PRACTICES THE DIFFERENT CELLS TYPES LIKE TARGET GENOTOXIC ENDPOINT IN MICRONUCLEUS ASSEY

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Apstract:

Introduction: The toxicological relevance of the micronucleus (MN) assey is well defined: it is a multi-target genotoxic endpoint, assessing not only clastogenic and aneugenic events but also some epigenetic effects, which is simple to score, accurate, applicable in different cell types. Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells. Aims of the study: To indicate the importance of the application of micronucleus test as standardized cytogenetic method as an important biomarker in detecting the impact of ionizing radiation on the entire genetic material in occupationally exposed health care workers. Matherial and methods: The study include health professionals who are directly and on daily basses exposed to ionizing radiation as physical agents and a control group that represents young and healthy population that is not exposed to any physical and chemical agents. Results. The major advantage of lymphocytes is that they are primary cells, easy to culture in suspension. The choice between whole blood and isolated lymphocytes depends upon the question addressed. The most important differences among the protocols are the hypotonic treatment (critical in particular for image analysis), fixation of the cells (dependent on laboratory preferences) and the final slide preparation. Conclusion: In recent years the in vitro micronucleus test has become an attractive tool for genotoxicity testing because of its simplicity of scoring and wide applicability in different cell types but the lymphocytes are the most reperesentative cells for this kind of research.

Keywords: cells cultures, lymphocytes, micronucleus, genotoxicology, radiation

Introduction: The in vitro micronucleus assay is a genotoxicity test for the detection of micronuclei (MN) in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic chemicals (Kirsch-Volders, M. (1997), Parry, J.M. and Sors, A. (1993), in cells that have undergone cell division during or after exposure to the test substance. The addition of cytoB prior to the targeted mitosis allows for the identification and selective analysis of micronucleus frequency in cells that have completed one mitosis because such cells are binucleate (Fenech, M. and Morley, A.A. (1985), Kirsch-Volders and all.(2000). The toxicological relevance of the micronucleus (MN) assey is well defined: it is a multi-target genotoxic endpoint, assessing not only clastogenic and aneugenic events but also some epigenetic effects, which is simple to score, accurate, applicable in different cell types. In addition, it is predictive for cancer, amenable for automation and allows good extrapolation for potential limits of exposure or thresholds and it is easily measured in experimental both *in vitro* and *in vivo* systems. Furthermore, the importance of adequate design of protocols is highlighted and new developments. We address future research perspectives including the possibility of a combined primary 3D human skin and primary human whole blood culture system, and the need for adaptation of the in vitro MN assays to assess the genotoxic potential of new materials, in particular nanomaterials. So many studies indicate that the use of cell lines can be recommended, it should be underlined that most of the cell lines are deficient in p53 or apoptosis controlling genes that lead to higher frequencies of MN and is thought to lead to positive results that are not confirmed in vivo in some cases. In contrast, for mechanistic studies, the use of cell lines can be very useful. The OECD guideline is not restricted to synchronised cells since it aims at maximising the probability of detecting an aneugen or clastogen acting at any stage of the cell cycle; therefore, a sufficient number of cells should be treated with the test substance during all the phases of their cell cycle (Volders and all., 2011). Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells, without extra in vitro cultivation step. MN observed in exfoliated cells are not induced when the cells are at the epithelial surface, but when they are in the basal layer. An ex vivo/ in vitro analysis of lymphocytes in the presence of cytochalasin-B (added 44 hours after the start of cultivation), an inhibitor of actins, allows to distinguish easily between mononucleated cells which did not divide and binucleated cells which completed nuclear division during in vitro culture. Indeed, in these conditions the frequencies of mononucleated cells provide an indication of the background level of chromosome/genome mutations accumulated *in vivo* and the frequencies of binucleated cells with MN a measure of the damage accumulated before cultivation plus mutations expressed during the first *in vitro* mitosis. In the absence of cytochalasin B, mononucleated cells are analyzed for the presence of micronuclei. In the presence of cytochalasin B, mononucleated cells are recommended to be harvested at 24 hours post-PHA stimulation as there can be no doubt at this time-point that MN within such a cell are a result of in vivo rather than ex vivo division. Binucleated cells are recommended to be harvested at 72 hours post-PHA. Moreover, 24 hour post-PHA time-point may be the right time to count apoptotic/necrotic cells (Kirsch-Volders et all., 2001). During or after exposure to the test substance, the cells are grown for a period sufficient to allow chromosome or spindle damage to lead to the formation of micronuclei in interphase cells. For induction of aneuploidy, the test substance should ordinarily be present during mitosis. Harvested and stained interphase cells are analysed for the presence of micronuclei. Ideally, micronuclei should only be scored in those cells that have completed mitosis during exposure to the test substance or during the post exposure period, if one is used. In cultures that have been treated with a cytokinesis blocker, this is achieved by scoring only binucleated cells. In the absence of a cytokinesis blocker, it is important to demonstrate that the cells analysed are likely to have undergone cell division during or after exposure to the test substance. For all protocols, it is important to demonstrate that cell proliferation has occurred in both the control and treated cultures, and the extent of test substance-induced cytotoxicity or cytostasis should be assessed in the cultures (or in parallel cultures) that are scored for micronuclei. The effects in early responding tissues are a very important issue in radiotherapy (Elyan, S.A. et all.1998). Normal tissue intrinsic radiosensitivity is the main limiting factor for total irradiation dose in clinical practice defining a normal tissue tolerance to irradiation (RefBurnet,et all. (1994), Peacock, J., Ashton, A., Bliss, J. et all. (2000); West, C.M., Davidson; S.E., Elyan, S.A. et all. (2001). Pelvic organs morbidity after irradiation of cancer patients remains a major problem, although new technologies have been developed and implemented. Multiple studies have shown that normal tissue morbidity after irradiation in cancer patients correlates with radiosensitivity of skin fibroblasts and peripheral blood lymphocytes of these patients. This finding stimulates the search of prognostic criteria for evaluating the intrinsic individual cellular radiosensitivity (Elyan, S.A. et all. (1998); Biete, A.,et all. (2010).

Aims of the study: To indicate the importance of the application of micronucleus test as standardized cytogenetic method as an important biomarker in detecting the impact of ionizing radiation on the entire genetic

material in occupationally exposed health care workers. The micronucleus test (MNT) determines the frequency of the radiation induced micronuclei (MN) in peripheral blood lymphocytes, which could serve as an indicator of intrinsic cell radiosensitivity. After mutagen attack, MN in interphase cells are formed by mitotic loss of acentric fragments or chromosomes which are not incorporated in the daughter cell nuclei (Fenech, M. and Neville, S. (1992).

Matherial and methods: The study include health professionals who are directly and on daily basses exposed to ionizing radiation as physical agents and a control group that represents young and healthy population that is not exposed to any physical and chemical agents. By applying the micronucleus test, quantitative and qualitative analysis are performed on binuclear lymphocytes and micronucleus as reliable indicators of possible chromosomal damage in the cells. For the scoring of micronuclei the following criteria were adopted from Fenech et all, (2003): the diameter of the MN should be less than one-third of the main nucleus, MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary, MN should have similar staining as the main nucleus. Whole blood treated with an anti-coagulant (e.g. heparin), or separated lymphocytes, are cultured at 37°C in the presence of a mitogen e.g. phytohaemagglutinin (PHA) prior to exposure to the test substance and cytoB.

Results. The number of micronucleus in the control group is in the normal reference values. The major advantage of lymphocytes is that they are primary cells, easy to culture in suspension. The choice between whole blood and isolated lymphocytes depends upon the question addressed. The most important differences among the protocols are the hypotonic treatment (critical in particular for image analysis), fixation of the cells (dependent on laboratory preferences) and the final slide preparation. These different parameters significantly influence cell density and cytoplasm preservation. A detailed protocol for isolated lymphocyte and whole blood culture MN assays was recently published and included detailed scoring criteria validated and recommended by protocol.

Conclusion: In recent years the in vitro micronucleus test has become an attractive tool for genotoxicity testing because of its simplicity of scoring and wide applicability in different cell types. Because of its reliability and easy performance, MN asseys could be a promising method for evaluating normal tissue morbidity in cancer patients during radiotherapy with results yielded in less than 2 weeks.

Refferences:

1. Biete, A., Valduvieco, I., Rovirosa, A., Farrus, B., Casas, F., and Conill, C. Whole abdominal radiotherapy in ovarian cancer. *Rep Prac Oncol Radiother*. 2010; 15: 27–30

2. Elyan, S.A. et al. The intrinsic radiosensitivity of normal and tumor cells. *Int J Radiat Biol*. 1998; 73: 409–413

3. Fenech, M. and Morley, A.A. (1985), Solutions to the kinetic problem in the micronucleus assay, Cytobios., 43, 233-246.

4. Fenech, M. and Neville, S. (1992): Conversion of excision-repairable DNA lesions to micronuclei one cell cycle in human lymphocytes. *Environ Mol Mutagen*. 1992; 19: 27–36

5. Kirsch-Volders, M. (1997), Towards a validation of the micronucleus test. Mutation Res., 392, 1- 4. (2) Parry, J.M. and Sors, A. (1993), The detection and assessment of the aneugenic potential of environmental chemicals: the European Community aneuploidy project, Mutation Res., 287, 3-15.

6. Kirsch-Volders, M., Sofuni, T., Aardema, M., Albertini, S, Eastmond, D., Fenech, M., Ishidate, M. Jr, Lorge, E., Norppa, H., Surralles, J., von der Hude, W. and Wakata, A. **(2000), Report** from the In Vitro Micronucleus Assay Working Group, Environ. Mol. Mutagen., 35, 167-172.

7. Micheline Kirsch-Volders, Ilse Decordier, Azeddine Elhajouji1, Gina Plas,Marilyn J. Aardema2 and Michael Fenech (2011):Mutagenesis vol. 26 no. 1 pp. 177–184, 2011 doi:10.1093/mutage/geq068 REVIEW In vitro genotoxicity testing using the micronucleus assay in cell lines, human lymphocytes and 3D human skin models

8. RefBurnet, N.G., Nyman, J., Turesson, I., Wurm, R., Yarnold, J.R., and Peacock, J.H. The relationship between cellular radiation sensitivity and tissue response may provide the basis for individualizing radiotherapy schedules. *Radiother Oncol*. 1994; 33: 228–238

9. Peacock, J., Ashton, A., Bliss, J. et al. Cellular radiosensitivity and complication risk after curative radiotherapy. *Radiother Oncol*. 2000; 55: 173–178

10. West, C.M., Davidson, S.E., Elyan, S.A. et al. Lymphocyte radiosensitivity is a significant prognostic factor for morbidity in carcinoma of the cervix. *Int J Radiat Oncol Biol Phys*. 2001; 51: 10–15

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