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Relationship between DNA replication and DNA repair in human lymphocytes proliferating in vitro in the presence and in absence of mutagen

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Abstract: The effects of mutagens on DNA replication and DNA repair were studied in peripheral blood lymphocytes (PBL) obtained from 21 healthy subjects, 2 samples from healthy heterozygote of *Xeroderma pigmentosum* (XP) and 2 samples from patient with clinically recognised XP. Inter-individual variations were found in DNA replication and in the level of spontaneous DNA repair measured under standard culture condition. Exposure of human PBL proliferating in vitro to B(a)P was followed by a partial inhibition of replicative DNA synthesis in all subjects and by an induction of DNA repair in healthy subjects. In XP patients DNA repair synthesis remained at the level attributed to spontaneous DNA repair. The response to mutagen varied individually. Results were analysed statistically. It was established that the studied indices of DNA synthesis correlate well with each other. The highest correlation was found between the levels of spontaneous and B(a)P-induced DNA repair. It is concluded that the level of spontaneous DNA repair is predictive for an estimation of cells ability to repair DNA damage. Inter-individual variations in the inhibition of DNA replication and in DNA repair synthesis are also dependent on the type of mutagen as shown by effects of other mutagens. Different effects of mutagen exposure on the inhibition of DNA replicative synthesis and induction of DNA repair can be explained by genetically controlled differences in the activity of enzymes responsible for mutagen processing and lesion removal.

Key words: DNA repair, DNA replication, genotoxicity, lypmhocyte proliferation.

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Genotoxicity of chemical mutagens is modulated by host factors (HARRIS 1989, PELKONEN 1992). Genetic factors emerge at all stages of mutagen interaction with an exposed cell or organism. Activation of carcinogen, detoxification of activated metabolite and lesion removal by DNA repair are enzymatic processes genetically controlled (HARRIS 1991). Investigation of genetic polymorphism in the mentioned processes remains in the frame of interest of molecular mutagenesis and carcinogenesis, attempting to estimate individual risk concerning the exposure to environmental mutagens (HEMMINKI 1991, PELKONEN 1992).

Because of a limited availability of human tissues to study molecular and cellular effects of mutagen exposure, PBL are the main object for investigations, despite their surrogate character as compared with target tissues (SZYFTER et al. 1993, PERERA, WHYATT 1994). Hence, lymphocytes/leukocytes were proposed to study several genotoxic effects such as formation of DNA adducts, single- and double-strand breaks in DNA molecule, DNA-DNA cross-links etc. followed by the induction of DNA repair, and the appropriate techniques were established (VENITT, PARRY 1984).

Recently, mechanisms of DNA repair have been extensively studied and it became clear that some enzymes such as helicase or DNA polymerases take part in both, DNA replication and DNA repair (HANAWALT 1991, STEWART 1992). Hence, it was of interest to study a relationship between DNA synthesis under standard conditions and in mutagen-exposed cultures taking into account the reported individual response of PBL to mitogen and mutagen (OESCH et al. 1987, KNUDSEN 1992). Benzo(a)pyrene (B(a)P) was chosen as a model carcinogen to induce changes in lymphocyte proliferation.

Material and methods

Study group. 21 blood donors were healthy adults of both sexes. To refer to cells known as DNA repair-deficient (CLEAVER 1989) the samples from *Xeroderma pigmentosum* (XP) were also included in the study. The XP donors were: an eight-year-old boy treated for XP at the Clinic of Dermatology, Pomeranian Medical University (Szczecin), and his heterozygous mother, an obligatory carrier of XP gene. Both donors were sampled twice within a 6-week period. The case of XP family will be reported separately.

Cell culture. Lymphocytes were isolated from heparinized peripheral blood by sedimentation through Ficoll/Uropolinum gradient (d = 1.077 g cm^{-3}) according to BOYUM (1977). Cells (1.0×10^5 per well) were cultured on 96-well U-bottom microplates (Sterilin, UK) in total volume of 0.2 ml Eagle's medium,

supplemented with 10% foetal calf serum (FCS, Serva) and gentamycin (20 μg ml^{-1}). Phytohemagglutinin (PHA, HA 17, Welcome, 1 μg ml^{-1}) administered at 0 hour was used as mitogen. All microcultures were performed in triplicates. PBL were cultured for 72 hours at 37°C in a humidified atmosphere containing 5% CO_2.

Carcinogen treatment. B(a)P (Serva) dissolved in methanol was added to the lymphocytes cultures at 48 hour up to the final concentration of 1 μM (SZYFTER et al. 1985). Stock solution of B(a)P provided solvent concentration in the cell culture medium below 2.5%. Methanol without mutagen was added in the same amount to the controls. Methyl methane sulphonate (MMN, ICN Pharmaceuticals) and N-nitrosodimethylamine (DMN, Merck) dissolved in culture medium were added at 48 hour up to the final concentration of 0.2 mM.

DNA synthesis measurements. DNA replication synthesis was estimated by measuring the amount of ^{3}H-dThd (spec. activ. 20 Ci/mmol, Amersham) incorporated to PBL following administration 5 hours before harvest. The DNA repair capacity was measured as unscheduled DNA synthesis (UDS) according to WATERS (1984) as incorporation of ^{3}H-dThd to the cultures blocked at 66 hour by addition of 10 mM hydroxyurea (HU) (Serva). ^{3}H-dThd was added for the last 5 hours of cell culture. Microplates were harvested at 72 hour on cell harvester. Cell were collected on glass fibre filters, washed with 5% trichloroacetic acid and water. Radioactivity was measured in a LKB Wallac 1217 Rackbeta liquid scintillation counter, and results were expressed as counts per minute (cpm).

Statistical analysis. Results were analysed by the t-test assuming unequal variances to calculate means, Pearson correlation coefficients and the level of significance. Descriptive statistics was applied to calculate standard deviations and the mode of distribution.

Results

DNA replicative synthesis in PBL stimulated to proliferation in vitro by mitogenic lectin PHA was measured as the amount of ^{3}H-dThd incorporated into lymphocytes. The mean values of DNA replicative synthesis were almost at the same level for healthy subjects, XP carriers and XP patients (Table 1). The exposure of proliferating lymphocytes to 1 mM B(a)P was followed by a partial inhibition of DNA replicative synthesis and by the induction of DNA repair synthesis. An inhibitory effect of B(a)P on DNA replicative synthesis in PBL from XP carriers (48.2% of ^{3}H-dThd incorporation in the standard

Table 1. DNA synthesis in in vitro proliferating PBL from normal and DNA repair-deficient subjects. The values are given in cpm/10^5 cell ($\bar{x} \pm SD$)

Group	n	DNA replication		DNA repair	
		PHA induced	BP-inhibited	spontaneous	BP-induced
Healthy	21	68 778 ± 28 220	44 581 ± 17 790	1 748 ± 1 024	4 511 ± 2 965
XP carriers	2	57 119 ± 4 898	27 536 ± 6 924	2 468 ± 353	4 232 ± 766
XP patients	2	59 699 ± 6 482	24 906 ± 3 502	861 ± 44	933 ± 1

conditions) and XP patients (41.7%) was more pronounced than that in healthy subjects (64.8%) but the differences remained in the range of standard deviation.

^{3}H-dThd incorporation into PBL after suppressing semiconservative replication by 10 mM HU was a measure of DNA repair synthesis. B(a)P-induced UDS in PBL exceeded the level of the spontaneous UDS 2.6-fold for healthy subjects and 1.7-fold for XP-carriers (Table 1). In the case of XP patients an induction of UDS was not observed at all. It is interesting to note that the level

Table 2. A statistical analysis of DNA replication and UDS in human PBL proliferating in vitro. The values given in parenthesies indicate the number of blood donors

Parameter 1	Mean cpm/well	Parameter 2	Mean cpm/well	Pearson correlation	Significance (P value)
PHA-induced replication	68 778 (21)	B(a)P-inhibited replication	44 562 (21)	0.879	0.00134
PHA-induced replication	68 778 (21)	spontaneous UDS	1 748 (21)	0.785	5.74×10^{-10}
PHA-induced replication	66 499 (20)	B(a)P-induced UDS	4 511 (20)	0.854	2.8×10^{-9}
B(a)P-inhibited replication	42 873 (20)	B(a)P-induced UDS	4 511 (20)	0.630	1.63×10^{-9}
Replication/PHA-induced – B(a)P-inhibited/	23 626 (20)	B(a))-induced UDS	4 511 (20)	0.824	1.6×10^{-5}
Spontaneous UDS	1 748 (20)	B(a)-induced UDS	4 511 (20)	0.937	0.0041
Spontaneous UDS (healthy)	1 748 (21)	spontaneous UDS (XP)	861 (2)	1	0.0006
B(a)P-induced UDS (healthy)	4 511 (20)	B(a)P-induced UDS (XP)	933 (2)	1	2.24×10^{-5}

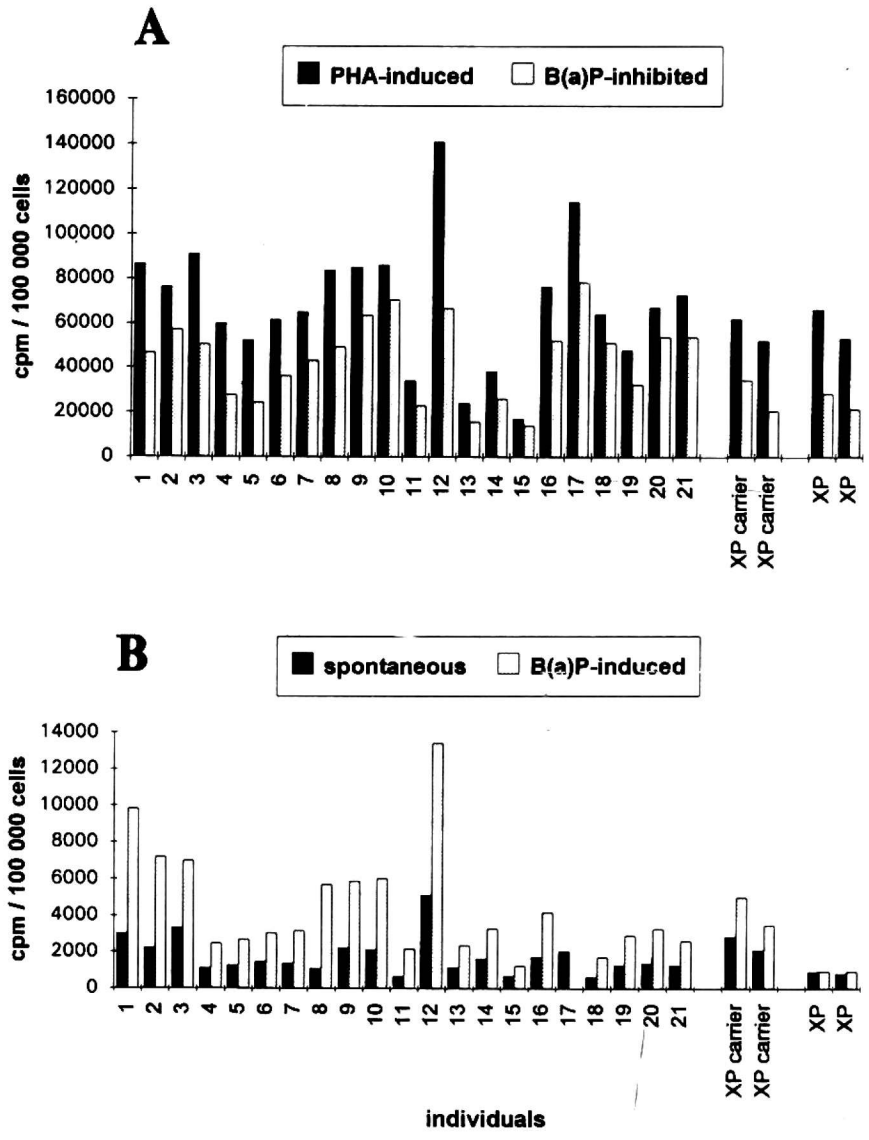

Fig. 1. Individual levels of DNA synthesis in human lymphocytes proliferating in vitro in standard culture conditions and in cultures affected by 1M B(a)P measured by ^{3}H-dThd incorporation
Upper part : DNA replicative synthesis. Lower part : DNA repair measured as UDS.

of spontaneous UDS in XP patient was 2.2-fold and 2.9-fold lower than the mean values established for healthy donors and XP carriers, respectively.

The levels of DNA replicative synthesis, B(a)P-inhibited replication (Fig. 1A), spontaneous and B(a)P-induced DNA repair synthesis (Fig. 1B) were found to be highly individual. In the group of healthy subjects the highest value exceeded the lowest one 8.3-fold for DNA replicative synthesis, 5.5-fold for B(a)P-inhibited DNA replicative synthesis, 8.1 for spontaneous DNA repair and 10.8 for B(a)P-induced DNA repair. Nevertheless, all the studied types of DNA synthesis appear to be normally distributed in healthy as well as in the group of all subjects.

Highly polymorphic response of PBL proliferating in vitro to the mitogen (PHA) and mutagen (B(a)P) raised a question concerning relationship between the studied indices. A statistical analysis has proven that there is a strong correlation between various types of DNA synthesis. As shown in Table 2 all differences between mean values of DNA synthesis in the group of healthy donors are statistically significant. B(a)P-induced disturbances in DNA replicative and repair syntheses are correlated with standard culture conditions. There is a gradual increase in Pearson correlation coefficient calculated for the dependence of B(a)P-induced UDS on B(a)P-inhibited DNA replication ($r = 0.63$), difference between DNA replication in standard and B(a)P-exposed culture ($r = 0.824$), PHA-induced DNA replication ($r = 0.854$) and spontaneous UDS ($r = 0.937$). Because of a high correlation in the latter case it can be inferred that the level of spontaneous UDS is a predictive parameter for estimation of PBL repair capacity to remove B(a)P-derived DNA lesions.

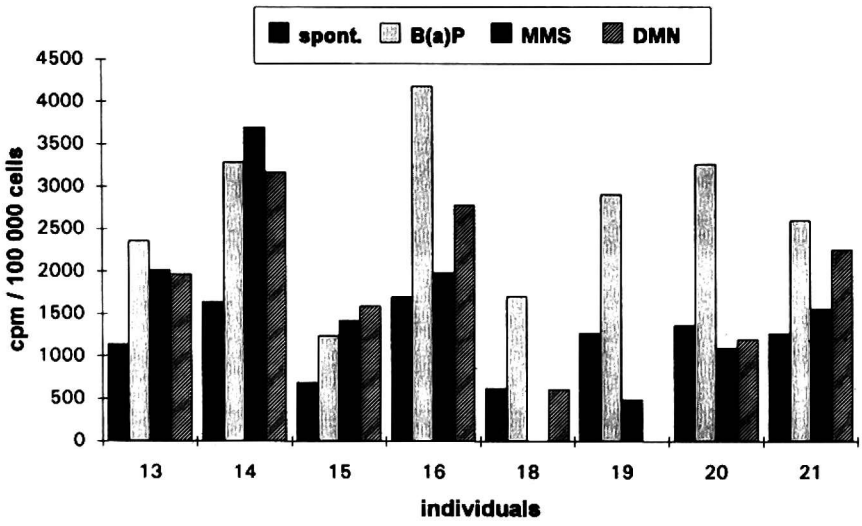

Fig. 2. A comparison of individual patterns of spontaneous and mutagen-induced DNA repair in human lymphocytes proliferating in vitro after stimulation by PHA.

Further, in a group of 8 healthy subjects two other mutagens were used to induce UDS to know if the observed inter-individual variability of DNA repair synthesis in PBL is dependent on the mutagen used. Following the same protocol, DNA repair synthesis induced by DNA methylating agents (MMS and DMN) was measured. Inter-individual variability of mutagen-induced DNA repair was observed but the variability patterns for B(a)P, MMS and DMN differed from one another (Fig. 2).

Discussion

DNA replication and repair are linked to some extent to each other because of (i) involvement of some enzymes (DNA polymerases, helicase, topoisomerase) in both processes and (ii) dependence on the cell cycle (HANAWALT 1991). In terms of molecular epidemiology an association between DNA replication and repair allows to explore DNA synthesis indices to study individual response of a cell/organism to genotoxic substances for estimation of individual sensitivity towards carcinogen (MAHER et al. 1990, POULSEN et al. 1993, ASAKURA et al. 1994).

The response of human lymphocytes proliferating in vitro to mitogens and mutagens varies individually (SZYFTER 1994). Variability of DNA repair was attributed to such factors as the type and dose of mutagen (CELOTTI et al. 1992, 1993) including smoking habits (CELOTTI et al. 1989), family cancer history (PERO et al. 1989), age (MAYER et al. 1989, GAUBATZ, TAN 1993) and seasonal variations (MUNCH-PETERSEN et al. 1985). The early papers concerning variability of mutagen-induced DNA repair were reviewed by SETLOW (1983). Next, changes in the activity of at least a few repair enzymes were associated with isoenzymes (TAKANO et al. 1991), cell differentiation (COHEN, LEUNG 1986) or cell cycle (GANGULY, DUKER 1990) giving a good background to study DNA repair variability in terms of genetic polymorphism. However, still there is no indication of different genotypes for slow and fast repair that is the case for mutagen activating or detoxifying enzymes (CAPORASO et al. 1991, POULSEN et al. 1993).

The results of this work clearly demonstrate inter-individual variability of spontaneous and mutagen-induced DNA repair in PBL proliferating under standard conditions and in cultures exposed to B(a)P. The results established throughout this work appear to be very similar to variations described by LAMBERT et al. (1979), OESCH et al. (1987) and CELOTTI et al. (1989) but they are slightly below the variability of DNA repair detected by KNUDSEN (1992), despite that all the quoted papers papers concern experiments with unstimulated PBL. To some extent it could be explained by limited accuracy of UDS estimation using liquid scintillation counting of incorporated ^{3}H-dThd (VENITT, PARRY 1984, ASHBY 1988). The next disadvantage of DNA repair estimation in PBL (both, proliferating or unstimulated) concerns application of HU to block G1 cells to enter S phase (CLARKSON 1978). HU itself may react with the microsomal activation mixture to produce DNA damage (WATERS 1984) and to deplete deoxyribonucleotide pool size (COLLINS, OATES 1987). Although HU concentration (10 mM) applied throughout this work is commonly used and has little effect on UDS induction we cannot exclude

variable cell response to this reagent. If that was a case it could interfere with the studied carcinogen effects.

The results of this work could be also interpreted against separate genotypes for fast and slow DNA repair as individual results of DNA repair were normally distributed. This is in contrast to the results of BENDER and SETLOW (1994) who claim Poisson distribution of UDS among healthy subjects. In any case, the existence of separate genotypes would rather be associated with multimodal distribution of individual indices of DNA repair.

Inter-individual differences in DNA replication in PBL proliferating under standard conditions and in B(a)P-exposed cultures are also evident. Differences between the mean values of DNA replication in PBL proliferating under standard conditions and following B(a)P exposure were statistically significant.

Further, to prove that the observed variability reflects differences in DNA repair instead of variable sensitivity to mutagens related to mutagen metabolic activation, three different mutagens were additionally used. Contrary to B(a)P and DMN requiring metabolic activation to exert their genotoxic potency, MMS acts directly (SINGER, GRUNBERGER 1983). Hence, differences observed in the latter case are a direct proof that individuals differ in their capacity to remove mutagen-induced DNA lesions. Moreover, DNA lesions induced by the used mutagens are processed in the course of different DNA repair mechanism. "Bulky" DNA adducts generated by B(a)P are removed by nucleotide-excision repair; methylation caused by nitrosating agents induces base-excision repair; simple methylation can be removed by the same mechanism or directly by methyltransferase (STEWART 1992). Variability patterns of DNA repair following exposure to all studied mutagens seem to indicate that polymorphic DNA repair is associated with most (all ?) DNA repair mechanisms.

Another intriguing question concerns mutual relationship between DNA replication and repair in standard and mutagen affected conditions. According to CELOTTI et al. (1989) there is a clear relationship between the ability to repair DNA and the inhibition of DNA replication. Such relationship was found also by us. However, we found even a better correlation between spontaneous and mutagen-induced DNA repair. Thus, it seems reasonable to determine the level of spontaneous DNA repair to predict the ability of cells to remove mutagen-induced DNA lesion. Another advantage of this relationship is that the level of spontaneous UDS could be used together with other indicators to estimate an individual risk towards carcinogens.

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