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Controlled drug delivery to the lung: Influence of hyaluronic acid solution conformation on its adsorption to hydrophobic drug particles

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Abstract

This work reports investigations into the interaction and adsorption of the hydrophilic polymer hyaluronic acid (HA) onto the surface of the hydrophobic corticosteroid drug fluticasone propionate (FP). The eventual aim is to formulate a bioadhesive pulmonary drug delivery system with prolonged action that avoids rapid clearance from the lungs by the mucociliary escalator.

Adsorption isotherms detailing the adsorption of HA from aqueous HA solution concentrations ranging from 0.14 to 0.0008% (w/v) to a fixed FP particle concentration of 0.1% (w/v) were investigated. The method of preparing FP particles with HA molecules adsorbed on their surfaces (FP/HA particles) involved suspension of the FP either in hydrated HA solution or in water followed by addition of solid HA, centrifugation of the solids to form a pellet, washing the pellet several times with water until no HA was found in the supernatant and then freeze drying the suspension obtained by dispersing the final pellet. The freeze dried powder was then analysed for adsorbed HA using a Stains-all assay. The influence of order of addition of HA to FP, time for the adsorption process, and temperature of preparation on the adsorption isotherms was investigated.

The non-equilibrium adsorption isotherms produced generally followed the same trend, in that as the HA solution concentration increased, the amount of HA adsorbed increased to a maximum at a solution concentration of ~0.1% (w/v) and then decreased. The maxima in the adsorption isotherms were close to the change from secondary to tertiary conformation in the HA solutions. Below the maxima, adsorption occurred via interaction of FP with the hydrophobic patches along the HA chains in the secondary structures. Above the maxima, secondary HA molecules aggregate in solution to form tertiary network structures. Adsorption from tertiary structure was reduced because strong interactions between the HA molecules limited the availability of hydrophobic patches for adsorption of HA onto FP. The influence of preparation variables on adsorption was also related to the availability of hydrophobic patches for adsorption.

Keywords: Hyaluronic acid; Fluticasone propionate; Corticosteroid; Adsorption isotherms; Bioadhesive; Pulmonary delivery

1. Introduction

Drug delivery to the lung for the local treatment of pulmonary disease with its two to four times daily dosing is far from ideal. The highly efficient clearance mechanisms that have evolved in the human respiratory tract give little time for drug action after dosing. Ideally, once daily therapy with prolonged drug action would allow more effective treatment of conditions such as asthma. An improved delivery system would allow for drug action during periods of the day where current therapies achieve none and the symptoms are most prevalent (Barnes, 1984). Recent research suggests that achieving sustained drug action in the lung is highly desirable (Surendrakumar et al., 2003; Cook et al., 2005).

Corticosteroid anti-inflammatory agents are widely used to prevent acute exacerbations of pulmonary disease. They reduce airway inflammation, oedema and microvascular leakage, secretion of mucus in the airways and also the hyperreactivity of bronchial smooth muscle. The mechanisms by which they act, however, is complex and still not fully understood. They are known to act on a subcellular level and on a variety of cells in the lung including mast cells, lymphocytes, neutrophils, macrophages, eosinophils and airway epithelial cells (Guyre and Munck, 1989; Schleimer, 1989). They are cleared rapidly from the site of deposition by the mucociliary escalator so that to achieve clinical efficacy regular two to four times daily dosing is

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required. Fluticasone propionate (FP) is a hydrophobic corticosteroid that has advantages over the earlier corticosteroids in that systemic side effects (such as skin thinning and osteoporosis) are reduced. The usual adult dose of FP is 100–250 μg twice daily increased to 1 mg in severe disease. A modified delivery system that provides constant levels of drug at the prime site of action for a prolonged time would enable greater control of the disease.

This work reports attempts to prepare a bioadhesive delivery system that avoids the mucociliary escalator by the adsorption of hyaluronic acid onto the surface of FP particles. The polysaccharide hyaluronan (hyaluronic acid, HA) was first isolated by Meyer and Palmer (1934) and has since been extensively investigated (Scott et al., 1991; Fraser et al., 1997; Esposito et al., 2005; Yamamoto et al., 2004). It plays many roles in mammals including regulation of water balance via osmotic pressure and flow resistance, interaction with plasma proteins by sieve and exclusion effects, lubrication through its rheological properties and stabilisation of structures by electrostatic and other interactions. HA was chosen for this work for a number of reasons. Firstly, because HA and the enzymes which metabolise it (mainly hyaluronidase) are endogenous to the pulmonary environment (Bollet et al., 1963). It has been isolated from the lungs of mammals (sheep, guinea pig, rat (Fraser et al., 1997), bovine (Westerman, 1972) and human lung parenchyma and pleura (Hallgren et al., 1985)). The quantity of HA in human lung secretions was found to be ∼66 ng/mL with values ranging from 34 to 423 ng/mL (Dentener et al., 2005).

Secondly, asthma is a disease characterised by immune hypersensitivity and the presence of inflammatory cells. HA has been shown to play a role in the function of various inflammatory mediators including the agglutination of alveolar macrophages (Shannon and Love, 1980) the function of lymphocytes (Darzynkiewicz and Balazs, 1971), monocytes, macrophages (Shannon and Love, 1980) and neutrophils (Forrester and Balazs, 1980). Although HA turnover in the lung has not been investigated, we anticipate that the use of small quantities of HA in a pulmonary delivery system is unlikely to lead to undesirable accumulation.

Finally, for a successful formulation, the delivery system must be bioadhesive, yet avoid clearance by the mucociliary escalator. We consider that high molecular weight HA may have these properties. It is bioadhesive (Barbault-Foucher et al., 2002) and it has been shown that high molecular weight HA present in the lung anchors proteins and enzymes preventing their removal by the mucociliary escalator, although HA of a lower molecular weight had the opposite effect in that it increased ciliary beat frequency to increase particle clearance in the lung (Forteza et al., 2001). High molecular weight HA was thus investigated in this work, in an attempt to anchor the FP long enough in the lung for a prolonged action.

Although HA is a hydrophilic molecule and FP particles are hydrophobic ($\log P = 4.6$), we hypothesised that interaction might occur under certain circumstances via hydrophobic patches within the HA chains. Other investigators have reported that HA can interact with the hydrophobic regions of molecules such as lecithin (Ghosh et al., 1994; Pasquali-Ronchetti et al., 1997) and the hydrophobic surface, graphite (Spagnoli et al., 2005) via such hydrophobic patches. In the current work, adsorption isotherms derived to characterise adsorption from different solution concentrations of HA under different experimental conditions are presented. The influence of adsorbed HA on the suspending properties of FP particles in water, and on the patterns obtained microscopically after freeze drying unwashed suspensions in situ is also reported.

2. Experimental

2.1. Materials

Micronised fluticasone propionate, FP, was donated by GlaxoSmithKline Research Laboratories, Ware, UK. Particle size analysis in 0.1% (w/v) Tween 80 using the Mastersizer 2000 (Malvern Instruments, UK) gave a volume based mean particle diameter of 1.88 μm, with a 10–90% range of 0.70–4.8 μm. Two samples of prokaryotic hyaluronic acid, HA (approximate molecular weight 1.49×10^6) produced by fermentation were donated by Vitrolife (Edinburgh, UK). Both samples of HA were from the same batch but either recrystallised from water (sample 1) or propan-2-ol (sample 2), respectively. The water content of the two HA samples determined by TGA (not shown) were 20.85% (w/w) and 7.54% (w/w), respectively. Reagents acetic acid (glacial GPR reagent 99.5% min), Stains-all (approximately 95%), and L-ascorbic acid (99.7%) were obtained from Sigma–Aldrich (Dorset, UK), 1,4 Dioxan (99.5% stabilised with ∼25 ppm of 2,6-di-*tert*-butyl-4-methylphenol) from BDH (Leicester, UK).

2.2. Assay for HA in the presence of FP

Initial experiments were performed to screen the suitability of several common assays for HA in the presence of FP particles. The assays investigated included Alcian Blue (Whiteman, 1973), uronic acid determination using microtiter plate assay (Hoogen et al., 1998) and fluorimetric determination with 1,4-aminothiophenol (Zhu and Nothnagel, 1991). All were unsuitable due to either interference by FP, especially at higher concentrations of HA, or incomplete dissociation of HA from the FP particles (Alcian Blue). Finally a spectrophotometric method based on the dye Stains-all (Benchetrit et al., 1977; Homer et al., 1993) was chosen due to its relative simplicity, lack of interference with FP and complete dissociation of the HA/dye complex from the FP particles which was confirmed microscopically.

Stains-all solution was prepared by dissolving the dye (final concentration 0.1 mM) in 50% (v/v) water and 50% (v/v) 1,4 Dioxan containing 1 mM acetic acid and 0.5 mM ascorbic acid. The 1,4 Dioxan was stabilised with approximately 25 ppm 2,6-di-*tert*-butyl-4-methylphenol. Dye solutions once prepared were stored in the dark at 25 °C and used within 14 days of preparation. A calibration line was prepared using HA solutions at concentrations ranging from 8×10^{-5} to 0.01% (w/v) by placing a 0.2 mL aliquot of HA solution in a 3 mL stoppered cuvette of 1 cm light path and adding 1.8 mL of dye solution and 1 mL of distilled water. The cuvette was inverted five times to mix