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In vitro primary human lymphocyte flow cytometry micronucleus assay: simultaneous assessment of cell proliferation, cell cycle changes, apoptosis and micronucleus induction

Abstract

Exposure to DNA damaging agents and mutagens as well as genomic instability may contribute to carcinogenesis. Therefore, determination of potential genotoxic hazard became one of the goals of the preclinical phase of the drug development process. The results obtained within the genotoxicity studies should help in the determination of a potential cancer risk in humans. International Conference on Harmonization (ICH) and Organization for Economic Co-operation and Development (OECD) as well as other organizations provide guidance on the test selection and data interpretation using multiple in vitro and in vivo approaches. In vitro tests, however, show, in some conditions, positive results that find no confirmation in rodent in vivo tests. Such results are referred to as false or irrelevant positive results and may be caused by the use of systems that are p53 and DNA repair deficient or are based on transformed cells of non-human origin. Moreover, the lack of fully metabolic competent test systems may as well contribute to the inconsistency of in vitro and in vivo results.

The in vitro micronucleus test is currently widely used in determination of genotoxic activity. The assay has the potential to detect both clastogenic and aneugenic chemicals. It has been shown that the primary human lymphocyte micronucleus test gives very promising results regarding reduction of irrelevant positive results if compared to standard rodent cell lines. Therefore, the goal of the presented project was to develop a human primary lymphocyte in vitro micronucleus test using flow cytometry, taking into
account cell division and other confounding factors related to apoptosis/necrosis or excessive cytotoxicity. The technology was based on sequential staining with carboxyfluorescein diacetate succinimidyl ester as a division control marker, ethidium monoazide to determine the apoptosis/necrosis level and 4-6-diaminodino-2-phenyl indole as a DNA marker. The assay was validated and miniaturized using genotoxic and non-genotoxic compounds with various modes of action. Moreover, application of flow cytometry technology provided the tool to analyze and understand the basic mechanisms of the compounds modes of action. Our data showed that the flow cytometry based micronucleus test is a sensitive and reliable tool for the determination of clastogenic and aneugenic activity of the tested compound with an indication of mode of action.