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In vitro genotoxic effects of the anticancer drug gemcitabine in human lymphocytes

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Abstract

This research was carried out to investigate in vitro genotoxic effects of the anticancer agent gemcitabine on the induction of chromosomal aberrations and sister-chromatid exchange in human lymphocytes.

Three doses of gemcitabine (0.001, 0.002 and 0.004 μg/ml) were applied to lymphocyte cultures from 15 donors. There was a significant increase in the induction of chromosome aberrations and in the occurrence of sister-chromatid exchange in these cells. In addition, gemcitabine significantly decreased the mitotic index and replicative index for all doses. Dose–response regression lines were used to compare the individual susceptibilities to gemcitabine with respect to the chromosome aberration and sister-chromatid exchange frequencies. Our results indicate that gemcitabine is able to induce both cytotoxic and genotoxic effects in human lymphocyte cultures in vitro in a dose-dependent manner.

Keywords: Gemcitabine; Sister-chromatid exchange; Chromosome aberrations; Genotoxic; Cytotoxic; In vitro; Human lymphocytes

1. Introduction

Nucleoside analogues belong to the most effective classes of drugs used in the treatment of specific types of malignancies and of some types of viral infections. The biological activity of most nucleoside analogues is due to their ability to inhibit the DNA synthesis process [1]. Gemcitabine is a member of the deoxycytidine family of nucleoside analogues and is used in the treatment of various solid tumors. Upon entering the cell, gemcitabine is phosphorylated to one of two active forms, gemcitabine diphosphate and triphosphate [2]. By mimicking the structure of the natural nucleoside, deoxycytidine, gemcitabine is inserted into DNA in base-pairing with deoxyguanosine, resulting in an arrest of DNA polymerization [2,3]. Likewise, gemcitabine inhibits RNA synthesis by incorporation into RNA chains in a similar manner. Furthermore, it also affects the deoxynucleotide pool by interfering with the enzyme ribonucleotide reductase [3]. Gemcitabine has shown a broad spectrum of activity against panels of solid murine tumors and human tumor xenografts [3].

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It has been used in the treatment of lung, pancreatic, breast, bladder and ovarian cancers [3–5].

In vitro, gemcitabine has been shown to induce apoptosis in a lymphoblastoid cell line, presumably by giving rise to DNA fragmentation [6]. In the study of Auer et al. [7], several doses of gemcitabine (from 0.6 to 32 ng/ml) were assessed for different cytogenetic parameters in low-passage fibroblast cells (LPF) and in Chinese hamster V79 cells. Gemcitabine induced high levels of chromosome aberrations (CA) and sister-chromatid exchange (SCE) in the V79 cells. However, these authors [7] were unable to observe any induction of CAs and SCEs in LPF cells, regardless of the dose. Aside from this report, no information is available at present on the in vitro genotoxic properties of gemcitabine in non-tumor cells. In a recent study by Aydemir and Bilaloğlu [8], it was reported that gemcitabine increased the frequency of CAs and micronuclei in mouse bone-marrow cells in vivo. However, the in vitro genotoxic effects of gemcitabine on human lymphocytes remain unclear.

In the past decade, interindividual variability in drug response has received much attention. The causes for this variation may be of genetic, physiologic and environmental origin [9]. One could envision that genetic factors could account for about 20–40% of the interindividual differences in drug metabolism and response. It is known that there are genotypic and, hence, phenotypic variations in DNA repair genes among individuals [10]. Thus, different levels of CA and SCE responses may occur in individuals exposed to genotoxic agents. In an in vitro study, it has been reported that several human malignant glioma cell lines are differently susceptible to the cytotoxic actions of gemcitabine at clinically relevant concentrations [11]. Although the different responses to cytotoxic effects of gemcitabine have been shown in vitro, no evidence of differences in susceptibility to the genotoxic effects of gemcitabine has been reported in the literature.

Genotoxic effects of anticancer drugs in non-tumor cells are of special significance due to the possibility that they may induce secondary tumors in cancer patients. Furthermore, the mutagenic and carcinogenic effects of antineoplastic agents on the health-care persons handling these drugs also need to be considered carefully. Therefore, it is important to determine the genotoxic potential of a drug that will be used in chemotherapy, particularly in native human cells. In general, CAs, SCEs and micronuclei are considered to be essential markers of genotoxicity in in vitro studies [12,13].

The present study was carried out to determine the in vitro genotoxic effects of gemcitabine on human lymphocytes using the CA and SCE assays. Additionally, we also sought to address the question whether interindividual differences in dose–response relationships to gemcitabine occur, by analyzing blood samples from 15 donors (9 women and 6 men).

2. Materials and methods

2.1. Chemicals

Gemcitabine (Cat. No.: 103882-84-4) was purchased from Lilly-Onkologie, Germany. 5-Bromodeoxyuridine was purchased from Sigma (Cat. No.: B 5002), and Mitomycin-C from Kyowa, Hakko, Japan.

2.2. Lymphocyte cultures and cell harvesting

Heparinized blood samples were collected from 15 healthy donors (6 males, 9 females, non-smokers, age range 20–40 years). Gemcitabine concentrations were 0.001, 0.002 and 0.004 μg/ml. As negative and positive controls, distilled water and Mitomycin-C (0.25 μg/ml) were used, respectively. Gemcitabine and Mitomycin-C (MMC) were dissolved in sterile distilled water and solutions were prepared immediately before use, to avoid degradation of the drugs.

Whole blood (0.5 ml) was added to 5 ml culture medium containing RPMI 1640 medium (pH 6.8–7.2), 20% fetal calf serum, 6 μg/ml phytohemagglutinin L (PHA-L), 0.5 mg/ml L-glutamine, and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin) for 72 h at 37 °C. For analysis of SCE, 10 μg/ml 5-bromodeoxyuridine was added 24 h after initiation of the culture. The cells were treated with gemcitabine for 24 h. Colcemid was added at 22 h at a final concentration of 0.2 μg/ml.

Chromosomes were prepared using standard procedures [14]. For analysis of SCE, the chromosome slides were stained according to the Fluorescence-plus-Giemsa technique [15].