Anti-Cancer Drugs 1997, **8**, pp. 529–532

Mitotic arrest and anaphase aberrations induced by vinorelbine in hamster cells *in vitro*

Marcela González-Cid, María Teresa Cuello and Irene Larripa
Departamento de Genética, Instituto de Investigaciones Hematológicas 'Mariano R Castex', Academia Nacional de Medicina. Buenos Aires, Argentina. Tel: (+54) 1 805-8803; Fax: (+54) 1 805-0712.

Aneugenic effects of the Vinca alkaloid vinorelbine (VRB) were evaluated *in vitro*, measuring sister chromatid exchanges (SCE), cell proliferation kinetics and anaphase–telophase aberrations in Chinese hamster ovary (CHO) cells. The highest dose of VRB (0.50 μg/ml) arrested cells at the first metaphase. An increase in abnormal anaphases was seen at 0.05–0.50 μg/ml of VRB, containing chiefly lagging chromosomes and multipolar spindles. No increase in SCE was found. These results indicate that VRB does not directly damage DNA, but acts on spindle microtubules, altering chromosome movement and causing aneuploidy.

Key words: Abnormal anaphase–telophase cells, aneugenic effect, spindle poison, vinorelbine.

Introduction

The Vinca alkaloids are antimitotic drugs used extensively to treat several forms of malignancy.[1] Vinorelbine (VRB) is a recently discovered semisynthetic Vinca alkaloid that differs chemically from the naturally occurring compounds (vinblastine and vincristine) in the configuration of the catharanthine ring structure.[2] This structural modification confers a high liposolubility to the molecule, and may be responsible for the lower toxicity of the drug and its increased antitumor activity compared with its analogs.[3]

VRB has demonstrated significant antitumor activity *in vitro* and *in vivo* experimental systems.[4] Anticancer effects have been documented in patients with non-Hodgkin's lymphoma, Hodgkin's disease, melanoma, head and neck cancer, breast cancer, and non-small cell lung cancer.[5,6] Toxicity has consisted mainly of neutropenia, with minimal non-hematologic toxicity.[7]

The antineoplastic activity of VRB is caused mainly by its ability to bind to tubulin, inhibiting tubulin polymerization and assembly of mitotic spindle microtubules.[5,8] Such effects may lead to aneuploidy, i.e. the production of daughter progeny with abnormal chromosome numbers during mitosis.

In the present work we studied the *in vitro* cytogenetic effect of VRB, analyzing sister chromatid exchanges (SCE), cell proliferation kinetics and anaphase–telophase aberrations in Chinese hamster ovary (CHO) cells.

Materials and methods

Chemicals

VRB, 5'-nor-anhydrovinblastine (CAS no. 71486-22-1), was commercially obtained as Navelbine® (Rontag, Argentina; Pierre Favre Médicament, Boulogne, France).

Colchicine (COL; Gibco, Grand Island, NY), considered the best known spindle-damaging agent, was included as a positive control compound.

Chinese hamster cell culture

CHO-K1 cells were grown as a monolayer in F-12 medium supplemented with 15% fetal calf serum in plastic culture flasks at 37°C. Trypsin (0.25%) was routinely used for subculture. For cell treatment, each flask received a suspension of 1×10^5 cells/ml. The average doubling time under these conditions was approximately 18 h. Two hours after seeding, cultures were treated with 0.01, 0.05, 0.10 or 0.50 μg/ml of VRB freshly dissolved in water. The drug was left in continuously in the cultures. Control and positive control (COL, 0.01 μg/ml) cultures were grown under identical conditions. Each dose was analyzed in three replicate cultures.

This work was supported by grants from the National Research Council (CONICET) and 'Alberto J Roemmers' Foundation.

Correspondence to M González-Cid

SCE, cell cycle kinetics and mitotic index

Cultures were incubated with bromodeoxyuridine (BrdU, 10 μg/ml) in complete darkness for 36 h. Colcemid (0.2 μg/ml) was added for the last 90 min of the culture period. The cells were resuspended in a prewarmed hypotonic solution of 0.075 M KCl for 20 min and fixed in methanol:acetic acid (3:1). Differential staining of sister chromatids was carried out by a modified fluorescence-plus-Giemsa technique.[9] The SCE average was taken from an analysis of 30 metaphases ($2n = 22 \pm 4$ chromosomes) during the second cycle of division.

For cell cycle analysis, 100 metaphases per culture were examined and the frequencies of first (M_1), second (M_2) or third and further (M_{3+}) divisions were determined. The replication index (RI) was calculated as follows: RI = 1 × (% of cells in M_1) + 2 × (% of cells in M_2) + 3 × (% of cells in M_{3+})/100.[10]

The mitotic index (MI) was scored as the percentage of metaphases among 1000 nuclei. Changes in the MI were expressed as a factor (f) of the mean MI from treated cultures over the mean MI from controls.[11]

Anaphase–telophase test

CHO-K1 cells were cultured in slide flasks (Nunc) and treatments were performed during 18 h. The cells were fixed in 96% ethanol and stained with hematoxylin & eosin.

For each dose, 100 anaphases were examined for the occurrence of chromatin bridges, lagging chromosomes or fragments and multipolar anaphases.

Statistical analysis

The means of the frequencies of SCE were statistically analyzed by the Kruskal–Wallis test. Differences between the values of the cell cycle kinetics and abnormal anaphases were evaluated by the χ^2 test. The mean MI frequencies were analyzed using the Student's t-test. The dose–response relationships were determined by means of the regression coefficients.

Results

The data of the frequencies of SCE in CHO cells treated with VRB are presented in Table 1. There was no significant increase in SCE in cultures treated with either VRB or COL ($p = 0.1079$). VRB could not be evaluated at 0.50 μg/ml since the cells had not progressed beyond the first metaphase.

Table 2 shows the cell cycle progression obtained at the different doses of VRB. A significant increase in M_1 and decrease in M_2 cells was observed at all the doses assayed, but the delay was similar at 0.01–0.10 μg/ml, then increased markedly at

Table 1. SCE in CHO cells treated with VRB

Chemical	Dose (μg/ml)	SCE/cell (mean ± SE)
Control		11.5 ± 1.5
VRB	0.01	10.3 ± 0.7
VRB	0.05	13.5 ± 2.1
VRB	0.10	13.1 ± 1.1
VRB	0.50	no M_2 cells
COL	0.01	12.1 ± 1.7

Table 2. Cell cycle kinetics and mitotic index in CHO cells treated with VRB

Chemical (μg/ml)	Percentage of cells at (mean ± SE)			RI	MI (%) (mean ± SE)	f
	M_1	M_2	M_{3+}			
Control	10.3 ± 2.9	83.7 ± 6.4	6.0 ± 2.3	1.96	1.4 ± 0.2	1.00
VRB 0.01	35.0 ± 1.4[b]	65.0 ± 1.4[b]	–	1.65	1.6 ± 0.1	1.14
VRB 0.05	32.0 ± 5.7[b]	68.0 ± 5.7[b]	–	1.68	1.8 ± 0.1	1.29
VRB 0.10	23.5 ± 2.1[a]	76.5 ± 2.1[a]	–	1.77	2.8 ± 0.5[c]	2.00
VRB 0.50	100.0 ± 0.0[b]	–	–	1.00	34.6 ± 2.1[d]	24.71
COL 0.01	18.5 ± 5.0	81.5 ± 5.0	–	1.82	2.0 ± 0.2	1.43

VRB, Vinorelbine
COL, Colchicine
M_1: first mitosis, M_2: second mitosis, M_{3+}: third and further mitosis.
RI: replication index. MI: mitotic index, f: factor
Significant differences with respect to controls: [a] $p < 0.02$, [b] $p < 0.001$, [c] $p < 0.05$, [d] $p < 0.01$.

0.50 μg/ml of VRB. RI shows the average number of times cells had divided in the presence of BrdU. At 0.50 μg/ml of VRB, the RI of treated cells decreased to 1.00 from 1.96 in the control culture.

The MI was used to determine the extent of mitotic arrest caused by VRB (Table 2). Accumulation of cells at metaphase was observed at 0.10 μg/ml of VRB ($p < 0.05$) and markedly at 0.50 μg/ml of VRB ($p < 0.01$). The changes in the MI from treated cultures are expressed by the factor *f*. At the dose of 0.50 μg/ml the MI was increased by a *f* of 24.71 over their control cultures considered as $f = 1.00$.

The alterations scored in anaphase–telophase cells are shown in Table 3. A significant increase in abnormal anaphases was found in VRB-treated cultures starting at 0.05 μg/ml. The principal aberrations observed were lagging chromosomes or fragments and multipolar anaphases. The frequencies of anaphases obtained decreased at higher concentrations, presumably because cells were arrested in metaphase.

Discussion

In our experiments VRB did not increase the frequency of SCE in the CHO cell line. SCE is a highly sensitive method for detection of certain DNA damaging agents.[12] There are conflicting reports on the induction of SCE by spindle poisons. Our findings agree with those reported by Morgan and Crossen[13] in human lymphocytes treated with vincristine and colcemid. In contrast, Banerjee and Benedict[14] found a slight increase in SCE at high concentrations of vincristine in a hamster cell line A(T_1)C1-3. On the other hand, Stoll *et al.*[15] described that vincristine caused a significant decrease in the numbers of SCE in human lymphocytes.

Our results show that VRB is a very efficient inducer of alterations of CHO cell division, ranging from delay to arrest at the M_1 stage. The ability of other Vinca derivatives (vincristine, vinblastine, vindesine, vinepidine and vinrosidine) to inhibit cell proliferation also closely coincided with their ability to cause accumulation of cells at a metaphase-like stage of mitosis.[16,17] Both delay and mitotic arrest appear to be related to the disturbance of chromosome segregation.[16,18]

The anaphase–telophase assay detects not only chemical clastogens but also spindle poisons. Treatment with VRB increased the frequency of anaphase cells with aberrations, especially lagging chromosomes or chromosomal fragments and multipolar spindles. Lagging chromosomes can originate from alterations of the mitotic spindle apparatus,[19] whereas lagging chromosomal fragments result from chromosome or chromatid breaks.[20] Taking into account the mechanism of action of VRB, we consider that the lagging elements were produced by whole chromosomes. No increase in the frequency of chromatin bridges was observed, indicating that exchange aberrations between chromatids or chromosomes were not induced by VRB. Hsu *et al.*[21] obtained similar results in a Chinese hamster cell line treated with a mitotic arrestant (diazepam).

In VRB-treated cultures, anaphases exhibited a high rate of multipolar spindles, similar to the effects of Vinblastine *in vitro*[22] and *in vivo*.[23] This phenomenon could be due to the absence of spindle tubules and the inability of centrioles to migrate to the opposite poles of treated cells.[24]

In conclusion, VRB is a potent spindle poison in CHO cells.

Table 3. Anaphase–telophase in CHO cells treated with VRB

Chemical (μg/ml)	Percent abnormal anaphases (mean ± SE)			
	Chromatin bridges	Lagging chromosomes or fragments	Multipolar spindles	Total
Control	4.3 ± 2.7	1.0 ± 0.7	1.7 ± 0.9	5.7 ± 0.4
VRB 0.01	7.0 ± 2.6	1.0 ± 0.7	1.3 ± 1.1	6.0 ± 2.6
VRB 0.05	3.3 ± 1.8	3.7 ± 2.7	5.3 ± 3.2	11.0 ± 3.1[a]
VRB 0.10	4.7 ± 2.1	7.0 ± 3.1	8.3 ± 2.2	15.7 ± 0.8[b]
VRB 0.50	9.0 ± 0.7	14.0 ± 6.4	21.7 ± 2.9	35.0 ± 7.5[b]
COL 0.01	4.0 ± 0.9	6.0 ± 2.9	4.3 ± 1.7	10.5 ± 1.7[a]

Significant differences with respect to controls: [a] $p < 0.02$, [b] $p < 0.001$.

References

1. Calabresi P, Parks RE. Quimioterapia de las enfermedades neoplásicas. In: Goodman Gilman A, Goodman LS, Rall TW, Murad F, eds. *Las bases farmacológicas de la terapéutica.* México: Editorial Médica Panamericana 1986: 1214–7.
2. Potier P. The synthesis of Navelbine® prototype of a new series of vinblastine derivatives. *Semin Oncol* 1989; **16**: 2–4.
3. Krikorian A, Rahmani R, Bromet M, Bore P, Cano JP. Pharmacokinetics and metabolism of Navelbine®. *Semin Oncol* 1989; **16**: 21–5.
4. Cros S, Wright M, Morimoto M, Lataste H, Couzinier JP, Krikorian A. Experimental antitumor activity of Navelbine®. *Semin Oncol* 1989; **16**: 15–20.
5. Boiron M, Niitani H, Queisser W. Navelbine®. Introduction. *Semin Oncol* 1989; **16**: 1.
6. Burris HA, Fields S. Summary of data from *in vitro* and phase I Vinorelbine (Navelbine) studies. *Semin Oncol* 1994; **21**: 14–20.
7. Besenval M, Delgado M, Demarez JP, Krikorian A. Safety and tolerance of Navelbine® in phase I–II clinical studies. *Semin Oncol* 1989; **16**: 37–40.
8. Lobert S, Vulevic B, Correia JJ. Interaction of Vinca alkaloids with tubulin: a comparison of vinblastine, vincristine, and vinorelbine. *Biochemistry* 1996; **35**: 6806–14.
9. Perry P, Wolff S. New Giemsa method for the differential staining of sister chromatids. *Nature* 1974; **251**: 156–8.
10. Schneider EL, Nakanishi Y, Lewis J, Sternberg H. Simultaneous examination of sister chromatid exchanges and cell replication kinetics in tumor and normal cells *in vivo*. *Cancer Res* 1981; **41**: 4973–5.
11. Miller BM, Adler ID. Suspect spindle poisons: analysis of c-mitotic effects in mouse bone. *Mutagenesis* 1989; **4**: 208–15.
12. Perry PE, Thomson EJ. The methodology of sister chromatid exchanges. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. *Handbook of mutagenicity test procedures*. Amsterdam: Elsevier 1984: 495–529.
13. Morgan WF, Crossen PE. Mitotic spindle inhibitors and sister-chromatid exchange in human chromosomes. *Mutat Res* 1980; **77**: 283–6.
14. Banerjee A, Benedict WF. Production of sister chromatid exchanges by various cancer chemotherapeutic agents. *Cancer Res* 1979; **39**: 797–9.
15. Stoll C, Borgaonkar DS, Levy JM. Effect of vincristine on sister chromatid exchanges of normal human lymphocytes. *Cancer Res* 1976; **36**: 2710–3.
16. Jordan MA, Thrower D, Wilson L. Mechanism of inhibition of cell proliferation by Vinca alkaloids. *Cancer Res* 1991; **51**: 2212–22.
17. Pauwels O, Kiss R, Pasteels JL, Atassi G. Cytotoxicity, cell cycle kinetics and morphonuclear-induced effects of Vinca alkaloid anticancer agents. *J Pharm Pharmacol* 1995; **47**: 870–5.
18. Pacchierotti F, Bassani B, Leopardi P, Zijno A. Origin of aneuploidy in relation to disturbances of cell-cycle progression. II: Cytogenetic analysis of various parameters in mouse bone marrow cells after colchicine or hydroquinone treatment. *Mutagenesis* 1991; **6**: 307–11.
19. Adler ID, Kliesch U, van Hummelen P, Kirsch-Volders M. Mouse micronucleus tests with known and suspect spindle poisons: results from two laboratories. *Mutagenesis* 1991; **6**: 47–53.
20. Dulout FN, Olivero OA. Anaphase–telophase analysis of chromosomal damage induced by chemicals. *Environ Mutagen* 1984; **6**: 299–310.
21. Hsu TC, Liang JC, Shirley LR. Aneuploidy induction by mitotic arrestants. Effects of diazepam on diploid Chinese hamster cells. *Mutat Res* 1983; **122**: 201–9.
22. Tucker RW, Owellen RJ, Harris SB. Correlation of cytotoxicity and mitotic spindle dissolution by vinblastine in mammalian cells. *Cancer Res* 1977; **37**: 4346–51.
23. Bogajewski J. The anaphase test. *Mutat Res* 1984; **130**: 189.
24. George P, Journey LJ, Goldstein MN. Effect of vincristine on the fine structure of HeLa cells during mitosis. *J Natl Cancer Inst* 1965; **35**: 355–75.

(Received 21 January 1997; revised form accepted 10 April 1997)