In-vivo Analysis of Lipid Impact on Intestinal Mucosa Barrier

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Statement of Purpose: Intestinal mucus is a highly viscoelastic semipermeable medium that provides a robust barrier against potentially hazardous microorganisms, yet allows efficient absorption of nutrients at the underlying epithelium. These conflicting roles are particularly important in the small intestine where the main absorption of nutrients and drugs occurs. However, the mechanisms of this selective barrier function have not been studied extensively. We have previously shown that food-associated exogenous lipids significantly impact particle transport in collected (scraped) porcine intestinal mucus and across intact mucus on a rat intestinal explant. In this investigation, the influence of lipids on model particulate drug carriers in rat intestinal mucus was studied after lipid and nanoparticle dosing to live rats to enable exploration of impact of lipids in-vivo. Results were analyzed in consideration of the significance of mucus barrier alteration upon food arrival.

Methods: 70-90 day old male Sprague-Dawley rats were dosed with 2 ml of water (control) or lipid (soybean oil) by oral gavage. Soybean oil was stained with 0.01% Sudan IV solution prior to dosing to enable observation in the GI tract using fluorescence microscopy. Rats were anesthetized using 2.5-3 % isoflurane vapor, and 200 nm carboxylate-modified microspheres diluted in PBS (0.0025 wt.-%) were injected into the duodenal lumen. Rats were kept anesthetized under 2.5 % isoflurane on a warm water blanket for 30 min to allow particle diffusion into the mucus. Intestinal tissues were harvested and cut open to allow imaging of mucus using an Olympus IX51 fluoresence microscope for multiple particle tracking experiments (MPT)[1] and laser scanning confocal microscope for analysis of particle distribution. Particle trajectories were used to calculate time-averaged mean squared displacements and effective diffusivities (D_{eff}): $MSD = [x(t+\tau)-x(t)]^2 + [y(t+\tau)-y(t)]^2$ and $D_{eff} = MSD/(4\tau)$ where x and y are positional data and τ is the time scale. Three independent experiments were done for each condition. A one tailed, unequal variance Student's t-test was used to determine significance ($P<0.05$). To further explore distribution of particles in mucus, histological staining was used. Intestinal tissues were fixed in Carnoy's solution, an effective fixative for high water content mucus[2], dehydrated, and embedded in paraffin. 5 µm thick sections were stained in 25 µg/ml lectin, Ulex europaeus agglutinin (UEA-1) conjugated with TRITC, to detect mucins and 10 µg/ml of Hoescht 33342 (nuclei).

Results:

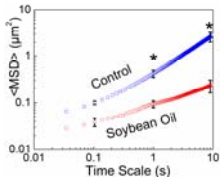

Figure1. Ensemble <MSD> versus time scale plot, experiments repeated three times and n≥100 for each experiment. Error bars denote standard error, * indicates statistically significant difference compared to soybean oil. ($P<0.05$)

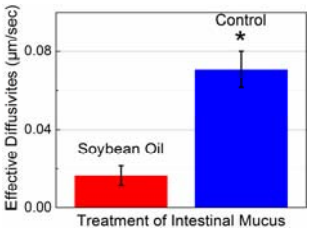

Figure2. Ensemble <Deff> at t = 10 s. Impact of lipids on particle transport rate is significant. Error bars = standard error, * = statistically significant difference compared to soybean oil. ($P<0.05$)

The transport rates of particles in control and soybean oil cases were quantified by their time scale-dependent mean- squared displacements (<MSD>). Samples treated with lipids displayed strongly hindered particle transport in mucus while they were able to diffuse in mucus exposed only to buffer (Fig.1). At a time scale of 10 s, transport rates of 200 nm carboxylate-modified particles after lipid dosing were nearly 5-fold lower than those of same-size particles in control (**Fig. 2**). This may result from altered intermolecular interactions, including possibly lipid-particle and/or lipid-mucus interactions. Furthermore, cross-section images of intestinal mucus Indicate particles were capable of penetrating the mucus barrier and reaching the underlying epithelium within control groups, where they were blocked and did not penetrate after oral dosing of soybean oil (**Fig. 3**), supporting mucus barrier alteration upon oral dosing of lipids.

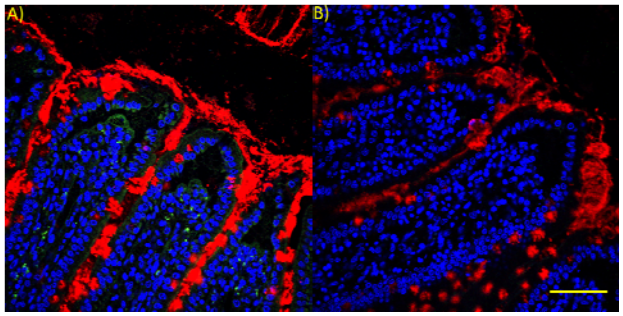

Figure 3. Confocal imaging reveals particle penetration across mucus barrier. Upon mucus exposure to A) Control; particles were able to penetrate through mucus barrier; B) Soybean oil; particles were hindered from penetrating across mucus structure. Bar = 20 µm

Conclusions: Food-associated lipids strengthen the intestinal mucosal barrier against model drug carriers. These results provide some insight into the impact of lipids on oral drug delivery, especially using particulate or lipid-based drug delivery systems.

References:

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2. Matsuo K, Ota H, Akamatsu T, Sugiyama A, Katsuyama T. Gut (1997) 40: 782–789.