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NUCLEOTIDIC PRODRUGS OF ANTI-HIV DIDEOXYNUCLEOSIDES

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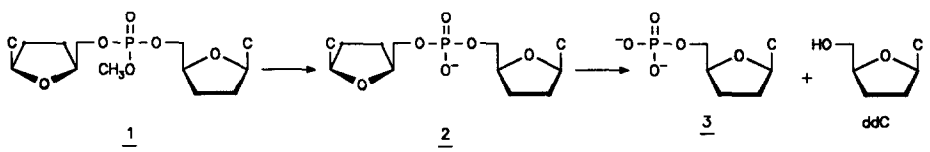
Abstract. Dinucleoside phosphodi- and triester of ddC have been studied as potential prodrugs of the 5'-monophosphate nucleoside. Their kinetics of decomposition have been determined by HPLC in biological media using the "On-Line ISRP Cleaning" technique. From the results, it can be concluded that the anti-HIV activities of the dimeric compounds are mainly related to extracellular decomposition.

For many years, numerous attempts have been proposed to use a masked-phosphate nucleotide in order to intracellularly deliver the first metabolite of bio-active nucleosides.[1] Recently, these prodrug approaches have been envisaged on mono-[2-6] and dinucleotidic[7-10] derivatives of anti-HIV dideoxynucleosides and most of the biological results have been related to the expected intracellular release of the 5'-monophosphate.
However, two key factors were usually neglected in those works, namely the fate of the starting prodrugs under incubation conditions and their intracellular behaviour. Nevertheless, the first point seems of the highest importance as the experimental conditions employed for determining the activity of anti-HIV drugs are very stringent since they imply about 4-5 days of incubation in media such as RPMI containing 10% of heat-inactivated fetal calf serum (FCS). We describe here our results obtained from studies on the dinucleoside phosphotriester 1 and phosphodiester 2 of ddC.[11] Both compounds 1 and 2 can be considered as "mutual prodrugs" susceptible to liberate ddC and its 5'-monophosphate 3 (Scheme 1).



Scheme 1

One can notice that we are dealing with a two-steps transformation : a chemical hydrolysis of 1 into 2 and then an enzymatic breakage of 2 into 3 and ddC. The first step is

restricted to a chemical process since no phosphotriesterase activity seems to have been reported in serum or in eukaryotic cells. Moreover, it is known that phosphotriesters as 1 can be slowly hydrolyzed at neutral pH through a classical C - O bond breakage mechanism (SN2 process, CH_3 > C_2H_5> $(CH_3)_2CH$)[12-13] which could insure selective methyl removal.

We examined the stability of 1 and 2 under various conditions. For that purpose, we used the "On-line ISRP Cleaning" technique that we recently described[14] and which allows to analyse biological samples without any pretreatment, by direct injection into the HPLC apparatus.

First, the stability of 1 was evaluated in culture medium (RPMI + 10 % of heat-inactivated FCS) and in cellular extract (from CEM cells) both at 37°C. Under such experimental conditions, a slight decomposition of 1 (6 % in 5 days) into 2 was observed (Figure 1a). It is noteworthy that this elimination reaction is selective since only the phosphodiester 2 was detected, but at a too low concentration to evaluate its decomposition pattern. Independently, we examined the fate of a chemically synthesized sample of 2 under the following conditions (Table 1) : i) in RPMI, in order to evaluate its chemical stability at pH 7.2; ii) in RPMI containing 10 % of FCS, heat-inactivated or not, in order to evaluate its stability in cell culture medium (Figures 1b and 1c); iii) in a cellular extract, as the cellular enzymatic content differs from the serum one.

RPMI	RPMI + 10 % FCS	RPMI + 10 % inactivated FCS	CEM Extract
Stable	$T_{1/2}$ = 22 h k = 5.2 10^{-4} min^{-1}	$T_{1/2}$ = 48 h k = 2.4 10^{-4} min^{-1}	Stable

Table I : Kinetic data of decomposition of 2 in various media (Concentration : 10^{-3} M, incubation at 37°C for 8 days).

The data presented on Table 1 deserve the following comments. i) As expected, the anionic phosphodiester 2 is very stable towards hydrolysis in RPMI. ii) Incubation of 2 in RPMI containing 10% FCS heat-inactivated or not gives rise to extensive degradation presumably through phosphodiesterase activities with concomitant formation of ddC and of the nucleotide 3. The latter is further transformed to the parent nucleoside through serum phosphatase activities (Figure 1b). In addition, the enzymatic activities are only slowed down but not suppressed if the FCS has been heat-inactivated (56°C, 30 min). iii) Surprisingly, in the cellular extract the phosphodiester 2 is stable under a 6 days incubation experiment. As the ratio of protein concentration (PC) cannot explain by itself the significant behaviour difference of 2 in cellular extract (PC ≈ 0.5 mg/mL) and culture medium (PC ≈ 7 mg/mL) , this absolute stability of 2 may be related to a lack of phosphodiesterase activities towards the unnatural dinucleotidyl-5',5' phosphodiester structure.

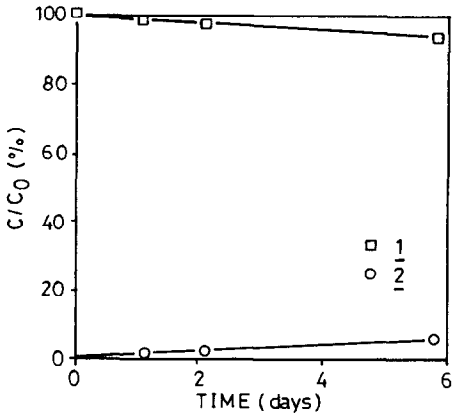


Fig. 1a

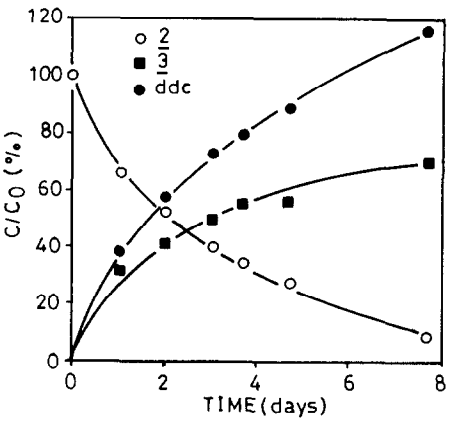


Fig. 1b

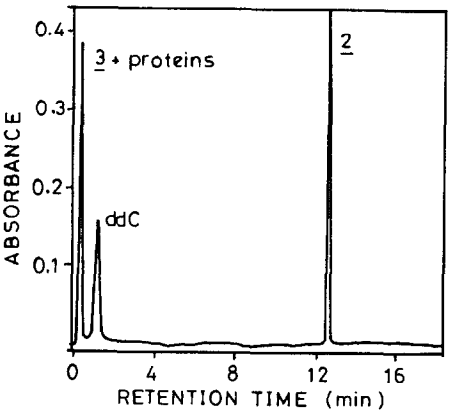


Fig. 1c

Fig. 1 : Decomposition of 1 (fig. 1a) and 2 (fig. 1b) in RPMI + 10 % inactivated FCS, upon incubation (C_0 = 10^{-3} M) with identification of the metabolites. Fig. 1c represents a typical HPLC chromatogram of 2 after 67 h of incubation under the previous conditions (the molar extinction coefficient of 2 is twice the one of ddC or 3).

From those data, one can conclude that the observed activity of 1 and 2 on HIV infected CEM cells (ED_{50} = 5 10^{-7} and 5 10^{-8} M respectively as compared to 10^{-8} M for ddC) may be mainly due to the release of ddC in the culture medium, whether there is or not an intracellular release of the 5'-monophosphate 3. The same conclusion may probably apply to various other data recently reported on analogous dinucleotidic prodrugs.

More generally, our present results deserve the following comments. Before reaching any conclusions on an eventual activity of prodrugs of anti-HIV nucleosidic compounds, one must determine their decomposition pattern in culture medium under incubation conditions. In many cases, the observed anti-HIV effect may be explained by the release of the nucleoside in culture medium.

This conclusion will be more documented in forthcoming full papers based on decomposition patterns of various mono- and dinucleotide phosphotriester prodrugs.

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