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FACULTY OF PHARMACEUTICAL SCIENCES

Screening methodology for chemical and metabolic stability of potential peptide drugs

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SUMMARY

Drug chemical stability and metabolism is an integrated part of drug discovery and drug development. Drug degradation products are less likely to exert the desired pharmacological effect, while another concern is their possible toxicity. Hence, early information on the chemical and metabolic stability is considered to be an essential factor in the evaluation of new lead compounds. Due to their structure, the stability is a critical key-determinant in the drugability of peptides, much more compared to the classic synthetic small molecule or large biotech drugs. Moreover, the qualification of possible related impurities, an important drug quality issue, is closely related to their occurrence as metabolites. During systemic circulation of potential peptide drugs, the most important tissue compartments concerning enzymatic degradation are blood and liver. Together with fast renal clearance, the peptide-instability frequently results in short biological plasma half-life times, limiting the exposure of the parent active peptide to its target tissue (e.g. brain) and thus impairing the desired functionality.

In order to obtain comprehensive information regarding the chemical stability and metabolic fate of peptide drug candidates, these compounds are tested *in vitro* by forced degradation and incubation in plasma and organ homogenates. A general screening procedure to evaluate peptide stability is used, where the potential peptide drug is incubated in the tissues matrices, with aliquots withdrawn at pre-determined time points and analysed by HPLC-DAD/Fl for quantitative information (kinetics) and ESI/iontrapMS^n for qualitative information (identification). Suitable controls and operational conditions have been developed, based upon our results with several existing and potential peptide drugs. The results of closely related peptides (extended and shortened), currently under investigation as NCEs, are presented as typical examples.

EXPERIMENTAL

1. ANALYTICS

Standard method of choice: RP-C18 HPLC

Alternative (currently most orthogonal; e.g. for hydrophilic peptides): HILIC-amide HPLC

Both systems: optimized 1.5 hrs gradient (0.1% m/V formic acid water + acetonitrile)

PDA (quantification at 195 nm) – Fluorescence and MS (Thermo LCQ ESI-iontrap)

2. IN-VITRO METABOLIZATION

Mouse plasma or liver-tissue extract + peptide stock solution in KH-buffer at 37°C

At different times (minute-scale up to maximally 240 min): stop + formic acid, 5 min at 95°C, cooled on ice: supernatans to HPLC.

RESULTS and DISCUSSION

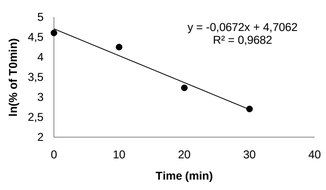
1. CHEMICAL STABILITY UNDER IN-VITRO METABOLIZATION TEST CONDITIONS

Control solutions: Immediate stop (T=0 min) compared to reference solution:

Wide differences between peptides: from no degradation (e.g. ganirelix) to over 10% degradation (e.g. buserelin)

2. PLASMA-STABILITY KINETICS AND METABOLIZATION

S N S S M | N S L | D | F S



$T_{1/2ext,pl}$ = 10.3 [6.6;23.0] min.

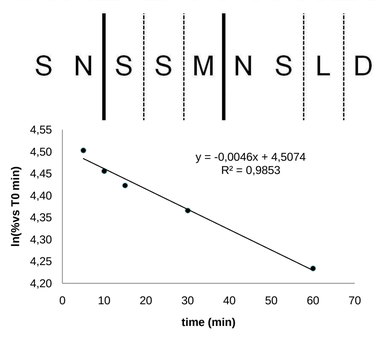
S N S S M N S L | D | F | S S

This is the short (sho) peptide originating from extended (ext) peptide, by truncation of one amino acid (S) at carboxyl-terminal end.

$T_{1/2sho,pl}$ = 42.8 [40.6;45.2] min.

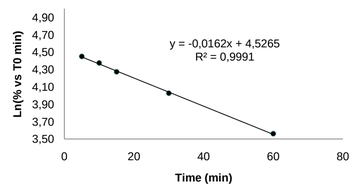
3. LIVER-STABILITY KINETICS AND METABOLIZATION

S N | S | S | M | N S L | D | F S S



$T_{1/2ext,liv}$ =149.6 [122.2;192.9] min.

S N | S | S | M | N | S L | D | F S



$T_{1/2sho,liv}$ = 42.8 [40.6;45.2] min.

CONCLUSION

Two peptides, differing only in C-terminal amino acid length (12 AAs versus 11 AAs): significantly different metabolic stability, as well as relative importance of cleavage bonds (not predicted by models, e.g. PeptideCutter).

References: **[1]** B. De Spiegeleer, V. Vergote, A. Pezeshki et al. Impurity profiling quality control testing of synthetic peptides using LC-PDA-Fl and LC-ESI-MS: the obestatin case. Anal. Biochem. 376 (2008) 229-234. **[2]** V. Vergote, S. Van Dorpe, B. De Spiegeleer et al. In vitro metabolic stability of obestatin: kinetics and identification of cleavage products. Peptides 29 (2008) 1740-1748.