

Micronucleus Based Genotoxicity Tests

Mikronükleus Temelli Genotoksiste Testleri

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ABSTRACT Genotoxicity assessment is an important process to keep human and animal health and aims to identify the safety of many kinds of substances. There are many in vitro mutagenicity prediction tests. The recent in vitro/in vivo genotoxicity assays and accepted common test methods may give critical information for the assessment of genotoxic and probable carcinogenic potential of suspected substances. Micronucleus (MN), MN-FISH, MN-based flow cytometry analysis done alone or done with chromosome instability assays and/or comet assay are some of assays that may be used to assess clastogenic and aneugenic effects of substances like pesticides, food additives, pharmaceuticals, chemicals used in cosmetics, veterinary drugs and living organisms like bacteria or virus. In this mini-review, the studies about the tests mentioned above are included. In summary, MN-based genotoxicity tests and accepted common test methods may provide important information for the assessment of genotoxic and probable carcinogenic potential of suspected substances.

Keywords: Genotoxicity tests; micronucleus tests; mutagens; carcinogens

ÖZET Genotoksiste tayini insan ve hayvan sağlığının korunmasında önemli bir procestir ve çeşitli maddelerin güvenliklerini tanımlamayı amaçlar. In vitroda genotoksiste tahminini sağlayan pek çok test mevcuttur. Mevcut in vitro/in vivo genotoksiste testleri ve kabul edilen test metodları şüphelenilen maddelerin genotoksik ve muhtemel karsinojenik potansiyellerini saptamada önemli bilgiler sağlayabilir. Mikronükleus (MN), MN-FISH, MN temelli akım sitometre testleri tek başına ya da beraberinde kromozom instabilite testleri ve/veya comet testi ile birlikte yapıldığında pestisit, gıda katkıları, farmasötikler, kozmetikte kullanılan kimyasallar, veteriner ilaçları ve bakteri veya virüs gibi yaşayan organizmaların klastojenik ve aneujenik etkilerini saptamak için kullanılabilir. Bu mini-derlemede, yukarıda bahsedilen testlerle ilgili yapılan çalışmalara yer verilmiştir. Özetle, MN temelli genotoksiste testleri ve kabul edilen diğer test metodları şüphelenilen kimyasalların genotoksik ve muhtemel karsinojenik potansiyellerini saptamada önemli bilgiler sağlayabilmektedir.

Anahtar Kelimeler: Genotoksiste testleri; mikronükleus testleri; mutajenler; karsinojenler

The chemicals and organisms that cause mutagenicity and carcinogenicity may be divided into two categories according to their pathogenic mechanism: non-genotoxic and genotoxic. Non-genotoxic ones do not directly interact with DNA; instead they react in metabolic or in epigenetic pathways like histone modification, chromatin remodelling, DNA methylation and miRNA.¹ Genotoxic chemicals and organisms directly react with DNA through formation of covalent bonds and/or giving direct damage to DNA. In the genome, chromosome number changes are called “aneuploidy”; chromosome rearrangements that change karyotype or particular sequence rearrangements are called “clastogenesis”.²

Genotoxic agents may cause two different kinds of DNA lesions: one is incorrect DNA repair and replication. Cell goes to death, apoptosis or senescence and accumulate mutations. Second one concerns genome and chromosomal rearrangements like base substitutions, aneuploidi, insertions and deletions. These kind of mutations are more important because they may cause carcinogenesis, many kinds of congenital diseases and also aging.³

So, genotoxicity assessment is an important process to keep human and animal health. Genotoxicity evaluation aims to identify the safety of many kinds of substances like food additives, pesticides, pharmaceuticals, chemicals used in cosmetics, veterinary drugs and living organisms like bacteria or virus. There are many *in vitro* genotoxicity prediction assays but they are insufficient to be used instead of animal tests. Worldwide, researchers have been trying to find the ways of optimising both *in vivo* and *in vitro* genotoxicity testing throughout the last decade.⁴ Micronucleus (MN) test, sister chromatid exchange (SCE) assay, chromosome aberration test and bacterial reverse mutation test are the officially approved genotoxicity tests included in the guideline of Organization for Economic Co-operation and Development (OECD). The MN test is seen in various genotoxic test guidelines in different groups of chemicals. The recognition of micronucleus was in the end of 19th century and recently *in vitro* MN test in human lymphocytes (OECD 487) and mammalian erythrocyte MN test (OECD TG 474) have been updated.⁵⁻⁷ Foresight of chromosome damage makes these tests widely used. Micronuclei are extranuclear, small objects that may be seen in cell divisions arise from whole chromosome/chromatids or chromosome/chromatid fragments without centromere that remand behind in anaphase and are excluded in telophase in the daughter nuclei.⁸ In this sense, high DNA damage levels validated by MN test may be a cancer risk biomarker.

The *in vitro* genotoxicity assays screen the substances and candidates to define their primer safety, but *in vivo* assays give more complicated data about biologic and physical properties of the substances. Moreover, *in vivo* genotoxicity tests

give opportunity to see the mutagenic effects in the complete biological system that are identified before by *in vitro* assays.⁹ As a result, *in vivo* assay data are more important than the *in vitro* ones because of their evidence weight. Presently, as mentioned above, the mammalian erythrocyte micronucleus test (OECD TG 474), the mammalian bone marrow chromosomal aberration test (OECD TG 475), the transgenic rodent somatic and germ cell gene mutation assay (OECD TG 488), and the *in vivo* mammalian alkaline comet assay are the most common *in vivo* tests that are currently used. Although, there is a variety of *in vitro* assays to define and detect hazardous effects of genotoxic substances, they can not be fully used instead of *in vivo* tests.⁴ For obtaining reliable and satisfactory outcomes, getting more information about chromosomal aberration types and having an idea about mechanism of DNA damage from *in vitro* assays, combination of two or more *in vitro* test systems may be used. In addition to MN, MN-FISH and chromosome instability assays, the comet assay may be used to assess the DNA single and double-stranded breaks in distinct tissues.² Matzenbacher et al. tried to assess the mutagenic and genotoxic effects of coal dust, fly and bottom ash using lung fibroblast cell line (V79) with the MN test and the comet assay together.¹⁰ Another investigation in human primary parotid gland cells, nicotine derived genotoxic effects were evaluated *in vitro* by MN test, comet assay and chromosome aberration test at the same time.¹¹

In one of our studies, we investigated the genotoxic effects of ¹⁸⁶Re-1,1-hydrox yethylidenediphosphonate (¹⁸⁶Re-HEDP), by using a micronucleus (MN)-fluorescence in-situ hybridization (FISH) assay, on the cultured peripheral blood lymphocytes.¹² Micronuclei seen as chromosomal fragments may be the result of DNA synthesis inhibition, conversion of single-strand breaks into double-strand breaks after cell replication, or direct double-strand DNA breakage.⁸ Biomonitoring the early genetic effects of human demands exact, delicate, and if possible, easy to do and not requiring a lot of time methods to assess mutations that are induced by radiation. Currently cytokinesis-

blocked MN (CBMN) assay is one of the most promising methods for evaluating DNA damage and measuring MN, which notices genome and chromosome mutations at the same time in binucleated cells.^{8,13} Human biomonitoring studies, in peripheral blood lymphocytes using MN assay, is mainly applied to: (i) consider the genetic damage rate of occupational, different environmental and lifestyle factors between exposed populations; (ii) compare radiosensitivity between cancer risk persons as a predictor of cancer risk and for optimization of radiotherapy at the same time; and (iii) evaluate the mutagenic potential of newly produced chemicals by the pharmaceutical and agrochemical industries.^{13,14} CBMN assay is an important assay because it can assess genomic instability at the chromosome/molecular level. It gives a considerate evaluation of chromosome loss, chromosome breakage, chromosome rearrangement, gene amplification, nondisjunction, apoptosis and necrosis.¹⁵ Direct correlation between genomic damage and micronuclei production makes CBMN assay a reliable method for determining genome-induced chromosome damages and/or genomic instability when applied to human blood peripheral lymphocytes.¹⁶

We also analysed the micronuclei origins by FISH using pancentromeric probes. Observed centromeric signal(s) in MN might mean that it includes a whole chromosome induced by an aneugenic agent involved in cell division and the mitotic spindle apparatus resulting loss or gain of whole chromosomes inducing an aneuploidy. On the other hand, if fluorescent signal in MN can not be seen, it means that MN originates as a result of chromosome breakage by clastogenic effects.^{17,18} Clastogenic agent may cause chromosomal breakage directly or indirectly by affecting replication of DNA.¹⁹ Molecules involved in chromatid attachment and separation (chromosome condensation, crossing over, kinetochores, chromatid glue proteins), part of essential DNA-containing structures (centromeres, telomeres) involved in cell cycle control (cyclins, cdks, p53), anaphase promoting complex, part of the spindle apparatus (tubuline, MAP, centrioles), and indirectly involved in the cell cycle (calmodulin, cellular, or nuclear mem-

brane) are the possible targets of aneugenic agents. Nondisjunction and chromosome loss are two classical processes leading to aneuploidy. They are results of the aneugens binded on centriolar tubulins or spindle, scaffolding, and nuclear proteins or kinetochore and centromere regions.²⁰

In the another study, we evaluated the genotoxic effects of hepatitis B virus (HBV) in the patients and carriers individually.²¹ We compared their results with a control to evaluate the genomic instability. Mutagenic effects of HBV within the host genome are detected by MN and breakage evaluations at cytologic and cytogenetic level. These methodologies are used to evaluate the effect of physical and chemical agents on chromosomal damage. By using these two tests, we observed damage within metaphase spreads and performed MN detection of cells in interphase at the same time, by this way a quick observation of large cell populations becomes possible.²²

Although characteristics of the MN test includes many advantages, it has some limitations such as false positive results, being hard to be seen in the peripheral blood erythrocytes of humans and rats, inadequate modes of action (MoA) information as may be experienced in other *in vitro* tests.²³ For high throughput screening different automated genotoxicity tests have been developed. Among these automated assays, the most widely used one is flow cytometry based MN test. When this test is used in erythrocytes, 4000 young erythrocytes and million of cells may be analysed easily by gating for young immunostained erythrocytes. Number 4000 is twice the number of cells that can be analysed in standard MN procedures.⁵ Muthusamy et al. used flow cytometry based MN assay in their study to show and screen the genotoxic potential of some environmental chemicals and metal mixtures in HepG2 cells. They mentioned about the usefulness of the test in efficient MN scoring and told how to get knowledge about cytotoxic effects of chemicals on cell cycle parameters.²⁴

Thus far, a brief explanation of MN-based of some short-term tests is given. Lately, in order to test the effects of spontaneous and induced muta-

gens, reporter genes are given to transgenic animals' target tissue or organs. These genes are sent to the different loci of animal genome, but this test does not give knowledge about sequence integrity of the whole genome, so it is indirect. Now by taking the advantages of next-generation sequencing (NGS) methods, it may be possible to examine the genetic material exactly in a whole genome with single nucleotide determination. The best part of these NGS methods is any type of genetic material derived from any part and any type of genom may be analyzed. Nevertheless, NGS methods about genotoxicity testing must be developed in application.³ Three-dimensional skin models are also developing and one of the *in vitro* assay in the testing of applied cosmetic and pharmaceutical compounds. In this model, human keratinocyte *in vitro* culture is used. The '*in silico*' methods that are using computationally derived structural similarities between known genotoxins and new compounds may be added to the developing *in vitro* genotoxicity assays.²

Taken together, MN-based genotoxicity tests and accepted common test methods may provide important information for the assessment of genotoxic and probable carcinogenic potential of suspected substances. Current *in vitro* genotoxicity methods have been modified and refined for more sensitivity and specificity, while new methods have been developed to be effective in pre-

venting usage of unanticipated genotoxic, carcinogenic pharmaceuticals, chemicals and so on. In the light of international/national institution guidelines, MN-based assays may be accepted as possible vehicles to assess the local genotoxicity and to understand positive relation between genotoxicity and carcinogenicity. The high throughput assays, next generation sequencing technologies, computer dependent technologies and all of these applied with new *in vitro* model systems will enable us to perfectly analyze genotoxic, mutagenic, carcinogenic end-points of substances.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

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