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Mutation Research 585 (2005) 156–169
Genetic Toxicology and Environmental Mutagenesis
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Detection of numerical chromosomal aberrations by flow cytometry: A novel process for identifying aneugenic agents

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Received 2 March 2005; received in revised form 4 May 2005; accepted 4 May 2005
Available online 29 June 2005

Abstract

Aneuploidy plays a significant role in adverse human health conditions including birth defects, pregnancy wastage, and cancer. Currently, there is no screening method sufficiently validated that can be used routinely to identify aneugenic agents in vitro because most conventional test systems rely on the labor-intensive microscopic assessment of the aneuploid cell population.

Our laboratory has recently developed a flow cytometry-based procedure for assessing numerical chromosomal aberrations in mitotic populations of lymphocytes on the basis of DNA content. Studies were conducted in 24 h treated human lymphocyte cultures to determine the sensitivity of this flow cytometry-based procedure to detect aneugenic agents. A comparison between the microscopic and the flow cytometry-based procedures for scoring polyploidy shows a strong agreement exists between the two methods.

Treatments with two known aneugenic agents, griseofulvin, and paclitaxel (taxol), resulted in a dose-related increase in the mitotic index, aneuploidy, and polyploidy. In contrast, results from the treatments with two known clastogenic agents, mitomycin-C, and etoposide, show a dose-related decrease in the mitotic index with a slight increase in the frequency of hypodiploidy at concentrations that produce severe chromosomal breakage. There were no increases in hyperdiploidy and polyploidy observed.

In conclusion, the reproducibility of the results obtained in this study indicates that this flow cytometry-based procedure for assessing numerical chromosomal effects in mitotic populations on the basis of DNA content is promising for the routine detection and characterization of aneugenic agents.

Keywords: Automation; Aneuploidy; Polyploidy; Mitotic index; Mitotic marker; Flow cytometry; Flow cytometry-based procedure

1. Introduction

Aneuploidy is a condition in which the chromosome number of a cell deviates from the normal diploid or haploid complement by one or more chromosomes; excluding the exact multiple of the haploid state known as polyploidy. Aneuploidy can result from subtle cell

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doi:10.1016/j.mrgentox.2005.05.002

cycle checkpoint errors, spindle, and/or kinetochore defects, or as a consequence of exposure to aneuploidy inducing agents. Current in vitro methods for the detection of aneuploidy inducing agents typically rely on the use of mammalian cell lines or stimulated primary human lymphocytes. Assessment of the aneuploidy condition is performed microscopically in either metaphase or interphase populations by differentiating the aneuploid and polyploid cells from the normal population.

Characterizing aneuploid cells in the metaphase population often includes the systematic scoring of individual metaphase cells for DNA content by performing either chromosomal counts [1] or a partial evaluation on a subset of chromosomes using pancentromeric DNA probes and/or chromosome paints [2]. Either way, scoring metaphase cells for the gain or loss of individual chromosomes can be time consuming and problematic since hypotonic treatment, fixation, and air-drying are all technical factors that can contribute to chromosomal loss during slide processing [3]. More commonly, the induction of polyploid cells in routine genotoxicity screening (i.e. the chromosome aberration test) is used as a marker for a chemical's ability to induce aneuploidy, as recommended by current OECD guidelines for the testing of chemicals [4], even though there is no relevant evidence to support the relationship.

A more popular and reliable technique for detecting chromosomal loss is measuring the induction of micronuclei (MN) formation in cytochalasin-B (CYB) blocked interphase cells in conjunction with either CREST serum [5,6] or fluorescence in situ hybridization (FISH) with centromeric specific DNA probes [7,8]. Increased sensitivity of the CYB blocked MN assay can be achieved by conducting the test in combination with FISH chromosome specific probes which allow for the measurement of both chromosomal loss and non-disjunction events at the same time [9]. While this test system is sensitive to measuring chromosome loss, it cannot detect chromosome gain, thus deciphering the inter-relationships between the aneuploid and polyploid populations is not possible.

The aim of this study is to evaluate the capability of a modified flow cytometry-based technique, as demonstrated in Muehlbauer and Schuler [10], to detect aneugenic agents by assessing numerical chromosomal effects in mitotic populations. Human lymphocytes treated with two known aneugens, griseofulvin, and taxol, and two known clastogens, mitomycin-C, and etoposide, were selected for evaluation. A comparison between the microscopic and the flow cytometry-based procedures for scoring polyploidy frequency was conducted. The inter-relationships between hypodiploid (chromosome loss), normal, hyperdiploid (chromosome gain), and polyploid mitotic populations were investigated. Lastly, structural chromosomal aberrations were scored microscopically for the clastogens, mitomycin-C, and etoposide, to examine the consequence(s) of severely damaged metaphase cells on results obtained using the flow methodology.

2. Material and methods

2.1. Cell culture

Peripheral human blood was collected in heparinized vacutainers from healthy male and female volunteers (<50 years of age). Approximately 10 ml of whole blood was dispensed into a Lymphoprep™ tube (Nycomed Pharma AS Diagnostic, Oslo, Norway) containing ~10 ml of sterile (1×) Dulbecco's phosphate buffered saline (PBS, GIBCO BRL Life Technologies Inc, Grand Island NY). Following centrifugation for 20 min at 900 × *g*, the lymphocyte population was isolated and washed (1×) in phosphate buffered saline (PBS). Stock cultures of isolated lymphocytes were established in the range of $3.6–5.8 \times 10^5$ cells/ml in RPMI 1640 culture medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM L-glutamine and 1.0% phytohemmaglutanin-M form (PHA, all reagents from Gibco BRL Life Technologies Inc., Grand Island, NY). The stock cultures were maintained at 37 °C in 5% CO_2 atmosphere for ~44 h.

2.2. Chemicals

Griseofulvin (GF, CAS no. 126-078), paclitaxel (Taxol, CAS no. 33069-62-4), and etoposide (ETOP, CAS no. 33419-42-0) were purchased from Sigma Chemicals Co., St. Louis, MO. Mitomycin-C (MIT, CAS no. 50-07-7) was purchased from Moltox Inc., Boone, NC. All chemicals were dissolved in dimethylsulfoxide (DMSO) so that the final concentration of the solvent did not exceed 1% in the culture medium. The concentration ranges used were: GF 1.0–100 μg/ml,