PHARMACOLOGY AND TOXICOLOGY

Effect of Regular Intake of Vitamin-Mineral Complex on Spontaneous and on Induced Clustogenesis in Humans

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Regular intake of a vitamin-mineral complex for 2 weeks had no effect on spontaneous clustogenesis in blood cells from healthy donors. Significant differences between the levels of chromosome aberrations induced by bleomycin and dioxidine *in vitro* before and after 2-week vitamin treatment indicate increased resistance of blood cells to clustogens.

Key Words: *spontaneous clustogenesis; induced clustogenesis; vitamin-mineral complex*

Reports about the effects of vitamins and trace elements on spontaneous and induced mutagenesis observed in mammalian cells *in vitro* and *in vivo* are contradictory. Some studies demonstrated antimutagenic effects of vitamins which were attributed to their capacity to inhibit induced free-radical oxidation [2, 9,13,14], while others detected comutagenic and even mutagenic effects of vitamins which were explained by their autooxidation, associated with initiation of free-radical oxidation [2,8,10,14,15]. The effect of vitamins and trace elements on human chromosomes is poorly studied.

In some recent studies of cell sensitivity to mutagenic effects was evaluated *in vitro*, depending on vitamin and trace element consumption by man [7,12]. It was discovered that ascorbic acid in a daily dose of 1 g decreased the sensitivity of cultured peripheral blood lymphocytes to clustogenic effect of bleomycin [12].

We analyzed chromosome aberrations (CrA) in native and bleomycin- and dioxidine-treated peripheral blood cells from donors before and after 2-week therapy with a multicomponent vitamin-mineral complex (VMC).

MATERIALS AND METHODS

The study was carried out on peripheral blood lymphocytes from healthy volunteers (9 women and 7 men) aged 23-54 years (mean age 32.5±2.3 years) who had not been x-rayed for 6 months before, had no respiratory viral diseases for 3 months, and had no contacts with chemical production.

Donors were treated with VMC Vitabalance-2000 (Vitamacs) containing vitamins in doses 2-20-fold surpassing the daily requirement and minerals and bioactive additives (one tablet 3 times a day orally for 2 weeks).

Peripheral blood for cytogenetic analysis was collected from the ulnar vein before and 2 weeks after VMC therapy. Blood was cultured routinely for 54 h.

Standard prooxidant clustogens bleomycin (Sigma) and dioxidine (Pharmacon) were added to blood cells after 50-hour culturing (G2 period) in doses of 1 U/ml and 0.1 mg/ml, respectively.

Cytogenetic preparations were prepared by the standard dry air method and analyzed as described

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previously [5,6]. Cells with single and paired fragments, exchanges, multiple chromosome aberrations (more than 5 CrA per metaphase spread), and destroyed metaphases (more than 20 CrA per metaphase spread) were counted (100-300 metaphase plates from each donor were analyzed).

The results were statistically processed using φ-test; the number of cells with CrA before and after VMC treatment was compared for each volunteer and the mean values were compared.

RESULTS

The number of destroyed cells before and after VMC was 3.2±0.3 and 3.4±0.3%, respectively (difference is insignificant, Table 1). Our data agree with the results obtained by N. P. Bochkov *et al.* in epidemiological studies characterizing chromosome variability in modern Russian population [1].

Hence, 2-week course of VMC does not affect spontaneous clustogenesis in blood cells from healthy donors.

Before VMC, the number of cells with CrA induced by bleomycin and dioxidine was significantly higher than in the control (Tables 2 and 3), which is in line with the reports about clustogenic activity of bleomycin and dioxidine *in vitro* [2,7,12]. The total number of abnormal metaphases after bleomycin treatment decreased significantly after 2-week therapy with VMC ($p<0.05$, Table 2), as did the number of paired fragments; no significant changes in other types of aberrations were observed.

Analysis of individual levels of CrA induced with bleomycin showed that the number of damaged metaphases after 2-week treatment with VMC tended to decrease (Table 2), but no significant differences between individual values before and after VMC were detected.

The mean percentage of dioxidine-damaged cells was significantly ($p<0.01$) decreased after VMC, the number of single and paired fragments being significantly decreased ($p<0.05$, Table 3).

Analysis of individual changes in donor 31 showed significantly ($p<0.05$) decreased number of damaged

TABLE 1. Effect of VMC on Spontaneous Clustogenesis in Donors

Parameter	Before VMC		After VMC	
	number of examined cells	percentage of damaged cells, *M±m*	number of examined cells	percentage of damaged cells, *M±m*
Individual values, code:				
11	300	4.3±1.2	300	4.0±1.1
13	300	3.6±1.1	300	4.3±1.2
16	300	3.3±1.0	300	3.0±1.0
17	300	1.7±0.6	258	1.6±0.7
18	300	4.7±1.2	300	4.7±1.2
20	291	3.8±1.1	300	5.3±1.3
21	300	1.6±0.7	115	4.4±1.9
22	194	5.2±1.6	121	2.5±1.4
23	292	3.4±1.1	300	2.3±0.9
24	204	2.0±1.0	300	4.0±1.1
30	200	2.0±0.9	300	2.3±0.9
31	200	1.5±0.9	300	2.6±0.9
37	300	4.3±1.2	300	2.3±0.9
38	300	3.0±1.0	300	3.3±1.0
39	300	4.0±1.1	300	4.7±1.2
Mean values	4081	3.2±0.3	4094	3.4±0.3
Number of CrA per 100 cells				
single fragments	3.1		3.3	
paired fragments	0.1		0.1	
exchanges	0.04		0.02	

TABLE 2. Effect of VMC on Clustogenic Effect of Bleomycin in Cultured Donor Lymphocytes

Parameter	Before VMC		After VMC	
	number of examined cells	percentage of damaged cells, $M\pm m$	number of examined cells	percentage of damaged cells, $M\pm m$
Individual values, code:				
11	300	24.0±2.5	300	22.9±2.4
13	300	35.0±2.7	300	30.6±2.7
16	300	20.6±2.3	300	20.0±2.3
17	300	23.3±2.4	106	18.5±3.8
20	106	21.6±4.0	300	15.9±2.1
21	260	19.7±2.5	120	15.8±3.3
30	150	16.7±3.0	300	12.7±1.9
31	200	20.0±2.8	300	18.3±2.2
37	160	15.6±2.9	159	12.6±2.6
38	136	15.4±3.1	300	19.1±2.3
39	300	17.7±2.2	300	17.7±2.2
Mean values	2512	20.9±0.8	2785	18.6±0.7*
Number of CrA per 100 cells				
single fragments	17.3		15.7	
paired fragments	1.5		0.9*	
exchanges	0.3		0.1	
multiple aberrations	1.3		1.1	
destructured metaphases	0.5		0.8	

Note. *$p<0.05$ *vs.* the level before VMC.

cells after VMC. The number of dioxidine-induced metaphase abnormalities in blood cells from this donor decreased by 42% after 2-week VMC course. In other donors no significant changes were detected in individual levels of aberrant cells, although the number of aberrations tended to decrease after vitamin course.

Hence, VMC did not affect the level of spontaneous clustogenesis in donor blood cells, which indicates genetic safety of this complex. No comutagenic effect of VMC was detected in experiments with prooxidants bleomycin and dioxidine. Studies of VMC effect on the clustogenic effects of bleomycin and dioxidine mediated by their prooxidant activity showed statistically significant differences between the groups and individuals attesting to improved resistance of donor lymphocytes to these clustogens after VMC treatment. The study confirmed that some vitamins and vitamin preparations improved the resistance of human cells to mutagens [3,4,11,12]. The antimutagenic effect of vitamins can be explained by inhibition of induced free-radical oxidation and maintenance of normal redox state of the cell. However other scientists demonstrated prooxidant effects of some vitamins, which showed, along with protective effects, comutagenic and even mutagenic properties, depending on the dose [2,3]. Therefore this report cannot be regarded as the final proof of the prophylactic effect of all vitamin complexes of different qualitative and quantitative composition with regard to mutagenesis. On the other hand, this study demonstrated that creation of genome-protective agents on the basis of vitamins and other bioactive compounds holds good promise.

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TABLE 3. Effect of VMC on Clustogenic Effect of Dioxidine in Cultured Donor Lymphocytes

Parameter	Before VMC		After VMC	
	number of examined cells	percentage of damaged cells, $M\pm m$	number of examined cells	percentage of damaged cells, $M\pm m$
Individual values, code:				
11	300	7.0±1.5	300	5.0±1.3
13	300	11.3±1.8	300	7.6±1.5
16	300	4.3±1.2	300	3.7±1.1
17	300	4.3±1.2	300	4.0±1.1
18	300	4.7±1.2	300	4.3±1.2
20	165	9.7±2.3	300	4.3±1.2
21	184	8.2±2.0	116	4.3±1.9
22	100	7.0±2.6	100	5.0±2.2
23	210	4.3±1.4	300	5.7±1.3
24	107	3.7±1.8	300	4.3±1.2
30	300	5.3±1.3	300	5.6±1.3
31	300	10.3±1.8	300	6.0±1.4*
37	227	7.5±1.7	191	6.2±1.7
38	176	5.7±1.7	300	5.7±1.3
39	300	6.0±1.4	300	5.0±1.3
Mean values	3569	6.6±0.4	4007	5.1±0.3*
Number of CrA per 100 cells				
single fragments		6.2		4.9**
paired fragments		0.4		0.2*
exchanges		—		0.02
multiple aberrations		0.04		—

Note. *$p<0.01$, **$p<0.05$ *vs.* the level before VMC.

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