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LC-MS Analysis of Various Food Intake Regulating Lipopeptides

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Introduction

A number of newly synthesized lipopeptides have demonstrated a great therapeutic potential for obesity treatment. Lipidization of neuropeptides usually brings various advantages including improved stability in plasma, improved ability to bind to plasmatic proteins, increased resistance to proteases, ability to cross blood-brain barrier, and modification of pharmacokinetic/pharmacodynamic profile. Our recently synthesized lipopeptides demonstrate high *in vivo* efficiency; lipidization is a prerequisite for their central effect after peripheral administration.

Plasma stability and pharmacokinetics of lipopeptides in blood plasma can be effectively studied by liquid chromatography-mass spectrometry (LC-MS).

Results and Discussion

Studied lipopeptides

Two groups of lipopeptides were studied; (a) prolactin-releasing peptide (PrRP) analogs, and (b) liver enriched antimicrobial peptide-2 (LEAP2) analogs. Their structures are summarized in Figure 1.

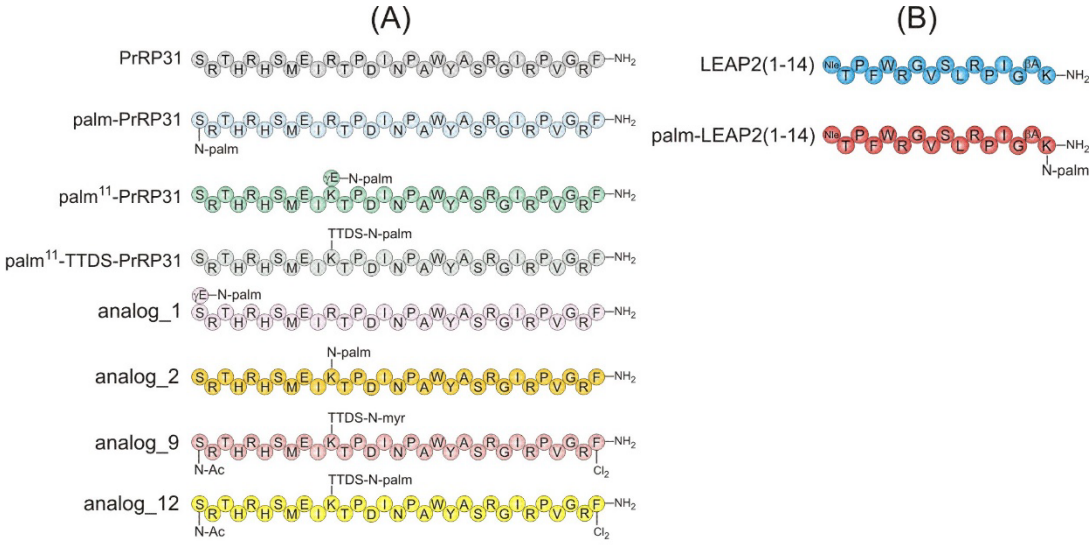

Fig. 1. Chemical structures of (A) PrRP and (B) LEAP2 analogs.

LC-MS instrumentation

For LC an UltiMate 3000 LC system (Thermo, USA) was used. MS was measured with a 3200 Q-TRAP mass spectrometer (AB Sciex, Canada). Separations were carried out with various reversed-phase columns, specifically, PrRP analogs: Chromolith RP-18e (Merck, Germany), a silica-based monolithic column (col. size 50 x 3 mm ID) and LEAP2(1-14) analogs: XBridge Premier BEH C18 (Waters, USA), particle size 2.5 µm, VanGuard Fit (col. size 50 x 2.1 mm ID). A gradient elution utilizing 0.1% HCOOH in H_2O and acetonitrile, respectively, was applied. MS signal was measured in multiple reaction monitoring (MRM) mode.

Presented lipidized peptides have been studied extensively in related projects for biological aspects focused on food intake regulation (see elsewhere). Here, the plasma stability of the above mentioned PrRP and LEAP2 analogs were determined by LC-MS. In this context it is important to mention that the sample preparation method preceding LC-MS measurement of the lipopeptide stability in plasma must be developed for each lipopeptide individually. Usually, protein precipitation with various aqueous organic solvents provides an acceptable lipopeptide recovery. We verified that in some cases, specifically utilizing monolitic column Chromolith RP-18e, a direct "dilute and shoot" approach can be applied [1-2].

As can be seen in Figures 2 and 3 *in-vitro* stability of all the studied lipopeptides in plasma has been significantly enhanced with respect to the corresponding non-lipidized analogs. Lipidization proved to be an efficient means of plasma stability protraction. Besides, it is evident that a chemical structure of the linker used for lipidization has a significant influence on the lipopeptide stability. Lipidized peptides with TTDS linker provided relatively low plasma stability being only slightly better than for PrRP3.

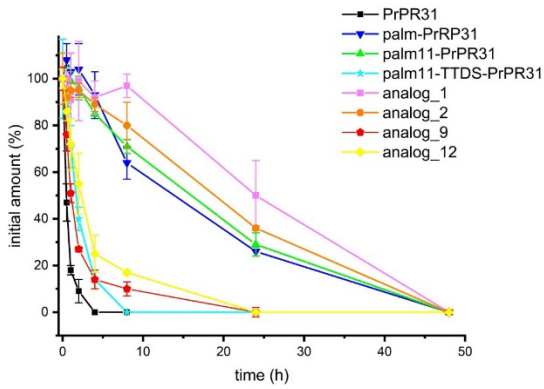

Fig. 2. Stability of PrRPanalogs in rat plasma.

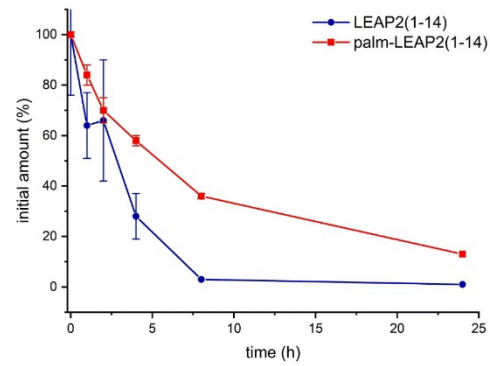

Fig. 3. Stability of LEAP2 analogs in rat plasma.

Acknowledgments

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References

1. Zemenová, J, Sýkora, D., Freislebenová ,A., Maletínská, L. *Bioanalysis* **9**, 1319-1328 (2017), http://dx.doi.org/ 10.4155/bio-2017-0125
2. Zemenova, J. Sykora, D., Adamkova, H., Maletinska, L., Elbert, T., Marek, A., Blechova, M. *J. Sep. Sci.* **40**, 1032-1039 (2017), http://dx.doi.org/10.1002/jssc.201601141