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RAPID COMMUNICATIONS

2',3'-DIDEOXY-2'-FLUORO-ARA-A. AN ACID-STABLE PURINE NUCLEOSIDE ACTIVE AGAINST HUMAN IMMUNODEFICIENCY VIRUS (HIV)

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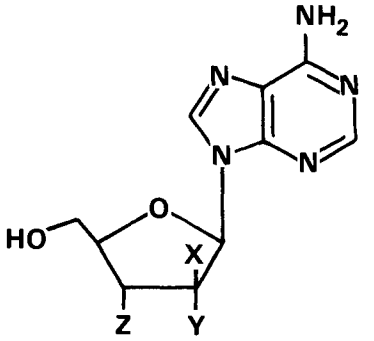
2',3'-Dideoxynucleosides are known to inhibit the cytopathic effect of human immunodeficiency virus (HIV), the causative agent of AIDS (1). Dideoxyadenosine (ddA; I) is a potent, effective member of this chemical family which is being considered as a possible clinical candidate by the NIH AIDS program. Because the activated form of dideoxynucleosides (5'-triphosphate) appears not to eradicate the virus but to inhibit its replication, it is most likely that a drug of this type must be taken continuously if the therapeutic effect is to be maintained. Since daily treatment for a long period might be required, oral drug administration is envisioned as the most practical route for a very large patient population.



I, X = H; Y = H; Z = H
II, X = H; Y = F; Z = H
III, X = F; Y = H; Z = H
IV, X = OH; Y = H; Z = H
V, X = H; Y = OH; Z = H
VI, X = F; Y = H; Z = OH

Drugs administered orally are exposed to a pH range of 1 to 2 in the human stomach for approximately 1 hr. This could result in a drug stability problem with ddA since this compound undergoes acid-catalyzed hydrolysis of the glycosidic bond at a rate 40,000 times faster than adenosine (2). In an attempt to produce an acid-stable, active anti-HIV agent, we undertook the synthesis of the two monofluoro 2'-diasteromers of ddA (II, III). Based on the mechanism proposed for the acid-catalyzed decomposition of dideoxy-

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nucleosides (2), it appeared that the introduction of an electronegative 2'-substituent might increase compound stability by destabilizing the oxonium ion which would result from the hydrolysis of the glycosidic bond. Fluorine was a particularly attractive substituent choice because of its electron-withdrawing properties and its similarity in size to the hydrogen atom being replaced.

MATERIALS AND METHODS

Acid-catalyzed decomposition studies. A pH 1 solution was prepared by dissolving 0.746 g KCl and 26.8 g 1.0 N HCl in sufficient distilled water to give 200 ml total volume. Aliquots of this solution (10 ml), prewarmed to 37°, were added to either 102 μg of ddA or 93 μg of the 2'-fluoro analog (II or III). Samples were shaken at 37°, and aliquots were removed at timed intervals and neutralized immediately with 0.1 N NaOH and chilled on ice. The amounts of I, II and III were determined by HPLC analysis using a 4.6 x 250 mm 5 μm Ultrasphere-ODS column protected by a guard column packed with 37-50 μm Vydac 201SC. Elution was with 12% acetonitrile in 0.01 M, pH 6.8, sodium phosphate buffer at 1.0 ml/min. Integrator peak areas were plotted as a function of time and the data fitted to a first order decomposition curve by a computer program (MLAB).

Antiviral experiments. The HIV cytopathic effect assay was performed using ATH8 cells as previously described (3). Briefly, 2 x 10^5 ATH8 cells were pre-exposed to polybrene, then exposed to HTLV-III_B virus (2000 virus particles/cell) for 45 min, resuspended in 1 ml of culture medium containing Interleukin 2 in the presence or absence of various concentrations of compounds, and incubated in culture tubes at 37° in 5% CO_2/95% air humidified atmosphere. Control cells were treated similarly but were not exposed to the virus. On day 5 of culture, the total viable cells were counted in a hemocytometer by the trypan blue dye exclusion method. In the HIV cytopathic effect assay using ATH8 cells, 0.5 to 5 virus particles per cell represent the minimum cytopathic dose of the virus (1,3).

Starting materials. 2',3'-Dideoxyadenosine (I) was obtained from Dr. Ven Narayanan, Drug Synthesis and Chemistry Branch (DS&CB), NCI. 3'-Deoxy-ara-A (IV) was synthesized by Dr. Terence C. Owen (University of South Florida) by the method of Hansske and Robins (4) under Contract NO1 CM 437639 to the DS&CB.

Synthesis of the 2'-F-ddA (II) and 2'-F-ara-ddA (III). Compound II [6-amino-9-(β-D-2',3'-dideoxy-2'-fluororibofuranosyl)-9-H-purine; 2'-F-ddA] was obtained in four steps from 3'-deoxy-ara-A (IV). This involved protection of the 5'-hydroxyl group with dimethoxytrityl chloride, activation of the 2'-hydroxyl group via formation of the corresponding triflate derivative, inversion of configuration at the 2'-position by an SN_2 displacement using tetra-n-butylammonium fluoride, and removal of the dimethoxytrityl protective group with dichloroacetic acid. The nucleophilic displacement of triflate by fluoride ion gave II as a lyophilized white powder, R_f 0.25 (SiO_2, $CHCl_3$:CH_3OH::9:1); m/z calc. $C_{10}H_{13}N_5O_2F$ (MH^+) 254.1052, found 254.1059 ± 0.0017. This compound was accompanied by a minor, olefin-containing product caused by elimination rather than displacement of the triflate group. Only elimination was observed when the same synthetic approach was applied to the preparation of the III from cordycepin (V).

The failure of the triflate displacement reaction required the development of an alternative approach to the III. This began with the synthesis of the previously reported compound 6-amino-9-(β-D-2'-deoxy-2'-fluoroarabinofuranosyl)-9H-purine (2'-F-ara-dA; VI) as a starting material. This compound, originally synthesized by Fox and co-

workers (5), was prepared using the improved, general procedure of Montgomery et al. (6), condensing 6-chloropurine with 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide. The required, functionalized halosugar was prepared in essentially the same manner as reported by Fox and coworkers (7) and, as expected, four isomers were obtained from the condensation reaction. After separation and characterization of the correct 6-chloro isomer, the required starting material, 2'-F-ara-dA, was obtained by ammonolysis with concentrated methanolic ammonia which simultaneously removed the protective groups. All the chemical, optical, and spectral properties of the compound matched those reported previously for 2'-F-ara-dA (5). Selective protection of the 5'-hydroxyl function of this compound with t-butyldimethylsilyl chloride gave a product that permitted the two-step reduction of the 3'-hydroxyl group (8). Treatment with phenyl chlorothionocarbonate, followed by reduction of the intermediate 3'-O-phenoxythiocarbonyl derivative with tri-n-butyl tin hydride, produced the desired 2',3'-dideoxy nucleoside. This required only the removal of the 5'-blocking group with tetra-n-butyl ammonium fluoride to give 6-amino-9-(β-D-2',3'-dideoxy-2'-fluoroarabinofuranosyl)-9-H-purine (2'-F-ara-ddA; III) as a white lyophilized product, R_f 0.18 (SiO_2, $CHCl_3$:CH_3OH::9:1); m/z calc. $C_{10}H_{13}N_5O_2F$ (MH^+) 254.1052, found 254.1031 ± 0.0018.

RESULTS

2',3'-Dideoxyadenosine (I) rapidly decomposed at pH 1.0 (37°). Under these conditions, the $t_{1/2}$ of I was 35 sec (Fig. 1). While 2'-F-ddA (II) was stable to acid-catalyzed decomposition at pH 1, the addition of a fluorine atom in this "down" configuration reduced the protective effect against HIV to 13% of that seen with ddA and produced a compound more toxic than the parent drug.

A change in the stereochemistry of fluorine at the 2'-position to the "up" configuration produced dramatically different activity results as well as acid stability. 2'-F-ara-ddA (III) was approximately as active and potent as AZT (1) or ddA (Fig. 2) in protecting

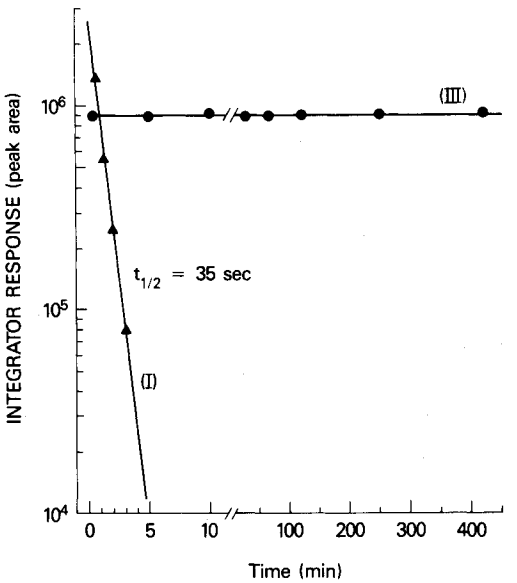

Fig. 1. Concentration versus time profile for 2',3'-dideoxyadenosine (▲) and 2'-F-ara-ddA (●) at pH 1 and 37°. The time scale for the first 10 min is greatly expanded. The initial concentration of I was 10.2 μg/ml, while that of III was 8.4 μg/ml.

ATH8 cells against the cytopathic effect of HIV under conditions of substantial viral excess. Furthermore, the antiviral effect of III was as durable as that of the parent compound, I. Dimethyl sulfoxide (DMSO) was used as a co-solvent in the preparation of the test samples of III. At the three highest concentrations of III tested (20, 100 and 200 μM), this resulted in 0.2, 1.0 and 2.0% DMSO, respectively, in the cell cultures. As seen in Fig. 2, the decrease in cell viability at 200 μM III can be ascribed to the effect of 2% DMSO alone. Full protection of the remaining viable cells, however, was maintained. It should be noted that III was completely unchanged after a 24 hr exposure in solution at pH 1 (Fig. 1).

In view of the favorable properties of 2'-F-ara-ddA, further antiviral and pharmacological studies are planned.

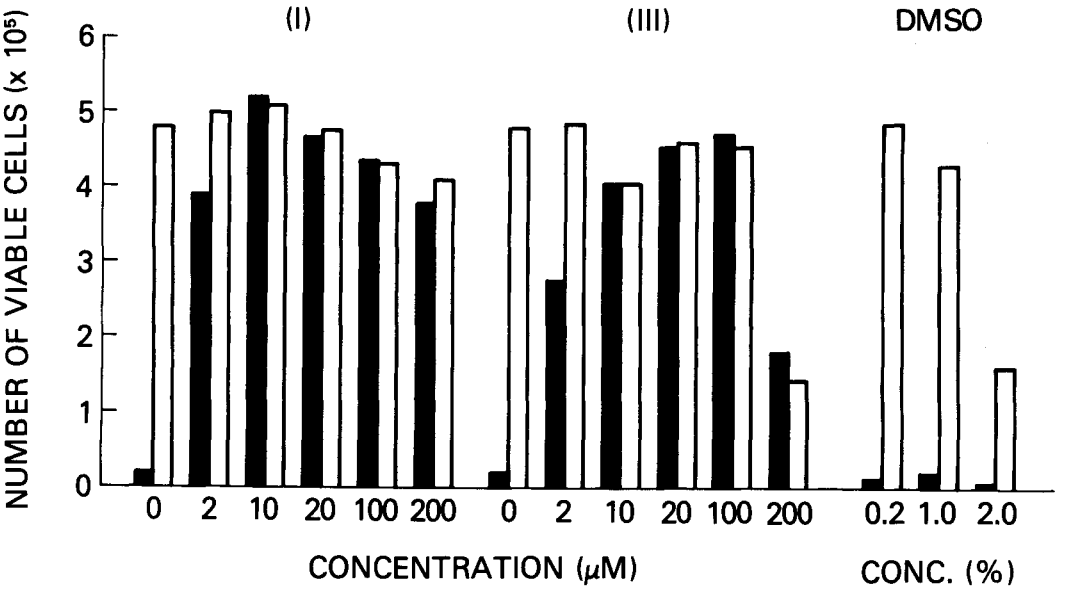

Fig. 2. Inhibition of the cytopathic effect of HIV by I, III and DMSO in ATH8 cells. ATH8 cells were exposed to HTLV-III$_B$ in culture tubes (solid columns) in the presence of various concentrations of the three test compounds. Control cells (open columns) were treated similarly, but were not exposed to the virus. On day 5, total viable cells were counted.

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