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Formulation, functional evaluation and *ex vivo* performance of thermoresponsive soluble gels - A platform for therapeutic delivery to mucosal sinus tissue

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ABSTRACT

Mucoadhesive *in situ* gelling systems (soluble gels) have received considerable attention recently as effective stimuli-transforming vectors for a range of drug delivery applications. Considering this fact, the present work involves systematic formulation development, optimization, functional evaluation and *ex vivo* performance of thermosensitive soluble gels containing dexamethasone 21-phosphate disodium salt (DXN) as the model therapeutic.

A series of *in situ* gel-forming systems comprising the thermoreversible polymer poloxamer-407 (P407), along with hydroxypropyl methyl cellulose (HPMC) and chitosan were first formulated. The optimized soluble gels were evaluated for their potential to promote greater retention at the mucosal surface, for improved therapeutic efficacy, compared to existing solution/suspension-based steroid formulations used clinically.

Optimized soluble gels demonstrated a desirable gelation temperature with Newtonian fluid behaviour observed under storage conditions (4–8 °C), and pseudoplastic fluid behaviour recorded at nasal cavity/sinus temperature (≈34 °C). The *in vitro* characterization of formulations including rheological evaluation, textural analysis and mucoadhesion studies of the gel form were investigated. Considerable improvement in mechanical properties and mucoadhesion was observed with incorporation of HPMC and chitosan into the gelling systems. The lead poloxamer-based soluble gels, PGHC4 and PGHC7, which were carried through to *ex vivo* permeation studies displayed extended drug release profiles in conditions mimicking the human nasal cavity, which indicates their suitability for treating a range of conditions affecting the nasal cavity/sinuses.

1. Introduction

The nasal airway passages play a crucial role in upper airway homeostasis with an abundance of air borne pathogens being drawn into the sinuses with each breath, resulting in a high probability of localised infection and chronic inflammation in susceptible patient groups (Parikh et al., 2014). Sinusitis or rhinosinusitis is an inflammatory condition of the nasal and paranasal sinus mucosa resulting in nasal discharge/congestion, nasal blockage, facial pain/pressure and reduction of sense of smell (Rosenfeld et al., 2007; Fokkens et al., 2007). Chronic rhinosinusitis (CRS), defined by the persistence of symptoms beyond 3 months, is among the commonest chronic medical complaints cited in the United States, affecting nearly 16% of the general population, with the increasing incidence and prevalence accounting for 13 million physician visits annually costing an estimated US$6 billion/year (Blackwell et al., 2014; Piromchai et al., 2013).

Treatment of CRS demands medical and surgical intervention, with the former often involving a combination of antibiotics, nasal decongestants, topical nasal/oral steroids as well as saline irrigation. That said the vast proportion of patients do not respond to medical treatment alone, and this invariably requires functional endoscopic sinus surgery (FESS) (Piromchai et al., 2013).

FESS is a well-established therapeutic option for intractable CRS, although success is hampered by poor wound healing rates, which can be complicated by postoperative adhesion/polyp formation, which ultimately leads to relapse (Georgalas et al., 2014; Kennedy, 1992; Lund and MacKay, 1994; Schaffhausen et al., 2008). In order to address these significant shortfalls several pharmaceutical formulations that include packing and gel-based materials have been

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proposed. However, these materials invariably lack sufficient residence time and physical integrity, limiting adherence to mucosal tissue and at best act as a very temporary physical barriers, with questionable therapeutic benefit (Schaffhausen et al., 2008). The efficacy of intranasal steroid sprays, drops and other conventional delivery methods are also compromised by poor drug delivery and retention, which is not helped by underlying postoperative oedema, crusting and secretions that ultimately lead to poor patient compliance (Rizan and Elhassan, 2016). Medical therapy remains the foundation of long-term care of chronic rhinosinusitis, particularly in surgically recalcitrant cases, although effective drug delivery remains a major obstacle to achieving this (Liang and Lane, 2013).

There is a need for formulations that possess optimum mucoadhesive properties that promote a moist wound healing environment, resist microbial colonisation, and release drug in a sustained manner to the paranasal sinuses. The stated aims can be achieved when an optimal formulation is coupled with a drug delivery device design providing optimal distribution of formulation across mucosal sinus surfaces (Liang and Lane, 2013). The proposed requirements are for a mucoadhesive *in situ* gelling system, otherwise termed as a soluble gel (sol-gel), that interacts with and adheres to the mucosal epithelial surface components and once administered in soluble form, rapidly undergoes *in situ* gelation (Miyazaki et al., 1999; Miyazaki et al., 2003). The gel swells at the site of administration forming an adherent layer that is capable of achieving prolonged residence time at mucosal surfaces (Rozier et al., 1989; Cohen et al., 1997; Srividya et al., 2001).

In the nasal context a sol-gel system should reduce anterior and posterior leakage, provide protection to the drug from enzymatic degradation, increase the rate of drug dissolution, improve contact of the formulation with the nasal mucosa while enhancing drug uptake across epithelium (Wang et al., 2008).

This study involves systematic formulation, optimization, functional evaluation and *ex vivo* drug delivery of thermo-responsive sol-gels containing the clinically relevant therapeutic, dexamethasone 21-phosphate disodium salt (DXN). DXN is a glucocorticoid used clinically for the topical or systemic treatment of chronic inflammatory disorders, severe allergies and other diseases requiring anti-inflammatory and immunosuppressive effects (Pignatello et al., 2007). The molecular weight of dexamethasone 21-phosphate disodium salt is 516.40 (log P = 1.83 and water solubility of 1.52 mg/mL). Poloxamer-407 (P407) was employed as the thermoresponsive polymer, with chitosan serving as a mucoadhesive polymer and permeation enhancer, while hydroxypropyl methyl cellulose (HPMC E4M) was added to deliver mechanical strength. The thermoreversible nasal sol-gels were systematically evaluated, with lead formulations carried through to *ex vivo* drug permeation studies using freshly excised human mucosal nasal tissue.

2. Materials and Methods

2.1. Chemicals

Dexamethasone 21-phosphate disodium salt (DXN) was purchased from Alfa Aesar, Ward Hill, MA, USA (PubChem CID: 16961). Poloxamer (P407) (PubChem CID: 24751), hydroxypropyl methyl cellulose (HPMC E4M), low molecular weight chitosan (50,000–190,000 Da, 75–85% deacetylated) (PubChem CID: 71853), glycerin (PubChem CID: 753), and propyl paraben (PubChem CID: 7175) were purchased from Sigma-Aldrich, Castle Hill, NSW, Australia. Acetic acid (PubChem CID: 176), sodium hydroxide (NaOH) (PubChem CID: 14798), potassium chloride (KCl) (PubChem CID: 4873), calcium chloride ($CaCl_2$) (PubChem CID: 5284359) and sodium chloride (NaCl) (PubChem CID: 5234) were of analytical grade and procured from Sigma-Aldrich, Castle Hill, NSW, Australia. Porcine stomach mucin type II was purchased from Sigma-Aldrich, Castle Hill, NSW, Australia. Deionized water was used as a formulation vehicle.

2.2. Preparation of Nasal Mucosa

Ex vivo permeation and mucoadhesion studies were carried out using freshly excised human nasal mucosa, which was obtained from patients undergoing surgery for nasal obstruction at the Greenslopes Hospital, Brisbane, Australia. The study was approved by the Greenslopes Hospital, and School of Pharmacy Human Ethics Committees. Mucosal tissue of variable thickness (700–1500 μm) was surgically removed from various regions of the nasal cavity and used immediately. By means of a scalpel, the human specimens were carefully dissected from most of the underlying bone and carefully removed of debris or blood (Schmidt et al., 2000). Next, specimens were equilibrated with simulated nasal fluid (SNF; KCl 1.29 mg/mL, NaCl 7.45 mg/mL, $CaCl_2 \cdot 2H_2O$ 0.32 mg/mL, adjusted to pH 5.5 with 0.1 M HCl/0.1 N NaOH) for a period of ~30 min before carrying out permeation studies (Cremaschi et al., 1996; Lang et al., 1996; Manda et al., 2011; Callens et al., 2003).

2.3. Preparation and Optimization of Soluble Gels

2.3.1. Optimization of P407 and Glycerin Concentrations

Sol-gel formulations were prepared using the cold method (Schmolka, 1972). Here, the concentrations of P407 (15–19% w/w) and glycerin (1–5% w/w) were first optimized such that a desirable $T_{sol/gel}$ was achieved. For this, P407 (15–19% w/w) was added to required amount of cold deionized water and mixed thoroughly (400 rpm) for 45 min at 4–8 °C and left to hydrate overnight at 2–8 °C. Next, glycerin (1–5% w/w) was added to fixed concentrations of poloxamer P407 solution, along with the preservative propyl paraben (0.05%). Then, formulations were adjusted to pH 5.5 (Lab Chem pH meter-Brendale, QLD, Australia) with 0.1 M HCl or 0.1 N NaOH, and made up to 10 g (~10 mL) with deionized water and stored overnight at 2–8 °C until required for determination of sol-to-gel transition temperature ($T_{sol/gel}$; see Section 2.4).

2.3.2. Preparation of Soluble Gels

On the basis of our target $T_{sol/gel}$ (34 °C), concentrations of P407 (15.5% w/w) and glycerin (3% w/w) were selected and used to prepare sol-gels with the remaining excipients. Briefly, P407 (15.5% w/w) was added to required amount of cold deionized water and mixed thoroughly (400 rpm) for 45 min at 2–8 °C and left to hydrate overnight at 2–8 °C. Separately, a stock solution of chitosan in (1% v/v) acetic acid was prepared. Then, different concentrations of HPMC (0.1%, 0.3%, 0.5% w/w) and chitosan solution (0.1%, 0.2%, 0.3% w/w) were added and mixed with deionized water containing DXN (0.1% w/w), at room temperature. Finally, this mixture was added to the P407 solution at 2–8 °C. Finally, the excipients, propyl paraben as preservative (0.05% w/w) and glycerin as humectant (3% w/w), were added to this cold mixture, which were adjusted to pH 5.5 with 0.1 M HCl or 0.1 N NaOH, and made up to 10 g (~10 mL) with deionized water and stored overnight at 2–8 °C (Nisha et al., 2012). Clarity of the formulations was assessed visually, behind a dark background.

2.4. Determination of Sol-to-gel Transition Temperature

Temperature of gelation of the thermosensitive gels was determined using a DHR-3 rheometer (TA Instruments, New Castle, DE, USA), with a 40 mm diameter parallel plate geometry. First, stress sweep measurements at 1 Hz (n = 1) were performed to determine the linear viscoelastic region (LVR) of measurement; that is the range of shear stress for which the elastic response (G′) of the sample does not change. The shear stress at which G′ deviates from a constant (plateau) value indicates deviation from linear viscoelastic behaviour. Further, gelation temperature measurement and rheological investigations were performed within this region.