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Citation	北海道大學理學部紀要, 15(3), 353-359
Issue Date	1964-12
Doc URL	https://hdl.handle.net/2115/27380
Type	departmental bulletin paper
File Information	15(3)_P353-359.pdf



Cytological Effects of Chemicals on Tumors, XXVI. The Action of Velban (Lilly) on Established Tumor Strains *in vivo* and *in vitro*^{1), 2)}

By

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(With 9 Text-figures and 3 Tables)

Velban (vinblastine sulfate, Lilly) is a new alkaloid extracted from the common perennial herb, *Vinca rosea* (Noble *et al.* 1958). The agent was found to have an empirical formula $C_{46}H_{58}O_9N_4$, and a new dimeric alkaloid containing both indole and dihydroindole moieties (Gorman *et al.* 1959). The mode of response of Velban to tumors is under investigation in the field of chemotherapy.

The present study was undertaken with a hope of obtaining some critical information on the cytological effect of this drug upon established transplantable ascites tumors *in vivo* and HeLa cells *in vitro*.

The authors wish to acknowledge with cordial thanks to Professor Sajiro Makino for his kind guidance and for going through the manuscript. Velban was kindly supplied through the courtesy of Mr. Tsuyoshi Takahashi, Shionogi and Co. Ltd. to whom the authors are much obliged.

Materials and Methods

1) *In vivo* experiment: Tumors used in the present experiment were MTK-sarcoma III, an ascites tumor of rats, and Ehrlich ascites carcinoma of mice. Rats of Wistar and Gifu strains weighing 90 to 130 g, and AKR and Swiss mice weighing 20 to 30g were selected as hosts for tumor transfers. On the 3rd day of tumor transfer, tumor-bearing animals received peritoneally the injection of Velban at doses of 0.1, 0.5 and 1.0mg per 100g of body weight. For general cytological studies, acetic dahlia squash-preparations were made at appropriate intervals following the injection of the drug. Some of the treated animals were sacrificed for examination of bone marrow cells.

2) *In vitro* experiment: Cells of HeLa strain of human cervical origin were used. They were seeded in Leighton tubes, containing coverslips. All cultures were incubated at 37°C for 48 to 72 hours and then the medium containing varying concentrations of Velban was added. For cytological observations, coverslips were fixed and stained according to the method of Jacobson and Webb (1952).

1) Contribution No. 655 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) Supported by a grant-in-aid for Fundamental Scientific Research from the Ministry of Education (No. 406000 in 1964).

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 15, 1964.

Results of Observations

In vivo experiment

Cytological effects on ascites tumor cells: The MTK-sarcoma III and the Ehrlich carcinoma were found to respond similarly to Velban. The following description can be applied to both. Increase in the number of mitotic cells was remarkable in the cell population under the influence of this agent. Within 3 hours after injection, the tumor ascites displayed a large number of metaphasic cells which were characterized by abnormal condensation and irregular scattering of the

Table 1. Frequency distribution of normal and abnormal cells of the MTK-sarcoma III following treatment with Velban (0.5 mg/100g)

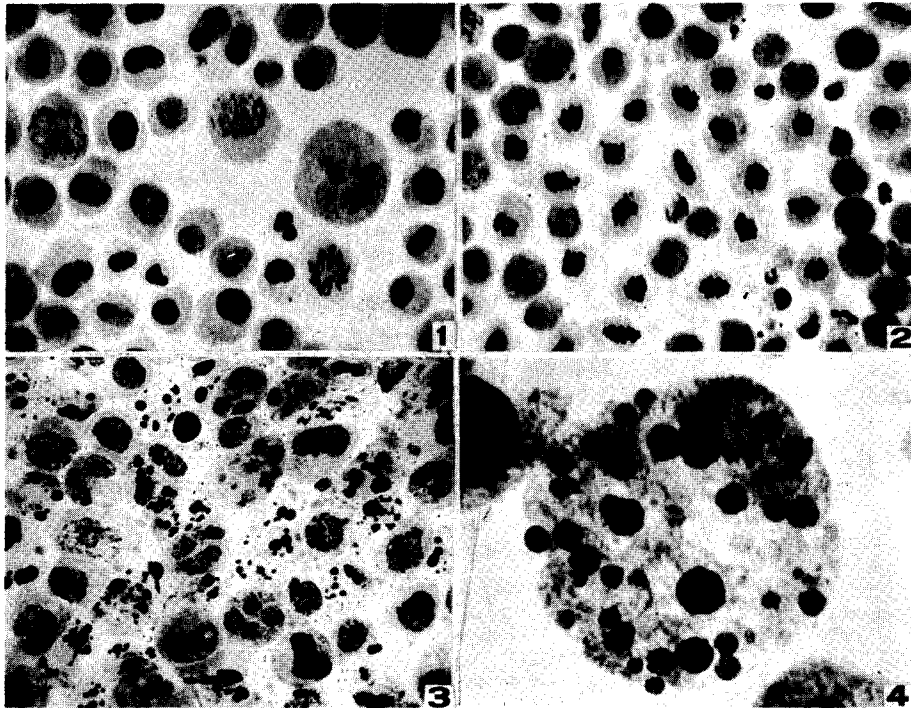
Hours after inj.	Mitotic index	% of dividing cells						Total no. of cells obs.
		Prophase		Metaphase		Ana-telophase		
		normal	abnormal	normal	abnormal	normal	abnormal	
0	3.4	19.0	0	59.7	0.4	20.8	0.1	2000
1	6.1	8.3	0.1	1.2	86.1	4.2	0.1	2011
3	18.0	3.9	0	0	94.8	0.9	0.4	2004
6	22.1	1.5	0.2	0	98.5	0	0	2008
9	27.6	1.7	0	0.2	97.7	0.2	0	2026
12	19.9	6.1	0	4.4	87.6	1.8	0.1	2001
24	4.9	16.7	0	42.1	30.2	7.4	3.6	2030
48	3.0	23.0	0	50.9	3.7	23.0	0.4	2002
72	3.2	15.6	0	56.6	0.5	27.1	0.2	2002

Table 2. Frequency distribution of normal and abnormal cells of the Ehrlich ascites carcinoma following treatment with Velban (0.5mg/100g)

Hours after inj.	Mitotic index	% of dividing cells						Total no. of cells obs.
		Prophase		Metaphase		Ana-telophase		
		normal	abnormal	normal	abnormal	normal	abnormal	
0	4.1	17.3	0	56.0	7.0	19.1	0.6	2000
1	7.3	13.7	0.3	37.4	33.5	12.0	3.1	2000
3	14.1	4.8	0	7.5	79.9	4.5	3.3	2061
6	29.3	0	0	0.3	97.1	1.1	1.5	2004
9	32.0	3.2	0	0	96.8	0	0	2000
12	20.9	8.3	0	4.8	81.1	5.7	0.1	2000
24	7.2	14.8	0	39.7	34.0	10.0	1.5	2020
48	3.3	20.3	0.7	49.8	10.0	18.9	0.3	2009
72	2.8	20.1	0.8	50.0	11.7	16.5	0.9	2000

chromosomes. The most marked effect was found 6 to 9 hours after injection (Fig. 2), and the mitotic index of affected tumor cells became seven to eight times as much

as that of untreated cells (Tables 1 and 2). During the period from 12 to 24 hours after injection, many pycnotic nuclei together with lobated nuclei appeared in samples (Figs. 3 and 4). The mitotic rate of the treated tumor cells was still considerably high in the 12 hour-sample, while it decreased in the 24 hour-sample. By 48 hours after injection, the number of tumor cells undergoing normal cell division increased, and cells showing pycnotic or lobated nuclei were rarely found.



Figs. 1-4. Successive stages of cellular damage in the MTK-sarcoma III following Velban-treatment (0.5 mg/100g).

Fig. 1. Untreated control; 3 days after transplantation.

Fig. 2. Metaphase block in cells of MTK-sarcoma III; 9 hours after treatment.

Fig. 3. Pycnotic disintegration of tumor cells; 12 hours after treatment.

Fig. 4. A resting cell with micronuclei; 24 hours after treatment.

Regrowth of the tumor occurred without exception within 72 hours after the drug application.

Some of the animals treated with the agent showed a considerable prolongation of their life span, and most of them lived as long as untreated animals (Table 3).

Cytological effects on bone marrow cells: In order to examine a possible effect of the agent on normal cells, 5 mice bearing the Ehrlich ascites carcinoma, as well as 1 rat bearing the MTK-sarcoma III, were sacrificed for examination of bone

Table 3. Life span of tumor-bearing animals after a single injection of Velban

Tumor	Dosage (mg/100g)	No. of animals treated	Life span (av. in days)
MTK-sarcoma III	control	10	8.7 (8-10)
	0.1	7	9.4 (8-13)
	0.5	5	16.8 (8-28)
	1.0	5	13.2 (6-16)
Ehrlich ascites carcinoma	control	10	16.1 (14-17)
	0.1	15	18.4 (14-22)
	0.5	15	19.6 (14-40)
	1.0	15	28.0 (16-42)

marrow cells following the drug application. The examination revealed that the intraperitoneal injection of Velban into tumor-bearing animals also exerted a striking inhibitory effect on bone marrow cells: mitotic cells were arrested at metaphase, showing abnormal condensation or scattering of the chromosomes.

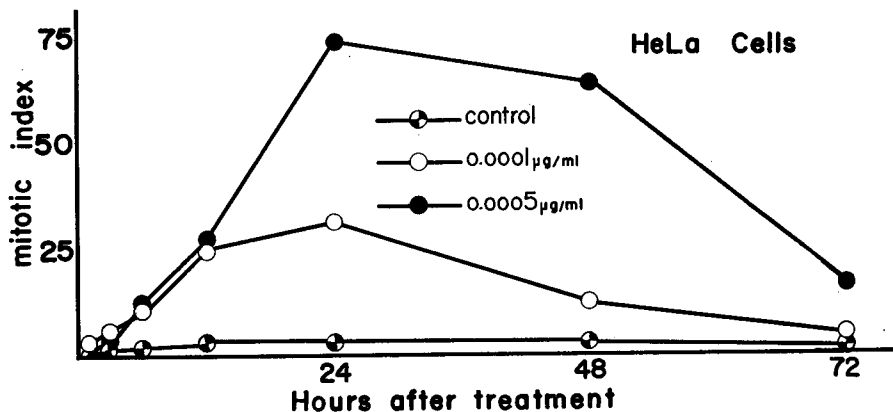
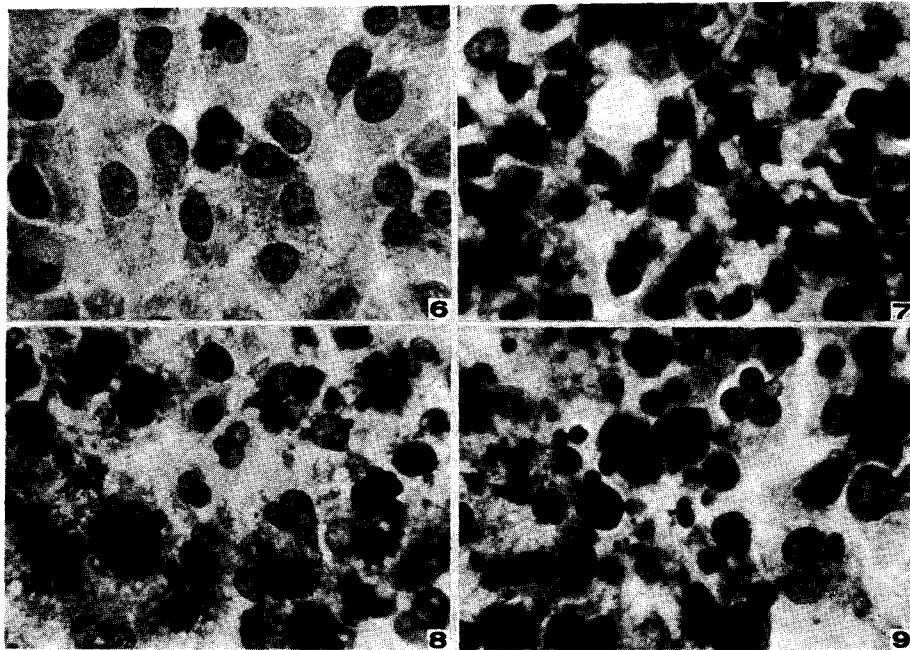


Fig. 5. Curves showing change of mitotic index of HeLa cells following Velban-treatment.

In vitro experiment

After several tests with various concentrations, it was found that 0.0001 µg/ml of Velban was the minimum dose to produce a detectable damage in HeLa cells. As was observed in *in vivo* experiments, mitotic cells were affected more severely than interphase cells. The first sign of cell damages appeared within an

hour after the exposure to the agent. The damage was represented by the metaphase block with like feature to that induced by colchicine: metaphase chromosomes were condensed irregularly, scattering throughout the cytoplasm. Such degenerative changes increased with the lapse of time. In the samples taken from 1 to 6 hours after exposure, the relative frequency of ana- and telophasic cells decreased in number with an increase of metaphasic cells. In Figure 5 is shown an accumulation of metaphasic cells. The number of dividing cells increased steadily by 24 hours, reaching the value to about 10 times as much as that of controls (Figs. 5 and 7).



Figs. 6-9. Successive changes in HeLa cells following Velban-treatment (0.0001 μ g/ml).

Fig. 6. Untreated control; 3 days after cultivation.

Fig. 7. Metaphase block of HeLa cells; 24 hours after treatment.

Fig. 8. Degenerating cells with lobated nuclei; 48 hours after treatment.

Fig. 9. Pycnotic disintegration of HeLa cells; 72 hours after treatment.

During 48 to 72 hours, mitotic incidence decreased to the control level, with concomitant appearance of degenerative cells at interphase. Cells with lobated nuclei were also abundant (Figs. 8 and 9).

In virtue of the above findings, it is possible to conclude that Velban exerts influence particularly upon mitotic cells *in vivo* and *in vitro*.

Discussion

Since the sensational discovery of colchicine by Blakeslee and Avery (1937), its effects on cells have been extensively investigated by a number of workers. Their results have uniformly indicated that colchicine inhibits the formation of a mitotic spindle, then resulting in an accumulation of arrested mitoses in cell population (Levan 1938). The results from the present study with Velban seem to nearly correspond to those obtained that colchicine, since cells under the influence of Velban are blocked at metaphase and then undergo pycnotic degeneration. Östergren (1944) described that the colchicine mitosis (c-mitosis) is not a solitary effect, but it is just one member of a large group of closely related effects (the narcotic effects), and that consequently, these other effects also will have to be considered when a deeper understanding is being sought. Biesele (1958) described that the chemicals known to have a c-mitotic activity can not be regarded as having identical modes of action, for their effects were not the same. The question whether the action of Velban is identical with that of colchicine or not is open for future study. However, it seems most probable that the agent has a c-mitotic action.

Recently, inhibitory effects of Velban upon tumor growth have been reported by some workers with particular regard to the prolongation of life of tumor-bearing animals. Reported evidence has shown that the effect of the agent is generally similar between *in vivo* and *in vitro* materials, and has a broad range of activity toward a variety of tumors which can be compared favorably with that of well established oncolytic agents (Cutts *et al.* 1960, Johnson *et al.* 1960, Hertz *et al.* 1960, Hodes *et al.* 1960, Warwick *et al.* 1960, Palmer *et al.* 1960). Palmer *et al.* (1960) are the first to report the action of this agent cytologically, and showed that the agent exerted typical c-mitotic changes on chromosomes of tissue culture cells derived from both malignant and normal sources. On the cytological basis, the present authors have also reached a similar conclusion in both *in vivo* and *in vitro* experiments. In addition, it was observed that Velban exerted strong antimitotic effects on bone marrow cells of tumor-hosts, as well as on tumor cells.

It seems to the authors that evidence presented suggests a necessity of careful cytological examinations in the application of drugs in cancer chemotherapy.

Summary

The present study deals with cytological effects of Velban on rat and mouse ascites tumors *in vivo*, and HeLa cells *in vitro*.

Velban exerted considerable influence upon tumor cells in the course of mitosis under both *in vivo* and *in vitro* conditions: tumor cells were arrested at metaphase in a manner similar to that induced by colchicine.

Some of the treated animals had a prolonged life span, almost two to four times as long as that of controls, although no complete regression of tumors was obtained in the present experiments.

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