Hydrophobic Ion Pair for the Oral Delivery of Leucine-Enkephalin

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1. Purpose

The global therapeutic peptides market was valued at US$26.98 billion in 2019 and is projected to reach US$51.24 billion by 2027, growing at a compound annual growth rate (CAGR) of 8.7% from 2020 to 2027 (Research 2021). The peptide market is bound to grow by the increase in metabolic diseases andcancers but their translation remains challenging due to pre-systemic degradation, short plasma half-lives, and poor permeability across physiological barriers. Ion-pairing has been proposed as a method for the oraldelivery of peptides (Griesser, Hetenyi et al. 2017) as it enables increase stability to gastrointestinal enzymedegradation and enhanced permeability across physiological barriers. In this study, we explore the formationof an ion-pair of leucine-enkephalin (LENK), an endogenous opioid pentapeptide with applications as an oraltherapeutic for the treatment of pain (Lalatsa, Lee et al. 2012), Chron's disease, and other gastrointestinalinflammatory conditions, as a strategy to enhance its oral bioavailability (Owczarek, Cibor et al. 2011).

2. Method

Standard orthogenic solid phase peptide synthesis was utilized to synthesize LENK (0.5 mmole scale) (Lalatsa, Lee et al. 2012) and the peptide was obtained in high yield (>85%) and highpurity (>95%) as determined by HPLC and LC-MS. LENK (2.73 mmole, 1.52 mg/mL, 3mL) andsodium docusate (3mL) in deionized water (pH: 2.9) were mixed at different molar ratio (1:1,1:3, 1:5 respectively) to understand optimal ratio for pair formation and vortexed over 1 minute prior centrifugation or ultracentrifugation for 90 minutes at 4ºC at 40,000 rpm (Hitachi Ultracentrifuge CP1000 NX). The supernatant was separated and the pellet was frozen withliquid nitrogen and lyophilized for 24 hours (Teslar, -50ºC, 0.2mbar pressure). The LENK content was characterized by a previously validated HPLC method (Lalatsa, Lee et al. 2012). Intestinal fluid (50mM phosphate buffer, pH 6.6) was prepared from excised mouse intestine(C57BL/6, 8 weeks old, male) as previously described and characterized for protein contentusing the Bradford assay and diluted (1mg mL) (Lalatsa, Lee et al. 2012). Stability studies (37ºC, 50 rpm) were undertaken and the remaining LENK was analyzed using HPLC after dilution in ice-cold acetonitrile (1:1) and centrifugation. Permeability studies across a Caco-2 cell monolayer were undertaken for LENK, the ion-paired LENK, and antipyrine with FITC dextran (3-5 kDa) as an internal control as previously described (Hubatsch, Ragnarsson et al. 2007). Caco-2 seed density was 10,000 cells/cm in this experiment.

3. Results

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The 1:1 and the 1:5 ratios resulted in low ion-pairing yields of 37% and 19%. The optimal ratio for pairing was identified to be 1:3 which resulted in an ion-pairing yield of 56% and this ratio was further tested in stability and permeability studies. Intestinal wash was selected as it more closely describes in vivo data scenarios (McConnell, Basit et al. 2008). LENK degraded rapidly in the intestinal wash (< 60 minutes) while the ion pair showed a 3.5-fold increase in half-life and showed levels that were significantly different after the first initial 10 minutes (Student t-test, $p < 0.05$) (Figure 1). Permeability across the Caco-2 cells indicate a trend for higher uptake for ion-pair LENK, but due to low TEER values obtained in our experiments due to low cell seed density (Figure 2.).

4. Conclusion

Ion-paired LENK has shown a significant enhancement in oral gastrointestinal stability and further studies are underway to assess its oral bioavailability across Caco-2 monolayers. Combining ion-pair technology with solid state or additively manufactured formulations can enable the production of an oral LENK formulation for the treatment of pain and inflammatory diseases such as Chron's disease.

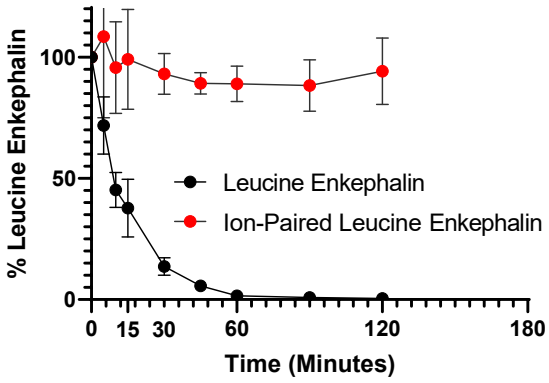

Figure 1. Leucine Enkephalin and Ion-Paired Leucine-Enkephalin stability comparison in mouse intestinal fluid over 2 hours (pH=6.6). Key: (-•-)Leucine-Enkephalin, (-•-) Ion-Paired Leucine Enkephalin.

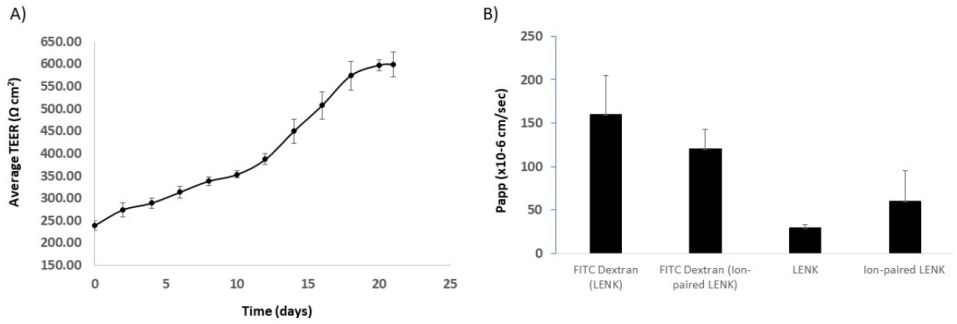

Figure 2. A) TEER values of the Caco-2 monolayers over 21 days of growing. B) Apparent permeability across Caco-2 cells.

5. References

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