PHARMACOLOGY AND TOXICOLOGY

Effect of Vitamin Therapy on Sensitivity of Peripheral Blood Lymphocytes to Clastogenic Effect of Mutagens *In Vitro*

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Spontaneous and *in vitro* induced (with 0.01 and 0.1 mg/ml dioxidine and 0.1 and 1.0 U/ml bleomycin) chromosome aberrations were counted in cultured peripheral blood cells from 11 donors before and after 2-week therapy with a vitamin complex. The complex contained the major vitamins in doses not surpassing the recommended daily doses. Vitamins had no effect on spontaneous mutations, but increased cell resistance to clastogenic effects of dioxidine in a concentration of 0.1, but not 0.01 mg/ml. Cell sensitivity to bleomycin notably increased after vitamin therapy in some donors and decreased in others, the mean parameters in the group remained virtually unchanged.

Key Words: *vitamins; chromosome aberrations; human cells*

The necessity for additional therapy with vitamins and trace nutrients aimed at liquidation of their deficiency observed in the majority of humans [5] and at prevention of cardiovascular diseases and cancer [6,8,12] is persuasively proven. On the other hand, the effect of vitamins and other essential nutrients on human heredity remains little studied. Only few direct observations were reported [4,11], while the results obtained in animal experiments suggest the possibility of both the positive antimutagenic and negative mutagenic or comutagenic effects of excessive vitamin intake on human heredity [3].

We investigated the sensitivity of human peripheral blood lymphocytes to the clastogenic effects of dioxidine and bleomycin before and after 2-week therapy with a polyvitamin complex.

MATERIALS AND METHODS

Peripheral blood lymphocytes were isolated from healthy volunteers (5 women and 6 men, mean age 26.8± 1.9 years), who were not X-rayed 6 months, had no viral diseases for 3 months before the study, and had no contacts with chemical production.

The donors received vitamin complex twice a day in doses not surpassing the recommended daily doses. Daily doses were 60 mg vitamin C, 1.2 mg vitamin B_1, 1.2 mg B_2, 1.2 mg B_6, 0.002 mg B_{12}, 13 mg PP, 1 mg A, 7 mg E, 300 I Units D_3, 6 mg pantotenic acid, 0.4 mg folic acid, 0.14 mg biotin, and 2.0 mg β-carotene.

Peripheral blood for cytogenetic study was collected from the ulnar vein before and after 2-week vitamin therapy and the cells were isolated and cultured routinely [1] for 54 h.

Bleomycin (Sigma) in concentrations 0.1 and 1.0 U/ml and dioxidine (Farmakon) in concentrations of 0.01 and 0.1 mg/ml were added 50 h before the end of culturing. The culturing was stopped without washout. All control and experimental samples were cultured in parallel.

Cytogenetic preparations were air dried and analyzed as recommended previously [1,9]. In each experiment 100-300 cells were examined. Chromosome aberrations (CA) of different types were counted. Cells

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with more than 5 chromosome aberrations were referred to cells with multiple aberrations (MA), cells with more than 15 CA were regarded as cells with chromosome destruction (CD).

The results were statistically processed using φ test; the counts of cells with CA, including those with MA and CS, before and after vitamin course in each subject and the mean values were compared.

RESULTS

Before vitamin therapy the metaphase material from 11 donors contained 2.4±0.3% cells with CA; after 2-week vitamin therapy this parameter was 2.8±0.3% (Table 1). Thus, the counts of cells with spontaneous CA before and after vitamin therapy were similar and corresponded to published data on chromosome variability in normal subjects in modern Russian population [2]. Individual parameters of chromosome variability before and after vitamin therapy also showed no significant differences.

Hence, 2-week vitamin therapy did not modulate spontaneous clastogenesis in normal human peripheral blood lymphocytes.

Comparison of the mean group values after cell treatment with dioxidine in a dose of 0.01 mg/ml showed no differences in cell sensitivity to the mutagen before and after vitamin therapy. Comparison of the values for each volunteer also showed no statistical differences (Table 2). The percentage of damaged cells in cultures treated with 0.1 mg/ml dioxidine significantly decreased after vitamin therapy compared to the initial values ($p<0.05$). The clastogenic effect of dioxidine significantly decreased in donors Nos. 10 and 14 (Table 2).

Thus, 2-week vitamin therapy improved cell resistance to the clastogenic effect of dioxidine in a concentration of 0.1 mg/ml, but not 0.01 mg/ml.

Comparison of the mean values revealed no significant differences in cultures treated with bleomycin in a dose of 0.1 U/ml. Comparison of individual values before and after vitamin treatment showed statistically significant differences in donors Nos. 15 (increase) and 36 (decrease, Table 3).

The clastogenic effect of bleomycin in a concentration of 1.0 U/ml was the same before and after vitamin therapy (Table 3). Comparison of individual values showed that vitamins increased cell sensitivity to the mutagen in donors Nos. 32 and 41 and decreased it in donors Nos. 34 and 35 (Table 3).

Hence, the mean group values characterizing the clastogenic effect of bleomycin did not change after vitamin course, but in some donors individual cell sensitivity to the mutagen decreased or increased.

The results of experiments with bleomycin are difficult to interpret. Usually the mean group values are the main parameter in evaluation of drug efficiency using specific biological markers, *e.g.* CA [7], and hence, our results indicate that vitamin therapy did not modify cell sensitivity to the mutagenic effect of bleomycin. However this approach ignores opposite effects produced by vitamin therapy in some donors. The possibility of opposite changes in mutagenic effects in genetically heterogeneous human population was previously hypothesized [3]. Our results directly

TABLE 1. Spontaneous Clastogenesis in Peripheral Blood Lymphocytes from Healthy Donors before/after Vitamin Therapy (*M*±*m*)

Examinee code	Number of examined cells	Per 100 cells			Percentage of damaged cells
		fragments		exchanges	
		solitary	paired		
10	300/300	3.3/3.0	—/—	—/—	3.0±1.0/3.0±1.0
12	300/300	1.7/1.3	0.3/—	—/—	2.0±0.8/1.3±0.7
14	300/300	3.3/4.0	—/—	—/—	3.3±1.0/4.0±1.1
15	168/300	1.3/1.7	0.3/0.3	—/—	1.3±0.9/2.0±0.8
32	300/155	2.3/3.9	—/—	—/—	2.6±0.9/3.9±1.6
33	300/100	2.7/2.0	—/—	—/—	2.7±0.9/2.0±1.4
34	300/300	2.7/2.7	—/—	—/—	2.7±0.9/2.7±0.9
35	300/300	2.0/2.7	—/—	—/—	2.0±0.8/2.7±0.9
36	300/152	4.0/2.0	—/—	—/—	4.0±1.1/2.0±1.1
40	300/300	1.0/3.7	—/—	0.3/—	1.3±0.7/3.7±1.1
41	300/300	1.7/2.7	—/0.3	—/—	1.7±0.7/3.0±1.0
Mean values for the group	3168/2807	2.4/2.7	0.05/0.05	0.03/—	2.4±0.3/2.8±0.3

confirm this hypothesis. The fact that vitamin therapy modulates the effect of 0.1 and 1.0 U/ml bleomycin in different donors does not contradict this hypothesis. The effect of prooxidants (bleomycin included) in biological systems is described by intricate concentration relationships [10] and depends on concentration of both the agent and antioxidant factors and is therefore specific in each individual case. This fact is directly confirmed by high variability of quantitative effects of mutagens before and after vitamin therapy and low variability of spontaneous mutagenesis.

Hence, vitamin therapy can specifically and oppositely modulate *in vitro* cell sensitivity to clastogenic effect of bleomycin in different individuals.

Our data indicate that vitamin therapy differently modulates the effects of the studied mutagens. Hence, the modulating influence of additional vitamin treatment on the effects of various trace mutagens can be highly specific, and it is difficult to evaluate the influence of vitamin treatment on human heredity from the benefits/risks viewpoint.

Our observations cannot be fully extrapolated to *in vivo* situation, but proved the need for more profound studies of wide-scale uncontrolled use of various vitamin complexes and bioactive nutrients on spontaneous and induced mutagenesis in humans.

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TABLE 2. Clastogenic Effect of Dioxidine on Donor Blood Cells before/after Vitamin Therapy (*M*±*m*)

Drug dose, mg/ml; examinee code	Number of examined cells	Per 100 cells			Percentage of damaged cells
		fragments		exchanges; MA for the dose of 0.1 mg/ml	
		solitary	paired		
0.01					
10	300/300	7.7/5.3	—/0.7	—/—	7.7±1.5/6.0±1.4
12	300/300	4.0/3.3	—/—	—/—	4.0±1.1/3.3±1.0
14	300/300	2.7/4.3	0.7/—	—/—	3.4±1.0/4.3±1.2
15	227/300	3.1/2.7	—/—	—/—	3.1±1.2/2.7±0.9
32	168/100	3.6/5.0	—/—	—/—	3.6±1.4/5.0±2.2
33	186/123	3.8/3.3	—/—	—/—	3.8±1.4/3.3±1.6
34	300/300	3.7/4.0	—/0.3	—/0.3	3.7±1.2/4.6±1.2
35	300/300	4.7/2.7	0.3/0.3	—/—	5.0±1.3/3.0±1.0
36	206/100	6.8/5.0	0.5/—	0.5/—	7.8±1.9/5.0±2.2
40	300/215	4.3/3.7	—/—	—/—	4.3±1.2/3.7±1.3
41	300/300	4.0/3.7	0.3/—	0.3/—	4.6±1.2/3.7±1.1
Mean values for the group	2887/2638	4.4/3.9	0.2/0.1	0.07/0.03	4.6±0.4/4.0±0.3
0.1					
10	300/300	11.7/7.0	—/—	0.3/—	12.0±1.9/7.0±1.5*
12	300/300	4.3/6.7	—/—	—/—	4.3±1.2/6.7±1.4
14	300/300	5.7/2.7	—/—	0.3/—	6.0±1.4/2.7±0.9*
15	300/300	5.7/4.3	—/0.3	—/—	5.7±1.3/4.6±1.2
32	300/200	5.0/6.5	—/0.5	—/—	5.0±1.3/7.0±1.8
33	147/100	6.3/6.0	—/—	—/—	6.3±2.0/6.0±2.4
34	300/300	7.3/5.7	1.3/—	0.3/—	8.9±1.6/5.7±1.3
35	300/300	8.0/4.3	0.3/0.3	—/—	8.3±1.6/4.6±1.2
36	143/263	7.0/6.5	0.7/0.4	—/—	7.7±2.2/6.9±1.6
40	300/300	4.3/4.7	0.3/—	—/—	4.6±1.2/4.7±1.2
41	300/300	4.0/4.0	—/—	—/—	4.0±1.1/4.0±1.1
Mean values for the group	2990/3263	6.3/5.3	0.2/0.1	0.1/—	6.6±0.5/5.4±0.4*

Note. Here and in Table 3: *$p<0.05$ compared to initial values.

TABLE 3. Clastogenic Effect of Bleomycin on Donor Blood Cells before/after Vitamin Therapy (*M*±*m*)

Drug dose, U/ml; volunteer's code	Number of examined cells	Per 100 cells					Percentage of damaged cells
		fragments		exchanges	MA	DC	
		solitary	paired				
0.1							
10	300/300	15.7/15.7	—/0.7	—/—	1.3/0.3	2.7/—	19.7±2.3/16.7±2.2
12	300/300	5.0/7.0	—/—	—/—	0.3/0.3	0.7/0.3	6.0±1.4/7.6±1.5
14	300/300	6.0/4.7	—/0.3	—/—	0.7/—	0.3/—	7.0±1.5/5.0±1.3
15	300/300	4.7/8.3	—/0.3	—/—	0.3/1.0	—/—	5.0±1.3/9.6±1.7*
32	300/165	6.3/10.9	1.7/1.2	—/—	—/—	0.3/1.2	8.3±1.6/13.3±2.6
33	300/100	8.0/8.0	1.7/1.0	—/—	1.0/—	—/—	10.7±1.8/9.0±2.9
34	300/285	6.3/4.6	0.3/0.4	—/—	0.7/—	—/—	7.3±1.5/5.0±1.3
35	300/300	6.0/4.3	1.3/0.7	0.3/0.3	—/—	0.3/0.3	7.9±1.6/5.6±1.3
36	192/300	11.5/6.5	—/—	—/—	1.6/0.5	0.5/0.5	13.6±2.5/7.5±1.9*
40	300/300	4.7/5.3	1.0/0.7	—/—	1.0/1.3	0.3/0.7	7.0±1.5/8.0±1.6
41	300/300	8.0/4.7	—/0.7	—/—	—/0.7	1.0/—	9.0±1.7/6.1±1.4
Mean values for the group	3192/2850	7.5/7.3	0.5/0.5	0.03/0.03	0.6/0.4	0.6/0.3	9.2±0.5/8.5±0.5
1.0							
10	300/300	21.3/21.0	1.7/1.0	—/—	1.0/4.0	—/2.0	24.0±2.5/28.0±2.6
12	300/300	17.3/11.7	—/—	—/—	0.7/1.3	0.7/1.0	18.7±2.3/14.0±2.0
14	300/168	15.3/9.5	—/0.6	—/—	0.7/—	—/1.8	16.0±2.1/11.9±1.9
15	300/300	17.0/17.7	1.3/—	—/0.3	0.3/1.7	—/0.3	18.6±2.2/20.0±2.3
32	300/100	10.0/17.0	2.0/2.0	—/—	1.3/2.0	—/1.0	13.3±2.0/22.0±4.1*
33	300/100	20.3/12.0	1.3/2.0	—/—	1.0/2.0	0.7/1.0	23.3±2.4/17.0±3.8
34	300/300	16.3/10.3	2.7/1.3	—/—	1.7/—	—/0.7	20.7±2.3/12.3±1.9*
35	300/300	22.0/11.7	1.3/1.0	—/—	1.3/0.3	0.7/—	25.3±2.5/13.0±1.9*
36	300/300	15.0/13.3	2.3/1.0	—/—	1.3/0.7	1.0/1.0	19.6±2.3/16.0±2.1
40	300/300	12.7/15.3	0.7/1.0	0.03/0.7	0.3/0.7	0.7/0.7	14.7±2.0/18.4±2.2
41	300/300	9.7/17.7	1.3/—	—/—	1.0/1.0	—/1.7	12.0±1.9/20.4±2.3*
Mean values for the group	3300/2768	16.1/14.3	1.3/0.9	0.03/0.1	1.0/1.2	0.3/1.0	18.7±0.7/17.5±0.7

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