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Effect of vincristine and vinblastine from *Vinca rosa* on microtubulues of tumor H_{22} cell line (hepatic cell line)

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Abstract: Vinca alkaloids (Vincristine and Vinblastine) are microtubular toxins of chemically similar nature that disrupt microtubule function by binding to a site on β-tubulin and suppressing microtubule dynamics. The study aims to evaluate biological activity of Vincristine and Vinblastine on microtubule H_{22} cell line using GF tubulin. Stable tumor cell line of H_{22} cell was used to investigate the action of vincristine and vinblastine on the microtubule network**.** An experimental work was conducted to determine the biological activity of vincristine and vinblastine on microtubule H_{22} cell line by used GF tubulin. Cells were treated with Vincristine and Vinblastine at various concentrations from 20 µg/ml to 400 µg/ml for 60 min.. Microtubules were detected by means of indirect immunofluorescence. No differences were found between the two cytostatics. As a conclusion, the cells showed changes in the arrangement of microtubules even at the 80 µg/ml concentration of cytostatics after 60-min exposition. Its damage increased with increasing concentration of cytostatics.

Keywords: Microtubule disruption, Cytoskeleton recovery, Vincristine, Vinblastine

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Introduction:

The cytoskeleton plays a specific role in cell division, maintenance and changes of cell shape, in intracellular contacts, interaction with membranes, extracellular matrix, and in cell motions(1).

The microtubular diameter measures about 25 nm. Microtubules are composed of 13 equally spaced protofilaments (2).Tubulin is the basic protein of the microtubules and molecules of tubulin are arranged in dimers containing both its forms: α-tubulin and β-tubulin. Microtubules are continuously changeable structures MTs(3) and polymerisation and depolymerisation of MTs is regulated by extracellular and intracellular factors(4). The presence of Guanine triphosphate (GTP)at microtubule ends is necessary to maintain the stability of the polymer (5).The cytoskeleton can be damaged through the effect of many external factors or chemical agents(5–11).

The opposite ends of free microtubules show different sensitivities to microtubule depolymerising agents such as low

temperature, Calcium or colchicines(12). The mitotic spindle is a self-organising structure that is constructed primarily from microtubules. Among the most important spindle microtubules are those that bind to kinetochores and form the fibres along with chromosomes movement.

Vinca alkaloids vincristine and vinblastine are microtubular toxins of chemically similar nature (13) that disrupt microtubule function by binding to a site on β-tubulin and suppressing microtubule dynamics. Although they are closely related in physical and chemical properties, they have various effects on the human body.

Vinca alkaloids perturb the kinetochore-microtubule attachment. This activates a checkpoint pathway that ensures proper attachment of chromosomes to the mitotic spindle (14–16) . When microtubules fail to attach to one or more kinetochores as a result of drug treatment, the checkpoint components continue to generate signals that inhibit the metaphase/anaphase transition that delays cell cycle progression and induces programmed cell death (17) .

At higher drug concentrations, vinca alkaloids induce the assembly of spiral filaments of tubulin, which, consequently, can interact laterally and form paracrystals (18).

This action is similar to the action of colchicine, but is different from that of paclitaxel, which promotes the polymerization of tubulin polymers to form abnormal stable microtubule structures (19).Vincristine and vinblastine have been widely used to treat cancer (e.g. acute leukaemia, Hodgkin's disease) (13).

Materials and Methods:

Cell line:

In this work the stable cell line of H_{22} cell which was used obtained from the Department of Biology, Faculty of Medicine, Huazhong University in Wuhan - China. The cell line were grown on uncoated coverslips in a Dulbecco's Minimal Essential Medium (DMEM) with 10 fetal bovine serum (PAA), 2 μM glutamine (PAA), 100 μ/ml penicillin, and 100 μg/ml streptomycin (China) (20).

Exposure to Vincristine and Vinblastine:

The first series of experiments, to investigate the action of Vincristine and Vinblastine onto the microtubule network, a solution containing 1 mg of Vincristini sulfas in 1 ml of the medium or an original solution containing 5 mg of vinblastini sulfate in 5 ml of medium was mixed with 3 ml of growth medium in each of the Petri dishes so that the final concentration of Vincristine, or vinblastine was 20, 40, 80, 100, 200 or 400 μg/ml, the second series of experiment the cell cultivated with extraction 1000 μg/ml and 2000 μg/ml (vincristine only). Each concentration was in two dishes. The second seris The cells were exposed to the drugs for 60 min at 37 °C. After the treatment, the samples were washed three times for 4 minutes concurrently with control samples in phosphate-buffered saline (PBS, pH 6.9) and processed for immunofluorescence microscopy in China.

In third experiments the cells were cultivated for, 6, 8, 10,hrs and 12 hrs with a final Vincristine concentration of 20 μg/ml or for 10 minutes in a medium

containing vincristine at a concentration of 400 μg/ml. The samples were washed three times for 4 minutes concurrently with control samples in PBS.

Statistical Analysis:

The differences are compared by using Data acquired with Duncun (21).

Results:

Results of Table-1 showed a considerable changes in the distribution of microtubules at high concentration in 60 min and Untreated control cells. Cells exposed to drugs at concentrations of 20 µg/ml and 40 µg/ml for 60 min did not show considerable changes in the distribution of microtubules but when cells exposed to drugs at concentrations of 80 µg/ml to 400 µg/ml for 60 min there were significant differences in all other groups. The quantity of microtubules in the cytoplasm was significantly high ($P<0.05$).

Table (1): Effect Vincristine at various concentrations of H_{22} cell line in mouse considerable changes in the distribution of microtubules.

concentration ml\gµ	Vanblastin		Vincristin	
	%	changes microtubules	%	changes microtubules.
control		0.00±4.20 a		0.00±4.20 a
20	13.33	0.56±0.56 f	7.14	0.11±0.30 f
40	30.09	0.11±1.60 e	26.19	0.20±1.10 e
80*	55.47	0.08±2.33 d	36.42	0.17±2.21 d
100*	79.28	0.08±3.33 c	50.00	0.11±2.53 c
*200	83.33	0.40±3.50 c	57.82	0.17±2.43 c
*400	91.19	0.03±3.83 abc	69.04	0.051±2.90 b

Different letters means the presence of significant different at ($P<0.05$).

Untreated control cells (Fig.-1) showed a microtubule network regularly distributed along the whole cell content. When cells exposed to drugs at concentrations of 20 µg/ml for 60 min did not show considerable changes in the distribution of microtubules.

Cells exposed to Vincristine or vinblastine at concentrations of 80,100, 200and 400 µg/ml for 60 min showed changes in the arrangement of the microtubular network.(Figs.-2, 3).

The network of cytoplasmatic microtubules at concentrations of 10, 80 µg/ml was thinned down, and individual fibres showed a wavelike shape. The network damage increased with increasing concentration of cytostatics. The microtubules were more thinned down and fragmentation of fibres occurred. At a higher concentration of 400 µg/ml, sometimes blebs were formed (Fig.-4). Cells exposed to Vincristine at concentrations of 1000 µg/ml and 2000 µg/ml formed paracrystals (Fig. 5), No significant difference was detected between vincristine and vinblastine treated cells.

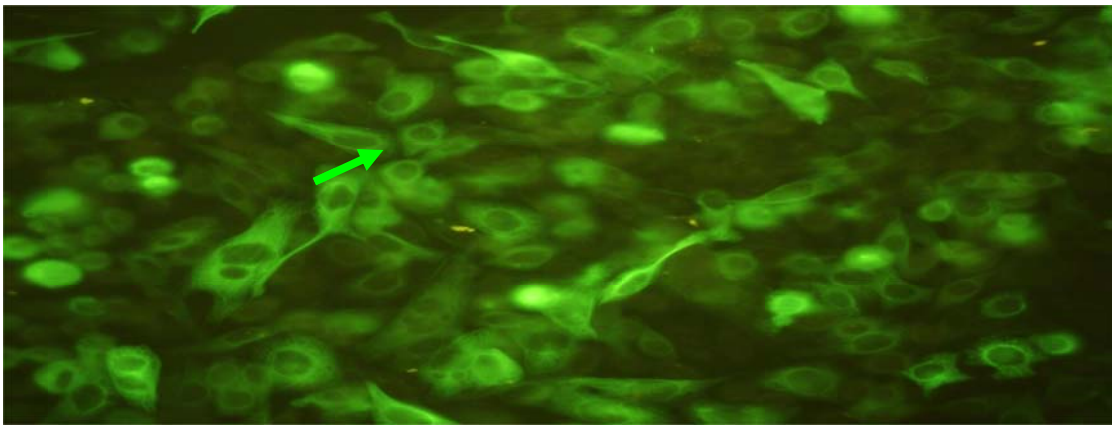

Figure (1): Microtubules of untreated control cells of H_{22} cell line. The network is regularly distributed along the whole cell content.

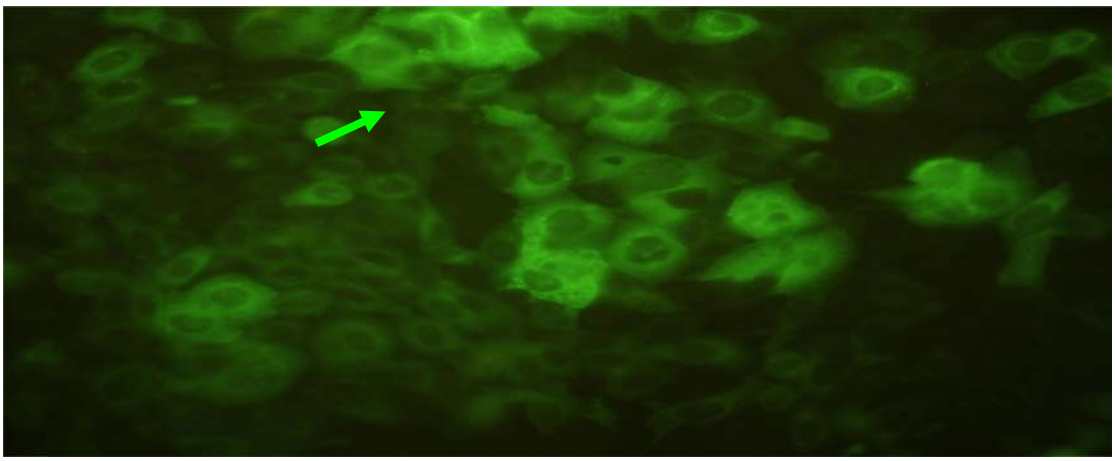

Figure (2): Microtubules of H_{22} cell line treated with vinblastine at a concentration of 80 µ/ml for 60 min. The network of cytoplasmatic microtubules is thinned down, and individual fibres have a wavelike shape.

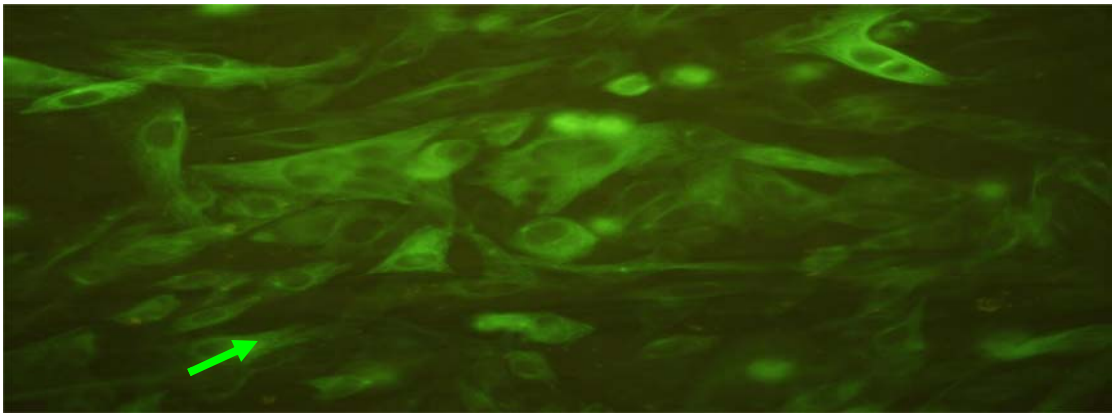

Figure (3): Microtubules of H_{22} cell line treated with Vincristine at a concentration of 400 µ/ml for 60 min. The network of cytoplasmatic microtubules is thinned down, and individual fibres have a wavelike shape.

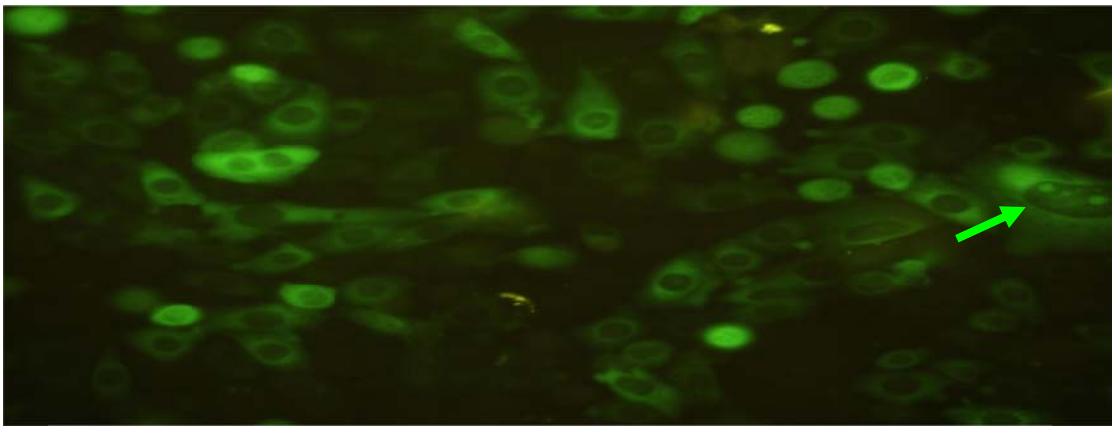

Figure (4): Cells of H_{22} cell line treated with Vincristine at a concentration of 400 µg/ml for 60 min. There are blebs formed on the periphery of the cells.

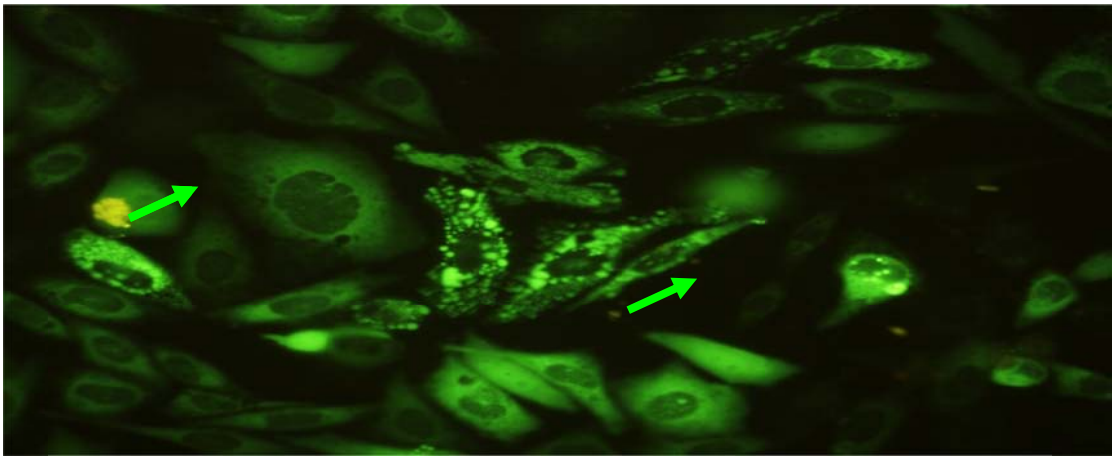

Figure (5): H_{22} cells treated with vincristine at a concentration of 1000 µg/ml for 60 min. Paracrystals are formed.

When cells were exposed to vincristine at a concentration of 20 µg/ml for 6, 8, or 10 hrs, no noticeable changes occurred in the microtubule network.but in the 12 hrs treatment at a concentration of 20 µg/ml caused disruption of microtubules. The network was thinned down, and individual fibres had a wavelike shape.

The cells exposed to Vincristine at a concentration of 400 µg/ml for 10 minutes showed a severely defective microtubular network. All the cells with the recovering period of 12 hrs in a drug-free growth medium following vincristine treatment showed damage of microtubules. The cells after a 12 hrs recovery period had their microtubular network either fully restored or still damaged. After recovery for an 12 hrs period, some cells showed a partly defective (thinned-down) network, but the majority of the cells showed restored microtubules.

When the cells were allowed to recover for 12 hrs, the microtubules were spread out comparably to those observed in untreated control cells. The

control cells showed their microtubule network regularly distributed along the whole cell volume. The microtubules were thinned down, and individual fibres had a wavelike shape. After a recovery period of 12 hrs, the network was also damaged (Fig. 6). When cells were recovered after treatment with Vincristine at a concentration of 400 µg/ml for 10 min, the cytoskeleton was partially restored afterwards (Fig. 7).

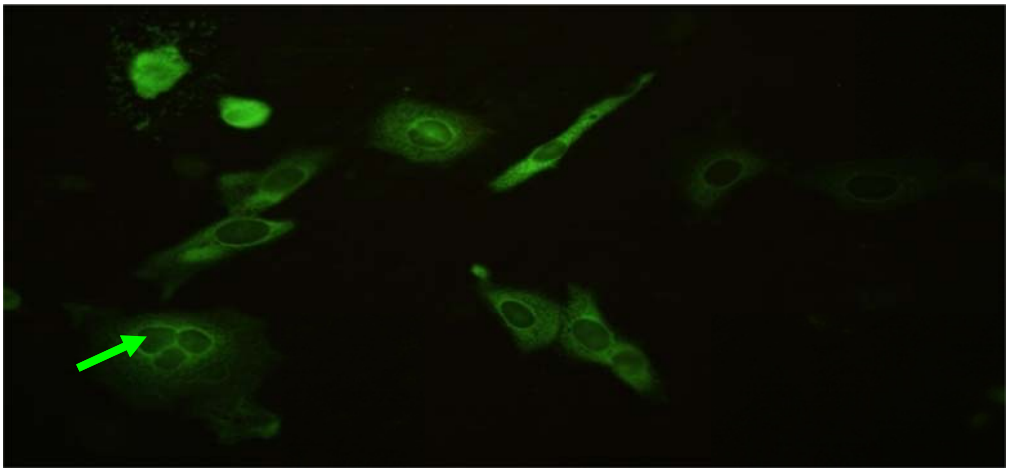

Figure (6): Microtubules of H_{22} cell line treated with Vincristine at a concentration of 20 µ/ml.Microtubules recovered for 12 hrs. The network is nearly restored.

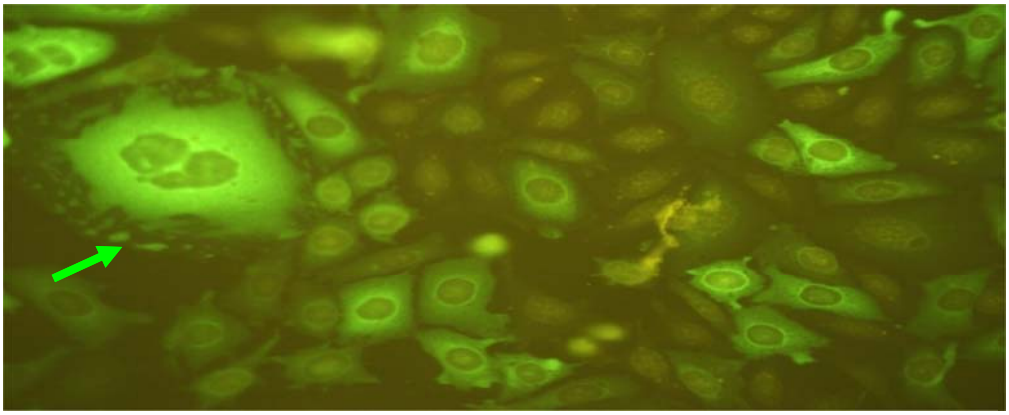

Figure (7): Microtubules of H_{22} cell line treated with Vincristine at a concentration of 400 µ/ml for 10 min.Microtubules were recovered and network is nearly restored.

Discussion:

The interaction of antitumour agents with compounds of the cytoskeleton is a theme studied in several studies (22, 26). Lobert (23) studied the interaction of Vinca alkaloids with tubulin, and compared Vinblastine and Vincristine.She studied e.g. the affinity of the drug for tubulin heterodimers.

Vincristine exhibited a higher overall affinity for porcine brain tubulin than vinblastine, but the affinity of the drug for tubulin heterodimers was identical for the two drugs. Under our experimental conditions we did not mark any differences between the two drugs. Some authors also studied the recovering processes of the cytoskeleton after treatment of cell cultures with physical factors or agents interfering with cytoskeleton compounds. Vincristine caused a sequence of morphological changes in sensitive cells from three pleiotropic resistant MCF-7 human breast carcinoma cell lines mixed with vaginal adenocarcinoma cells. The cells were selected in serially increasing drug concentrations.

The changes occurred initially within 6 hrs of incubation, but were expressed in all cells after 12 hrs. If, after 6 hrs of drug exposure, the cells were subcultured in drug-free media, the cytoskeletal structure reformed within 12 hrs. The maximal recovery of the cytoskeletal structure occurred 12 hrs after drug removal and was sustained up to 12 hrs eliminated the microtubule bundles, leaving only tubulin paracrystals. Within 12 hrs after washing out the vincristine, (24).

Treatment with Vincristine (25) the microtubule bundles repolymerised in cultured hippocampal neurons**.** (27) they investigated quantitative changes after application of the microtubule inhibitor nocodazole. They presented a decrease of the assembled tubulin after treatment with nocodazole and a less delicate structure of the remaining microtubules. This was indicated by a reduction of the parameters used. They also showed significant differences between the high and low metastatic cell lines. They used confocal laser scanning microscopy.

We have no possibility of comparing the mathematical approach because the description of image analysis is not detailed in this paper, but we suppose to have probably a more appropriate approach on account of using histogram expansion, gamma correction (28).

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