atopic dermatitis has yet to be confidently established, however it is believed to be impacted by both genetic and environmental factors. Two schools of thought persist on the cause of atopic dermatitis: either immunological dysfunction creates epidermal inflammation that compromises the skin barrier, allowing entry of irritants and pathogens, or inherent epithelial cell dysfunction disrupts the skin barrier and promotes immunological reactivity to external stimuli and allergens.[1,2,4,7,8] Regardless of etiology, the focus is on ameliorating the skin barrier defect issue, as a recent genetic study found mutations in the filaggrin gene (FLG) impair epidermal integrity and are present in a significant portion of atopic dermatitis patients, although not widespread.[1,2,7,8] To do so, hydration of the skin is an important part of treatment for patients with atopic dermatitis, along with avoidance of potential irritants or triggers and maintenance of proper hygiene to prevent frequent skin infections.[1]

Since scratching irritated skin can further exacerbate the disease, it is imperative to target inflammation and itching, where topical corticosteroids remains the primary modality of treatment.[1] Although widely used, there are adverse side effects associated with long-term use of topical steroids. The most common local side effect of steroid application is skin atrophy, characterized by epidermal thinning as cell layers degenerate, and loss of connective tissue and structural integrity of the dermis. Other unwanted local effects include hypopigmentation,

delayed wound healing, premature aging, and aggravation of skin infections. Pediatric populations are also at higher risk for developing systemic side effects, such as suppressed adrenal function, due to their softer, more permeable skin and body surface area to weight ratios.[9]

Topical steroids in conjunction with other biologics, such as monoclonal antibodies, show effectiveness in treating skin related diseases like atopic dermatitis, however at substantial costs for consumers. Newer and more expensive biologic drugs drive up the cost of generic dermatologic drugs,[10,11] and the February 2019 KFF Health Tracking Poll estimated that 1 in 4 adults struggle to pay for prescription medications.[12] In addition, out-of-pocket treatment costs for those with eczematous diseases are markedly higher than those for other diseases, such as diabetes mellitus or hypertension.[6] Taken together, it appears more cost considerate medications that are safer than current topical therapies are needed for the treatment of atopic dermatitis.

Skin Characteristics

Skin is the largest human organ and functions to prevent water and heat loss, protect against external physical or chemical damages, and is the body's primary barrier to pathogen and allergen entry.[8,13–16] While this defensive property is integral to bodily homeostasis and external protection, it makes it challenging to utilize skin as a mode of drug delivery. A detailed structure of skin and its components can be observed in Figure 1.1. These different skin layers have varying physicochemical properties, and by modifying topical or transdermal formulations around those properties, molecules can successfully penetrate the barrier for skin targeted delivery or systemic circulation.

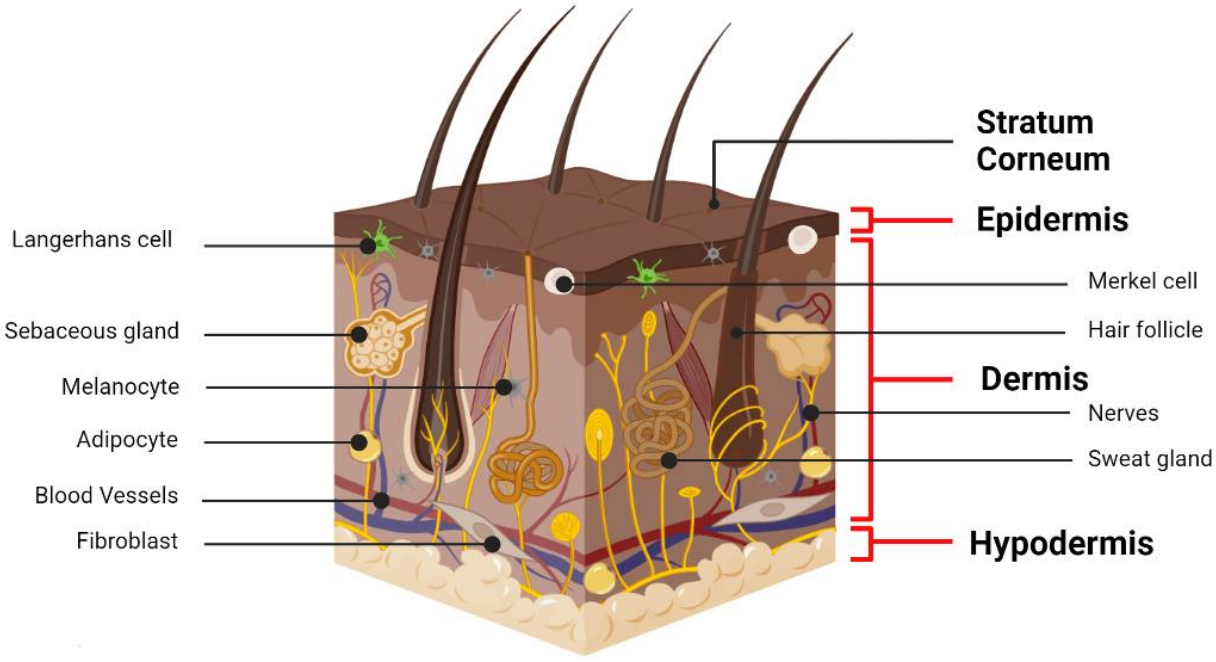

Figure 1.1. Detailed diagram of human skin structure and its various discussed constituents designed using BioRender

The outermost layer of skin, the stratum corneum, is made up of 15-30 layers of corneocytes that renew and migrate to the surface every 4 weeks.[16] These corneocytes are dead and terminally differentiated keratinocytes that have lost their nucleus and other cellular organs, resulting in rigidity and minimal hydration. In fact, the stratum corneum composition is 70-80% keratin and lipids and 15-20% water, whereas most body tissues contain approximately 70% water.[14,15] Desmosomes, or adhesive protein complexes, and lipid lamellae made of ceramides, fatty acids, and triglycerides contribute to the cohesion of corneocytes and the increased lipid content of the stratum corneum.[14–16] The stratum corneum composition is not homogenous throughout the entire layer, and it has been found that the primary lipid barrier and water loss function is localized to the lower stratum corneum.[17] This combined with overall low hydration

results in the most superficial layer of skin being highly lipophilic, and the "brick-and-mortar" organization of corneocytes bars entry of large molecules.[14–17]

Beneath the stratum corneum are 4 more layers that comprise the viable epidermis.[15,17] These layers of epidermis have no vascularization, and instead rely on diffusion of nutrients from the circulating blood of the dermis, which also contributes to epidermal waste removal.[13,15] While "viable" is then a questionable designation, the remaining 4 layers of epidermis contain keratinocytes, melanocytes, sensory Merkel cells, and Langerhans cells, or the dendritic cells of the skin.[13,15,16] In the most basement layer, the stratum basale, stem cells divide and differentiate into keratinocytes, where gradual cornification and loss of organelles during migration to the surface results in the dead corneocyte layer of the stratum corneum.[15,16] The stratum basale also has direct contact with the next deepest skin layer, the dermis, through collagen fibers.[16]

The dermis is vascularized and thus a good target for systemic delivery of drugs via skin. This system of blood vessels enables the dermis to regulate thermal homeostasis, oxygen and nutrient diffusion, and the removal of toxins from surrounding tissues.[15,18] There are also lymph vessels, nerves, adipocytes, melanocytes, and fibroblasts that make collagen, the primary component of the dermis.[13,15,16] Collagen allows for elasticity and flexibility in movement, while mast cells, which also reside in the dermal layer, provide the primary inflammatory and immune response of the skin alongside other phagocytes and leukocytes.[14,15] The presence of triacylglycerols and phospholipids are observed uniformly throughout the dermis, while the deeper portions may contain lingering adipocytes originating from the subcutaneous tissue.[19] The 3 main appendages of the skin also originate from the dermis: hair follicles, sebaceous glands, and sweat glands.[15,16] Sebaceous glands help keep skin lubricated and at sufficient pH, while sweat glands help modulate body temperature in response to stress or exercise, with both

excretions having antimicrobial properties.[13,15] A subset of sweat glands, the apocrine glands, are responsible for "milk" production in few areas of skin.[15,18] These appendages and overall dermal structure afford channels that make the dermis more hydrophilic and susceptible to drug permeation, though it can limit penetration of more lipophilic drugs.[14,18]

The final and deepest layer of skin is the subcutaneous tissue, or the hypodermis. The hypodermis contains the entry routes for blood and lymph vessels and nerves to the dermis, and is primarily composed of adipose tissue.[13,15,17] These fat lobules are connected with a network of collagen and elastin fibers and function to store energy, insulate the body, and protect it from external shock.[15–18]

Cromolyn Sodium

Cromolyn sodium, or disodium;5-[3-(2-carboxylato-4-oxochromen-5-yl)oxy-2-hydroxypropoxy]-4-oxochromene-2-carboxylate, is the salt form of the drug cromolyn which was derived from the plant chromone khellin.[20] Also known as disodium cromoglycate, sodium cromoglicate, cromoglycic acid, or more commercially as Nasalcrom or Opticrom, cromolyn sodium has been used over the past 50 years to orally or nasally treat a variety of allergic diseases such as allergic rhinitis, inflammatory bowel disease, bronchial asthma, allergic conjunctivitis, food allergy, and systemic mastocytosis.[21–28]

Mechanism

While the precise mechanism of action is still unclear, studies have shown cromolyn sodium operates diversely for immune response in allergy related diseases and has seen success inhibiting both early and late phase allergy responses. Primarily, research has suggested in early response cromolyn sodium acts to stabilize mast cells, preventing degranulation and subsequent

release of pro-inflammatory mediators like histamine.[22–25,29,30] One explanation could be the ability of cromolyn sodium to suppress calcium currents, suggesting that it blocks calcium channels on the surface of mast cells to prevent degranulation.[26,28,30,31] Additionally, cromolyn sodium does not appear to interfere with mast cell sensitization to antibodies or the antigen-mast cell interaction, indicating it mediates mast cell response post-antigen exposure.[22,29] In terms of late phase allergy, use of cromolyn sodium has shown to inhibit degranulation, activation, and chemotaxis of other granulocytes like neutrophils and eosinophils.[23,27,28]

Cromolyn sodium has also proven to inhibit tachykinin-mediated airway leakage in asthmatic diseases, suggesting that it could also act on nerve endings.[32] *In vivo* investigations have actually suggested cromolyn sodium fails to inhibit histamine release from mast cells in human skin-test models.[33] A study designed to assess the topical effects of cromolyn sodium found no reduction in wheal formation after histamine injection, suggesting cromolyn sodium does not prevent mast cell degranulation, as these mediators facilitate the occurrence of wheals in allergic skin reactions.[34] Furthermore, monitoring of local and systemic circulatory effects of injected cromolyn sodium provide evidence that the drug stimulates human nerves directly, as warm, burning sensations of the face and chest were reported without correlating increases in skin temperature or blood flow.[35] These studies suggest that cromolyn sodium likely acts on sensory nerves rather than mast cells.

Other theories for the exact molecular mechanism exist, such as G-coupled protein receptor 35 signaling, stimulating mast cell secretion of anti-inflammatory mediators like Annexin-A1,[24] and inhibition of neutrophil NADPH oxidase assembly,[23] however no conclusive evidence has indicated one singular mechanism is responsible for cromolyn sodium's anti-inflammatory and anti-histamine effects.

Clinical Use in Atopic Dermatitis

While cromolyn sodium has previously been used for treatment of respiratory, ocular, and food-related allergic diseases, its anti-inflammatory properties have made it a valuable option for the treatment of atopic dermatitis. Topical use of cromolyn sodium for atopic dermatitis first gained attention in the 1970s, with a clinical trial designed to assess a 10% cromolyn sodium paraffin ointment in the clinical presentation of children diagnosed with atopic eczema, which significantly reduced disease severity when compared to placebo.[36]

Later, a clinical study was conducted utilizing a nebulized 1% cromolyn sodium solution on children younger than 3 years old and found improved sleep and itching scores in patients having moderate to severe atopic dermatitis.[37] Another study evaluating a cromolyn sodium oil-in-water cream on atopic dermatitis found no significant difference in active drug usage or placebo, suggesting such a vehicle was not ideal for therapeutic treatment.[38] Focus continued on establishing ideal cromolyn sodium content in formulations for atopic dermatitis treatment, where studies employing a 4% cromolyn sodium cream[39] and a 0.21% cromolyn sodium cream[40] saw successes in improving disease symptoms.

In 2004, efficacy of the Altoderm 4% cromolyn sodium aqueous cream was investigated on children with atopic dermatitis and showed to significantly improve skin condition and itching in patients with active treatment.[41] Furthermore, a sensory study on skin condition following intradermal histamine injection found that 2% and 4% cromolyn sodium emulsions, or liquid-in-liquid suspensions, significantly inhibited itching and reduced area of erythematous skin in atopic and non-atopic individuals.[34]

Reemergence of interest in topical cromolyn sodium treatment next occurred in mid-2010, with the following clinical studies all utilizing 4% cromolyn sodium emulsions. A meta-analysis of three clinical trials testing the same 4% cromolyn sodium emulsion established significant improvements in SCORAD scores, a clinical tool used to assess individual disease severity and symptoms.[42] In one such study, active treatment applied over 12 weeks showed improved skin condition and reduced topical steroid use in children 2 to 12 years old.[43] Another study focused on children aged 1 to 12 found treatment with the emulsion to be well-tolerated over 64 weeks of use and proposed trends for improved quality of life and self-reported skin condition.[44]

Despite the noted successes in using 4% cromolyn sodium formulations for the treatment of atopic dermatitis, no gel formulation has been investigated to date. Additionally, no studies investigating formulation optimization have been published.

Properties for Skin Permeation

The molecules that function best to cross the stratum corneum and penetrate skin are small (≤ 500 Da) and have partition coefficient values (log *P*) approximately between 2 to 3, which suggests great enough lipophilicity to bypass the stratum corneum without failing to permeate the more hydrophilic layers. Ideal molecules also have low ionizability, as unionized compounds tend to have higher potential to permeate through skin. While all the aforementioned factors affect a drug's ability to cross the stratum corneum, ionizability and partition coefficient are the primary determinants.[15] Following these indicators, cromolyn sodium is considered a large molecule with a molecular weight of 512.3 Da, and highly ionizable with a pKa of 1.9.[45] Additionally, the partition coefficient for cromolyn sodium falls around -5 to -4.8 at pH 7.4, indicating cromolyn sodium's hydrophilicity and main obstacle for skin permeation.[46] With

cromolyn sodium's hydrophilicity, high ionizability, and large molecular weight, passive delivery of the drug through the lipophilic stratum corneum and into the deeper skin layers poses a challenge. In order to overcome the physiochemical properties of cromolyn sodium, various types of enhancements can be used to increase the drug's ability to partition into skin.

Transdermal Drug Enhancement

Topical and transdermal applications for drug delivery are designed to penetrate into the deeper skin layers and allow for systemic circulation, respectively.[15,47] Skin-based therapeutics offer many advantages, such as sustained drug delivery and circulation while avoiding first-pass metabolism. Administration through skin also allows for local and systemic treatments that are non-invasive, likely to be more patient compliant, and easier for use in pediatric and geriatric populations.[47–50] Due to the skin's barrier-like and highly protective qualities, however, not all drugs are ideal candidates for either system of delivery. In response, various penetration enhancers have been developed to increase cutaneous absorption for drug delivery.[15,16,47–50]

Chemical Enhancers

One category of skin penetration enhancers are chemical enhancers, which are chemical additives incorporated into drug formulations. Chemical enhancers are generally considered to be a passive method of enhancement, as they can be used to modify stratum corneum function or influence drug and/or vehicle properties in formulation.[14,16,47,49] These enhancers can temporarily alter the composition or organization of the stratum corneum by disordering the lipid bilayer and increasing its fluidity, interacting with skin proteins, or increasing its overall hydration, making the stratum corneum more permeable to drug penetration.[48,51,52] They can also act on the target drug by increasing its solubility in skin or thermodynamic activity, allowing

for easier partitioning into skin.[15,16,48,49] Chemical enhancers can be classified into different groups based on chemical structure, as most tend to use multiple mechanisms to increase skin penetration.[48]

Oleic acid. Oleic acid is a free fatty acid that occurs commonly in nature and is even found in human skin.[52] This fatty acid has a long, unsaturated hydrocarbon tail[52,53] and has frequently been shown *in vitro* to improve skin permeation of both large and small molecules,[52–57] with its structure classifying it as a lipophilic enhancer. Oleic acid has shown to interfere with the stratum corneum in various ways by incorporating into the solid lipid matrix in a liquid phase and disordering stratum corneum lipids.[52,54,56,57] Other studies have demonstrated oleic acid incorporation increases stretching of lipid bonds[55,56] and decreases lipid transition temperatures,[54,55] suggesting oleic acid fluidizes and phase-separates the stratum corneum barrier to reduce its resistance to penetrating drugs.[56] This enhancement activity has also been observed to work best with hydrophilic molecules, as indicated by higher enhancement ratios when compared to its administration with lipophilic molecules.[52]

Salcaprozate sodium. Another enhancer, salcaprozate sodium, has chiefly been used to increase oral or intestinal absorption of target drugs. A synthetic derivative of salicylic acid, salcaprozate sodium is amphiphilic, but relies primarily on its hydrophilic surface area for permeation into lipid membranes,[58] implying its use as a hydrophilic enhancer. It has been investigated over the past 30 years for its effect on intestinal permeation, and has even been employed in clinical trials for increasing oral delivery of large molecules.[58] Numerous *ex vivo* and *in vitro* studies have proven salcaprozate sodium functions to increase intestinal permeation of charged and uncharged molecules beyond mucosal membranes of the stomach, intestines, and colon from rats and humans,[59,60] as well as across Caco-2 human colorectal cells.[61,62] Li *et al.*

noted that these effects seem to be more prominent with charged drugs.[62] Though the mechanism of action behind salcaprozate sodium's enhancement is unknown, several theories persist around membrane fluidization or perturbation, molecular interactions that increase the drug's lipophilicity or alter its solubility, or tight junction opening formation.[58] Studies have investigated the permeation effects of salcaprozate sodium on oral absorption of cromolyn sodium specifically, and have found no decisive evidence on its mechanism, though claims are placed in either non-covalent complexation that increases cromolyn sodium's lipophilicity[63] or disruption of the mucosal membrane.[64]

Physical Enhancers

A more active method of enhancing skin permeation involves physical enhancers, or methods that employ energy or mechanical disruption of the stratum corneum for drug diffusion.[16,48,49] These enhancers can operate either indirectly or directly. Indirect physical enhancers apply electrical current or an energy source through the skin to drive drug partitioning, such as iontophoresis, sonophoresis, magnetophoresis, and electroporation. Direct physical enhancers form micropores, or channels, in skin or reduce stratum corneum thickness to allow drug to permeate deeper layers, such as micro needling, thermal ablation, and microdermabrasion.[15,16,47–49] Physical enhancers are useful for more immediate and reproducible delivery of drug through skin or systemic circulation and increase permeation for a larger range of drugs compared to chemical enhancers.[48,49]

Microneedles. As a subset of direct physical enhancers, the use of microneedles has been shown to be an effective and minimally invasive method for enhancing the skin-specific or circulatory drug delivery of small and large hydrophilic molecules. Microneedle arrays pierce the stratum corneum without penetrating the deeper dermis, allowing for painless and more

patient compliant administration when compared to hypodermic or subcutaneous injections.[15,16,47–49,65,66] Commonly, microneedle arrays line transdermal patches and can be applied to skin with thumb pressure or applicators, with success of application consistent between consumer and professional usage.[47] Although depth, width, shape, material, and type of microneedles vary, microneedles should be strong enough to overcome the skin's elasticity without breaking and structured so as to avoid contact with nerves and blood vessels.[16,48,66] One such type of microneedle fabrication, dissolving microneedles that dissolve drug directly into the skin, have already proven successful in treating skin lesions in mice models of atopic dermatitis using poly-γ-glutamate microneedles with[67] or without epigallocatechin gallate.[68] Other dissolving microneedles incorporating high dose topical steroids have also shown to relieve atopic dermatitis symptoms in mice.[69]

Another type of microneedle fabrication is solid microneedles, which are primarily used as a skin pretreatment to create conduits designed to allow drug into skin. Once removed from skin, appropriate drug formulations or patches can be applied on top of the newly created channels for drug delivery.[47–49,65,66] Dermapens® have become widely available on the market, and are automated, hand-held machines that can microporate skin at various sustained intensities and depths.[70] These commercially available instruments have been used largely in cosmetics[70] and have shown success at mimicking solid microneedle pretreatment of skin in recent *in vitro* permeation studies for delivery of niacinamide,[71] naloxone hydrochloride,[72] and ketamine.[73]

Goals and Hypothesis

With the long-term intention of creating a commercially available treatment for atopic dermatitis that is more cost-effective and safer than those currently used, our objective was to investigate the formulation of semisolid gels for cromolyn sodium using clinically tested doses

and optimize those gels for maximum drug delivery. Chemical enhancers oleic acid and salcaprozate sodium were tested along with microneedle pen pretreatment as a physical enhancer to evaluate their potential to increase drug delivery of cromolyn sodium. On the basis of its proposed mechanisms of action, cromolyn sodium delivery was targeted to the dermis for direct access with mast cells and nerve endings of the skin, where enhanced drug delivery would be indicated by marked increases in drug deposited into the dermis. With its novel use in skin permeation studies, the enhancement capabilities of salcaprozate sodium were also investigated to potentially clarify its mechanism of action with cromolyn sodium. To further investigate the feasibility of a cromolyn sodium gel to be sold commercially, the drug content and stability of the gels were monitored over 30 days. We hypothesized that all forms of enhancers would increase cutaneous absorption of cromolyn sodium, likely seeing the greatest improvement in drug delivery to the dermis with microneedle pretreatment.

CHAPTER 2. MANUSCRIPT

Journal of Pharmaceutical Sciences

Evaluation of Chemical and Physical Enhancers on the Skin Permeation of Cromolyn Sodium

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Abstract

Cromolyn sodium (CS), a suspected mast cell stabilizer, has reduced atopic dermatitis disease severity when used topically. Despite this, the physical properties of CS make passive drug delivery through skin challenging. The present study aimed to optimize CS gel formulations utilizing different chemical or physical enhancers in order to provide the maximum therapeutic effect targeted to the dermis. *In vitro* permeation studies were performed using dermatomed porcine ear skin mounted between vertical Franz diffusion cells with subsequent skin separation and drug extraction. Effects of salcaprozate sodium (SNAC), oleic acid, and microneedles as enhancers were investigated. A reverse permeation study was performed to explore the mechanistic action of SNAC and all skin extraction data were corrected using experimental recovery values. Gels incorporating oleic acid failed to enhance drug delivery to the dermis, while a 2.5% SNAC gel improved drug delivery compared to the control. Microneedle application improved overall drug delivery, although no synergistic effect was observed when combined with SNAC treatment. Findings of the reverse permeation study suggest further investigation on the action of SNAC enhancement. The results indicate microneedle treatment with a 4% CS control gel may be the most effective for delivery of CS to the dermis.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory disorder characterized by red, itchy skin. Since the beginning of industrialization, AD has become the most prevalent disease affecting children: it's estimated that 15-20% of children worldwide suffer from atopic dermatitis, with incidence in the United States being 10.7% for children.[1] Although it can develop in adulthood, AD most often presents before adolescence, and 10-30% of children with AD never have resolution of symptoms, suffering later into their adult years. Current treatments for AD include the use of topical steroids, which have shown adverse side effects such as decreased immunity and skin atrophy when used long term,[2] as well as biologics that are costly to the average patient.[3]

Cromolyn sodium, disodium 5- [3-(2-carboxylato-4-oxochromen-5-yl)oxy-2-hydroxypropoxy]-4-oxochromene-2-carboxylate (CS), is an anti-inflammatory drug that has been used as a therapeutic agent for various allergic diseases since the 1970's, presenting as a possible treatment for AD.[1,2,4-6] Clinical studies have already been performed testing whether topical application of CS could work to lessen symptoms and disease severity, and have shown success in limiting pruritus and sleep loss in individuals with AD.[7-11] CS likely acts as a mast cell stabilizer, preventing release of pro-inflammatory mediators,[12,13] or targets cutaneous sensory nerves directly to reduce itching.[14,15] Despite this, no commercial topical formulation has been made and is still inaccessible to the public. Although CS has shown success in limited treatment, this study aims to develop an effective topical gel formulation to provide individuals with AD a less expensive and safer alternative to their current therapeutics. A skin-based delivery method would also sustain its therapeutic effect by targeting the dermal layer of the skin, providing direct contact with skin mast cells, nerves, and blood vessels, allowing for targeted treatment and systemic circulation of CS.[11,16]

Owing to the physical properties of CS as a large (MW = 512.133 Da) and hydrophilic (logD = -3 to -5) drug, passive permeation through skin is expected to be low.[17] To combat this issue for topical and transdermal drug delivery, chemical enhancers have been used as formulation additives to increase drug permeation, along with physical enhancers designed to manually disrupt the stratum corneum.[18,19] Oleic acid (OA) is a well-known and widely used chemical enhancer in skin formulations due to its ability to increase permeation of target drugs by fluidizing the lipid membrane.[20-23] The mechanisms of OA interaction with skin lipids have shown that OA enhancement is primarily caused by phase separation of the stratum corneum, where OA remains in a fluid state as it partitions into the solid lipid matrix of skin, allowing administered drugs to bypass the stratum corneum.[20,24] OA has also successfully enhanced cutaneous penetration of molecules like caffeine,[25] tretinoin and isotrentinoin,[26] and heparin sodium[27] using *in vitro* permeation studies across human or rat skin. Salcaprozate sodium (SNAC) is another chemical enhancer that has shown success in increasing oral absorption of molecules such as heparin or vitamin B12 in recent clinical trials.[28-30] Through investigation, the enhancement strategy of SNAC could act directly on the intestinal barrier through membrane perturbation, fluidization, or tight junction openings, or it might interact with target drugs as a carrier or by altering a drug's physiochemical properties.[28] While its exact mechanism of action is still a topic of controversy, the use of SNAC in skin permeation enhancement has yet to be tested. Microneedles (MN) are an emerging treatment option for the delivery of micro- and macromolecules by minimally piercing the stratum corneum and creating channels for drug delivery into the deeper skin layers.[19,31-34] A particular type of MN, solid MNs, are intended to be applied to skin prior to topical formulation use. In this way, microchannels are already formed in the skin and can facilitate drug penetration after application of skin therapeutics. Automated MN instruments have been used to pretreat skin

similar to solid MN application for *in vitro* permeation studies. These MN pretreatment techniques have successfully shown increased permeation of niacinamide[35] and naloxone hydrochloride[36] through porcine ear skin.

OA, SNAC, and use of MNs were investigated as potential modes of enhancement to increase delivery of CS to the dermal layer in order to optimize the drug's therapeutic effect and sustain it over time. Permeation studies utilizing dermatomed porcine ear skin were run over 24 h to determine how each enhancer would impact CS permeation profiles. Following 24 h permeation, skin layers were separated, and drug extracted was analyzed using efficiency corrected values to assess amount of drug deposited into the dermis. Gel formulation stability was also evaluated 30 days following application for each gel, and a reverse permeation study was performed to investigate the mechanism of action involved in SNAC skin permeation enhancement.

Materials and Methods

Materials

CS, 0.2 M potassium phosphate monobasic (KH_2PO_4), 85% o-phosphoric acid, phosphate buffered saline, pH 7.4 (PBS 10X), were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Analytical grade methanol was purchased from Concord Technology (Beichen, Tianjin, China). Deionized (DI) water was acquired from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Oleic acid was purchased from Croda International (Snaith, UK) and sodium laureth sulfate used is from MedChemExpress (Monmouth Junction, NJ, USA). All SNAC was also gifted and manufactured by Quality Horizons International, L.L.C. (Fairfield, NJ, USA).

Instrumentation and HPLC Analysis

All HPLC samples were run on a Waters 2695 Separations Module including a column heater with a Waters 2996 Photodiode Array detector (Milford, MA, USA). The HPLC column used was a Kinetex® 5 µm, 100 Å, 250 X 4.6 mm C18 from Phenomenex (Torrance, CA, USA). The column was maintained at 45°C with mobile phase (KH_2PO_4, adjusted to pH 4 using phosphoric acid: methanol = 75:25) set at a flow rate of 1 mL/min, and UV detection of CS at 236 nm. The limits of detection and quantitation were established at 0.02 and 0.05 µg/mL, respectively. Intra- and inter-day accuracy and precision of the method were also within acceptable limits as outlined by the International Conference on Harmonisation Guidelines.[37]

Preparation of Gel Formulations

Table 2.1 shows the compositions of the gel formulations. CS solutions (4%) were prepared by dissolving the appropriate amount of CS in propylene glycol (PG) (w/w). The corresponding amounts of SNAC were then added to create 2.5%, 4.5% and 9% SNAC test gels, corresponding to 1:1, 2:1, and 4:1 molar ratios of SNAC to CS. A control solution was prepared that dissolved only CS in PG. These solutions were left to shake overnight at 200 rpm (Orbi Shaker TM Jr. Benchmark, Edison, NJ, USA). Hydroxypropyl cellulose was added as a gelling agent to all solutions the next day which were again left to shake overnight at 150 rpm. All gels were sonicated for 30 min prior to application. In the microneedle evaluation studies, only control and 2.5% SNAC gels were used. Following solubility determination, OA drug solutions were prepared by dissolving 1% CS in an OA:PG solution (5:95, v/v) in appropriate amounts. A second 1% CS control solution was made, and preparation followed the above SNAC gel procedure.

Table 2.1
Percent Composition of Gel Formulations.

	Gel Formulations (w/w)					
Components	4% Control	2.5% SNAC	4.5% SNAC	9% SNAC	1% Control	1% CS OA
CS	4	4	4	4	1	1
SNAC	-	2.5	4.5	9	-	-
PG	95	92.5	90.5	86	98	-
OA:PG (5:95, v/v)	-	-	-	-	-	98
HPC	1	1	1	1	1	1

Numbers shown are percentages. w/w, weight to weight; CS, cromolyn sodium; SNAC, salcaprozate sodium; PG, propylene glycol; OA, oleic acid; v/v, volume to volume; HPC, hydroxypropyl cellulose.

Preparation of Skin Samples for Permeation Studies

Porcine ears were obtained from Animal Technologies (Tyler, TX, USA) and damage-free skin was excised for use. Hairs were trimmed and skin was dermatomed using a Nouvag AG 75 mm dermatome (Goldach, Switzerland) to remove any remaining subcutaneous tissue and the lower portion of the dermis. The skin was then cut into suitable sized pieces and stored in a freezer at -20°C. When ready for use, skin pieces were thawed at room temperature, blotted dry, and average thickness was measured by using a thickness gauge (Cedarhurst, NY, USA).

Skin Resistance Assessment

Vertical Franz diffusion cell receptor compartments were filled with 5 mL 1x PBS, pH 7.4, and skin pieces were mounted with the epidermis facing upward. Once mounted, donor compartments were clamped on top of the skin and 300 µL of PBS were added to the donor compartment. Skin resistance was evaluated by running a constant current through skin using Agilent 34410A 6 ½ Digit Digital Multimeter and 33220A 20 MHz function/arbitrary waveform

generator (Agilent Technologies, CA, USA). Silver chloride electrodes were placed in the receptor, and silver electrodes were submerged in the donor to measure the voltage drop across the skin. Skin resistance was calculated following a previously established formula.[31,38] Skin pieces with resistance less than 10 kΩ were not included in the study, and pieces were chosen so resistances between groups and experiments were comparable.[31] Following measurement, PBS in the donor compartment was removed in preparation for gel application.

In Vitro Permeation Studies

SNAC and OA Enhancement

In vitro permeation of 4% CS control, 2.5% SNAC, 4.5% SNAC, and 9% SNAC (n = 3-5) gels were evaluated using static vertical Franz diffusion cells with a diffusion area of 0.64 cm^2. Cells were washed with ethanol and water prior to use and filled with PBS to maintain sink conditions. Skin temperature was kept at 32°C using a circulating water bath for the duration of the study.[30,38] Following skin mounting, resistance measurement, and temperature equilibration, 100 µL of each 4% CS gel formulation was applied to the donor compartment of respective cells. Receptor solution was mixed and sampled (300 µL) at predetermined timepoints over 24 h. Following each sampling, receptor solution was replaced with 300 µL of PBS, and each sample was filtered through 0.22 µm nylon membrane syringe filters (New Oxford, PA, USA). Filtered samples were analyzed using HPLC. Similar permeation studies were performed using the 1% CS control gel (n = 3) and 1% CS OA gel (n = 5) formulations.

Microneedle Enhancement

In vitro permeation of 4% CS gel formulations with and without MN pretreatment was evaluated following the same protocol used for the previous enhancement studies. MN application was achieved by using the Dr. Pen Ultima A6 microneedling pen (Dr. Pen Inc., San Jose, CA, USA) on dermatomed porcine ear skin following resistance measuring at a depth of 500 µm for 2 min at 13000 ins/min. Based on observations and results from the SNAC enhancement study, 2.5% SNAC gel (n = 5) was used in this study alongside the control gel (n = 4) to investigate potential synergistic effects.

Reverse Permeation

Franz cells were prepared similarly as in previous studies, though skin was mounted inversely with the dermis facing upwards, and resistance measurements were taken as described above. After removal of PBS from the donor compartments, either a control gel (n=3) or 2.5% SNAC gel (n=3) was applied to the empty donors, and the permeation study continued for 24 h using the same protocol.

Extraction of CS from Skin

Following each 24 h permeation study, excess gel was removed using Kimwipes and skin was cleaned thoroughly with cotton swabs soaked in sodium laureth sulfate (10%) twice and water twice. Skin was then dried with a single dry cotton swab, and skin layers were manually separated using forceps and minced into individual 20 mL vials. Methanol (2 mL) was added to each vial as an extraction solvent, and vials were kept on the shaker at 150 rpm for 4 h. From each vial, 1

mL was filtered, and 100 µL of filtered solution was held under vacuum to dry overnight.[39] Samples were reconstituted with PBS and analyzed using HPLC.

Extraction Efficiency Study

Seeing as absolute extraction of drug permeated into skin is not entirely feasible due to complex skin component and drug interactions,[39-42] the aim of this study was to determine an efficiency factor to be applied to the skin extraction data to more accurately assess amounts of CS permeated into the skin layers. Frozen dermatomed porcine ear skin was thawed in fresh PBS at room temperature for 2 h. Following previous studies, epidermis was separated from the dermis using forceps, and both skin layers were cut into small pieces.[31] CS standard solutions were prepared in methanol at concentrations 100, 200, 500, 1000, and 2000 µg/mL. Minced pieces of each layer of skin (50 mg) were placed into centrifuge vials and 50 µL of respective standard solutions were applied (n=3). All vials were centrifuged at room temperature for 15 min to ensure full contact of the skin with the standard and then left to incubate overnight at 37°C. After incubation, 500 µL methanol was added to each vial and vortexed for 30 seconds. Supernatants (400 µL) were removed and vacuum dried overnight to be reconstituted with PBS and filtered. Samples were then analyzed using HPLC to determine amount of drug absorbed by the different skin layers. Following supernatant removal, previous extraction protocol described was performed on the remaining skin pieces to determine how much drug could be extracted from the skin using this method.

Gel Stability Studies

Following the initial use and application of the control and 2.5% SNAC gels in permeation studies, gels were diluted in PBS and analyzed using HPLC in triplicate to confirm drug content. Remaining gel was split and stored separately at ambient room temperature and at 4°C to be analyzed after 30 days (n = 3) for drug content reassessment and determination of gel stability.

Statistical Analysis

Data for all studies were tested for statistical significance ($p \leq 0.05$) using Student's t test with Welch's correction or one sample t test against zero where appropriate using GraphPad Prism version 9.3.1 and Microsoft Excel. For SNAC and OA studies, each test gel was evaluated against its respective control, and for the MN study, control gel with or without MN pretreatment and 2.5% SNAC gel with or without MN pretreatment were compared separately. In the extraction efficiency study, linear trendlines were fitted to the data and R^2 values were reported as a goodness-of-fit measure. All data are reported as means with standard error (SE).

Results

Extraction Efficiency

Drug extraction protocols might not accurately assess how much drug permeated the different skin layers. Therefore, the amount of drug recovered was plotted against the amount of drug actually absorbed by skin for both the epidermal and dermal layers to establish extraction recovery equations (Fig. 2.1). These equations were used to correct all epidermis and dermis CS extraction data to more accurately evaluate the amount of drug permeated into the separate skin layers.

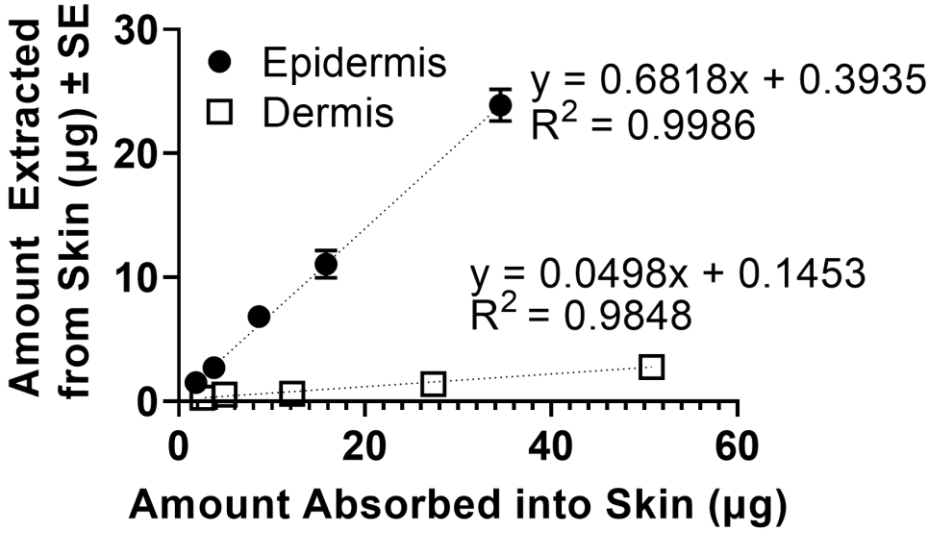

Fig. 2.1. Recovery of cromolyn sodium (CS) from epidermis and dermis (n = 3).

Effect of SNAC on Skin Permeation of CS

The cutaneous permeation of CS through dermatomed porcine ear skin was evaluated using SNAC enhancement over 24 h and is shown in Figure 2.2. All groups experienced increases in amount of drug delivered to the receptor by 24 h (Fig. 2.2A). Receptor solution for the control (5.02 ± 1.53 µg/cm^2) was not significantly different from the receptor solutions of 2.5% (3.28 ± 0.62 µg/cm^2), 4.5% (5.67 ± 1.46 µg/cm^2), or 9% (5.74 ± 0.63 µg/cm^2) SNAC groups ($p > 0.05$).

CS recovery from the epidermis displayed no significant difference in delivery between groups when compared to the control (18.41 ± 4.78 µg/cm^2), as observed in Figure 2.2B ($p > 0.1$), however all gel groups incorporating SNAC showed enhanced drug delivery to the dermis where the control gel performed negligibly (0 µg/cm^2) (Fig. 2.2C). The dermis retained significantly more CS with use of 2.5% (36.26 ± 13.05 µg/cm^2, $p = 0.05$), 4.5% (11.64 ± 1.45 µg/cm^2, $p = 0.001$), and 9% (35.87 ± 2.23 µg/cm^2, $p = 0.004$) SNAC gels as compared to the control. No

remarkable differences in drug delivery to the dermis were observed between 2.5% and 9% SNAC groups ($p = 0.977$).

Based on these results, which show the 2.5% SNAC gel was able to deliver more drug to the dermis with the lowest enhancer ratio, 4.5% and 9% SNAC gels were not evaluated in microneedle enhancement or reverse permeation studies.

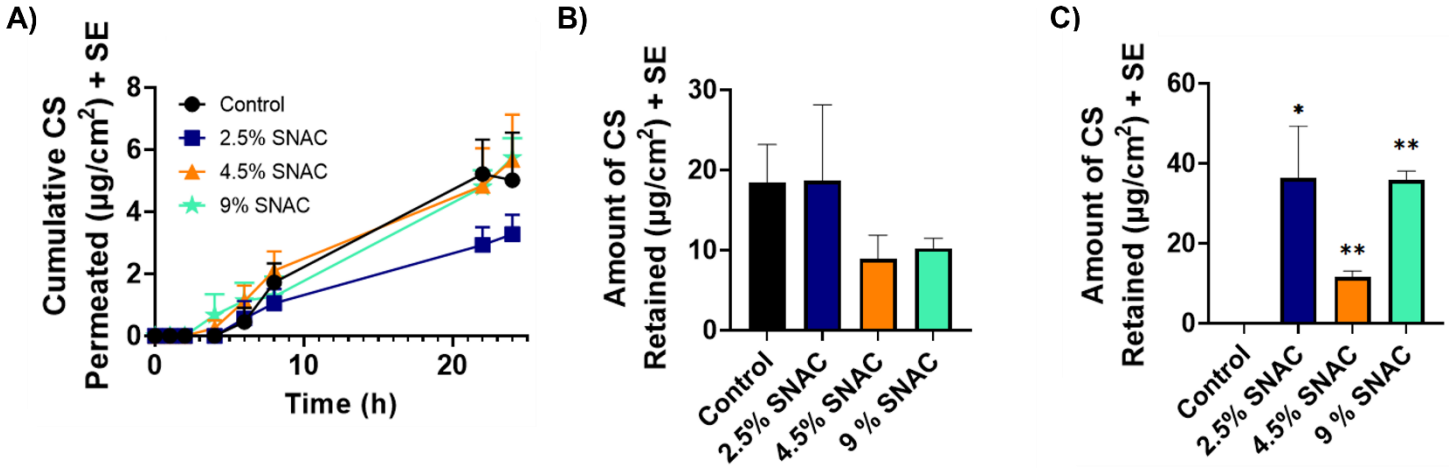

Fig. 2.2. Effect of salcaprozate sodium (SNAC) on the *in vitro* skin permeation of cromolyn sodium (CS) through dermatomed porcine ear skin. The cumulative amount of CS in receptor solution at (A) 24 hours and the average amount of CS delivered to the (B) epidermis and (C) dermis were measured for control (n = 4), 2.5% SNAC (n = 5), 4.5% SNAC (n = 4), and 9% SNAC (n = 3) gels. Data shown as mean with standard error (SE); * $p \leq 0.05$; ** $p \leq 0.01$.

Effect of OA on Skin Permeation of CS

An additional study was performed to investigate a second chemical enhancer, OA, and its impact on CS permeation. Receptor solution exhibited no significant differences at 24 h permeation in cumulative drug amount between control (6.09 ± 1.49 µg/cm^2) and

OA (2.79 ± 0.74 µg/cm^2, p =0.142) groups (Fig. 2.3A), although the control gel showed a more prominent trend of drug delivery to the receptor as time progressed.

Skin permeation showed the control gel delivered greater amount of drug into the epidermis (9.48 ± 1.51 µg/cm^2) when compared to the OA gel (4.68 ± 1.62 µg/cm^2), as observed in Figure 2.3B, although not significantly (p = 0.077). The dermis of skin treated with the control gel (26.01 ± 4.74 µg/cm^2) also had markedly higher amounts of CS recovered than the skin treated with OA (7.95 ± 4.87 µg/cm^2, p = 0.042) (Fig. 2.3C).

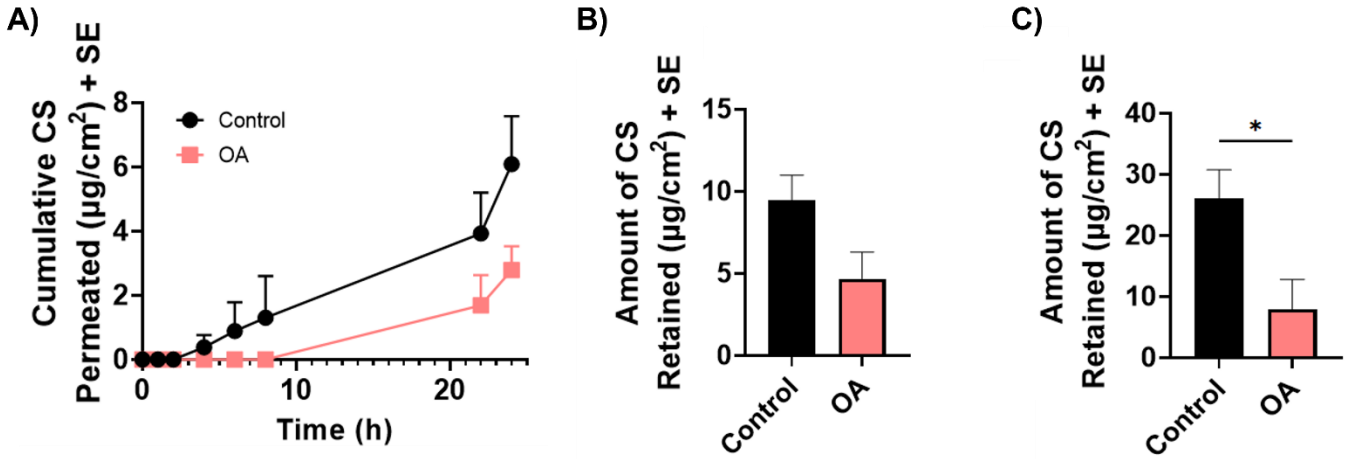

Fig. 2.3. Effect of oleic acid (OA) on the *in vitro* skin permeation of cromolyn sodium (CS) through dermatomed porcine ear skin. The cumulative amount of CS in receptor solution at (A) 24 hours and the average amount of CS delivered to the (B) epidermis and (C) dermis were measured for 1% CS control (n = 3) and 5% (v/v) OA in PG (n = 5) gels. Data shown as mean with standard error (SE); * $p \leq 0.05$.

Effect of Microneedles on Skin Permeation of CS

The relationship between skin microporation and additional SNAC enhancement was explored using further permeation and skin distribution studies. Figures 2.4A and 2.4B show pretreatment of skin with MNs significantly increased permeation for the control gel at both 8 h (221.17 ± 44.16 μg/cm^2, $p = 0.016$) and 24 h (692.17 ± 143.66 μg/cm^2, $p = 0.017$), respectively. No significant differences were observed in drug retained in the epidermis for the control gel, with (21.91 ± 3.76 μg/cm^2) or without MN pretreatment ($p = 0.588$) (Fig. 2.4C). Figure 2.4D, however, shows that MN pretreatment remarkably increased drug retained in the dermis (170.87 ± 16.78 μg/cm^2) compared to control gel only ($p = 0.002$).

Similar trends were observed for MN pretreatment combined with 2.5% SNAC gel application. MN pretreatment showed increased drug delivery to the receptor at 8 h (221.31 ± 45.72 μg/cm^2, $p = 0.009$) and 24 h (668.95 ± 138.09 μg/cm^2, $p = 0.009$) permeation when compared to 2.5% SNAC gel treatment alone (Figs. 2.4A and 2.4B, respectively). Comparable to the control gel, MN pretreatment combined with 2.5% SNAC gel application (16.12 ± 2.92 μg/cm^2) did not significantly affect CS delivery to the epidermis ($p = 0.81$) (Fig. 2.4C) while it significantly increased CS delivery to the dermis (107.98 ± 20.70 μg/cm^2, $p = 0.023$) as compared to 2.5% SNAC gel application only (Fig. 2.4D).

Importantly, no significant differences were observed between the control gel with MN pretreatment and the 2.5% SNAC gel with MN pretreatment for receptor solutions at 8 h ($p = 0.998$) or 24 h ($p = 0.911$) (Figs. 2.4A and 2.4B, respectively). Additionally, no marked differences were seen between the two groups for amount of drug retained in the epidermis ($p = 0.27$) or dermis ($p = 0.051$) (Figs. 2.4C and 2.4D, respectively).

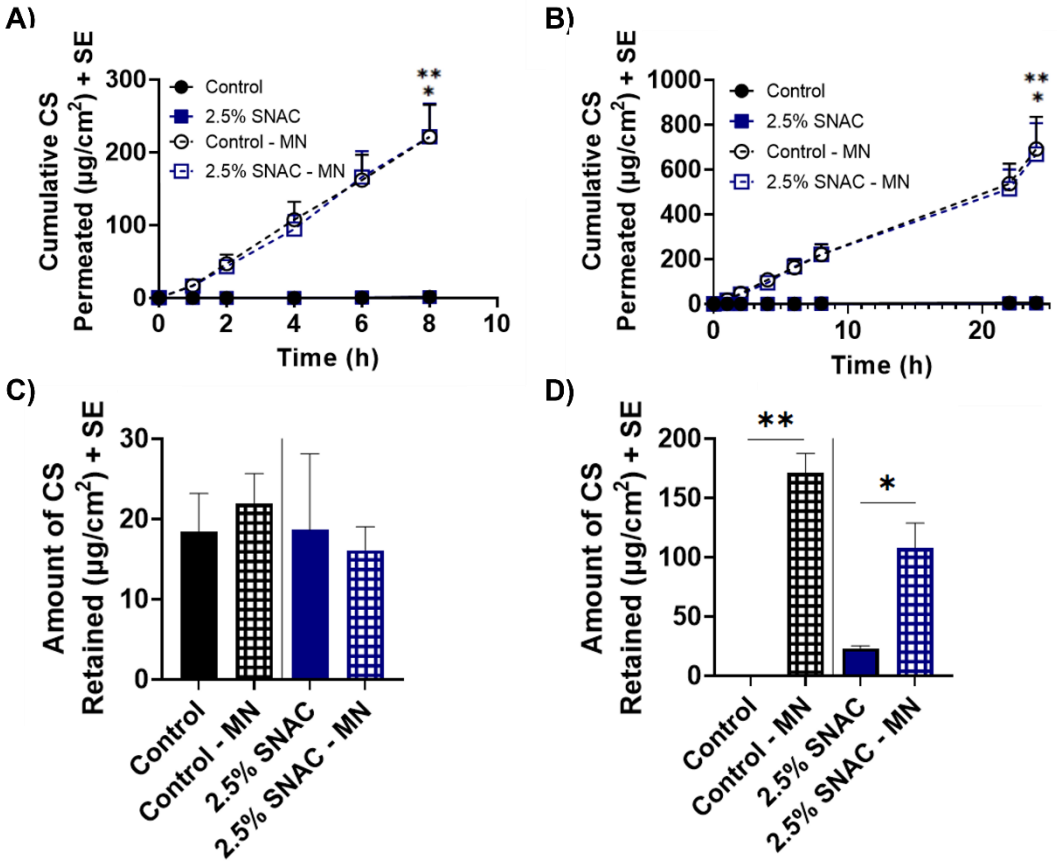

Fig. 2.4. Effect of microneedle (MN) pretreatment on the *in vitro* skin permeation of cromolyn sodium (CS) through dermatomed porcine ear skin. The cumulative amount of CS in receptor solution at (A) 8 hours and (B) 24 hours and the average amount of CS delivered to the (C) epidermis and (D) dermis were measured for the control gel, with or without MN treatment (n = 4), and the 2.5% SNAC gel, with or without MN treatment (n = 5). Data shown as mean with standard error (SE); * $p < 0.05$; ** $p \leq 0.01$.

Evaluating the Mechanism of SNAC

In order to further understand the mechanism of action behind SNAC enhancement, a reverse permeation study was performed where skin was mounted upside down on the Franz diffusion cells. Control and 2.5% SNAC gels were applied and allowed to permeate through skin over 24h. At 24 h permeation, cumulative amount of CS found in the receptor seemed to trend

higher for the control (12.09 ± 4.43 µg/cm^2) when compared to 2.5% SNAC (7.92 ± 2.48 µg/cm^2) group, but this difference was not significant (p = 0.469) (Fig. 2.5A).

As can be seen in Figure 2.5B, the amount of drug delivered to the epidermis did not vary significantly between control (35.23 ± 10.82 µg/cm^2) and 2.5% SNAC (22.98 ± 10.89 µg/cm^2, p = 0.51). Similarly, there was not a considerable difference between the control (12917.06 ± 811.96 µg/cm^2) and 2.5% SNAC (12890.59 ± 1787.77 µg/cm^2) groups regarding amount of CS recovered from the dermis (p = 0.99) (Fig. 2.5C).

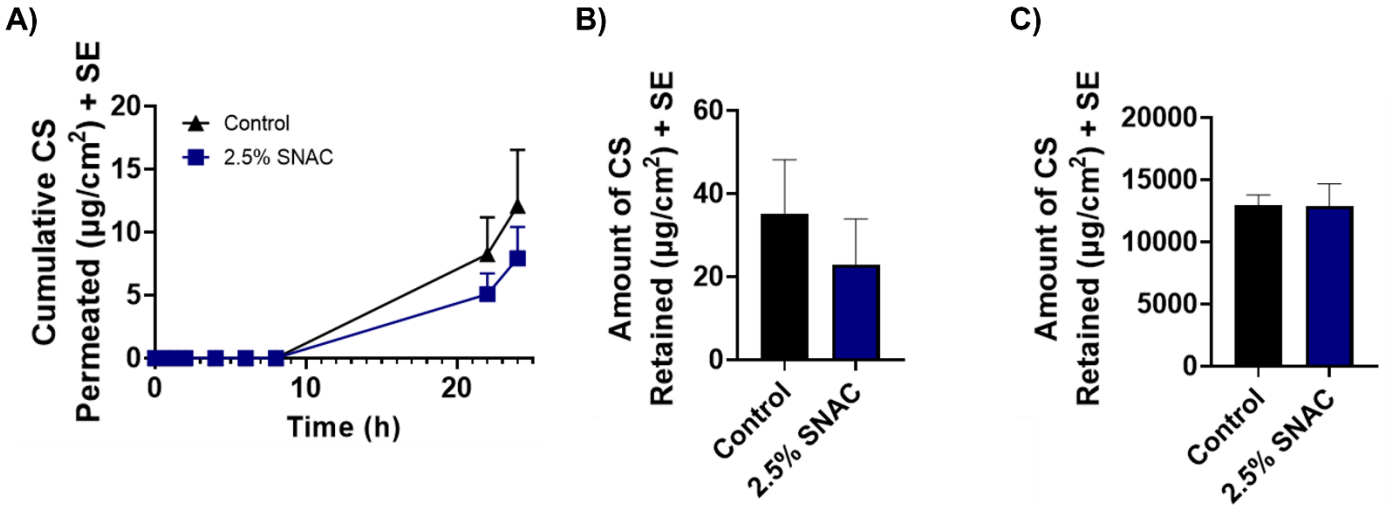

Fig. 2.5. The reverse permeation of cromolyn sodium (CS) through dermatomed porcine ear skin using salcaprozate sodium (SNAC) enhancement. The cumulative amount of CS in receptor solution at (A) 24 hours and the average amount of CS delivered to the (B) epidermis and (C) dermis were measured for control (n = 3) and 2.5% SNAC (n = 3) gels. Data shown as mean with standard error (SE).

SNAC Gel Stability

After initial application, it was established that the control gel had a drug content of 4.57 ± 0.19%, and the 4% CS 2.5% SNAC gel had a drug content of 4.13 ± 0.05%. Both gels were split and stored separately at room temperature and 4°C. After 30 days, the control gel stored at room temperature contained 87.03 ± 2.6% CS compared to day 1 ($p = 0.071$), while the control gel stored at 4°C contained 87.3 ± 2% CS ($p = 0.076$) (Fig. 2.6). Regarding the 2.5% SNAC gel, when stored at room temperature and 4°C the gels maintained 77.71 ± 3.85% and 84.96% ± 2.11% of initial drug content, respectively. The drug content of the control gels stored for 30 days were not significantly different from the initial drug content, but the 2.5% SNAC gels stored at room temperature ($p = 0.023$) and 4°C ($p = 0.007$) had markedly lower drug content compared to day 1. All gels after 30 days of storage were clear and did not show any color change or drug precipitation.

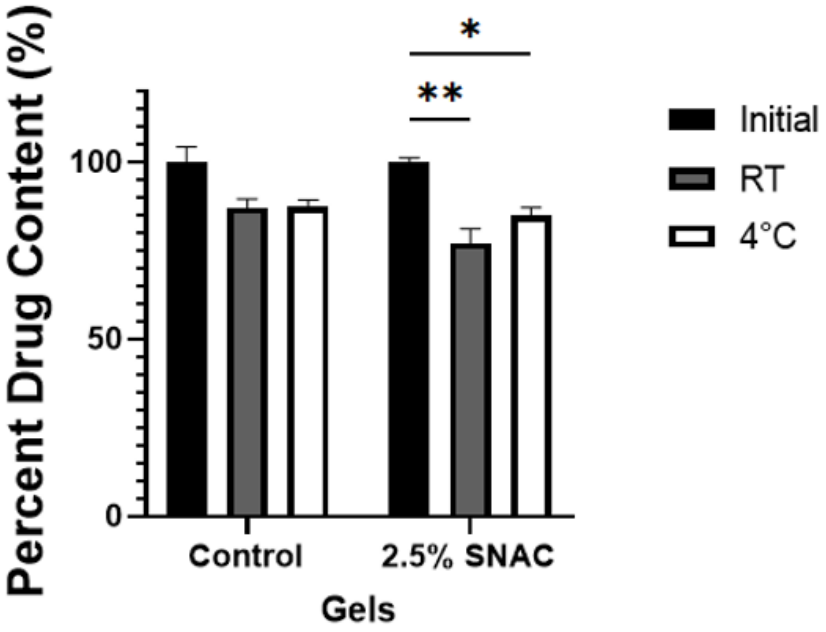

Fig. 2.6. Effect of storage conditions on the drug content of cromolyn sodium (CS) gels. RT: room temperature; Control: 4% CS control gel; 2.5% SNAC: 4% CS 2.5% SNAC gel. Data shown as mean with standard error (n = 3); * $p < 0.05$; ** $p \leq 0.01$.

Discussion

Cromolyn sodium (CS) has been used in clinical settings to topically treat atopic dermatitis. These recently tested formulations primarily had drug content of 4% and were a solution or emulsion.[14,15,43] To date, no such formulation has entered the market and become available for commercial use on skin. Additionally, CS is a large hydrophilic drug and does not easily permeate beyond the lipophilic stratum corneum. This outer dead layer of skin prevents CS entry into the deeper, hydrophilic dermal layer, where CS deposition would likely see the most therapeutic effect. Thus, this work offers one of the first investigations into formulation optimization for a semisolid CS gel utilizing different methods of enhancement.

Of the chemical enhancers with well-established use in skin, oleic acid (OA) was evaluated for its effects in CS gels. The 5% (v/v) OA:PG mixture showed difficulty in solubilizing CS, and only 1% CS formulations were capable of making gels without drug precipitating out. Surprisingly, the OA gel did not improve CS delivery to the receptor or different skin layers (Fig. 2.3). The 1% CS control gel seemingly outperformed the OA gel in all areas and had significantly more drug permeated into the dermis. Likely, OA's lipophilicity made it difficult for hydrophilic CS to remain in solution while concurrently preventing dissolution of ideal amounts of OA for permeation enhancement. A study by Yamane *et al.* was performed using 5% OA in PG as a chemical enhancer for the hydrophilic drug 5-fluorouracil, however the skin they used had been hydrated for 3 days in an aqueous sodium azide solution and enhancer was applied to skin samples 1-12 h before drug was applied separately.[44] Increased hydration of skin is known to promote drug delivery,[45] and by pretreating skin with OA they were not limited by drug solubility in the enhancer solution. They also observed a plateau in enhancement capabilities of OA pretreatment, where the enhancement effect was saturated 6 h after application. Similar to our work, Pierre *et al.* investigated the enhancement effects of OA in PG at increasing concentrations on the skin

permeation of 1% 5-aminolevulinic acid (5-ALA). While all ratios of enhancer improved drug delivery of the highly hydrophilic 5-ALA to the receptor, 2.5% and 5% OA:PG solutions showed significant reduction in drug retained in the stratum corneum and slightly less drug retention in the remaining skin layers for 2.5% OA:PG compared to control.[46] They did not report on 5-ALA solubility in the OA enhancer solutions, however their results provide evidence of OA showing reduced delivery of a hydrophilic drug that might support the unusual observations in this study.

Additionally, when comparing the difference between chemical enhancers tested, the 2.5% SNAC gel delivered more drug to the receptor and skin layers, though these differences were not statistically significant ($p > 0.08$) (refer to Appendix A). These results do not show any evidence for the use of OA in CS gel formulations designed to enhance drug delivery to the dermis; therefore, studies using the OA gel were discontinued. The most striking result to emerge from Figure 2.3 is the 1% CS control gel was able to deliver CS to the dermis whereas the 4% CS gels from the SNAC study did not ($p = 0.008$) (Fig. 2.2D), suggesting higher concentration of CS prevented entry into the dermis. This could be related to the increased proportion of PG in the OA gel, which also acts as a skin permeation enhancer in formulation,[45] and the amphiphilic nature of CS that allows for drug molecules to self-aggregate.[17] Previous work by Tavano *et al.* showed that the skin permeation of a CS-water solution was dependent upon drug concentration and is negatively correlated with the ability of CS to form aggregates, wherein smaller self-aggregates enhance permeation while larger phases decrease permeation. Furthermore, the authors showed that *ex vivo* permeation of CS through rabbit ear skin was highest using a niosomal solution of 0.5% CS (w/w). The increase in CS delivery to the dermis with the 1% formulation may then be explained by the formation of niosomes, or nanosized vesicle structures, that allow CS to act as drug and as its own carrier when partitioning into lipid membranes. It is possible that at higher

clinically used concentrations of 4%, CS forms other structures that are too large to move efficiently across the epidermis and into the dermis. Further work needs to be done to evaluate the optimum CS content in gels for maximum therapeutic effect while taking noisome formation or self-aggregation into consideration.

Although it has not been studied for its enhancement capabilities in skin, salcaprozate sodium (SNAC) has shown success in increasing intestinal permeability and oral absorption of CS.[38-40] For this reason, SNAC was also chosen for incorporation into CS gels at varying molar ratios. Interestingly, increasing the SNAC composition across gels did not correlate to an increase in drug permeation into the receptor solution (Fig. 2.2A). Similar trends could be observed in the epidermis and dermis (Figs. 2.2B and C respectively), where drug delivery varied across SNAC enhancement. All SNAC gels were, however, able to sufficiently deliver drug into the dermis, whereas CS alone was unable to passively permeate beyond the epidermis. Furthermore, the 2.5% SNAC gel was able to deliver similar amounts of CS to the dermis when compared to the 9% SNAC gel, although with a wider error margin. Despite this, with the goal of creating a less expensive, safer therapeutic alternative for atopic dermatitis to be sold commercially, the 2.5% SNAC gel appears to be the more logistical option for moving forward. Subsequently, only the 2.5% SNAC gel was investigated in the microneedle and reverse permeation studies, with continuing interest in the gel's stability.

The variation in drug delivery through skin observed in Figure 2.2 could be contributed to the molecular interactions of SNAC with CS. SNAC has shown to form noncovalent complexes with CS in a 1:1 molar ratio through weak forces that are thought to be a product of ring-stacking between the two, which also plays a large role in CS self-aggregation.[47,48] Ding *et al.* were able to demonstrate that SNAC interrupted CS self-binding, suggesting there might exist competition for

similar binding sites between SNAC and CS. This competition could contribute to the varied effect observed between all SNAC gel groups, where the increased enhancement capabilities of the 2.5% CS gel could be explained by SNAC outcompeting CS for binding sites. If 2.5% SNAC, which corresponds to a 1:1 molar ratio to CS, completely saturates CS in formulation, then it would be possible to observe its enhancement effects. This effect could then be lost when using the 4.5% SNAC gel, which corresponds to a 2:1 molar ratio to CS, where this excess SNAC might in some way impede CS delivery. This suggests that some other mechanism might be responsible for the increased delivery of CS by the 9% SNAC gel, a 4:1 molar ratio to CS, and should be investigated further. Additionally, the binding interactions between SNAC and CS particularly in skin requires continued work.

An additional study was performed to help elucidate the mechanism of action behind the enhancement properties of SNAC. Previous work investigating oral absorption of CS has suggested that the weak, noncovalent interaction between SNAC and CS enhances the lipophilicity of CS, thereby making it easier to permeate through the intestinal barrier.[47,48] Later work contested these findings by arguing that such an interaction is too weak to alter the physiochemical properties of CS, and proposed that SNAC instead helped to fluidize the membrane to allow drug through.[49] In an attempt to further justify the mechanism of SNAC, an *in vitro* permeation study employing upside down skin was used with gel application to the dermis. If SNAC increased the lipophilicity of CS, then there would be an observed decrease in dermal permeation when compared to the reverse permeation control gel, considering the dermis is highly hydrophilic. While there were no statistically significant differences between treatments (Fig. 2.5), no such decrease in permeation was observed and SNAC treatment maintained comparable levels of CS in the dermis, possibly indicating that the complexation of SNAC and CS is not strong enough to increase the lipophilicity

of CS. Drug from the control gel was also able to permeate the receptor and epidermis in larger quantities than the 2.5% SNAC gel was. Further, when preparing all SNAC gels, it was observed that increasing amount of SNAC seemed to increase CS solubility in PG, as indicated by the reduced effort and time required for complete dissolution. This combined with the skin data might suggest that SNAC enhances permeation by interacting with lipid structures and fluidizing the membrane as opposed to increasing drug lipophilicity; however, the data was inconsistent and not significant. Not enough solid evidence was provided to confirm the mechanism of SNAC, although it is possible that with a larger sample size results might become clearer. Additional methods should be explored when investigating drug and SNAC interactions within skin to ensure all complexities are considered.

Skin permeation studies employ physical enhancers in order to disrupt the skin surface and allow for large, hydrophilic drugs like CS to permeate deeper skin layers. Microneedles create hydrophilic conduits that deliver drug directly into the skin, bypassing the need to overcome the stratum corneum barrier. Use of a microneedling pen allowed for the evaluation of microneedle application on CS permeation into the dermis, with or without additional enhancement from SNAC. Microporation of skin distinctly showed greater delivery of CS for both gels to the receptor and dermis (Fig. 2.4), suggesting that microneedle pretreatment alone was enough to promote CS permeation across skin. Interestingly, combined enhanced effects were not observed with the 2.5% SNAC gel when used together with microneedle pretreatment, and in fact appeared to deliver less CS to the dermis when compared to the control gel with microneedle pretreatment, although not significantly. This could be a result of CS's inherent hydrophilicity, which might make CS more likely to be retained in the hydrophilic dermis compared to SNAC, which is more hydrophobic than CS. With the assistance of microneedles, the control gel does not seem to have as much

difficulty permeating through the epidermis and into the dermis over 24 hours. Moreover, applying the control gel to microneedle pretreated skin delivered the most CS to the dermal layer over all other treatments or formulations.

To accurately assess skin data obtained by extraction protocol from all enhancement studies, an experiment evaluating drug recovery from the epidermis and dermis was performed. CS extraction from the epidermis displayed greater overall recovery when compared to the amount absorbed into skin. Extraction of drug from the dermis by methanol proved to be more difficult, as indicated by the lines plotted in Figure 2.1, although sufficient correlation was still observed. All skin data were corrected using their respective equation to establish reliable amounts of drug in the different skin layers. Even with difference in correction values, previously observed trends remained the same for all studies, and all corrected data and statistics were reported in this paper. Similar extraction values were observed by Puri *et al.* where ethanol was used to extract EGCG from porcine ear skin (24-29%).[31] EGCG, a polyphenol found in green tea, is also highly hydrophilic,[50] which could suggest alcohol extraction methods have difficulty recovering hydrophilic molecules like CS from the more hydrophilic dermis. Additionally, Demertas *et al.* using 69:31 methanol:water (v/v) as extraction solvent observed less than 70% extraction in canine skin, but were able to recover >90% of drug after replacing methanol with acetonitrile.[51] Further supporting acetonitrile use for dermis extraction specifically, Padula *et al.* utilized methanol for porcine ear stratum corneum and epidermis extraction of benzophenone-3, vitamin A, and vitamin A acetate and acetonitrile for extraction from the dermis with greater than 90% recovery in all three skin layers.[52] Evaluating the efficacy of acetonitrile in extraction protocols could improve recovery values of this method.

The control and 2.5% SNAC gels displayed moderate stability over the course of 30 days (Fig. 2.6.). While the control gels stored at room temperature and 4°C maintained similar drug content, the 2.5% SNAC gels stored at room temperature appeared to be less stable and contain less drug than the gel stored at 4°C, although this difference was not significant. Still, both 2.5% SNAC gels had markedly lower drug content compared to the initial measurement on day 1. This suggests that CS is relatively stable in gels, while SNAC itself or the CS-SNAC complex formed appears to be less stable overall and especially so at room temperature. These trends were similarly observed with the 4.5% SNAC gel and the 1% CS OA gel at 4°C, while drug precipitated out of the 9% SNAC gel in both environments (refer to Appendix B). Likely, when gel formulations for commercial use are considered, a CS gel without SNAC would be more stable. Investigation into the degradation of CS in PG only and in conjunction with SNAC should be performed. It might be possible after identifying the cause of the gels' instability for the addition of stabilizing agents like antioxidants, antimicrobial preservatives, or other excipients or co-solvents to ameliorate this issue.[53]

Conclusion

The present study aimed to evaluate the optimum formulation and enhancement techniques for delivery of cromolyn sodium (CS) into the dermal layer of skin. Gels incorporating CS have yet to be evaluated clinically for the treatment of atopic dermatitis, and this paper presents the first investigation into the optimization of any such formulation for potential therapeutic treatment. Taken together, these findings suggest that the 4% CS control gel applied to microneedle pretreated skin serves as the best enhancement strategy to increase drug delivery to the dermis and maintain a more stable gel. For commercial sale, microneedle fabrications and patches incorporating CS

could be investigated, which would streamline therapeutic applications. Additionally, novel use of SNAC in skin permeation studies and as a potential skin enhancer for CS was demonstrated. SNAC formulations showed significant enhancement of CS delivery to the dermis compared to the control, and for its ease of application, the 2.5% SNAC gel would still be worth considering in commercial markets for short-term treatments. While this study aimed to identify the mechanism of SNAC enhancement in skin, results were inconclusive and needs to be explored further.

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CHAPTER 3. CONCLUSIONS AND FUTURE DIRECTIONS

Due to rising financial burdens and cost of therapeutics for those diagnosed with eczematous diseases and the associated risks with topical corticosteroid use, an investigation was performed to optimize a cromolyn sodium gel for the potential treatment of atopic dermatitis. The dermis was chosen as the target skin layer for drug delivery as it hosts the mast cells and nerve endings of the skin, either of which cromolyn sodium likely targets to induce its anti-inflammatory and anti-itch properties.

Semisolid gels were created using oleic acid or salcaprozate sodium as chemical enhancers, and formulations were applied to skin with or without microneedle pretreatment to determine how the enhancers performed in increasing drug delivery to the dermis. Oleic acid did not improve drug delivery overall, while the 2.5% salcaprozate sodium formulation significantly enhanced drug delivery to the dermis. Microneedle pretreatment of skin was determined to be the ideal enhancement for cromolyn sodium, as it significantly increased drug permeation into the dermis when compared to individual oleic acid and salcaprozate sodium use. This increase was observed for each 4% cromolyn sodium gel tested, however the control gel without chemical enhancement seemingly delivered more drug compared to the 2.5% salcaprozate sodium gel, though this difference was not significant. The control gel without salcaprozate sodium incorporation also appeared to be the most stable over 30 days of storage at room temperature and 4°C.

While this study did determine the best method of enhancement for drug delivery to the dermis out of the methodologies tested, it does not determine how these treatments would perform on eczematous skin. Atopic dermatitis results in a compromised epidermal barrier and

skin lesions, inherently increasing the stratum corneum's permeability in affected areas. All gels investigated were tested on what was considered healthy porcine ear skin; therefore, for a more accurate indication of how the enhancers and gels used would partition drug into skin, *in vitro* permeation studies should be performed on skin deemed an appropriate model for atopic dermatitis. The compromised skin barrier could likely lead to greater skin permeation overall. Since this study's approach for microneedle enhancement utilized a microneedling pen for simulation of a solid microneedle patch, further studies could employ dissolving microneedle patches incorporating cromolyn sodium, as their use has already been tested for efficacy in atopic dermatitis mice models. Considering microneedle applications are successful in increasing lateral diffusion of drugs in skin,[74] *in vitro* permeation studies on atopic dermatitis model skin might not be necessary, as formulated microneedle patches could be applied adjacent to affected areas. If intended for such use, work specifically monitoring lateral drug diffusion would need to be explored. Additionally, to evaluate the circulatory and remedial effects of cromolyn sodium gels, *in vivo* studies should be considered in established mice models for human atopic dermatitis. Although the gels were formulated to target the dermis for improvement of disease symptoms, only *in vivo* analyses would provide information on the effectiveness of the gels for treating atopic dermatitis.

Concurrently, this work attempted to determine by what mechanism salcaprozate sodium enhances cromolyn sodium permeation observed in oral absorption studies. The *in vitro* reverse permeation study did not provide conclusive evidence for the enhancement interactions of salcaprozate sodium, but other quantitative or qualitative analyses such as Fourier-transform infrared spectroscopy could provide a better idea of how salcaprozate sodium might impact the structure and ordering of lipids in skin. Shifts in stretching vibrations could indicate salcaprozate

sodium perturbs or fluidizes the membrane by acting on skin lipids. Additionally, fluorescence anisotropy studies used by previous authors attempting to confirm salcaprozate sodium's mechanism in colorectal cell lines could be used in excised skin. Using a fluorescent probe designed as a measure of membrane fluidity could provide further evidence for such a mechanism. Vehicle interactions between salcaprozate sodium and propylene glycol should also be explored to separate enhancement by salcaprozate sodium only from the synergistic enhancement effects commonly observed with propylene glycol solutions.[75]

Finally, further research should be involved in determining a sufficient minimum dosage of cromolyn sodium in gel formulations. Topical formulations for cromolyn sodium assessed clinically have chiefly been 4% drug emulsions, and the permeation results of the 1% cromolyn sodium gel indicate 4% cromolyn sodium might prevent passive delivery to the dermis. Again, while a 1% cromolyn sodium gel did show sufficient skin permeation, the curative effects of such a dose would need to be evaluated *in vivo*. Furthermore, with this knowledge it would be of value to investigate salcaprozate sodium enhancement effects on 1% cromolyn sodium gels to further improve skin targeted drug delivery.

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APPENDICES

Appendix A: OA vs SNAC Comparison

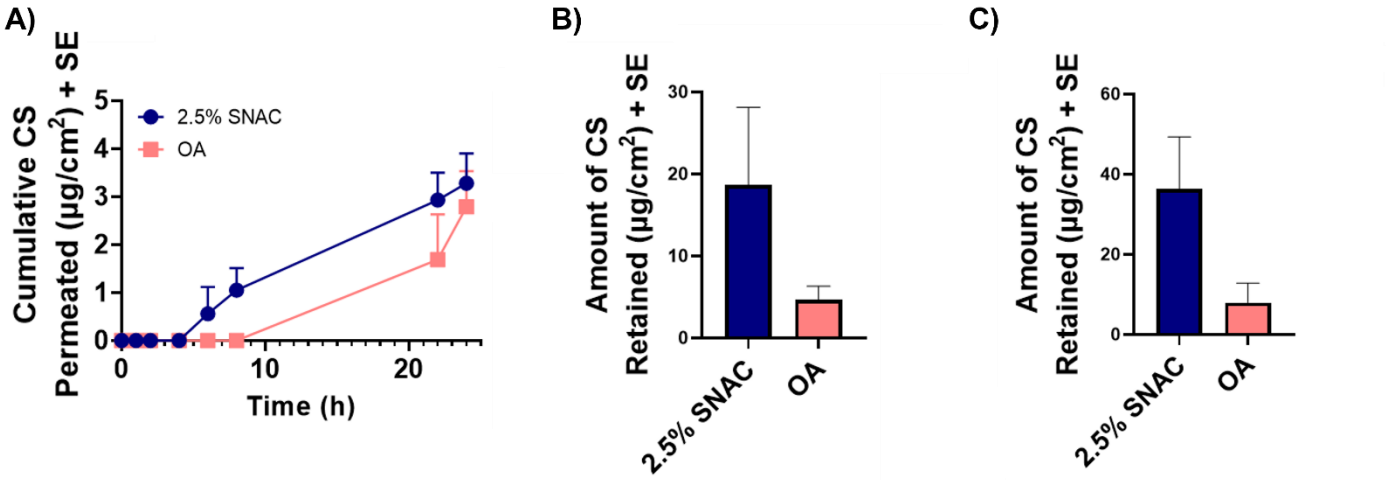

Fig. 1. Comparison of salcaprozate sodium (SNAC) and oleic acid (OA) effects on the permeation of cromolyn sodium (CS) through dermatomed porcine ear skin. The cumulative amount of CS in receptor solution at (A) 8 hours and (B) 24 hours and the average amount of CS delivered to the (C) epidermis and (D) dermis were measured for 4% CS 2.5% SNAC (n = 5) and 1% CS 5:95 OA:PG (n = 5) gels. Data shown as mean with standard error (SE).

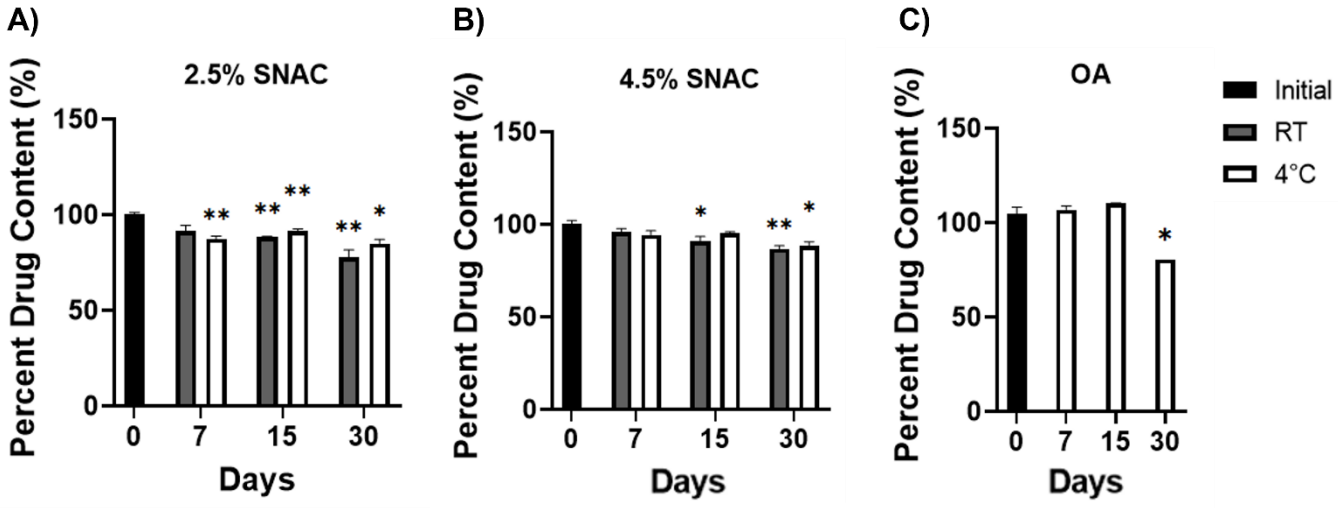

Fig. 2. Effect of storage conditions on the drug content of all formulated cromolyn sodium (CS) gels. Gel content measured over 30 days for (A) 2.5% SNAC, (B) 4.5% SNAC, and (C) 5% OA gels at RT and 4°C. The OA gel stored at RT experienced fungal growth at day 7 and was discarded. 9% SNAC gels showed drug precipitation after 7 days and were not included for further analysis. RT: room temperature; Control: 4% CS control gel; 2.5% SNAC: 4% CS 2.5% SNAC gel; 4.5% SNAC: 4% CS 4.5% SNAC gel; OA: 1% CS 5:95 OA:PG gel. Data shown as mean with standard error (n = 3); * $p < 0.05$; ** $p \leq 0.01$.

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