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Antiviral activity of plant extracts against Herpes simplex virus type 1: Inhibition of viral adsorption by polyphenolic compounds

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Herpes Simplex virus type 1 (HSV-1) is the prototype member of the herpes virus subfamily Alphaherpesvirinae. It is extremely widespread and causes a broad range of diseases ranging from localised skin infections to serious infections of the central nervous system. After primary infection HSV-1 persists in sensory neurons, establishes latency and can be periodically reactivated. The therapeutic gold standard acyclovir effects the DNA replication of HSV-1, so aim of this work was to identify compounds which work in a different way and inhibit the initial step of infection, the adsorption of HSV-1 to cells.

Various secondary compounds of different classes of natural products and miscellaneous plant-extracts were tested for antiviral activity against herpes simplex virus typ-1 (HSV-1). The tested flavan-3-ols and the oligomeric proanthocyanidins exhibited strong antiviral activity, especially the galloylated ones. Compounds belonging to other classes of natural products (oligo-/polysaccharides, monoterpenes, saponines, peptides) were ineffective. Polyphenol-enriched extracts from the aerial parts of *Rumex acetosa* L. (R2), *Myrothamnus flabellifolia Welw*. (MF) and *Rhododendron ferrugineum* L. (RF) could be identified as extracts with the strongest antiviral activity. By plaque reduction test as well as MTT assay a concentration-dependent activity of these extracts could be identified und the HSV-1 specific inhibitory concentration (IC(50)) and the cytotoxic concentration (CC(50)) were determined. R2 exhibited an IC(50) of 0.8 µg/mL, MF of 0.4 µg/mL and RF of 7,4 µg/mL, the corresponding CC(50) were 78.6 µg/mL (R2), 50.2 µg/mL (MF) and 472.8 µg/ml (RF). These results were summarized in one term, the selectivity index (SI) (ratio of IC(50) to CC(50)), which was approximately 100 for R2, 120 for MF and 64 for RF. To determine the mechanism of these antiviral effects, the extracts were added at different stages during the viral replication cycle. With an adsorption assay it was clearly verified that the attachment of virus to host cells was inhibited by the extracts. The effective compounds of the extracts were shown to interact directly with viral particles leading to the oligomerisation of envelope proteins as demonstrated in immunoblot experiments for the essential viral glycoprotein D (gD). The oligomerisation of proteins takes place because polyphenols which are ingredients of the extracts have adstringent properties. The interaction of proanthocyanidins of R2 with viral structures was verified via HPLC analysis.

Using raft cultures with three-dimensional organotypic human skin equivalents it was shown by immunostaining and RT-PCR that treatment of cultures with R2 or MF after infection with HSV-1 resulted in a reduced viral spread.

The replication of adenovirus type 3, in contrast to HSV-1 a virus without envelope, was neither effected by R2, MF and RF nor by any other tested extract or compound. These results suggest that compounds of the effective extracts interact with viral envelope proteins and so a selective effect of proanthocyanidins isolated from R2, MF and RF against viruses with envelope is given.