HUMAN ECOLOGY

Individual Sensitivity to Genotoxic Effects of Nickel and Antimutagenic Activity of Ascorbic Acid

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Cytotoxicity of nickel compounds was studied by stimulating the repair synthesis of DNA and counting lymphocyte micronuclei in workers of smelting shop of copper-nickel sulfide processing plant. Nickel content in the organism was evaluated by its concentrations in hair. Therapy with ascorbic acid (1 g/day for 1 month) led to a significant decrease in the number of micronuclei. The number of micronuclei before and after ascorbic acid treatment varied within a wide range in different individuals.

Key Words: *nickel; professional exposure; micronuclei; repair DNA synthesis; ascorbic acid; individual sensitivity*

Variability of individual sensitivity to mutagen is determined by hereditary factors, age, sex, and environmental factors to which humans are exposed during life, starting from intrauterine development. Individual sensitivity to genotoxic effects of chemical factors largely depends on activity of enzymes involved in xenobiotic metabolism, activity of repair systems, nutrition (consumption of vegetables, fruits, coffee, alcohol, *etc.*), and genotype. Four percents individuals are heterozygotes for chromosomal instability syndromes [10,11]. For instance, zero glutathione-S transferase genotype determines high sensitivity to diepoxybutane, as this enzyme is involved in detoxification of this mutagen.

Recent studies were focused on individual sensitivity in chronic occupational exposures to various mutagens. Workers chronically exposed to low-dose radiation are less sensitive to high doses of bleomycin than controls [7]. This phenomenon was called adaptive response. Interestingly, that sensitivity to bleomycin was heterogeneous in the irradiated population, but this heterogeneity was less pronounced than in intact population, in which mutagen sensitivity varied within a greater range. Enhanced free-radical oxidation processes underlie the genotoxic effects of many mutagens, including metals [8,9]. Therefore, quantitative and qualitative characteristics of genotoxic effects depend on the antioxidant system phenotype, sex, and age.

We evaluated the possibility of modifying mutagenesis and repair in workers engaged in nickel smelting with ascorbic acid (AA) and studied the relationship between genetic response and nickel content in the body.

MATERIALS AND METHODS

The content of micronuclei (MN) and repair synthesis (RS) of DNA in lymphocytes were studied in smelting workers exposed to nickel compounds and the content of nickel in hair was measured. In order to reduce the mutagenic effect of nickel, the workers were treated with AA characterized by antioxidant activity [1,6] for 1 month (1 g/day). Control group consisted of workers of other professions working at the same plant.

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Cells were cultured 24 h after blood collection. Blood samples were stored at 0-4°C.

In order to evaluate spontaneous level of sister chromatid exchanges (SCE) and the rate of renewal, peripheral blood lymphocytes from healthy donors were cultured in RPMI 1640 with 10% ETC (both from Biological Industries). Lymphocyte culture included leukocytes and growth medium in 1:9 ratio. Cell division was stimulated by phytohemagglutinin (Difco M) in a dose of 1 μg/ml culture medium. During 2 cycles the cells were incubated with 5-bromo-2-deoxyuridin (Sigma) in a concentration of 5 μg/ml. Fixation was carried out 72-74 h after phytohemagglutinin stimulation by the standard method [4]. The preparations were stained by a modified method [2], and the cell cycle kinetics was evaluated by the mean duration of generation, determined by a previously described method [3].

Micronuclei were counted in 24-h blood lymphocytes. Cells were cultured routinely, as for evaluation of SCE. For making preparations with MN, fixation was carried out 68-72 h after addition of phytohemagglutinin. The preparations were stained by a modified method [2]. At least 2000 cells with intact cytoplasm per point were counted and the number of MN per 1000 cells was estimated by the method modified by G. P. Makedonov *et al.*

For evaluating DNA RS, 24-h blood lymphocytes were cultured in RPMI 1640 with 10% ETC (1:4 cells-medium ratio) and cell division was stimulated with phytohemagglutinin (10 μl/ml, 24 h, 37°C). After 24 h, hydroxyurea (10^{-3} M, Boehringer Hannhein GmbH) was added for 40 min, after which the mixture was centrifuged on the cold for 10 min at 1500 rpm. DNA RS induced by 4-nitrocholine-1-oxide (4NCO) (10^{-7} M) or $NiSO_4$ (10^{-4} M) was investigated by the liquid scintillation method by ^{3}H-thymidine incorporation (7.4×10^3 Bq/ml, Sigma) for 2 h in phosphate buffer (pH 6.8) [4]. The cells were precipitated on nitrocellulose filters (Sartorius, 1.2 μ) and washed (in succession) in Versain solution, distilled water, cold 5% trichloroacetic acid, and ethanol. Filters were put into flasks with scintillation fluid. The intensity of DNA RS was evaluated by the ratio of radioactivity in mutagen-treated cells to radioactivity of control samples. The count was 1300-3000 cpm on a toluene scintillator.

Analysis of hair for trace and macroelements was carried out by atomic emission spectrometry with inductive bound argon plasma on an ICAP-9000 device (Thermo Jarrell Ash).

The data were processed using Microsoft Excel 95 software.

Analysis of mutagenic activity of nickel compounds was carried out with consideration for the length of service in smelting shop, nickel content in hair, and tobacco smoking status.

RESULTS

The content of nickel in the hair of smelting workers was significantly higher than in workers of other professions (18.1 and 7.6 μg/g, respectively). High level of nickel in smelters' hair (27-31 μg/g) correlated with longer length of service in smelting shops (Fig. 1). The content of MN in lymphocytes of workers of other professions was approximately the same (9.2-11.1 NM/10^3 cells), while in smelters this parameter varied from 6.7 to 11.6 MN/10^3 cells. No positive or negative

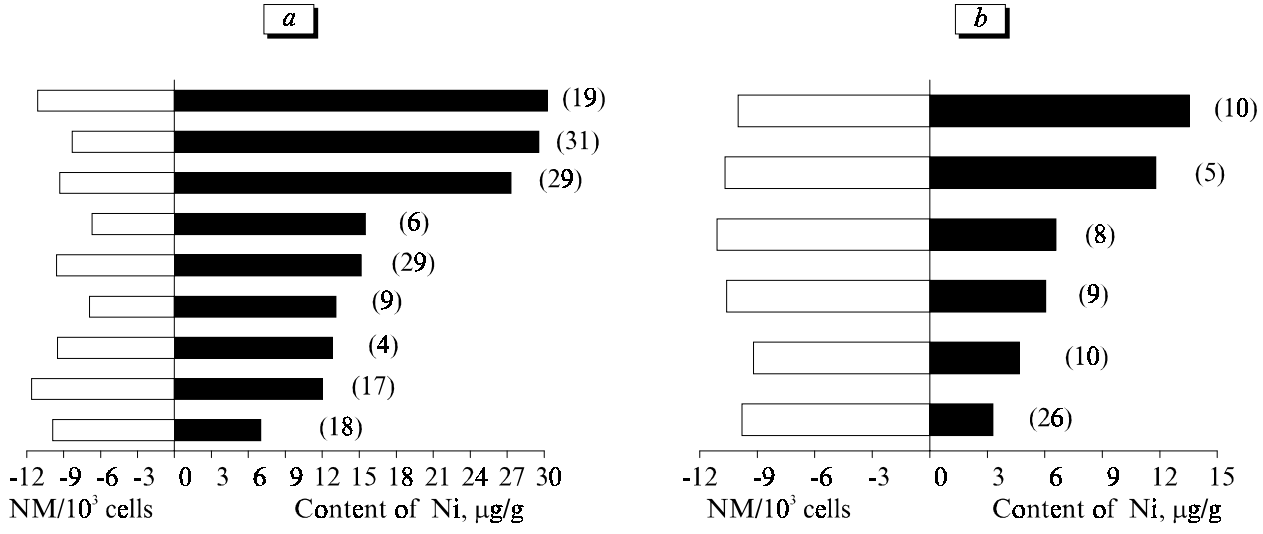

Fig. 1. Variability of individual numbers of micronuclei (NM) in lymphocytes of workers of smelting shops (*a*, *n*=9) and workers of other professions (*b*, *n*=6) depending on the content of nickel in hair. Length of service at copper and nickel sulfide ore processing plant (in years) is shown in parentheses.

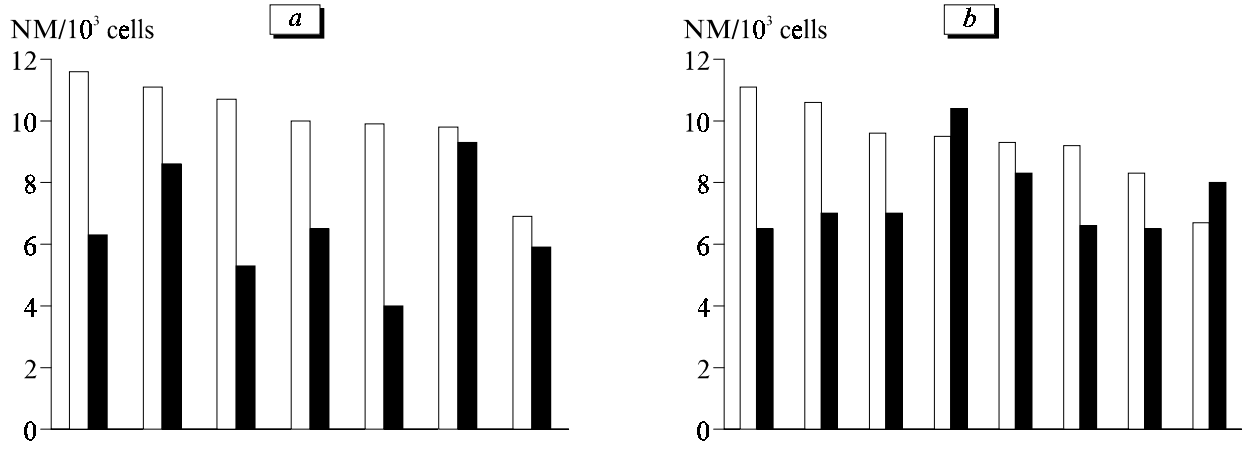

Fig. 2. Individual sensitivity of smoking (*a*) and non-smoking (*b*) workers of smelting shop of nickel processing plant to nickel compounds and ascorbic acid. Here and in Fig. 3: light bars: initial values; dark bars: after a course of ascorbic acid.

correlation between the index of structural chromosome changes and nickel accumulation in the hair was detected in any group.

In the control, nickel accumulation in the hair varied from 0.07 to 0.90 μg/g, while in smelters it ranged from 3.29 to 30.86 μg/g, which corresponded to the difference between the external exposure doses of professional risk groups and of the population not exposed to atmospheric pollution with this production waste.

The initial level of MN was increased virtually in all tobacco smokers in comparison with nonsmokers (Fig. 2) and significantly surpassed the mean population value (3-4 MN/10^3 cells). After a course of AA treatment the level of cytogenetic damage increased by 34% in smokers and by 20% in nonsmokers. In addition, the numbers of structural chromosome aberrations in smokers working in smelting shop varied in a broader range compared to nonsmokers. On the other hand, AA treatment was ineffective in two non-smoking smelters (length of service 4 and 6 years), while DNA RS was notably stimulated. Hence, 13% examines were indifferent to this vitamin; this is in line with previous data that 15-18% normal subjects are incapable of AA assimilation [5], which was believed to be the cause of suppurative complications in the postoperative period.

The relationship between the decrease in the number of MN and nickel concentration in the organism is worthy of note. The efficiency of AA treatment decreased with decreasing nickel content in the body (and virtually the same levels of chromosome aberrations): at nickel accumulation of 4-7 μg/g this decrease was 33%, at 11-16 μg/g 25%, and at 27-31 μg/g 19%.

Analysis of the level of cytogenetic damage with consideration for profession and smoking status showed that the higher was the initial level of structural chromosome aberrations, the higher was the antimutagenic effect of AA treatment (Fig. 3, *b*).

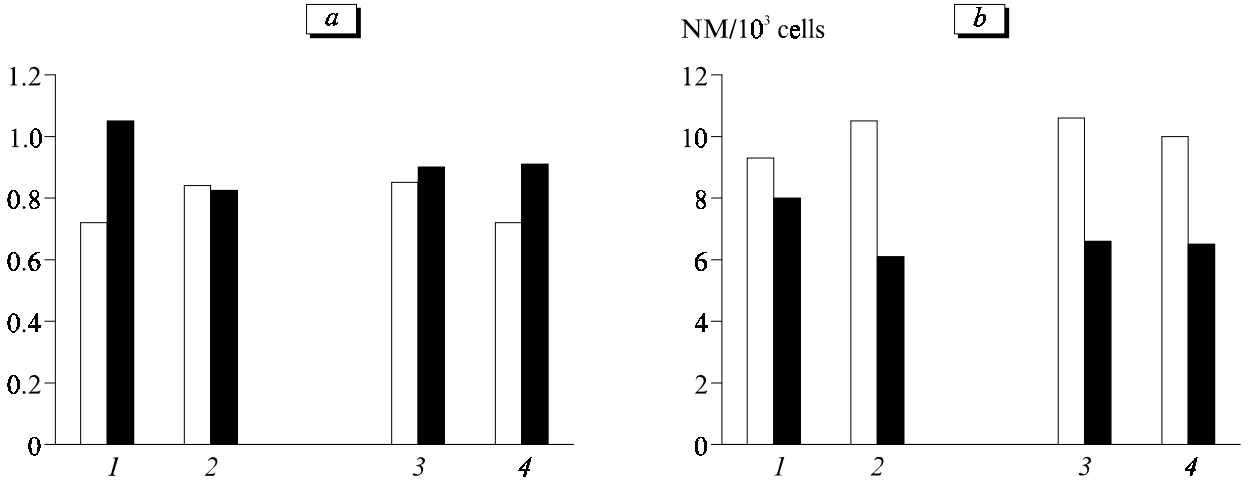

Fig. 3. Modification of genotoxic effect of nickel compounds by ascorbic acid in lymphocytes of smelters (*1, 2*) and workers of other professions (*3, 4*) of smelting shops of nickel plant. *a*) index of DNA reparative synthesis stimulation; *b*) number of micronuclei (median values). *1, 3*) nonsmokers; *2, 4*) tobacco smokers.

Analysis of the other criterion of nickel mutagenic activity, DNA RS, showed virtually complete (95%) inhibition of repair activity of lymphocytes in smelters. This is presumably due to the fact that nickel compounds inhibit DNA repair. Index of DNA RS stimulation induced by 4NCO increased from 0.83±0.06 to 0.91±0.04 after a course of AA (medians 0.79 and 0.90, respectively, n=15). The number of MN and index of lymphocyte repair activity were in positive correlation (Fig. 3, *a*). A slight increase of RS in cells from smelter workers could be due to stimulation of some enzyme systems responsible for repair of induced DNA damage. On the other hand, an essential decrease in the number of MN after AA treatment could be explained by the activity of other reparation stages, which does not rule out the possibility of DNA structure stabilization with participation of AA. Moreover, it is known that AA possessing antioxidant activity realizes its protective function by directly capturing free radicals without pronounced stimulation of DNA RS. The latter assumption is in line with the data on the inverse relationship between antioxidant effect of AA and the level of nickel accumulation in hair.

In the controls with normal level of cytogenetic damage (6-8 SCE/cell) AA treatment decreased this parameter just negligibly (by 6%). Cell capacity to repair increased essentially (from 0.76±0.16 to 1.13± 0.19, median 0.63 and 0.96, respectively, n=6) in experiments with 4NCO, while in experiments with $NiSO_4$ the index of DNA RS stimulation notably decreased (from 1.32±0.22 to 0.99±0.14, median 1.30 and 1.00, respectively, n=7). Differences in the initial levels of 4NCO- and $NiSO_4$-induced DNA RS can be due to different mechanisms of their mutagenic effects; a small sampling does not allow us to draw the final conclusion, though the differences were statistically significant.

Hence, individual genotoxic sensitivity to nickel was characterized by high variability. The genetic effect seemed to be determined by the individual genotype, as it did not depend on the length of service or nickel concentration in the hair. Published reports about the relationship between the genotype and sensitivity to mutagen and even tobacco smoking are scanty [12]. For example, it was revealed that aldehyde dehydrogenase 1-2 genotype was associated with increased frequency of SCE.

In general, the results indicate that in cases when low level of nickel in the hair is associated with a high level of cytogenetic damage, the subjects can be referred to a risk group. These workers are characterized by high sensitivity to mutagenic effect of nickel and are in need of protective and adaptive measures. AA can be used as an effective antimutagen in subjects exposed to nickel.

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