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Can individual repair kinetics of UVC-induced DNA damage in human lymphocytes be assessed through the comet assay?

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Abstract

The suitability of the comet assay for quantifying DNA repair capacity at individual level was studied following the kinetics of nucleotide excision repair (NER) in human lymphocytes from four healthy donors, at various time steps after a single dose of UVC. A significant increase of DNA migration was seen as soon as 20 min after UV exposure, reaching the peak within 60–90 min. Afterwards, a rapid decline was observed, approaching the basal level at 180–240 min. The increase could be ascribed to excision activity, while the reduction to gap filling and rejoining, as demonstrated by the effects of phase-specific inhibitors, novobiocin and aphidicolin. Therefore, the comet assay should allow following the biphasic kinetics of NER. Wide inter-individual differences were observed, although repeated tests on the same donor cells revealed a large experimental variation. To quantitatively compare the individual patterns, a mathematical model was developed that adequately fitted the experimental results and estimated appropriate descriptors for each phase and for each donor. A second approach was also used to directly compare the distributions of damaged cells and to assess the differences between donors and between experiments visualizing them as reciprocal distances on a two-dimensional space computed with a principal component analysis (PCA). The results confirmed the inter-individual differences, but also the strong influence of experimental factors of the comet assay.

The two approaches provided the means of accurately comparing DNA repair kinetics at individual level, taking also into account the experimental variability which poses serious doubts on the suitability of the comet assay. Nevertheless, since this methodology allows a detailed analysis of repair kinetics and it is potentially very useful for identifying individual with reduced repair capacity, further efforts have to be addressed to improve the reproducibility of the comet assay.

Keywords: UVC irradiation; DNA repair capacity; Comet assay; DNA repair inhibitors; Experimental variability

1. Introduction

An important source of interindividual variability in cancer susceptibility is related to DNA repair capacity. In fact, individuals may widely differ in their capacity to repair DNA damage induced by both exogenous

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agents, such as tobacco smoke and sunlight exposure, and endogenous agents, such as oxidative stress by-products. For this reason, a number of epidemiological studies have been conducted to compare DNA repair capacity of cancer patients with controls [1–4]. There are interesting evidences that many polymorphisms of genes involved in different DNA repair pathways, such as base excision repair (BER), nucleotide excision repair (NER), recombination repair and mismatch repair (MMR) can modulate cancer susceptibility. This is not surprising because it is well known that genetic defects in DNA repair systems are responsible for an extremely high cancer proneness in several diseases, such as xeroderma pigmentosum (XP), hereditary non-polyposis colorectal cancer (HNPCC) or Li-Fraumeni syndrome.

NER is a very versatile and complex pathway, controlling the removal of UV-induced DNA damage and bulky adducts [5]. It is a highly conserved process among eukaryotes and is accomplished by at least 20–30 proteins [3]. NER pathway consists of the following four steps: recognition of the DNA lesion; excision of a 24–32 nucleotide stretch containing the lesion by dual incision of the damaged DNA strand on both sides; filling in of the resulting gap by DNA polymerase and ligation of the nick [6]. Therefore, during the process, DNA breaks are produced as intermediates, which can be visualized as DNA migration by the comet assay [7,8]. Polymerization and ligation steps subsequently rejoin the broken ends, determining a reduction of DNA breaks. Thus, the comet assay should allow following in details the kinetics of NER process, so that this test is potentially more informative than other cytogenetic assays (such as micronuclei, chromosome aberration and sister chromatid exchanges) which detect only indirectly the induction and repair of genetic damage [9–13].

Some chemicals are known to inhibit enzymes taking part in NER pathway. Novobiocin (NOV), interacting with the ATPase subunit of topoisomerase II, inhibits the incision step, thus markedly reducing repair-specific DNA cleavage [14–16]. On the other side, aphidicolin (APC), an inhibitor of DNA polymerase α, can block the rejoining of DNA ends, thus causing the persistence of DNA breaks [14,16,17]. Therefore, these two inhibitors can be used to separately suppress the two NER steps (incision step and polymerisation–ligation step) in UV-exposed human lymphocytes and their effects can be monitored by the comet assay [18,19].

The aim of this work is to assess whether the comet assay may be a reliable method for quantifying the two main phases of NER, i.e. DNA excision and DNA synthesis-ligation, controlled by several polymorphic genes. This would allow a careful assessment of the functional effect of specific alleles and allelic associations at individual level.

2. Materials and methods

2.1. Lymphocyte isolation

Heparinized venous blood samples were collected from two female and two male non-smoking healthy donors of similar age (25–27 years). Donors had never experienced photosensitivity or other unusual reactions consequent to sun exposure during their life. Three milliliters of whole blood and phosphate buffered saline (PBS, pH 7.4) (1:1) were carefully layered on 6 ml of Histopaque 1077 (Sigma, Milan, Italy) and centrifuged at 2100 rpm for 30 min. The upper layer was removed, the lymphocyte-containing buffy coat was carefully aspirated and the cells were washed in RPMI 1640 (Gibco, Invitrogen, s.r.l., San Giuliano Milanese, Italy), then resuspended in RPMI medium with antibiotics (100 IU penicillin/ml and 100 μg/ml streptomycin) and phytohemagglutinin (PHA, Gibco, Invitrogen, s.r.l., San Giuliano Milanese, Italy) for 20 h before UV exposure [20]. For lymphocyte cryopreservation and recovery, the procedures described by Visvardis et al. were followed [21].

2.2. UV irradiation and treatment with DNA repair inhibitors

Lymphocytes, suspended in PBS (pH 7.4), were irradiated for 15 s with a 254-nm UVC germicidal lamp (Philips, Milan, Italy) at a dose rate of 0.1 J/m^2/s. The intensity was measured with a short-wave ultraviolet intensity meter (UVP, USA). UVC irradiated cells were then suspended in RPMI 1640 medium supplemented with 10^{-4} M thymidine (Invitrogen) and incubated for eight time lengths (0, 20, 40, 60, 90, 120, 180 and 240 min) before proceeding to the comet assay. NOV (Sigma, Milan, Italy) was dissolved in dimethylsulfoxide (DMSO) and added to the cell suspension (900 μM) 1 h before UV irradiation and maintained in the medium until the end of the culture [16]. A stock solution of APC (Sigma, Milan, Italy), 2 mg/ml in ethanol, was stored at 4 °C [18,19]. APC was supplemented to the cell suspension (5 μM) immediately after UV irradiation and then kept in the culture for 1 or 4 h. After incubation, cells were washed in PBS. Lymphocytes to be processed for the assessment of DNA damage by means of the comet assay were divided into four vials as follows: (1) control, unirradiated cells; (2) unirradiated cells added with APC or NOV; (3) UV irradiated cells; (4) UV irradiated cells added with APC or NOV.

2.3. Single-cell gel electrophoresis (comet assay)

The alkaline (pH > 13) comet assay was performed according to Tice et al. [22]. Briefly, cells were suspended in pre-warmed low melting point agarose (LMA). Two solutions, 1%