RESEARCH NOTE

A cytogenetic methodology to evaluate *in vitro* the G2-chromosomal radiosensitization induced by chemicals at non-clastogenic doses

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Introduction

Efforts to identify chemicals that are likely to pose a potential cancer threat to humans have intensified in recent years. Since radiation is a well known carcinogenic and leukemogenic agent, the chemically-induced radiosensitization may have important implications for human health. In this study, we propose an experimental methodology to evaluate *in vitro* the G2-chromosomal radiosensitization induced by chemicals at non-clastogenic doses and elucidate the mechanism involved. The proposed approach is based on a combination of cytogenetic methods, such as the G2-chromosomal radiosensitivity assay (G2-assay) and the premature chromosome condensation (PCC) via calyculin-A induction or cell fusion.

The constantly growing research regarding the clastogenic and carcinogenic potential of a variety of harmful physical and chemical agents includes the monitoring of cellular alterations linked with carcinogenesis such as (i) induction of chromosomal damage, (ii) disturbances of the DNA-repair machinery, and (iii) alterations in cell-cycle control mechanisms (Luch 2002; Sakata *et al*. 2007). However, most assessments of possible deleterious outcomes from environmental and occupational exposures are directed mainly towards single agents (either chemicals or radiation) and thus combined effects that may cause synergism often remain unclear. Since ionizing radiation has been associated with cancer (Finch 2007), chemically-induced-chromosomal radiosensitization may escalate the risk of genetic diseases and carcinogenesis (Scott *et al*. 1996). In some occupational environment (e.g., diagnostic radiology laboratories), the extensive exposure to both chemicals and radiation increases the necessity of evaluating the chemically-induced chromosomal radiosensitization.

In this work, we propose an experimental methodology to evaluate *in vitro* the G2-chromosomal radiosensitization induced by chemicals at non-clastogenic doses in PHA-stimulated healthy human peripheral blood lymphocytes (PBL) and also to elucidate the mechanism involved. This methodology is based on a combination of cytogenetic methods such as: (i) conventional chromosomal aberration analysis at metaphase and direct chromatid type aberration analysis in the G2-phase using calyculin-A; (ii) the G2-chromosomal radiosensitivity assay (G2-assay) after irradiation of cells in the G2-phase of the cell-cycle; and (iii) the PCC method via calyculin-A induction in cycling lymphocytes or cell fusion in G0-lymphocytes. The proposed protocol involves three steps.

The first step (figure 1a), involves the application of standard clastogenicity tests on healthy human PBL to evaluate *in vitro* the clastogenicity levels and the non-clastogenic doses of the tested chemical (Jacobson-Kram and Contrera 2007).

The second step (figure 1b) includes the pre-irradiation exposure of PBL cultures to non-clastogenic doses of the tested chemical and the evaluation of the chemically-induced-chromosomal radiosensitization as quantified by means of the G2-assay. This assay is based on the exposure of PBL cultures to ionizing radiation at a dose of 1 Gy (dose rate: 1 Gy per min) in the G2-phase of the cell-cycle and the microscopic analysis of chromatid type aberrations at the subsequent metaphase (Scott *et al*. 1996). Since an increase in the chromatid type aberrations by means of the G2-assay has been linked with chromosomal instability and cancer proneness (Scott *et al*. 1996; Terzoudi *et al*. 2000; Scott 2004), any statistically significant increase in the radiation-

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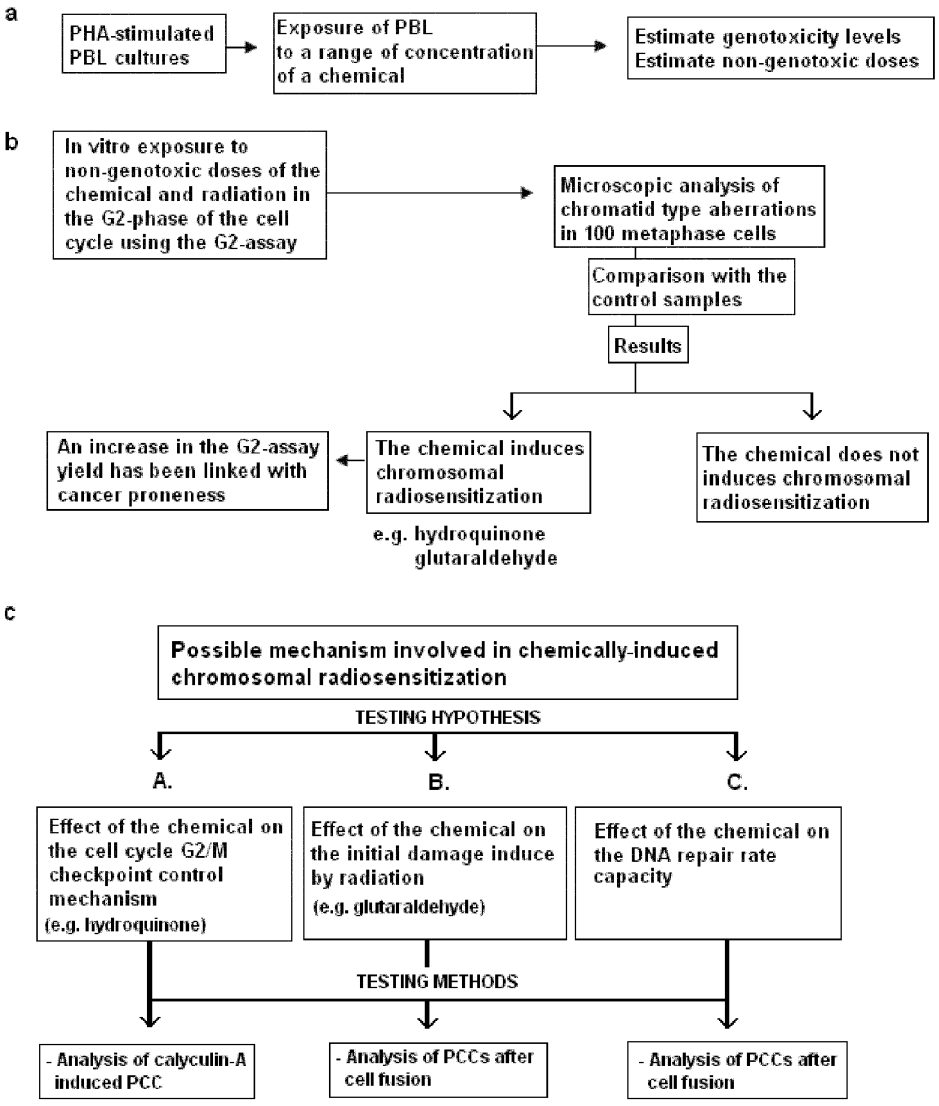

PBL. peripheral blood lymphocytes;
PHA. phytohemagglutinin; PCC. premature chromosome condensation.

Figure 1. Experimental methodology for the *in vitro* evaluation of chemically-induced G2-chromosomal radiosensitization at non-clastogenic doses and elucidation of the mechanism involved.

induced chromatid damage due to the chemical exposure, could reveal its carcinogenic potential.The third step (figure 1c), involves the *in vitro* evaluation of the mechanism involved in chemically-induced-chromosomal radiosensitization. This mechanism could be associated with an effect of the tested chemical on either: (i) the initial radiation induced DNA damage, (ii) the cellular DNA repair rate capacity, or (iii) the cell-cycle proliferation kinetics and the G2/M checkpoint control mechanism. These three disturbances are tested using three set of experiments based on the PCC method by means of either calyculin-A induction or polyethylene glycol mediated cell fusion (Pantelias and Maillie 1983; Terzoudi *et al*. 2003; Hatzi *et al*. 2006). Calyculin-A induced PCC method allows the classification of chromosomes in the G2-phase of the cell-cycle and the monitoring of any effect on cell-cycle proliferation kinetics and the G2/M checkpoint control mechanism resulting from an exposure to a tested chemical (Hatzi *et al*. 2007). Cell fusion mediated PCC method enables the direct analysis of chromosomal damage in G0-lymphocytes after exposure to clastogenic agents as well as the monitoring of the DNA repair rate of chromosomal damage (Pantelias and Maillie 1985).

The proposed methodology has been applied successfully in our recent work (Hatzi *et al*. 2007; Hatzi 2008; Hatzi *et al*. 2008). It was shown that glutaraldehyde and hydroquinone that are widely used by workers in diagnos-

tic radiology laboratories (Makropoulos and Alexopoulos 2006), induce G2-chromosomal radiosensitization in healthy human PBL even at non-clastogenic doses, thus highlighting their potential carcinogenic profile. The mechanism of the chemically-induced-chromosomal radiosensitization was further elucidated in each case. Specifically, it was found that pre-irradiation exposure of PBLs to glutaraldehyde induces radiosensitization by increasing the initial yield of chromatid type aberrations following irradiation (Hatzi *et al*. 2008) while hydroquinone induces chromosomal radiosensitization by a different mechanism. In particular, hydroquinone affects the cell-cycle proliferation kinetics inducing a less efficient G2-M checkpoint, which results from the increased G2-chromosomal radiosensitization thus facilitating the transition of damaged cells from G2-phase to M-phase (Hatzi *et al*. 2007).

Future applications of the proposed experimental methodology could contribute to test *in vitro* the G2-chromosomal radiosensitizing potential of other chemicals used in occupational environments where simultaneous exposure to ionizing radiation may also occur. The evaluation of the capacity of a given chemical to induce chromosomal radiosensitization will enrich our knowledge about its hazardous and/or carcinogenic potential. Therefore, the radiosensitizing capacities of given chemicals should be regarded as critical parameters in the process of chemical carcinogenesis. In the field of cancer therapy, the application of the proposed methodology to elucidate the mechanism of action of the radiosensitizers used in cancer treatment (Shenoy and Singh 1992) could contribute to modulating the administered dose (of the radiosensitizer and/or ionizing radiation), thus optimizing radiotherapy protocols.

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