Surface-Dependent Mechanism for Transcellular Permeation of Polymeric Nanoparticles Across Intestinal Monolayers

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Introduction

Nanomedicines generally utilise 2 approaches; orally delivered solid drug nanoparticles (SDNs) or injected drug carriers[1-3]. Oral delivery remains the preferred route of administration in the treatment of certain chronic diseases largely due to practicality. However, nanocarriers are rarely administered orally. This is in-part due to the challenges associated with the diffusion of intact particulates from the gut and into the systemic circulation. Delivery of intact particulates is often a prerequisite for subsequent downstream targeting strategies and a greater understanding of the mechanisms that underpin these processes are required for informed particle design[4-6]. Orally delivered nanocarriers may be worth exploring further for certain diseases, like HIV, where daily injectables have failed to meet clinical needs. **Aims:** To investigate the suitability of polymeric nanoparticles with differing surface functionalities for the oral delivery of a poorly water-soluble compound and investigate the mechanisms that underpin the translocation of nanocarriers across Caco-2 monolayers as a model for the intestinal epithelium, utlising various pharmacological inhibitors.

Results

Nanoparticle characteristics

Polymeric nanocarriers were prepared using a nanoprecipitation technique and surface functionalised with either PEG or amine functional dendron. The lipophilic dye, Fluoresceinamine was encapsulated into the nanocarriers and used as a traceable surrogate for insoluble drugs. The characteristics of the particles used in the study are outlined in Figure 1.

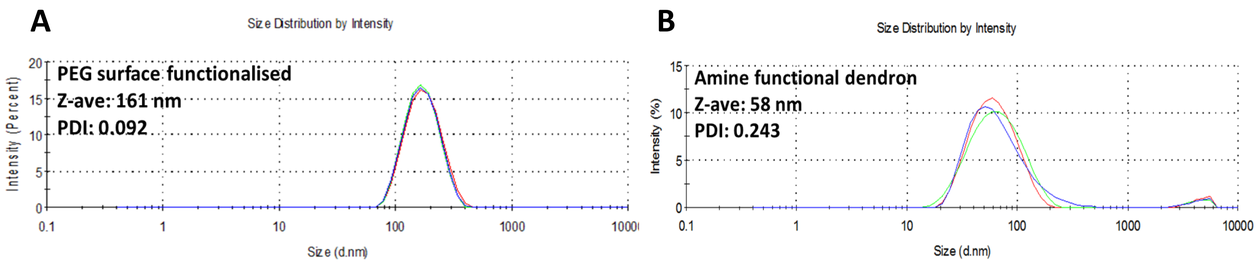

Figure 1. Dynamic light scattering analysis of (A) PEG surface functionalised and (B) amine functional dendron nanocarrier suspensions (Malvern Instruments, UK).

Cellular Accumulation

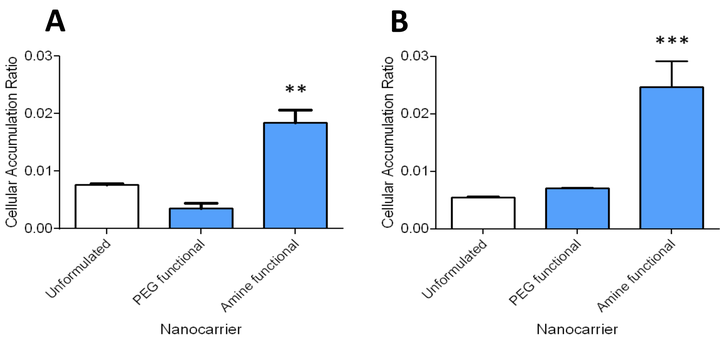

Figure 2. CAR of Fluoresceinamine in (A) Caco-2 and (B) ATHP-1 cells following 4 h incubation at 37°C 5% CO_2 with unformulated or nanocarrier encapsulated Fluoresceinamine.
**, P<0.01; and ***, P<0.001 (ANOVA) (n=4)

The Cellular Accumulation Ratio (CAR) of both unformulated and nanocarrier encapsulated Fluoresceinamine was determined in (A) Caco-2 and (B) ATHP-1 cells. Figure 2, suggests up to a 2- and 4.5-fold increase in the accumulation of Fluoresceinamine in Caco-2 and ATHP-1 cells, respectively, when treated with the amine functionalised dendron nanocarriers, compared with the unformulated preparation. The Caco-2 and ATHP-1 cells treated with PEG functionalised nanocarriers displayed comparable or reduced accumulation compared to the unformulated control.

Transcellular permeation

The results in Figure 3 indicate that the PEG and amine functional dendron nanocarriers increased the apparent oral absorption of Fluoresceinamine over 5- and 3-fold, respectively, compared to the unformulated equivalent. Suggesting an overall increase in the absorption of Fluoresceinamine from the gut and into the systemic circulation.

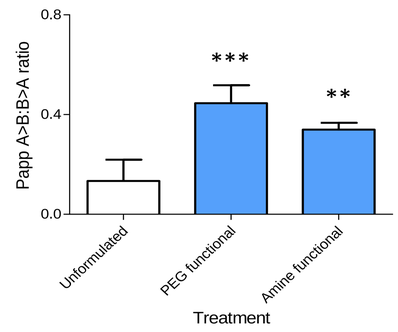

Figure 3. The Papp ratio of unformulated and nanocarrier encapsulated Fluoresceinamine. Monolayers were incubated for 4 h at 37°C 5% CO_2. **, P<0.01; and ***, P<0.001 (ANOVA) (n=4)

Endocytosis inhibition

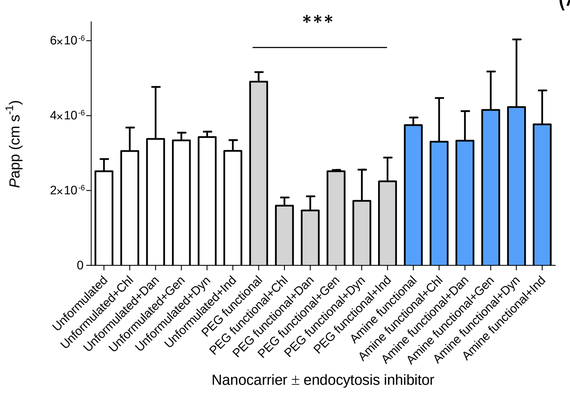

Figure 4. The Papp of Fluoresceinamine following incubation with the inhibitors: chlorpromazine (Chl); dansylcadaverine (Dan); genistein (Gen) or Indomethacin (Ind) with unformulated or nanocarrier encapsulated equivalents. Monolayers were pre-incubated for 30 min with the outlined inhibitors and co-incubated for 4 h at 37°C 5% CO_2.***, P <0.001 (ANOVA) (n=4).

An investigation into the effects of co-incubating Caco-2 monolayers with both clathrin- and caveolae-mediated endocytosis inhibitors and unformulated or nanocarrier encapsulated Fluoresceinamine revealed significant differences in the dyes ability to transverse the monolayer (Figure 4):

- All the inhibitors caused a significant reduction in Fluoresceinamine Papp when delivered via the PEG-functionalised nanocarriers. Up to a 70% reduction in Papp was observed.
- Variable Fluoresceinamine Papp was observed when the monolayers were incubated with the amine functional dendron nanocarriers, regardless of inhibitor.
- Co-incubation of the monolayers with the unformulated preparation and endocytosis inhibitors led to an overall increase in Fluoresceinamine Papp.

Discussion

- Two distinct polymeric nanocarriers were developed with either PEG or dendron surface functionalisation and loaded with the traceable hydrophobic dye Fluoresceinamine.
- Differences in Fluoresceinamine's ability to accumulate and transverse Caco-2 monolayers suggested distinct mechanisms of particle uptake and translocation between delivery platforms.
- Co-incubation of the nanocarriers with various clathrin- and caveolae-mediated endocytosis inhibitors suggested the PEG surface functionalised nanocarriers utilised a predominantly endocytic process to allow Fluoresceinamine to translocate across the monolayer. However, translocation was not effected when delivered using the amine functional dendron nanocarriers suggesting an alternative translocation route, possibly involving paracellular processes.
- These data provide *in vitro* support for permeation of intact nanocarriers across the intestinal epithelium via distinct mechanisms that appear to be highly dependent on surface composition.
- Tuneable oral absorption of intact nanocarriers potentially allows for drug targeting that is adaptable for specific disease requirements and therefore warrants further investigation.

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