Biosimilar Intestinal Mucus Protects Caco-2 Epithelial Monolayers from Damage Caused by Fed State Intestinal Components and Alters Permeation Enhancer Efficacy

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**Purpose**
In order to thoroughly assess the potential of orally administered drugs, in vitro models such as the Caco-2 cell culture model is often utilized to assess the propensity of a drug to translocate across a biological membrane. When cultured on permeable filter inserts, Caco-2 cells form a monolayer of absorptive enterocytes that mimics the characteristics of the intestinal membrane. However, a limitation for the utilization of the Caco-2 cell culture model is the absence of a mucus layer. The mucus in the small intestine acts as a protective barrier to detrimental effects by intestinal fluid components (e.g. bile salts, lipids) and pathogens. As such, the absence of the mucus layer prevents drug delivery studies in the presence of highly concentrated fluids (e.g. during the fed state). By addition of a biosimilar mucus layer, recently developed by Bøgh et al., the mucin barrier properties of the mucus can be introduced to the model. The objective of this study was to assess the effect of biosimilar mucus (BM) as a protectant against detrimental damage caused by intestinal fluid components under fed state conditions. Further, the aim was to elucidate to which extent the delivery propensity of fatty acid permeation enhancers would be affected by the presence of mucus for delivery enhancement of both small and large molecular weight (Mw) drugs.

**Methods**
Caco-2 cell culture monoculture and HT29 cell culture monoculture and Caco-2/HT29 co-culture models were cultured on permeable filter inserts for 18-20 days. The cells were then kept with or without BM, as well as test solutions containing fed state simulated intestinal medium, fatty acids or a combination hereof, for 4 hours. The permeation rate of small and large test moleculars, transepithelial-electrical resistance (TEER) and relative viability of the cells following exposure to test solutions was utilized to determine the effect of BM.

**Results**
Caco-2 cell monolayers kept with BM and buffer displayed no effect on TEER, viability or permeation rate of a small Mw paracellular marker as compared to cells kept without BM. No changes to this were observed for Caco-2 cell monolayers kept with BM and fed state simulated medium. In contrast, cells kept with fed state medium in the absence of mucus was associated with a > 90 % decrease in TEER and viability, and a significant increase in permeation rate via both the transcellular and paracellular pathway. Similar observations were done for HT29 cell monocultures and Caco-2/HT29 cell co-cultures, albeit to a less severe degree. Cells kept with BM in the presence of fatty acids displayed an increased permeation rate for paracellular markers at non-cytotoxic concentrations. The effect was more pronounced when co-incubated with fed state simulated medium as compared to buffer. In comparison, Caco-2 cell monolayers kept without BM, HT29 cell monolayers or Caco-2/HT29 co-cultures were susceptible to detrimental effects caused by the fatty acid permeation enhancers at significantly lower concentrations.

**Conclusion**
This study revealed that BM protects Caco-2 cell epithelium from detrimental effects caused by the presence of fed state intestinal components for up to 4 hours of exposure. Furthermore, an increased paracellular permeation rate can be obtained in the presence of fatty acid without causing detrimental effects to the cells. In addition, drugs permeating by the transcellular route is affected to a degree, dependent on drug properties. Comparing the BM to other mucus-containing models, it was observed that cells kept with BM suffered from less detrimental effects when incubated with an array of permeation enhancers or membrane active compounds, as compared to established mucus models.

**References**
1 Hidalgo et al. Gastroenterology. 1989 Mar;96(3):736-49