Enzymatic and Chemical Stability of 2′,3′-Dideoxy-2′,3′-didehydropyrimidine Nucleosides: Potential Anti-acquired Immunodeficiency Syndrome Agents

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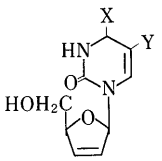
The enzymatic and chemical stability of three 2′,3′-dideoxy-2′,3′-didehydropyrimidine nucleosides has been studied. Chemical degradtion of the analogues was measured in the pH range of 1.0—9.0. 2′,3′-Dideoxy-2′,3′-didehydrocytidine (DDCN) degraded rapidly under acidic conditions, but the chemical stability was greater under basic conditions. The chemical degradation of 2′,3′-dideoxy-2′,3′-didehydrouridine (DDUN) and 2′,3′-dideoxy-2′,3′-didehydrothymidine (DDTN) was not pH dependent and was faster than that of cytarabine. Enzymatic degradation of DDCN, DDUN and DDTN was not observed in human plasma, though cytarabine was degraded enzymatically under the same conditions. DDCN was also not degraded in the presence of mouse kidney cytidine deaminase.

Keywords 2′,3′-dideoxy-2′,3′-didehydropyrimidine nucleoside; stability; acquired immunodeficiency syndrome

Introduction

2′,3′-Dideoxy-2′,3′-didehydropyrimidine nucleosides (Fig. 1) are potent inhibitors of the reverse transcriptase of the human immunodeficiency virus (HIV) isolated from patients with acquired immunodeficiency syndrome (AIDS).[1,2] Some of these analogues have a superior inhibitory effect to that of zidovudine (2′,3′-dideoxy-3′-azidothymidine, AZT), which seems to be the most potent anti-AIDS drug currently available. Since these nucleoside analogues work as metabolic antagonists against the reverse transcriptase of the virus, their anti-viral effect can be time dependent or require long retention of the drug in the body.

In order to develop an effective formulation and/or chemical modification of the molecules, information on the chemical and enzymatic stability of the compounds is needed. In this report, the stability of the 2′,3′-dideoxy-2′,3′-didehydropyrimidine nucleosides under various pH conditions or in the presence of enzymes was examined and compared with that of cytarabine, an unstable nucleoside analogue.[3,4]



	X	Y
DDCN	$-NH_2$	$-H$
DDUN	$=O$	$-H$
DDTN	$=O$	$-CH_3$

Fig. 1. Structure of 2′,3′-Dideoxy-2′,3′-didehydropyrimidine Nucleosides

Experimental

2′,3′-Dideoxy-2′,3′-didehydrocytidine (DDCN), 2′,3′-dideoxy-2′,3′-didehydrothymidine (DDTN) and 2′,3′-dideoxy-2′,3′-didehydrouridine (DDUN) were synthesized from the corresponding deoxynucleosides according to the procedure reported by Horwitz *et al.*[5,6] Cytarabine (cytosine arabinoside, AraC) was a gift from Nippon Shinyaku Co. (Kyoto, Japan). All other chemicals were of reagent grade. Stock solutions of the nucleoside analogues were prepared in methanol to give a concentration of 500 μg/ml and were stored at −40 °C. A stock solution of AraC was prepared in distilled water to give a concentration of 500 μg/ml and was stored at 5 °C.

Human blood was collected from a volunteer into a heparinized syringe. The blood was centrifuged at 1000 × *g* for 15 min and freshly prepared plasma was used for each experiment.

Cytidine deaminase was prepared from a mouse kidney homogenate by denaturation at 60 °C, fractionation with $(NH_4)_2SO_4$, treatment with alumina gel Cr(pH 7.5), and dialysis against 0.02 M acetate buffer (pH 4.5) according to the procedure described by Tomchick *et al.*[7]

The chemical stability of the analogues was measured in 0.1 N HCl (pH 1.0) and phosphate buffer solutions (pH 3—9, 10 mM) at 37 °C. Each reaction was initiated by adding a stock solution (20 μl) to a preheated solution (2 ml) in a glass tube with a screw cap. A 100 μl portion of the reaction mixture was periodically withdrawn and stored at −40 °C until high performance liquid chromatographic (HPLC) analysis.

The enzymatic stability was measured in human plasma. The experiments were performed at 37 °C and initiated by adding the stock solution (16 μl) to fresh human plasma (1.6 ml). Two hundred microliter portions of the mixture were periodically withdrawn and immediately mixed with 400 μl of acetonitrile for deproteinization. The mixture was centrifuged for 10 min at 3000 rpm and the supernatant fluid was collected. Then 200 μl of distilled water and 400 μl of methylene chloride were added, mixed and centrifuged. The aqueous phase was collected and methylene chloride dissolved in the aqueous phase was evaporated. The residual solution was introduced into the HPLC column. 5-Fluoro-2′-deoxyuridine dissolved in acetonitrile for deproteinization was used as the common internal standard for the 2′,3′-dideoxy-2′,3′-didehydopyrimidine nucleosides and AraC. The pH changes of the human plasma during the experiments were measured; the pH values were in the range of 7.8—8.2. Enzymatic deamination rates of DDCN and AraC were measured in the presence of a mouse kidney cytidine deaminase preparation. The experiments were performed at 37 °C and initiated by adding the stock solution (10 μl) to the mouse kidney cytidine deaminase preparation (5 mg of protein/1 ml phosphate buffer, pH 7.4) to give a final drug concentration of 5 μg/ml. The decreases in concentrations of DDCN and AraC were followed by HPLC analysis of samples taken periodically from the reaction mixture.

For HPLC analysis of DDCN, DDTN, DDUN, and AraC, a mixture of methanol and 0.02 M phosphate buffer (1—4 : 96—99), pH 7.0, was used as a mobile phase on a C18 reversed-phase (Superspher RP-18, Merck-Kanto Chemicals) 300 × 4.6 mm column at a flow rate of 1.0 ml/min. The wavelength of the spectrophotometer was set at 269 nm with an attenuation of 0.01 AUFS.

Results and Discussion

Typical time courses of disappearance of the nucleoside analogues in various buffer solutions are shown in Fig. 2. The slopes of semilogarithmic plots of the concentration against time gave the half-lives ($t_{1/2}$). Table I shows the chemical stability ($t_{1/2}$) of the nucleoside analogues and AraC at 37 °C under various pH conditions. DDCN degraded rapidly under acidic conditions (pH 1 and 3) with half lives of less than 30 min. The degradation product of DDCN in the acidic media has been identified as cytosine by HPLC analysis. DDCN showed greater stability under the higher pH conditions (pH 7 and 9). Both DDUN and DDTN showed greater stability than DDCN in the acidic media (pH 1—5) and the chemical stability was less pH-dependent. Degradation products of DDUN and DDTN

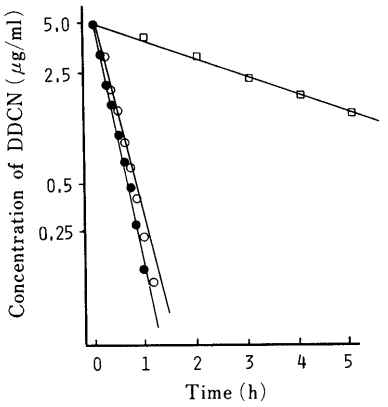


Fig. 2. Chemical Degradation of DDCN
(□), pH 5.0; (○), pH 3.0; (●), pH 1.0.

TABLE I. Chemical Stability of 2′,3′-Dideoxy-2′,3′-didehydropyrimidine Nucleosides and Cytarabine

pH	$t_{1/2}$ (h) (Mean ± S.E.M. $n=3$)			
	DDCN	DDUN	DDTN	AraC
1.0	0.20 ± 0.02	15.03 ± 0.16	26.37 ± 2.46	534.16 ± 114.85
3.0	0.22 ± 0.00	18.06 ± 2.01	91.95 ± 20.66	>2000
5.0	2.89 ± 0.28	29.89 ± 3.43	105.31 ± 3.67	>2000
7.0	171.08 ± 36.43	33.00 ± 3.62	102.06 ± 2.23	>2000
9.0	>2000	33.38 ± 0.54	96.86 ± 17.29	>2000

were the corresponding pyrimidine bases (*i.e.* uracil and thymine) under all the pH conditions investigated. AraC was quite stable compared with DDCN, another cytidine analogue; degradation of AraC was not detected in the experimental period (10 d) except at the lowest pH (pH 1.0).

Table II shows the enzymatic stability of the analogues in human plasma and in the presence of the mouse kidney cytidine deaminase preparation. Though AraC degraded enzymatically in the presence of human plasma, enzymatic degradation was not observed with the other analogues, *i.e.*, the half lives of DDCN, DDTN and DDUN in the human plasma were quite similar to or longer than those in the absence of the enzyme (at pH 7—8). Since DDCN is a cytidine analogue, the susceptibility of DDCN to cytidine deaminase was measured and compared with that of AraC. Though AraC was degraded rapidly ($t_{1/2}=8.6$ min) by the cytidine deaminase preparation, no enzymatic degradation was observed in the case of DDCN.

TABLE II. Enzymatic Stability of 2′,3′-Dideoxy-2′,3′-didehydropyrimidine Nucleosides and Cytarabine

Compound	$t_{1/2}$ (h) (Mean ± S.E.M.)	
	Human plasma ($n=3$)	Mouse kidney cytidine deaminase ($n=5$)
DDCN	>300	>300
DDUN	47.6 ± 9.3	—[a]
DDTN	>300	—[a]
AraC	3.4 ± 0.2	0.143 ± 0.003

a) Not done.

The above results show that 2′,3′-dideoxy-2′,3′-didehydropyrimidine nucleosides are enzymatically stable but are chemically less stable compared with AraC. The chemical lability of DDCN under the acidic conditions is especially noteworthy; the rapid degradation below pH 3 ($t_{1/2}=20$ min) suggests that careful dosage form design (*e.g.* enteric coating) and/or a prodrug approach will be required for the oral administration of DDCN.

References

1) T. S. Lin, R. F. Schinazi, M. S. Chen, E. Kinney-Thomas and W. H. Prusoff, *Biochem. Pharmacol.*, **36**, 311 (1987).
2) P. Herdewijn, J. Balzarini, E. De Clercq, R. Pauwels, M. Baba, S. Broder and H. Vanderhaeghe, *J. Med. Chem.*, **30**, 1270 (1987).
3) R. E. Notari, M. L. Chin and R. Wittebort, *J. Pharm. Sci.*, **61**, 1189 (1972).
4) W. A. Creasey, R. J. Papac, M. E. Markiw, P. Calabresi and A. D. Welch, *Biochem. Pharmacol.*, **15**, 1417 (1966).
5) J. P. Horwitz, J. Chua, J. M. Noel, *J. Org. Chem.*, **29**, 2076 (1964).
6) J. P. Horwitz, J. Chua, J. M. Noel and J. T. Donatti, *J. Org. Chem.*, **32**, 817 (1967).
7) R. Tomchick, L. D. Saslaw and V. S. Waravdekar, *J. Biol. Chem.*, **234**, 2534 (1968).