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Negatively Charged Human Serum Albumins: A Novel Class of Polyanionic Proteins with a Potent Anti-HIV-I Activity

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In antiviral therapy some of the serious side effects of nucleoside analogues may be reduced by coupling the drugs to a macromolecular vector which is selectively taken up by the target cells. In this way anti-HIV agents can be delivered to T-lymphocytes and macrophages. Recently we discovered that some (glyco)proteins designed as a drug carrier for anti-HIV agents already exhibited an intrinsic antiviral activity even without coupling of the antiviral drug AZT. This activity was shown to be caused by an increased negative charge of the particular (glyco)protein.
An extremely high anti-viral activity was found after derivatization of human serum albumin (HSA) using anhydrides of succinic acid or cis-aconitic acid, producing strongly negatively charged proteins. The in vitro IC_{50} values were in the nanomolar concentration range and they showed favorable pharmacokinetics, very low acute toxicity and immunogenicity. The products showed neither anticoagulant activity. The negatively charged albumins exhibited potent activity against SI inducing clinical HIV isolates. We postulate a high affinity binding to the V3 loupe of gp120, explaining the potent effect on HIV-I replication and syncytium formation.

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Preparation and Characterization of Conjugates of (Modified) Human Serum Albumin Liposomes: Drug Carriers with an Intrinsic Anti-HIV Activity

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In order to combine the carrier quality of liposomes with the intrinsic antiviral activity against HIV 1 of negatively charged human serum albumins, the covalent coupling of these albumins to small type liposomes, using the heterobifunctional reagent N-succinimidyl-S-acetylthioacetate (SATA), was characterized. Coupling to liposomes of HSA or HSA which was partially derivatized with cis-aconitic anhydride (Aco-HSA), significantly increases the liposome diameter. The amount of HSA that can be coupled to liposomes depends on the degree of derivatization of the HSA and ranged from 75 μg HSA / μmol total lipid for native HSA to 20 μg HSA / μmol total lipid for Aco_{54}-HSA. The in vivo behaviour of liposome-(modified)HSA conjugates can be modulated by modification of the HSA coupled to the liposomes. With liposome-native HSA conjugates more than 70% of the conjugate is found in the blood circulation after 30 minutes, while liposome-Aco-HSA conjugates are cleared from the blood completely in less than 30 minutes. The rapid clearance of these conjugates is related to a high liver uptake. The circulation time of these conjugates can be extended by coating the liposomes with poly-ethylene glycol (PEG-PE); 3 hours after injection more than 60% of PEG-liposome-ACOHSA conjugates are remaining in the blood. Both liposome-Aco_{54}-HSA and PEG-liposome-Aco_{54}-HSA conjugates show potent antiviral activity in an in vitro anti-HIV 1 assay. In conclusion, HSA which is derivatized to a high degree can still be coupled covalently to liposomes using the reagent SATA. The in vivo disposition of liposome-(modified)HSA conjugates can be modulated by modification of the HSA coupled to the liposomes and by modification of the liposome surface with PEG. This allows for the development of a potent antiviral drug carrier system which might act simultaneously on two stages of the HIV 1 life cycle; on the virus binding as a result of the intrinsic antiviral activity of the carrier and for example on reverse transcriptase by encapsulated nucleoside analogues.