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Effect of storage conditions of blood on radiation-induced chromosomal aberrations and apoptosis in human lymphocytes

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ABSTRACT

To evaluate the effect of storage conditions of blood on the direct relationship between radiation-induced chromosome aberrations and apoptosis in human peripheral blood lymphocytes, whole blood was irradiated with 3 Gy X-rays. Directly after irradiation, a sample of blood was analyzed for chromosome damage and proliferation index, after phytohaemagglutinin stimulation and incubation at 37 °C for 56 h. Blood samples were stored for 48 h at 4 and 20 °C with or without phytohaemagglutinin and analyzed for cell viability and apoptosis at 0, 24 and 48 h storage time. After 48 h of storage, unstimulated cultures were stimulated to proliferate. These samples and cultures stimulated immediately before storage were incubated at 37 °C for 56 h and analyzed for chromosome damage and proliferation index. Metaphases were examined for the presence of dicentric, excess acentric, and rings. Storage at 20 °C without phytohaemagglutinin for 48 h increases significantly the yield of apoptosis and decreases significantly the yield of dicentric. During 48 h of storage time the presence of phytohaemagglutinin and the temperature of 4 °C protected the irradiated lymphocytes from apoptosis allowing accurate estimation of the real yield of radiation-induced chromosome damage. Therefore these blood-storage conditions enable analysis in metaphase and may offer some advantages for biodosimetry of absorbed radiation dose.

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1. Introduction

Extensive studies have demonstrated that living cells react in different ways when subject to radiation injury causing DNA damage, depending on both the cell type and radiation dose. If the damage is modest, it may be completely handled by the specific survival-response network, while if the damage is excessive and/or irreparable, the cell undergoes apoptosis through activation of the programmed cell-death pathway. In case of escape from these mechanisms, a transformed tumour cell is possibly generated [1,2].

Since the outcome of genotoxic damage in cells resistant to apoptosis will be entirely different from that found in apoptosis-prone cells, apoptosis appears to play an important role in physio-pathological processes, particularly within the blood system. In fact, human peripheral blood lymphocytes (PBLs) provide a convenient and readily available source of human material and are routinely used experimentally to assess the extent of cytogenetic damage induced by physical and chemical agents. PBLs represent the most common source in radiation-biodosimetry studies. It is known that culture conditions, cell-cycle phase and differentiation stage can influence their sensitivity to radiation-induced apoptosis. In particular, addition of mitogens or growth factors can enhance

their radio-resistance [3–6] and furthermore, mitogen-stimulated cells immediately after irradiation have a higher frequency of chromosome aberrations than cells resting in G0-phase before the addition of mitogens [7,8].

The relationship between radiation-induced unstable chromosomal aberrations and apoptosis is known [8–10]; in particular, after 48 h of storage lymphocytes carrying dicentric chromosomes are preferentially eliminated by the apoptotic process [11]. These findings have implications for biomonitoring and biodosimetry studies using human lymphocytes, usually analyzed after a period of storage (involved in collection and transportation). In a collaborative exercise on cytogenetic dosimetry for simulated accidental irradiation, the transit times of blood samples from the dispatching laboratory to the recipients' laboratories ranged from 24 to 100 h [12]. Therefore, storage conditions of blood and lymphocytes are important for a proper reflection of the effects of exposure to genotoxic chemicals or ionizing radiation (e.g. in the case of accidents) and it is important to decide if the storage of human blood samples influences the outcome of the cytogenetic endpoint studied.

This problem has been partially addressed in a previous study [13], which concluded that storage of human whole blood in the presence of phytohaemagglutinin (PHA) at 4 °C up to 4 days was optimal to maintain maximum viability of the lymphocytes. Since the choice of suitable blood-storage conditions could be a critical factor for biological dosimetry purposes, the aim of the present study was to assess whether the blood-storage conditions, i.e., tem-

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