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RAPID COMMUNICATION

EQUAL INHIBITION OF THE REPLICATION OF HUMAN IMMUNODEFICIENCY VIRUS IN HUMAN T-CELL CULTURE BY ddA BIS(SATE)PHOSPHOTRIESTER AND 3'-AZIDO-2',3'-DIDEOXYTHYMIDINE

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Abstract–It is shown that ddA bis(SATE)phosphotriester is one of the most potent anti-HIV agents in cell culture. Compared with the parent nucleoside, ddA, an increase of 3 orders of magnitude was observed in the EC_{50}, which makes this compound as active as AZT. This can be tentatively explained if one considers that direct ddAMP intracellular delivery shunts the well established ddA/ddI metabolism pathway.

Key words: prodrugs, nucleoside phosphotriesters, anti-HIV nucleoside derivatives, didanosine

We have reported previously that DTE† or SATE phosphate bioreversible protecting groups can be used for intracellular nucleotide delivery [1-4].

When applied to an anti-HIV nucleoside, such an approach, which bypasses the first activating phosphorylation step, may have further consequences that could transform the behaviour of the parent nucleoside in terms of metabolism, biodistribution, toxicity and biological response. That one can thus obtain an entirely different anti-HIV drug, regardless of the antiviral activity of the parent nucleoside, is well established.

We have examined this concept by transforming two well-known anti-HIV drugs that are considered equivalent with regard to their *in vitro* activity [5], namely ddA and ddI (Didanosine, Videx^R), to their bis(SATE)phosphotriesters and have shown that the corresponding ddA derivative is as active as AZT against HIV-1 replication in human T-lymphoblastoid cell culture.

The synthesis of the bis(SATE)phosphotriester of ddA and of ddI is straightforward: condensation of the corresponding diisopropyl aminophosphite diester with the unprotected nucleoside, followed by subsequent *in situ* oxidation, as shown in Fig. 1 [3,4].

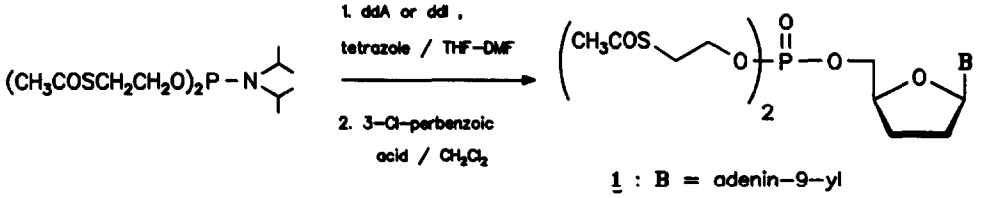

Fig. 1. Synthesis of bis(SATE)phosphotriester derivatives.

The bis(SATE)phosphotriesters 1 and 2 were fully characterized by the usual analytical methods (^1H and ^{31}P NMR, UV, and mass spectrometry), and their purity was verified by analytical HPLC.

The anti-HIV effects of 1 and 2 were evaluated as previously described [2] on two HIV-1 infected T-cell lines, along with the parent nucleosides and AZT as reference compounds. As shown in Table 1, the bis(SATE)phosphotriester of ddA 1 exhibited a very potent antiviral effect in the same range as AZT; compared with ddA, one can observe an increase of 3 orders of magnitude in the EC_{50} values. The ddI derivative 2 did not show a corresponding potent anti-HIV effect.

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† Abbreviations: DTE, [S-(2-hydroxyethylsulfidyl)-2-thioethyl]; SATE, S-acetyl-2-thioethyl; ddA, 2',3'-dideoxyadenosine; ddI, 2',3'-dideoxyinosine; AZT, 3'-azido-2',3'-dideoxythymidine; HIV-1, human immunodeficiency virus type 1; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; AK, adenosine kinase; 5Nuc, 5'-nucleotidase; AMPdA, AMP deaminase; AMPS, adenylosuccinate.

Table 1. Antiviral activity and cytotoxicity of ddA and ddI compared with their corresponding bis(SATE)phosphotriester derivatives

	Nucleoside				Bis(SATE)phosphotriester			
	CEM-SS		MT-4		CEM-SS		MT-4	
	EC_{50}[a,b] (M)	IC_{50}[a,c] (M)	EC_{50} (M)	IC_{50} (M)	EC_{50} (M)	IC_{50} (M)	EC_{50} (M)	IC_{50} (M)
ddA	$5.4 \pm 1.1 \times 10^{-7}$	$>10^{-4}$	$1.0 \pm 0.1 \times 10^{-5}$	$>10^{-4}$	$5.6 \pm 3.4 \times 10^{-10}$	$2.4 \pm 0.1 \times 10^{-5}$	$1.1 \pm 0.8 \times 10^{-8}$	$1.6 \pm 0.9 \times 10^{-5}$
ddI	$4.3 \pm 2.0 \times 10^{-6}$	$>10^{-4}$	$1.1 \pm 0.2 \times 10^{-5}$	$>10^{-4}$	$1.2 \pm 0.6 \times 10^{-6}$	$>10^{-4}$	$3.4 \pm 1.1 \times 10^{-6}$	$>10^{-4}$
AZT	$4.8 \pm 2.4 \times 10^{-9}$	$>10^{-4}$	$1.8 \pm 0.6 \times 10^{-8}$	$>10^{-4}$	ND[d]	ND	ND	ND

[a]Values are means of data obtained from triplicate experiments (± standard error of the mean); [b]EC_{50}: 50% effective concentration or molar concentration required to inhibit the replication of HIV by 50%; [c]IC_{50}: 50% inhibitory concentration or molar concentration required to reduce the viability of the cell by 50%; [d]Not determined.

Although dideoxynucleoside triphosphates show similar *in vitro* inhibitory effects on HIV reverse transcriptase (i.e. Ki for ddAZTTP 0.10 mM vs 0.22 mM for ddATP [5]), striking differences were observed for the EC_{50} of their parent nucleosides in cell culture (4.8×10^{-9} M for AZT and 5.4×10^{-7} M for ddA in CEM-SS, see Table 1). This may be related to the capacity of the cell to enzymatically phosphorylate the corresponding nucleoside to its triphosphate. As the first phosphorylating step is expected to be the most selective, account must be taken of the fact that some intracellular enzymes are highly dependent on host species (human or animal), cell type and the stage in the cell cycle [6].

Considering the data presented in Table 1 and the well-established ddA/ddI cellular metabolic pathway [6-11] (Fig. 2), the following points should be noted: (i) ddA is degraded rapidly to ddI by the ubiquitous cellular enzyme ADA, but not readily transformed to ddAMP by cellular kinases [10,11]; (ii) ddI seems to be phosphorylated to ddIMP by 5'-nucleotidase [10,11], a cellular enzyme whose activity remains relatively constant during all phases of the cell cycle [6, 12]; and (iii) ddIMP anabolism to ddAMP by AMPS synthase and adenylosuccinase does not seem to be very efficient [13, 14], which makes this the rate-limiting step for ddAMP formation.

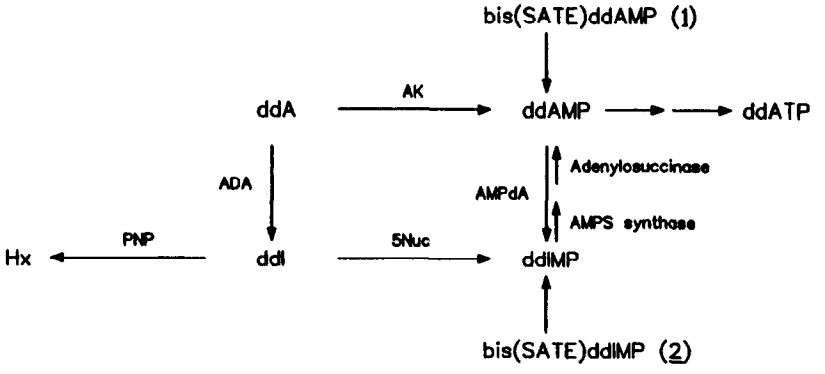

Fig. 2. Metabolic pathways of ddI, ddA and expected metabolism of their bis(SATE) phosphotriester derivatives.

Regarding the behaviour of the bis(SATE)phosphotriesters, the following considerations are relevant: (i) we have shown previously that nucleoside bis(SATE)- or bis(DTE)-phosphotriesters are transformed intracellularly to the corresponding nucleoside monophosphate, indicating that such neutral molecules are absorbed by cells [2-4]. Once inside the cells, probably through carboxyesterase activation, the bis(SATE)phosphotriester decomposes to the corresponding phosphodiester, which is further cleaved

(carboxyesterase and/or phosphodiesterase) to the corresponding nucleoside monophosphate [4]. It is noteworthy that different bioreversible phosphate protecting groups, also based on carboxyesterase activation, have been proposed previously [15]; (ii) substrate deamination of 1 and ddA was compared using calf intestinal ADA. We found that 1 was not a substrate for ADA after a 6-hr incubation at 25° in the presence of 12.5 units of enzyme/mL; in contrast, ddA was readily deaminated under the same conditions. In addition, ddAMP has been shown not to be a substrate for ADA [14]. These observations are significant since one of the reasons that ddI was selected for clinical evaluation is the ease of enzymatic conversion of ddA to ddI. In this respect, ddA has been considered a prodrug of ddI [7,9]; and (iii) also noteworthy is the fact that the ddI phosphotriester 2 is not a good substrate of calf spleen PNP and that the ddA phosphotriester 1 is not a substrate for AMPdA.

The antiviral data obtained with 1 and 2 (Table 1) may be explained as follows: (i) ddA, ddI and 2 exhibit roughly the same biological response due to the low efficiency of ddAMP formation from ddIMP, even when the latter is delivered intracellularly. This point is in agreement with the assumptions of Nair and Sells [14]; and (ii) the bis(SATE)phosphotriester of ddA 1 leads to direct intracellular delivery of ddAMP, thus shunting the ddI pathway. This may result in an increase of ddAMP concentration, which is subsequently transformed to ddATP.

To corroborate those points, the cellular pharmacology of 1 and 2 will be investigated using the corresponding radiolabelled derivatives.

An important property of the purine dideoxynucleosides is their facile acid hydrolysis (glycosidic bond breakage), thus liberating the corresponding purine bases. As a consequence, after oral administration of ddA, its hydrolysis product, adenine, has been shown to produce nephropathy [7,8]. This is another reason that influenced the choice of ddI over ddA for clinical development, even though ddI is degraded faster than ddA (hypoxanthine, unlike adenine, is relatively non-toxic).

On the basis of our previous work on the synthesis of abasic sites in DNA and ddA incorporation, we expected that phosphorylation of a 5'-nucleoside hydroxyl group would induce stabilization of the glycosidic bond [16]. Therefore the half-lives of phosphotriesters 1 and 2 were determined at pH 2 and compared with those of their parent nucleosides. As shown in Table 2, in both cases a stabilization effect of more than 1 order of magnitude was observed. These encouraging stability data reinforce interest in the bis(SATE)phosphotriesters.

Table 2. Half-lives of 1 and 2 compared with their parent nucleosides at pH 2 (glycine-HCl buffer)‡

	Half-lives	
	Nucleoside	Bis(SATE)phosphotriester
ddA	28 min	7.4 hr
ddI	8 min	1.16 hr

‡ Data were obtained by HPLC monitoring.

The preliminary data on bioreversible bis(SATE)phosphotriesters open a wide field of exploration for the intracellular delivery of nucleoside monophosphate derivatives. The most specific, often cell-cycle-dependent, enzyme-activating step may thus be bypassed [6, 9, 12]. The fact that we observed an important increase in the antiviral efficacy of ddA does not imply that this approach will lead to the same consequences when applied to other nucleosides. However, since it is possible to modify the nature of the bioreversible protecting groups, one can expect to modulate the bioavailability of any nucleoside drug (i.e. AZT and ddI), provided its pharmacokinetic parameters are appropriate. Work along these lines is in progress in our group.

In conclusion, the ddA and ddI bis(SATE)phosphotriesters 1 and 2 have been evaluated as potential anti-HIV agents. We have shown that 1 is much more active *in vitro* (3 orders of magnitude) than its parent nucleoside ddA, giving rise to an EC_{50} comparable to that of AZT. In addition, the enzymatic stability of 1 towards ADA degradation and its enhanced stability in acidic medium make this compound worthy of further evaluation as a new anti-HIV agent.

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