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Novel polyanionic albumin derivatives are potent and selective *in vitro* inhibitors of HIV, FIV and SIV cell fusion and giant cell formation.

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The positively charged NH^+_3 groups of lysine residues of human serum albumin (HSA) were derivatized, introducing one or more negatively charged COO^- groups. This led to a series of modified albumins (mHSA) with a polyanionic character. The in vitro anti-HIV activity of these compounds was positively correlated to their net negative charge. The IC_{50} of the most potent mHSA was 74 pM (0.006 μg/ml), which is about 30 times more potent than AZT on a molar basis. No toxicity was observed at concentrations up to 1 mg/ml, resulting in a selectivity index of at least 100.000. The compounds did not have a significant anticoagulant activity in contrast to sulphated polysaccharides. From the combined data on anti-gp120 mAb-, anti-CD4 mAb- and virus-cell binding, as well as on giant cell formation assays, we conclude that the mHSA's interfere with virus-cell fusion and syncytium formation, but not primarily with the gp120-CD4 interaction. mHSA's did not effect replication or cell fusion of 14 other envelope viruses tested (except that of influenza virus). Kinetic studies and immunohistochemistry showed that the mHSA's are endocytosed in non parenchymal liver cells via the scavenger receptor with a high affinity, low capacity uptake system. Therefore, in intact rats, plasma concentrations well above the IC_{50} could be obtained after i.v. administration.

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Cellular Metabolism and Enzymatic Phosphorylation of (2R,5R)-9-[2,5-Dihydro-5-(phosphonomethoxy)-2-furanyl]adenine (D4AMPI), An Anti-HIV Agent

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D4AMPI, a phosphonate isostere of D4A-monophosphate[1], demonstrated in vitro anti-HIV activity similar to that of DDI and PMEA (IC_{50}=10-28μM) and inhibited CEM cell growth by 50% at 200μM[2]. Cellular metabolism of D4AMPI and the potential target enzymes involved were investigated to elucidate the mechanism of action of this agent. After incubating CEM cells with 7 μM (2,8-^{3}H)D4AMPI for 24 hr, HPLC analysis of the 60% MeOH cell extract revealed the presence of D4AMPI mono- and diphosphate. (^{3}H)D4AMPI diphosphate accounted for 50% of the total radioactivity detected in the cell extract. The uptake and phosphorylation of D4AMPI in CEM cells was time- and dose-dependent, with the concentration of the diphosphorylated D4AMPI reaching as high as 30% of that of drug (5-100 μM) in the media. Formation of the mono- and diphosphorylated D4AMPI was also observed in quiescent and PHA-stimulated human PBMCs. When supplied with ATP and PEP, D4AMPI was phosphorylated to its mono- and diphosphate in a cell free system containing adenylate kinase and pyruvate kinase. D4AMPI diphosphate inhibited HIV-RT (K_i=0.64 μM) competitively with respect to dATP (K_m=0.45 μM), while unphosphorylated D4AMPI showed no significant inhibition at a concentration as high as 500 μM. After removal of D4AMPI from the media, D4AMPI diphosphate concentration decayed with a half-life around 13 hr in CEM cells and around 30 hrs in resting PBMCs. These results suggested that D4AMPI is taken up by cells and metabolized to its diphosphorylated form effectively. The high intracellular level of diphosphorylated D4AMPI and its long half-life may also contribute to the efficiency with which D4AMPI diphosphate competes against the natural substrate, dATP, for HIV-RT in infected cells, thus inhibiting viral DNA synthesis.

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