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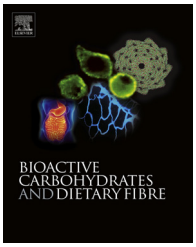

Guluronate oligosaccharides as enhancers of nanoparticle drug delivery in the oral cavity

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ABSTRACT

Guluronate oligomers have been shown to modify the gastrointestinal mucus barrier and improve nanoparticle penetration. Here we investigate the effect of guluronate oligomers on nanoparticle mobility in saliva, a central extracellular barrier in the oral cavity. Guluronate oligomers reduced nanoparticle aggregation in saliva and improved nanoparticle mobility over and above that which would be expected to result from the decrease in size. As such guluronate oligomers may be of interest for nanomedicine delivery in the oral cavity.

1. Introduction

Drug delivery is a central facet of drug development. Even the most potent new molecules (new molecular entities or NME) can only be developed into successful drugs if they can be satisfactorily delivered to their site of action (Shi, Votruba, Farokhzad, & Langer, 2010).

Nanoparticle drug delivery is a rapidly expanding area of research potentially offering significant improvements over traditional delivery methods (Singh & Lillard, 2009). Benefits of nanoparticle drug delivery may include (Farokhzad & Langer, 2009) improved delivery of poorly water soluble drugs targeted delivery to specific cells or tissues; transcytotic access across tight epithelial or endothelial barriers, taking advantage of existing cellular machinery; and protection and delivery of large macromolecular drugs to intracellular sites of action.

Intravenous administration of nanomedicines (suspensions of drug carrying nanoparticles) such as Doxil and Abraxane has shown improved clinical outcome over traditional formulations of the same drug (Adair, Parette, Altinoglu, & Kester, 2010). However, there may be even great benefits to nanomedicines when other, less invasive, delivery routes are considered, for example mucosal drug delivery. Such routes offer benefits over the parenteral route, requiring no injection (and therefore no trained staff), being associated with lower hygiene requirements and infection risk and having better patient compliance (Babiuch, Gottschaldt, Werz, & Schubert, 2012). The challenges associated with mucosal drug delivery are not trivial, involving both potential degradation in harsh environments (particularly in the gastrointestinal tract) and the ubiquitous defensive mucus secretions of mucosal surfaces. And here again, nanotechnologies offer potential solutions to these problems, both

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through encapsulation, and thus protection, of the drug moiety, and surface modifications to overcome the biological barriers. Ideal characteristics for a nanoparticle for drug delivery have been widely discussed (Adair et al., 2010). The nanoparticle should be composed of non-toxic materials and the degradation products of those materials should also be non-toxic; there should exist a physiological clearance mechanism for the nanoparticle to prevent long term accumulation in the patient and the nanoparticle should have a sufficiently long circulation time to effectively deliver its cargo. The nanoparticle must also be colloidally stable in suspension before administration and in biological media. It is preferable if the nanoparticle encapsulates therapeutic cargo (as surface loading exposes drug to degradation), is able to be targeted to the cell or tissue of interest and can achieve controlled release of therapeutic cargo.

When considering mucosal drug delivery, colloidal stability in the relevant mucosal secretions is inherently important, as is the ability to escape mucus entrapment and cross epithelial barriers.

The oral cavity has been investigated as a site for both local and systemic drug delivery with local indications including gingivitis, oral candidosis and oral cancers (Smart, 2005). In terms of systemic drug delivery the oral cavity, most specifically the sublingual and buccal mucosae, offers a number of benefits; they are easily accessible, do not have the harsh enzymatic and pH environment found further down the GI tract and avoid the problems of 1st pass metabolism (Smart, 2005). Additionally these mucosae have been estimated to be 4–4000 times more permeable than another easily accessible surface, the skin (Roblegg et al., 2012). The sublingual mucosa is also of interest as a site for mucosal vaccination (Kweon, 2011). Recently, there has been increased interest in the buccal mucosa as a site for delivery of nanoparticle drug formulations (Morales & McConville, 2014; Teubl et al., 2013; Yuan, Fu, Kao, Janigro, & Yang, 2011), increasing the need for understanding of how nanoparticles interact with the oral environment.

The primary barriers of the oral mucosa are the saliva, the mucus layer, cell junctions in the epithelia and membrane coating granules found within the epithelia (Roblegg et al., 2012; Tabak, 1995). In this paper we will address the extracellular barriers of the saliva and the mucus layer in relation to nanoparticle drug delivery.

Saliva is a mucous secretion containing water, salts, enzymes and glycoproteins, including the secreted mucins MUC5B (MG1) and MUC7 (MG2)(Roblegg et al., 2012; Tabak, 1995). MUC5B (MG1) is a high molecular weight polymeric mucin and is the main constituent of the mucus layer, or salivary pellicle, whereas the monomeric MUC7 (MG2) is predominantly found in the lower viscosity salivary fluid (Tabak, 1995) where it contributes to the aggregative properties of saliva (Ericson, Pruitt, & Wedel, 1975; Tabak, 1995; Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005), which form a primary defence against bacteria and viruses. The mucus layer of the oral cavity is similar to the mucus layer covering other mucosal sites within the body (Roblegg et al., 2012) however with an estimated thickness of 70–100 μm (Collins & Dawes, 1987), it is somewhat thinner than the mucus layers found in the rest of the GI tract. These extracellular barriers pose a challenge to nanoparticle drug delivery that is dependent on size, charge and surface structures (Florence & Hussain, 2001; Glantz, Friberg, Christersson, & Baier, 1995; Roblegg et al., 2012). Particularly, it has been shown that 200 nm diameter negatively charged particles are both substantially aggregated by saliva components and unable to penetrate the buccal mucus layer (Roblegg et al., 2012).

Mucus is well recognized as a challenge for mucosal delivery of nanomedicines in the GI, respiratory and genitourinary tracts, where it acts as both steric and interactive barrier to nanoparticle mobility and hindering nanoparticle access to the cell surface and thereby cellular uptake. We have previously shown that guluronate oligomers, isolated from alginate, are able to modify the barrier properties of mucus (Nordgård, Nonstad, Olderøy, Espevik, & Draget, 2014), and these oligomers have also been shown to modify bacterial biofilms (Powell et al., 2013). It is therefore of interest to investigate whether these oligomers are able to modulate the extracellular barriers to nanoparticle drug delivery in the oral cavity.

2. Materials and methods

2.1. *Guluronate oligomers (G-blocks)*

The low molecular weight guluronate oligomer samples were obtained by acid hydrolysis of high molecular weight alginates with a high content of guluronic acid residues as previously reported (Haug, Larsen, & Smidsrod, 1966,1967). Chemical composition and sequence were determined by ^{1}H NMR spectroscopy (Grasdalen, 1983), and revealed that the fractions of guluronate containing monad (F_G), diad (F_{GG}) and triad (F_{GGG}) were 0.94, 0.83 and 0.80, respectively. The distribution as well as the number-average degree of polymerization (DP_n) of the this oligoG sample used was quantified as described earlier (Gimmestad et al., 2009) applying anionic exchange chromatography, HPAEC-PAD (Dionex BioLC System, Dionex Corporation, Sunnyvale, Ca). The chromatographic spectrum revealed an average $DP_n = 12$ with 40% of the molecular population in both the DP range of less than 10 as well as in the range 10–20. No high molecular weight tail was observed.

2.2. *Saliva*

Non-stimulated human saliva was collected from a healthy volunteer at least 2 h after eating or drinking and used immediately.

2.3. *Nanoparticles*

Yellow–green fluorescent FluoSpheres with 200 nm diameter and a carboxylate modified surface (2% solids in distilled water containing 2 mM sodium azide) were purchased from Invitrogen Molecular Probes.

2.4. *Confocal microscopy and particle tracking*

Samples of saliva (200 μl) were placed in the chambers of an 8- chambered coverglass (Nunc Lab-Tek). For control samples