RATIONALLY DESIGNED PEPTIDES ALTER INTESTINAL TIGHT JUNCTION COMPOSITION AND INCREASE PERMEABILITY TO POORLY ABSORBED DRUGS

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Background: Tight junctions (TJs) form a semi permeable barrier between epithelial cells, and in the intestine represent a major barrier to drug absorption. TJs consist of a number of transcellular proteins, such as occludin and claudins, which are anchored to the actin cytoskeleton and interact with TJ proteins from adjacent cells. TJ proteins have different barrier properties and the make up of TJs affects their permeability. TJs can be opened by phosphorylation of myosin light chain (MLC), which is controlled by MLC kinase (MLCK) and MLC phosphatase (MLCP). MLCP is formed by a protein phosphatase subunit (PP1) associating with a targeting subunit (MYPT1) via an interaction at a specific binding motif. We have designed **P**ermeable **I**nhibitor of **P**hosphatase (PIP) peptides that bind PP1 and inhibit MLCP to increase TJ permeability.

Methods: PIP peptides were applied to the apical surface of Caco-2 monolayers in addition to 4 kDa fluorescent dextran (FD4). Basal concentration of FD4 was measured and used to calculate apparent permeability (P_{APP}) with and without PIP peptides. TEER measurements were taken before and after PIP application. At the end of experiments, Caco-2 cells were lysed and levels of tight junction proteins were analysed by western blot. PIP peptides (20mM) were injected into the intestinal lumen of rats with 200 mg/kg exenatide (Ex4) or salmon calcitonin (sCT). Blood samples were taken and EX4 and sCT concentration was measured by ELISA. Injection sites were fixed and stained for visualization of tight junction proteins.

Results: Two lead peptides were tested, PIP250 and PIP640. Both peptides increased pMLC and increased P_{APP} of FD4 from 0.11×10^{-6} cm/s to 0.315 and 0.453×10^{-6} cm/s respectively. Positively charge dextran had a higher P_{APP} with both PIP640 (0.72×10^{-6} cm/s) and PIP250 (0.48×10^{-6} cm/s). PIP250 and PIP640 reduced TEER to 35% and 49% of baseline respectively. PIP640 increased claudin-2 1.6x and PIP250 increased it 1.2x in Caco-2 cells. PIP250 reduced occludin levels to 60% of baseline in caco-2 cells, whereas PIP640 had no effect. Intraluminal injection of Ex4 or sCT alone did not produce detectable serum concentrations. PIP250 increased Ex4 and sCT serum concentrations to 10.4 ng/mL and 10.5 ng/mL respectively. PIP640 increased serum concentrations to 10.14 ng/mL and 14.6 ng/mL respectively. PIP250 treated tissue showed a redistribution of occludin away from TJ localisation to the lateral membrane of epithelial cells.

Conclusions: Both peptides increased permeability in *in vitro* and *in vivo* models, demonstrating potential as permeation enhancers. Both also increase pMLC but also appear to have different permeation enhancement profiles. PIP640 produced a more rapid increase in permeability than PIP250. Permeability mediated by both peptides was preferential to positively charged molecules, but the effect was considerably greater with PIP640. PIP640 also increased claudin-2 levels more than PIP250. Claudin-2 is associated with cation selectivity at tight junctions. This suggests that PIP mediated claudin-2 upregulation contributes to cation selectivity of permeability. PIP250 also causes occludin down-regulation, which increases permeability. PIP640 does not have an effect on occludin. PIP250 occludin down-regulation takes longer to happen than pMLC increase, which may explain why PIP250 mediated increase in permeability is slower than PIP640. We have demonstrated two peptides that can enhance intestinal drug delivery *in vitro* and *in vivo*. The difference in permeability characteristics means there is a potential to use either peptide with specific drugs to enhance oral bioavailability.