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SHORT COMMUNICATIONS

Stathmokinetic and therapeutic effects of maytansine in mice bearing P388 and L1210 leukemias

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Maytansine (NSC 153858), an ansa macrolide isolated from *Maytenus serrata* by the late S. M. Kupchan *et al.* [1], is currently undergoing Phase I–Phase II clinical trials for the treatment of cancer [2, 3]. Its effects in a variety of systems resemble those of colchicine and the Vinca alkaloids, since maytansine was shown to arrest cultured mammalian cells in mitosis [4, 5], to bind to isolated brain tubulin [6, 7], and to block polymerization of tubulin into microtubules [7, 8].

The purpose of this study was to evaluate the ability of maytansine to produce mitotic arrest of tumor cells *in vivo* and to determine whether there was a relationship between extent and/or duration of strathmokinesis and the therapeutic efficacy of maytansine. The murine P388 and L1210 leukemias were utilized in this study because earlier studies [9, 10] had indicated that maytansine markedly prolonged the lifespan of mice bearing P388 leukemia, whereas little or no increase in lifespan was observed in mice bearing L1210 leukemia. A further consideration was the relative ease and accuracy in determination of a mitotic index in these ascitic leukemia cell lines.

The effects of maytansine on the survival of $B6D2F_1$ mice bearing either L1210 or P388 leukemia are shown in Table 1. Over a dosage range of 0.0648 to 0.300 mg/kg, administered on days 1, 5 and 9 after i.p. tumor inoculation, maytansine significantly increased the lifespan of mice with L1210 leukemia by 39 and 33 per cent at 0.180 and 0.300 mg/kg respectively. In previous therapeutic studies of maytansine against L1210 leukemia [9, 10], a daily treatment schedule was utilized and the drug was not found to be significantly active (increase in lifespan ≤25 per cent). Thus, maytansine does have activity against L1210 leukemia when administered intermittently, and its activity in this tumor system is similar to that obtained with the Vinca alkaloids. Using the same treatment regimen over a slightly lower dosage range (0.0540 to 0.250 mg/kg/injection), the lifespan of mice bearing P388 leukemia was increased by over 100 per cent at the three highest dose levels of maytansine. A dose of 0.300 mg/kg of maytansine administered i.p. q4D × 3 is approximately an LD_{10} for $B6D2F_1$ mice based on previous studies and unpublished screening data of the National Cancer Institute.

Table 1. Effects of maytansine on the survival of mice bearing ascitic L1210 and P388 leukemias*

Tumor system	Dose (mg/kg/day, i.p., days 1, 5 and 9)	MST (days ± S.E.)	ILS (%)	LTS
L1210	Untreated control	8.3 ± 0.5		1/16
	0.300	11.0 ± 0.5	33	0/8
	0.180	11.5 ± 0.8	39	0/8
	0.108	9.6 ± 0.6	16	0/8
	0.0648	9.4 ± 0.5	13	0/8
P388	Untreated control	13.2 ± 0.6		0/16
	0.250	29.1 ± 3.0	121	0/8
	0.150	27.6 ± 2.7	109	1/8
	0.090	26.6 ± 3.0	102	0/8
	0.054	23.9 ± 1.1	81	0/8

*Viable cells (10^6) were inoculated i.p. in groups of male $B6D2F_1$ mice (19–23 g) on day zero. MST = mean survival time (excluding survivors). ILS = increase in lifespan. LTS = long-term tumor-free survivors on day 60.

In order to determine the effects of maytansine on the mitotic index of both cell lines, large groups of $B6D2F_1$ mice were inoculated i.p. with either 10^5 L1210 cells or 10^6 P388 cells. Six days later maytansine was administered as a single i.p. dose at either 0.410 mg/kg, which is a single dose LD_{10}, or at a 10-fold lower dose of 0.041 mg/kg. Groups of three mice with each tumor and at each dose level were killed at the following intervals after maytansine administration: 0 (untreated control), 2, 4, 6, 8, 10, 12, 16, 18, 20, 24, 48, 72 and 96 hr. Ascites cells were aspirated and a smear was made with the cells from each mouse. The smears were fixed with methanol and stained with Giemsa stain. Mitotic counts were determined microscopically by randomly counting 1000 cells on each slide. The slides were counted blindly; that is, the person counting the slides was not apprised of which treatment group or time point the slides represented.

As shown in Fig. 1, maytansine was extremely effective in producing mitotic arrest of the leukemia cells *in vivo*. The peak in mitotic arrest occurred between 12 and 20 hr with 60–70 per cent of the cells in mitosis. The mitotic index of cell populations from untreated animals was 2–3 per cent. Profound mitotic arrest was evident for both L1210 leukemia (panel A) and P388 leukemia (panel B). The mitotic figures of treated cells were often bizarre, with chromatin clumped or scattered throughout the cells. Generally, a similar degree of mitotic arrest was achieved with the 10-fold different doses of maytansine. The duration of mitotic arrest was somewhat longer; that is, the peak of mitotic index was broader at the higher dose of the drug. At 24 hr and thereafter, when the mitotic index was returning to control levels, the proportion in both leukemic cell populations of giant, bizarre-shaped and multinucleated cells was greatly increased by maytansine treatment.

The results show that maytansine is considerably more effective in producing mitotic arrest of leukemia cells *in vivo* than either the Vinca alkaloids [11, 12] or oncodazole (methyl[5-(2-thienyl-carbonyl)-1H-benzimidazol-2-yl]carbamate, NSC 238159) [13], a relatively new synthetic antitumor agent, which also interferes with the structure and function of microtubules [14]. The peak mitotic arrest of L1210 leukemia after exposure to these agents *in vivo* occurs at 8–10 hr, and the duration of arrest is shorter than that produced by maytansine, with the mitotic index returning to

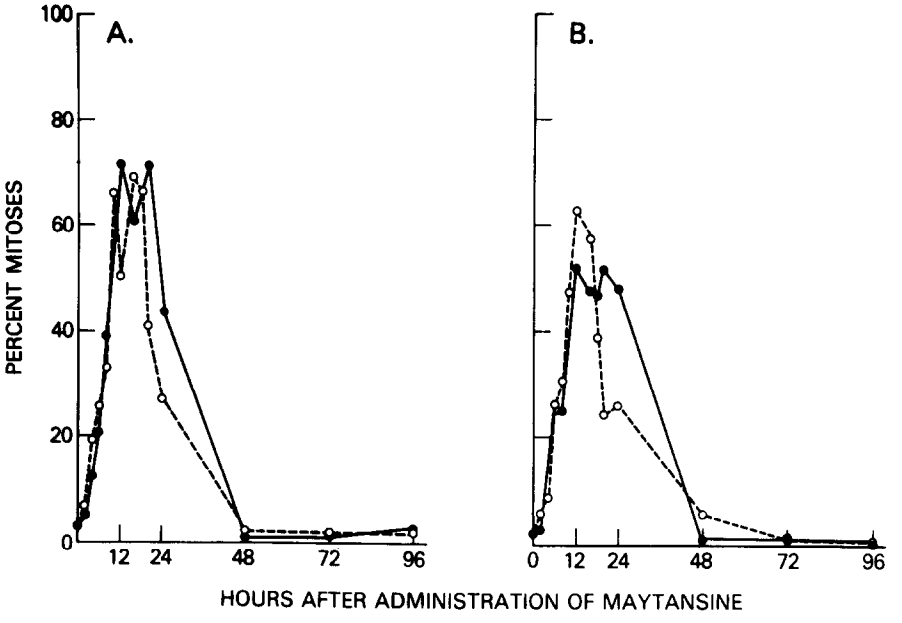

Fig. 1. Stathmokinetic effect of maytansine on L1210 (panel A) and P388 leukemia (panel B) *in vivo*. Maytansine was administered as a single treatment, i.p., on day 6 after tumor implantation at a dose equivalent to the LD_{10} for $B6D2F_1$ mice, 0.410 mg/kg (●——●), and at one-tenth of the LD_{10} dose, 0.0410 mg/kg (○- - -○). Points represent the mean values for three mice at each time point.

control levels by 12 hr. The Vinca alkaloids or oncodazole are capable of increasing the mitotic index of L1210 leukemia *in vivo* to about 30 per cent compared to 70 per cent after maytansine administration. These findings suggest that maytansine is more effective than the other mitotic spindle poisons in arresting the growth of cells. At least two factors could explain these results: greater retention of maytansine by leukemic cells or a longer plasma half-life. The latter would result in a prolonged maintenance of effective concentrations of the drug, thus enabling a greater proportion of the cells to traverse the cell cycle and be arrested in mitosis. Pharmacokinetic studies with labeled maytansine might provide an explanation for these observations. The longer duration of the stathmokinetic effect of maytansine might be put to use in current combination regimens that are based on synchronization of tumor cells with Vinca alkaloids, followed by a second cell-cycle-specific agent. After the demonstration that bleomycin was most cytotoxic to cultured cells in mitosis [15], a number of clinical protocols were generated in which bleomycin was administered 6 hr after either vincristine or vinblastine [16, 17]. Substituting maytansine for the Vinca alkaloids and lengthening the interval to bleomycin administration might result in improved therapeutic response in such combinations.

In this study, sensitivity of a tumor to maytansine did not correlate with mitotic arrest. This is not entirely surprising since it was shown [18] that vinblastine and vincristine had a similar stathmokinetic effect in human bone marrow, although vinblastine is much more myelosuppressive than vincristine. The relationship between antimitotic action and cytotoxicity has not been clearly defined. Without the use of time-lapse cinematography, it is difficult to ascertain the fate of individual cells after treatment, i.e. whether a particular cell died or recovered. Although maytansine may kill cells by more than one mechanism, it is known that prolonged mitotic arrest can result in eventual cell death [19]. It is also known from tissue culture studies that cells can recover from the effects of a mitotic arresting agent if the exposure time and concentration of the agent are limited [19, 20]. The increased incidence of bizarre multinucleated cells at later time points suggests that cells can escape maytansine-induced mitotic arrest and proceed to interphase. Whether these cytologically abnormal cells are capable of continued prolferation is uncertain.

In summary, maytansine produced profound mitotic arrest of L1210 and P388 leukemias *in vivo* over a broad dosage range. The extent and duration of stathmokinesis in these cell lines were greater than those shown in previous studies for other mitotic spindle poisons. The ability of maytansine to produce mitotic arrest was not related to its therapeutic activity in the two cell lines studied.

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Characterization of cell-surface alterations produced by NSC 208642 (Lymphosarcin)

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The poylene antibiotic Lymphosarcin, NSC 208642, has been found active* against a wide spectrum of experimental animal tumors, including the P388 lymphocytic leukemia, B16 melanoma, colon 38 and Danny Martin mammary carcinoma. The studies described here were designed to assess the effects of this agent on some cell-surface and membrane properties of the L1210 murine leukemia which, unlike P388, can readily be grown in culture. Properties of new antitumor agents are usually characterized with regard to inhibition of RNA, DNA and protein synthesis. We report here a different type of drug toxicity resulting from selective inhibition of certain membrane functions.

NSC 208642 was provided by the Drug Development Branch, National Cancer Institute; aqueous drug solutions were stored at −20°. Methods for maintenance of L1210 cells in culture, together with procedures for partitioning, transport and cell viability studies, have been described [1, 2]. Two-phase partitioning systems contained 5% (w/v) Dextran T500, lot 7839 (Pharmacia, Piscataway, NJ) and 3.6% polyethylene glycol (PEG), mol. wt 6000 (Pierce Chemical Co., Rockland, IL), as described by Walter [3]. A phosphate-rich mixture was made up in 70 mM NaCl + 60 mM NaH_2PO_4 at pH 7.0. A phosphate-poor mixture was made up in 140 mM NaCl + 10 mM NaH_2PO_4 at pH 7.0, and contained 1 μg/ml of PEG-palmitate (PEG-p) prepared as described in Ref. 4; 55–60 per cent of the total OH groups were esterified.

Suspensions of 7×10^6 cells/ml were treated with specified levels of drug for 10 min at 37°. To minimize drug–substrate interactions, the cells were suspended in fresh medium (5×10^6/ml) for further studies. Viability was determined, as described in Ref. 1, at 24- and 48-hr intervals after drug treatment; cell number was measured with a model Z_F Coulter Electronic Cell Counter. Accumulation of labeled actinomycin D and cycloleucine was measured over 5-min intervals at 37°; uridine accumulation was measured during a 1-min interval at 10° (to minimize subsequent incorporation of label into RNA). In one series of experiments, the non-metabolized nucleoside 5′-deoxyadenosine [5] was substituted for uridine, without altering any result. Partitioning studies were carried out in 10-ml portions (5 ml of each phase) containing 10^6 cells. After the phases had separated, the cell density in the upper phase was measured, and the partition coefficient was expressed as per cent of total cells found in the upper phase. When control cells were partitioned, 40 per cent were found in the upper phase of the phosphate-rich mixture and 10 per cent in the upper phase of the system containing PEG-p.

Transport data are reported in terms of per cent control rates; partitioning results are shown as per cent control values. In general, these data are reproducible to ± 10 per cent of indicated numbers.

A summary of the results is shown in Fig. 1, and is described below. Treatment of L1210 cells with NSC 208642 caused an initial increase in the number of cells partitioning into the upper phase of the phosphate-rich system (Ph), followed by a fall in partition coefficient at higher drug levels. In contrast, the number of cells partitioning into the upper phase of the low-phosphate mixture containing PEG-p increased with increasing drug concentration.

No inhibition of rate of uptake of cycloleucine or uridine was found at drug levels of 3 μg/ml or less; at higher drug concentrations, impaired uptake of both cycloleucine and uridine was not significantly different in this study. The rate of actinomycin D uptake was in-

*John Douros, National Cancer Institute, personal communication.