GENOTOXIC EFFECTS OF ENVIRONMENTAL POLLUTANTS GENOTOXIC MONITORING AND DETECTION OF ANTIGENOTOXIC EFFECTS

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ABSTRACT

The control of genotoxic agents mass release, which can adversely affect the ecosystem stability and human health is of the greatest importance. Therefore, it is necessary to seriously elaborate the strategy of genotoxic monitoring and relevant legislation. Additional approach is the study and dietary use of antigenotoxic plant substances for prevention of mutation-related diseases.

Key words: environmental pollution, genotoxic monitoring, genotoxicity/ antigenotoxicity tests

Introduction

Environmental pollution is one of the critical factors that determine the destiny of life on Earth. All organisms are continuously exposed to chemical and physical pollutants, with different consequences. The problem of uncontrolled pollution of the environment is especially severe in the developing countries. In addition, our country has been exposed to war destruction and in some areas the environment has been heavily polluted (1, 2). Many pollutants are toxic and their effect is rapidly seen at the physiological level. However, many environmental pollutants have genotoxic effects i.e. they cause mutations and other genetic changes in the cells. In contrast to toxic compounds, genotoxic agents have long-term effects, with serious health and environmental consequences.

Genotoxic agents cause genetic damage in all organisms, including human. Changes in the genotype, which may influence the reproductive capacities of species, are of the great importance for every population. However, genetic changes in human cells, either somatic or germ, are very important for every individual. Mutations in germ cells can cause abnormal development of embryo, prenatal death or genetically defective offspring .

Somatic mutations and rearrangements in DNA molecule (gene conversion, reciprocal recombination, and chromosomal aberration) can lead to development of degenerative diseases including arteriosclerosis, autoimmune defects and some forms of diabetes. Moreover, the relation between somatic mutations and induction, promotion and progression of some forms of cancer is undoubtedly proved (3 -8).
The main arguments for development and application of genotoxic monitoring strategy
The risks from uncontrolled presence of genotoxing agents in the environment are numerous (9-11).

- Genotoxic agents significantly increase the frequency of mutations in germ cells and therefore seriously endanger the reproduction of different species, including human.
- Genotoxic agents significantly increase the frequency of mutations in somatic cells and cause the neoplasia and other genetic diseases.
- By increasing the mutation rate, genotoxic agents cause quantitative and qualitative changes in the genetic structure of populations. The induced mutations are usually harmful and they are subjected to intensive natural selection. Adaptively harmful dominant mutations are rapidly eliminated from population gene pool, while recessive mutations, which are more frequent, are eliminated slowly, increasing the population genetic load.
- The mutational events are unpredictable and mostly harmful, even after a very short or/and a low dose exposure.

Following war destruction our environment is additionally contaminated with genotoxic and carcinogenic pollutants, which calls for intensive genotoxic monitoring in this area.

Development of biomonitoring system for the detection of genotoxic agents - genotoxic monitoring

In last decades, many methods for detection of mutagenic/ carcinogenic effect of environmental pollutants have been developed. The study of potential consequences of the exposure to genotoxic agents is multidisciplinary and includes many scientific fields, such as DNA repair, mutagenesis, population genetics, mathematical modeling, statistical analysis of experimental data, etc.
Genotoxic monitoring has two major approaches (12):

1. *in situ* monitoring of genetic changes in somatic cells of organisms from polluted environment. The current methodologies include plants as air pollution bioindicators, shells, amphibian and fish as water pollution bioindicators, etc, and need further development and standardization.
2. Laboratory examination of water samples, air particulates and soil extracts with specially constructed and verified tests for qualitative and quantitative detection of genotoxic effects. This approach has been of great importance for detection of substances with mutagenic/carcinogenic effects and their classification. The databases obtained are useful for further strategy and legislation.

The numbers of countries in the modern world are taking extensive measures for environmental protection, as an imperative of the quality of life. Since our country has

adopted the use of genotoxicity tests recommended by OECD (Organization for Economic Co-operation and Development) (13,14) in the legislation concerning toxic chemicals, it is necessary to include detection of genotoxicity in the biomonitoring program.

Laboratory tests for detecting genotoxic agents

Commission of the European Communities (CEC) and OECD, in co-operation with experts for chemical mutagenesis from European Environmental Mutagen Society (EEMS), American Environmental Mutagen Society (AEMS) and World Health Organization (WHO) has made the instructions for test selection and application (13, 14). The guidelines recommend 15 tests, placed into three major categories on the basis of the genetic effects they detect:

1) Tests for detection of genetic mutations
- Reversion mutation test in *Salmonella typhimurium* (Ames test)
- Reversion mutation test in *Escherichia coli*
- *Bacillus subtilis rec* test
- Gene mutation test in *Saccharomyces cerevisiae*
- Sex-linked recessive lethal mutations (SLRL) test in *Drosophila melanogaster*
- Spot test in mice

2) Tests for detection of chromosome mutations
- *In vivo* citogenetic test
- *In vitro* citogenetic test
- Micronucleus test
- Dominant lethal mutation test
- Heredity translocation test
- Citogenetic test in mammalian germ cells

3) Tests for detection of the effects on DNA molecule
- Damage and repair of DNA; unscheduled DNA synthesis *in vitro*
- Mitotic recombination test in *Saccharomyces cerevisiae*
- Sister chromatide exchange (SCE) *in vitro*

To bring a definitive conclusion about the genotoxicity of the agent it is necessary to use a battery of tests. There are two possible strategies: a) hierarchical (phase) approach and b) matrix approach (12, 14).
In the hierarchical approach the genotoxicity testing is conducted at three levels ("three-tier" system). The initial phase is **screening** involving microbial and other short-term tests *in vitro*. The second phase is **confirmatory** involving *in vivo* and *in vitro* tests in higher eukaryotes, including mammals. The selection of tests is often determined by results in the initial phase. The third phase is essential for **evaluation** of human risk and involves *in vivo* tests in mammals.
In the matrix approach an agent is tested through the battery of tests, (not more then five) with different levels of detection: usually one microbial test, one test in *D. melanogaster* and one or two *in vivo* tests in mammals.

When the result is positive in either of the test systems, e.g. when an agent shows a genotoxic effect, it is easiest to forbid it for further use. However, it is not that simple. For example, if the substance is of important use and no adequate substitute is available, then making decision is more complex and inevitably includes assessment of risk and benefit.
Most of the examples reported favor hierarchical approach which should finally lead to the following classes of compounds: *in vitro* genotoxins (positive only *in vitro*), *in vivo* genotoxins (at somatic and/or germ cell level), and the non-genotoxins.

Detection of antigenotoxic effect

Since it is impossible to exclude the exposure of human population to genotoxic agents in the environment, the new research area of antimutagenesis/anticarcinogenesis is primarily directed to the detection of factors that lower the risk of exposure to genotoxic agents. Antimutagens are substances that reduce the frequency of spontaneous and induced mutations, and thereby can be used in primary prevention of cancer and other mutation-related diseases (15).
Isolated substances with antigenotoxic effects belong to a number of different chemical classes, and are mostly of biological origin. Especially interesting genotoxic inhibitors are natural plant substances. They differ in chemical structure and the mechanism of action, and they are often present in human diet (vitamins, natural phenols, flavonoids, terpenoids, etc.) (16).
Microbial and other tests, routinely used for detection of mutagens in the environment are recommended for identification of substances with antigenotoxic/anticarcinogenic properties. In addition, a new bacterial test-system, which detects both antimutagenic activity and the mechanisms of antimutagenesis is constructed and validated in our laboratory (17). The test-system has been successfully used for screening of antimutagenic potential of differently prepared extracts from medicinal and aromatic plants (16).
The study of antigenotoxic substances and the mechanisms of their activity are of great importance in creating an "anti-risk" strategy for protection of human health.

Conclusions

On the basis of above mentioned considerations and revolving the current state of genotoxicity/antimutagenicity testing in our country, it is highly desirable:

- To define methods and testing strategies for evaluating the genotoxic properties of environmental pollutants and their inhibitors. Genotoxicity testing should be oriented towards detection of both potential germ cell mutagens (due to their involvement in heritable genetic defects) and somatic cell mutagens (involved in processes such as malignant transformations).
- To harmonize, within the context of regulatory activities, the national and international legislation (14).
- To promote networking of validated units for testing, education and research and their financial management.

Establishment of the Agency for Environmental Protection could be seen as a condition for a more efficient and organized approach in creating new guidelines in Yugoslavia.

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