Hes·so
Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
University of Applied Sciences and Arts Western Switzerland

Master of Science HES-SO in Life Sciences

Bio-analytical methods to monitor and quantify peptides in biological matrices

Nadège Gsponer

Applied Biosciences
HES-SO Valais

Advisor: Dr. Origène Nyanguile

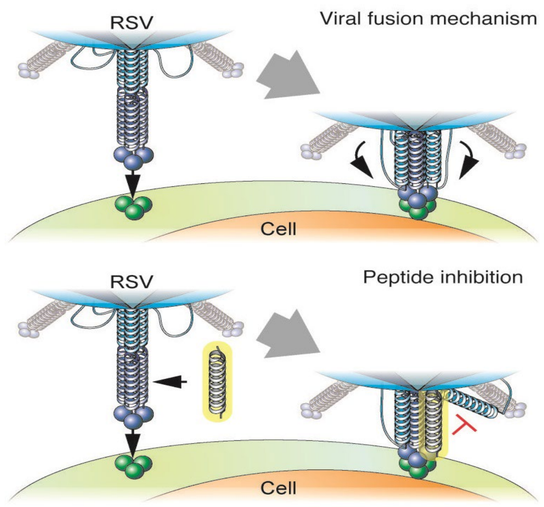
DESCRIPTION

RSV (Respiratory Syncytial Virus) is a single-stranded, polarity-negative RNA virus belonging to the large family of *Paramyxoviridae*. It is responsible for a large number of lower respiratory tract infections. This is mainly in young children. This virus can cause serious complications and even death.

While an effective vaccine against **RSV** is still lacking, one possible strategy to combat the virus is to stop it from fusing with the cells it seeks to infect. It has been discovered that this can be achieved by using inhibitory peptides.

In the Peptide Chemistry Laboratory of the HES-SO Valais, a large number of peptides for inhibiting the RSV fusion process were synthesised and tested. Three particularly effective lead peptides, named A, B and C, were identified.

To further develop a potential drug, it is important to characterise the behaviour of these peptides *in vivo*, in particular through pharmokinetic experiments.



Mechanism of action of RSV inhibiting peptides

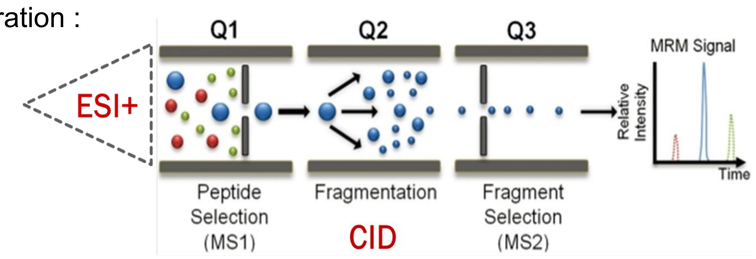
OBJECTIVES AND ACHIEVEMENT

The main aim of this thesis was to develop analytical methods for monitoring and quantifying the three lead peptides in complex biological matrices.

These methods were to be used to perform proteolytic degradation assays and to determine the stability of the peptides in CD-1 mouse plasma. Finally, once their reliability had been demonstrated, they would allow pharmacokinetics of the peptide leads to be performed *in vivo* in mouse.

To achieve these objectives, it was first necessary to develop an efficient extraction method. **Selective precipitation** of plasma proteins by addition of acetonitrile was chosen to fulfil this role. The samples were then to be measured by **HPLC-MS/MS**.

The mass spectrometer chosen for these experiments was a **triple quadrupole** used in the following configuration :

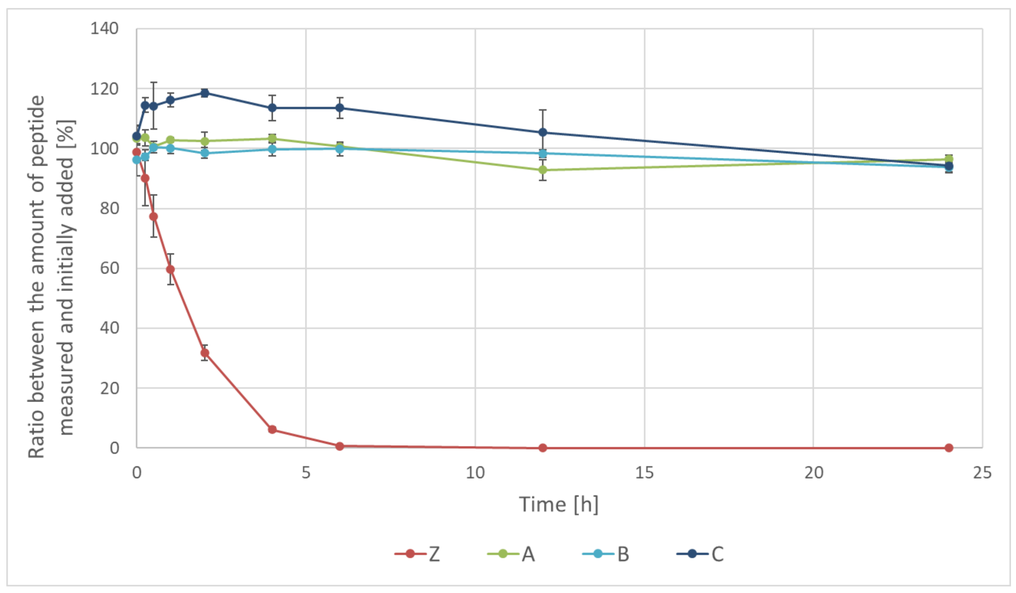


Configuration of the equipment used for QQQ analysis

RESULTS

In vitro stability in mouse plasma

In the plasma stability tests, the three lead peptides were compared to the Z peptide. The latter represents the initial unmodified sequence from which the A, B and C peptides were inspired.

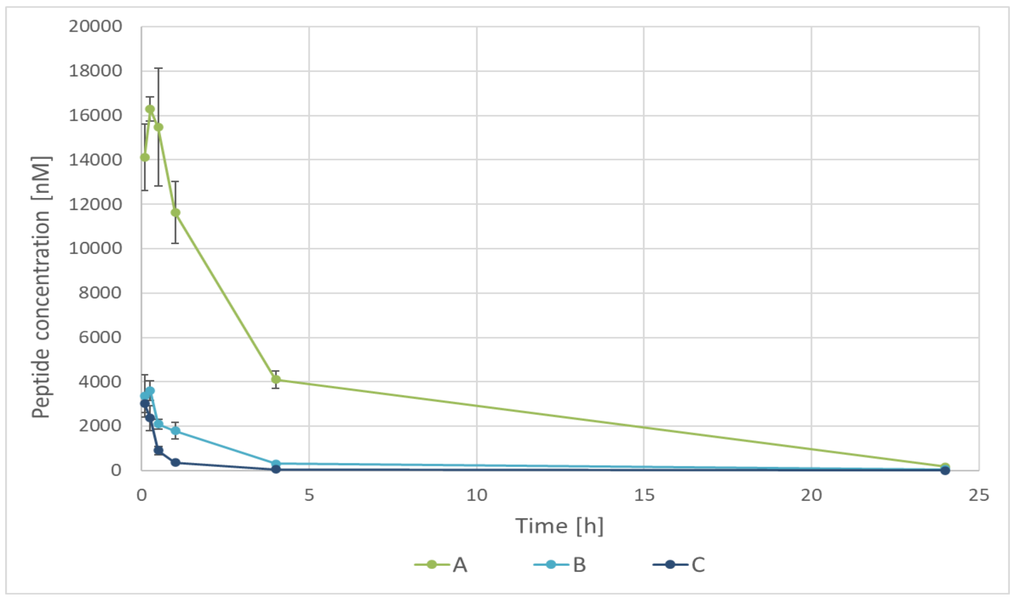


In vitro plasma stability curves for each peptide studied

All three peptide leads behave similarly. However, Z is much less resistant. This is mainly due to the fact that its three-dimensional α -helix conformation is not stabilised. Whereas in the lead peptides, it is maintained by hydrocarbon bridges between some amino acids.

In vivo pharmacokinetics in mouse

In the pharmacokinetic experiments, the A peptide clearly showed the most interesting behaviour.



Pharmacokinetic profiles of A, B and C peptides after intravenous administration at an initial concentration of 98 µM

CONCLUSION

Bio-analytical methods for monitoring and quantifying peptides in the complex matrix of mouse plasma have been developed.

Thanks to these methods, *in vitro* stability and *in vivo* pharmacokinetic experiments could be performed. They demonstrated the importance of α-helix stabilisation on peptide resistance. They also allowed to designate A as the most interesting peptide so far for the future development of a treatment against RSV.

The next experiments to be carried out should allow the peptide to be followed in the mouse body to see if it is indeed located in the lung, which is its target of action. The search for the right formulation can then be pursued. These experiments will make it possible to determine whether the formulant used should also act as a vehicle or not.

The methods that will be used for this are Fluorescence Molecular Tomography and analysis of mouse lung tissue.

SWISS UNIVERSITIES APPLIED SCIENCES AND ARTS

MLS | MASTER OF SCIENCE IN LIFE SCIENCES