Acylation of Therapeutic Peptides: Interaction with Lipid Membranes and its Implications in Oral Delivery

Oral administration of therapeutic peptides could benefit millions of chronically ill people worldwide, through easier and less stigmatized therapy, and likely improve the long-term effects of currently widespread disease mismanagement. However, oral peptide delivery is a formidable task due to the harsh and selective gastrointestinal system, and development has lacked far behind injection therapy.

Peptide acylation is a powerful tool to alter the pharmacokinetics, biophysical properties and chemical stability of injectable peptide drugs, primarily used to prolong blood circulation, but it is not widely studied in an oral context. As acylation furthermore increases interactions with the lipid membranes of mammalian cells, it offers several potential benefits for oral delivery of therapeutic peptides, and we hypothesize that tailoring the acylation may be used to optimize intestinal translocation.

This work aims to characterize acylated analogues of two therapeutic peptides by systematically increasing acyl chain length in order to elucidate its influence on membrane interaction and intestinal cell translocation in vitro. The studied peptides are the 33 amino acid Glucagon-like peptide-2 (GLP-2), which promotes intestinal growth and is used to treat bowel disorders such as inflammatory bowel diseases and short bowel syndrome, and the 32 amino acid salmon calcitonin (sCT), which lowers blood calcium and is employed in the treatment of post-menopausal osteoporosis and hypercalcemia. The two peptides are similar in size and structure, but oppositely charged at physiological pH. Both peptides were acylated with linear acyl chains of systematically increasing length, where sCT was furthermore acylated at two different positions on the peptide backbone.

For GLP-2, we found that increasing acyl chain length caused increased self-association and binding to lipid and cell membranes, whereas translocation across intestinal cells displayed a nonlinear dependence on chain length. Short and medium chains improved translocation compared to the native peptide, whereas long chain acylation displayed no improvement in translocation. This indicates an initial translocation benefit for shorter chains through increased interaction with the cell membrane, which reverts to a hindrance for long chains, i.e. the analogues get stuck in the cell membrane. Co-administration of a paracellular absorption enhancer was found to increase translocation similarly for each analogue while retaining acyl chain length dependence. A transcellular enhancer displayed increased synergy with the long chain acylation, consistent with increased membrane fluidization ‘liberating’ bound peptide, although medium chain acylation remained optimal overall. The results indicate that rational acylation of GLP-2 can increase its in vitro intestinal absorption, alone or in combination with permeation enhancers, and are consistent with the initial project hypothesis.

For sCT, an unpredicted effect of acylation largely superseded the anticipated membrane interactions; i.e. acylated sCT acted as its own in vitro intestinal permeation enhancer. Acylated analogues permeabilized lipid membranes, causing drastically increased peptide permeability through reversible cell effect similar to transcellular permeation enhancers. The effect likely stems from a synergy between the positive peptide charge and membrane-active acyl moiety, supported by its pH-dependency, whereby the effect increased with decreasing pH and concomitant charge increase. The extent of permeation enhancing effect was highly dependent on acylation chain length and position, with highest peptide permeability for short chain N-terminal acylation or medium/long chain Lys18 acylation, whereas permeability and cell membrane binding appeared correlated only for some analogues. However, prolonged heating and/or solution storage of certain acylated sCT analogues caused aggregation in physiological buffer solutions, potentially forming fibril-like structures. Lys18 acylation appeared superior to N-terminal acylation, most clearly exemplified by the short chain analogues, however, no systematic dependence on acylation chain length was apparent. All analogues could be monomerized by addition of cycloextrin, however, their separate permeability enhancing effects were reduced in the mixtures, and the additive was not investigated further. Thus, acylation of sCT for oral delivery purposes may not be indiscriminately applicable, and requires rational choices of acylation details and/or additives.

Further investigations of the most cell-permeable sCT analogues unveiled quite distinct permeation enhancer effects, ranging from high membrane binding / high permeability and non-specific enhancing effect on model compounds, to very low membrane binding / high permeability and very limited unspecific permeation enhancer effects, i.e. selective and efficient translocation. Peptide receptor potency was retained for GLP-2 analogues following acylation, whereas sCT analogues displayed substantially reduced potency, depending on acylation position and length. Overall, rational acylation of the studied peptides can increase in vitro intestinal permeability, modestly for GLP-2 and drastically for sCT, and might benefit oral delivery. GLP-2 results provide a well-founded predictive power for future peptide analogues, whereas sCT results hold great promise for future analogues, albeit with a larger uncertainty in predictions.