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Review

Molecular mechanisms by which *in vivo* exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes *in vivo* and *ex vivo* in humans

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ABSTRACT

The purpose of this review is to summarise current knowledge on the molecular mechanisms by which *in vivo* exposure to exogenous chemical genotoxins in humans induces micronuclei (MNi) and other nuclear anomalies in lymphocytes *in vivo* and *ex vivo* after nuclear division *in vitro*. MNi originate from acentric chromosome fragments and/or whole chromosomes that are unable to engage with the mitotic spindle and/or fail to segregate properly to the daughter nuclei during anaphase. The lagging fragments or whole chromosomes are surrounded by membrane and become MNi. Acentric fragments are caused by failure of repair or mis-repair of DNA strand breaks which may be induced by chemicals that (i) damage the phosphodiester backbone of DNA, and/or (ii) inhibit the DNA damage response mechanisms or repair of DNA strand breaks and/or (iii) cause DNA replication stress due to DNA adduct or cross-link formation. MNi originating from lagging whole chromosomes may be induced by chemicals that cause defects in centromeres or the mitotic machinery. Mis-repair of chemically-induced DNA breaks may also cause formation of dicentric chromosomes and nucleoplasmic bridges (NPBs) between daughter nuclei in mitosis. NPBs may break and initiate recurring breakage-fusion-bridge cycles and chromosomal instability. The review also explores knowledge on (i) the routes by which lymphocytes in the human body may be exposed to genotoxic chemicals, (ii) kinetics of MNi expression in lymphocytes *in vivo* and *ex vivo* in the lymphocyte cytokinesis-block micronucleus (L-CBMN) assay and (iii) current evidence on the efficiency of the L-CBMN assay in detecting *in vivo* exposure to chemical genotoxins and its concordance with MNi expression in epithelial tissues. The review also identifies important knowledge gaps (e.g. effect of nanomaterials; interactions with nutritional deficiencies etc.) regarding mechanisms by which *in vivo* chemical genotoxin exposure may cause MNi formation in lymphocytes *in vivo* and *ex vivo* in lymphocytes.

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1. Introduction

The lymphocyte cytokinesis-block micronucleus cytome (L-CBMN) assay is one of the most widely used methods to measure genome damage in humans which may be induced by endogenous and environmental genotoxins [1]. It detects both structural and numerical chromosome aberrations [1–4]. The L-CBMN assay has the distinct feature that it allows (i) micronuclei (MNi) expressed *in vivo* to be observed in lymphocytes without the need of completing nuclear division *ex vivo* as well as (ii) micronucleus (MN) expression *ex vivo* in lymphocytes that have been stimulated to divide in *in vitro* culture [2]. The most commonly used mitogen in this assay is phytohaemagglutinin (PHA) which predominantly stimulates T-lymphocytes to divide, however other mitogens can be used that stimulate other lymphocyte subsets to divide. The use of lymphocytes is particularly important because it allows the L-CBMN assay to be performed both *in vivo* and *ex vivo* for biomonitoring studies and also for the purpose of *in vitro* genetic toxicology testing of chemicals and pharmaceuticals [5].

The *ex vivo* step in the assay is important because it allows persistent DNA or protein damage accumulated during maturation in and after exit from the bone-marrow to be converted to structural chromosome aberrations and malsegregated chromosomes during mitosis from which MN and other associated nuclear anomalies (nucleoplasmic bridges, NPB, and nuclear buds, NBUD) may ultimately arise [1–4]. For example a DNA strand break may lead to an acentric chromosome fragment that cannot engage the spindle, and protein damage to kinetochore or spindle proteins could lead to a whole chromosome lagging during anaphase. The exclusion of the acentric fragment and/or whole chromosome from the daughter nuclei during anaphase/telophase ultimately results in their conversion to MNi (Fig. 1). Misrepair of DNA strand breaks or impairment of chromatid separation due to defects in cohesins

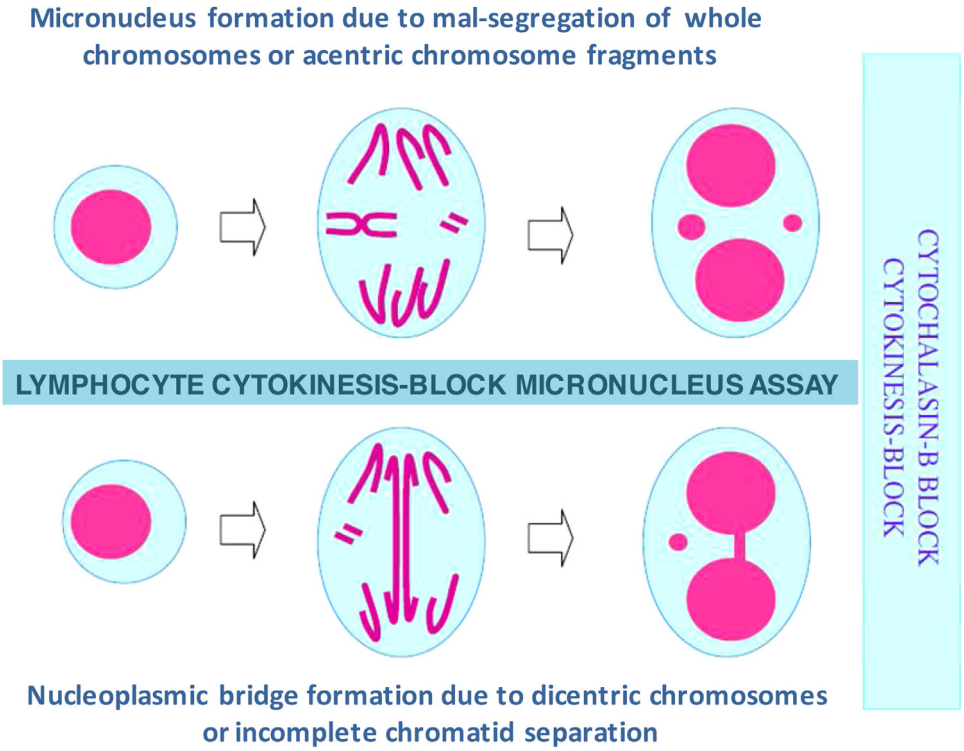

Fig. 1. Schematic diagram describing the origin of micronuclei and nucleoplasmic bridges and their detection using the cytokinesis-block micronucleus assay in lymphocyte cultures. Nucleoplasmic bridges may be accompanied with a micronucleus if the dicentric chromosome is a result of mis-repair of double stranded DNA breaks which also yields acentric chromosome fragments from which micronuclei may arise. The figure was adapted from (A) in *Fenech M, The in vitro micronucleus technique. Mutat Res 200; 455: 81–95*, with permission from Elsevier.