Event Abstract

Comet assay as a human biomonitoring tool: application in occupational exposure to antineoplastic drugs

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Antineoplastic drugs are a heterogeneous group of chemicals used in the treatment of cancer, and have been proved by IARC to be mutagens, carcinogens and teratogens agents (Fucic et al., 1998; Burgaz et al., 1999; Sessink & Bos, 1999; Bouraoui et al., 2011; Gulten et al., 2011; Buschini et al., 2013). In general, chemicals that interact directly with DNA by biding covalently or by intercalating, or indirectly by interfering with DNA synthesis, were among the first chemotherapeutics developed (Jackson et al., 1996). Also, these drugs can induce reactive oxygen species that can lead to DNA damage and, consequently, mutations (Rombaldi et al., 2008). These drugs are often used in combination to achieve synergistic effects on tumour cells resulting from their differing modes of action. However, most if not all of these chemical agents are generally nonselective and, along with tumour cells, normal cells may undergo cytotoxic/genotoxic damage (Connor, 2006; Kopjar et al., 2006; Villarini et al., 2012). The in vivo exposure to antineoplastic drugs has been shown to induce different types of lesions in DNA, depending on the particular stage of cell cycle at the time of treatment. Besides the patients that use these drugs as a treatment, workers that handle and/or administer these drugs can be exposed to these substances; namely pharmacy, and nursing personnel in hospital context. The comet assay identifies injuries which are still reparable, such as single and double-strand DNA breaks, alkali labile lesions that are converted to strand breaks under alkaline conditions and single-strand breaks associated with incomplete excision repair sites (Villarini et al., 2012), thus providing information about recent exposures (Laffon et al., 2005). In particular, the comet assay with the use of enzymes, which recognizes and cuts specifically oxidized DNA bases allows for the evaluation of oxidative DNA damage (Collins, 1999). It is one of the most used methods in biomonitoring studies of genotoxicity on blood lymphocytes (Cavallo et al., 2009), and is widely used to evaluate the genotoxic effects of exposure to specific antineoplastic drugs in several in vitro and in vivo studies (Digue et al., 1999; Blasiak et al., 2000; Brahnam et al., 2004). DNA glycosylase (OGG1) represents the main mechanism of protecting the integrity of the human DNA with respect to 8-OHdG (Hu & Ahrendt, 2005; Jiao et al., 2007), the most well studied biomarker of oxidative damage. Some findings indicate that the inactivation of OGG1 plays a role in the multistage process of carcinogenesis. The human OGG1 gene is located on chromosome 3 (3p26), and encodes a bifunctional DNA glycolylase

endowed with an AP lyase activity. This is a region frequently lost in various types of cancer, especially in small-cell lung cancers where loss of heterozygosity in nearly 100% of the cases can be observed. Loss of one OGG1 allele may lead to a moderate generation of 8-OHdG in DNA. However, loss of both alleles would abrogate OGG1 activity imposing an increased risk of mutagenicity on the cell due to the imbalance of oxidative burden and accumulation of 8-OHdG in DNA (Pilger & Rüdiger, 2006). The product of OGG1 gene exhibits specificity and activity for the excision of 8-OHdG (Boiteux & Radicella, 1999; Cooke et al., 2003; Au et al., 2004), and has a major role in the prevention of ROS-induced carcinogenesis (Cooke et al., 2003). A common polymorphism is Ser326Cys, which affects over 50% of Chinese and Japanese and approximately 33-41% of Caucasian population (Hu & Ahrendt, 2005). The OGG1 has a C→G polymorphism at position 1245 in exon 7 which causes the substitution of serine by cysteine at codon 326 (Kohno et al., 1998) and it is associated with increased risk for cancer (Macpherson et al., 2005). The study population consisted of 46 exposed subjects working as pharmacists, pharmacy technicians, and nurses that handle antineoplastic drugs of two hospital units, and 46 unexposed control subjects. Isolated lymphocytes were cryopreserved following the protocols of Duthie et al. (2002) and Singh & Lai, (2009). Briefly, isolated lymphocytes suspended in RPMI medium with L-glutamine were either centrifuged (600g, 10 min) and ressuspended in freezing mix (90% v/v heat-inactivated fetal calf serum and 10% v/v DMSO), frozen at -1°C/min in polystyrene and stored at -80°C. For analysis of DNA damage and oxidative damage a modification of the comet assay (originally described by (Singh et al., 1988) was used to measure the basal level of DNA oxidation in lymphocytes (Collins, 2009). We measured also DNA oxidative damage by using FPG [kindly donated by Prof. Andrew Collins (Department of Nutrition, University of Oslo, Norway)]. The slides were dried at room temperature, stained with 25 µl DAPI (1 µg/mL) and visualized. Percentage of DNA in the tail and oxidative damage parameters were measured using Zeiss AxioScope.A1 fluorescence microscope and Comet Assay IV capture system (Perceptive Instruments ® software) and 50 nucleoids were scored per gel. The genotype of the polymorphisms OGG1 (rs1052133) was studied by Real Time PC R using the iCycler iQ®Multicolor Real-Time PCR. It was found higher mean levels of % DNA in tail and DNA oxidative damage in the exposed group (15.18±1.40 and 5.32±0.54, respectively) in comparison with controls (12.42±1.24 and 4.59±0.59, respectively). However, no statiscally significant differences (Mann-Whitney test, $p>0.05$) were found between subjects with and without exposure. No significant differences were observed in genotypic and allelic frequencies in OGG1 polymorphisms under study between exposed and controls (Fisher's exact test, $p>0.05$) and no consistent trend regarding the variation of biomarkers measured with alkaline comet assay and OGG1 polymorphisms was found (Kruskal-Wallis test, $p>0.05$). Our results suggest that occupational exposure to antineoplastic drugs in healthcare workers induces DNA damage but the increase compared to controls was not significant, being in line with studies from Ursini et al., (2006) and Buchini et al. (2013). A possible explanation may have to do with comet assay predominantly detecting single-strand breaks and alkali-labile sites, which are induced by antineoplastic drugs (Kopjar et al., 2009). Since both types of DNA damage are continuously and efficiently repaired, the measured damage level is a result of equilibrium between the amount of DNA damage inflicted and the speed of repair (Kopjar & Garaj-Vrhovac, 2001). An important aspect to consider is the fact of antineoplastic drugs are well-known cross-linking agents, which can increase the effective molecular weight of DNA, are thereby are known to reduce the ability of DNA containing

strand breaks to migrate in an electric field (Villarini et al., 2011). Occupational exposure to antineoplastic drugs is associated with genotoxic effects, although comet assay analyzed parameters were higher in exposed comparing with controls, were not significant. Also the study of the susceptibility biomarkers did not show statistical significant differences, the small size of our sample hampered the finding of a possible association, let alone a causality relationship.

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