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Negatively Charged Human Serum Albumins: Inhibition of Replication of Primary HIV-1 Isolates and Biomolecular Interaction with Gp120

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Recently a novel class of polyanionic proteins, the negatively charged albumins (with the prototypes Suc-HSA and Aco-HSA), that lack an effect on the blood coagulation, but have an potent anti-viral activity on HIV-1 IIIB and other laboratory strains have been described. Here the anti-viral effect of these compounds on replication of phenotypically distinct patient HIV-1 isolates that differ with respect to syncytium inducing (SI) capacity and cell tropism is reported. In addition the interaction of these compounds with the gp120 envelope protein of the distinct HIV-1 isolates is described. Both Suc-HSA and Aco-HSA potently inhibited replication of primary HIV-1 variants with IC_{50} values in the nanomolar concentration range. Moreover, Suc-HSA and Aco-HSA IC_{50} values for replication of a macrophage-tropic HIV-1 variant in either peripheral blood mononuclear cells or primary macrophages was identical. The inhibition of the formation of syncytia and the absence of early products of reverse transcription, in cells inoculated with HIV-1 in the presence of Suc-HSA or Aco-HSA indicated that these agents interfere at an early level in the virus replication cycle, most likely at the level of virus entry. Experiments in which binding of Suc-HSA and Aco-HSA with a series of gp120-peptides was studied demonstrated binding affinities that were comparable with such a mechanism of action: Interaction was only observed for the V3 loop and the C-terminal part of gp120. These peptide sequences have been reported to be involved in the viral fusion and/or syncytium formation process, subsequent to CD4 binding. Competition studies with heparin and dextran sulphate indicated that the interaction between HIV-1 gp120 peptides and the negatively charged albumins is predominantly caused by electrostatic interactions, but that hydrophobic interactions are also involved. The inhibitory capacity of Suc-HSA and Aco-HSA on primary HIV-1 variants and the inhibition of the virus/cell fusion as well as syncytium formation, may imply that these agents are potential candidates for antiviral therapy in vivo.

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Pharmacokinetics and anti-HIV-1 Efficacy of Negatively Charged Human Serum Albumins in Mice

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Negatively charged albumins (NCAs, with the prototypes Suc-HSA and Aco-HSA), modified proteins with a potent anti-HIV-1 activity *in vitro*, were studied for their pharmacokinetic behaviour in mice and their *in vivo* anti-HIV-1 efficacy in an HIV-1 infection model in mice. I.v. injections of 1 mg to 300 mg/kg for both NCAs showed a lineair correlation between the area under the curve (AUC) and the dose with t½s of 44 min (± 21 min) and 56 min (± 11 min) for Suc-HSA and Aco-HSA respectively. This pattern appeared to be quite different with the first order kinetic profile of native HSA in mice (t½: 124 ± 31 min). In contrast with the pharmacokinetic profiles of NCAs in rats, a saturable elimination process could not be demonstrated. Plasma levels were significantly influenced by preinjections of formaldehyde treated albumin (F-HSA) or polyinosinic acid (PIA), used to demonstrate scavenger receptor mediated elimination. Injection of the inhibitors resulted in plasma levels that were 3 to 4 times higher for Aco-HSA and Suc-HSA respectively, as compared to controls. Organ distributions showed an accumulation in liver (Suc-HSA:15.6±6.3 % of the dose, Aco-HSA 20.0±2.0%), lungs (Suc-HSA 12.1±9.9%, Aco-HSA 14.3±13.5%) and bone (Suc-HSA 10.7±6.0%, Aco-HSA 17.4±4.8%). Uptake in liver and spleen could not be reduced by preadministration of an excess of F-HSA or PIA.. This excludes the involvement of scavenger receptors in liver and spleen. Intraperitoneal injections of 300 mg/kg Suc-HSA demonstrated a rapid absorption into the general circulation. One hour after i.p. injection, the bioavailability was close to 100 %. Next, Suc-HSA was tested in an HIV-1 infection model, developed by us in mice. Immunosuppressed B cell-deficient CBA/N mice were i.p. injected with human PBL. Suc-HSA (300 mg/kg) was i.p. injected one hour before the mice were triggered with the HIV-1 IIIb strain. The viral load in the human lymphocytes, isolated from the ascites fluid, was determined after 1 and 7 days. It appeared that Suc-HSA was able to completely protect the mice against the HIV-1 virus. These results imply that not only *in vitro* but also in an *in vivo* HIV-1 infection model the NCAs are antiviraly active. These findings render these conjugates promising compounds for antiviral therapy in humans. NCAs can be used as carriers for AZT-like compounds, resulting in dual-targeting preparations, with inhibitory effects on virus entry as well as on RNA/DNA transcription, which might have beneficial effects with regard to antiviral synergism and prevention of drug resistance at long term administration.