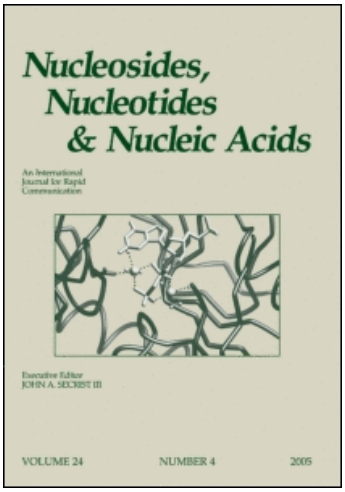

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Comparison of the Inhlbitory Effects of 2-Chloro-2'-deoxyadenosine and 9-β-D-Arabinosyl-2-fluoro-adenine on Metabolism of Deoxyadenosine in Human Lymphocytes and Erythrocytes

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COMPARISON OF THE INHIBITORY EFFECTS OF 2-CHLORO-2'-DEOXY-ADENOSINE AND 9-β -D-ARABINOSYL-2-FLUORO-ADENINE ON METABOLISM OF DEOXYADENOSINE IN HUMAN LYMPHOCYTES AND ERYTHROCYTES.

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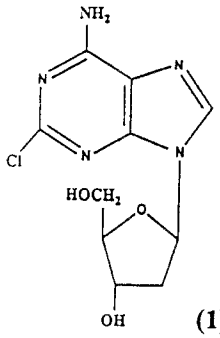
Abstract: The effect of 2-chloro-2'-deoxyadenosine and 9-β-D-arabinosyl-2-fluoro-adenine on metabolism of deoxyadenosine in human lymphocytes or erythrocytes was estimated. These drugs demonstrate different effects; 2CdA blocks both the dAdo phosphorylation and deamination (at 95% and 55%, respectively), while F-ara-A inhibits dAdo phosphorylation only at 40% and remains without effect on ADA activity.

The two 2-halogenated adenosine analogues: 2-chloro-2'-deoxyadenosine (**1**)(Cladribine, 2CdA) and 9-β -D-arabinosyl-2-fluoro-adenine (**2**)(Fludarabine, F-ara-A) have found clinical application for the treatment of lymphocytic and lymphoid leukemias [1,2]. According to published data, 2CdA and F-ara-A result in apoptosis of cells and it has been established that the mechanisms of action of both drugs lead (via their phosphorylated derivatives) to inhibition of DNA synthesis. In the case of 2CdA [3], this involves inhibition of ribonucleotide reductase, DNA polymerases and DNA repair. Whereas F-ara-A [3] blocks the action of DNA primase, DNA ligase, RNA and protein synthesis. Recently, we reported the block of dAdo metabolism by 2CdA in lysate of CNS lymphoma cells [4]. A complete inhibition of phosphorylating activity for dAdo, and 60% of inhibition of adenosine deaminase (ADA) activity have been observed in *in vitro* studies. The findings suggested that the toxic effect of 2CdA (depletion of the NAD level and imbalance of the pool of deoxynucleotides [5]) results from the inhibitory effect of 2CdA on enzymes involved in the metabolism of dAdo. Moreover, based on previous results of studies *ex vivo* of the inhibitory effect of 2CdA on S-adenozylhomocysteine (SAH) hydrolase activity [6], it was proposed that the mechanism of 2CdA toxicity includes a blocking of dAdo metabolism with inactivation of SAH-hydrolase as a natural consequence, secondary to dAdo accumulation.

Knowing the importance of undisturbed dAdo degradation for normal function of immune system cells [7], and knowing from previous studies that 2CdA contributes to perturbation of dAdo metabolism [4], we decided to compare the effect of 2CdA with that of F-ara-A, which is a 2-halogenated analogue of Ara-A. Additionally, this comparison of biomolecular mechanism of toxicity of both drugs, including an effect on synthesis of adenine DNA precursors seems to be worth elucidation, because the efficacy of both drugs against CLL, (chronic lymphocytic leukemia) is very similar while it is different in the case of HCL (hairy cell leukemia) [8]

In the present study, the effect of 2CdA and F-ara-A on the phosphorylating activity for dAdo in lysates of human lymphocytes and activity of ADA and SAH-hydrolase in lysed erythrocytes was investigated. Moreover, the studies included estimation of phosphorylating activity for deoxycytidine (dCyt) in lymphocytes, because deoxycytidine kinase, is the enzyme responsible for phosphorylation of: dCyt, dAdo, 2CdA and F-ara-A.



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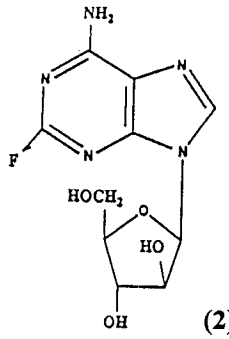

(2)

We found that 2CdA and F-ara-A had several unreported effects on the activity of some enzymes of adenine salvage pathway, differing both compounds considerably. Contrary to 2CdA, F-ara-A has: no effect on deamination of dAdo, a slight influence on phosphorylation of both dAdo and dCyt, and potent inhibitory activity against SAH-hydrolase.

Materials and methods. The activity of deoxycitidine kinase for dAdo (or dCyt), ADA and SAH-hydrolase was assayed using radiochemical methods with paper chromatography to separate substrates and products of reaction mixtures.

dAdo phosphorylating activity: incubation mixture (240µl) contained: 50 mM Tris/HCl (pH 7.4), 10 mM ATP, 5 mM $MgCl_2$, 10 µM deoxycoformycin (inhibitor of ADA), 0.05 mM [8-^{14}C] dAdo or dCyt (0.05 µCi per sample), 0.01-0.2 mM inhibitors (i.e. 2CdA or F-ara-A), and supernatant of lysed human lymphocytes (80-100 µg of protein per sample). (Lymphocytes were isolated by Histopaque-1077 density gradient centrifugation). Incubation was carried out for 60 min. at 37°C. Reaction was terminated by immersion of the tubes for 2 min. in boiling water. Formation of radioactivity dAMP, dADP and dATP (or dCMP,d CDP and dCTP) was determined by measuring radioactivity of spots separated by descending chromatography. (Whatman No.1) in 1 M ammonia acetate: 95% etanol, 3:7, (pH 7.5) with R_f for dAdo, dAMP, dADP and dATP of: 0.75; 0.25; 0.18; and 0,125. (or R_f for dCyt, dCMP, dCDP and dCTP of: 0.87; 0.51; 0.31 and 0.21). The spots corresponding to standars were counted in liquid scintillation counter.

ADA activity: incubation mixture (240 µl) contained: 30 mM phosphate buffer (pH 7.4), 0.1 mM [8-^{14}C] dAdo (0.05 µ Ci per sample), 0.01-0.2 mM inhibitors (i.e. 2CdA or F-ara- A), and lysate of human erythrocytes (0.15-0.20 mg of hemoglobin per sample). Incubation was carried out for 30 min. at 37°C. Reaction was terminated in the way described for dAdo phosphorylation. Formation of radioactivity of deoxyinosine (dIno) and hypoxantine was determined by measuring radioactivity of spots separated by descending chromatography. (Whatman DE 81) in 2 M ammonium formate with R_f for dAdo, dIno and hypoxantine of 0.67; 0.25 and 0.19.

TABLE 1. Effect of 2CdA and F-ara-A on the phosphorylating activity for dAdo (or dCyt) in lysate of human lymphocytes

Phosphorylating activity for	Human Lymphocytes Activity [nmol/mg of protein/h]	Inhibition [%] 2CdA	F-ara-A
dAdo	5.0 ± 0.3	95 (K_i=2.5x10^{-6}M)	40 (K_i=5.3x10^{-4}M)
dCyt	7.8 ± 0.6	70	15

TABLE 2. Effect of 2CdA and F-ara-A on the activity of ADA and SAH-hydrolase in lysed human erythrocytes

Enzyme	Human erythrocytes Activity nmol/mg of Hb/h	Inhibition [%] 2CdA	F-ara-A
ADA	62.4 ± 4.4	55 (K_i=3.1x10^{-4}M)	0
SAH-hydrolase	5.3 ± 0.4	25	85

SAH-hydrolase activity: incubation mixture (300µl) contained: 30 mM phosphate buffer (pH 7.4), 0.5 mM [8-^{14}C] Ado (0.05 µCi per sample), 1mM DTT, 6 mM homocysteine, 10 µM deoxycoformycin, inhibitors (i.e. 2CdA or F-ara-A) at equal concentrations of substrate (Ado), and lysate of human erythrocytes (0.8-1.2 mg of hemoglobin per sample). Incubation was carried out for 60 min. at 37°C. Termination of reaction proceeded in the way described above. Formation of radioactivity of SAH was determined by measuring radioactivity of spots separated by descending chromatography. (Whatman DE 81) in 2 mM ammonium formate with R_f for Ado and SAH of: 0.66 and 0.18.

Chemicals: D,L-homocysteine, adenosine, dAdo, ATP, F-ara-A were obtained from Sigma Chemical Co. Radiolabelled substrates: [8-^{14}C] dAdo, [8-^{14}C] Ado, and [5-^{3}H] dCyt were obtained from Amersham, Life Science; 2CdA was kindly donated by Prof. Z. Kazimierczuk (Dept. Biophys., Inst. Experimental Physics, Univ. of Warsaw, Poland)

Results and discussion. The inhibitory effects of 2CdA and F-ara-A on dAdo and dCyt phosphorylating activity in lysed human lymphocytes are summarized in Table 1, while the effects of both drugs on ADA and SAH-hydrolase activity in lysed human erythrocytes are showed in Table 2.

The results show that 2CdA competitively inhibited phosphorylation of dAdo in lysed human lymphocytes (by almost 100%)(Table 1) and the activity of ADA in lysates of human erythrocytes was also reduced to 45% by the drug. (Table 2). K_i values for 2CdA against dAdo phosphorylating activity and against ADA were estimated to be 2.5×10^{-6}M and 3.1×10^{-4} M, respectively. However, 2CdA, in vitro studies, showed only slight effect on the activity of SAH hydrolase (not more than 25%) in lysate of erythrocytes. The F-ara-A effect on metabolism of dAdo was diffrent from that of 2CdA. F-ara-A caused only 40% inhibition of deoxycytidine kinase with dAdo as substrate, and did not inhibit ADA activity. In contrast to 2CdA, F-ara-A directly inhibited SAH-hydrolase activity (at 85%) in lysates of human erythrocytes.

The present studies whose aim was the comparison of biochemical activity of two new antileukemic drugs (i.e. 2CdA and F-ara-A) on metabolism of dAdo, clearly demonstrate distinct properties of both nucleosides. Contrary to 2CdA, F-ara-A does not reveal any or significantly less inhibitory effects on important enzymes for dAdo metabolism. It seems that showed differences can be one of the reasons of distinct sensitivity of leukemic cells to treatment with these new antimetabolited drugs.

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