Effect of occupational exposure to cytostatics and nucleotide excision repair polymorphism on chromosomal aberrations frequency

Ľudovít MUŠÁK 1,2, Veronika POLÁKOVÁ 3, Erika HALAŠOVÁ 1, Oto OSINA 2, Ludmila VODIČKOVÁ 3,4, Janka BUCHANCOVÁ 5, Henrieta HUDEČKOVÁ 5, Pavel VODIČKA 4

1 Institute of Medical Biology, Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia
2 Clinic of Occupational Medicine and Toxicology, Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia
3 Institute of Experimental Medicine Academy of Sciences in Prague, Czech Republic
4 National Institute of Public Health in Prague, Czech Republic
5 Institute of Public Health, Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia

ABSTRACT
Authors evaluated the incidence of total chromosomal aberrations (CA) and their types – chromatid-type (CTA) and chromosome-type (CSA) in peripheral blood lymphocytes from 72 oncologic unit’s workers occupationally exposed to cytostatics in relationship to polymorphisms of DNA repair genes XPD, XPG and XPC. The cytogenetic analysis was used for determination of chromosomal aberrations frequency and PCR-RFLP method for polymorphisms of genes. Statistically higher frequency of total CA was detected in exposed group as compared to control (1.90±1.34 % vs. 1.26±0.93%; Mann-Whitney U-test, p=0.001). There was not detected any difference between CTA and CSA (0.92±1.04% vs. 0.98±1.17%). Similarly, in genes XPD exon 23 and XPC exon 1 5 wasn’t detected any difference neither in total chromosomal aberrations nor in CTA and CSA types. Statistically significant decrease of total chromosomal aberrations and CTA-type with presence of variant allele C was detected in gene XPG exon 15. Authors pointed out the importance of individual susceptibility factors in evaluation of effects of genotoxic agents, in that event, when the concentration does not meet the occupational exposure limit.

KEY WORDS: chromosomal aberrations; cytostatics; occupational exposure; DNA repair genes XPD, XPG, XPC; polymorphisms of genes

Introduction
Workers in oncologic units are regularly, in the long term occupationally exposed to low doses of cytostatics. There are agents with antineoplastic effect, which are used in chemotherapy in malignant tumour’s therapy. These agents have direct toxic or cytotoxic effect, and can act as an important risk factors through their indirect impact (mutagenic, carcinogenic and teratogenic). Antineoplastic agents forestall in irregular division of cells, damage intercellular surroundings and induce the cell’s death. Connor (2006) reports that the skin is the first meeting point of antineoplastic agents’ contamination. The contamination of the skin was repeatedly discovered even the protective gloves were used (Fransman et al., 2005). Fransman et al. (2007b) followed also the contamination of bed sheets by antineoplastic drugs (cyclophosphamide, ifosfamide, methotrexate, 5-fluourouracil, etoposide, cytarabine, gemicitabine and chlorambucil). Mentioned cytostatics were found on workers’ skin of hands or in any of the air samples. The increased frequency of chromosomal aberrations, sister chromatid exchanges (SCE) and micrornuclei (MN) after occupational exposure was described by many authors (Pilger et al., 2000; Burgaz et al., 2002; Musak et al., 2006). Maluf and Erdtmann (2000) learned higher incidence of MN and positive comet assay. Rubes et al., (1998) detected noticeable higher number of translocations and unstable CA. Major et al. (1999) determined evidently higher frequency of premature divided centromeres. Burgaz et al. (2002) discovered 2.5-times higher number of CA within exposed workers in comparison to unexposed persons. Exposure to methotrexate (MTX) caused detectable
but slight increase of MN (Deng et al., 2005). Rekhadevi et al. (2007) and Cavallo et al. (2007) detected positive comet assay test, higher frequency of micronuclei in bucal mucosa and peripheral blood and cyclophosphamide (CP) level in urine. Musak et al. (2006) published the higher frequency of CA in variant type of alleles in genes XRCC1 exon 10 and XRCC3 exon 7. Testa et al. (2007) found out not significant relationship between polymorphisms of genes for GST and higher frequency of CAs. Tuimala et al. (2002) detected higher number of CTA-type aberrations in peripheral lymphocytes in individuals with variant allele in gene XRCC1, codon 280 exposed to bleomycine. The impact of mitomycine C (MMC) a bleomycine (BLM) in terminal segments of chromosomes was evaluated by measure of telomere length. Exposure to MMC cases an important influence in genotype also of polymorphism of gene XPD in healthy individuals (Angelini et al., 2008). Indirect determination of efficiency of DNA repair mechanisms was stated on the frequency of CAs induced by bleomycine (BLM). Laczmanska et al. (2007) published the correlation between CAs frequency and polymorphisms of DNA repair genes for base excision repair (BER) – XRCCI, nucleotide excision repair (NER) – XPA, XPC, XPG, XPD, XPF, ERCCI and homologous recombination (HR) – NBSI, RAD51, XRCC2, XRCC3, BRCA1. Only the gene XPC exon 15 and intron 11 was found to be in the relationship with increased frequency of CAs induced by BLM. It point out on importance of NER in CAs reparation. Rombaldi et al. (2009) analysed the genotoxicity using comet assay, and MN test as well as the level of oxidative stress. The exposed workers presented increased DNA damage levels by the comet assay as compared to the controls. The comet assay results have also shown significant positive correlation with the length of exposure and with the amount of consumed alcohol. MN frequency was significantly higher in the exposed workers and presented noteworthy correlation with age and working time. In the oxidative stress parameters, only CAT presented a significant increase.

Cytostatics genotoxicity is expressed especially by undirected damage of genetic material. The most of mutagens are electrophilic agents, or can be metabolised by electrophilic intermediators. There are able to create covalent bonds with nucleophile structures of cells. Cells dispose of mechanism for elimination of genotoxic effect on DNA as well as for reparation of created lesions. The terminal manifestation of mutation is the results of primary genetic damage and the effect of repair processes in cell. Individual efficiency in repair process can be one of primary attribute for inherited predisposition to tumours creation.

### Material and methods

In the present work 148 exposed and control individuals were analysed for frequency of chromosomal aberrations and polymorphisms of DNA repair genes. All of them completed anamnestic questionnaire about length and way of exposure, job categorize, exogenous factors (smoke, drug usage, exposure to radiation, alcohol consumption and dietary) before blood collection and give an agreement to be involved in the study.

Exposed group consists 72 workers from specialized oncologic departments occupationally exposed to cytostatics. They were from three hospitals in north part middle Slovakia region. 28 workers were from Faculty Hospital in Martin, 31 from Central Military Hospital in Ružomberok and 13 from Hospital with polyclinic in Trstená. All of workers were regularly in contact with cytostatics that dilute and apply to patients. By job grade are they nurses and physicians. There were predominantly females in both groups. Smokers form 26.39%. Control group consisted from medical workers and workers from factory Biotika. They were not exposed to any genotoxic agents. Characteristics of exposed and control groups are present in Table 1. We microscopically analysed 100 mitoses per person. We evaluated the frequency of total chromosomal aberrations, and then subdivided them to CTA and CSA types. Methodology of cytogenetic analysis was performed according to AHEM (2007). Polymorphisms of DNA repair genes were performed by PCR-RFLP. Amplified fragments were pre-digested by restriction endonucleases and analyzed. Genotypes were determined in direct process sequence of amplified fragments. Gene XPD exon 23 (A→C), kodon 751 (Lys751Gln); primers – F (forward): 5’-CCC CTC TCC CTT TTC TCT GTT-3’; R (reverse): 5’-GCT GCC TTC TCC TGC GAT TA-3’; restriction enzyme PstI; size of fragments: 1) normal homozygote (Lys751Lys) – 290+146 bp; 2) heterozygote (Lys751Gln)

<table>
<thead>
<tr>
<th>Table 1. Characteristics of exposed groups and control.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposed group</strong></td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years±S.D.)</td>
</tr>
<tr>
<td>Exposure (years±S.D.)</td>
</tr>
<tr>
<td>Sex (N) M/F</td>
</tr>
<tr>
<td>Smoking (N) S/NS</td>
</tr>
<tr>
<td>Job (N) physician/nurse</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Number of total chromosomal aberrations, chromatid-type (CTA) and chromosome-type (CSA) in exposed group and control.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total CA</strong></td>
</tr>
<tr>
<td>Exposed group</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

***p=0.001 Total CA exposed vs. control
– 290+227+146+63 bp; 3) variant homozygote (Gln751Gln) – 227+146+63 bp.

Gene XPG exon 15 (G→C), codon 1104 (Asp1104His): primers – F (forward): 5’T-GTG ATT TTT GGG GGA GAC CT-3; R (reverse): 5’-CGG GAG CTT CCT TCA CTG AGT-3; restriction enzyme Hsp92II; size of fragments: 1) normal homozygote (Asp1104Asp) – 159 bp; 2) heterozygote (Asp1104His) – 59+100+159 bp; 3) variant homozygote (His1104His) – 59+100 bp.

Gene XPC exon 15 (A→C), codon 939 (Lys939Gln): primers – F (forward): 5’-GAT GCA GGA GGT GGA CTC TCT-3; R (reverse): 5’-GTA GTG GGG CAG CAG CAA CT-3; restriction enzyme PvuII; size of fragments: 1) normal homozygote (Lys939Lys) – 281 bp; 2) heterozygote (Lys939Gln) – 150+131+281 bp; 3) variant homozygote (Gln939Gln) – 150+131 bp. In this paper are accepted all principles for protection personnel data, for health care. The design of the study was approved by the Ethical Committee of Jessenius Medical Faculty in Martin.

The peripheral blood sampling was realized within the specialised medical examinations.

Statistical analysis was performed using program Statgraphics, version 7 (Manugistics, Cambridge, MA). We used nonparametric Mann-Whitney U-test for testing differences between groups and analysis of variance (ANOVA) for testing relationships between biomarkers and genotype. The values in tables are presented as average ± S.D.

**Results**

We detected higher frequency of total chromosomal aberrations (CAs) in exposed group in comparison to control (1.90±1.34% vs. 1.26±0.93%, Mann-Whitney U-test, P=0.001). In exposed group we did not detect any difference between chromatid-type (CTA-type) and chromosome-type (CSA-type) – 0.92±1.04% vs. 0.98±1.17% (Table 2). Evaluating the role of XPD gene, exon 23 we stated, that the exposition did not influence the total CAs. The frequency of chromatid-type aberrations was faintly lower in the presence the variant allele and contrary, the frequency of chromosome-type aberrations was faintly higher. The differences were not statistically significant (Figure 1). XPG gene exon 15 polymorphisms showed the significant decrease of total CAs and CTA-type aberrations frequency if the variant allele was present (p<0.05), the frequency of CSA-type aberrations was lower but not significant (Figure 2). In XPC gene exon 15 we observed similar trends like in gene XPD; i.e. in frequency of total CAs we did not detect any difference between alleles (Figure 3).

**Discussion**

Cytostatics are mutagenic and carcinogenic agents (Vorlíček et al., 2000). They cause variously DNA damages. Burgaz et al. (2002) followed the effect of 9 cytostatics and Cavallo et al. (2005) of 5 cytostatics in nurses and pharmaceutical workers that prepared, diluted or applied cytostatics. The authors examined also workers that were in contact with...
contaminated bed sheets, possibly excrements of treated patients. They both observed the higher frequency of CAs and MN in buccal mucosa cells in exposed workers. Burgaz et al. (2002) detected 2.5-time higher frequency of CAs in exposed persons.

Our findings are in accordance with previously mentioned; we detected higher frequency of total chromosomal aberrations (CAs) in exposed group in comparison to control. However, we did not find any difference between chromatid-type (CTA-type) and chromosome-type (CSA-type) of aberrations in exposed group. Numerous authors determined in their explorations that the increased risk to exposure is related with inconsistent observance of safety regulations. The higher exposures to cytostatics were observed at workplaces, where the personnel were not adequately informed about risk associated with this job and where were not strictly keep the safety directives (Maluf and Erdtmann, 2000; Connor, 2006; Fransman et al., 2007a). The human genome is permanently repaired protecting the cells and organism life. It is proved that the DNA repair can influence the CAs frequencies (Skjelbred et al., 2006). We can presume certain selection drift of heterozygous in comparison to homozygous. The interest in polymorphisms that are related with modifications in human genome is permanent. There are many discussions pointed on gene-environmental interaction that lead to formation of disease in some individuals. To determine the CAs frequency in human lymphocytes we analysed specific polymorphisms of DNA repair genes. We looked for the relation between genes polymorphism for nucleotid excision repair XPD, XPG and XPC and CAs frequency. In genes XP2 and XPC we did not detect any difference neither in frequency of total CAs nor in their separate types. In gene XPG we found out statistically significant decrease of total CAs and CTAs with presence of variant allele. The decrease of CSA-type was not statistically significant. The polymorphism of many genes included in BER or NER, and repair of double strand breaks is tightly connected with increased risk of tumours and DNA damages (Hemminki et al., 2001, Hou et al., 2002, Kumar et al., 2003). Genotoxic impact of gamma radiation reduces the ability of DNA repair process (Suéprasert et al., 2006). The reduced ability of DNA repair was detected after exposure to UV light and mitomycin C in malignant cells of prostate. Variant alleles AA of gene XRCC1 in codon 280 and alleles TT in codon 194 tightly connected with increased ability of the repair DNA chain that was detected in urban population (Tuimala et al., 2002). Gene XRCC3 is needed in repair of breaks by homologous recombination and its polymorphism probably decrease the number of sister chromatid exchanges and chromosomal aberrations (Tuimala et al., 2004). These authors did not confirm the association between polymorphism of gene XRCC3 in codon 241 (Thr241Met) and frequency of chromosomal aberrations after treatment of bleomycine. The sensitivity of bleomycine depends on individual ability to DNA repair and is particularly impacted by genetic polymorphism of XRCC1 gene that affect on DNA repair in vitro (Tuimala et al., 2002; Tuimala et al., 2004). Vodička et al. (2004) evaluated the relationship between polymorphisms of DNA repair genes XRCCI, XRCC3, XPD, XPG and XPC and frequency of CAs and SCE. They detected the higher frequency of CAs in individuals with allele A in XPD gene exon 23, e.i. in individuals with genotype AA a AC. Musak et al. (2006) detected higher frequency of CAs in variant type of alleles in genes XRCC1 exon 10 and XRCC3 exon 7. Since polymorphisms influence many metabolic processes and detoxification of toxic chemical agents, DNA changes could increase the risk of cancer.

Relativedy insufficiently is examined the part of interactions of DNA repair genes. It is caused by the fact, that in DNA repair process are present more than 100 genes, and almost 40 are polymorphic (Mohrenweiser et al., 2002). Currently is not sufficient explanation for the relationship between genes for xenobiotics metabolism, DNA repair genes and their function, the manifestation in phenotype. The great attention is spending in genotypes combinations, their inter-genes interactions in evaluation of genotoxic effects of agents in exposed individuals.

From literature is known, that variously genetic polymorphisms can have the influence on the incidence of variety diseases, and particularly tumours and also on genotoxic effects induced by occupational exposure to genotoxic agents (Norppa, 2003; Norppa, 2004). There was optimal to evaluate the individual susceptibility, and to determine "positive” and "negative” genotypes in order to minimize the risk of exposure in sensitive individuals. It is not possible because we have insufficient informations about genotypes, and interactions of genes.

We evaluated the frequency of total chromosomal aberrations and individual type of them, e.i. CTA-type and CSA-type of workers in oncologic units occupationally exposed to antineoplastic agents in compared to polymorphism of DNA repair genes for nucleotid excision repair XP2, XPG and XPC. Presented results refer to importance of individual susceptibility’s factors in assessment of genotoxic effects, in these cases, when concentration of genotoxic agents usually not exceeds the occupational exposure limit.

Acknowledgement

This work was supported by the grants MZ SR 2007/48-UK-13 (SR) and IGA MZ CR 8563-5/2005 (CR).

REFERENCES


