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DNA damage in peripheral blood lymphocytes in patients during combined chemotherapy for breast cancer

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Abstract

Combined chemotherapy is used for the treatment of a number of malignancies such as breast cancer. The target of these antineoplastic agents is nuclear DNA, although it is not restricted to malignant cells. The aim of the present study was to assess DNA damage in peripheral blood lymphocytes (PBLs) of breast cancer patients subjected to combined adjuvant chemotherapy (5-fluorouracil, epirubicin and cyclophosphamide, FEC), using a modified comet assay to detect DNA single-strand breaks (SSB) and double-strand breaks (DSB).

Forty-one female patients with advanced breast cancer before and after chemotherapy and 60 healthy females participated in the study. Alkaline and neutral comet assays were performed in PBLs according to a standard protocol, and DNA tail moment was measured by a computer-based image analysis system.

Breast cancer patients before treatment had higher increased background levels of SSB and DSB as compared to healthy women. During treatment, a significant increase in DNA damage was observed after the 2nd cycle, which persisted until the end of treatment. Eighty days after the end of treatment the percentage of PBLs with SSB and DSB remained elevated, but the magnitude of DNA damage (tail moment) returned to baseline levels. There was no correlation between PBL DNA damage and response to chemotherapy.

DNA-SSB and DSB in PBLs are present in cancer patients before treatment and increase significantly after combined chemotherapy. No correlation with response to adjuvant chemotherapy was found. Biomonitoring DNA damage in PBLs of cancer patients could help prevent secondary effects and the potential risks of developing secondary cancers.

Keywords: Breast cancer; Chemotherapy; Comet assay; DNA damage; DNA repair; Lymphocytes

1. Introduction

Antineoplastic drugs have been widely used in the treatment of several types of human malignancies [1]. In general, approximately half of breast cancer patients receive chemotherapy as part of their treatment [2]. The goal of this therapy is to destroy malignant cells while trying to maintain the integrity of normal cells. The difference between the amount of drug needed to induce successful tumorigenic action and the amount that produces toxicity in normal cells is small [3]. Carcinogenicity of

Abbreviations: SSB, DNA single-strand breaks; DSB, double-strand breaks; PBLs, peripheral blood lymphocytes; AP, apurinic sites; ROS, reactive oxygen species; TM, tail moment; FAP, 5-fluorouracil, adriamycin and cisplatin; FEC, 5-fluorouracil, epirubicin and cyclophosphamide; AO, acridine orange; CR, complete responders; PR, partial response; NR, nonresponders.

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many chemotherapy agents is partly dependent on their ability to induce mutagenic and clastogenic DNA damage, including base adducts, replication errors, strand breaks and crosslinks [4,5]. Also, a number of studies provide evidence that chemotherapeutic agents induce apoptosis in different cell lines and tumors [6,7].

In this context, repair of DNA damage is an important mechanism that protects against the deleterious effects of carcinogenic therapies. Activation of DNA repair pathways is intimately linked with other cellular pathways including transcription, cell cycle checkpoint arrest and apoptosis so that damaged cells respond appropriately to DNA damage and are either repaired or eliminated [8–10]. Loss or attenuation of DNA repair can lead to genomic instability and it has been suggested as a mechanism that operates in the pathogenesis of some therapy-induced cancers [11].

A number of *in vitro* and *in vivo* studies [12–21] have been published on DNA damage effect and alterations of DNA repair efficiency before and after treatment with different antineoplastic drugs and/or radiation in peripheral blood lymphocytes (PBLs). These studies have demonstrated that administration of antineoplastic drugs produces significant DNA damage in PBLs and displays decreased DNA repair efficacy only hours after drug administration. Our patients received the alkylating drug cyclophosphamide in combination with the 5-fluorouracil (5FU) and epirubicin (FEC). These drugs are known to produce a variety of DNA damage including DNA strand breaks and alkylating base modifications. Antimetabolite drugs such as 5FU work by inhibiting essential biosynthetic processes or by being incorporated into macromolecules such as DNA and RNA and inhibiting their normal function [22]. Intracellular production of free radicals along with intercalation with DNA and subsequent inhibition of topoisomerase II is generally accepted as the major mechanism of cytotoxicity by epirubicin [19]. Cyclophosphamide is a bifunctional alkylating nitrogen mustard analogue capable of inducing various types of primary DNA damage, gene mutations and chromosomal aberrations and also forms crosslinks that are translated into DNA strand breaks during excision repair [23]. Most of these combinations offer the advantage of producing additive or synergistic cancer cell killing while decreasing the probability for the emergence of drug-resistant cells.

The comet Assay or single-cell gel electrophoresis (SCGE) technique is a sensitive method for detecting the presence of DNA single-strand breaks, double-strand breaks, DNA interstrand crosslinks and alkaline-labile sites [24]. Two different versions of the comet assay have been developed: the alkaline method used for detecting of single-strand breaks according to Singh et al. [25], and the neutral assay modified by Olive et al., to visualize double-strand breaks [26]. SCGE has been applied to characterize individual's capacity in nucleotide excision repair and in rejoining of ionizing radiation-induced strand breaks in lymphocytes of cancer patients who developed secondary thyroid tumors after exposure to therapeutic irradiation [27]. Comet assay offers an opportunity for correlating levels of therapy-induced DNA damage with administered dose and for modulating the dose schedule to reduce genotoxic damage [28,29]. It has been suggested that the degree of PBL DNA damage might be indicative of response to chemotherapy [17,18,30,31].

It is customary that in combined chemotherapy protocols such as FEC, antineoplastic agents are administrated in four to six cycles, usually separated by 3 weeks to allow recovery from bone marrow damage and leukopenia [32]. It has not been established how much DNA damage persists in PBLs after each cycle and at the end of treatment. It would be useful to investigate the extent of DNA damage occurring in PBLs of patients undergoing combined chemotherapy during the 12-week treatment duration and to correlate these findings with chemotherapy response. It the present study we assessed DNA damage in PBLs from breast cancer patients after each cycle in the course of therapy with FEC, using the alkaline and neutral comet assay in PBLs from breast cancer patients to detect DNA SSB and DSB and to correlate these findings with response to chemotherapy.

2. Methods

2.1. Patients and samples studied

Blood samples were obtained from 41 subjects with ductal carcinoma of the breast at clinical stage III. Patients' ages were between 32 and 75 years. Patients were programmed for chemotherapy with the following protocol: 165 mg/m^2 of 5-fluorouracil, 70 mg/m^2 of epirubicin and 1875 mg/m^2 of cyclophosphamide (FEC) administrated in four cycles every 21 days [2]. All patients had not been subjected to any other therapy and were non-smokers. A full clinical evaluation 1 month after the termination of chemotherapy permitted the separation of three groups according to the response observed: Group 1 patients were complete responders (CR) in which the tumor disappeared; Group 2 patients had partial response (PR) in which >50% of tumor disappeared, and Group 3 patients were nonresponders (NR) where <50% of the tumor disappeared and lymph nodes remained unchanged or progressed. Response to chemotherapy was established by clinical assessment of the breast mass according to the International Union against Cancer criteria. Patient characteristics are shown in Table 1. The control group consisted of 60 healthy volunteers age-matched, who were non-smokers without history of radiation exposure, drug intake, or relevant alcohol consumption (median 39 and range 25–63 years).

Blood samples were collected 21 days after each intravenous (IV) administration of antineoplastic agents. From each patient, one pre-treatment blood sample was obtained prior to the first chemotherapy cycle, and a final sample was collected 80 days after treatment completion.

2.2. Cell preparation

PBLs were obtained from heparinized blood and separated using HP-1077 according to the method of Boyum [33]. PBLs were washed and resuspended with PBS buffer and adjusted at 1×10^6 cell/ml. After washing cells in PBS, they were tested for viability using trypan blue 0.05% exclusion test. Cytological smears were stained with Giemsa for morphological observation. Apoptotic morphology was scored according to the criteria suggested by Wyllie et al. [34] and counted per 500 cells.

2.3. Comet assay

Lymphocytes were processed following two different methods. The alkaline denaturating comet assay allows migration of unwinded DNA SSB [24], whereas the modified neutral non-denaturating method preferentially permits migration of DNA DSB [26]. Briefly, cell suspensions adjusted at 10^4 cells were embedded in 1% normal-melting agarose at 40 °C. The content was immediately pipetted onto frosted microscopic slides and allowed to gel.