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S15.8

Polymeric Sialosides as Inhibitors of Influenza Virus-to-Cell Adhesion

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Influenza virus attachment to host cells is mediated by specific interaction of viral hemagglutinin with the cell-surface sialooligosaccharides. In effort to produce potent synthetic inhibitors of viral attachment a number of polyacrylates and dextrans bearing α-benzylsialoside residue (Neu5Acα2-$OCH_2C_6H_4NHCOCH_2NH$-) were prepared and tested as inhibitors of virus binding activity and multiplication in tissue culture. 20 various strains of influenza A and B virus were tested.

The greatest affinity for inhibitors was shown mostly by H3 strains emerged after 1977, while H1, H2 and type B strains were of lower affinity. Polyacrylic acid containing 12 mole % of sialoside (P_{12}) was found to be the best inhibitor, those containing 5 and 20 mole % were significantly less effective. Elongation of spacer by aminocaproyl led only to 2–4 fold higher inhibition activity for H1, H2 and type B viruses and had no effect on H3 high affinity strains. The influence of carrier on inhibitory activity was demonstrated by comparison of polyacrylates. Polyacrylic acid as carrier was the best for H1 strains, some H3 and type B viruses have higher affinity for polyacrylamide and -ethanolamide type polymers, in case of H3 (after 1977) strains all three polymers had equal binding constants. Additional substituents (alkyls, Gal, aminoacids) of carrier did not increase the binding affinity of polysialoside. Hydrophobic (*n*-cetyl) or strongly ionized (sulfonic acid) substituents lowered inhibition ability. The polymer-virus binding was amplified with the increase of polymer MW. For A/Philippines/2/82 the 1000 kDa polymer affinity was 500-fold higher comparing to 20 kDa. Further increase of MW resulted in decrease of affinity. Activity of dextran based inhibitors increased tenfold when going from D70 to D500.

Potency of polysialosides as inhibitors of A/Philippines/2/82 receptor-binding activity and infection ability (MDCK cells)

	equine α_2MG	P	D70	D500
K_{aff}, mM SA	0.007	0.05	1.5	0.16
ED_{50}, mM SA	0.06	0.2	1.0	0.5

S15.9

Characterization and Antiviral Function of Sialyloligosaccharides from Egg Yolk

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The role of various sialyloligosaccharides is being explored to create novel pharmaceutical products and functional foods. Recently, a number of sialyloligosaccharides have been identified to modulate the interaction of various pathogenic viruses. Human rotavirus is known as a major pathogen of infectious gastroenteritis in infants and is also a cause of traveler's diarrhea. Previously, we reported an economical process of large scale preparation of sialic acid from egg. In the present study, we isolated, characterized sialyloligosaccharides of egg yolk origin and studied their interaction with rotavirus. The oligosaccharides of egg yolk were liberated from protein by enzymatic treatment. Sialyloligosaccharides were separated from neutral oligosaccharides, peptides and salts by column chromatography at industrial scale. The sialyloligosaccharides labeled with ABEE were fractionated by anion exchange, normal phase and reversed phase HPLC. Their structures were determined by 400 MHz NMR. Aliquots of sialic acid and sialyloligosaccharides were added to rotavirus (SA11, 1×10^5 FCFU/ml) and absorbed onto MA-104 cells. The ability of sialic acid and its oligosaccharides to inhibit rotavirus replication was measured by viral neutralization analysis. Various sets of mice were orally administered with sialyloligosaccharides and inoculated with SA11 virus. The product having sialyloligosaccharides completely inhibited rotavirus infection *in vitro* and it also showed encouraging results *in vivo*.

S15.10

Anti-Tumor Effects of Sphingolipids

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The anti-tumor activity of seven sphingolipids, two ceramides and five glycosphingolipids against the syngeneic murine ascitic tumors MH134 and MM102 in C3H mice was examined. Five of these compounds showed anti-tumor activity against the tumors, ceramides type-IV (Cer-IV) having the highest activity without cytotoxic or cytostatic activity. These results indicate that the fatty acid in ceramide and sugar chains binding to it affect the anti-tumor activity *in vitro*. The anti-tumor activity of Cer-IV depended on the time of treatment. Mice treated with Cer-IV one day after tumor implantation showed the highest rate of survival. The cured mice were resistant to rechallenge with the same tumor (MH134→MH134, MM102→MM102) but not with a heterologous tumor (MH134→X5563, MM102→X5563), indicating that the effects of Cer-IV may be due to *in vivo* induction of specific immunity. Studies with various antibodies demonstrated that the anti-tumor effect of Cer-IV was inhibited by all the antibodies tested (L3T4, Lyt-2, and Thy-1,2 T cells, macrophages, and TNFα) in the induction phage (before Cer-IV administration) and by the antibodies of L3T4 and TNFα in the effector phage (after Cer-IV administration). Therefore, the anti-tumor effect of cytotoxic