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Intracellular Metabolism and Action of an Antitumor Nucleoside, 2'-C-Cyano-2'-Deoxy-1- β -d-arabinofuranosylcytosine (CNDAC)

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INTRACELLULAR METABOLISM AND ACTION OF AN ANTITUMOR NUCLEOSIDE, 2'-C-CYANO-2'-DEOXY-1- β -D-ARABINOFURANOSYLCYTOSINE (CNDAC).

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ABSTRACT: The mechanism of antitumor activity of 2'-C-cyano-2'-deoxy-1- β -D-arabinofuranosylcytosine (CNDAC) has been examined. Intracellular metabolism of CNDAC using human leukemia cell lines are described. Incorporation of CNDAC triphosphate into DNA and the consequence of this incorporation have been evaluated *in vitro* using DNA primer extension assay with purified human DNA polymerase α and defined DNA primer/templates.

We have designed and synthesized CNDAC as a potential mechanism-based DNA strand-breaking nucleoside¹⁻³. CNDAC showed potent growth inhibitory activity against human tumor cell lines *in vitro* and *in vivo*⁴⁻⁵. We have demonstrated that single-strand DNA strand-breaks are actually occurring after CNDAC is incorporated into the elongating DNA strand by DNA polymerase in primer extension assays⁶.

The human T cell leukemia line, CCRF-CEM, and the human myeloid line, ML-1, were used for investigation of mechanisms of antitumor activity of CNDAC. The IC₅₀ values were 30 nM and 100 nM after a 72 h incubation of CNDAC for CCRF-CEM and ML-1 cells, respectively. The mono-, di-, and triphosphate of [³H]CNDAC were readily detected by HPLC analysis. Intracellular accumulation of CNDAC triphosphate (CNDACTP), the major metabolite, in CCRF-CEM was greater than in ML-1, and reached a plateau at 4 h

in both cell lines. Elimination of CNDACTP from CCRF-CEM was faster than from ML-1; half-life periods of intracellular CNDACTP were 1.6 h and 4.8 h for CCRF-CEM and ML-1, respectively. dNTP pools of both cell lines were not affected by CNDAC treatment. Radioactivity from [^3H]CNDAC-labeled cells was associated mainly with cellular DNA and, to a much less extent, with RNA. [^3H]Thymidine incorporation into DNA in both cell lines were inhibited by CNDAC. The IC_{50} values of thymidine incorporation were 1.1 μM and 0.8 μM cells after a 4 h incubation of CNDAC with CCRF-CEM and ML-1 cells, respectively. Furthermore, CNDAC caused the cells to arrest in the G2 phase of cell cycle, which is an unique effect on cell cycle when compared with other nucleoside analogues. Two other analogues of deoxycytidine, ara-C and dFdC, blocked cells at the S phase of the cell cycle.

We demonstrated CNDACTP was an effective substrate for incorporation into the elongating DNA strand by purified human DNA polymerase α using the DNA primer extension assays; the K_m was 0.2 μM compared to 2.0 μM for dCTP. CNDACTP competed with dCTP for incorporation into DNA (K_i , 0.02 μM). Elongation of the DNA primer was potently abrogated by the incorporated CNDACMP at the 3'-end of the primer.

These results indicate that CNDAC is metabolized to mono-, di-, triphosphates by nucleoside and nucleotide kinases in tumor cells, and then CNDACTP inhibits cellular DNA synthesis after its incorporation into DNA. Furthermore, the formation of the phosphodiester linkage after incorporation of the analogue into DNA would trigger a β -elimination reaction that causes a single strand break 3' to CNDAC monophosphate. The antitumor activity of CNDAC would be enhanced by this DNA strand-breaking reaction.

REFERENCES

- (1) Matsuda, A.; Nakajima, Y.; Azuma, A.; Tanaka, M.; Sasaki, T. *J. Med. Chem.*, **34**, 2917 (1991).

- (2) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Saski, T.; Matsuda, A. *J. Med. Chem.*, **36**, 4183 (1993).
- (3) Matsuda, A.; Azuma, A.; Nakajima, Y.; Takenuki, K.; Dan, A.; Iino, T.; Yoshimura, Y.; Minakawa, N.; Tanaka, M.; Sasaki, T. *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*: Chu, C. K.: Baker, D. C. Eds.: Plenum Publishing Co.: New York, 1993, pp1-22.
- (4) Tanaka, M.; Matsuda, A.; Terao, T.; Saski, T. *Cancer Lett.*, **64**, 67 (1992).
- (5) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem.*, **38**, 3391 (1995).
- (6) Matsuda, A.; Azuma, A. *Nucleosides Nucleotides*, **14** (3-5), 461 (1995).