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PRELIMINARY COMMUNICATIONS

INITIAL STUDIES ON MAYTANSINE-INDUCED METAPHASE ARREST IN L1210 MURINE LEUKEMIA CELLS

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Maytansine is a novel ansa macrolide recently isolated by Kupchan and coworkers from Maytenus serrata and Maytenus buchananii (1,2). It is the first ansamycin to show significant antitumor activity, as measured by prolonged survival of mice bearing the P388 lymphocytic leukemia, the B16 melanocarcinoma, and the Lewis lung carcinoma (1,2). In our initial studies on the cytotoxic action of maytansine, we observed an increase in the mitotic index of exponentially growing cultures of L1210 cells after exposure to concentrations of maytansine as low as 10^{-8} M (3). In this report we provide an analysis of the stathmokinetic properties of maytansine.

Murine L1210 leukemia cells were grown in spinner culture (250 ml in 500 ml bottles) at a density of 10^5 cells/ml in RPMI 1630 media supplemented with 5% fetal calf serum, penicillin and streptomycin. Aliquots were removed at designated times for cell counts on a model B Coulter counter, histological examination and DNA content analysis by flow microfluorometry (FMF analysis) (4). For histological examination, cells were suspended in fetal calf serum, layered on duplicate microscope slides with a Shandon Elliott cytocentrifuge, fixed in methanol for 10 min, and then stained with Giemsa (azure B type) (Harleco; Arthur H. Thomas Co., Philadelphia, Pa.). Cells for FMF analysis were washed, fixed with formaldehyde, and stained with the fluorescent dye benzoflavin using a modified Feulgen procedure (5). The frequency distribution of fluorescence emission per cell (proportional

to DNA content) was measured for samples of 10^5 cells using a Cytofluorograph (Biophysics Systems, Inc., Baldwin Place, N. Y.) and analyzed with a multichannel analyzer (Nuclear Data Inc., Madison, Wis.). The results are expressed as a histogram representing the relative number of cells with a given DNA content. Maytansine (NSC 153858) was provided by Dr. Harry Wood, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute. It was dissolved in ethanol and its concentration was determined by uv spectroscopy (ε 27,200 at 254 nm) (1). Either ethanol solutions of maytansine diluted in water, or an equal volume of water, was added in volumes of 0.1 ml per 5 ml of cell culture. Cells were never exposed to ethanol concentrations in excess of 0.01%.

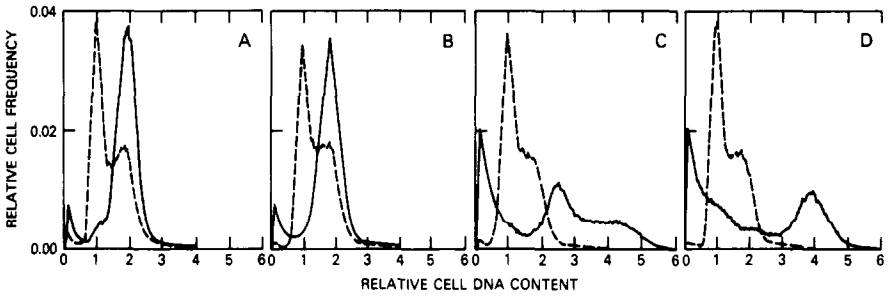

Fig. 1. Histograms showing DNA distributions in control (-----) and maytansine-treated (———) cell populations. Each panel shows FMF analysis after a different treatment time: A, after 6 hr; B, after 12 hr; C, after 24 hr; and D, after 36 hr.

Using FMF analysis, one can determine the phase of the cell cycle in which a cell resides on the basis of its cellular DNA content, for a G_2 cell has twice the DNA content of a G_1 cell. In control cultures of exponentially growing L1210 cells, as indicated by the dashed lines in each panel of Fig. 1, the large peak at 1 on the abscissa represents cells with a G_1 DNA content, while the smaller peak at 2 includes cells in G_2 and M (mitosis), both phases of which have double the DNA content of G_1 cells. Cells in S phase (DNA replication phase) are found in the interval between 1 and 2. Since cells in S phase have replicated variable amounts of DNA, late S phase cells are near the G_2 + M DNA peak, while early S phase cells are near the G_1 DNA peak. After 6 hr of exposure to 10^{-8} M maytansine, the proportion of cells with a G_1 content of DNA markedly declined, as indicated by the solid line in Fig. 1A, while the peak representing cells with a G_2 + M content correspondingly increased. By 12 hr virtually the entire treated population had shifted to a G_2 + M content of DNA (Fig. 1B). After 24 hr the proportion of dead cells increased, as indicated by the rise in the number of cells with a relative DNA content of less than 1. In addition, the population of cells shifted to include many cells with a DNA content greater than 2.

By 36 hr a new peak representing cells with a relative DNA content of 4 appeared (Fig. 1D). This new peak indicates that a second wave of DNA synthesis has occurred and that these cells have endoreduplicated, i.e. they have doubled their content of DNA but have failed to divide into two daughter cells.

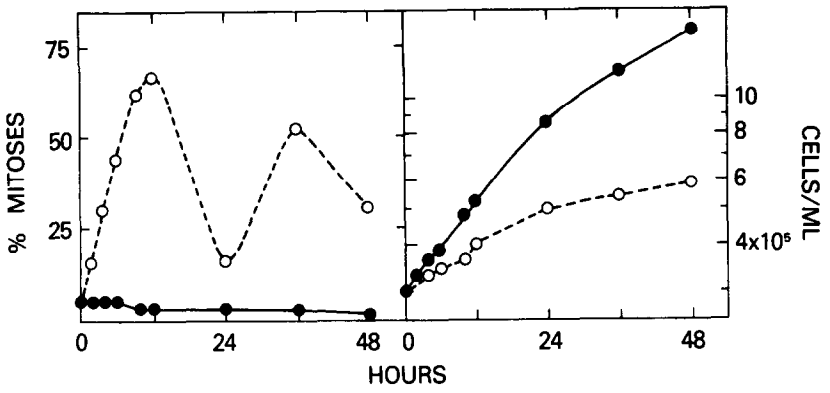

Fig. 2. Cell growth and mitotic index of L1210 cells. The mitotic index is expressed as a percentage. Each data point is the average mitotic index after counting 1000 control cells (●——●) or 1400 maytansine-treated cells (O- - -O) from 4 separate experiments. Each data point on the growth curve is the mean of 3 separate experiments.

Because FMF analysis cannot distinguish a G_2 cell from a mitotic cell since both contain identical amounts of DNA, cells were examined histologically after exposure to maytansine as shown in Fig. 2. Control L1210 cells had a generation time of 14 hr during exponential growth and a mitotic index which ranged between 3.2 ± 0.4% and 5.8 ± 0.8%. After 24 hr the cells entered stationary phase and the mitotic index decreased to as low as 0.8 ± 0.2% at 48 hr. By contrast, in the maytansine-treated cultures the mitotic index reached 66.8 ± 2.4% after 12 hr, the peak of the points examined. Although in control cultures all stages of mitoses could be found, normal anaphase and telophase patterns were missing after 2 hr of maytansine treatment. Cells exposed to maytansine showed complete disruption of chromosomal organization very similar to the metaphase arrest or "C" mitosis produced by colchicine (6) and the Vinca alkaloids (7,8). By 24 hr the number of cells in mitosis had dropped to 16.2 ± 1.1% as many cells resumed the interphase state. Thus, under these conditions maytansine did not permanently block all cells in metaphase. At 36 hr another peak of mitosis was observed; however, by this time many cells appeared heavily vacuolated and pyknotic. In addition, many polyploid cells were seen, which corroborates the finding in Fig. 1D of a peak at 4 which is not seen in control cells. By 48 hr further cell degeneration was apparent, for there was considerable cellular debris in the

slide preparations.

In summary, our results using FMF analysis show that within 12 hr of exposure to 10^{-8} M maytansine, the distribution of the DNA content in a population of exponentially growing L1210 cells shifts to a single peak which corresponds to cells with a G_2 + M DNA content. Histologic examination of these cells reveals that the majority are arrested in metaphase, and suggests that maytansine impairs the function of mitotic spindles (9). The mitotic inhibition may be related to the antitumor activity of maytansine, for Vinca alkaloids such as vincristine also cause metaphase arrest and are clinically useful anti-cancer agents. It is of interest in this connection that in our original report we observed cross-resistance between vincristine and maytansine in a study of the effect of both agents on the survival of mice bearing a vincristine-resistant P388 lymphocytic leukemia (3).

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REFERENCES

1. S.M. Kupchan, Y. Komoda, W.A. Court, G.J. Thomas, R.M. Smith, A. Karim, C.G. Gilmore, R.C. Haltiwanger and R.F. Bryan, J. Am. Chem. Soc. 94, 1354 (1972).

2. S.M. Kupchan, Y. Komoda, A.R. Branfman, R.G. Dailey, Jr. and V.A. Zimmerly, J. Am. Chem. Soc. 96, 3706 (1974).

3. M.K. Wolpert-DeFilippes, R.H. Adamson, R.L. Cysyk and D.G. Johns, Biochem. Pharmac. 24, 751 (1975).

4. R.A. Tobey, H.A. Crissman and P.M. Kraemer, J. Cell Biol. 54, 638 (1972).

5. P.V. Woolley, III, R.L. Dion and V.H. Bono, Jr., Cancer Res. 34, 1010 (1974).

6. E. Stubblefield, in Cytogenetics of Cells in Culture (Ed. R.J.C. Harris), Vol. III, p. 223. Academic Press, New York (1964).

7. G. Cardinali, G. Cardinali and M.A. Enein, Blood 21, 102 (1963).

8. J.H. Cutts, Cancer Res. 21, 168 (1960).

9. L. Wilson and J. Bryan, in Advances in Cell and Molecular Biology (Ed. E.J. DuPraw), Vol. III, p. 21. Academic Press, New York (1974).