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PERSISTENCE OF MICRONUCLEI IN HUMAN LYMPHOCYTES AFTER FRACTIONATED IRRADIATION IN VITRO

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Abstract

The study evaluate relationship between incidence of micronuclei and chromosomal aberrations after fractionated irradiation of human lymphocytes in vitro. Obtained results have shown that incidence of chromosome aberrations aberrations declined faster than micronuclei at all given doses if the time between two irradiations is longer than 2 hours. The study confirmed that CBMN test is very sensitive assay for estimation of effects of ionizing radiation in the case of fractionated irradiation. This observation could be of interest for radiotherapy, particularly for applying micronucleus test as predictive test for hypersensitivity to ionizing radiation.

Introduction

The two most common in vivo cytogenetic assays, the micronucleus (MN) and metaphase aberration assays (CA) are inconsistent among countries worldwide [2]. Since there is a mechanistic link between chromosome breaks and micronuclei, it is generally agreed that these two endpoints detect similar types of chromosomal damage. Micronuclei are chromatin-containing structures in cytoplasm surrounded by a membrane without any detectable link to the cell nucleus. Micronuclei originate from chromosome fragments or whole chromosomes that lag at anaphase because they lack a centromere, or centromere is defective, or there is a defect in mechanism that enables the chromosome to distribute correctly to the poles of the cell at anaphase. Despite the increased popularity and use of the micronucleus test little has been reported on the comparative outcomes of the CA and MN tests, particularly in the cases of fractionated or prolonged conditions of irradiation. The aim of this study is to establish persistence of micronuclei in circulating human lymphocytes after fractionated irradiation in vitro and compare it with incidence of chromosomal aberrations.

Methods

Subjects: blood samples were taken from three donors of mean age 38 and were irradiated using X-rays 300 kVp, 10mA, 2.7mm CuHVT. The radiation dose employed were 1-4 Gy. Each dose was given in two equal fractions with four different time intervals between two irradiations: 30 minutes, 1 hour, 2 and 3 hours.
Blood culture and micronuclei analysis: The micronuclei analysis was carried out according to method of Fenech et al [1].

Results and Discussion

The results of the study are presented in Figure 1.

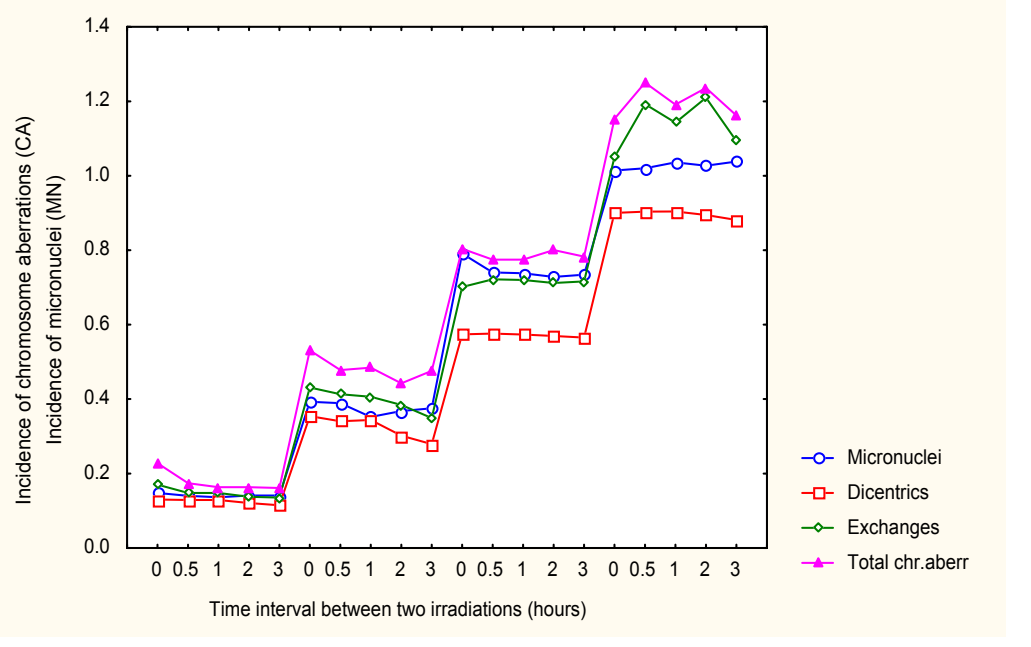

Figure 1: Incidence of chromosome aberrations and micronuclei in human lymphocytes after acute and fractionated irradiation employing doses of 1,2,3 and 4 Gy (X-rays)

Dose fractionated experiments for studying the interaction of lesions are based on the premise that the breaks induced by the first dose remain open to interact with the breaks induced by the second dose and the kinetics of disappearance of this possible interaction are similar to the repair kinetics of DSBs. Dose-fractionated experiments have indicated that the time during which the lesions are available for further interactions.

The comparison of the yields of chromosome aberrations and micronuclei in our previous investigation has shown that the best correlation is between the incidence of exchange aberrations and micronuclei [2]. At dose of 1 Gy incidence of chromosome aberrations decline slowly as a function of time interval between two irradiations. At the longest time interval between two irradiations (3 hours) incidence of dicentrics is lowered for 8%. The same tendency of declining aberrations is observed at dose of 2 Gy , where percent of dicentrics is lowered for 17%. In contrast to findings at doses of 1 and 2 Gy, incidence of dicentrics radiation doses of 3 and 4 Gy is almost the same to the incidence found in samples acute irradiated with the same doses. Considering incidence of total chromosome aberrations, at doses of that decline significantly and reach 70.6% of the value found in acute irradiated samples. At dose of 4 Gy incidence of exchages is slightly higher compared with acute irradiated sample.

Total incidence of chromosomal aberrations declined significantly at dose of 1 Gy, particularly at the longest time interval between two irradiations. At dose of 2 Gy they decline much slowly, wheras at dose of 3 Gy they reach the incidence found in acute irradiated sample. At dose of 4 Gy the incidence of aberrations are higher than found in acute irradiated sample.Micronuclei findigs are following: at dose of 1 and 2Gy the incidence of micronuclei decline slowly, as dicentrics, while at dose of 3 and 4 Gy their incidence is almost constant as to those values counted in acute irradiated samples. Mainly types of lesions that are induced in the DNA by ionizing radiation are single- and double strand breaks (DSBs), base damages (BDs) and DNA-protein cross-links. DSBs and BDs are considered to be the most possible lesions. The yield of exchange type of aberrations after low LET radiation follows a linear-quadratic model ($y=\alpha D+\beta D^2$)+C. The α component is contributed from lesions probably DSBs, occurring close together arising from a single track and increasing linearly with the dose. The repair of these lesions is very fast (less than 20 minutes). The β component comes from two independently induced lesions interacting with each other from a an exchange aberration and therefore follows two-hit kinetics. The presence of micronuclei indicates that a cell has suffered chromosome damage. Micronucleus arise after cell division when a chromosome or chromatid fragment is lost from the nucleus but retained by the cytoplasm. Although is clear that relation between micronucleus yield and yield of chromosomal damage (as measure as number of dicentrics) is complex, the present investigation support the CBMN test as simple and sensitive method for monitoring the sensitivity of persons undergoing radiotherapy.

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