

Genotoxic Effects Induced by Fotemustine and Vinorelbine in Human Lymphocytes

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The aim of this study was to investigate the *in vitro* genotoxic effects of the anticancer drugs fotemustine and vinorelbine on human lymphocytes and to determine individual and sex-related responses to these drugs. Fotemustine is a DNA-alkylating drug while vinorelbine is a semi-synthetic *Vinca* alkaloid. The study was carried out with twenty independent healthy donors for each drug. We have tested the ability of these drugs to induce chromosome aberrations (CAs) and sister chromatid exchanges (SCEs) as well as effect on the mitotic index (MI) in cultured human lymphocytes. Fotemustine was shown to induce CAs and SCEs at all concentrations tested (2, 4 and 8 $\mu\text{g/ml}$) in a dose-dependent manner. Additionally it also decreased the mitotic index in a similar dose-dependent manner. Vinorelbine had no effect on structural CAs, but it significantly increased the numerical CAs at all doses tested (0.5, 1 and 2 $\mu\text{g/ml}$). Vinorelbine also induced SCE events and increased the MI values.

Two-way analyses of variance were used to compare the individual and gender-related susceptibilities to fotemustine and vinorelbine with respect to the CA, SCE and MI values. The results indicated that individuals in fotemustine treatment groups showed different genotoxic responses with respect to CA and SCE induction and additional findings indicated a gender-specific response in this group. Individuals in the vinorelbine test group also exhibited statistically significant numerical CA, SCE and MI responses to vinorelbine. A statistically significant gender-related SCE response to this drug was also evident. This study indicates that these drugs have potentially harmful effects on human health.

Key words: Fotemustine, Vinorelbine (Navelbine), Genotoxicity

Introduction

Fotemustine is a DNA-alkylating 2-chloroethyl-substituted *N*-nitrosourea (CENU) drug (Vermeulen *et al.*, 1998). Among antitumour agents, nitrosoureas belong to an extremely active class of alkylating compounds that have widespread clinical application in the treatment of brain tumours, melanomas and various leukemias (Winum *et al.*, 2003). The nitrosoureas induce DNA interstrand cross-links starting with a carbonium ion attack at the O⁶-position of the guanine (Vermeulen *et al.*, 1998; Gander *et al.*, 1999). There is a good correlation between the number of interstrand cross-links and the cyto- and geno-toxicity of nitrosoureas (Marzolini *et al.*, 1998; Gander *et al.*, 1999). In general, alkylating agents are known to cause a number of biological effects in living organisms including mutation induction, carcinogenesis, cell death, chromosome damage biosynthetic pathway inhibition, cell cycle arrest and teratogenicity (Margison and Santibanez-Koref, 2002). Previously, Ashby *et*

al. (1993) have shown that fotemustine is a base-pair mutagen to *Salmonella*. Additionally, it was also observed in this study that fotemustine is a somatic and germ cell mutagen to *Drosophila*. Fotemustine also had a potent clastogenic effect in mouse bone marrow cells *in vivo*, following either oral gavage or intraperitoneal injection in that study (Ashby *et al.*, 1993). However, to our knowledge, no studies have been performed regarding the genotoxic effects of fotemustine on human lymphocytes *in vitro*.

Vinca alkaloids, including the natural products vincristine (VCR) and vinblastine (VBL) and the semi-synthetic derivatives vindesine (VDS) and vinorelbine (Navelbine, VRB), are antimitotic drugs that have been widely used in the treatment of cancer for more than 30 years. In spite of being chemically related, these mitotic spindle poisons exhibit significant differences concerning cytotoxicity, as well as antitumour activity (Zhou and Rahmani, 1992). In VRB the catharantine moiety contains an eight-membered ring in place of the

nine-membered ring that is present in all naturally occurring members of the VBL group (Ngan *et al.*, 2001; Gonzalez-Cid *et al.*, 1997). It is effective in non-small cell lung cancer, metastatic breast cancer, and ovarian cancer, head and neck cancer, melanoma, non-Hodgkin's lymphomas and Hodgkin's disease and shows promise in the treatment of esophageal cancer and prostatic carcinoma (Bunn and Kelly, 1998).

The mechanism of the *Vinca* alkaloids is known to involve depolymerization of spindle microtubules and induction of paracrystalline tubulin *Vinca* alkaloid arrays. Similar to the other *Vinca* alkaloids, VRB binds tubulin, in effect, negating assembly/disassembly of microtubules by inhibiting tubulin polymerization (Gonzalez-Cid *et al.*, 1997).

The effects caused by the *Vinca* alkaloids in various *in vitro* and *in vivo* experimental systems point to a significant aneuploidogenic activity of VCR, VBL and VRB as well as the lesser ability of these chemicals presented with regards of the inducement of clastogenic events. Tiburi *et al.* (2002) have shown VCR, VBL and VRB to be recombinagenically potent using *Drosophila* somatic mutation and recombination tests (SMART) implying a significant genotoxicity of the chemicals. An effect on the proliferative activity induced by VRB was also demonstrated in that the MI (mitotic index) in different normal and isolated lymphocyte sub-populations was significantly increased (Gonzalez-Cid *et al.*, 1997). Moreover, to compare the aneugenic effect of VRB and VCR, a micronuclei (MN) test was used in cultured human lymphocytes using the cytokinesis block method. Both chemicals increased the MN frequency when rates were compared to a negative control (Gonzalez-Cid *et al.*, 1999). In the present work, we have examined the induction of CAs (chromosome aberrations) and SCEs (sister chromatid exchanges) *in vitro* by fotemustine and vinorelbine using lymphocytes. Similarly, the effect of these drugs on MI under like conditions was also measured.

In recent years interindividual differences in drugs and toxicant reactions have received significant emphasis (Ingelman-Sundberg, 2001). In particular, gender-specific toxicant response is a naturally important subset in predicting overall risk factors of toxicant exposure on humans (Aldridge *et al.*, 2003). Thus, we have tried to investigate the interindividual and gender-specific differences in

genotoxic response to fotemustine and vinorelbine in relatively larger donor groups (20 donors for each drug).

Materials and Methods

Chemicals

Fotemustine (muphoran, CAS No. 92118-27-9) and vinorelbine (navelbine, CAS No. 125317-39-7) were kindly provided by Department of Radiation Oncology, Faculty of Medicine, Uludağ University, 5-Bromodeoxyuridine (BrdU) was purchased from Sigma and mitomycin-C from Kyowa (Hakko, Japan), respectively.

Lymphocyte cultures and cell harvesting

Heparinized peripheral blood used in all experiments was obtained from 20 healthy, non-smoking donors (for fotemustine 8 males and 12 females, age: 18–34; for vinorelbine 6 males and 14 females, age: 19–40) for each drug with two replicates, independently. The cultures were set up by adding 0.3 ml of whole blood to RPMI 1640 medium (10X, Sigma) supplemented with 20% fetal calf serum (Biochrom AG), 100 IU/ml penicillin, 100 µg/ml streptomycin (Biological Industries), 0.5 mg/ml L-glutamine and 6 µg/ml phytohemagglutinin (PHA-L; Biochrom AG). The whole blood cultures for all treatments of each donor were done as two replicates. We have tested several doses of fotemustine (25, 20, 15, 10, 7.5, 5, 2 and 0.5 µg/ml) and have also tested several doses of vinorelbine (15, 10, 7.5, 5, 2 and 0.5 µg/ml) prior to settling upon the doses used in this study. From these preliminary studies, we have chosen the 2, 4 and 8 µg/ml doses for fotemustine and the 0.5, 1 and 2 µg/ml doses for vinorelbine. Lymphocytes were incubated at 37 °C for 72 h. As negative and positive controls, distilled water and mitomycin-C (0.25 µg/ml) were used, respectively. Fotemustine, vinorelbine and mitomycin-C (MMC) were dissolved in sterile, distilled water and drug solutions were prepared immediately before use, to avoid degradation of the drugs. The cells were treated with fotemustine and vinorelbine for 24 h prior to harvest. Cultures for SCE analyses were incubated in the dark in the presence of 10 µg/ml 5-bromodeoxyuridine. Metaphases were obtained by adding colcemid (0.2 µg/ml final concentration; Sigma) 2 h prior to harvest.

Cell harvesting and slide preparation were performed according to Sbrana *et al.* (1993). To visu-

alize CAs, slides were stained with 5% aqueous Giemsa solution for 15 min. Slides to be used for analysis of SCEs were stained using a modified fluorescence plus Giemsa (FPG) method (Parry and Wolf, 1974). 100 metaphases per treatment were analyzed for the presence of CAs and 30 metaphases were counted for SCEs (Bolzan and Bianchi, 2002). The MI was estimated by counting 1000 cells for each treatment and replicate.

Statistical analysis

The frequencies of CAs and SCEs in treated cultures were compared by use of one-way analysis of variance (ANOVA) and Tukey honest significant difference (HSD) tests with 95% confidence intervals. Dose-response relationships were determined by regression analyses.

The individual susceptibility to fotemustine and vinorelbine was determined by two-way analysis of variance (MANOVA) and Tukey HSD tests. These analyses were carried out with the commercial software programs SPSS 11.5 and Statistica 6.0, respectively.

Results

The results of the CA analysis and MI values induced by fotemustine in lymphocyte cultures are shown in Tables. As seen in Tables I and II, fotemustine significantly increased CAs when gap and pulverized metaphase were both included and excluded compared with the negative control group ($p < 0.0005$). This effect of fotemustine was dose-dependent (for the total CAs: $r = 0.879$, $p < 0.001$,

and for the total CAs excluding gaps and pulverizations: $r = 0.890$, $p < 0.001$). Chromatid breaks, iso-chromatid breaks, gaps and sub-chromatid aberrations were the most common chromosomal abnormalities. A significant and dose-dependent decrease ($p < 0.001$) in the frequency of mitoses was detected for the treatment with fotemustine ($r = -0.491$, $p < 0.001$). The results on the induction of SCEs and HFCs (high frequency SCE cells) at all dose groups are shown in Tables III and IV. Specifically, induction of SCEs is significantly increased by fotemustine compared with the negative control ($p < 0.0005$). This effect of fotemustine was further found to be dose-dependent ($r = 0.903$, $p < 0.001$).

Vinorelbine only increased the numerical CAs significantly ($p < 0.001$) and this effect was found as dose-dependent ($r = 0.757$, $p < 0.0005$, Table V). Although vinorelbine slightly increased the structural CA frequency, this increase was not statistically significant ($p > 0.05$). A significant increase in the MI values was observed in all doses of vinorelbine ($p < 0.001$).

Induction of SCEs is significantly ($p < 0.001$) and dose-dependently increased by vinorelbine compared with the negative control ($r = 0.823$, $p < 0.001$) as seen in Tables III and VI.

Two-way ANOVA was done to determine different interindividual and gender-specific genotoxic responses to fotemustine and vinorelbine. For this aim, we used the CA, SCE and MI data resulting from fotemustine and vinorelbine treatment in each donor and gender. Individual responses to fotemustine were analyzed utilizing the

Table I. Frequencies of chromosome aberrations and mitotic index in cultured human lymphocytes treated with fotemustine and its statistical evaluations.

Test substance	Dose [μg/ml]	Mitotic index	Total abnormalities ^a										TCA/Cell ^b	TCA-(G+P)/Cell ^b
			CB	ICB	G	SCA	E	ACF	P	DS	R			
Distilled water		5.45 ± 1.33	17	4	50	0	1	0	0	0	0	0.07 ± 0.03	0.02 ± 0.02	
Fotemustine	2	4.99 ± 1.51	211	52	237	111	26	2	2	0	0	0.63 ± 0.18***	0.39 ± 0.12***	
	4	4.45 ± 1.19	369	135	331	173	103	2	2	2	0	1.11 ± 0.24***	0.79 ± 0.21***	
	8	3.58 ± 0.96**	565	251	381	200	205	4	4	0	0	1.59 ± 0.47***	1.21 ± 0.36***	
Mitomycine-C	0.25	2.15 ± 0.19	125	76	37	4	32	37	7	5	2	2.13 ± 0.86	1.84 ± 0.73	

^a A total of 4000 cells was recorded for analysis of chromosome aberrations. CB, chromatid break; ICB, iso-chromatid break; G, gap; SCA, sub-chromatid aberrations; E, exchange; ACF, A-centric fragments; P, pulverization; DS, dicentric chromosome; R, ring chromosome.

^b TCA, total chromosome aberrations; TCA-(G+P), total chromosome aberrations excluding gaps and pulverizations.

** $p < 0.001$; *** $p < 0.0005$.

Means \pm standard deviation.

Table II. Induction of chromosome aberrations and the mitotic index values in cultured lymphocytes of each donor treated with fotemustine.

Gender ^d	Age	Fotemustine dose									
		Control					2 µg/ml				
		MI ^a (%)	TCA +(G+P) ^b	TCA -(G+P) ^c	MI ^a (%)	TCA +(G+P) ^b	TCA -(G+P) ^c	MI ^a (%)	TCA +(G+P) ^b	TCA -(G+P) ^c	MI ^a (%)
M	18	5.23	0.06	0.02	4.38	0.49	0.35	3.75	0.77	0.57	2.76
M	25	6.63	0.06	0.03	6.93	0.63	0.43	4.18	1.20	1.01	4.32
F	22	4.39	0.04	0.00	5.04	0.82	0.54	6.02	1.05	0.76	4.48
M	21	4.95	0.11	0.05	3.76	0.87	0.48	5.83	1.20	0.72	2.66
F	19	6.37	0.09	0.03	4.12	0.75	0.37	3.90	1.25	0.75	3.25
M	19	6.64	0.04	0.02	6.54	0.38	0.26	5.20	1.00	0.62	4.96
M	21	5.71	0.10	0.03	4.23	0.86	0.50	4.33	1.38	0.85	2.63
M	25	8.29	0.08	0.01	6.53	0.52	0.29	5.07	0.65	0.33	4.83
F	20	3.16	0.10	0.03	2.87	0.90	0.59	2.28	1.68	1.36	2.58
F	34	5.45	0.06	0.02	3.77	0.56	0.38	3.92	0.91	0.57	2.57
M	20	5.25	0.05	0.01	5.41	0.36	0.19	4.16	1.30	0.85	3.04
F	31	5.65	0.14	0.04	5.41	0.46	0.27	5.34	1.00	0.75	3.64
F	21	5.72	0.06	0.02	5.43	0.62	0.45	4.85	0.97	0.67	3.69
F	20	2.68	0.08	0.02	2.02	0.60	0.52	1.68	1.22	0.92	1.87
F	20	5.94	0.06	0.00	4.76	0.60	0.34	5.26	1.04	0.80	5.20
F	18	7.48	0.04	0.00	8.54	0.72	0.40	6.53	0.89	0.68	4.24
M	29	4.97	0.04	0.00	6.20	0.47	0.19	4.82	1.04	0.78	4.78
F	22	3.95	0.09	0.02	4.00	0.52	0.34	3.43	1.48	1.04	3.23
F	18	4.74	0.10	0.08	4.16	0.47	0.35	3.90	1.18	0.94	3.69
F	21	5.76	0.04	0.00	5.72	0.96	0.54	4.33	1.08	0.76	3.24
Mean ± S.D.		5.45 ± 1.33	0.07 ± 0.03	0.02 ± 0.02	4.99 ± 1.51	0.63 ± 0.18	0.39 ± 0.12	4.45 ± 1.19	1.11 ± 0.24	0.79 ± 0.21	3.58 ± 0.96
											1.59 ± 0.47
											1.21 ± 0.36

^a MI, mitotic index.^b TCA+(G+P), total chromosome aberrations including gaps and pulverizations.^c TCA-(G+P), total chromosome aberrations excluding gaps and pulverizations.^d F, female; M, male; *n* = 20.

Test substance	Dose [$\mu\text{g/ml}$]	Min.-Max. SCE ^a	SCE/Cell ^b	HFC ^c
Control		0– 10	3.08 \pm 0.81	0.00 \pm 0.00
Fotemustine	2	6– 41	18.46 \pm 6.65***	0.00 \pm 0.00
	4	14– 83	39.48 \pm 12.28***	0.00 \pm 0.00
	8	32–118	66.10 \pm 17.64***	0.07 \pm 0.15*
Control		0– 10	2.51 \pm 0.93***	0.00 \pm 0.00
Vinorelbine	0.5	0– 16	4.97 \pm 1.80***	0.00 \pm 0.00
	1	2– 18	7.23 \pm 2.01***	0.00 \pm 0.00
	2	4– 21	9.63 \pm 2.42***	0.00 \pm 0.00
Mitomycine-C	0.25	40–115	73.04 \pm 7.03	0.01 \pm 0.04

Table III. Effects of fotemustine and vinorelbine on SCE induction and HFC proportions in human lymphocyte cultures. SCE/Cell and HFC values are percentage averages. (Each treated group $n = 20$; means \pm standard deviation.)

^a SCE, sister chromatid exchange.

^b SCE/Cell, sister chromatid exchange per cell.

^c HFC, high frequency SCE cell.

* $p < 0.05$; *** $p < 0.0005$.

Donor	Gender ^a	Age	Control	Fotemustine dose		
				2 $\mu\text{g/ml}$	4 $\mu\text{g/ml}$	8 $\mu\text{g/ml}$
1	M	18	2.12	12.46	30.80	47.72
2	M	25	4.30	27.24	62.76	72.46
3	F	22	2.80	23.14	36.00	46.94
4	M	21	3.50	15.94	37.56	84.38
5	F	19	3.10	14.24	32.08	43.66
6	M	19	3.88	13.68	26.08	48.36
7	M	21	3.20	12.36	35.64	50.52
8	M	25	3.12	12.56	23.56	59.38
9	F	20	2.62	18.28	38.28	77.52
10	F	34	2.32	16.06	41.56	92.60
11	M	20	2.50	10.40	26.06	38.88
12	F	31	3.68	15.08	36.00	59.24
13	F	21	2.96	20.20	38.04	57.26
14	F	20	3.20	35.32	60.24	86.13
15	F	20	5.33	16.76	40.28	90.24
16	F	18	1.80	12.56	35.32	57.26
17	M	29	2.76	20.88	35.84	65.66
18	F	22	2.64	25.76	51.56	78.24
19	F	18	2.36	16.72	32.80	71.24
20	F	21	3.32	29.32	69.08	94.28

Table IV. Induction of sister chromatid exchanges per cell in cultured lymphocytes of each donor treated with fotemustine.

^a F, female; M, male.

total aberrations data yielding significantly different responses among individuals ($F = 2.715$, $p < 0.001$). Likewise, a significant difference was found among individuals with respect to the results of total aberrations excluding gaps and pulverizations ($F = 2.802$, $p < 0.001$). When we compared the total aberrations between genders we did not find any significantly different overall response to fotemustine ($F = 2.486$, $p > 0.05$). However, each gender exhibited a different response when we excluded the gaps and pulverizations from the total aberration data ($F = 5.855$, $p < 0.05$).

We used numerical CA values for the comparison of interindividual variation differences following vinorelbine treatment. Statistically significant differences in the numerical CAs in each donor

were found ($F = 1.949$, $p < 0.05$). However, there was no significant difference between two genders according to numerical CA values.

Moreover, interindividual variations in the MI values were significantly different within the fotemustine treatments ($F = 8.987$, $p < 0.001$). Additionally, significantly different MI responses in each gender were also found ($F = 4.262$, $p < 0.05$).

Although individuals showed different MI responses to vinorelbine ($F = 4.019$, $p < 0.001$), we did not find any significant variation among male and female donors according to MI values ($F = 0.140$, $p > 0.05$).

Highly significant variations in the SCE response to fotemustine were obtained among individuals ($F = 3.467$, $p < 0.001$) and also in each gender ($F = 6.250$, $p < 0.05$). Similarly, with re-

Table V. Frequencies of structural chromosome aberrations and mitotic index in cultured human lymphocytes treated with vinorelbine and corresponding statistical evaluations ($n = 20$).

Test subs. ^a	Dose [$\mu\text{g/ml}$]	Mitotic index	Total abnormalities ^b							TCA/Cell ^c TCA – (G+P) /Cell ^c	Numerical CA ^d		Total numerical CA ^d	
			CB	ICB	G	SCA	E	ACF	P		DS	R		Hypo
D.W. VRB		5.02 ± 1.24	15	0	42	0	0	0	0	0	0.06 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	0.5	9.51 ± 2.75**	28	2	20	0	0	1	3	0	0.04 ± 0.03	0.03 ± 0.03	0.33 ± 0.11**	0.36 ± 0.11***
	1	10.00 ± 4.51**	36	1	13	0	0	1	2	0	0.05 ± 0.05	0.03 ± 0.04	0.39 ± 0.14**	0.44 ± 0.13***
	2	8.08 ± 4.71**	38	1	27	0	1	3	3	0	0.06 ± 0.08	0.04 ± 0.06	0.42 ± 0.14**	0.46 ± 0.15***
MMC	0.25	2.15 ± 0.19	125	76	37	4	32	37	7	5	2	2.13 ± 0.86	1.84 ± 0.73	

^a D.W., distilled water; VRB, vinorelbine; MMC, mitomycin-C.^b A total of 4000 cells was recorded for analysis of chromosome aberrations in each dose and control. CB, chromatid break; ICB, iso-chromatid break; G, gap; SCA, sub-chromatid aberrations; E, exchange; ACF, A-centric fragments; P, pulverization; DS, dicentric chromosome; R, ring chromosome.^c TCA, total chromosome aberrations.^d CA, chromosome aberrations.** $p < 0.001$; *** $p < 0.0005$.Means \pm standard deviation.

spect to the SCE response to vinorelbine, there was a significant interindividual variation ($F = 7.667$, $p < 0.001$). Lastly, a significant gender-specific SCE response to vinorelbine was evident ($F = 15.176$, $p < 0.001$).

Discussion and Conclusion

Chromosome aberrations (Natarajan, 2002; Lopez-Nigro *et al.*, 2003), SCEs, HFCs (Wolff, 1982) and aneuploidy (Kirsch-Volders and Fenech, 2001) have long been recognized to be important biomarkers of human exposure to ionizing radiation and genotoxic chemicals, and sensitive parameters for describing induced DNA and spindle damage. These events can lead to the loss of chromosomal material at mitosis and may lead to cell death through loss of chromosomal DNA (Tucker and Preston, 1996). In this investigation, we assessed the induction of CAs, SCEs and aneuploidy, and its cytotoxic effect using the MI parameter in human lymphocytes *in vitro*. The results of our study indicated that fotemustine caused a significant increase in the SCE frequency and CAs in human lymphocytes. These effects of fotemustine may be cross-links in DNA as an alkylating agent. It was shown that vinorelbine produced hypo- and hyperploidy. These findings are in agreement with those observed in previous studies in which aneuploidy was determined using micronucleus assays with fluorescence in *in situ* hybridization (Gonzalez-Cid *et al.*, 1999).

The MI was decreased significantly by fotemustine treatment. This effect of fotemustine may cause cell death and DNA damage. The mitotic arresting activity of vinorelbine was also manifested in a high number of cells blocked at metaphase, in that substantial increase in the MI was observed in all tests. Similar results were also found in Chinese hamster cells (Rainaldi *et al.*, 1987) and in mice exposed to *Vinca* alkaloids or other antimitotic drugs (Pacchierotti *et al.*, 1991).

In the past decade, inter-individual and gender-related variability in drug response have received much attention. In this study, we have set out to determine these variabilities in genotoxic response to fotemustine and vinorelbine. We found statistically significant differences in the induction of CAs, SCEs, aneuploidy cells and MI among individuals for fotemustine and vinorelbine. These different genotoxic responses were also determined between genders. Individual and gender-related

Donor	Gender ^a	Age	Control	Vinorelbine doses		
				0.5 µg/ml	1 µg/ml	2 µg/ml
1	F	25	2.43	5.91	9.00	11.56
2	F	23	3.87	8.96	12.02	16.87
3	F	22	2.78	6.65	8.83	8.63
4	F	22	3.12	5.84	6.87	9.31
5	M	25	1.06	2.43	3.75	6.18
6	M	27	0.97	2.64	5.07	9.80
7	F	19	2.04	4.12	4.24	6.89
8	F	22	2.67	5.14	7.26	8.20
9	F	22	2.85	4.57	6.80	9.20
10	F	21	2.54	6.09	9.32	13.43
11	F	22	2.44	4.21	7.54	10.57
12	F	21	2.87	5.50	6.89	7.46
13	F	22	3.41	6.19	8.74	10.13
14	M	29	2.76	4.08	5.52	7.59
15	F	31	3.40	4.94	6.82	7.66
16	M	28	0.52	1.68	4.78	9.11
17	F	21	2.56	4.85	6.89	10.13
18	M	26	1.20	2.29	6.86	9.47
19	F	20	3.74	6.48	7.70	9.02
20	M	40	3.04	6.82	9.64	11.36

Table VI. Induction of sister chromatid exchanges per cell in cultured lymphocytes from each donor treated with vinorelbine.

^a F, female; M, male.

variations can be caused by genetic polymorphisms, age and even gender associated with hormonal function (Aldridge *et al.*, 2003).

Many chemotherapeutic drugs have unintentional side effects during the treatment of patients and on the health-care personnel exposed chronically to these drugs. However, such favorable survival rate brings additional concern about the long-term adverse effects of chemotherapy, markedly on fertility and in the incidence of secondary malignancies (Tiburi *et al.*, 2002; Terracini *et al.*, 1987). Therefore, it is essential that effective anti-cancer drugs should be tested not only for their

cytotoxic potential but also for their ability to disturb genomic integrity, in order to render a deeper understanding of the potential risks related to exposing in anytime. Different individual and gender responses to fotemustine and vinorelbine demonstrated may also be indicative of a dose selection in chemotherapy.

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