Optimization of vCCL2 based CXCR4 inhibitors by Phage Display and Rational Design

Virginie Fievez¹, Martyna Szpakowska², Karthik Arumugam³, Amor Mosbah³, Sabrina Deroo³, Xavier Dervillez³, Pierre-Arnaud Gauthier², Michele Baudy-Floch², Carole Devaux¹, Andy Chevigné¹

¹Department of infection and immunity, Luxembourg Institute of Health, 84, Val Fleuri, L-1526 Luxembourg - (andy.chevigne@frh.lu)
²Université de Rennes 1, Sciences Chimiques de Rennes U.M.R., 6226, Avenue du Général Leclerc, R-35042 France

BACKGROUND

The viral broad spectrum chemokine vCCL2 binds to the chemokine receptors CXCR4 and CXCR5 and inhibits HIV-1 entry protecting host cells against infection [1]. Peptide derived from vCCL2 N-terminus (vCCL2(1-21)) displays modest CXCR4 and CXCR7 binding affinity and antiviral properties compared to the parental chemokine but mutations within its CC motif has been shown to enhance its antiviral properties, for review see [2]. In this study, we explored these observations to develop highly potent and selective Mimokine antagonists.

AIM

The aim of the current study was to develop a new class of short and selective CXCR4 inhibitors inspired by the binding mode of natural chemokines (Mimokines). The N-terminus of vCCL2 (vCCL2(1-21)), a viral chemokine displaying antagonist properties on CXCR4, was used as scaffold to introduce controlled randomizations and to create phage library of peptide variants.

METHODS

A Mimokine phage display library, in which C16-C16 amino acids within the N-terminus vCCL2(20-21) peptide were fully randomized (GKSWHRPPDXXQGQYKNPLP), was engineered by direct cloning of degenerated oligonucleotides into a filamentous phage vector. The isolation of Mimokines specific to CXCR4 was then performed by phage display biopanning on CXCR4 displayed at the surface of proteoliposomes.

RESULTS

Screening of Mimokine phage library on CXCR4 magnetic proteoliposomes -

- Identification of 30 Mimokines
- In contrast to the N-terminus vCCL2(21) peptide, in which C16-C16 residues allow dimer formation, selected Mimokines are exclusively monomeric
- Bivalent ligands have been shown to be stronger binders than their monomeric counterparts, we engineered and used a N-terminus vCCL2(21) C16-5 C5-5 mutant as monomeric control for the further characterization of the selected Mimokines

CXCR4 binding affinities of monomeric Mimokines

Selected Mimokines were evaluated for their ability to bind to CXCR4 in competitive assay using a fluorescently labelled OCL12 (AF647-OCL12) with Mimokine display (Figure 1A).

- Mimokines VR, SR, FR and WL were the most potent inhibitors displaying 3- to 4-fold higher affinity for CXCR4 than Mimokine SS (Figure 1B).

Antiviral properties of monomeric Mimokines -

Selected Mimokines were also tested for their ability to inhibit the infection of MT-4 cells by the laboratory-adapted OX4-HIV-1 strain (IB8).

- At a final concentration of 100 µM, only Mimokine WL fully protected cells from virus cytolytic effect while Mimokine FR, LR, QR, VR and SR displayed 50, 22, 21, 18 and 17% protection, respectively (Figure 2A).
- Mimokine SS displayed a 60% protection at 300 µM while Mimokines VR, SR and FR showed an IC50 value of 114, 120 and 90 µM, respectively (Figure 2B).
- Mimokine WL displayed the most potent antiviral properties exhibiting an IC50 value of 49 µM (Figure 2B).

Molecular modelling and docking into CXCR4 3D structure -

- Higher affinity of Mimokines FR, SR and VR for CXCR4 might be due to the formation of an additional salt bridge between the Arginine 12 of the Mimokine and the sulfated tyrosine 21 of the receptor (Figure 3).

Development of Dimeric and D-amino acids Mimokine analogues -

Taking advantage of the higher binding affinity and potency observed for bivalent D-amino acids ligands, we further enhanced the potency of the selected Mimokines by combining dimerization and D-amino acids substitution.

- Bivalent Mimokines SR, VR, FR and WL in D-format exhibited 10 to 20-fold increase in CXCR4 binding affinity (Figure 4A) and 20 to 100-fold increase in antiviral activities when compared to their respective monomeric L-counterparts (Figure 4B).

CONCLUSIONS

Optimized bivalent D-Mimokines displayed 10 to 20-fold enhanced CXCR4 affinity and 20 to 100-fold improved antiviral properties when compared to the initial monomeric L-Mimokines.

These bivalent D-Mimokines showed a reduced affinity for CXCR7 and may therefore serve as lead compounds for the further development of more selective CXCR4 HIV-1 inhibitors.

REFERENCES


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