In Vitro Metabolic Stability Evaluation of Peptides

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Abstract

Peptides are rapidly degraded by endogenous proteases in the bloodstream, stomach, intestine, liver, and kidney, hindering their entry into systemic circulation after oral administration. This study evaluated peptide metabolic stability across gastrointestinal, hepatic, renal, and circulatory systems to identify optimal *in vitro* models for guiding structural optimization.

Objective

The objective of this study was to establish an *in vitro* metabolic platform to determine the fate of the peptide after incubations with plasma, gastrointestinal enzymes, and hepatic and renal matrices.

Methods

The commercially available peptides were purchased from credible vendors. The frozen plasma, human liver S9, human liver homogenate, human hepatocyte, human kidney homogenate and human kidney S9 were purchased from BioIVT. Chymotrypsin, trypsin, pancreatin, pepsin and elastase were purchased from sigma or other credible vendors.

Matrix
- Gastrointestinal enzyme: Chymotrypsin, trypsin, pancreatin, pepsin and elastase
- Circulatory system: Frozen or fresh plasma, **EDTA-K_2 or heparin** anticoagulant, serum.
- Liver-derived: homogenate, hepatocyte, S9, microsome.
- Kidney-derived: homogenate, S9

Incubation
- Peptide and different matrix were mixed thoroughly.
- Samples of 100 µL were incubated in a water bath for different timepoints.

Bioanalysis
- Peptide was semi-quantitated by LC-MS/MS

Results

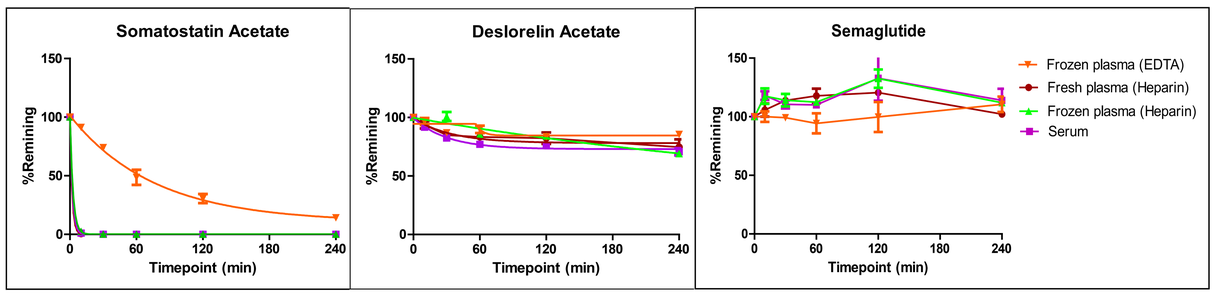

Figure 1 The plasma stability of somatostatin acetate, deslorelin acetate and semaglutide under different conditions. Plasma stability was tested using 3 reference peptides (fast, medium and slow metabolism) to compare the effects of anticoagulants (EDTA-K_2 vs. heparin sodium), plasma status (fresh vs. frozen), and plasma vs. serum. It revealed shorter peptide half-lives in heparin sodium-anticoagulated plasma versus EDTA-K_2 plasma (from the data of somatostatin acetate and deslorelin acetate), likely because EDTA chelates the metal ions required for some enzyme reactions. No significant differences were observed between fresh and frozen plasma, plasma and serum (especially from the data of deslorelin acetate**,** because somatostatin acetate and semaglutide are metabolized extremely fast and extremely slow, respectively).

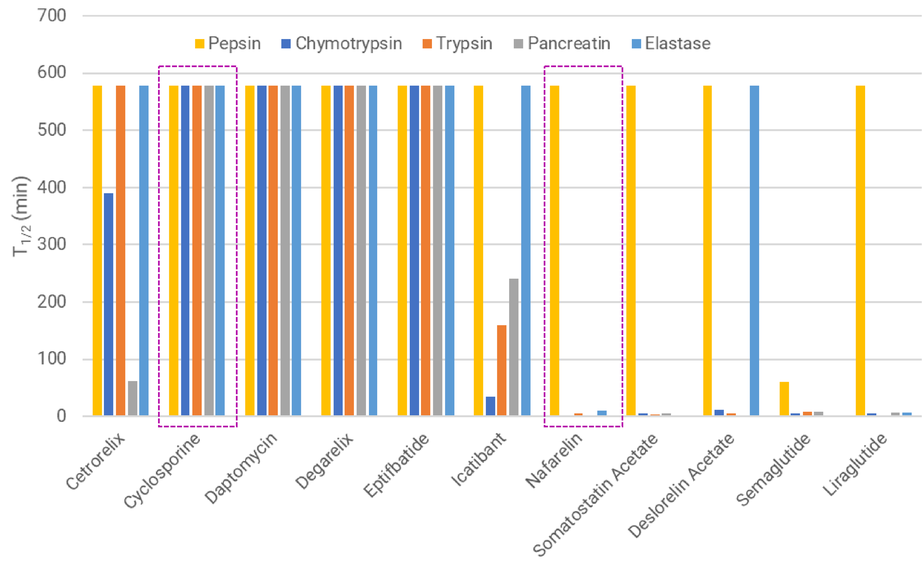

Figure 2. The stability of 11 peptides in gastrointestinal proteases. Gastrointestinal degradation profiles of cyclosporine and nafarelin aligned with literature data. (Cyclosporine is highly stable towards gastrointestinal peptidases, with more than 90% remaining intact after 2 hours of incubation. In contrast, nafarelin is stable towards human pepsin but is rapidly hydrolyzed by intestinal peptidases[1]). Additionally, the metabolic rates of other compounds vary, indicating that these enzymes can differentiate metabolic rate of compound, thus proving the feasibility of the system.

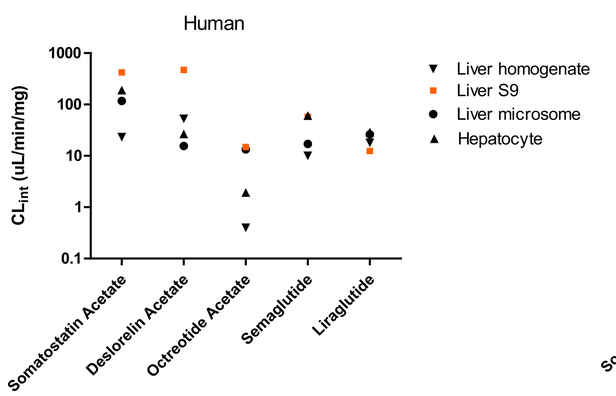

Figure 3 The metabolism of 5 peptides in liver-derived subcellular and cellular incubation systems. S9 fraction exhibited higher or similar intrinsic clearance for 5 peptides [2].

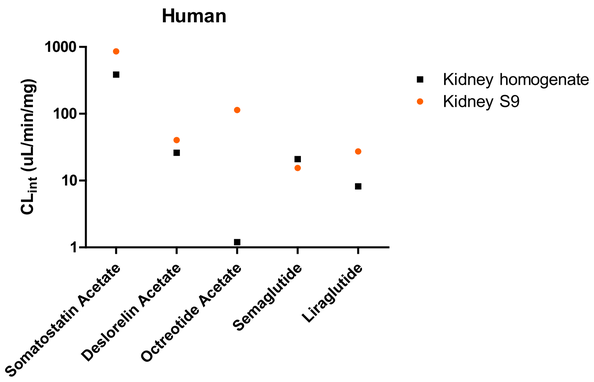

Figure 4 The metabolism of 5 peptides in kidney homogenate and S9. S9 fraction exhibited higher or similar intrinsic clearance for 5 peptides [3].

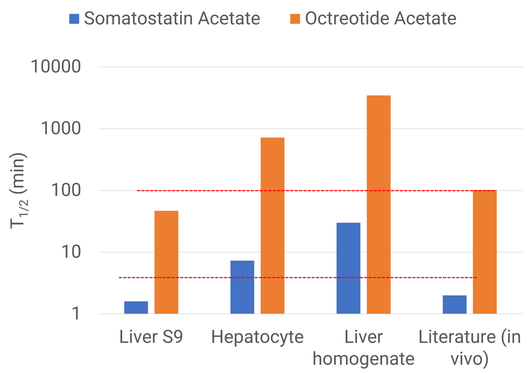

Figure 5 The correction between *in vitro* and *in vivo*. Half-life values of somatostatin acetate and octreotide acetate in liver S9 were better correlated with *in vivo* data[1] than in other matrices. (Half-life of somatostatin acetate in S9, hepatocyte, homogenate, *in vivo*=1.6, 7.3, 30, 2 min; half-life of octreotide acetate in S9, hepatocyte, homogenate, *in vivo*=46.9, 720, 3468, 102 min;)

Conclusions

- An *in vitro* platform for peptide metabolism was established, identifying heparin sodium plasma as preferable for circulatory stability testing due to EDTA's interference.
- Hepatic and renal S9 fractions emerged as cost-effective, physiologically relevant models for metabolic studies, offering predictive clearance values critical for early-stage structural optimization.

References

[1] International Journal of Peptide Research and Therapeutics. 27:1397–1418
[2] Journal of Pharmaceutical and Biomedical Analysis. 196: 113921
[3] Journal of Pharmaceutical and Biomedical Analysis. 225: 115219

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