Labyrinthopeptin A1 demonstrates potent and dual anti-HIV and anti-HSV activity

G. Férir[1], M.I. Petrova[2], G. Andrei[1], R. Snoeck[1], J. Vanderleyden[2], M. Brönstrup[3], R.D. Süssmuth[4] and D. Schols[1]

[1] Rega Institute for Medical Research, University of Leuven, Belgium; [2] Centre of Microbial and Plant genetics, University of Leuven, Belgium;

[3] Sanofi R&D, Frankfurt am Main, Germany; [4] Technische Universität Berlin, Berlin, Germany.

Background

Lantibiotics are peptides, produced by bacteria, that contain the noncanonical amino acid lanthionine and many of them exhibit antibacterial activities. The most well studied lantibiotic peptide to date is nisin, which is widely used as a food preservative (food additive number E234) for >40 years **(Fig. 1)**. However, labyrinthopeptin A1 (LabyA1) is a prototype peptide of a novel class of carbacyclic lantibiotics, which contain the posttranslationally modified triaminoacid labionin (Lab). Originally, it was isolated from the actinomycete *Actinomadura namibiensis* DSM 6313 (Meindl et al., 2010). Its biosynthesis was further investigated in subsequent studies (Krawczyk et al., 2013; Müller et al., 2010; Müller et al., 2011). Here, we extensively studied its broad-spectrum anti-HIV and anti-HSV activity *in vitro* in cell-free replication assays **(Fig. 2)**, and it ability to inhibit HIV cell-cell transmission in DC-SIGN dependent and independent cocultivation models **(Fig. 3)**. Time-of-drug addition (TOA) studies were performed to investigate its mechanism of action **(Fig. 4)** and its safety for potential microbicidal applications was investigated in various cellular assays and a *Lactobacillus* growth assay **(Figs. 5-7)**. The last years, there is more and more evidence that HIV and HSV are sexually transmitted co-pathogens, enhancing each other's infectivity (Thurman & Doncel, 2012). Therefore, LabyA1 could be an ideal microbicidal candidate to reduce the sexual transmission of HIV and HSV.

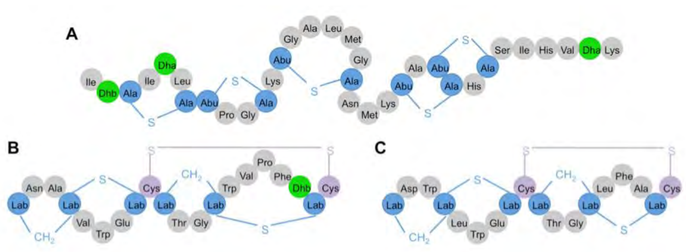

Fig. 1: Amino acid structures of the lantibiotics used in this study. (A) nisin, (B) labyrinthopeptin A1 (LabyA1), (C) labyrinthopeptin A2 (LabyA2). Lab, Dhb and Dha are the abbreviations of the unusual amino acids labionin, didehydrobutyrine and didehydroalanine. Ala-S-ala stands for lanthionine and Abu-S-Ala in nisin is methyllanthionine.

Methods and Results

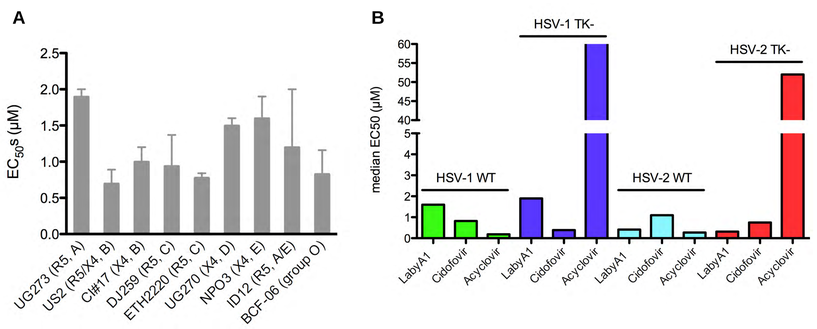

Fig. 2: Anti-HIV and anti-HSV activity of LabyA1. (A) Broad-spectrum anti-HIV-1 activity against various HIV-1 clinical isolates in PHA-stimulated PBMCs. HIV-1 coreceptor tropism (X4 and/or R5) and subtype is shown between brackets. Mean EC_{50} ± SEM is shown. (B) Anti-HSV-1 and anti-HSV-2 activity of LabyA1. Median EC_{50} is represented. Cidofovir and acyclovir were included as reference compounds. TK$^-$ or thymidine kinase-deficient HSV strains are resistant to acyclovir.

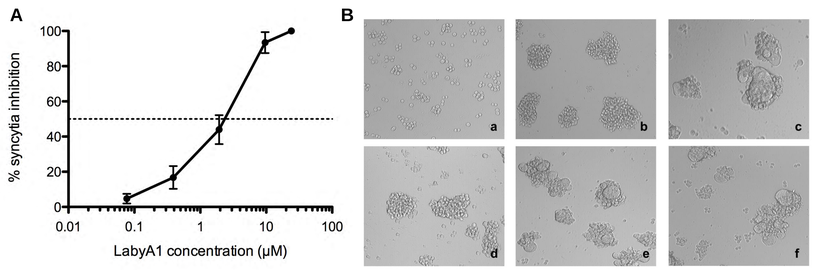

Fig. 3: LabyA1 inhibits the HIV-1 cell-to-cell transmission. (A) Persistently HIV-infected HUT-78/IIIB cells were cocultivated with non-infected CD4$^+$ target SupT1 T cells at 37° C. After 24h of coculture, the percentage of inhibition of target T cell destruction was quantified by flow cytometry. LabyA1 profoundly inhibited this process with an EC_{50} of 2.5 ± 0.6 µM (mean ± SEM; *n*=4). (B) HIV-1 was bound to the attachment receptor DC-SIGN (panel a) and these cells were cocultivated with CD4$^+$ T cells (panel b). After 24h of coculture, this resulted in the formation of multinucleated giant cells (panel c). LabyA1 inhibited the transmission of HIV captured by DC-SIGN$^+$ cells to uninfected CD4$^+$ T cells dose-dependently with an EC_{50} of 4.2 ± 02. µM (mean ± SEM; *n*=2)

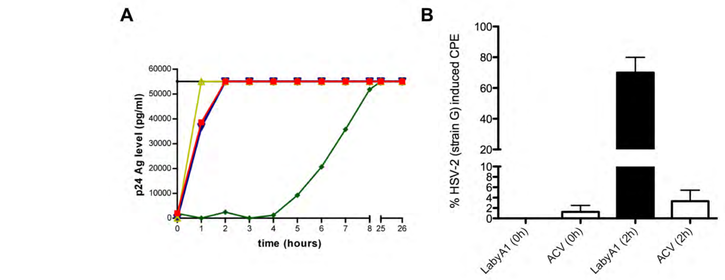

Fig. 4: LabyA1 interferes with the viral replication at an early time point. (A) MT-4 cells were infected with HIV-1 IIIB and test compounds were added at different time points post-infection. After 31h, viral replication was measured by HIV-1 p24 Ag ELISA. The colored lines represent respectively the positive control in black, 48 µM LabyA1 in red, 421 µM dextran sulfate in yellow, 6 µM AMD3100 in blue and 7.5 µM nevirapine in green. (B) HEL cells were infected with HSV-2 strain G and test compounds were added at 0h and 2h post-infection. After 3 days, CPE was scored microscopically. Black and white bars represent the effect of 19 µM LabyA1 and 35 µM acyclovir (ACV) respectively.

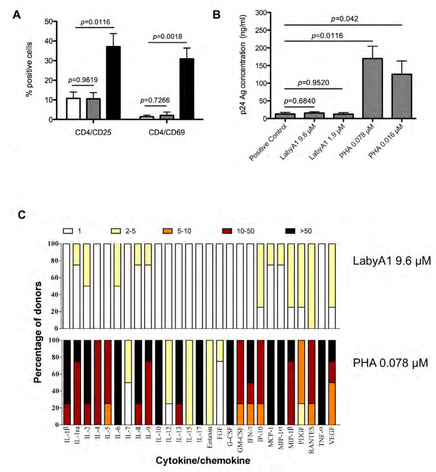

Fig. 5: Lack of stimulatory activity of LabyA1 on freshly isolated PBMCs. (A) The cells were incubated with LabyA1 or PHA for 3 days and afterwards CD25 and CD69 expression was analyzed using flow cytometry. (B) Cells were pre-treated with LabyA1 or PHA for 24h. After extensive washing, the cells were infected with HIV-1 BaL in the absence of compound. Viral replication was measured 7 days post-infection using HIV-1 p24 Ag ELISA. (C) PBMCs were incubated for 24h with medium or compound. Supernatant (SN) was collected and cytokine/chemokine production was determined using the Bio-Plex system (Bio-Rad). The fold-increase of the cytokine/chemokine concentration in the SN of treated- with respect to the concentrations in the SN of untreated PBMCs was determined from 4 individual donors. The fold-increase is divided in 5 groups: 1-fold (white), 2-5-fold (yellow), 5-10-fold (orange), 10-50-fold (dark red) and >50-fold (black). The amount of –fold increase for each cytokine is given as a percantage in the total amount of donors.

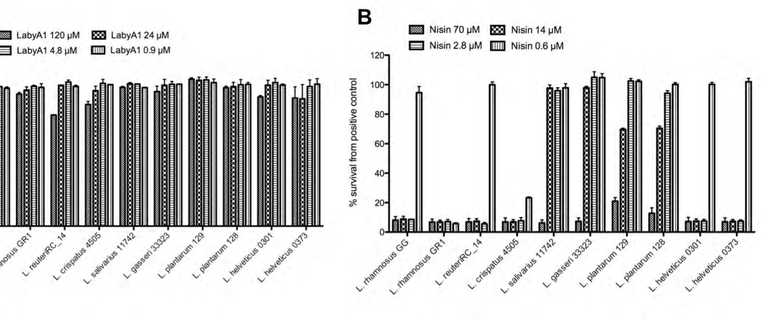

Fig. 6: Lack of toxicity of LabyA1 on the growth of vaginal *Lactobacilli* sp. Dose-dependent effects of the lantibiotics LabyA1 (A) and nisin (B) on the growth capacity of ten (one gastrointestinal (L. Rhamnosus GG) and nine vaginal) *Lactobacillus* strains. The results are expressed in percentage compared to the growth of each strain in MRS medium without LabyA1 and nisin (positive controls), which were taken as 100%. Mean ± SD out of 3 experiments is shown.

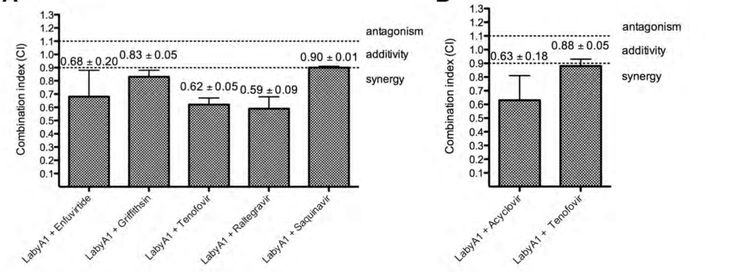

Fig. 7: Synergistic activity of LabyA1 with various anti-HIV and anti-HSV compounds. (A) Evaluation of LabyA1 in combination with selected anti-HIV inhibitors of different classes against HIV-1 strains ETH2220 (clade C) and BaL (clade B) in PHA-stimulated PBMCs. (B) Evaluation of LabyA1/acyclovir and LabyA1/tenofovir combinations against HSV-2 strain G induced CPE in HEL cells. The combination indices (CI) were calculated at the EC_{95}-level, using CalcuSyn software (BioSoft, UK) based on the median-effect principle of Chou and Talalay (Chou & Talalay, 1984) whereby CI>1.1 are antagonistic; 0.9<CI<1.1 are additive and CI<0.9 are synergistic.

Labyrinthopeptin A1:

- inhibits HIV and HSV replication by interfering with the viral entry process
- potently inhibits the HIV cell-cell transmission and the DC-SIGN mediated viral transfer
- shows no side-effects on PBMCs, vaginal epithelial cells and various *Lactobacilli*
- All LabyA1/antiviral drug combinations display synergistic to additive effects.

- **These data are encouraging for LabyA1 as an ingredient in a multi-targeted microbicide.**

This work was supported by the KU Leuven (GOA nos. 10/014 and PF/10/018), the FWO (no. G485.08), and the European Commission (FP7/2007-2013) (CHAARM no. 242135), COST action CM0804, the Fondation Dormeur, Vaduz, the Deutsche Forschungsgemeinschaft (DFG grant SU 239/9-2, the Cluster of Excellence "Unifying Concepts in Catalysis" coordinated by the Technische Universität Berlin.

Suggested reference see Férir *et al.*, PLoS One (2013)