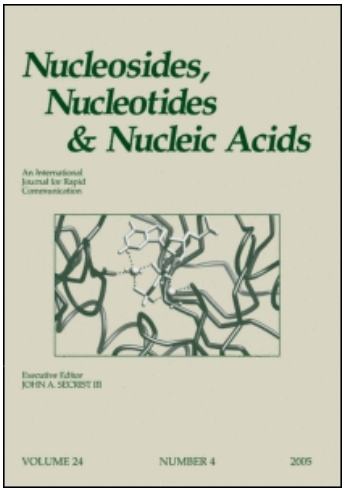

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Biological and Biochemical Studies on Ro31-6840 (2'βFddC), a Dideoxynucleoside Analogue Active Against Human Imhunodeficiency Virus Type 1 (HIV-1)

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BIOLOGICAL AND BIOCHEMICAL STUDIES ON Ro 31-6840 (2'ßFddC), A DIDEOXYNUCLEOSIDE ANALOGUE ACTIVE AGAINST HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1).

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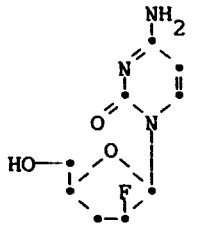
Abstract

Ro 31-6840 (2'ßFddC) has potent and selective anti-HIV-1 activity in cell culture. The mean antiviral IC_{50} was 0.61 µM (n=15) with no adverse effect on host cells at concentrations of up to 100 µM. Biochemical studies with Ro 31-6840 triphosphate indicate a high degree of selectivity for HIV-1 RT (Ki = 0.071 - 0.27 µM) when compared with inhibition of cellular DNA polymerases α, ß and γ.

A number of nucleoside analogues especially in the dideoxynucleoside series are now known to inhibit the replication of HIV-1 in vitro. These analogues have a similar mode of action. They are activated through phosphorylation by cellular kinases and as triphosphates are substrates for incorporation into viral DNA by the HIV-coded reverse transcriptase (RT) resulting in premature chain termination. To date only 3'-azidothymidine (AZT, Zidovudine) has been approved for the treatment of acquired immunodeficiency syndrome (AIDS) and although significant benefits can be achieved with AZT therapy[1-3] its use has been limited by severe side effects, notably, bone marrow suppression[4,5]. Dideoxycytidine (ddC) is another nucleoside analogue currently undergoing extensive clinical trials. Early evaluation with relatively high doses in man revealed a somewhat different and unexpected toxicity profile in that ddC was largely free of the bone marrow toxicity seen with AZT but caused a characteristic painful peripheral neuropathy, the onset and duration of which appeared to be dose related[6]. Clearly compounds of this

class are useful anti-HIV agents and there is a need to develop potent but safer analogues for the treatment of AIDS. To this end, we have synthesised a number of fluorinated analogues of dideoxynucleosides and evaluated them against HIV-1 in cell culture. As a result of these studies, Ro 31-6840 was identified for further biological and biochemical evaluation on the basis of its potent and selective anti-HIV-1 activity.

Ro 31-6840



[1-(2',3'-dideoxy-2'-fluoro-beta-D-threopentofuranosyl)cytosine]

Results and Discussion

Ro 31-6840 shows good antiviral activity against several strains of HIV-1 grown in a number of T-cell lines. In a comparative study using HIV-1 (RF strain) grown in C8166 cells, Ro 31-6840 was approximately 3-fold more active than ddA (dideoxyadenosine) but 4-fold and 23-fold less active than ddC and AZT respectively (Table 1). In this cell line, none of the compounds was toxic at concentrations of up to 100 μM after 3 days incubation.

To further assess the biochemical properties of Ro 31-6840, we synthesised the triphosphate derivative (Ro 31-6840TP) and evaluated its activity alongside ddC triphosphate (ddCTP) against purified HIV-1 RT and Hela cell polymerases α, ß and γ. Selectivity for the viral enzyme with respect to the host cell polymerases is of obvious importance and likely to contribute to the overall therapeutic potential of these agents. The results (Table 2) show that Ro 31-6840TP was slightly less potent (2 to 3-fold) than ddCTP as an inhibitor of HIV-1 RT with Ki's of 0.071 μM (RNA template) and 0.27 μM (DNA template). However, ddCTP was also a very potent inhibitor of cellular γ-polymerase with a Ki of 0.003 μM. In contrast, only a weak inhibition (Ki = 24 μM) was observed with Ro 31-6840TP. Neither compound was active against α polymerase (Ki = >100 μM) and both displayed moderate inhibition (Ki = 0.75 μM & 1.4 μM) of ß-polymerase.

TABLE 1:

Anti HIV-1 activity of Ro 31-6840 compared with AZT, ddC and ddA

		Mean IC_{50} [μM]
Ro 31-6840	(n=15)	0.61
AZT	(n=9)	0.026
ddC	(n=9)	0.15
ddA	(single test)	2.0

TABLE 2:

Inhibition of HIV-1 RT and Hela cell polymerases

		Ki (μM)	
Enzyme	Template	Ro 31-6840TP	ddCTP
HIV-1 RT	RNA▲	0.071	0.038
HIV-1 RT	DNA*	0.27	0.08
α-Polymerase	DNA*	>100	>100
ß-Polymerase	DNA*	1.4	0.75
γ-Polymerase	DNA*	24	0.003

▲ Oligo(dT)-primed rat liver m-RNA,
* Activated salmon sperm DNA.

Studies on the uptake and phosphorylation of Ro 31-6840 was carried out in mitogen-stimulated human peripheral blood lymphocytes (PBL's) and in several established human lymphoid cell lines. In all instances, Ro 31-6840 was efficiently phosphorylated to its triphosphate form. In contrast to AZT which accumulated to high concentrations as its monophosphate derivative, the intracellular levels of Ro 31-6840 mono-, di- and triphosphates were more or less equivalent. In this respect, the profile of Ro 31-6840 metabolites resolved by HPLC was very similar to that of ddC. A comparison of the

rates of intracellular triphosphate decay in C8166 cells indicates a similar half-life of ~3 hours for Ro 31-6840TP and ddCTP compared with 45 minutes for AZTTP. Using a modification of the dideoxy sequencing method with HIV-1 RT, we have also shown that Ro 31-6840TP was an efficient chain terminator giving an identical pattern of autoradiographic bands as ddCTP.

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