MAYTANSINE BINDING TO THE VINBLASTINE SITES OF TUBULIN

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Received 4 January 1977

1. Introduction

Tubulin possesses two distinct binding sites for vinblastine; one of high affinity ($K_a = 6.2 \times 10^6 M^{-1}$) occupancy of which prevents polymerization of tubulin and a second, lower affinity site ($K_a = 8 \times 10^4 M^{-1}$) occupancy of which correlates with the aggregating effects of the alkaloid on tubulin [1]. The recent report [2] that maytansine, a new antitumor agent of plant origin [3,4], was a potent competitive inhibitor of ^3H vincristine binding in rat brain homogenates, led us to investigate which of the two vinblastine-binding sites would be involved in this effect. The formulae of these two compounds are shown below (fig.1).

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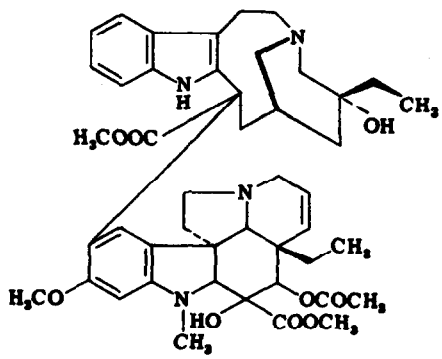
2. Materials and methods

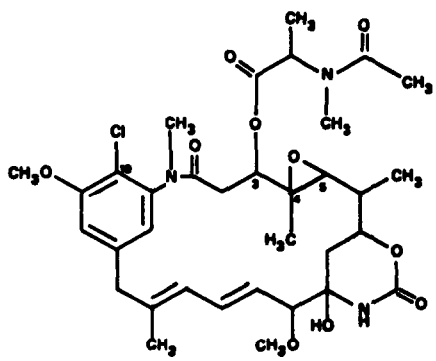
For polymerization experiments, tubulin was purified from rat brain extracts by three cycles of polymerization and depolymerization, according to the method of Shelanski et al. [5]. The kinetics of tubule assembly have been studied by turbidimetric measurements at 400 nm as described by Gaskin et al. [6] in a temperature-controlled chamber of a Cary spectrophotometer (model 14) at 37°C. The polymerization buffer contained 0.1 M 2-(*N*-morpholino-ethane–sulfonic acid) (Mes) buffer, pH 6.4, 1 mM ethylene glycol–bis(β-amino-ethyl ether)–*N,N'*-tetraacetic acid (EGTA), 1 mM GTP and 0.5 mM $MgCl_2$. Colchicine binding was determined by a modification of the DEAE-filter paper method [7].

2.1. *Vinblastine binding assay*

The DEAE-filter paper disc assay for colchicine had to be modified to make it suitable for vinblastine-



VINBLASTINE



MAYTANSINE

Fig.1.

binding. Two DE-81 paper discs (Whatman) were washed with cold PMG buffer (10 mM sodium phosphate, pH 6.8, 10 mM $MgCl_2$, 0.1 mM GTP solution) 4°C), by mild suction, taking care not to dry the paper. The sample (100 μl) was applied and was absorbed on to filters over a period of 1–2 min. The filters were then rinsed four times with 4 ml of cold (4°C) PMG buffer by mild suction. The radioactivity of the filter papers was determined in 10 ml of Hydromix (Yorktown). In all cases, controls were run in the absence of tubulin and this blank value was subtracted from the quantity of vinblastine bound in the presence of tubulin. Binding results of duplicate experiments agreed to within 10%. The concentration of protein was determined by the method of Lowry et al. [8] with crystalline bovine albumin as a standard.

GTP (Grade II-S) was obtained from Sigma. Tritiated colchicine (ring C, [^{3}H]methoxy) a product of New England Nuclear Corp., had a specific activity of 18.45 Ci/mmol. [^{3}H]Vinblastine was prepared as described and had a specific activity about 19 Ci/mmol and about 95% radiochemical purity [9] or was purchased from Amersham-Searle (13.5 Ci/mmol). Vinblastine was a gift of Eli Lilly Laboratories. Maytansine was generously supplied by Dr David G. Johns of the National Cancer Institute.

3. Results

Since vinblastine-binding can be indirectly measured by its effect on the state of aggregation of tubulin [1], we compared the effects of maytansine and vinblastine on the polymerization of rat brain tubulin. As shown in fig.2, both drugs prevented polymerization in the micromolar range. The concentration for 50% inhibition was 0.35×10^{-6} M for maytansine and 0.20×10^{-6} M for vinblastine. While the relative potencies were more different than expected [2], the effects were parallel throughout the concentration range.

In contrast to the similarity between the drug effects at low concentrations, when the concentration range for low affinity vinblastine-binding [1] was investigated, marked differences between these two drugs were observed (fig.3). As shown previously [1], vinblastine caused tubulin aggregation with a half-maximal concentration of 2×10^{-5} M (followed by precipitation at concentrations $> 1 \times 10^{-3}$ M). In marked contrast, maytansine was totally unable to promote tubulin polymerization up to concentrations $> 1 \times 10^{-3}$ M. Nevertheless, preincubation of tubulin preparations with 3×10^{-4} M maytansine prevented the subsequent effect of vinblastine at this site and suggested that vinblastine could bind to the

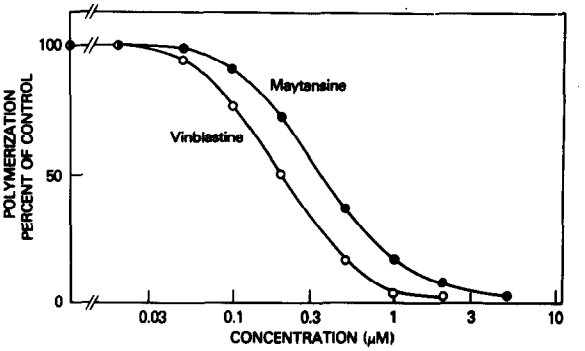


Fig.2. Effects of antimitotic drugs on tubulin polymerization. Aliquots of tubulin (2.1 mg/ml) in polymerization buffer were mixed with different concentrations of maytansine and vinblastine immediately before incubation at 37°C. The polymerization was monitored by optical density at 400 nm in a temperature-controlled recording spectrophotometer. Data are expressed as the percent of the maximal plateau level reached without drug.

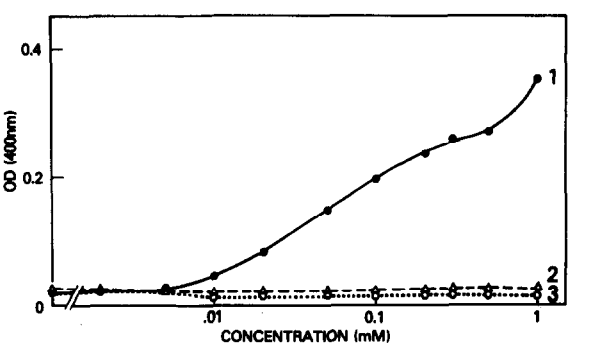
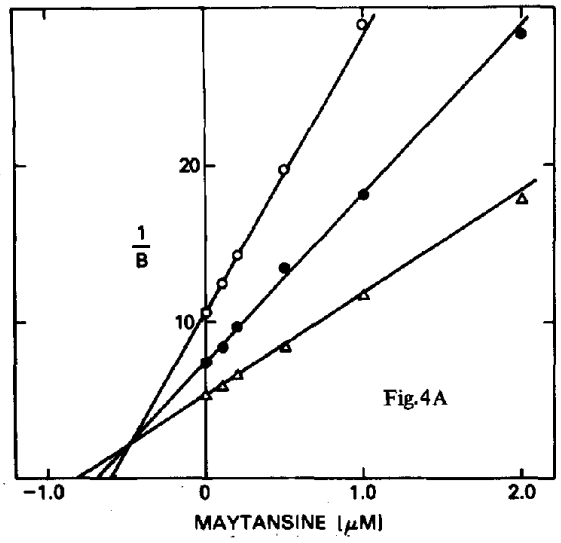


Fig.3. Effects of antimitotic drugs on tubulin aggregation. Tubulin (0.75 mg/ml) in polymerization buffer was titrated with increasing concentrations of drugs as follows: curve 1, vinblastine, curve 2, maytansine and curve 3, first preincubated with 3×10^{-4} M maytansine and then titrated with vinblastine. After each addition of drug, the solution was incubated for 15 min at 37°C, after which the optical density was measured at 400 nm.



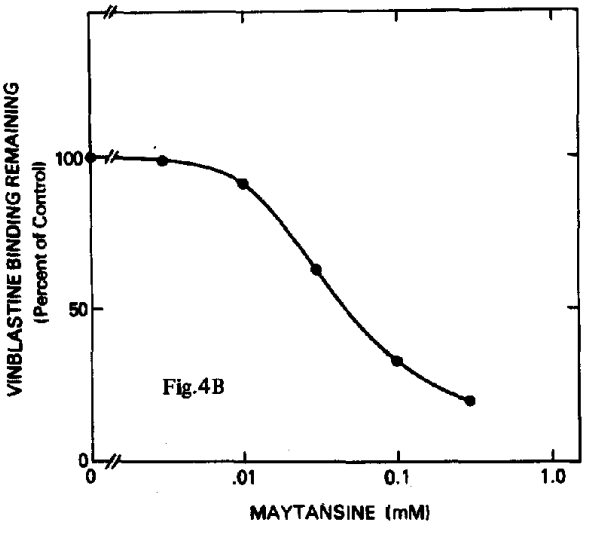


Fig.4.A. Left panel. Dixon plot demonstrating the effect of maytansine on the vinblastine-binding reaction. The concentration of maytansine present is plotted against the reciprocal of the moles of vinblastine bound per mole of tubulin, B, at several concentrations of vinblastine: (△) 1×10^{-6}, (●) 0.75×10^{-6}, (○) 0.5×10^{-6}. The reaction mixtures in PMG buffer, pH 6.8, were incubated at 37°C for 30 min and binding was assayed by the DEAE-filter disc method. Fig.4.B. Right panel. Inhibition of vinblastine-binding to the low affinity site of tubulin by maytansine. The aged tubulin used in this experiment was devoid of vinblastine-binding to the high affinity site of tubulin. The reaction mixtures contained 5×10^{-6} M tubulin and 2.5×10^{-5} M vinblastine in PMG buffer and were incubated at 37°C for 30 min.

low affinity site but was not able to promote aggregation.

Binding of maytansine to the two vinblastine-binding sites was measured directly as the blocking of [^{3}H]vinblastine by the macrolide. As shown by the Dixon plot in fig.4, the high affinity site is blocked competitively by maytansine yielding an apparent $K_i \sim 0.5 \times 10^{-6}$ M. Similarly, the low affinity site could be blocked competitively by maytansine. Since, however, displacement from this second site can be confused by displacement from the high affinity site, we chose another method to investigate the low affinity vinblastine site.

Tubulin can be aged at 4°C until the high affinity vinblastine-binding site is entirely lost with little damage in binding to the low affinity site [1]. Such tubulin preparations were used in fig.4B. The results again show that maytansine is a competitive inhibitor of [^{3}H]vinblastine-binding (apparent $K_i = 4.4 \times 10^{-5}$ M). Clearly, therefore, despite rather different properties of the two vinblastine-binding sites, both bind maytansine competitively.

Vinblastine is well known for its ability to protect

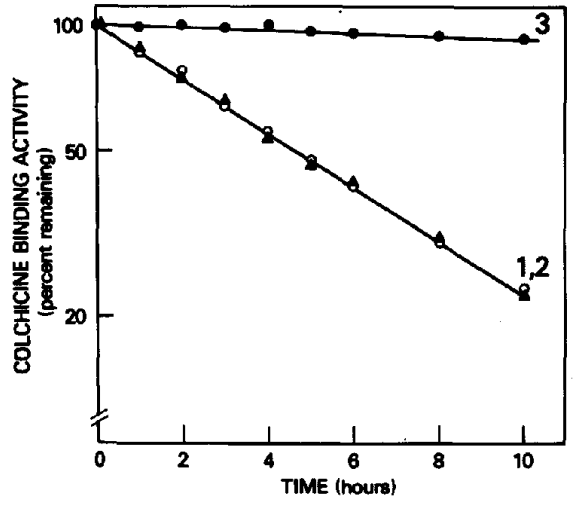


Fig.5. Effect of preincubation at 37°C of rat brain tubulin on colchicine binding. Tubulin was incubated at 37°C for the indicated period. The conditions of preincubation are: curve 1 tubulin in PMG buffer (○). Curve 2, tubulin in PMG buffer containing 3×10^{-4} M maytansine (▲). Curve 3, tubulin in PMG buffer containing 3×10^{-4} M vinblastine (●). Samples were preincubated at 37°C and the colchicine-binding activity was measured for 1 h at 37°C.

the colchicine binding site of tubulin [10] although the nature of this protective effect is not yet understood. Maytansine (0.3 mM) was not, however, able to elicit a similar stabilization of the colchicine-binding site (fig.5).

Maytansine is a potent antileukemic ansa macrolide isolated from *Maytemus buchananii* and *Maytemus serrata* [3,4]. Preliminary studies had shown an increased mitotic index and a DNA content characteristic of the G_2 + M phases of the cell cycle [11,12]. Furthermore, low concentrations of maytansine completely inhibited cleavage of sea urchin eggs [13]. Since there was cross resistance between vincristine and maytansine in certain cell lines [11], the effect of the macrolide on vincristine-binding to tubulin was investigated [2]. In the present study we confirm the conclusion that maytansine is a competitive inhibitor of binding for the vinca alkaloids. This effect is exerted at both the high and low affinity binding sites and occurs with a potency (K_i) in the range of vinblastine [1]. Interaction with the high affinity site is accompanied by depolymerization of microtubules, as also shown by Remillard et al. [13]. However, interaction at low affinity site, while easily measured by binding studies, is not accompanied by aggregation and prevents the aggregating effect of vinblastine. Moreover, unlike vinblastine, maytansine does not protect the independent colchicine-binding site. Whether this implies that the aggregating effect of the vinca alkaloids is the result of their dimeric nature remains to be determined.

Acknowledgements

We should like to thank Drs M. K. Wolpert-DeFilippes and D. G. Johns for their advice and criticism.

References

[1] Bhattacharyya, B. and Wolff, J. (1976) Proc. Natl. Acad. Sci. USA 73, 2375–2378.
[2] Mandelbaum-Shavit, F., Wolpert-DeFilippes, M. K. and Johns, D. G. (1976) Biochem. Biophys. Res. Commun. 72, 47–54.
[3] Kupchan, S. M., Komoda, Y., Court, G. J., Thomas, G. J., Smith, R. M., Karim, A., Gilmore, C. J., Haltiwanger, R. C. and Bryan, R. F. (1972) J. Amer. Chem. Soc. 94, 1354–1356.
[4] Kupchan, S. M., Komoda, Y., Branfman, A. R., Bailey, R. G., Jr. and Zimmerly, V. A. (1974) J. Amer. Chem. Soc. 96, 3706–3708.
[5] Shelanski, M. L., Gaskin, F. and Cantor, C. R. (1973) Proc. Natl. Acad. Sci. USA 70, 765–769.
[6] Gaskin, F., Cantor, C. R. and Shelanski, M. L. (1974) J. Mol. Biol. 89, 737–758.
[7] Weisenberg, R. C., Borisy, G. G. and Taylor, S. W. (1968) Biochemistry 7, 4466–4478.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Bhattacharyya, B. and Wolff, J. (1975) J. Biol. Chem. 250, 7639–7646.
[10] Wilson, L. (1970) Biochemistry 9, 4999–5007.
[11] Wolpert-DeFilippes, M. K., Adamson, R. H., Cysyk, R. L. and Johns, D. G. (1975) Biochem. Pharmacol. 24, 751–754.
[12] Wolpert-DeFilippes, M. K., Bono, V. H., Dion, R. L. and Johns, D. G. (1975) Biochem. Pharmacol. 24, 1735–1738.
[13] Remillard, S., Rebhun, L. I., Howie, G. A. and Kupchan, S. M. (1975) Science 189, 1002–1005.