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# Thw Glycobiology of Human Adenovirus Infections: implications for tropism and treatment

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Akademisk avhandling

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**Abstract:** Human adenoviruses (HAdVs) are common human pathogens, causing gastrointestinal, ocular, and respiratory infections on a regular basis. Epidemic keratoconjunctivitis (EKC) is a severe ocular infection for which no approved antivirals are available. HAdV-D37 is one of the causative agents of EKC and uses sialic acid (SA)-containing glycans as cellular receptors. HAdV-D37 interacts with SA via the knob domain of the trimeric virus fiber protein, containing three SA-binding sites. HAdV-D37 also bind to glycosaminoglycans (GAGs), but the outcome of this interaction remains unknown. Here, using biochemical and cell-based assays, the impact of GAGs on HAdV-D37 infection (paper I) was investigated. We found that HAdV-D37 interacts with both soluble and cell-surface sulfated GAGs via the knob domain of the viral fiber protein. Remarkably, removal of heparan sulfate (HS; a type of GAG) from human corneal epithelial (HCE) cells by heparinase III enhanced HAdV-D37 infection. We propose that sulfated GAGs in bodily secretions and on plasma membranes function as decoy receptors that prevent the virus from binding to SA-containing receptors and inhibit subsequent virus infection. We also found abundant HS in the basement membrane of the human corneal epithelium. We suggest that this layer of HS functions as a barrier to sub-epithelial infection of HAdV-D37. Based on this finding, we hypothesized that GAG-mimetics may act as artificial decoy receptors and inhibit HAdV-D37 infection. Here, the antiviral effect of suramin (a known GAG-mimetic) and its analogs against HAdV-D37 (paper II) was evaluated. Interestingly, all compounds displayed antiviral effects by inhibiting the binding of HAdV-D37 to HCE cells. The antiviral effect of suramin was HAdV species-specific. We report for the first time that virus binding to cell-surface decoy receptor constitutes a potential target for antiviral drug development.

HAdVs are the major cause of infectious conjunctivitis, constituting up to 75% of all conjunctivitis cases worldwide. Species B HAdV type 3 (HAdV-B3) causes pharyngoconjunctival fever (PCF), whereas HAdV-D8, -D37, and -D64 cause EKC. Recently, HAdV-D53, -D54, and -D56 have emerged as new EKC-causing agents. HAdV-E4 causes both PCF and EKC. SA-containing glycans have been established as cellular receptors for HAdV-D37. By means of cell-based assays, we investigated if ocular HAdVs other than HAdV-D37 also use SA-containing glycans as receptors on HCE cells (paper III). It was found that SA-containing glycans function as cellular receptors for five (HAdV-D8, -D37, -D53, -D54, and -D64) out of six EKC-causing species D HAdVs. We showed that these viruses interact with SAs via the knob domain of the viral fiber protein. HAdV-E4 and -D56 infection of cells was independent of SAs. Surprisingly, HCE cells were completely refractory to HAdV-B3 infection. A trivalent sialic acid (TSA) derivative ME0462 (compound 17a in paper II), designed to bind to SA-binding sites on HAdV-D37 fiber knob, also showed potent antiviral activity against several EKC-causing HAdVs. This suggests that ME0462 can be used as a broad-spectrum antiviral against known and emerging EKC-causing HAdVs. Surface plasmon resonance (SPR) analysis confirmed a direct interaction between ME0462 and fiber knobs of EKC-causing HAdVs.

Recently, a TSA derivative (ME0322; designed to bind to SA-binding sites on HAdV-D37 fiber knob) was shown potent antiviral against HAdV-D37 *in vitro*. To improve the antiviral potency of this compound, six new TSA derivatives were synthesized and their inhibitory effects were evaluated against HAdV-D37 (paper IV). Interestingly, the best compound 17a was found approximately three orders of magnitude more potent ($IC_{50\ (binding)}$ = 1.4 nM, $IC_{50\ (infection)}$ = 2.9 nM) than ME0322 ($IC_{50}$ in µM range). SPR data showed that HAdV-D37 fiber knob binds to TSA compounds with high affinities. Structural data revealed the trivalent binding mode of all newly synthesized TSA compounds to HAdV-D37 fiber knob. Ophthalmic toxicity of compound 17a (best compound) was also investigated in rabbits without any sign of toxicity.

HAdV-D36 is a member of species D HAdV and has the ability to infect a broad range of animals, which is unusual for HAdVs. Another remarkable feature of HAdV-D36 is that this virus induces obesity in experimental animals. Several epidemiological studies highlighted a link between HAdV-D36 and human obesity. There is no information about the cellular receptor usage by HAdV-D36. Using structural biology and cell-based approaches, we investigated the cellular receptor(s) for HAdV-D36 (paper V). We show that HAdV-D36 attaches to host cells (via the fiber knob) using the coxsackie and adenovirus receptor (CAR), SA-containing glycans, and one or more unknown proteins or glycoproteins. Using glycan microarray, we found that HAdV-D36 displays binding preference to a rare SA-variant: 4-*O*,5-N-diacetylneuraminic acid (Neu4,5$Ac_2$), over the more common SA (in humans) i.e. 5-N-acetylneuraminic acid (Neu5Ac). Structural analysis of HAdV-D36 fiber knob:Neu4,5$Ac_2$ complex explained this preference. To date, Neu4,5$Ac_2$ has not been detected in humans, although it is synthesized by many domestic and livestock animals. Our results indicate that HAdV-D36 has evolved to utilize a specialized set of cellular receptors that coincide with a unique host range and pathogenicity profile.

These studies provide insights into multiple roles of glycans in HAdV infection cycle and highlight the therapeutic potential of glycans/glycan-mimetics in HAdV-D37 infection.

**Keywords**
Adenovirus, cellular receptor, glycosaminoglycan, sialic acid, decoy receptors, tropism, epidemic keratoconjunctivitis, antiviral drugs, GAG-mimetic, obesity

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