GENETICS

Effects of Vitamin Supply on Spontaneous and Chemically Induced Mutagenesis in Human Cells

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Chromosome aberrations in donor peripheral blood lymphocyte culture were evaluated before, 14 and 30 days after treatment with a vitamin/mineral complex. Treatment with the complex had no effect on spontaneous level of aberrant cells. The number of chromosome aberrations induced by dioxidine or cadmium chloride *in vitro* at the G_2 stage decreased on days 14 and 30 of treatment with the complex.

Key Words: *chromosome aberrations; blood lymphocytes; mutagens; vitamins*

Vitamins, vitamin-mineral complexes (VMC), vitamin-rich bioactive additives and foodstuffs are widely used, but their use is not studied from genotoxicological viewpoint [7,9,10]. It remains unclear whether extra vitamin consumption modulates induced and spontaneous mutagenesis in humans. Published reports are extremely contradictory and, as a rule, present the data on only individual vitamins. For example, mutagenic, comutagenic, and antimutagenic effects of vitamin C were reported [7,13]. Sometimes the data characterizing the genotoxic or antimutagenic effects of vitamins and their complexes with trace elements were obtained by using inadequate approaches and cannot be extrapolated to humans [5,7].

Only few reports evaluate the effects of extra vitamin or VMC intake on the resistance of human cells to the mutagenic effects or the relationship between this parameter and vitamin status of the donor [6,8, 12]. These results are not sufficient for quantitative conclusions on the effects of extra intake of VMC on the level of mutagenesis in human cells.

We evaluated the intensity of chemically induced mutagenesis in human cells depending on the vitamin status.

MATERIALS AND METHODS

The study was carried out in 15 donors (8 females and 7 males). All donors gave informed consent to participation in the study. The mean age of women was 32.4±2.1 years, of men 32.7±2.7 years. Before the experiment the donors were not X-rayed for 6 months, had no viral infections and no occupational contacts with hazardous chemicals for 3 months. During the study all of them did not change the diet, working schedule, and individual life schedule.

Blood samples were collected after overnight fast from the ulnar vein into standard tubes with heparin (Vacutainer) 3 times: before and after 14 and 30 days of VMC treatment.

Vitamin mineral complex (Table 1; the dose divided by half for morning and evening intake) included ginkgo biloba, garlic extract, Q10 coenzyme, grape

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TABLE 1. Daily Doses of Vitamins and Trace Elements Received by Donors during Treatment with VMC

Vitamin	Content	Mineral	Content
A (β-carotene)		Chromium	13 μg
	3200 IU	Calcium	300 mg
C	90 mg	Copper	2 mg
E	98.2 IU	Iodine	300 μg
B_1	20 mg	Iron	8 mg
B_2	20 mg	Magnesium	100 mg
B_5	30 mg	Molybdenum	50 μg
B_6	12 mg	Nickel	2.5 μg
B_{12}	100 μg	Potassium	60 mg
D	600 IU	Selenium	40 μg
K	20 μg	Silicon	20 μg
Niacinamide	50 мг	Vanadium	20 μg
Biotin	60 μg	Manganese	3 mg
Folic acid	800 μg	Phosphorus	230 mg
		Zinc	15 mg

seed extract, ginseng root, guarana, echinacea, spirulina, royal jelly, Alfalfa powder, and chlorella.

Blood was cultured by the standard method in a medium containing 75% (7.5 ml) RPMI-1640 (Sigma), 15% (1.5 ml) fetal serum (BioClot), 10% (1 ml) blood, and 0.02 ml phytohemagglutinin (Difco).

Mutagens were added after 50-h culturing: dioxidine (0.03 mg/ml, 0.1 mg/ml); cadmium chloride (0.02 mg/ml). The total duration of culturing was 54 h. Colchicine was added 2 h before cell fixation.

Cytogenetic preparations were prepared by the standard dry air method and stained with Azur eosin. Chromosome aberrations (CA) were analyzed as recommended [1,11] without metaphase karyotyping. A total of 300-600 cells from each donor were examined at each term and in each variant of the experiment.

Plasma concentrations of vitamins and their metabolites were measured at Laboratory of Vitamins and Mineral Substances of Institute of Nutrition.

The results were statistically processed using DataFit software.

RESULTS

The increase in vitamins concentrations was observed not for all vitamins. The concentrations of antioxidant vitamins (C, E, β-carotene, and carotenoid sum) increased over 30 days (Table 2).

The results of cytogenetic studies in 15 donors are summed up in this paper. At least 4500 cells were analyzed for each point, this allowing detection of 20% alteration of CA level [3].

The detected values and range of values did not differ between each other and virtually did not differ from the results characterizing chromosome variability in normal subjects, residents of Russia (Table 3, Fig. 1) [2].

Each point in the figure means individual measurement. If the results coincide, one point corresponds to two and more individuals. Thus, 30-day treatment with VMC did not modify the level of spontaneous chromosome aberrations in the peripheral blood lymphocytes of donors. The majority of aberrant cells contained one aberration. The distribution of aberration types corresponded to the control summarized in laboratory [4].

Comparison of the mean group values evaluated after cadmium treatment of the cultures showed no differences in donor cell sensitivity to the mutagen before and after 14 days of VMC treatment. The value recorded after 30 days of VMC treatment was significantly lower than before the treatment (Table 3, Fig. 1, *b*).

The results of dioxidine treatment (0.03 mg/kg) are presented in Table 3 and Fig. 1, *c*. Cytogenetic analysis of cell material from donors collected before VMC treatment showed 9.9±0.4% aberrant metaphases. After 14- and 30-day treatment with VMC the number of aberrant cells was 8.1±0.4 and 7.2±0.4%, respectively. Both these values are significantly lower than before VMC treatment.

TABLE 2. Vitamin Supply of Donors during Experiment (*M*±*m*)

Parameter	Normal value	Before VMC treatment	14 days of treatment	30 days of treatment
Vitamin B_2, ng/ml	6.0-20.0	6.3±1.3	11.1±1.5*	10.2±1.9
Vitamin C, mg/ml	0.4-1.5	0.98±0.06	1.11±0.06	1.18±0.06*
Vitamin E, mg/dl	0.8-1.5	0.68±0.03	0.80±0.04*	1.28±0.08***
Retinol, μg/dl	30-80	49.9±3.2	52.9±3.3	54.8±3.9
β-Carotene, μg/dl	10-85	11.5±1.6	32.8±4.3***	59.8±6.5***
Carotenoid sum, μg/dl	80-230	35.7±3.3	56.6±5.7**	92.2±8.4***

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the level before VMC treatment.

TABLE 3. Characteristics of Chromosome Aberrations in Intact (Control) and Mutagen-Treated Cultures

Parameter	Control			Cadmium chloride			Dioxidine (0.03 mg/ml)			Dioxidine (0.1 mg/ml)		
	before VMC	after 14 days of VMC	after 30 days of VMC	before VMC	after 14 days of VMC	after 30 days of VMC	before VMC	after 14 days of VMC	after 30 days of VMC	before VMC	after 14 days of VMC	after 30 days of VMC
Cells with aberrations, % (range of values)	2.8±0.2 (1.3-4.3)	3.0±0.2 (1.7-4.3)	2.7±0.2 (2.0-4.7)	5.5±0.3 (4.0-7.0)	5.2±0.4 (2.3-7.3)	4.3±0.3* (2.0-6.3)	10.0±0.6 (6.0-13.8)	8.1±0.4** (6.3-11.3)	7.2±0.5*** (4.7-11.0)	17.1±0.8 (10.8-24.0)	16.3±0.6 (13.0-20.0)	14.8±0.6* (11.8-20.0)
Aberrations, per 100 cells	3.0±0.2	3.1±0.3	2.8±0.2	5.9±0.3	5.5±0.3	4.4±0.3*	10.6±0.4	8.6±0.4***	7.5±0.4***	19.0±0.5	18.3±0.6	16.5±0.5**
Solitary fragments, per 100 cells	2.4	2.5	2.4	5.0	4.7	3.9*	9.5	7.8	7.0***	17.6	16.6	15.6
Paired fragments, per 100 cells	0.3	0.4	0.3	0.6	0.5	0.4	0.7	0.7	0.4	1.1	1.1	0.6*
Chromatid exchanges, per 100 cells	0.04	0.1	0.04	0.2	0.09	0.04	0.2	0.08	0.09	0.1	0.3	0.1
Chromosome exchanges, per 100 cells	0.1	0.1	0.04	0.3	0.2	0.1	0.2	0.06	0.09*	0.2	0.4	0.2
Number of cells analyzed	4800	4500	4800	4800	4800	4600	4800	4600	4600	5100	4500	5100

Note. *p<0.01, **p<0.01, ***p<0.001compared to the control.

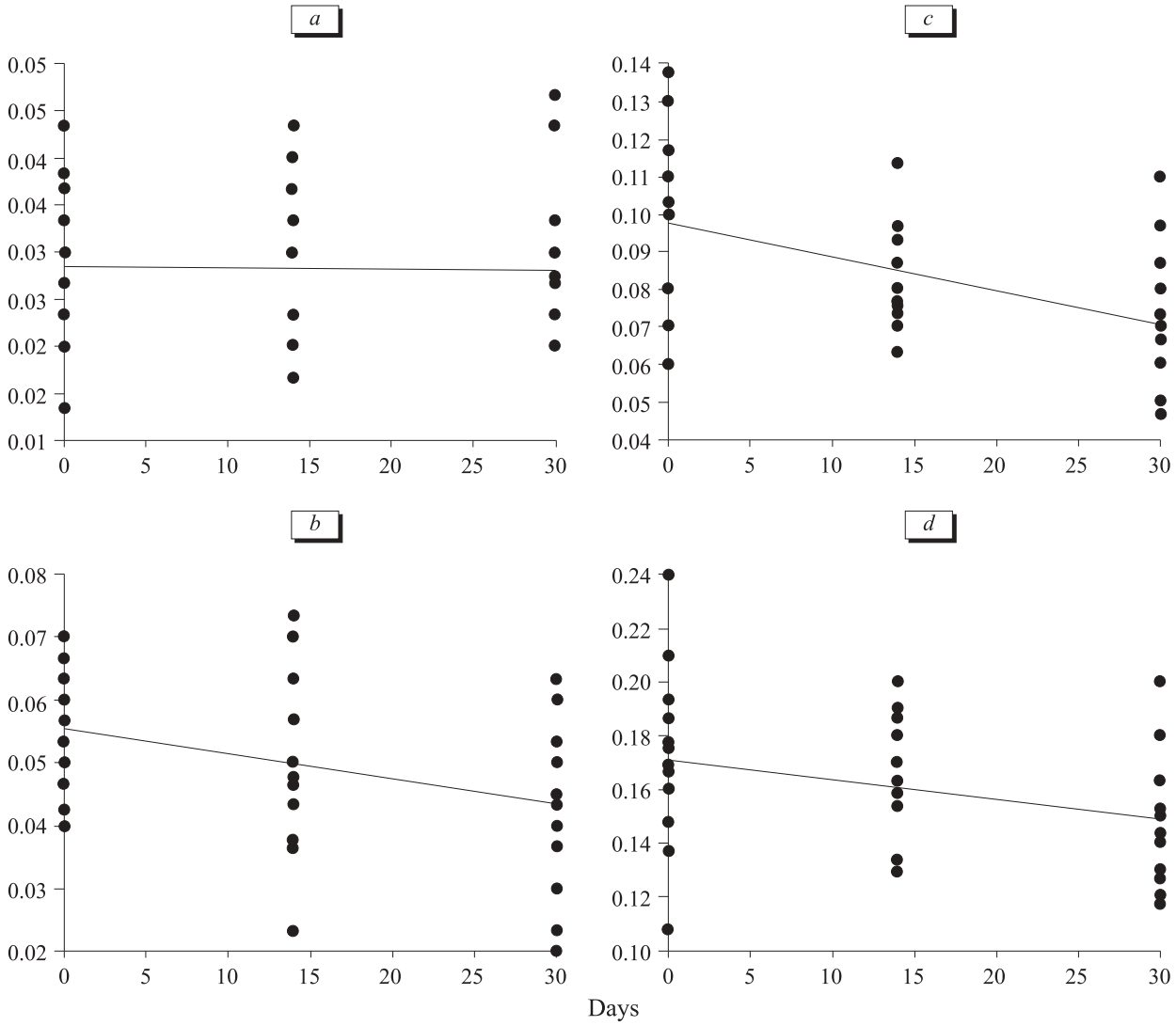

Fig. 1. Dynamics of cell count with chromosome aberrations. *a*) spontaneous level; *b*) cadmium chloride; *c*) dioxidine (0.03 mg/ml); *d*) dioxidine (0.1 mg/ml). Abscissa: duration of VMC treatment; ordinate: number of aberrant cells per 100 metaphases studied.

Increasing dioxidine concentration to 0.1 mg/ml notably increased its cytogenetic effect. Two-week treatment with vitamins did not modulate the mutagenic effect, while after 30-day VMC treatment the incidence of aberrant cells was significantly lower than before the treatment (Table 3, Fig. 1, *d*).

Hence, additional treatment with VMC of the above-mentioned composition appreciably increases the resistance of human cells to some chemical mutagens. The protective effect depends on the duration of treatment with VMC (positive effect was detected after 30 days of treatment in the majority of cases) and on the extent of the cytogenetic effect. It is obvious that weak effect of dioxidine in a dose of 0.03 mg/ml was easier modified than the effect of the same mutagen in a higher concentration.

No increase of the cytogenetic effect of the mutagens was detected in any variant of the experiment. This confirms that purposeful modification of mutagenesis in humans is largely determined by the qualitative and quantitative composition of the complexes [5,6,8]. We can assert that the protective effect of vitamins is higher when they are used by long courses, though the doses of vitamins are also essential.

From the practical viewpoint it is important that the presented VMC composition can be used for improving the resistance to environmental mutagenic exposure.

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