TABLE 2: ACUTE ANTIINFLAMMATORY ACTIVITY OF OAHD COMPARED WITH OA WITH MULTIPLE DOSES

Group	Dose mg/kg	The degree of oedema (mg)		
		ip	sc	ig
Control		12.6±2.72	13.0±2.47	12.5±1.99
OA	25	8.93±1.87*	8.94±2.09*	10.3±1.49
OA	50	6.80±1.10*	7.60±1.67*	9.23±2.41*
OA	100	6.00±1.57*	6.25±1.95*	8.00±3.37*
OAHPDS	25	10.0±3.74	10.5±3.58	7.45±2.18*
OAHPDS	50	8.05±2.64*	8.12±2.37*	6.60±2.83*
OAHPDS	100	7.50±1.81*	7.42±1.59*	5.64±2.10*
HYD	20	4.52±2.08*	4.44±1.97*	4.41±2.09*

Ip, sc and ig represent intraperitoneally, subcutaneously and intra-gastrically administration, respectively. Each value represents mean±standard error of the mean of 10, Each group consisted of 10 animals. Asterisk indicate level of significance compared with control, *p<0.05 (student' test). HYD was intramuscularly administration in every group.

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Antitumour Property of Vinblastine Monohydrazide

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Accepted 29 October 2003

Revised 1 August 2003

Received 13 February 2003

The bioconverted product of vinblastine (alkaloid from *Vinca rosea*), vinblastine monohydrazide was administered to cell line-induced solid tumour in mice and the changes in life span and tumour size were noted. It was found that the bioconverted product was an antitumour agent as it

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helps in the reduction of solid tumour and also in increasing the life span of tumour induced mice when compared to the crude alkaloid vinblastine.

Vinca alkaloids are employed for the treatment of various types of cancers[1]. Malignant cells show several biochemical differences when compared to normal cells, which can be exploited for studies[2]. Solid tumour reduction studies have been conducted to explain the anticancer effects of vinblastine and its bioconverted product vinblastine monohydrazide. Various experimental models for testing the antitumour property of different compounds had been suggested by Conners and Johns[3] and the present study was conducted in tumour bearing mice. The antitumour property of vinblastine is well established and is therapeutically used in human leukemia and other forms of malignancies as a cytotoxic agent by its ability to perform as a mitotic poison arresting cell division[4]. Reports are available on the antitumour activity of vinblastine and its biotransformed products namely vindoline, dihydro vindoline, O-demethyl vindoline and other hydroxylated products[5] but no report seem to be available on the anti-tumour property of vinblastine monohydrazide. The antitumour property of vinblastine and its bioconverted product vinblastine monohydrazide were studied using Ehrlich's ascites lymphosarcoma system in mice.

All the experimental protocols described below were approved by the Institutional Animal Ethics Committee. Inbred male Balb/c mice, 7 w old, weighing 15-18 g were used for the study. The mice were obtained from Veterinary College, Kerala Agriculture University, Thrissur. Mice with an average weight 15.5±1.5 g were selected and divided into three groups consisting of six mice in each group. Solid tumour was induced by subcutaneous injections of $5x10^4$ Ehrlich's Ascites Cells (EAC) on the flank region. All operations were done aseptically. Vinblastine was biotransformed to vinblastine monohydrazide with a Pseudomonas sp. and purified. This preparation was dissolved in sterile normal saline containing 0.9% benzyl alcohol (IP) aseptically. Velban was also dissolved in normal saline containing 0.9% benzyl alcohol. Group 1 was kept as control; group 2 was injected with vinblastine and group 3 with vinblastine monohydrazide. Into the tumour mass, vinblastine preparation and vinblastine monohydrazide were injected subcutaneously in a single dose and in three intermittent doses with an interval of 24 h between each administration. Controls were treated with the same quantity of sterile saline. On the 12th d, the diameter of subcutaneous mass i.e. the average of two measurements at right angles was measured. The diminution of tumour size indicates the antitumour activity, which is expressed as mean change in tumour size. A comparison of vinblastine and vinblastine monohydrazide in the survival of tumour bearing mice was also studied and the results are discussed.

The effect of vinblastine on EAC-induced sarcoma was also studied. Mice having an average weight 15.5±1.5 g were selected and divided into three groups consisting of six mice in each group. They were inoculated intraperitoneally with $1x10^5$ tumour cells. Group 1 was kept as control group, which were treated with the same amount of normal sterile saline. Group 2 was injected with vinblastine and group 3 with vinblastine monohydrazide the bioconverted product in different doses (2, 5, 10 and 20 μg/mouse). All injections were given through the same route in a single dose at three intermittent doses with an interval of 24 h between each administration. The survival times of the treated groups were compared with that of the control, after administering several different dose levels. There was an inverse relation between survival time and number of viable cells injected. The percentage increase in life span of treated groups was compared with that of control groups as a direct measure of tumour cell kill[6]. Statistical analyses were done by Student's 't' test. The values which have $p \leq 0.001$ were considered to be significant.

The antitumour property of vinblastine monohydrazide, the bioconverted product was compared with that of vinblastine (Table 1). There was a reduction in growth rate of tumour when 1 mg of vinblastine monohydrazide was injected subcutaneously when compared to control group. The tumour size could be brought down with 2 μg/mouse of the drug. Almost complete regression of the tumour size was obtained by injecting 10 μg of the drug subcutaneously into the tumour mass. The average reduction in tumour size was more with increase in the dose of the drug.

The effect of vinblastine monohydrazide on Ehrlich's ascetic sarcoma bearing mice is given in Table 2. The duration of the experiment was 60 d after tumor implantation. All animals that received 5 μg/mouse of vinblastine monohydrazide in 3 intermittent doses at 24 h intervals survived to the last day of the experiment. Injecting the same amount of the drug in intermittent doses rather than a single

TABLE 1: ANTINEOPLASTIC ACTIVITY OF VINBLASTINE AND VINBLASTINE MONOHYDRAZIDE

Dose of the drug (μg)	Mean change in tumour size (mm)**		
	Vinblastine	Vinblastine monohydrazide	Control without drug
1	-1.4 ±0.19*	-1.5±0.14*	+8.3±0.34
2	-3.1±0.30*	-2.9±0.15*	
5	-5.8±0.17*	-5.4±0.09*	
10	-7.9±0.21*	-7.6±0.13*	
3 intermittent doses of 2 μg each	-8.1±0.20*	-8.0±0.22*	

*Denotes statistical significance at $p<0.05$. **Diameter of the tumour mass, 6 mice in each group. Injections were given 7 d after tumour implantation and the effect was measured on the 12th d.

dose could increase the average survival period. When large quantities of the drug was administered intraperitoneally, there was a reduction in the survival period, i.e., a single dose of 20 μg of the drug could not protect the tumour bearing mice to survive to the last day of experiment. The average survival period of Ehrlich's ascites tumor bearing mice was increased by the administration of 3 intermittent dose of the drug, which can be concluded from the percentage of survivors. A comparison of vinblastine and vinblastine monohydrazide in the survival of tumour bearing mice is given in Table 3. Both vinblastine and vinblastine monohydrazide were curative at a dose level of 5 μg.

Both vinblastine monohydrazide and vinblastine showed a decrease in tumour size. Tissue necrosis was very little and complete disappearance was observed with 3 doses of 2 μg each of vinblastine monohydrazide. This clearly establishes the antineoplastic effect of vinblastine

TABLE 2: EFFECT OF VINBLASTINE MONOHYDRAZIDE ON THE SURVIVAL PERIOD OF SARCOMA BEARING MICE

Dose (μg)	Injections	Survivors*	Percentage survivors**	Day of death	Average of the days ± SD
Control	1	0	0	13,15,17,20, 21,23	18.16±3.85
Vinblastine					
2	1	2	33.3	35, 38, 42, 57	43±2.94
5	1	4	66.6	40,53	46.5±9.19
10	1	3	50	43,54, 56	51±1.14
20	1	1	16.6	36,45,49,55, 58	97.2±8.68
Three intermittent doses of 5 μg each	3	6	100	65, 79, 73, 80, 90, 93	80±10.43

Control-Not treated with the drug and given sterile normal saline. *Mice that survived up to the 60th d of experiment. **Survivors at the time of death of controls. Vinblastine monohydrazide was administered intraperitoneally in a single dose and in three intermittent doses of 24 h after tumour implantation.

TABLE 3: COMPARISON OF SURVIVAL PERIOD IN TUMOUR BEARING MICE TREATED WITH VINBLASTINE AND VINBLASTINE MONOHYDRAZIDE

Dose of the drug (μg)	Vinblastine		Vinblastine monohydrazide	
	Average survival time (d)	Increase in life span (%)	Average survival time (d)	Increase in life span (%)
Control (untreated)	17.8±2.92	-	17.8±2.92	-
Treated with-				
2	56.7±2.30[a]	318.5	55.9±3.40[a]	314
5	60.7±3.19[a]	341.0*	63.9±2.59[a]	358.98*
10	64.1±1.98[a]	360.1*	61.3±1.65[a]	344.38*
20	61.9±1.94[a]	347.8*	60.5±2.75[a]	339.88*
Three intermittent doses of 5 μg each	86.7±2.25[a]	487.1*	82.9±2.06[a]	465.73*

a- $p \leq 0.05$. *Mice, which survived up to the end of the experiment (60 d).

monohydrazide with fewer side effects, which reveals the cytotoxic effect on EAC tumour cells but not on normal cells. Tumour reduction studies and survival period studies hence prove the antitumour property of vinblastine monohydrazide, it can be used as an antituour agent with less side effects. Anti tumour property of vinblastine monohydrazide might be due to free radical scavenging property of the compound, which coincides, with the report of Ruby[7,8].

ACKNOWLEDGEMENTS

G. Jayashree is thankful to M. G. University for providing the JRF during the work. S. Sudarslal and G. Vasundhara are thankful to CSIR and UGC respectively for the SRF provided during the work.

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