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Suppression of TNF-α- and HIV-1 gp120-induced neural cell death by an anti-inflammatory alkaloid, cepharanthine

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Human immunodeficiency virus type 1 (HIV-1) gp120 and tumor necrosis factor (TNF)-α are considered to play an important role in the pathogenesis of HIV-1- associated central nervous system (CNS) disorders. These substances are produced predominantly by HIV-1-infected macrophages and microglia in the brain and induce neural cell death. Cepharanthine is a biscoclaurine alkaloid isolated from *Stephania cepharantha* HAYATA and has been shown to have anti-inflammatory, anti-allergic, and immunomodulatory activities *in vivo*. We recently reported that it could inhibit TNF-α- or phorbol 12-myristate 13-acetate-induced HIV-1 gene expression in chronically infected cells through the inhibition of nuclear factor-κB. In this study, we have demonstrated that cepharanthine suppresses TNF-α- or gp120-induced neural cell death *in vitro*. The significant suppression of neural cell death was observed with cepharanthine at a concentration of 0.2 μg/ml. Propidum iodide staining and terminal deoxynucleotidyl transferase-mediated dUTP nick and labeling staining of TNF-α- or gp120-treated cells revealed that the cell death was due to apoptosis. Consequently, cepharanthine proved inhibitory to apoptotic cell death of neural cells. It penetrates to the blood-brain barrier, and the drug containing cepharanthine as a major component has been used for the treatment of patients with chronic inflammatory diseases in Japan. Thus, cepharanthine should be further pursued for its therapeutic and prophylactic potential in HIV-1 associated CNS disorders.

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INTRACELLULAR METABOLISM OF 3-METHYL-*CYCLO*SAL-D4TMP AND 3-METHYL-*CYCLO*SAL-AZTMP: SAME CONCEPT BUT DIFFERENT ANTIVIRAL OUTCOME

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In previous work we observed a striking difference in biological activity of *cyclo*Sal-pronucleotides of the antivirally active nucleoside analogues d4T and AZT: while the former compounds exhibited pronounced antiviral activity in thymidine-kinase deficient CEM/TK$^-$ cells as a proof for the kinase bypass concept, the latter compounds were surprisingly less active in this cell system. Therefore, tritium-labeled 3-methyl-*cyclo*Sal-d4TMP and 3-methyl-*cyclo*Sal-AZTMP have been synthesized to study the intracellular metabolism of these compounds in wild-type CEM/O as well as in CEM/TK$^-$ cells. Exposure of CEM/TK$^-$ cells to the lipophilic d4TMP prodrug resulted in an efficient intracellular release of d4TMP and subsequent formation of d4TDP and d4TTP while d4T failed to generate any significant amounts of d4TTP levels. In contrast, both 3-methyl-*cyclo*Sal-AZTMP and AZT failed to generate significant levels of phosphorylated AZT metabolites in the TK$^-$ cells. This can be attributed to relatively high hydrolysis rate of AZTMP to the parent nucleoside AZT, combined with the inability of the CEM/TK$^-$ cells to phosphorylate AZT to AZTMP through the cytosolic salvage enzyme thymidine kinase. The effect of the different stereochemistry of the *cyclo*Sal-compounds on their antiviral and metabolic properties will be discussed.

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Treatment of human T-lymphoid cell lines with pharmacological relevant cytarabine concentrations results in decreased CD4 expression and reduced HIV-1 susceptibility

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HIV-1 infected patients often suffer from a broad spectrum of malignant tumours affecting various organ systems. The fact that these HIV-1 infected patients were simultaneously treated with antiretroviral and antitumoral chemotherapeutic agents arise the question of possible interactions of both therapy regimens. We found that continuous cultivation of T-lymphoid cell lines in the presence of pharmacological relevant concentrations of cytarabine (Ara-C) results in decreased expression of CD4 molecule, the major cellular receptor for HIV, as demonstrated by FACS and RT-PCR analysis. HI-virus titers ($TCID_{50}$) were significantly reduced in Ara-C-resistant cells in comparison to parental cells, whereas cell growth rate was unchanged. Ara-C-resistant cells were significantly less sensitive against cytotoxic effects of cytidine analogs such as Ara-C and gemcitabine (dFdC). Enzymatic observations showed that in addition to decreased gene expression of deoxycytidine kinase (dCK) also thymidine kinase (TK) mRNA was decreased in Ara-C resistant cells in comparison to parental cells. The lower mRNA levels of both enzymes dCK and TK correlated significantly with lower enzyme activities. These cellular resistance mechanisms result in diminished antiretroviral activity of cytidine analogs such as zalcitabine (ddC), lamivudine (3TC) and also in cross-resistance against zidovudine (AZT). The results demonstrate that *in vitro* selection of T-lymphoid cells with antitumoral agents such as Ara-C results in down-regulation of CD4 molecules and therefore decreased susceptibility of HIV-1. Furthermore, decreased dCK and TK function in Ara-C resistant cells leads to decreased anti-HIV-1 activity of cytidine and thymidine reverse transcriptase inhibitors in these cells.

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Effects of ADA on Intracellular Nucleoside Triphosphate Pools and Implications for Use in Combination Therapy

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Azodicarbonamide (ADA)is a novel antiviral compound that has *in vitro* activity and virucidal activity against HIV-1 and all other retroviruses except spumaretroviruses. It has shown evidence of activity in initial clinical studies in man. Initial studies to elucidate mechanism of action were unproductive. Due to its structural similarity to hydroxyurea (HU), a ribonucleotide reductase inhibitor, we began to evaluate ADA effects on intracellular nucleoside triphosphates. Initial studies in a continuous T cell line, A3.01 cells, showed a specific reduction in dCTP pools to 51% of untreated controls at 10 and 100 uM. At 200 uM cellular toxicity was observed with pools of TTP, dCTP and dATP reduced. At 100 uM HU, the same three pools were inhibited without evidence of toxicity. In PBMC, the triphosphate pools are much lower, and ADA had a greater effect on dCTP pools with inhibition ranging from 50% to 99% at 50 uM ADA. In continuous T cells, high concentrations of deoxycytidine reversed the antiviral effects of ADA. ADA showed subsynergistic antiviral interactions with ddC, 3TC, or abacavir. While these studies were ongoing, Rice, et al. reported that ADA targets HIV NCp7 zinc fingers. It remains unclear whether both mechanisms contribute to the antiviral activity of ADA.