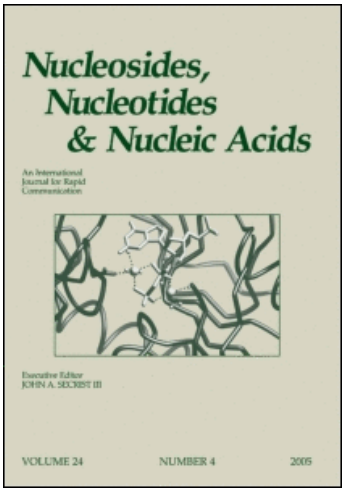

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Synthesis, In Vitro Anti-HIV and Anti-hepatitis B Activities and Pharmacokinetic Properties of Amphiphilic Heterodinucleoside Phosphates Containing ddC and AZT

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SYNTHESIS, *IN VITRO* ANTI-HIV AND ANTI-HEPATITIS B ACTIVITIES AND PHARMACOKINETIC PROPERTIES OF AMPHIPHILIC HETERODINUCLEOSIDE PHOSPHATES CONTAINING ddC AND AZT

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ABSTRACT: Amphiphilic heterodinucleoside phosphates containing AZT and ddC as antiviral monomer were synthesized according to the hydrogenphosphonate method and evaluated *in vitro* against HIV. dT-N^4-pamddC was the most active (IC_{50} = 40 μM, EC_{50} = 80 nM) and least toxic (TI = 524) dimer and it exhibited also strong antiviral effects against eight AZT-resistant HIV strains. The ddC-containing heterodimers additionally inhibited HBV replication by 50-80 % at 50 μM in Hep G2 2.2.15 cells.

INTRODUCTION: Antiviral efficacy, pharmacokinetics and pharmacological properties of the dideoxynucleosides (ddNs) presently used for the treatment of HIV and HBV infections still need to be improved. We have synthesized the following amphiphilic heterodinucleoside phosphates which contain AZT or ddC as the antiviral monomer unit: N^4-hexadecyl-2'-deoxyribocytidylyl- (3'→5') -3'-azido-2',3'-deoxythymidine (N^4-hxddC-AZT), N^4-palmitoyl-2′-deoxyribocytidylyl- (3′→5′) -3′-azido-2′,3′-deoxythymidine (N^4-pamdC-AZT), N^4-hexadecyl-2'-deoxycytidylyl-(3'→5')-2',3'-dideoxycytidine (N^4-hxddC-ddC) and 2'-deoxythymidylyl-(3'→5')-N^4-palmitoyl-2',3'-dideoxycytidine (dT-N^4-pamddC). These dimers include the 5'-monophosphates of AZT and ddC or of a lipophilic ddC derivative, masked by a phosphodiester linkage. In this study we analyzed the pharmacokinetic properties and the antiviral activities of these heterodimers against various resistant HIV-strains and in the HBV-infected Hep G2 2.2.15 cell line.

MATERIAL AND METHODS: The amphiphilic heterodimers were synthesized as described[1,2]. The heterodimers were tested *in vitro* on CEM-SS cells infected with HIV-1 wild type or resistant variants (AEB panel) in the NCI anti-AIDS drug discovery program and on HBV-infected Hep G2.2.15 cells as described by Peghini et al.[3]

RESULTS: The inhibitory concentrations of the most active heterodimer dT-N^4-pamddC in HIV infected CEM-SS cells were as follows: IC_{50} = 40 μM; EC_{50} = 76 nM and IC_{50}/EC_{50} = 524. The EC_{50} values of the other dimers were about 20-fold and the IC_{50} values 2-4-fold higher. dT-N^4-pamddC had strong inhibitory effects against a panel

TABLE 1: Distribution of AZT, N^4-pamdC-AZT and N^4-hxddC-AZT in H9 cells

Cell fraction	Drug distribution (percentage of total uptake)		
	[^{3}H]-AZT	N^4-pamdC-[^{3}H]-AZT	N^4-hxdC-[^{3}H]-AZT
Total uptake (nmol)	2.82 ± 0.16	3.76 ± 0.27	2.70 ± 0.20
Membranes	0.4 %	30.2 %	44.0 %
Cytoplasm	99.3 %	45.7 %	30.3 %
Microsomes	0.3 %	24.1 %	25.7 %

H9 cells ($5x10^6$/sample) were incubated for 4 h at 37 °C with 400 µM of the drugs. AZT in 0.9% NaCl and the dimers in liposomes suspended in 0.9% NaCl. The cells were separated from medium and free drug by centrifugation through bromododecane. Cellular drug distribution in subfractions of membranes, cytoplasma and microsomes was determined as described[3].

of resistant HIV strains with IC_{50} = 38 – 49 µM, EC_{50} = 30 – 526 nM and IC/EC = 82 - 1660. dT-N^4-pamddC and N^4-hxddC-ddC were also found to be effective against HBV with an inhibition of HBV replication by 50 to 80 % at 50 µM drug concentration given daily for 9d. Differences in pharmacokinetic properties, cellular uptake and distribution between the antiviral monomers and the heterodimers were evaluated using as model compounds liposomal formulations of the tritium labeled AZT heterodimers. In mice the blood levels of the heterodimers decreased at a 2-3-fold slower rate than AZT and the areas under the curves were 5-7 fold higher for N^4-pamdC-AZT and N^4-hxddC-AZT, respectively. Compared to AZT, the peak levels of the dimers were 3-4 times higher in blood and 5-6 times higher in the liver. The distribution of the dimers in blood 30 min after i.v. injection showed that they were bound to a higher degree to plasma proteins, whereas AZT had a 2-5 times higher affinity to erythrocytes. TABLE 1 summarizes the distribution in H9 cells of N^4-pamdC-AZT and N^4-hxddC-AZT in comparison to AZT. In contrast to AZT, which was located predominantly in the cytoplasm, the heterodimers were distributed more evenly in all cell compartments. Concentration dependent uptake experiments with H9 cells showed that uptake of N^4-pamdC-AZT was about 2-3-fold higher than N^4-hxddC-AZT. Due to the susceptibility of N^4-pamdC-AZT to enzymatic hydrolysis, the amount of drug determined in the cytoplasm might also contain AZT or AZT-5'-monophosphate.

The dimerization of dideoxynucleosides to amphiphilic heteronucleoside phosphates extends the antiviral potency of the corresponding monomers and offers new therapeutic modalities for the treatment of viral infections.

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