37th EPS European Peptide Symposium
14th IPS International Peptide Symposium
25 - 29 August 2024
Satellite Workshop 30 - 31 August 2024
Florence, Italy- 2024

Development of a retro-inverso tetrapeptide collagen modulator as anti-aging active principle

Fosca Errante [a,b,c], Marco Pallecchi [b], Gianluca Bartolucci [b], Elena Frediani [d], Francesca Margheri [d], Lisa Giovannelli [e], Anna M. Papini [a,f], Paolo Rovero [a,b*]

a) Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, University of Florence, Sesto Fiorentino (FI), 50019, Italy
b) Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Sesto Fiorentino (FI), 50019, Italy
c) Espikem s.r.l., Prato (PO), 59100, Italy
d) Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Firenze (FI), 50139, Italy
e) Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Firenze (FI), 50139, Italy
f) Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino (FI), 50019, Italy

https://doi.org/10.17952/37EPS.2024.P2071

UNIVERSITÀ DEGLI STUDI FIRENZE
PeptLab
INTERDEPARTMENTAL RESEARCH UNIT OF PEPTIDE AND PROTEIN CHEMISTRY AND BIOLOGY

fosca.errante@unifi.it

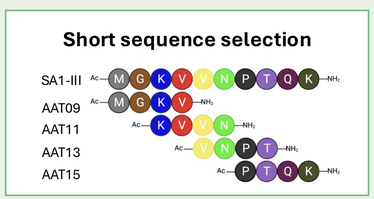
BACKGROUND

Serpin A1 or Alpha1-antitrypsin is the physiological inhibitor of elastase, one of the main enzymes involved in collagen degradation. Congote et al.[1] have shown that the C-terminal portion of Serpin A1 is able to stimulate type I collagen production.
We previously showed that a shorter portion of Serpin A1, termed SA1-III[2], is a collagen turnover modulator and the activity of SA1-III is based on inhibition of collagen degradation, as described by Cipriani et al.[3].

RESULTS

We developed new peptide analogues, acting on the following parameters:
- Size of the molecule (n° of amino acids);
- Chemical stability (presence of Met);
- Enzymatic stability (retro-inversion).

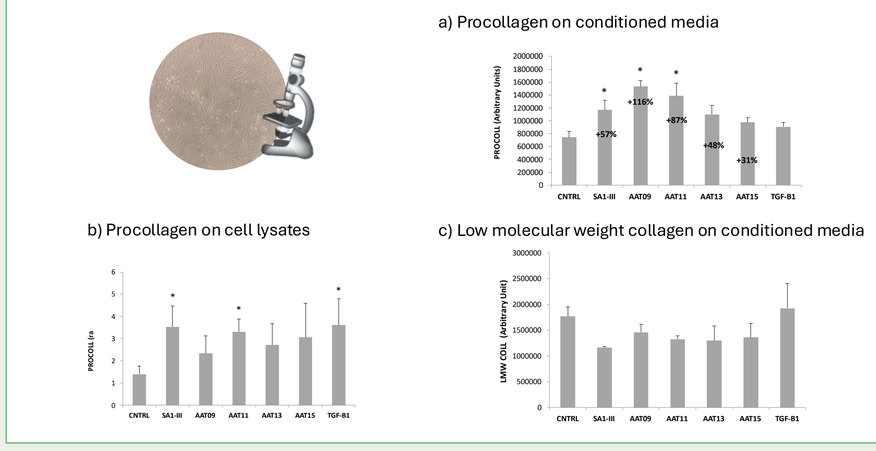
Short sequence selection



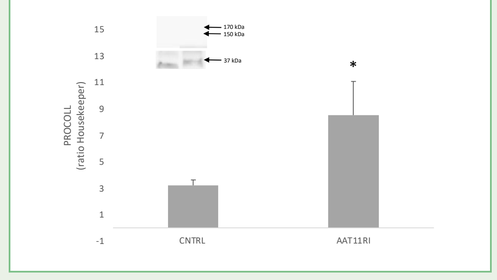
Among all the tested sequences, peptide **AAT11RI** was found to be the most active and a family of peptides was patented[4].

The activity of peptide AAT11RI in **decreasing collagen degradation** was evaluated trough Western Blot experiments (procollagen measurement) and Invasion experiments (migration of fibroblasts in Boyden chambers)[5].

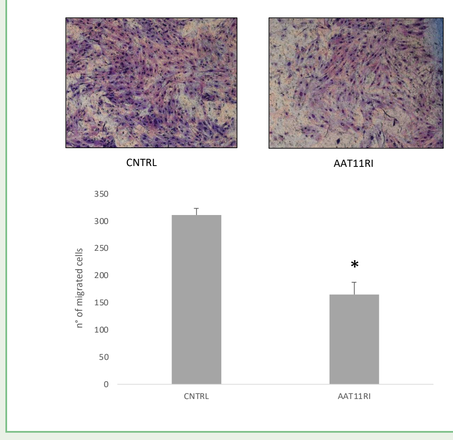
72-h treatment of NHDFs with peptides (20 µM) Followed by Western blot measurement[5]



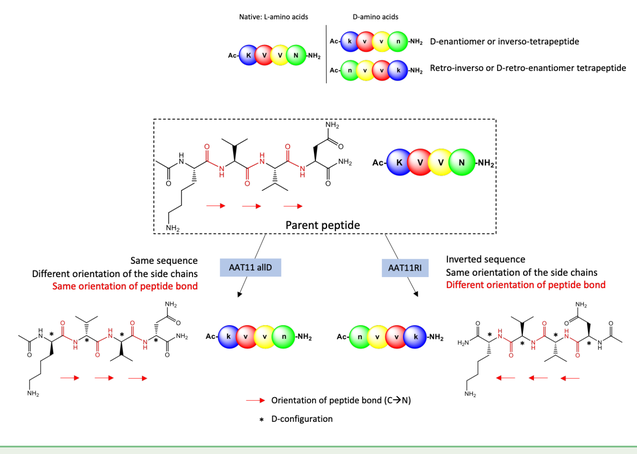
Procollagen on cell lysates[5]



Number of NHDFs migrated trough the Boyden chamber[5]

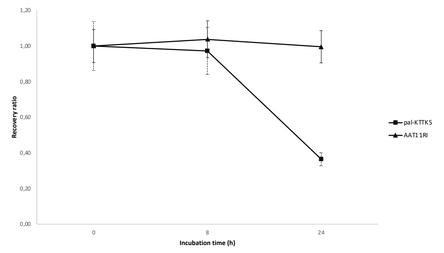


Design of novel analogues[5]



Peptide **enzymatic stability** towards skin proteases was evaluated in ex-vivo experiments on human skin homogenates in comparison with a well-known cosmeceutical peptide[5].

Degradation profile observed for AAT11RI and peptide pal-KTTKS at different time of incubation[5]



METHODS

Peptide synthesis:
Solid Phase Peptide Synthesis (SPPS)
Flash chromatography and semi-preparative HPLC
UHPLC-MS characterization
Cell treatments:
In Neonatal Normal Human Dermal Fibroblasts (Neo-NHDFs)
Collagen quantification:
Western Blot
Stability evaluation:
LC-MS/MS using human skin homogenates (HSH)
Invasion assay:
Boyden chambers

CONCLUSIONS

Second-generation peptides were designed, starting from SA1-III, as novel cosmeceutical ingredients. A *retro-inverso* peptide, named AAT11RI was synthetized, tested and patented. Further studies described AAT11RI mechanism of action as a collagen degradation protector[5]. Moreover, the use of D-amino acids induced in peptide AAT11RI high stability to dermal proteases. Besides the use in cosmeceuticals, there is evidence indicating that serine proteases play a pathogenic role in some diseases (e.g. chronic inflammatory lung disorders) characterized by elevated protease activity. This suggests a potential interest in further investigating the pharmaceutical applications of short sequences derived from serpin A1 such as AAT11RI.

(1) Congote, L. F.; Temmel, N.; Sadvakassova, G.; Dobocan, M. C. Comparison of the Effects of Serpin A1, a Recombinant Serpin A1-IGF Chimera and Serpin A1 C-Terminal Peptide on Wound Healing. *Peptides* 2008, *29* (1), 39–46. https://doi.org/10.1016/j.peptides.2007.10.011.
(2) Pascarella, S.; Tiberi, C.; Sabatino, G.; Nuti, F.; Papini, A. M.; Giovannelli, L.; Rovero, P. Serpin A1 C-Terminal Peptides as Collagen Turnover Modulators. *ChemMedChem* 2016, *11* (16), 1850–1855. https://doi.org/10.1002/cmdc.201500472.
(3) Cipriani, C.; Pascarella, S.; **Errante, F.**; Menicacci, B.; Magnelli, L.; Mocali, A.; Rovero, P.; Giovannelli, L. Serpin A1 and the Modulation of Type I Collagen Turnover: Effect of the C-Terminal Peptide 409-418 (SA1-III) in Human Dermal Fibroblasts: Serpin-A1 C-Terminal Modulates Collagen Levels. *Cell Biol. Int.* 2018, *42* (10), 1340-1348. https://doi.org/10.1002/cbin.11018.
(4) **Errante, F.**; Giovannelli, L.; Papini, A. M.; Rovero, P. Bioactive Peptides and Compositions Comprising Them. WO/2020/245772, December 10, 2020.
(5) **Errante F.**, Pallecchi M., Bartolucci G., Frediani E., Margheri F., Giovannelli L., Papini A.M., Rovero P., Retro-Inverso Collagen Modulator Peptide Derived from Serpin A1 with Enhanced Stability and Activity In Vitro. *J. Med. Chem.* 2024 *67* (6), 5053-5063. https://doi.org/10.1021/acs.jmedchem.4c00137

Regione Toscana
This work was supported by Tuscany Region [PORCREOFESR 2007-2013]
European Peptide Society is kindly acknoledged for the awarded registration grant to Dr. Fosca Errante