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Mechanism of mitotic block and inhibition of cell proliferation by alkaloids extraction from *Convolvulus Scammonia* at low concentrations

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ميكانيكية وقف الانقسام وتثبيط تكاثر الخلايا السرطانية CHO باستخدام القلويد المستخلص من نبات *Convolvulus Scammonia* بتراكيز واطئة

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كلمات مفتاحية : النبيبات الدقيقة : خطوط الخلايا: المجهر المتالق

المستخلص

صمم هذا البحث لدراسة تأثير المستخلص القلويدي الخام لنبات السقومانيا على النبيبات الدقيقة لخلايا الهامستر الصيني المعلمة GFP tubulin الطبيعية لدراسةالتغيرات المحتملة عليها وذلك لأهميتها في الانقسام الخلوي للخلايا السرطانية اظهرت نتائج المعاملة الاولى بالمستخلص الخام لنبات السقومونيا قدرة المستخلص على وقف الانقسام الخلوي في الطور الاستوائي بالتركيز 8 nM وبالتالي التاثير على على النبيبات الدقيقة تثبيط الانقسام اقترن مع التغير في شكل النبيبات الدقيقة مشابه الى المركب الفانبلاستين وغيرها من المركبات المضادة للانقسام واعتمد اظهار التأثير على النبيبات الدقيقة للخلايا السرطانية على استخدام مجهر Immunofluroresence.لا زيادة في كمية النبيبات عند التركيز10 نانو مول من القلويد هذه النتيجة مشابها للفانبلاستين وان المعاملة بالتركيز الواطئ يوقف الانقسام من خلال وقف او تثبيت النبيبات ولا تغيير في شكلها ولا كميتها.

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ABSTRACT

Alkaloids extraction from *Convolvulus Scammonia* inhibited CHO cell line (**china hamster**) proliferation by inducing a sustained mitotic block at the metaphase/ anaphase boundary. Half-maximal inhibition of cell proliferation occurred at 8 nM alkaloids, and mitosis was half-maximally block 8 nM alkaloids.

Inhibition of mitosis was associated with formation of an incomplete metaphase plate of chromosomes and an altered arrangement of spindle microtubules that strongly resembled the abnormal organization that occurs with low concentrations of vinblastine and other antimitotic compounds. No increase in microtubule polymer mass occurred below 10 nM alkaloids. The results indicate that alkaloids shares a common antiproliferative mechanism with vinblastine. At its lowest effective concentrations, alkaloids appears to block mitosis by kinetically stabilizing spindle microtubules and not by changing the mass of polymerized microtubule

Keyword: microtubules : cell line: Immunofluroresence

Introduction

The antimitotic antitumor drug Taxol and Vanblastin has undergone extensive clinical development as a result of its efficacy in the treatment of refractory ovarian cancer and its potential value for the treatment of breast, lung, and other cancers (1).

The mechanism of action of Taxol has been considered to be unique. The target for taxol appears to be microtubules, and in contrast to Vinblastine, Colchicine, and compounds that can inhibit microtubule polymerization both in vitro and in cells, taxol can enhance microtubule polymerization (2, 3). Taxol can block mitosis, induce extensive formation of microtubule bundles in cells, and induce multinucleation of cells during interphase (4-6). Enhanced microtubule polymerization has been suggested to be responsible for the antitumor activity of the drug .

Vinblastine and Vincristine inhibit mitosis and cell proliferation in HeLa cells without decreasing the mass of microtubules and with only subtle changes in the organization of the mitotic spindles.(7-9) also found that low concentrations of vinblastine inhibit dynamic instability and treadmilling of reassembled bovine brain microtubules in vitro without appreciably affecting the microtubule polymer mass (10, 11).

In the present study, we found that low concentrations of Alkaloids inhibited mitosis in CHO cells line inhibition of mitosis was associated with an altered organization of mitotic spindles that was strikingly similar to that induced by the vinca alkaloids and several other antimitotic drugs, suggesting that mitotic block and inhibition.

proliferation by all of these drugs, including Alkaloids from *Convolvulus Scammonia* , at their lowest effective concentrations, involves kinetic stabilization of mitotic spindle microtubule dynamics rather than alterations in microtubule polymer mass .

MATERIALS AND METHODS

CHO cells line (from the Department of Biology, Faculty of Medicine, wuhan University - china) were grown in monolayers at 37°C without antibiotics in 5%C02/95% air (7). Cell proliferation was determined by countingcells by hemocytometer at the time of alkaloids addition and20 h later. Mitotic index, cell morphology, and spindle interpolar distances were determined by immunofluorescence microscopy (8). Levels of polymerized tubulin in cells were determined by measuring the tubulin content of isolated stabilized cytoskeletons by an ELISA (two to six determinations per alkaloids concentration) (13).

RESULTS

Relationship of Inhibition of Cell Proliferation, Mitotic Block, and Enhancement of Microtubule Polymer Levels by alkaloids.CHO cells were incubated for the duration of one cell cycle with alkalois over a broad range of concentrations. After 20 h, cytoskeletons were isolated to determine the mass of tubulin in the form of microtubules. In parallel experiments, inhibition of proliferation and mitotic indices were determined. Alkaloids inhibited cell proliferation half-maximally at a concentration of 8 nM, and inhibition was complete at concentrations 33 nM (Fig. 1A). alkaloids induced the accumulation of cells in mitotic metaphase half-maximally at a concentration of -8 nM, and maximal mitotic accumulation (80-95%) occurred at alkaloid concentrations of 33 nM and above. Thus mitotic accumulation occurred in parallel with inhibition of proliferation. No increase in microtubule polymer mass occurred at 10 nM alkaloids. The mass of microtubules then increased as the alkaloids concentration was raised, attaining a half-maximal increase at a concentration of 80 nM and a maximal increase of 500% of the normal level at 33 nM alkaloid. Effects of alkaloids on Spindle Organization. The arrangements of microtubules, chromosomes, and centrosomes of control cells and of cells incubated with alkaloids are shown in Fig. 2.Mitotic spindles of cells blocked in metaphase by low concentrations of alkaloids strongly resembled spindles of cells blocked by low concentrations of other antimitotic drugs including vinblastine, vincristine, colchicine, and podophyllotoxin (7, 8). Other spindles (20-32% with 0.33-10 nM alkaloids) were blocked in a nearly normal configuration; these spindles were bipolar with a compact metaphase plate of chromosomes but with some chromosomes located near the spindle poles (Fig.2 d-J) (Table 1) .

With increasing concentrations of alkaloids, spindle morphology became more abnormal; increasing numbers of chromosomes were located near the poles of bipolar spindles rather than in the metaphase plate. Also as the alkaloids concentration was increased, many spindles had no bipolar organization but were ball-shaped aggregations of condensed chromosomes containing one or more asters of microtubules (Fig. 2 g-i). These resembled classical olchicine mitoses or type III aberrant spindles (8).The morphological changes in spindle structure induced by alkaloids were nearly identical to those that occurred with other antimitotic drugs (7, 8). However, there were some minor differences. Some bipolar spindles blocked by low concentrations of alkaloids appeared to have reduced numbers of interpolar microtubules in contrast with the bipolar spindles induced by low concentrations of other antimitotic drugs that appeared to have normal numbers of interpolar microtubules. This observation with alkaloids is consistent with the finding that addition of 10-20 M alkaloid to

PtKl cells in early anaphase caused the disappearance of most interzonal microtubules within 5 min (15). In addition, in the present work upon incubation with 10-100 nM alkaloid, some mitotic asters contained no centrosomes (data not shown). Similar results were obtained in PtK2 cells after incubation with micromolar concentrations of alkaloids (16, 17). Induction of Microtubule Bundling. Microtubule bundlesdid not form in the alkaloid concentration range in which little
or no increase in microtubule mass occurred (1-10 nM). However, with 10 nM alkaloids, microtubules often became oriented in parallel fashion. (Compare the meshwork of microtubules of a control cell in interphase in Fig. 3a with the array of parallel microtubules radiating out from the nucleus after incubation with 10 nM alkaloids in Fig. 3b.) Loosely packed bundles of microtubules were observed in a few cells incubated with 33 nM alkaloids (Fig. 3c), a concentration at which the microtubule polymer mass was double that of control cells. Massive bundles of microtubules formed at higher alkaloids concentrations (e.g., 1 ,M, Fig.3d).

DISCUSSION

Very low concentrations of alkaloids are sufficient to inhibit proliferation of CHO cells. Both half-maximal inhibition ofproliferation and 50% blockage in mitotic metaphase occurred at 8 nM taxol. The degree of metaphase block by alkaloids paralleled the degree of inhibition of cell proliferation at all alkaloids concentrations. Inhibition was associated with formation of an incomplete metaphase plate of chromosomes and an arrangement of spindle microtubules that strongly resembled the abnormal organization that occurs with low concentrations of vinblastine and other antimitotic drugs (8). The most sensitive inhibitory effects of alkaloids on proliferation were not associated with an increase in microtubule polymer mass or with the formation of microtubule bundles, actions of alkaloids that occur at relatively high drug concentrations. No increase in microtubule polymer mass occurred below a alkaloids concentration of 10 nM (Fig. 1A), and 80 nM taxol was required to induce a half-maximal increase in the microtubule polymer mass. Thus these results indicate that the most sensitive action of alkaloids on CHO cell proliferation involves blockage of cell cycle progression at the metaphase/anaphase transition in the presence of a normal mass of microtubule polymer.

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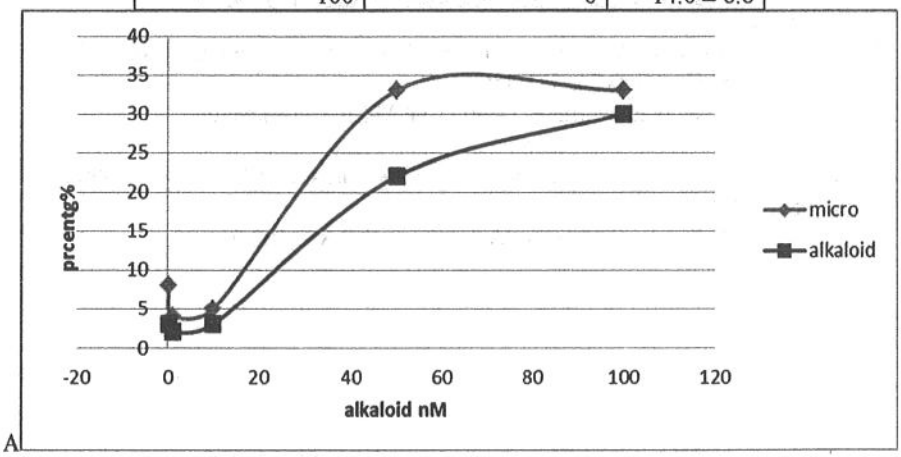
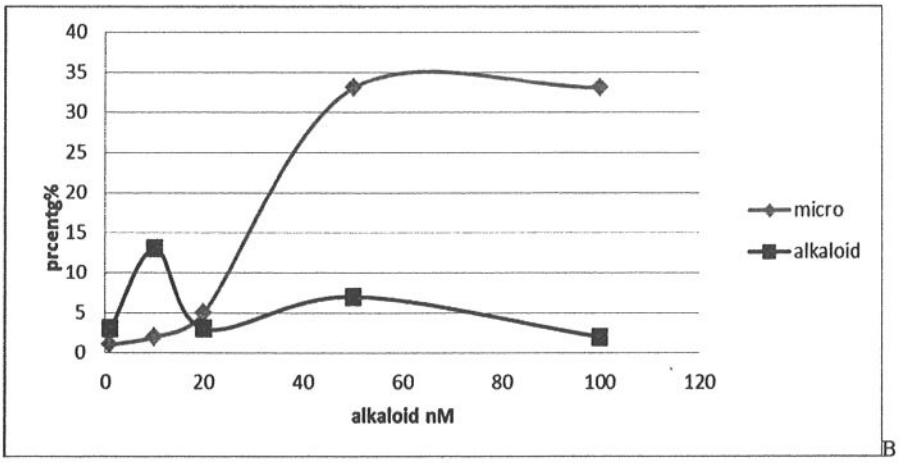
Table 1. Effects of alkalois (20-h incubation) on metaphase/anaphase transition, and spindle.cells in metaphase

Alkaloid con. nM	Cells in anaphase/ , cells in metaphase,	Multinucleated interphase cells, %
0	0.14 ± 0.03	2.7 ± 0.7
0.3	0.09 ± 0.04	3.2 ± 1.2
1	0.11 ± 0.05	11.6 ± 4.0
3	0.04 ± 0.04	31.2 ± 17.9
6	0.007 ± 0.004	32.6 ± 2.6
10	0	37.7 ±11.5
33	0	16.5± 4.0
100	0	14.0 ± 6.8



A



B

FIG. 1. (A) alkaloids concentration dependence for metaphase arrest , for inhibition of proliferation (and for the increase in microtubule mass. (B) alkaloids concentration dependence of spindle organization in CHO cells after incubation with alkaloids for one cell cycle. (A) Accumulation of cells in metaphase was concomitant with inhibition of proliferation but accompanied by little or no increase in the mass of microtubule. (B)Percentages of metaphases that were normal abnormal bipolar Arrows denote control values for normal.

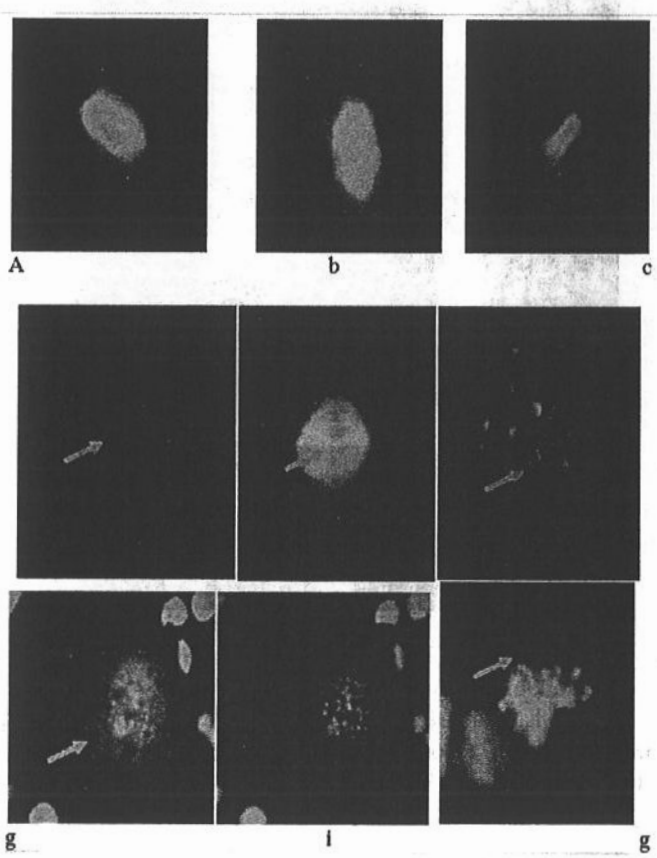


FIG. 2. Microtubules (a, d,and g), chromosomes (b, e, andh), and centrosomes (c,f, and i) ofCHO cell mitotic spindles after incubation for 18-20 h with alkaloid.(a-c) Control cell spindle with few astral microtubules and a welldefined compact metaphase plate of chromosomes. (d-f) At 6 nM alkaloids, an abnormal bipolar spindle(type I) with prominent astral microtubules (arrow in d) and chromosomes near the spindle poles (arrows in e). (g-i) At 1 ,uM alkaloids, a ball-shaped chromosomal mass with a monopolar microtubule and centrosome arrangement

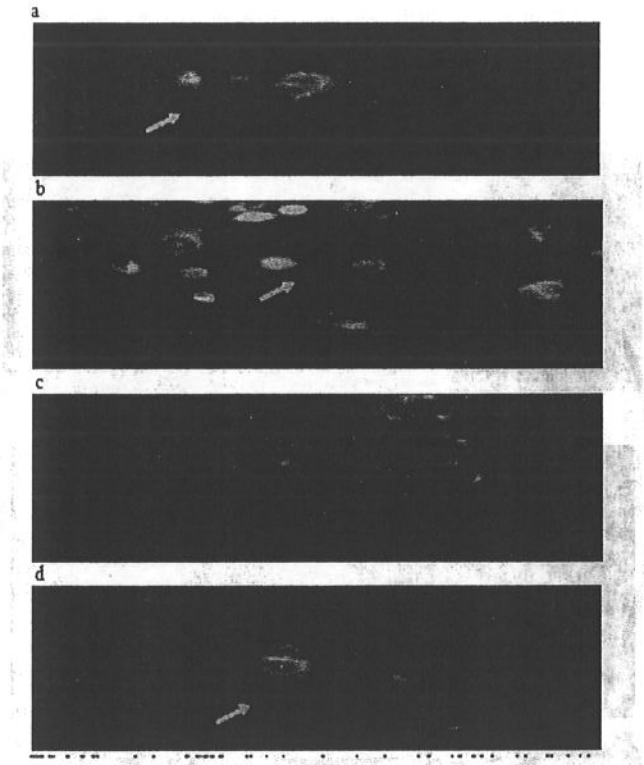


FIG. 3. Microtubules of CHO cells in interphase incubated for 18-20 h with alkaloids. (a) Control irregular meshwork of microtubules. (b) At
10 nM alkaloids, a somewhat parallel alignment of microtubules but absence of microtubule bundles. (c) At 33 nM alkaloids, a loosely packed bundle
of microtubules. (d) At 1 ,uM alkaloids, three compact bundles