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Structure-Activity Relationship Studies of Nucleoside Analogs with Anti-HIV Activity Using Structure-Activity Maps

R. R. Parakulam, M. L. Lesniewski, and C.-c. Tsai
Department of Chemistry, Kent State University, Kent, Ohio 44242 USA

The structure-activity relationships of nucleoside analogs with anti-HIV activity were studied utilizing structure-activity maps (SAMs). SAMs are chemical structures, quantified by molecular descriptors, plotted against their biological activities. The molecular descriptor number of atoms and bonds of a molecule (NAB) was used to quantify the chemical structures. The cytotoxicity measurement used in this study was the 50% cytotoxic dose (CD_{50}) in μM, and the antiviral activity measurement used in this study was the 50% effective dose (ED_{50}) in μM. The SAMs were examined for compounds grouped according to NAB. The topoisomer groups (based on NAB) were examined to determine important activity trends utilizing structural orderings and structural transformations. SAMs were used to systematically identify the effects of chemical modification on the antiviral activity of nucleoside analogs and to determine the site and type of modification for improved activity and reduced toxicity of potential anti-HIV agents.

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High Cellular Uptake of Lipophilic Oligonucleotide Prodrugs

J.-L. Imbach, J.-C. Bologna, J.-C. Brès, T. Lioux, I. Lefebvre, J.-J. Vasseur, E. Vivès*, and F. Morvan.

Laboratoire de Chimie Bio-organique, UMR 5625, Université de Montpellier II, CC008, Place E. Bataillon, 34095 Montpellier Cedex 5, France.
*Institut de Génétique Moléculaire de Montpellier, UMR 5535 CNRS, Université Montpellier II, 1919, Route de Mende, BP 5051, 34293 Montpellier Cedex 5, France.

The main drawbacks to the use of oligonucleotides (ON) as therapeutics is the negatively charged phosphate backbone. As a consequence ON exhibit inefficient cellular uptake, instability in biological fluids, rapid elimination, very low oral availability and lack of entry into the nerve central system. To overcome such hurdles we have been applying the prodrug concept to ON to yield prooligonucleotide approach. The prooligonucleotides are constituted with several phosphodiesters (or phosphorothioates) transitorily masked with carboxyesterase labile S-Acyl-Thio-Ethyl (SATE) protecting groups [1,2]. These prooligos exhibit relative high lipophilicity and solubility in aqueous media. By fluorescence microscopy we observed fluorescence in the whole cell (without any particular cytoplasmic localization) and an important accumulation in nucleus [3]. In addition we showed by MALDI-Tof analysis that Me-SATE dT_{12} are fully demasked in total CEM cell extract to the parent oligo. The use of the prooligo approach for oligo delivery could be of the greatest interest for their use as therapeutics.

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***In Vitro* Cytotoxicity of Adefovir (ADV) Mediated by the Human Renal Organic Anion Transporter 1 (hOAT1) Is Efficiently Reduced in the Presence of Various Nonsteroidal Anti-Inflammatory Drugs (NSAIDs).**

T. Cihlar, A. S. Mulato, E. S. Ho, and D. C. Lin. Gilead Sciences, Foster City, CA, USA.

ADV is an HIV reverse transcriptase inhibitor with unique resistance profile. Some patients on prolonged ADV therapy may develop drug-associated nephrotoxicity manifested by changes in laboratory markers of renal tubular functions that are reversible upon drug discontinuation. hOAT1, a membrane transport protein localized specifically in the kidney, has recently been shown to play a role in the etiology of ADV nephrotoxicity by mediating its accumulation in renal proximal tubules. In an effort to look for potential inhibitors of this transport process, we established an *in vitro* model by stable transfection of hOAT1 cDNA into Chinese hamster ovary cells (CHO). The resulting CHO^{hOAT} cells showed hOAT1-specific transport of ADV (K_m = 23 μM) and were significantly more susceptible to ADV cytotoxicity than the control CHO^{pIRES} cells transfected with the empty expression vector. Among a variety of tested compounds, several NSAIDs efficiently inhibited hOAT1-specific transport of ADV at clinically relevant concentrations. Ketoprofen, diflunisal, flurbiprofen, indomethacin, naproxen, and ibuprofen were equally or more effective (IC_{50} values ranging from 0.85 to 8 μM) than probenecid or betamipron, two known potent inhibitors of hOAT1 (IC_{50} = 8 and 6 μM, respectively) with *in vivo* nephroprotective effects. Transport experiments with [^{3}H]ketoprofen and [^{3}H]ibuprofen demonstrated that NSAIDs themselves were not efficiently transported by hOAT1. Accordingly, the cytotoxicity of NSAIDs in the absence of ADV did not increase upon hOAT1 expression. Importantly however, NSAIDs significantly reduced the cytotoxicity of ADV in CHO^{hOAT} cells with ketoprofen, naproxen, and diflunisal being 2- to 3-times more effective than probenecid. None of the NSAIDs tested showed any effect on the cytotoxicity of ADV in control CHO^{pIRES} cells nor did they interfere with the anti-HIV activity of ADV in MT-2 T-cells. In conclusion, these observations suggest that NSAIDs may reduce or delay the emergence of nephrotoxicity in HIV-infected patients on ADV therapy.

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HIV drug resistant quasispecies analysis in plasma, PBMC and viral isolates from HAART experienced patients

F. Baldanti, S. Paolucci, D. Lilleri, M. Zavattoni, E. Cattaneo, L. Dossena, G. Gerna.
Servizio di Virologia, IRCCS Policlinico S. Matteo, Pavia, Italy.

The pattern of HIV reverse transcriptase (RT) and protese (PR) gene mutations conferring drug resistance were studied in 5 HIV-infected patients receiving HAART (2RTI + 1PI) for ≥1yr. Direct sequencing was performed on plasma HIV-RNA, viral isolates RNA and HIV-DNA from PBMC. In addition, RT and PR sequences from PBMC HIV-DNA and from viral isolate HIV-RNA were cloned in a plasmid vector to study the quasispecies distribution of drug-resistance associated mutations. Sequencing of HIV plasma RNA showed a total of 6 mutations in 1 patient, 11 mutations in 2 patients and 12 mutations in 2 patients, respectively (see table). A similar pattern of mutations was found in PBMC HIV-DNA and viral isolates RNA. On the contrary, viral quasispecies mutation analysis obtained by sequencing plasmid clones from PBMC HIV-DNA and HIV-RNA from viral isolates, showed several additional changes in RT and PR compared to the pattern obtained by direct sequencing of PBMC HIV DNA and viral isolate RNA (see table). In detail, 1-5 additional mutations were identified in all patients by analysis of multiple clones from PBMC HIV DNA, whereas additional mutations were identified in two patients only by cloning of HIV-RNA from viral isolates. Most additional changes were found in a minor number of analyzed clones, suggesting that direct sequencing cannot evidence minor viral quasispecies. In conclusion, the presence of additional mutations found by cloning HIV RT and PR only, might represent new emerging drug resistant strains not detectable by direct sequencing. Quasispecies analysis of plasma HIV-RNA is currently being performed.

patients	Total number of mutations								
	plasma	PBMC		HIV isolates		PBMC clones*		HIV isolates clones*	
		+	—	+	—	+	—	+	—
1	6	0	0	0	0	4	0	0	0
2	11	0	1	0	0	1	0	0	0
3	11	0	0	1	0	5	0	4	0
4	12	2	2	1	1	6	2	3	0
5	12	1	1	0	0	5	0	0	0

+, additional mutations; —, missing mutations; *, number of clones examined=10.