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doi:10.1016/j.antiviral.2011.03.076

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Mechanistic Studies on a Novel Hydrophobic Derivative of Aglycoristocetin with Potent and Broad Activity Against Influenza Viruses

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We previously reported on the in vitro anti-influenza virus activity and structure-activity relationship of hydrophobic derivatives of the glycopeptide compound aglycoristocetin [Naesens et al., Antiviral Res., 2009]. In Madin-Darby canine kidney (MDCK) cells infected with different strains of influenza A/H1N1, A/H3N2 and B viruses, the lead compound 8e displayed an antivirally effective concentration of 0.4 μM. The concentration producing 50% inhibition of cell proliferation was 67 μM, yielding an antiviral selectivity index of 167. Virus yield at 72 h p.i. was reduced by 3 logs at 5 μM 8e. Inhibition of virus replication by 8e was confirmed in A549 and Vero cells. Influenza virus fully retained its sensitivity to 8e after 11 sequential virus passages in MDCK cells in the presence of 8e (at concentrations up to 25 μM). In time-of-addition studies, 8e lost activity when added 1 h or later p.i., showing that 8e inhibits an early step in virus replication. 8e produced no inhibitory effect on binding of virus to MDCK cells at 4 °C. Inhibition of hemagglutinin (HA)-mediated membrane fusion was excluded, since 8e had no effect on virus-induced red blood cell hemolysis at low pH, nor on polykaryon formation of HA-expressing cells exposed to low pH. Besides, 8e did not inhibit the conformational change of the HA at low pH, as observed in a tryptic digestion assay. Confocal microscopy on influenza virus-infected MDCK cells stained with anti-nucleoprotein antibody at 1 h p.i., revealed that 8e causes a marked inhibition of the nuclear entry of the virus. Studies with reference compounds are ongoing to unravel precisely how 8e interferes with the influenza virus entry process. The aglycoristocetin derivative 8e represents a new class of potent and broad-acting influenza virus inhibitors with potential therapeutic relevance.

Supported by the Flemish Fonds voor Wetenschappelijk Onderzoek, the Geconcerteerde Onderzoeksacties and the International Consortium on Anti-Virals

doi:10.1016/j.antiviral.2011.03.077

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Avoidance of Coxsackievirus Drug Resistance by Using a Novel Scheme of Combining Anti-Enteroviral Inhibitors *In Vivo*

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We present a novel scheme for combined application of anti-enteroviral substances in coxsackievirus infections in mice, which consists of a consecutive alternating, not simultaneous, administration of the substances in combination. This study summarizes the results from the experiments with two coxsackieviruses – CVB1 (neurotropic) and CVB3, as the latter is represented by two strains – cardiotropic strain Woodruff and neurotropic strain Nancy. A triple combination of enteroviral replication inhibitors showing good efficacy was selected – its effectiveness is expressed in lengthening of the mean survival time and about 50–60% reduction of mortality rate in infected mice as compared both to the placebo group, partner compounds used alone every day, and to the same combination applied simultaneously every day. Studies of the drug sensitivity of viral brain isolates from mice, treated with this combination and the combination partners indicate that virus isolates from the group treated with the alternating combination not only preserve, but even increase their sensitivity to the drugs.

doi:10.1016/j.antiviral.2011.03.078

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Inhibition of Enveloped Virus Infection of Cultured Cells by Valproic Acid

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Valproic acid (VPA) is a short chain fatty acid commonly used for treatment of neurological disorders. As VPA can interfere with cellular lipid metabolism, its effect on the infection of cultured cells by viruses of seven viral families relevant to human and animal health, including eight enveloped and four non-enveloped viruses, was analyzed. VPA drastically inhibited multiplication of all the enveloped viruses tested, including the zoonotic lymphocytic choriomeningitis virus and West Nile virus (WNV), while it did not affect infection by the non-enveloped viruses assayed. VPA reduced vesicular stomatitis virus infection yield without exerting a major blockage of either viral RNA or protein synthesis. In contrast, VPA drastically abolished WNV RNA and protein synthesis, indicating that this drug can interfere at different steps of enveloped virus infection. Thus, VPA can contribute to understand crucial steps on viral maturation and to develop future strategies against infections associated with enveloped viruses.

doi:10.1016/j.antiviral.2011.03.079

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Antiviral Effect of Molluscan Haemocyanines

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Hemocyanins (Hcs) are oxygen-binding glycoproteins, freely dissolved in the hemolymph, of many arthropods and mollusks. The structure and oligosaccharide moieties of the molluscan Hcs *Rapana venosa* and *Helix lucorum* have been determined and recently received particular interest due to their immunostimulatory properties. Hemocyanins also have been found to show antiviral activity. In the present study the antiviral effect is tested against the *in vitro* replication of human respiratory syncytial virus (hRSV) and influenza virus A/Aichi/2/68/H3N2 by the CPE-inhibition assay. The complete molecules of Hcs do not show antiviral effect. But a marked antiviral activity of the structural subunits and the functional units is found against the replication of hRSV. Their effect