The cytokinesis-block micronucleus assay: experimental procedure and application in bioelectromagnetics research

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Abstract

The cytokinesis-block micronucleus assay is a sensitive and simple indicator of chromosome damage, both chromosome loss and chromosome breakage, which also provides information on cell cycle progression and cytotoxicity. It was introduced in 1985 and has been successfully employed in many laboratories on different cell types to monitor exposed population, to identify mutagen-sensitive individuals and for in vitro studies. This cytogenetic technique has been largely employed to evaluate the induction of genotoxic effects of electromagnetic fields, including radiofrequency radiation.
The experimental procedure to evaluate the induction of micronuclei is described and its application in bioelectromagnetics research is reviewed.

1. Introduction

Observation of chromosomes and counting of aberrations in metaphases is the most detailed analysis to measure chromosome damage, but the complexity and time consuming of this technique has stimulated the development of a simpler method of measuring chromosome damage. Schmid [1] and Heddle [2] proposed to measure micronuclei (MNi) as a simpler alternative to assess chromosome damage.
MN are small nuclei separated from the main nucleus and contain chromosomes or chromosome fragments, derived from mitotic spindle dysfunction or acentric fragments. Because they are expressed in cells that have completed nuclear division they are ideally scored in the binucleated stage of the cell cycle. Therefore, several methods have been proposed that, within a cell population, could distinguish between cells that are not dividing and cells that are undergoing mitosis. Among them, the citokinesis block (CB) method was the most suitable due to its versatility, simplicity and lack of effects on base-line genetic damage [3].

2. The cytokinesis-block micronucleus (CBMN) assay

This technique consists in adding to cell cultures cytochalasin-B (Cyt-B), an inhibitor of the mitotic spindle that prevents cytokinesis. As a consequence, cells that have completed one nuclear division are identified by their binucleated appearance. The CBMN assay was proposed in 1986 for human lymphocytes [4] and since then it has been successfully introduced in many laboratories all over the world and is applied on different cell types, relevant for human biomonitoring. It is a very sensitive and simple indicator of chromosome damage, which also provides information on cell cycle progression, it is less time-consuming respect to chromosomal aberrations and slides scoring is relatively easy [5]. In addition, by classifying cells according to the number of nuclei, this assay provides an index of cytotoxicity (binucleate cell index, BCI) [6] and information on the cell cycle kinetics (cytokinesis-block proliferation index, CBPI) [7].
Because of world-wide interest in the MN method to assess environmental effects on chromosome damage in blood and epithelial tissues in human populations, in 1997 an International Collaborative Project on MN Frequency in Human Population (HUMN) was launched. 34 laboratories in 21 countries participate to the Project, with the aim to compare the baseline MN frequency from different labs and among different populations, to compare different techniques to define a standard protocol and to evaluate the suitability of MN as biomarker of risk for diseases such as cancer [8]. Main results of the HUMN project concern the evaluation of factors influencing MN frequency (such as age, sex, smoking, alcohol consumption, diet, drugs, X-rays), the criteria for slides scoring [9] and the recommendations for performing the MN assay on several cell types [7].

3. Molecular techniques for measuring chromosome loss in micronuclei

The combination of the CBMN assay with staining techniques of the centromeric region of the chromosomes allows discrimination between micronuclei containing a whole chromosome (centromere positive MN) and an acentric chromosome fragment (centromere negative MN). This is possible by using antibodies from scleroderma patients of the CREST subtype that bind to kinetochore proteins assembled at the centromeric regions, not specific for single chromosomes (CREST) or by applying the fluorescence in situ hybridisation (FISH) with a DNA probe specific to the centromeric regions [5].

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4. Experimental procedure

Here is described the experimental procedure currently in use in our laboratory to perform the CBMN assay in human lymphocytes and in several cell lines: cells are seeded in complete culture medium and cultures are left in a CO_2 incubator until the time of Cytochalasin-B addition (4 hours after the first cell division), that is variable on the basis of the cell cycle duration. At the end of the second cell division, 1 ml of culture is collected from each sample, transferred in coded tubes and centrifuged at 3000 rpm for 1 minute. Supernatant is discarded and 3 washing steps are performed with a wash medium composed by culture medium additioned with 2% Foetal Bovine Serum (FBS). Cells are then swollen for 15 min with an hypotonic solution composed by one part of wash medium and four parts of distilled water and slides are prepared by using a cytospin. Coded slides are located in appropriate holder equipped with a filter and a chamber for cell suspension. Cells in hypotonic solution are added to the chamber and cytospinned for 7 minutes. Than, slides are recovered, air dried, fixed and stained in a Giemsa solution.

5. Application of CBMN assay in Bioelectromagnetics research

In the last years the CBMN technique has been largely employed to evaluate the induction of genotoxic effects in several cell types in *in vitro* and *in vivo* experiments following exposure to electromagnetic fields, including radiofrequency radiation (RFR). The induction of MN was investigated after exposure to RFR alone or in combination with well known chemical or physical agents. Several experiments have been carried out by adopting different exposure conditions and by using different cell types. Most of the data reported in the literature failed to demonstrate induction of genotoxic effects due to RF exposure in the frequency range of 800-2000 MHz. The results obtained have been reviewed by several authors and the prevalent opinion is that available data do not suggest a direct mutagenic effect of RF radiation [10, 11]. Nevertheless, even if the general consensus is that RF radiation signals are not directly mutagenic because of the very low associated quantum energy, some positive findings have been reported, often associated with the type of modulation of the RF signal employed.

7. References

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