gama-irradiation-induced DNA single- and double-strand breaks and their repair in chronic lymphocytic leukemia cells of variable radiosensitivity


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Abstract

Abstract. gama-Irradiation-induced DNA single- and double-strand break (SSB and DSB) formation and their repair kinetics in normal hematopoietic cells and in leukemic lymphocytes was investigated using alkaline and neutral comet assays. The cells were isolated by density gradient centrifugation from peripheral blood of patients with chronic lymphocytic leukemia (CLL) and from healthy study subjects. Furthermore, CD34+ progenitor cells isolated with immunomagnetic beads from bone marrow of non-leukemic persons were investigated. The cytotoxicity of 137Cs irradiation was determined in vitro in peripheral blood mononuclear lymphocytes from 36 CLL patients and from 8 healthy donors using radioactive leucine incorporation assay in 4-day culture. A dose-dependent increase in DNA migration was observed in alkaline (SSBs) and neutral (DSBs) gel electrophoresis when the cells were exposed to γ-irradiation doses up to 10.4 Gy. After irradiation with doses of 2.4 and 5.4 Gy, the cells repaired their single- and double-strand breaks almost completely. The formation and repair of DNA strand breaks were essentially similar in all normal cell populations investigated and in CLL cells. The gama-irradiation-induced cytotoxicity did not correlate with DNA strand break formation and repair capacity. According to these results, the differences of gama-irradiation tolerance among individual CLL cases and among healthy persons are explicable in terms other than DNA strand break formation or repair.

Resistance to radiation and chemotherapeutic drugs represents a major obstacle in human cancer therapy. A variety of different mechanisms can contribute to cellular resistance against DNA-reactive anticancer drugs and irradiation. One such mechanism may be enhanced repair of drug- or irradiation-induced DNA lesions. However, very little is known about the DNA repair capacity of malignant cells derived from patients. This is mainly because of a lack of sufficiently sensitive methods for quantifying DNA lesions and the rate of their repair in relatively small cell samples or in individual cells available from patients [1].

A few attempts have been made to investigate the role of DNA repair in drug resistance in chronic lymphocytic leukemia (CLL) cells. No correlation was observed between the clinical outcome and the DNA repair activity of leukemic cells when the latter was indicated by the removal of cross-links or by the activity of a central DNA repair enzyme, O6-alkylguanine-DNA alkyltransferase [2, 3]. On the other hand, increased expression of a DNA excision repair gene (ERCC1) in cell extracts and enhanced repair of DNA cross-links in cells from therapy-resistant CLL patients have been reported [4, 5]. In addition, alterations in the mismatch repair pathway and a loss of stringent control of the DNA repair process appear to be peculiar to CLL cells [6].
It has also been demonstrated that resistance to irradiation may develop in parallel with resistance to chlorambucil [8].

Survival of CLL cells after ionizing irradiation varies widely among samples. It has been reported that the in vitro kinetics of cell death of lethally-irradiated cells varies independently of susceptibility to lethal injury [9]. In order to investigate the formation of DNA single- and double-strand breaks (SSBs and DSBs) and their rejoining pathway in radiation-resistant and radiation-sensitive cases of CLL, we set up experiments to study the extent of break formation and joining kinetics, using alkaline and neutral single cell gel electrophoresis, or comet assay [10-17]. For comparison, similar studies were performed with peripheral blood lymphocytes from healthy volunteer donors as well as with bone marrow CD34+ progenitor cells obtained from patients with no malignant hematological diseases.

Materials and methods

Subjects

Clinical specimens were obtained after informed consent from 36 consecutive patients referred to the CLL outpatient clinic of Tampere University Hospital. The diagnosis and staging of CLL were based on standard clinical, morphological and immunophenotyping criteria, as illustrated in Table 1. CLL cells were isolated from peripheral blood samples by centrifugation over a Lymphoprep layer (Nycomed, Oslo, Norway) of density 1.077 g/ml. The CLL cells were washed twice with phosphate-buffered saline (PBS, pH 7.4) and once with complete CLL medium consisting of RPMI 1640 (20 mM Hepes, ICN Biomedicals, Costa Mesa, CA), 10% heat-inactivated fetal calf serum (Gibco BRL, Paisley, UK), 2 mM L-glutamine and antibiotics (Gibco; 50 U penicillin/ml and 50 µg streptomycin/ml). Thereafter, the cells were washed once with complete CLL medium without L-glutamine and antibiotics and resuspended in RPMI 1640, 50% heat-inactivated fetal calf and 10% dimethyl sulfoxide (DMSO). The cells were placed at -70°C overnight and moved into liquid nitrogen the following day. For the comet assay experiments, cells were thawed rapidly at +37°C, washed twice and resuspended in complete CLL medium, where the cell viability was > 98% as determined by trypan blue exclusion. The numbers of polyclonal monocytes, B cells and T cells were determined by flow cytometry. Their number was always less than 10%, indicating that the clonal B-CLL cells represented > 90% of the isolated cells. Blood was also obtained from healthy donors and mononuclear cells (MNCs) were isolated as described above.