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Formulation of olfactory-targeted microparticles with tamarind seed polysaccharide to improve nose-to-brain transport of drugs

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ABSTRACT

Targeted delivery and retention of drug formulations in the olfactory mucosa, the target site for nose-to-brain drug absorption is a major challenge due to the geometrical complexity of the nose and nasal clearance. Recent modelling data indicates that 10 μm-sized microparticles show maximum deposition in the olfactory mucosa. In the present study we tested the hypothesis that 10 μm-sized mucoadhesive microparticles would preferentially deposit on, and increase retention of drug on, the olfactory mucosa in a novel 3D-printed human nasal-replica cast under simulated breathing. The naturally occurring mucoadhesive polymer, tamarind seed polysaccharide (TSP) was used to formulate the microparticles using a spray drying technique. Physicochemical properties of microparticles such as size, morphology and mucoadhesiveness was investigated using a combination of laser diffraction, electron microscopy and texture-analysis. Furthermore, FITC-dextrans (5–40 kDa) were incorporated in TSP-microparticles as model drugs. Size-dependent permeability of the FITC-dextrans was observed *ex vivo* using porcine nasal mucosa. Using the human nasal-replica cast, greater deposition of 10 μm TSP-microparticles in the olfactory region was observed compared to TSP-microparticles 2 μm in size. Collectively, these findings support our hypothesis that 10 μm-sized mucoadhesive microparticles can achieve selective deposition and retention of drug in the olfactory mucosa.

1. Introduction

Evidence of direct nose-to-brain transport of a variety of compounds in recent years has rejuvenated interest in intranasal delivery as an approach that can circumvent the blood-brain barrier (BBB) and deliver therapeutic molecules to the brain (Lochhead & Thorne, 2012). Studies in rats (Thorne, Pronk, Padmanabhan, & Frey, 2004) and monkeys (Thorne, Hanson, Ross, Tung, & Frey, 2008) suggest that direct nose-to-brain transport of molecules into the brain occurs via the olfactory or the trigeminal nerve pathways. A direct nose-to-brain route also offers potential advantages such as rapid onset of drug action, avoidance of systemic side effects, avoidance of "first-pass" metabolism in the liver and reduced doses of drug for an equal or better therapeutic effect (Lochhead & Thorne, 2012).

Targeted delivery and retention of drug formulations on the olfactory mucosa, the target site for nose-to-brain drug absorption, however, is a major challenge due to the geometrical complexity of the nose and active nasal clearance (Djupesland, 2013). Furthermore, the volumes of aqueous solution required to dissolve most drug molecules often cannot be accommodated by the upper, neuron-containing, region of the nasal passage and are instead swallowed or lost to dripping (Kapoor, Cloyd, & Siegel, 2016). The percentage of drug shown to reach the brain via the nose in animal experiments is thus typically very small (much less than 1%) (Casettari & Illum, 2014). Often powder formulations are preferred to liquids for intranasal drug delivery as they are less readily cleared from the nasal cavity compared to liquid preparations.

Mucoadhesion is crucial to increasing the residence time of deposited particles in the nasal cavity. Consequently, a common approach to increase the residence time of drugs on the olfactory

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epithelium has been to incorporate them into particulate-carriers which have been formulated with polymers that can interact with mucin. Given that mucin is a major constituent of mucous, formulations prepared using polymers which can interact with mucin enable mucoadhesion and retention of the formulation on the olfactory mucosa (Ugwoke, Agu, Verbeke, & Kinget, 2005).

Deposition of particles within the geometrically complex human nasal cavity is influenced by the size of the constituting particles. Particles larger than 20 μm show preferential deposition in the anterior part of the nasal cavity due to high inertial impaction, while particles less than 5 μm escape the nasal cavity, following the air stream lines, and deposit in the lungs (El-Sherbiny, El-Baz, & Yacoub, 2015; Shi, Kleinstreuer, & Zhang, 2007). Recent modelling data indicates that particles around 10 μm in size show maximum deposition in the olfactory region when administered intranasally at normal inhalation rates of around 20 L/min (Schroeter, Tewksbury, Wong, & Kimbell, 2015; Shi et al., 2007). The above findings suggest that formulating drug into *mucoadhesive microparticles which are 10 μm in size* can potentially target and increase the residence time of drug on the olfactory mucosa after intranasal administration. This proof-of-concept study tests this hypothesis with 10 μm-sized microparticles prepared using the naturally occurring mucoadhesive polymer, tamarind seed polysaccharide (TSP) and fluorescently-labelled dextrans (3–40 kDa) as model drugs in *in vitro* and *ex vivo* models. To the best of our knowledge this combined formulative approach has not reported elsewhere in the literature.

Tamarind seed polysaccharide (TSP) is a highly branched polysaccharide with a molecular weight of 720–880 kDa and is obtained from the endosperm of *Tamarindus indica* seeds. TSP is composed of a (1 → 4)-β-D-glucan backbone substituted with side chains of α-D-xylopyranose and b-D-galactopyranosyl (1 → 2)-α-D-xylopyranose linked (1 → 6) to glucose residues (Kaur, Yadav, Ahuja, & Dilbaghi, 2012). TSP is GRAS approved by the FDA and is commonly used as an excipient in food and pharmaceutical preparations mostly as a thickener or stabiliser due to its gelling properties (Gupta, Puri, Gupta, Jain, & Rao, 2010; Kulkarni, Dwivedi, & Singh, 1998). TSP has also been investigated for a number of drug delivery applications (Avachat, Gujar, & Wagh, 2013; Pal & Nayak, 2012) (Sumathi & Ray, 2003), largely due to biodegradability and stability under acidic pH conditions.

Microparticles prepared with other naturally occurring mucoadhesive polymers such as chitosan, have shown promising nose-to-brain transfer of several drugs (Casettari & Illum, 2014). The mucoadhesive properties of polymers such chitosan is elicited mainly through the interaction of positively charged amino groups with sialic acid in the mucous layers. Chitosan is also able to transiently open the tight junctions between epithelial cells and increase epithelial membrane permeability, which can cause acute mucosal toxicity (Croisier & Jérôme, 2013). However, very little is known about the effect of long-term exposure of the nasal mucosa to chitosan. Unlike chitosan, TSP is uncharged under physiological conditions (Gupta et al., 2010) and therefore less likely to cause nasal toxicity. Mucoadhesion of TSP, like other polysaccharides most likely occurs as a result of the hydroxyl groups interacting with mucin through hydrogen bonds and van-der-Waals attraction (Smart, 2005).

TSP is already available in the market as an eye drop for treating for dry eyes. Clinically, TSP-containing eye drop formulations have demonstrated efficacy and tolerability with increased retention on the eye surface when compared to a number of competitor products (Jacobi, Kruse, & Cursiefen, 2012; Rolando & Valente, 2007). The increased residence time of TSP-containing formulations on the eye after intraocular administration has been attributed to the structural similarity between TSP and the transmembrane glycoprotein mucin 1, which is thought to protect and wet the corneal surface of the eye (Gupta et al., 2010; Rolando & Valente, 2007).

Table 1
A 4 factor (independent factors) 3 level (low, base and high) Box-Bhenken design used to determine the effect of independent factors on mode size of the microparticles. A P-value of <0.05 was taken to be significant.

Independent factor	Coded level			P-value
	Low (−1)	Base (0)	High (+1)	
Atomizing air flow (L/h)	246	494	742	0.0005
Aspiration air flow rate (%)	50	60	70	0.0190
TSP:FITC-dextran ratio (%w/w)	9:1	3:1	1:1	0.4877
Inlet temperature (°C)	100	110	120	0.3652

2. Materials and methods

2.1. Materials

Tamarind gum powder was purchased from Xi'an Jiatian Biotechnology (China). Mucin (from bovine submaxillary glands Type I-S, M3895) and fluorescein isothiocyanate (FITC)-labelled dextrans with molecular weights of 3–5, 10, 20 and 40 kDa were purchased from Sigma-Aldrich (New Zealand). All water used in this study was ion exchanged distilled and passed through a MilliQ water purification system (Millipore, Bedford, MA, USA).

2.2. Isolation of TSP

TSP was isolated from tamarind gum powder using methods described by Rao and Krishna (1947). Briefly, 20 g of tamarind gum powder was dispersed in 200 mL of MilliQ water to obtain a slurry, which was then mixed with a further 800 mL of MilliQ water and boiled for 20 min under constant stirring (800 rpm). This dispersion was kept overnight to allow proteins and fibres to sediment and subsequently centrifuged at 5000 rpm for 20 min, the supernatant was separated and mixed with twice the volume of absolute ethanol under constant stirring. This led to a precipitate of TSP, which was washed with MilliQ water and dried at 60 °C for 12 h to obtain a film of TSP. The film was crushed to a fine powder and stored in a desiccator until required.

2.3. Preparation of TSP-microparticles

All microparticle formulations in the study were prepared by spray drying using a laboratory-scale Mini Spray Dryer (Büchi B-290, Büchi Labortechnik AG, Switzerland). The effect of spray drying process variables; atomizing air flow, aspiration air flow rate, TSP:FITC-dextran ratio and inlet temperature (independent factors) on the size of microparticles (response) was determined using a 4 factor, 3 level (low (-1), base (0) and high (+1)) Box-Bhenken design as shown in Table 1 in order to identify the parameters required to produce microparticles with an average size of 10 μm. A total of 29 experiments were designed and analysed using Design-Expert® Software Version 9 (Stat-Ease Inc., MN, USA). The effect of independent variables upon the response was modelled using a linear mathematical model. One-way Analysis of Variance (ANOVA) was applied to determine the significance of the model ($P < 0.05$). Contour plots were analysed and are reported to show the effect of independent factors on microparticle size.

Feed solutions for spray drying were prepared as follows. TSP was dissolved in water under constant stirring at 60 °C for 2 h and cooled to room temperature. To this solution, different amounts of FITC-dextrans were added and mixed for a further 1 h. The solutions were spray-dried with a standard nozzle cap with an orifice diameter of 0.7 mm and feed solution flow rate of 2 mL/min. The