Environmental Susceptibility and Resilience Due to Nuclear Anomalies in The Buccal Cells of Children and Adults From Technogenically - Loaded Regions of Ukraine

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Abstract

The micronucleus (MN) assay as a method for testing of the environmental conditions and the effectiveness of natural adaptogens has been applied on exfoliated buccal cells of mining workers and also children living on the coal and uranium-mining and processing territories of Ukraine (Dnepropetrovsk area). A positive modifying cytogenetic influence of natural adaptogens has been established.

Keywords: environment, mutagens, genotoxicity, cytogenetic human health, exfoliated buccal cells, MN assay.

Introduction

The accumulation in different environmental objects of many toxicants defines the real risks for biota and human health [1]. An especially serious risk for all living organisms is presented by environmental mutagens that can affect the hereditary apparatus of somatic and sex cells, resulting in cancer increases and other ecologically dependent pathologies [2-4]. Therefore, an investigation of mutagenesis in cells at genetic level is very urgent and real. Biological monitoring, which includes genetic and cytogenetic monitoring, provides a useful tool for estimating the genetic risks deriving from integrated exposure to a complex mixture of chemical, physical and biological environmental agents [5, 6].

Damage to the genome is probably the most important and fundamental cause of the development of anomalies and degenerative diseases. It has been established that genomic damage is produced by exposure to genotoxic substances, medical procedures (such as from radiation and chemicals), micronutrient deficiency (from folic acid), life styles (relating to alcohol, smoking, drugs, and stress, for example), and genetic factors (such as defects in metabolism and/or in the repair of DNA). Hence, it is essential to perform biomonitoring with minimally invasive markers.

One of the important tests for cytogenetic monitoring used for estimating the general mutagenic background of the urban environment is a micronucleus (MN) assay on exfoliated human buccal cells. The test involves a non-invasive, screening express method to measure DNA damage in humans.

Cells of buccal mucosa in the oral cavity are comfortable objects for estimating the physiology state of the organism [7] and the influence of environmental factors [8]. Thus, the MN-assay in human buccal cells has been widely used to detect the genotoxic effects of pesticides [3, 9-11], automobile exhaust-gases [12, 13], oil products [14], chemotherapy [15, 16], genotoxicity agents [17-20], as well as lifestyle habits [21-23].

As a criterion for estimation of negative influence of different environmental agents the MN-assay allows also evaluating the presence and frequency of occurrence other nuclear anomalies, such as cells with micronuclei and "broken eggs" (biomarkers of DNA damages), binucleated

cells (biomarkers of pathological cytocinesis) and pyknotic, karyorrhectic and karyolytic cells (biomarkers of apoptosis) [8, 24].

High correlation is set between the increase of number of chromosomal aberrations, activity of mitosis process and induction of micronuclei [8, 10, 25]. Micronuclei are formed from acentric fragments or whole chromosomes because of abnormalities of processes of cell division [24]. The presence of micronuclei in cells is considered to be as the marker of genetic instability [25].

Study Area

Considering the above mentioned, we have used the MN-test to determine the genotoxic effects of harmful ecological and/or occupational factors as well as the efficiency of rehabilitation measures on the basis of application of natural adaptogenes carrying out the test in exfoliated buccal cells of mining and smelting workers and also children living in the cities of Ukraine with the high technogenical loading.

Methods

The subjects of the research were the cytogenetic status of human organism and the general mutagenicity in the Dnepropetrovsk region which has cities of different types and technogenic load levels, namely Marganets, Zholtyye Vody (Yellow Waters), Nikopol and Dnepropetrovsk. These cities are characterized by high levels of development of such branches of industry as mining, metallurgical and chemical manufacturing, ore production and uranium ore dressing. In the capacity of a local "control" an area with a low technogenic load was chosen, namely the area of the medically-improving complex "Solyony Liman (Silted Estuary)" located in the Novomoskovsk part of the Dnepropetrovsk region.

In a group used in the cytogenetic survey the healthy and practically healthy children of 5-7-years-old or adults working at mining or metallurgical enterprises were selected by a special questionnaire [24].

Subjects were required to rinse their mouths with water before sampling. Exfoliated epithelial cells of buccal mucosa were obtained by scraping the middle part of the inner cheek with a wadded tampon on a spatula. The epithelial cells collected from buccal mucosa were smeared onto clean microscope glass slides which were then air-dried and fixed with a mixture of ethanol and glacial acetic acid (3:1) within one hour. Then the slides were stained with aceto-orcein.

A light microscope "Olympus" using 100-times magnification on coded slides was used for MN analysis. At least 1,000 cells per child were analyzed to determine MN frequency.

The scoring criteria used are mainly based on those originally described by [26, 24]. Normal differentiated cells have a uniformly stained nucleus which is oval or round in shape. They are distinguished from basal cells by their larger size and by a smaller nucleus-to-cytoplasm ratio. No other DNA-containing structures apart from the nucleus are observed in these cells. These cells are considered to be terminally differentiated relative to basal cells because no mitotic cells are observed in this population group [24].

The micronuclei are round or oval in shape and their diameter should range between 1/3 and 1/16 of the main nucleus. Also, they have the same staining intensity and texture as the main nucleus. Most cells with MNi will contain only one MN but it is possible to find cells with two or more MNi.

MN-index was calculated in accordance with frequency of cells with MNi. The received experimental data were used for the calculation of conditional indices of damage (CID) for biosystems. On this basis an estimation was made of the ecological situation on the mutagen background [27, 28]. Using statistical analyses all the data were expressed as the mean ± standard error of the mean and results with $p < 0.05$ were considered significant.

Results and Discussions

Analysis of results of cytogenetic survey of pre-school age children living in the technogenic loaded cities of the Dnepropetrovsk area (Table 1) showed an increase in frequency of micronuclei in buccal cells that is 2.8-4.2 times that found in those who live in the "conditionally clear" territory of the medically-improving complex "Solyony Liman".

In accordance with our methodology used [27, 28], the calculated conditional indices of damageability (CID) of child organisms for cytogenetic parameters (considering the minimal (P comfortable) and maximal (P critical) values of the investigated parameter) testify that the total mutagen background in the Dnepropetrovsk region has to be considered "unsatisfactory" on the basis of a "threatening" condition for children's organism, and that the level of damage to their cells is "above average". Of the four investigated cities in the Dnepropetrovsk region the greatest index of micronuclei in epithelial cells of children is defined in the center of the uranium-extractive and uranium-processing mining industry, namely the city of Zholtyye Vody. In the control area there was defined a "low" level of genetic damages in epithelial cells and a "safe" condition of organism in respect of the cytogenetic status. It has allowed considering as "favorable" the ecological condition of the control territories of the medical-and-health improving complex „Solyony Liman".

In the investigated cities there were defined groups of children with an increased genetic risk having values of the MN-index equal to or exceeding 0.100. In Zholtyye Vody an elevated risk group included 48.4% of the examined children, while in Dnepropetrovsk it was 35.9%, in Marganets 40%, and in Nikopol 36.5%. As to the 'control' area, there are undetectable representatives with an elevated level of genetic disorders in somatic cells.

The raised mutagen background of the researched territories has caused a necessity of carrying out the rehabilitation measures directed at an increase of protective functions of organism. In our researches for rehabilitation of child organisms the rehabilitation program held at the base of Pulmonary Sanatorium of Dnepropetrovsk and Marganets included the combined oral administration of humic substances (humics), carotene oil (pro-vitamin A), enterosorbents (pectin), and probiotics (acidophilus). All were officially approved and previously tested for food supplements. A humic food additive in the form of a 0.05% solution of humic acid was used for 21 days according to instructions from the Pharmaceutical Committee of the Ukraine Ministry of Health (Table 1).

Table 1. Influence of natural adaptogenes on decreasing of number of MNi in cells of adults and children of Dnepropetrovsk region ($p < 0.05$)

Groups of cytogenetic examination	Number	MN-index before rehabilitation courses, per cell	MN-index after rehabilitation courses, per cel
Children			
Dnepropetrovsk	24	0.067±0.006	0.046±0.005*
Zholtyye Vody	140	0.100±0.010	-
Nikopol	52	0.089±0.003	-
Marganets	37	0.085±0.010	0.043±0.004**
Local control "Solony Liman"	53	0.024±0.004	-
Workers of the harmful enterprises			
Dnepropetrovsk	36	0.094±0.004	0.043±0.002***
Pavlograd	40	0.140±0.013	0.085±0.013***

** pectine's pill and humate; ** vitamins complex, pectine's paste and probiotics; *** humate, carotene oil and pectine's paste.*

Treatment was provided for 2 months to children suffering recurring bronchitis. All food supplement dosage levels were age-appropriate and reassessments were conducted at the end of the 2-month treatment period. An anti-mutagenic effect was observed in 87.5% of cases (i.e. $p < 0.01$). In addition, experiments displayed normalization of the immune system condition of child organisms and a reduction in the level of respiratory diseases by 1.3 times within the next autumn and winter period.

The NM-test was also applied for cytogenetic estimation of adult organism in the conditions of harmful production factors (Table 1). Such researches were made together with medics of the Therapy Department of the Medical Academy in the Tire Works in Dnepropetrovsk region. The cytogenetic health status of the workers of the car tires producing Division was investigated. There was observed an increase by 2.5 times in the frequency of occurrence of MNi (0.094+0.004) in comparison with control (0.038+0.002) made by a group of students of the Dnepropetrovsk Medical Academy. The rehabilitation courses made at this enterprise including the taking of an adaptogene complex (humics, carotene oil and pectic paste) have improved the cytogenetic status of organism of the workers involved in the harmful manufacture.

Researches made in the group of the miners suffering from chronic bronchitis have shown that this illness is accompanied by an increase of the frequency of occurrence of the cells with micronuclei by 3.7 times, and a decrease of the immunological status in comparison with a control group of practically healthy people living in the same area. After a complex treatment with natural adaptogens, there was observed an improvement of the clinical condition of the patients, normalization of their immune system, as well as a decrease in the frequency of occurrence of cytogenetic disorders to the control level.

It is necessary to note that high physiologic activity of the humic substances and their anti-toxic and anti-mutagen properties are due to favorable influence on the protein, the synthesizing cell system, biological membranes, sub-cell structures and the cell division processes making the base for ontogenesis. Their unique structure and sorptive properties provide for limitation of ecological toxicant migration within the soil-to-plant system especially within industrial areas, increase resistance rates of the plants and other organisms with regard to the variety of unfavorable environmental factors. This determines their protective, etc ecological functions at the level of the biogeocenosis. Decrease of the genetic damages in the biota and human cells makes the humic substances promising for protection of the genetic fund and prevention of genetic consequences of the technogenesis [29].

Conclusions

Thus, a decrease of cytogenetic disorders in human organisms, induced by the action of harmful ecological and productive factors, is possible on the basis of therapeutic actions including the use of physiologically active preparations of the natural origin. The obtained data represent a theoretical basis for the formation of rehabilitation Programs for the population health status in technogenically-loaded territories.

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