Summary

Infections with *Herpes simplex* virus (HSV) can cause severe ocular diseases and encephalitis. Special risk groups are immunocompromised patients, newborns and neurodermatitis patients. Most drugs available inhibit viral DNA synthesis, often possessing tolerability problems and increasingly leading to drug resistant HSV strains and cross-resistances in risk groups. There is still an unmet need for new drug discovery with novel modes of action that are less sensitive for the development of resistances.

Fusion and subsequent entry of HSV-1 into the host cell can be mimicked in a virus-free eukaryotic cell culture system by co-expression of HSV-1 glycoproteins D, H, L and B (gD, gH, gL and gB) in presence of a gD receptor, resulting into excessive membrane fusion and polykaryocyte formation. The following project aimed to reliably and effectively identify natural products as inhibitors of HSV-1 induced membrane fusion with two virus-free cell-cell fusion assays, which have to be established and validated.

In a microscopical fusion assay, visual read-out was used for the screening of potential inhibitors, whereas luminometric quantification of cell-cell fusion was used in a reporter fusion assay. HSV-1 gB was tagged at its C-terminus with mCherry to express mCherry-gB in both assay systems for the visualisation of polykaryocyte formation. In the reporter fusion assay, reporter protein expression of SEAP was regulated by Tet-On 3G system. The plasmids for the expression of the transactivator Tet-On 3G and the reporter protein were stably integrated into the Vero Tet3G and Vero SEAP cell lines, respectively. The microscopical fusion assay was validated concerning (1) the functionality of mCherry-gB, (2) the linearity between effector cell number and number of syncytia, (3) the linearity between the concentration of the fusion inhibiting antibody α-gB-2c and the relative syncytia formation as well as (4) the reproducibility of relative fusion activity within four control groups. The reporter fusion assay was validated concerning (1) the signal-to-background ratio in case of mCherry-gB in comparison to wild type gB as well as (2) the reproducibility of relative fusion activity and cell viability within three control groups. The Tet-On 3G reporter fusion assay revealed higher sensitivity and robustness compared to the T7-promoter/T7 RNA polymerase-regulated reporter fusion assay described in literature.

Out of 32 selected test compounds from different natural product groups, three

triterpenoid saponin mixtures were identified as specific and potent inhibitors of HSV-1 gD, gH, gL and mCherry-gB induced membrane fusion: aescin with IC_{50} 7.2 µM, CC_{50} 25 µM and SI 3.5; *Quillaja saponaria* saponin extract 4 (QS 4) with IC_{50} 1.9 µg/mL, CC_{50} 29.5 µg/mL and SI 15.5; *Anagallis arvensis* saponin extract (ASE) with IC_{50} 1.4 µg/mL, CC_{50} 18.7 µg/mL and SI 13.4. Within HSV-1 plaque reduction assays on Vero cells, it was shown that the fusion inhibiting effects of aescin, as identified within the virus-free cell-cell fusion assays, reduced HSV-1 entry into the host-cell and HSV-1 cell-to-cell spread significantly in a dose-dependent manner. 15 µM aescin decreased relative plaque counts to 41% and 10 µM aescin resulted in a residual plaque size of 11% (HSV-1 17 syn^+) and 2% (HSV-1 ANG^{path}). Additionally, the release of HSV-1 progeny virus was reduced by one log step in the presence of 15 µM aescin, whereas treatment post infection had no influence. Thus, later steps in the HSV-1 life cycle seemed to be unaffected by aescin. Virus particle integrity was unaffected up to 20 µM aescin, so that antiviral and fusion inhibiting effects are not attributed to a disruption of HSV-1 particles.

Investigation of two different aescin batches revealed significantly different effects on fusion activity and cell viability, resulting in SIs of 3.7 (batch 1) and 2.8 (batch 2), which is most likely induced by differences in the composition of triterpene saponins, as evidenced by LC-MS analysis. The aescin isomers aescin IA, IB and isoaescin IA, IB exerted a different degree of cytotoxicity and inhibition of HSV-1 induced membrane fusion with following SIs: 4.8, 1.6, 3.7 and 2.5, respectively.

QS4 (5 µg/mL) had no effect on HSV-1 plaque counts, but the relative plaque size was decreased significantly to 22% (HSV-1 17 syn^+) and 6% (HSV-1 ANG^{path}). Bioassay-guided fractionation of QS 4 did not lead to preservation or enhancement of the inhibitory effect on HSV-1 induced membrane fusion. ASE up to 10 µg/mL did not reduce HSV-1 plaque counts, whereas HSV-1 cell-to-cell spread was strongly reduced by 2 µg/mL ASE resulting into a residual plaque size of 25% (HSV-1 17 syn^+) and 3% (HSV-1 ANG^{path}). 6 out of 12 reported Anagallis saponin isomers and one new Anagallis saponin structure were found in ASE by LC-MS analysis next to several accompanying substances.

Saponins isolates from aescin, *Quillaja saponaria* and *Anagallis arvensis* as well as two aescin isomers (aescin IA and isoaescin IA) are promising antiviral agents with antiviral activity against HSV-1. Besides the inhibition of HSV-1 induced membrane fusion, the detailed mode of action has to be discovered in further investigations.