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Mechanism of inhibitory effect of polyanionic polymers on human cytomegalovirus infectivity

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Several sulfated polymers [dextran sulfate, pentosan polysulfate, heparin, co-polymers of acrylic acid with vinylalcohol sulfate (PAVAS), sulfated α-cyclodextrins] as well as polyhydroxycarboxylates derived from phenolic (PDP) compounds and polycarboxylates based on aurintricarboxylic acid proved to be potent inhibitors of human cytomegalovirus (CMV) infectivity *in vitro*. A close correlation was found between the 50% inhibitory concentrations of the sulfated polymers and poly(hydroxy)carboxylates for CMV cytopathicity, virus-cell binding and expression of immediate early antigens (IEA) in human embryonic lung (HEL) cells. CMV particles were bound to heparin immobilized on a sepharose matrix and the polyanionic polymers specifically eluted the virus particles from this matrix. Enzymatic digestion of cell surface heparan sulfate, but not chondroitin sulfate A, B, C, prevented the cells from being infected with CMV. In a preliminary experiment it was demonstrated that radiolabeled CMV was not able to bind to mutant derivatives of Chinese hamster ovary (CHO) cells deficient in synthesis of all glycosaminoglycans (kindly provided by Dr. J. Esko, University of Alabama) while CMV bound efficiently to wild-type CHO cells. The mechanism of action of the sulfated polymers and poly(hydroxy)carboxylates can be attributed to an inhibitory effect on the binding of CMV particles to the host cells. Presumably the polyanionic polymers interact with the viral envelope site(s) involved in the attachment of the CMV virions to the cell surface constituent heparan sulfate.

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Mutant Varicella-Zoster Virus Thymidine Kinase: Clinical Resistance Correlates with Enzyme Impairment.

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Varicella-zoster virus (VZV) encodes a thymidine kinase (TK, EC 2.7.2.21) which phosphorylates several antiviral nucleoside analogs including acyclovir (ACV). A mutation in the VZV TK coding sequence, resulting in an arginine-to-glutamine substitution at amino acid residue 130 (R130Q), is associated with clinical resistance to ACV. We have expressed the wild-type and the mutant enzymes in bacteria, and have studied the kinetic characteristics of the purified enzymes. The arginine-to-glutamine substitution resulted in decreased catalytic activity and altered substrate specificity. The most striking effect was a decrease in the rates of nucleoside phosphorylation to less than 2% of the rates with the wild-type enzyme. This was accompanied by increased apparent K_m values for thymidine and deoxycytidine. Acyclovir was not detectably phosphorylated by the R130Q enzyme but still competed with thymidine for the enzyme. The inability of the R130Q enzyme to catalyze the phosphorylation of ACV correlates with the high degree of resistance to ACV noted with a clinical isolate of VZV. (Some of these data have been published in the *Journal of Virology*, December 1991.)