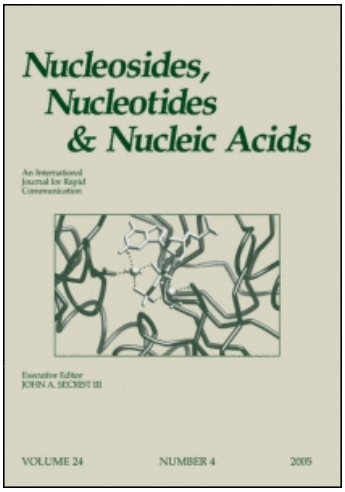

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Synergistic Combinations of Nucleoside Analog Drugs with Other Drugs Induce Greater Apoptosis in Human Leukemic T-Cells

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SYNERGISTIC COMBINATIONS OF NUCLEOSIDE ANALOG DRUGS WITH OTHER DRUGS INDUCE GREATER APOPTOSIS IN HUMAN LEUKEMIC T-CELLS.

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ABSTRACT. Combinations of nucleoside analog drugs such as 6-MP, ara-C, or F-araA are synergistic against human leukemic T-cells and induce apoptotic cell death. Addition of Taxotere or PEG-ASNase to the synergistic combination of nucleoside analog drugs augments the synergism several fold by enhancing cellular apoptosis.

Introduction: Apoptotic cell death generally occurs in response to any genetic instability induced by chemical agents or environmental factors. Apoptosis is characterized by cytoplasmic boiling, condensation of chromatin and oligonucleosomal DNA fragmentation[1]. It has been shown that nucleoside analog drugs like fludarabine (F-araA), cytosine arabinoside (ara-C), and Gemcitabine induce apoptosis in leukemic T-cells as evidenced by the small and large molecular weight DNA fragmentation[2,3]. We have shown that combination of 6-mercaptopurine (6-MP) or F-araA and ara-C in a sequence specific manner is highly synergistic against leukemic T-cells[4,5]. We have observed that addition of PEG-Asparaginase (PEG-ASNase) or Taxotere to these synergistic nucleoside drug combination increases the synergism by several fold. We report here that the drug synergism observed by the combination of 6-MP + ara-C or F-araA + ara-C is correlated to increased apoptotic cell death by the combination regimen over either drug alone. Also, the increased synergism, following the addition of a third drug (Taxotere or PEG-ASNase) to the nucleoside analog drug combination, is due to enhanced apoptotic cell death.

Materials and Methods: *Cell lines:* The CEM/0 cell line was obtained from the DCT tumor bank, NCI-NIH, Fredrick, MD, and the CEM/ara-C/7A, a cell line that is 50% resistant to ara-C was developed in our laboratory[4].

Chemicals: Fludarabine was obtained from NCI-NIH and Taxotere and PEG-ASNase were provided by Rhone-Poulenc Rorer. Ara-C and 6-MP were purchased from Pharmacia-Upjohn and Sigma, respectively. *Drug synergism studies and Cell viability:* Drug synergism studies were conducted as described elsewhere[6]. Cell viability was determined by the trypan-blue dye exclusion test or the clonogenic method[4]. *Extraction and quantitation of DNA:* DNA extraction and gel electrophoresis was performed according to the reported methodology[3]. Apoptotic DNA was quantitated by the modified diphenylamine reaction[7].

Results and Discussion: The results show that all the nucleoside analog drugs like 6-MP, F-araA, or ara-C induce apoptosis in leukemic T-cell lines in a concentration (0.1-10 μM) and time-dependent manner. However, PEG-ASNase exhibited optimum apoptosis at 1 IU/ml. When two nucleoside analog drugs were combined in a sequence specific manner they exhibited synergism[4,5]. The combination of 6-MP followed by ara-C and PEG-ASNase against CEM/0 and CEM/ara-C/7A cell line was highly synergistic as seen by the Combination Index plot (FIG.1). The three drug combination exhibited 15.6-fold synergism over the 6-MP + ara-C combination regimen against CEM/0 cells. This increased drug synergism correlated well with the augmented apoptotic cell death (FIG.2). Similarly, the combination of F-araA followed by ara-C and Taxotere showed a 2-fold and a 9-fold synergism over the F-araA + ara-C combination regimen against CEM/0 and CEM/ara-C/7A cells, respectively[8]. The addition of PEG-ASNase at the end of the F-araA + ara-C regimen also enhanced apoptotic cell death as compared to the combination of two drugs against CEM/0 cells (FIG.3). Electrophoretic separation of DNA extracted from the same number of cells treated with F-araA + ara-C + Taxotere resulted in greater DNA laddering with dose escalation at the therapeutic range (1-10 μM), suggesting increased apoptotic cell death, hence more DNA fragmentation (FIG.4). Sub-therapeutic concentrations of the drugs did not induce this phenomenon. Increasing the dose of these three drugs by 10-fold did not exhibit an additional effect in CEM/0 cells. Optimum DNA fragmentation in CEM/ara-C/7A cells was observed after treatment with 10 μM F-araA + 10 μM ara-C + 1 μM taxotere for which DNA fragmentation was significantly greater for this drug combination than the one $\log_{10}$ lower concentrations. This cell line exhibits an example of cross-resistance to Taxotere which when

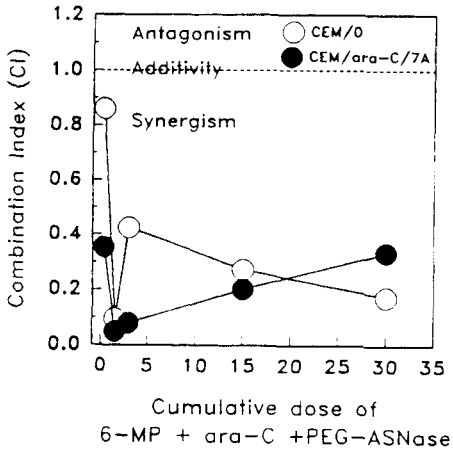

FIG.1. Synergistic effect of 6-MP + ara-C + PEG-ASNase against CEM/0 and CEM/ara-C/7A. All of the CI values were less than 1 indicating a strong drug synergism.

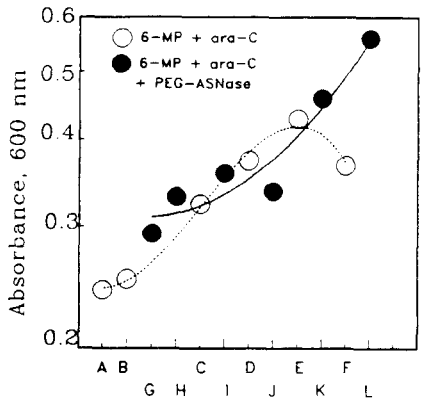

FIG.2. Quantitation of small MW fragmented DNA after sequential treatment of CEM/0 cells with 6-MP+ara-C (○) or 6-MP+ara-C+PEG-ASNase (●). A. 0.01 μM 6-MP+0.01 μM ara-C; B. 0.1 μM 6-MP+ 0.1 μM ara-C; C. 0.5 μM 6-MP+0.5 μM ara-C; D. 1 μM 6-MP+1 μM ara-C; E. 5 μM 6-MP+5 μM ara-C; F. 10 μM 6-MP+5 μM ara-C; G. 0.1 μM 6-MP+ 0.1 μM ara-C+0.1 IU/ml PEG-ASNase; H. 0.1 μM 6-MP +0.1 μM ara-C+0.5 IU/ml PEG-ASNase; I. 0.1 μM 6-MP+0.1 μM ara-C+1 IU/ml PEG-ASNase; J. 1 μM 6-MP+1 μM ara-C+1 IU/ml PEG-ASNase; K. 5 μM 6-MP+5 μM ara-C+1 IU/ml PEG-ASNase; L. 10 μM 6-MP+10 μM ara-C+1 IU/ml PEG-ASNase.

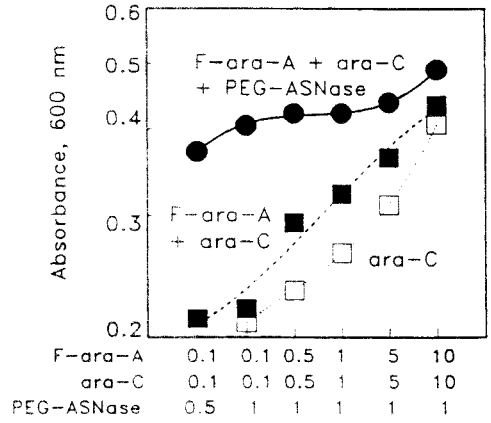

FIG.3. Quantitation of small MW fragmented DNA after sequential treatment of CEM/0 cells with ara-C alone (□), F-ara-A + ara-C (■), and F-ara-A + ara-C + PEG-ASNase (○). The addition of PEG-ASNase at the end of the F-araA + ara-C regimen enhanced apoptotic cell death as compared to the combination of two drugs.

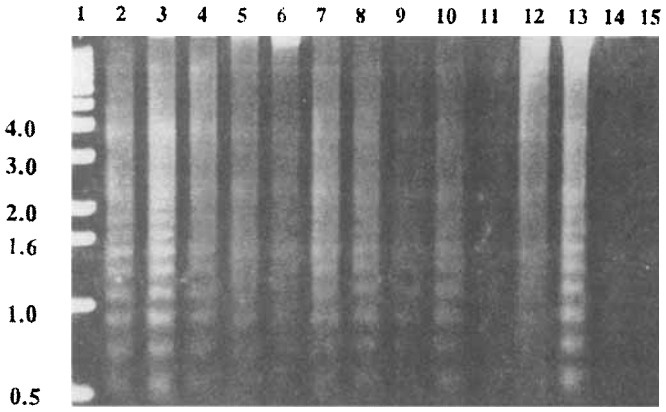

FIG. 4. DNA ladder after treatment of CEM/0 (lanes 2-8, 14) and CEM/ara-C/7A (lanes 9-13, 15) cells with F-araA, ara-C, Taxotere or combinations of these drugs. Lane 1. DNA MW markers; Lanes 2, 3, 4. 0.1, 10, 1 μM Taxotere, resp., 24 hrs; Lane 5. 0.01 μM FaraA + 0.001 μM ara-C + 0.01 μM Taxotere; Lane 6. 0.1 μM F-araA + 0.01 μM ara-C + 0.1 μM Taxotere; Lane 7. 1 μM F-araA + 0.1 μM ara-C + 1 μM Taxotere; Lane 8. 10 μM F-araA + 1 μM ara-C + 10 μM Taxotere; Lanes 9, 10, 11. 0.1, 10, 1 μM Taxotere, resp., 24 hrs; Lane 12. 0.1 μM F-araA + 0.01 μM ara-C + 0.1 μM Taxotere; Lane 13. 10 μM F-araA +1 μM ara-C + 10 μM Taxotere; Lanes 14, 15. CEM/0, CEM/ara-C/7A, control resp.

combined with F-araA + ara-C causes a case of collateral sensitivity. The combination of 6-MP + ara-C + PEG-ASNase against CEM/0 and CEM/ara-C/7A cell lines also showed a dose-dependent cellular apoptosis[9]. In conclusion, this study shows that the increased synergism of these three drug combinations over the combination of two nucleoside analog drugs is correlated with enhanced apoptotic cell death.

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