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Antiviral Effects of Milk Proteins: Acylation results in Polyanionic Compounds with Potent Activity against HIV type I and II *in vitro*.

P.J. Swart[1*], M.E. Kuipers[1], C. Smit[1], E. De Clercq[2], H. Huisman[3] and D.K.F. Meijer[1]

[1]Department of Pharmacokinetics and Drug Delivery, University Centre for Pharmacy, A. Deusinglaan 2, 9713 AW Groningen, The Netherlands [2]Rega Institute for Medical research, Katholieke Universiteit Leuven, B-3000 Leuven Belgium. [3]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Department of Delevopmental Research, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.

A number of native and modified proteins from bovine or human sources were analyzed for their anti-HIV-1 and anti-HIV-2 effect *in vitro* in a MT4 cell test system. The proteins investigated were lactoferrin, α-lactalbumin, ß-lactoglobulin A and ß-lactoglobulin B. Of all milk proteins tested only native lactoferrin originated from bovine milk, bovine colostrum, human milk and human plasma was able to completely inhibit HIV-1 replication. The in vitro IC_{50} values ranged between 500 and 1500 nM. The other native proteins had no effect. By acylation of the amino function of the amino acid lysin in the protein using anhydrides of succinic acid or *cis*-aconitic acid, proteins were synthesized that all showed a strong antiviral activity against the human immunodeficiency virus type 1 and/or 2. The *in vitro* IC_{50} values of the aconitylated proteins were in concentration range of 0.3 to 3 nM. Peptide scanning studies indicated that the native lactoferrin as well as the charged modified proteins strongly bind to the V3 loop of the gp120 envelope protein in the same concentration range as the above mentioned IC_{50}. Therefore, shielding of this domain, resulting in inhibition of the cellular fusion and entry of the virus in MT4 cells is the likely underlying mechanism. Succinylation or aconitylation of α-lactalbumin and ß-lactoglobulin A/B also produced strong anti-HIV-2 activity with IC_{50} values in the order 500 to 3000 nM. All compounds showed virtually no cytotoxicity in the concentration range studied.

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Thiadiazole derivatives were highly potent inhibitors of HIV-1

[1]Masatoshi Fujiwara, [1]Katsushi Ijichi, [2]Yasuaki Hanasaki, [2]Teruhiko Ide,[2]Kimio Katsuura, [3]Hiromitsu Takayama, [3]Norio Aimi, [4]Shiro Shigeta, [1]Kenji Konno, [1]Tomoyuki Yokota and [5]Masanori Baba

[1]Rational Drug Design Laboratories, [2]Tosoh Co. Ltd., [3]Faculty of Pharmaceutical Sciences, Chiba University, [4]Fukushima Medical Collage, [5]Faculty of Medicine, Kagoshima Univercity, Japan.

We have recently reported that thiadiazole (TDA) derivatives are highly potent inhibitors of HIV-1 replication. These compounds belong to the family of nonnucleoside reverse transcriptase inhibitors (NNRTIs). Furthermore, novel TDA derivatives have been synthesized in an attempt at developing the compound as effective anti-HIV-1 agents. Among them, RD4-2217 was found to be the most potent inhibitor of HIV-1 replication. RD4-2217 inhibited the replication of HTLV-III_B strain in MT-4 cells at a concentration of 6 nM. It was also highly inhibitory to clinical isolates and AZT-resistant mutants of HIV-1. However, NNRTI-resistant mutants and HIV-2 were less sensitive or insensitive to the compound. The combination of RD4-2217 with AZT or the protease inhibitor A-75925 exerted synergistic anti-HIV-1 activity. Studies on the emergence of drug-resistant mutants revealed that, although much higher concentrations (1-10 μM) were required, RD4-2217 could completely suppress the breakthrough of the virus in culture supernatants during its long-term treatment. These results suggest that the TDA derivatives are worth pursuing as candidate drugs for the chemotherapy of HIV-1 infections.

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ADA, A Potential Anti-HIV Drug

M. Witvrouw[1], M. Vandevelde[2], J.-P. Tassignon[3], J.-C. Schmit[1], J. Desmyter[1] and E. De Clercq[1]

[1]Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium; [2]Hubriphar SPRL, Brussels, Belgium; [3]Tassignon & Partners S.A., Brussels, Belgium

ADA (1,1'-azobisformamide), an azoic compound with the following structural formula:

$H_2N\text{-}OC\text{-}N{=}N\text{-}CO\text{-}NH_2$

has been recently introduced in a phase I/II clinical trial in combination with nucleoside reverse transcriptase (RT) inhibitors in advanced AIDS patients. Here we report for the first time preclinical results of studies showing that ADA is inhibitory to the replication of HIV *in vitro*. ADA dose-dependently inhibited HIV-1 replication in acutely infected peripheral blood mononuclear cells (PBMC). The 50% inhibitory concentration (IC_{50}) was about 5 μg/ml against HIV-1/III_B and 10 μg/ml against patient isolates, whether the virus was resistant to AZT or other nucleoside RT inhibitors. ADA also proved inhibitory to HIV-1 and HIV-2 replication in MT-4 cells, its IC_{50} being 10 μg/ml, and its CC_{50} 25 μg/ml, thus resulting in a selectivity index of 2.5. The only catabolite of ADA, biurea, did not inhibit HIV replication in MT-4 cells; hence, the double bond of the azo moiety is essential for anti-HIV activity. Sub-synergistic effects on the inhibition of HIV-1 replication have been observed *in vitro* with combinations of ADA and didanosine (DDI) or stavudine (d4T), with a minimum fractional inhibitory concentration (FIC) of approximately 0.65. ADA did not inhibit (i) binding of concentrated HIV-1/III_B particles or recombinant gp120 to $CD4^+$ cells, (ii) giant cell (syncytium) formation between persistently infected HUT-78/III_B and $CD4^+$ MOLT-4 (clone 8) cells, (iii) Tat transactivation of HIV-1 LTR-driven gene expression; (iv) RT activity using poly(C).oligo(dG) as template primer. Its mechanism of anti-HIV action remains to be clarified. In conclusion, ADA represents an interesting new investigational compound for the treatment of AIDS in combination with nucleoside RT inhibitors.

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Liposome Encapsulated Nucleoside 5'-Phosphate Prodrugs: a Strategy to Introduce 5'-Nucleotides into Cells.

S. Khan, Y. Zou, R. Perez-Soler and D. Farquhar. Department of Clinical Investigation, The Univ. of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Bis(pivaloyloxymethyl) 2',3'-dideoxyuridine 5'-monophosphate (PIV_2-ddUMP) is effective at inhibiting the replication of HIV type 1 infection in human T cells in culture. Mechanistic studies have shown that PIV_2-ddUMP is taken up by the cells and converted to ddUTP, a potent inhibitor of reverse transcriptase. However, a potential shortcoming of PIV_2-ddUMP as a clinical therapeutic agent is that it is rapidly degraded (half-life approx. 4 minutes) in human plasma by carboxylate esterases. To circumvent this limitation, we have investigated three different liposomal formulations of PIV_2-ddUMP: (1) DPPC/DMPG (4:1 molar ratio), (2) DMPC/DMPG (4:1 molar ratio), and (3) Egg PC/DMPG/CHOL (4:1:1 molar ratio). The drug encapsulation efficiency for the these three formulations varied from 60 to 65%. Based upon physicochemical and drug stability characteristics, the DPPC/DMPG preparation was selected for further studies. The half-life of the encapsulated drug in human plasma was 108 min. In vitro studies of this preparation in HIV infected cells is in progress and will be reported.