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Cationic liposome-mediated delivery of anti-HIV-1 ribozyme to chronically infected cells.

K. Konopka[1], J.J. Rossi[2], P. Felgner[3] and N. Düzgünes[1]
[1]University of the Pacific, School of Dentistry, San Francisco, CA 94115; [2]Loma Linda University Medical School, CA 92357; [3]Vical, Inc., San Diego, CA 92121; USA.

Human cell lines stably expressing ribozymes targeted to HIV-1 RNAs exhibit markedly decreased viral production after infection with HIV-1. Here we have investigated cytoplasmic delivery of the fluorescein-labeled chimeric DNA-RNA ribozyme targeted to the HIV-1 5' LTR, using three cationic liposomal preparations, Lipofectamine, Lipofectin and 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide, DMRIE:DOPE, (1:1). Uninfected monocytic THP-1 cells and chronically infected THP-1/HIV-1_{IIIB} cells (K. Konopka et al., Virology 1993, **72,** 877) differentiated with phorbol 12-myristate 13-acetate (PMA) were transfected with liposome-ribozyme complexes, in the presence of 8% FBS. After lipofection the cells were viewed by phase contrast and fluorescence microscopy. Toxicity was evaluated by the Alamar Blue Assay and infection was monitored by the p24 level in the supernatant. Different fluorescence patterns were observed when differentiated THP-1 cells were exposed to Lipofectamine at 3 or 8 μM and to Lipofectin and DMRIE at 15 or 40 μM at a ratio of 1.3:1 (lipid:RNA), for 4 or 24 h at 37°C. With Lipofectamine intense cell-associated fluorescence was found. Transfection with Lipofectin resulted in less intense diffuse fluorescence, while with DMRIE an intense but sporadic fluorescence was observed. Only transfection with 8 μM Lipofectamine was cytoxic, causing disintegration of PMA-treated THP-1 cells. Similar fluorescence patterns were observed when differentiated THP-1/HIV-1_{IIIB} cells were transfected with Lipofectamine at 3 μM and Lipofectin or DMRIE at 15 μM at ratios of 1.3:1 or 4:1, for 4 h at 37°C. Transfection with 3 μM Lipofectamine was cytotoxic at the ratio of lipid:RNA, 1.3:1. In spite of their intracellular accumulation, liposome-ribozyme complexes did not affect p24 production at non-cytotoxic concentrations. Whether ribozyme/cationic liposome complexes inhibit virus production in acutely infected cells is being investigated currently.

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Metabolism of the carbocyclic analog of 2'-deoxyguanosine (CdG) in human cells. Allan, P.W., W.B. Parker, G. Arnett, L.M. Rose, S.C. Shaddix, D.S. Shewach, I. Fourel, J.A. Secrist III, J.A. Montgomery, Y.F. Shealy and L.L. Bennett, Jr.; Southern Research Institute, Birmingham, AL; Fox Chase Cancer Center, Philadelphia, PA; and University of Michigan Cancer Center, Ann Arbor, MI.

CdG is a potent inhibitor of herpes simplex virus (HSV), hepatitis-B virus (HBV), and cytomegalo virus (CMV) DNA replication. In HSV-infected cells it is activated (phosphorylated) by the HSV-encoded thymidine kinase. Previously, we have shown that both enantiomers of CdG are phosphorylated in human cells. Because HBV and CMV do not express nucleoside kinases, it was of interest to identify the enzymes that are responsible for the phosphorylation of CdG in human cells and to determine the effect of virus infection on the metabolism of CdG. D-CdG was phosphorylated by deoxycytidine (dCyd) kinase isolated from Molt-4 cells with a K_m of 2.0 mM. Like other substrates for dCyd kinase, the L enantiomer of CdG (K_m of 600 μM) was a better substrate than D-CdG. The maximal velocity of the dCyd kinase reaction was similar using either D- or L- CdG as substrate. D- and L-CdG were also good substrates for 5'-nucleotidase isolated from HEp-2 cells. The K_m for D-CdG was 2 to 3-fold greater than the K_m for L-CdG and its V_{max} was approximately 10-times that for L-CdG. Combination studies in intact cells indicated that dCyd kinase was primarily responsible for the metabolism of both D- and L-CdG in CEM cells and that 5'-nucleotidase was primarily responsible for their metabolism in HEp-2 and MRC-5 cells. These results indicate that both enzymes are important in the metabolism of CdG in human cells and that the enzyme responsible for its metabolism in any given tissue depends on the relative abundance of dCyd kinase and 5'-nucleotidase. Infection of duck hepatocytes with duck HBV did not affect the phosphorylation of D-CdG, whereas, CMV infection of a human fibroblast cell line (MRC-5) resulted in a 10 to 20-fold increase in the metabolism of CdG. The identity of the enzyme that phosphorylates CdG in CMV-infected cells is under study. Supported by NIH grants CA-34200 and AI-18641, and a fellowship from the French Association for Research on Cancer.