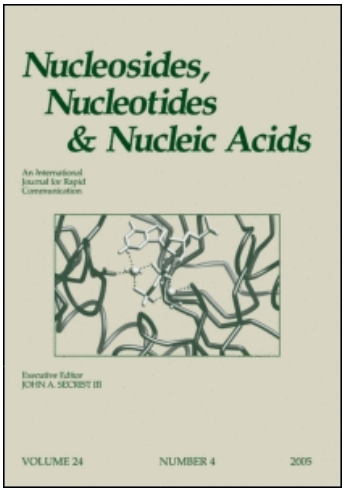

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Antileukemic Activities and Mechanism of Action of 2'-Deoxy-4'-methylcytidine and Related Nucleosides

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ANTILEUKEMIC ACTIVITIES AND MECHANISM OF ACTION OF 2'-DEOXY-4'-METHYLCYTIDINE AND RELATED NUCLEOSIDES

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ABSTRACT: Antileukemic activity of several analogues containing 2'-deoxy-4'-methylcytidine and its araC counterpart were evaluated against murine leukemic P388 cells *in vitro* and *in vivo*. Both compounds showed significant cytostatic activity (both IC_{50}= 0.4 μM) *in vitro* and the former compound administered intraperitoneally at a dose of 3 mg/kg/day x 5 showed high activity (T/C = 175%) *in vivo*. The mechanism of action of these 5'-triphosphates on DNA polymerases in detail will be also described.

It has been reported that several 4'-azido-2'-deoxyribonucleosides, such as 4'-azido-2'-deoxythymidine and 4'-azido-2'-deoxyadenosine showed to exert inhibitory effects on HIV in A3.10 cells [1]. On the other hand, cytosine and guanine counterparts have been shown remarkable cytostatic effects on mammalian cells *in vitro* [1]. This has stimulated intense efforts to discover other 4'-substituted 2'-deoxyribonucleosides with equal or better inhibitory activity on proliferating cells. The proposed mechanism of action of such nucleosides involves phosphorylation to their 5'-triphosphates, which can act as potent inhibitors of cellular DNA polymerases, especially those of the α-family, and/or be incorporated into 3'-terminus of DNA where they induce chain termination. 4'-Substituted -2'-deoxyribonucleosides are unique among antitumor nucleoside analogues because they retain the 3'-down hydroxyl group and have an extra functional group at the 4'-position. Due to the presence of the 3'-down hydroxyl group, those analogues may have strong affinity for deoxynucleoside kinases at the nucleoside level and also for the DNA polymerase-α family in their 5'-triphosphate form. However, in spite of the presence of the 3'-hydroxyl group, they might be able to act as chain terminators as well as polymerase inhibitors, since the conversion of chemical structure of sugar

moiety may cause the conformational change of its sugar skeleton. Therefore, the synthesis and evaluation of biological activities of some 4'-methyl-2'-deoxyribonucleoside analogues were considered.

Waga et al. synthesized 2'-deoxy-4'-methyladenosine (4'-Me-dA), 2'-deoxy-4'-methylthymidine (4'-Me-dT), 2'-deoxy-4'-methylcytidine (4'-Me-dC), and 1-(4-methyl-β-D-arabinofuranosyl)cytosine (4'-Me-araC) [2]. The results of their antileukemic activities against murine leukemic P388 cells *in vi*tro were shown in FIG. 1. In these compounds, both IC_{50} values of 4'-Me-dC and its araC counterpart (4'-Me-araC) were 0.4 μM. That of araC was 0.04 μM in the same system. Interestingly, in an *in vivo* system with the same cells in mice, 4'-Me-dC and 4'-Me-araC administered intraperitoneally at a dose of 3 mg/kg/day x 5, showed higher activity than the positive control araC, i. e. the percent values of T/C of 4'-Me-dC, 4'-Me-araC and araC were 175, 138 and 130, respectively. These results suggest that 4'-Me-dC may be a candidate as antileukemic agent.

In order to elucidate a mechanism of action, the inhibitory effects of their 5'-triphosphates on mammalian DNA polymerases were examined. 4'-Me-dC and 4'-Me-araC were chemically phosphorylated to the corresponding 5'-triphosphates as following. Amino group of 4'-Me-dC was selectively protected with benzoyl group by the reaction with benzoic anhydride in aqueous dioxane [3]. The protected nucleoside were converted into the corresponding 5'-monophosphate derivative by phosphorylation with $POCl_3$ [4] and then the nucleotide was further converted to its 5'-triphosphate using the phosphoroimidazolidate method [5]. After the deprotection of the nucleotide by the treatment with 1 M ammonium hydroxide at room temperature for overnight, desired compound (4'-Me-dCTP) was obtained. 4'-Me-araCTP was obtained in a similar fashion.

We examined the inhibitory effects of 4'-Me-dCTP and 4'-Me-araCTP on calf thymus DNA polymerase α and recombinant rat DNA polymerase β with activated calf thymus DNA as the template-primer. As shown in FIG. 2, 4'-Me-dCTP inhibited DNA polymerase α as strongly as araCTP. From the double reciprocal plots analyses, the mode of inhibition of 4'-Me-dCTP was competitive with respect to dCTP. The *K*i values of 4'-Me-dCTP and araCTP and *K*m value of dCTP were determined to be 1.0, 1.2 and 1.4 μM, respectively. On the other hand, 4'-Me-araCTP was weak inhibitor of both DNA polymerases α and β. It is interesting that 4'-Me-araC exhibited antileukemic activity as potent as araC *in vivo*.

Primer extension reactions by DNA polymerase α on synthetic template-primer of defined sequence were examined using 4'-Me-dCTP and 4'-Me-araCTP as substrates. Radiolabeled products were analyzed by polyacrylamide gel electrophoresis (20 % acrylamide/7 M urea) followed by autoradiography. Incorporation of 4'-Me-dCTP or

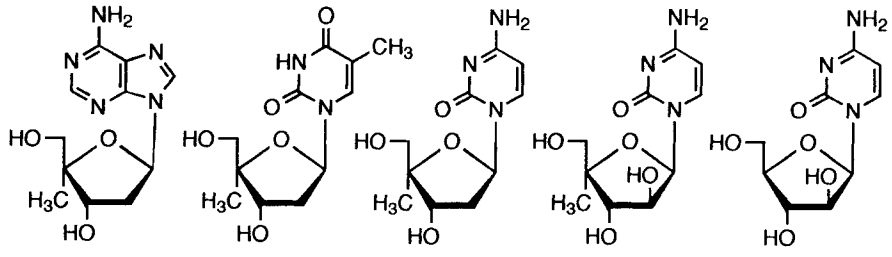

FIG. 1. Chemical structures of 2'-deoxy-4'-methylnucleoside analogues and their inhibitory effects on the growth of P388 leukemic cells *in vitro*.

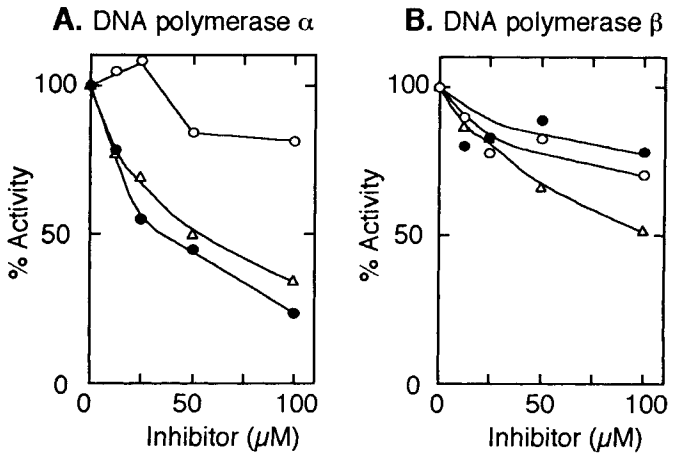

FIG. 2. Inhibitory effects of 4'-Me-dCTP (●–●), 4'-Me-araCTP (○–○) and araCTP (△–△) on eukaryotic DNA polymerases α (panel A) and β (panel B). Reactions were carried out for 20 min at 37°C with activated calf thymus DNA as the template-primer in the presence of 50 μM [^{3}H]dCTP.

4'-Me-araCTP catalyzed by DNA polymerase α resulted in immediate chain termination and similar result was obtained using araCTP (data not shown).

In conclusion, 4'-Me-dCTP was found to inhibit DNA polymerase α strongly in a competitive manner, and as expected was also a chain terminator for DNA polymerase α-catalyzed chain elongation of the DNA strand. Our results suggest a possible mechanism for the antileukemic activity of 4'-methyl-2'-deoxycytidine analogues.

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REFERENCES

(1) Prisbe, E. J.; Maag, H.; Verheyden, P. H.; Rydzewski, R. M. In *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K. and Baker, D. C., Eds.; Plenum Press: New York, 1993, pp 101-113.

(2) Waga, T.; Ohrui, H.; Meguro, H. *Nucleosides Nucleotides* **1996**, *15*, 287-304.

(3) Akiyama, M.; Oh-ishi, J.; Shirai, T.; Akashi, K.; Yoshida, K.; Nishikido, J.; Hayashi, H.; Usubuchi, Y.; Nishimura, D.; Itoh, H.; Shibuya, C.; Ishida, T. *Chem. Pharm. Bull.* **1978**, *26*, 981-984.

(4) Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505-3508.

(5) Maeda, M.; Patel, A. D.; Hampton, A. *Nucleic Acids Res.* **1977**, *4*, 2843-2853.