[Chem. Pharm. Bull. 36(5)1899—1901(1988)]

Inhibition of Phosphorolytic Degradation of 5-Fluoro-2′-deoxyuridine by Pyrimidine Acyclonucleosides and Normal Pyrimidine Metabolites in Rat and Beagle Tissue Homogenates

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(Received November 11, 1987)

Inhibition constants (K_i) of pyrimidine acyclonucleosides and several normal pyrimidine metabolites for the phosphorolytic degradation of 5-fluoro-2′-deoxyuridine (FUdR) in rat and beagle tissue homogenates (liver and small intestine) were measured. Acyclothymidine (AcycTdR) showed the lowest K_i value for all the homogenates. The K_i/K_m values of AcycTdR and acyclouridine (AcycUdR) depended on the homogenates, and the values (K_i/K_m) in the rat liver homogenate were higher than those in all other homogenates.

Keywords——phosphorylase; inhibitor; acyclonucleoside; 5-fluoro-2′-deoxyuridine; K_m; K_i

Introduction

The antitumor activity of 5-fluoro-2′-deoxyuridine (FUdR) is limited *in vivo*,[2,3] because of its rapid degradation by pyrimidine nucleoside phosphorylases.[4] Potentiation of the antitumor activity of FUdR by using phosphorylase inhibitors has been suggested by many investigators[5-7] and several successes have been reported in cultured cells.[8,9] In a recent study, we have achieved potentiation in the survival of mice bearing L1210 by oral coadministration of acyclothymidine (5-methyl-1-(2′-hydroxyethoxymethyl)uracil) esters and FUdR esters.[10] We have also reported the significant inhibitory effect of acyclothymidine (AcycTdR) on FUdR degradation in various tissue homogenates, and the much weaker effect of acyclouridine (AcycUdR).[11] In the present study, the inhibitory effects of the acyclonucleosides and related normal metabolites on various tissue homogenates were quantitatively investigated.

Experimental

Pyrimidine acyclo-nucleosides were prepared from trimethylsilylated pyrimidines and 2-(benzoxy)ethoxymethyl chloride according to the procedure described by Kelley and Baker.[11] All other chemicals were purchased from Sigma Chemical Co. (MO, U.S.A.).

Male beagles (9.5—10.0 kg) and Sprague Dawley rats (250—280 g) were sacrificed to obtain livers and small intestines. The tissues were homogenized with pH 7.0 isotonic phosphate buffer (0.1 M) containing 0.19 M sucrose at 0 °C to give a concentration of 5.0%, w/v. One milliliter portions of the homogenates were transferred to small glass tubes and stored at −80 °C until the enzyme assays.

The enzyme assays were carried out at 37 °C by using 1 ml of the homogenates diluted with the phosphate buffer to give final concentrations of 0.05—1.2%, w/v. Initial degradation rates of FUdR (velocity) were measured by the high performance liquid chromatography (HPLC) method described previously.[13] Kinetic constants (K_m) for the homogenates were determined with five levels of FUdR (in the range $0.5—7.0 \times K_m$ of FUdR) and three concentrations of homogenates. The K_m values were obtained from double-reciprocal plots of velocity *vs.* FUdR level. Studies of inhibition kinetics were made with five levels of FUdR (0.0125, 0.025, 0.05, 0.1 and 0.2 mM) for each of three inhibitor levels and control mixtures lacking inhibitor. Inhibition constants (K_i) were obtained from replots

of inhibitor concentrations *vs.* slopes of double-reciprocal plots of velocity *vs.* FUdR level. All of the latter plots were linear.

Results

AcycTdR, AcycUdR, thymidine (TdR), deoxyuridine (UdR), thymine and uracil were tested for inhibition of FUdR phosphorolytic activity of liver and intestinal homogenates from rat and beagle tissues. Three appropriate concentrations of an inhibitor were employed to estimate the inhibition constant (K_i); competitive inhibition was observed in all cases. The K_m values of FUdR and the K_i values for the homogenates are given in Tables I and II.

Since the K_m values depended on the homogenates, K_i/K_m values were used for the evaluation. A higher inhibitory effect of 5-methyl-substituted compounds (*i.e.* thymine and its analogues) over the corresponding uracil and its analogues was observed in all the homogenates; the differences were more significant in the beagle homogenates (10—20 times) than in the rat's (1—8 times). The acyclonucleosides showed much higher inhibitory effects than the normal pyrimidine metabolites, and again the tendency was more evident in the beagle homogenates (14—17 times) than in the rat's (1—6 times). The intestinal homogenates were more sensitive to the inhibition by AcycTdR than the liver homogenates (1.6 times in the beagle and 4.1 times in the rat). The K_i/K_m values for TdR and thymine also depended on the homogenates; TdR was a better inhibitor than thymine in the rat liver homogenate, but the K_i/K_m values of TdR were higher than those of thymine in all other homogenates.

TABLE I. Kinetic Constants (K_m) and Inhibition Constants (K_i) for Rat Tissue Homogenates

Compound	Liver (0.5%, w/v)		Intestine (0.09%, w/v)	
	K_i (mM)	K_i/K_m [a]	K_i (mM)	K_i/K_m [b]
AcycTdR	0.021	0.22	0.0037	0.053
AcycUdR	0.17	1.77	0.014	0.20
TdR	0.019	0.20	0.024	0.34
UdR	0.27	2.81	0.13	1.86
Thymine	0.044	0.46	0.011	0.16
Uracil	0.16	1.67	0.056	0.80

a) K_m of FUdR = 0.096 mM. Three levels of the homogenate (0.25, 0.5 and 1.0%) were used. *b*) K_m of FUdR = 0.070 mM. Three levels of the homogenate (0.02, 0.045 and 0.09%) were used.

TABLE II. Kinetic Constants (K_m) and Inhibition Constants (K_i) for Beagle Tissue Homogenates

Compound	Liver (0.8%, w/v)		Intestine (0.8%, w/v)	
	K_i (mM)	K_i/K_m [a]	K_i (mM)	K_i/K_m [b]
AcycTdR	0.00043	0.013	0.00046	0.0079
AcycUdR	0.0048	0.15	0.0090	0.16
TdR	0.0072	0.23	0.010	0.17
UdR	0.10	3.13	0.19	3.28
Thymine	0.0046	0.14	0.0058	0.10
Uracil	0.048	1.50	0.063	1.09

a) K_m of FUdR = 0.032 mM. Three levels of the homogenate (0.2, 0.4 and 0.8%) were used. *b*) K_m of FUdR = 0.058 mM. Three levels of the homogenate (0.2, 0.4 and 0.8%) were used.

Discussion

Among several types of pyrimidine nucleoside phosphorylases, non-specific uridine phosphorylase, which cleaves 5-substituted pyrimidine deoxynucleosides, is known to be responsible for FUdR degradation.[14,15] Since the non-specific phosphorylase is widely distributed and plays crucial roles in the body,[15] the inhibition of the enzyme should be reversible and limited to target tissues. The results of this study indicate a species specificity (rat–beagle) and a tissue specificity (liver–intestine) of the phosphorolytic reaction. AcycTdR showed the highest inhibitory effect in all the homogenates, but the K_i/K_m value in the rat liver homogenate is remarkably higher than in the other homogenates. The response of the rat liver homogenate to AcycUdR and TdR is also characteristic. These results indicate that tissue-specific inhibition of the FUdR-degrading enzyme may be feasible by using an inhibitor suitable for the administration route and tumor location.

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