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Review

Use of the lymphocyte cytokinesis-block micronucleus assay in occupational biomonitoring of genome damage caused by in vivo exposure to chemical genotoxins: Past, present and future

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ABSTRACT

This article concerns the use of the lymphocyte cytokinesis-block micronucleus (CBMN) assay in biomonitoring of occupational and environmental exposures to genotoxic agents. Furthermore, we evaluated the use of this method in different exposure scenarios, in comparison to other DNA damage biomarkers and its regional distribution. So far ca. 400 studies have been published and the number increased substantially in the last years. The most frequently investigated groups are hospital personnel, followed by workers in the chemical industry and agricultural workers. The lymphocyte CBMN assay is more frequently used in occupational studies than MN assays with epithelial cells and other methods, such as chromosomal aberration analyses, Comet assay and DNA-adduct measurements. The use of probes which enable the discrimination between MN containing chromosome fragments and whole chromosomes allows the identification of the molecular mechanisms of MN formation. Most studies were performed in Europe and Asia (ca. 65% and 25%, respectively). Important future developments will be the evaluation of the biological consequences of formation of additional nuclear endpoints (e.g. nucleoplasmic bridges), the improvement of the understanding of the health consequences of their formation, and the use of automated scoring devices. Future applications of the CBMN assay should address new emerging problems, e.g. the potential genotoxic damage induced by the use of nanoparticles and mobile phones. The control of occupational exposures to chemical genotoxins is currently based on chemical measurements which do not reflect interactions of individual factors and the inclusion of the lymphocyte CBMN assay in routine surveillance of workers could contribute substantially to the prevention of adverse health effects.

Contents

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1. Introduction

This chapter provides an overview on important historical milestones of the development of the cytokinesis-block micronucleus (CBMN) assay and its increasing use in occupational studies in different countries in comparison to other biomonitoring approaches. Further subchapters concern the use of this method in regard to induction of genetic damage found at different workplaces and by different groups of industrial and environmental chemicals. The last paragraphs describe recent developments and improvements as well as future trends and challenges.

2. Search strategy

The data which are described in the present paper concerning the use of the micronucleus (MN) assay and other genotoxicity tests in different areas and occupational settings are based on a computer aided literature search through the electronic databases Scopus, MedLine/PubMed, TOXLINE, Web of Science was carried out up to March of 2016. Additionally, a manual search of the reference list of studies and of review articles was performed. References of retrieved articles were analyzed to identify articles which were potentially missed in the initial search. Only studies in English where the full text was available were considered. The keywords and the combinations of search terms used were: micronucleus, micronuclei, micronucleus, lymphocytes, erythrocytes, reticulocytes, workers, occupation, occupational exposure, chemicals, chemical industry. Other key words were: air; alcohol; buccal; cancer; cervix; chromosomal aberrations; comet assay; diseases; DNA adducts; ENDOIII; environment; fluorescence in situ hybridization; FISH; FPG; food; gene polymorphism; lifestyle; mobile phone; nanoparticles; nasal; pancentromeric probe; SCGE; single cell gel electrophoresis assay; SCE; sister chromatid exchange; smoking; urothelial; vitamins; water.

3. Milestones of the development of the lymphocyte CBMN assay

MN were detected by the Swiss cytogeneticists Matter and Schmid [1] who worked at the Department of Paediatrics in the University of Zürich. They detected these extracellular DNA containing bodies in bone marrow cells of mice and hamsters and it became soon clear that they are formed as a consequence of structural and numerical chromosomal aberrations (CA) and can be scored much faster than these structures in metaphase cells. In the following years, a variety of tests was developed which are based on the quantification of MN [2,3]. A overview of these methods can be found in a special issue of Mutagenesis [4]. The routine screening of chemicals involves the use of a battery of *in vitro* tests, including the MN test. The *in vitro* MN test with lymphocytes and the *in vivo* bone marrow MN assay in rodents are currently included in the OECD guidelines for screening of chemicals [5,6].

Only a few publications which concerned the formation of MN in occupationally exposed workers were published before the protocol for the lymphocyte CBMN assay using cytochalasin-B was developed in 1985 [7,8]. The development of this protocol was an important milestone because it was evident from *in vitro* studies with genotoxins that the frequency of MN varies depending on the proportion of dividing cells [8,9]. This is because MN are mainly expressed during mitosis when chromosome fragments or whole chromosomes which cannot engage with the mitotic spindle, lag behind at anaphase and are excluded from the main nuclei and subsequently surrounded by a nuclear membrane to form MN. To overcome this kinetic problem, Fenech and Morley in 1985 [7] developed a method to identify cells that have completed nuclear division by their binucleated appearance by using cytochalasin-B, an inhibitor of cytokinesis. The development of the CBMN method made the assay much more robust by eliminating false negative results caused by inhibition of nuclear division by the agent being tested or as a result of host factors (such as ageing) that reduce lymphocyte responsiveness to mitogen. In addition, the CBMN assay allowed the proportion of dividing cells to be measured and it became possible to score nucleoplasmic bridges (a biomarker of DNA strand break misrepair or telomere end-fusions) in binucleated cells which enabled a more comprehensive assessment of genotoxicity.

Another problem in the early years of the use of the lymphocyte CBMN assay was the variety of fixation techniques, staining procedures, scoring methods and scoring criteria which was used [10]. For example, the number of binucleated cells scored per sample differed strongly among the studies [10]. In order to reduce this heterogeneity, which led to substantial intra- and inter-laboratory variations, the HUMN consortium was formed in 1997 [11]. The major achievements which emerged from the activities of this group are described in a number of articles (for details see www.humn.org, [12]). Amongst the most important milestones of this initiative are the publication of a standard protocol [13,14] which was developed on the basis of results of scoring exercises [15], the identification of the main confounding factors as well as results of statistical analyses which indicate that MN are a reliable biomarker for the detection of increased human cancer risks [16,17].

A substantial achievement which allows to draw conclusions on the molecular mechanisms leading to MN formation was the development of the fluorescence *in situ* hybridisation technique (FISH) [18,19]. By use of "pancentromeric probes" it became possible to discriminate between MN which contain chromosomal fragments (C^-) and MN with centromeres (C^+) which contain of entire chromosomes.

Another important step was the development of automated scoring devices which enable to evaluate larger sample sizes and to avoid inter-individual scorer variations. In total, about 20 studies have been published in which such systems were used, however, none of them concerned occupational exposures. A detailed description of different approaches can be found in a recent review published by the HUMN project [20].

In 2004, the first occupational study was published which concerned polymorphisms of genes encoding for gluthatione-*S*-