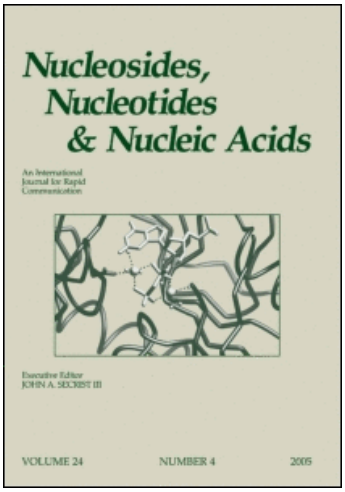

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Distinct Inhibitory Effects of 2-Chloro-2'-deoxyadenosine and 9-β-D-Arabinosyl-2-fluoroadenine on DNA Methyltransferase in Human T-Lymphocytes

Dorota Wyczechowska[a]; Katarzyna Ruckemann[b]; John A. Duley[b]; Anne H. Simmonds[b]; Krystyna Fabianowska-majewska[a]
[a] Department of General Chemistry, Medical University of Lodz, Lodz, Poland [b] Purine Research Laboratory, UMDS Guy's Hospital, London, UK

To cite this Article Wyczechowska, Dorota , Ruckemann, Katarzyna , Duley, John A. , Simmonds, Anne H. and Fabianowska-majewska, Krystyna(1999) 'Distinct Inhibitory Effects of 2-Chloro-2'-deoxyadenosine and 9-β-D-Arabinosyl-2-fluoroadenine on DNA Methyltransferase in Human T-Lymphocytes', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 831 — 834
To link to this Article: DOI: 10.1080/15257779908041572
URL: http://dx.doi.org/10.1080/15257779908041572

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 831–834 (1999)

DISTINCT INHIBITORY EFFECTS OF 2-CHLORO-2'-DEOXYADENOSINE AND 9-β-D-ARABINOSYL-2-FLUOROADENINE ON DNA METHYLTRANSFERASE IN HUMAN T- LYMPHOCYTES

Dorota Wyczechowska[1], Katarzyna Ruckemann[2], John A.Duley[2], Anne H.Simmonds[2], Krystyna Fabianowska-Majewska[1*]

[1] Department of General Chemistry, Medical University of Lodz,Lindleya 6, 90-131 Lodz Poland; [2] Purine Research Laboratory UMDS Guy's Hospital, London SE1 9RT, UK

ABSTRACT: The effect of 2-chloro-2'-deoxyadenosine and 9-β-D-arabinosyl-2-fluoro-adenine on DNA methyltransferase activity in stimulated human T-lymphocytes was estimated. In comparative studies 5-aza-deoxycytidine and deoxyadenosine plus deoxycoformycin were used. These antileukemic compounds demonstrated different effects; both 2CdA and dAdo plus dCF, like 5-aza-dCyt, inhibited the enzyme activity by 85-90% after 72 hours activation of lymphocytes, while the effect of F-ara-A, under the same conditions, was insignificant.

In eukaryotes DNA methyltransferase (C-5 MT-ase, EC 2.1.1.37) catalyzes the transfer of methyl groups from the cofactor S-adenosyl-L-methionine (SAM) to cytosine C5 in dinucleotide target sequences (CpG) of double-stranded DNA [1]. The enzyme is involved, among others, in differentation of cells and regulation of gene expression[1].

Some adenosine analogues have inhibitory effect on the activity of S-adenosylhomocysteine (SAH) hydrolase [2], which suggest perturbation of SAM dependent DNA methylation. More recently, it was reported, that there is a close relationship between DNA methylation and DNA repair in which poly(ADP-ribose) polymerase plays an important role in repair of DNA fragmentation [3].

2-Chloro-2'-deoxyadenosine (2CdA, Cladribine) and 9-β-D-arabinosyl-2-fluoro-adenine (F-Ara-A, Fludarabine) are drugs which a few years ago were introduced to treat indolent lymphoid malignancies [4,5]. Their efficacy results from inhibition of DNA synthesis which leads to fragmentation of DNA at internucleosomal sites and apoptosis [6].

As the sensitivity of leukemic cells to treatment with these two antimetabolite drugs is different, we have previously studied differences in the mechanisms of their cytotoxic action. We have reported blockage of deoxyadenosine (dAdo) metabolism in extracts of human lymphocytes [7] and CNS lymphoma cells [8] by 2CdA but not F-ara-A. Recently, we have shown that 2CdA, in contrast to F-ara-A, caused progressive depletion of all nucleotide pools and dramatic inhibition of SAM production during 72 hours in activated human lymphocytes [9]. Although F-ara-A is a very potent direct inactivator of SAH hydrolase (in studies *in vitro*) in murine leukemic cells, L1210, it did not influence its activity during the growth of the cells [10].

In the present studies the effects of 2CdA and F-ara-A on C-5 MT-ase activity in cultured, stimulated human T-lymphocytes were investigated. Additionally, we included comparative assays using 5-aza-deoxycytidine (5-aza-dCyt), a potent inhibitor of DNA methylation, and dAdo plus deoxycoformycin (dCF) (the latter compounds mimic a deficiency of adenosine deaminase).

Methods. T-lymphocytes were separated from the peripheral blood according to Bofill's method [11]. T-lymphocytes were purified by monocyte, B-lymphocyte, NK-cell and reticulocyte depletion using, antibodies (i,e. α HLA-DR, anti-glycophorin A and CD16) and magnetic beads. C-5 MT-ase activity was analysed according to Issa's method[12]. The amount of methyl group incorporated from [^{3}H] SAM to deoxycytidine of poly[dI-dC]• poly[dI-dC], used as acceptor of the methyl groups, was determined.

Results and discussion. The inhibitory effect of 2CdA, F-ara-A, 5-aza-dCyt, and dAdo plus dCF on the activity of C-5 MT-ase at 24h intervals in extracts of human T-lymphocytes is documented in Fig. 1. The results show that 2CdA at 1 μM concentration in culture medium inhibited incorporation of the radiolabelled methyl group from SAM by 50% and 90% at 48h and 72h of growth, respectively. The level of inhibition by 2CdA is similar to the inhibitory effect of 5-aza-dCyt, a known potent inhibitor of DNA methylation reactions. Analogous changes of C-5 MT-ase activity were observed when culture medium was supplemented with dAdo (10 μM) plus dCF (60μM). Under the same conditions, the effect of F-ara-A (1μM) was insignificant. However, a 5-fold higher concentration of F-ara-A (5 μM) caused decrease of the enzyme activity to approximately 35%.

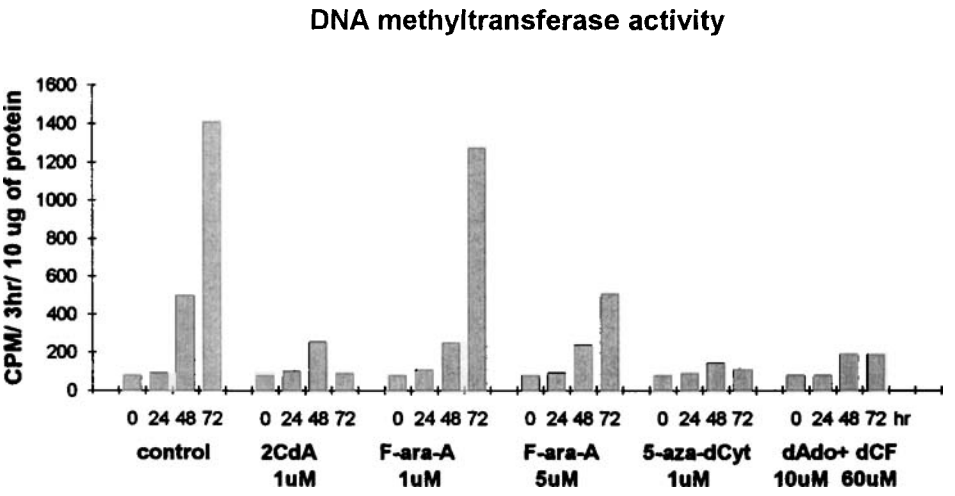

FIG. 1. Effects of 2CdA, F-ara-A, 5-aza-dCyt and dAdo plus dCF on C-5 MT-ase activity in stimulated human lymphocytes.

Similar changes of C-5 MT-ase activity under the influence of 2CdA, F-ara-A and 5-aza-dCyt to the above findings have been noted in extracts of murine leukemic cells, L1210 [10]. Moreover, the consistency of the results for 2CdA and dAdo plus dCF, with time, confirms previous literature data that 2CdA on its own action simulates the lymphotoxicity observed for ADA deficiency [13].

Obviously, the cytotoxic effectiveness of the tested adenosine analogues, which leads to internucleosomal fragmentation of DNA, required in the first place their phosphorylation to triphosphate derivatives [14]. However, involvement of inhibition of DNA C-5 MT-ase in the lymphotoxicity of 2CdA is a new finding, providing evidence for additional, different mechanisms of action of the two antileukemic drugs, 2CdA and F-ara-A.

Acknowledgments. One of us (D. W.) gratefully acknowledges the support of Fellowship Award from The International Union Against Cancer ICRETT, grant No. 652/1997. We would like to thank The Special Trustees of Guy's Hospital and The Medical University of Lodz, grant No 502-11-332 (103) for support.

REFERENCES

(1) Labahn, J.; Granzin, J.; Schluckebier, G.; Robinson, D.P.; Jack, W.E.; Schildkraut, I.; Saenger, W. *Proc.Natl.Acad.Sci.USA*, **1994**, 91, 10957-10961.

(2) Kim, I-K.; Li, C-C.H.; Young, H.A.; Lee, J-H.; Kim, H-S.; Pardhasaradhi, K.; Garcia, G.E.; Chiang, P.K. *J.Biomed.Sci.*, **1997**, 4, 83-90
(3) Zardo, G.; D'Erme, M.; Reale, A.; Strom, R.; Perilli, M.; Caiafa, P. *Biochemistry*, **1997**, 36, 7937-7943
(4) Saven A.; Piro L.P. *Ann.Intern.Med.*, **1994**, 120, 784-791
(5) Wright S.J.; Robertson L.E.; O'Brien S.; Plunkett W.; Keating M.J. *Blood Rev.*, **1994**, 8, 125-134
(6) Robertson, L.E.; Chubb, S.; Meyn, R.E.; Story, M.; Ford, R.; Hittelman, W.N.; Plunkett, W. *Blood*, **1993**, 1, 143-150
(7) Fabianowska-Majewska, K.; Wyczechowska, D. *Nucleos.Nucleot.*, **1997**, 16, 1283-1286
(8) Fabianowska-Majewska, K.; Tybor, K.; Duley, J.A.; Simmonds, H. A. *Biochem. Pharmacol.*, **1995**, 9, 1379-1383
(9) Fabianowska-Majewska K.; Ruckemann K.; Duley J.A.; Simmonds H.A. *Adv.Exp. Med.Biol.*, **1998**, 431, 531-535
(10) Wyczechowska, D.; Fabianowska-Majewska, K. *Adv.Exp.Med.Biol.*, **1998**, 431, 595-598
(11) Bofill, M.; Fairbanks, L.D.; Ruckemann, K.; Lipman, M.; Simmonds, A. *J.Biol. Chem.*, **1995**, 270, 2969-29697
(12) Issa, J-P.; Vertino, P.M.; Wu, J.; Sazawal, S.; Celano, P.; Nelkin, B.D.; Hamilton, S.R.; Baylin, S.B. *J.Nat.Cancer Inst.*, **1993**, 85, 1235-1240
(13) Beutler, E.; Carson, D.E. *Blood Cells*, **1993**, 19, 559-568
(14) Benveniste, P.; Cohen A. *Proc.Natl.Acad.Sci.USA*, **1995**, 92, 8373-8377