TOWARDS ORAL DELIVERY OF ARTIFICIALLY INTELLIGENCE-PREDICTED PEPTIDES

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Background: Research into oral delivery of peptides has been encouraged by the FDA-approval of an oral peptide tablet formulation of the GLP-1 agonist, semaglutide (Rybelsus®, Novo Nordisk). The preparation of oral peptide formulations is challenging and is challenged by gastrointestinal enzymes and the intestinal epithelial barrier leading to poor oral bioavailability. Production of stable potent peptides of a molecular weight < 5 kDA is a key factor in progress of oral formulations. A sustainable production by employing machine learning and *in vitro* laboratory approaches has created a library of food source-inspired proprietary peptides with potential health benefits at Nuritas Ltd. The novel bioactive peptides are still subject to the challenges of low intestinal permeability and require investigation for strategies to improve intestinal permeability and oral bioavailability. This study focusses on screening a selection of bioactive peptides using the *in vitro* Caco-2 monolayer assay in Transwells® and *ex vivo* rat jejunum assay using Ussing chambers.

Methods: The Caco-2 monolayer assay is typically performed after incubating the cells on polycarbonate Transwell® filter inserts for 21 days in 12 well plates. Male Wistar rats weighing 250-350 g are used to source tissue sections of jejunal mucosae. The muscle-stripped tissues are mounted on Ussing chambers bathed in oxygenated Krebs buffer on the apical and basolateral sides. Peptides are added to the donor side of monolayers and mucosae and are sampled over 120 min. Samples collected from the assays are analysed by LC-MS/MS.

Results: Transepithelial electrical resistance (TEER) data have been recorded to see effects on epithelial integrity and tight junction openings. Selected peptides have been studied for fluxes in combination with an established intestinal permeation enhancer.

Conclusions: Artificial intelligence-predicted novel bioactive peptides have potential for oral administration for nutraceutical and pharmaceutical applications. Combination of permeation enhancers with selected peptides in in vitro studies can offer an indication for specific formulation approaches to take for rat intestinal instillation and oral gavage experiments.