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EXPERIMENTAL / LABORATORY STUDIES

The Effect of Glutathione S-transferase M1 Genotype on Benzo[α]pyrene-Induced Sister Chromatid Exchanges and Chromosomal Aberrations in Peripheral Blood Lymphocytes

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Abstract: Sister chromatid exchange (SCE) and chromosomal aberration (CA) in peripheral lymphocytes have been widely used in assessing exposure to mutagens and carcinogens. One of the extensively studied genotoxins is benzo[α]pyrene (BaP). The aim of the present study was to examine the ability of BaP to induce different individual cytogenetic response measured by SCE and CA frequency. The possible influence of genetic polymorphism was also taken into account, by including donors representing positive or null glutathione S-transferase M1 (GSTM1) genotypes. SCEs and CAs were analyzed from 72-h whole-blood lymphocyte cultures of 16 GSTM1 positive and 15 GSTM1 null donors after treatment with 5 μM BaP, which do not decrease cell viability. We found no influence of the GSTM1 genotype on SCE or CA frequency. The rates of chromatid and chromosome-type gaps and breaks induced in vitro by BaP were similar in all the groups. In the GSTM1 null genotype, however, chromatid-type breaks were seen more frequently than chromosome-type breaks after a 48 h treatment with BaP. These findings suggest that SCEs and CAs induced in vitro by BaP are not influenced by the genotype GSTM1.

Key Words: Sister chromatid exchanges, Chromosome aberrations, Benzo[α] pyrene, Glutathione S-transferase M1

Introduction

Cytogenetic tests such as chromosome aberration (CA) and sister chromatid exchange (SCE) are most often applied in the biomonitoring of the genotoxicity of potentially carcinogenic chemicals in peripheral blood lymphocytes. One of the polycyclic aromatic hydrocarbons, benzo[α] pyrene (BaP), is considered a classic DNA-damaging carcinogen (1) and is commonly found in tobacco smoke and in the ambient environment. Epidemiologic studies proved that exposure to BaP increases the risk of cancer in the lungs, stomach, bladder and skin (2,3). Because not all exposed individuals develop cancer, genetically determined host factors may contribute to predisposition to DNA damage (4) and therefore modulate the risk of cancer.

BaP is a procarcinogen requiring metabolic activation (5). In phytohemagglutinin-stimulated human lymphocytes, a large number of metabolites of BaP are produced, including 4,5-dihydroxy-4,5-dihydrobenzo[α]pyrene, and 7b,8α-dihydroxy-9α,10a-epoxy-7,8,9,10-tetrahydrobenzo [α]pyrene (BPDE) (6). The BaP metabolites produced by metabolic activation are highly variable and probably depend on specific activation and detoxification enzymes present in BaP-exposed cells.

Glutathione S-transferases (GSTs) belong to a superfamily of multifunctional isoenzymes that contribute to the detoxification process through several different mechanisms (7). Based on sequence similarities, mammalian cytosolic GSTs have been grouped into at least 6 classes called Alpha, Mu, Pi, Theta, Sigma and Zeta. As GTSs function widely in the metabolic detoxification of xenobiotics, their genetic polymorphism could play an important role in determining individual sensitivity to various reactive chemicals. One member of the GST Mu, GSTM1, is polymorphic in humans. Approximately half of the Caucasian populations are

homozygous deleted for the GSTM1 gene and fail to express the protein (8). The results of experimental studies indicated that GSTM1 is a marker of susceptibility to the induction of cytogenetic damage by a certain class of mutagens (9,10). Lack of the GSTM1 isoform is associated with reduced efficiency in binding genotoxic substrates, including epoxides deriving from PAHs and aflatoxin (7). One of the consequences of inheriting a nonfunctional GSTM1 allele appears to be an increased risk of lung cancer and some other malignancies (11,12). To our knowledge, no satisfactory data on the effect of GSTM1 deficiency on the cytogenetic damage induction by BaP in vitro are available.

The aim of the present study was to examine the ability of BaP to induce different individual cytogenetic responses measured by SCE and CA frequency, taking into account the possible influence of GSTM1 polymorphism.

Materials and Methods

Subjects

The experiments were conducted on human whole blood lymphocytes obtained by venipuncture from 31 volunteers, 16 having the GSTM1 gene and 15 lacking it. The group consisted of 16 women and 15 men. The mean age of the group was 23.4 (range 18-35) years. The group was matched precisely according to the confounding factors (age, sex, smoking habits, coffee and alcohol consumption and occupation) that might influence the cytogenetic results. They all were healthy, non-smokers, and not coffee or alcohol addicts. None of them was exposed to any known mutagens (e.g., X-rays, medicines) for 3 months prior to the cytogenetic examination.

Laboratory Reagents

Standard lymphocyte culture reagents were purchased from Gibco Laboratory (Grand Island, NY, USA). 5'-Bromo-2'-deoxyuridine (BrdU), benzo[α]pyrene and other standard laboratory reagents were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals used in the study were of the highest purity available from commercial sources. Taq DNA polymerase and 2'-deoxyribonucleoside-5'-triphosphates were purchased from Epicentre Technologies (Madison, WI, USA). The primers were purchased from Operon Technologies (Alameda, CA, USA).

GSTM1 polymorphism

For the determination of the genetic status, DNA was prepared from peripheral lymphocytes of anticoagulated blood (EDTA) by proteinase K digestion and a salting-out procedure with a saturated NaCl solution as described by Miller et al. (13). A polymerase chain reaction (PCR) method was used to detect the presence or absence of the GSTM1 (12). The GSTM1 primers used were forward, 5'-GAACTCCCTGA AAAGCTAAAGC-3'; reverse, 5'-GTTGGGCTCAAA TATACGGT GG-3'. The beta-globulin primers used were forward, 5'-CAACTTCATCC ACGTTCACC-3'; reverse, 5'-GAAGAGCCAAGGACAGGTAC-3'. DNA from individuals with positive GSTM1 and beta-globulin alleles yielded 215 and 268-bp products, respectively. The absence of amplifiable GSTM1 (in the presence of beta-globulin PCR product) indicates that GSTM1 has a null genotype.

SCE analysis

Whole heparinized blood (0.5 ml) was added to 4.5 ml of RPMI 1640 culture medium containing L-glutamine (1%), fetal calf serum (20%), penicillin (100 UI/ml), streptomycin (100 µg/ml), phytohemagglutinin (1.5%) and 5-bromo-2-deoxyuridine (10 µM) (BrdU). The cultures were incubated for 72 h at 37 °C. Colchicine in a final concentration of 0.2 µg/ml was added 2 h prior to the harvesting.

After 24 h of incubation, the cultures were treated with BaP for 48 h. BaP was solubilized in DMSO giving the final 5 mM concentration of the chemical in the cultures. Exposure of cells to this concentration revealed insignificant changes in cell death rate. At 24 h after culture initiation, DMSO was added to the cultures set up as a control. The final concentration of DMSO did not exceed 0.1%. Techniques for cell harvest and slide preparation followed conventional procedures (14). The cells were harvested by centrifugation, and then given a hypotonic shock in 0.075 M KCl and fixed in 3:1 methanol:acetic acid. From a suspension of the fixed cells, slides were prepared and stained with 5% Giemsa solution in a freshly made Sörenson's pH 10.4 buffer for 12 min. The frequency of SCE was assessed by scoring the number of SCEs in 25 complete second metaphases per donor.

CA analysis

Metaphase chromosome analysis for the detection of CAs was performed according to the conventional

technique (15). The whole blood cultures were established as described above. No BrdU was added. Lymphocytes were treated with BaP (5 mM) for 24 and 48 h. Control cultures, set up in parallel with the treated cultures, received DMSO. After harvesting, air-dried slides were G-banded and scored for chromosome aberrations. A total of 50 well-spread metaphases were analyzed per treatment per duration for all types of chromatid and chromosome type of aberrations.

Statistical analysis

All tests were performed in duplicate, and the results were expressed as means ± S.D. The results were evaluated using Student's t-test for dependent and independent groups. The level of significance was set at 0.05.

Results

SCE results

The effect of BaP on the induction of SCEs in lymphocytes is presented in Table 1. In both genotype groups, treatment of blood lymphocytes with BaP caused a significant increase in SCE frequency compared with the controls ($P < 0.001$). The SCE response of the GSTM1 positive and the GSTM1 null donors did not differ from each other in the control or treated cultures ($P > 0.05$). Slightly higher individual SCE responses were observed in control ($P = 0.11$) and BaP-treated ($P = 0.13$) cultures for the GSTM1-null donors compared with the GSTM1-positive donors. These differences were not, however, statistically significant.

CA results

The chromosomal aberration frequencies in lymphocytes induced by BaP are presented in Table 2. The total numbers of aberrations and numbers of individual types of structural aberrations were statistically compared with those in the controls. In both genotype groups, BaP induced statistically significant increases in structural CAs such as chromatid and chromosome-type gaps and breaks when compared to the controls ($P < 0.001$). There was a time-dependent increase in chromatid-type gaps and breaks in both GSTM1 positive and null individuals; however, the differences were not statistically significant ($P > 0.05$). The rates of chromatid and chromosome-type gaps and breaks were similar in all the groups ($P > 0.05$), but the chromatid-type gaps and breaks were more prevalent than the chromosome-type gaps and breaks ($P < 0.05$) when lymphocytes from individuals with the GSTM1 null genotype were treated with BaP for 48 h.

In the next step of our study, we analyzed the influence of GSTM1 polymorphism on the induction of CAs by BaP. There were no differences between GSTM1 null and positive individuals either in the level of control CA or in the level of CA induction by BaP for 24 and 48 h ($P > 0.05$).

Discussion

In the present study, we studied the association between GSTM1 polymorphism and cytogenetic damage induced in vitro by BaP. Two cytogenetic endpoints (CA and SCE) were used as indicators of the cytogenetic damage in peripheral blood lymphocytes of healthy individuals.

BaP is relatively non-toxic, but its bioactivation in vivo by cytochrome P450 and peroxidases generates highly toxic electrophilic and free radical reactive intermediates, such as BPDE. BPDE can irreversibly damage DNA by covalent binding or oxidation (16), and has high specificity for GSTM1 (17). Lymphocytes have been used

Table 1. nfluence of GSTM1 genotype on sister chromatid exchange induction by BaP.

Genotype	No. of subjects	Number of SCEs per metaphase (mean ± SD)	
		DMSO	BaP
GSTM1 Positive	16	2.96 ± 0.35	5.56 ± 0.83 *
GSTM1 Null	15	3.23 ± 0.56	6.09 ± 1.11 *

when compared with DMSO-treated control cultures, statistically significant at *P < 0.001

Table 2. Influence of GSTM1 genotype on chromosome aberration induction by BaP.

Genotype	No. of subjects	Treatment	Duration (h)	Aberration/metaphase (mean ± SD)		Total Aberration	Aberration/metaphase (mean ± SD)
				Chromatid type	Chromosome type		
GSTM1 Positive	16	BaP	24	0.036 ± 0.027 $^{\phi,*}$	0.047 ± 0.047 $^{\phi}$	67	0.083 ± 0.059 $^{\phi}$
	16	BaP	48	0.050 ± 0.041 $^{\phi\phi,*}$	0.042 ± 0.037 $^{\phi\phi}$	74	0.092 ± 0.057 $^{\phi\phi}$
	16	DMSO	24	0.009 ± 0.012 *	0.008 ± 0.012	13	0.017 ± 0.022
	16	DMSO	48	0.009 ± 0.016 *	0.008 ± 0.016	13	0.017 ± 0.025
GSTM1 Null	15	BaP	24	0.045 ± 0.039 $^{\phi,*}$	0.044 ± 0.025 $^{\phi}$	69	0.090 ± 0.058 $^{\phi}$
	15	BaP	48	0.064 ± 0.039 $^{\phi\phi,**}$	0.032 ± 0.027 $^{\phi\phi}$	72	0.096 ± 0.050 $^{\phi\phi}$
	15	DMSO	24	0.009 ± 0.016 *	0.009 ± 0.016	14	0.018 ± 0.027
	15	DMSO	48	0.008 ± 0.012 *	0.008 ± 0.014	12	0.016 ± 0.025

when compared with DMSO-treated control cultures, statistically significant at $^{\phi}P < 0.001$ (the 24h treatment period), $^{\phi\phi}P < 0.001$ (the 48h treatment period)

when compared with chromosome type aberration, statistically significant at $*P > 0.05$, $**P < 0$.

extensively as a convenient tissue source for investigating the cytotoxicity of xenobiotics. Lymphocytes also possess GSTM1 enzymic activity and are subject to oxidative stress when exposed to various factors (18). If the GSTM1 isotype is involved in defences against oxidative stress then lymphocytes having the GSTM1 null genotype could be more susceptible to genotoxic and cytotoxic damage. However, we were unable to show any significance of GSTM1 deficiency on SCE- and CA-induction caused by BaP. These results agree with our earlier reports suggesting that lack of the GSTM1 gene does not influence the induction of micronuclei in human lymphocyte cultures by BaP (19).

A limited number of studies have been performed using cytogenetic biomarkers to investigate the role of GSTM1 polymorphism on sensitivity to BaP in vitro. We can compare our results with 2 cytogenetic studies measuring cytogenetic markers in the in vitro genotoxic effects of BaP. One of the studies suggested an increase in BaP-induced CAs, but not in SCEs, in lymphocytes of GSTM1-null donors (20). In another study investigating the role of null GSTM1 and GSTT1 genotypes in BPDE-induced chromosomal aberrations in peripheral blood lymphocytes from breast cancer and matched controls, Xiong et al. suggested that the GSTM1 null genotype was not associated with an increased trend to form chromosomal aberrations in either control or case cultures (21). These contradictory results obtained with cytogenetic assays may be due to the different experimental conditions such as concentration and duration of BaP-treatment, the use of BPDE instead of BaP (21) and the detection methods used (the use of FISH assay for CA in the study by Salama et al. (20)), and the influence of combined genotypes involved in BaP metabolism (i.e. GSTM1nulll/EH4 genotypes on CA in the study by Salama et al. (20)) and DNA repair. A recent study demonstrated that the XPD genotype in combination with the GSTM1 null genotype significantly influenced percent detectability and levels of BPDE-DNA in white blood cells (22).

Other studies association with BaP have addressed the relationship between GSTM1 polymorphism and some biomarkers of effect in humans exposed to PAH. However, it remains unclear if GSTM1 polymorphism modulates the genotoxic or carcinogenic effects of BaP in humans in vivo. In accordance with our findings, most of the studies that used cytogenetic techniques did not report differences in cytogenetic marker levels between subjects with the GSTM1 null and GSTM1 active genotypes analyzing occupationally or environmentally PAH-exposed subjects (23-25). In other studies dealing with DNA adducts in exposure to PAH, contradictory results have been reported. Some studies showed the influence of the genotype GSTM1 on DNA adduct levels, alone or in combination with other genotypes (26,27), whereas some other studies showed no difference in

adduct levels dependent on the GSTM1 genotype (23,28).

In accordance with our findings, BaP has been shown to elevate chromosomal aberrations in the lymphocytes from healthy individuals (29). Not only the increase in the frequency of structural CA but also their type and distribution are important. It is well known that the type of aberrations induced by genotoxic agents are cell cycle dependent. Most of the chemically induced aberrations are formed only during the DNA synthesis phase (probably due to misreplication). Such chemical agents induce mainly chromatid-type aberrations and are also very efficient in inducing SCEs (30). The exact mechanism of how BaP induces chromosomal aberrations remains to be determined. BaP-induced DNA damages are repaired mainly by nucleotide excision repair (NER). During the repair process, it is likely that delayed completion of initial nicking of DNA strands by NER may induce DNA strand breaks that eventually lead to chromatid breaks (31). In our study, a detailed analysis of structural chromosomal aberrations showed that the rates of chromatid- and chromosome-type gaps and breaks induced in vitro by BaP were similar in all the groups. In the GSTM1 null genotype, however, chromatid-type breaks were seen more frequently than chromosome-type breaks after a 48 h treatment with BaP. This result suggests that because the conversion of BaP into DNA-reactive metabolites is dependent on a cascade of biotransformations, BaP metabolites, which are substrates for GSTM1, might be active during long-term treatment to BaP and thus subjects who are null for GSTM1 may have higher levels of chromatid-type breaks than those with the gene. Indeed, it has been reported that the amount of BaP metabolized by incubating mitogen-stimulated lymphocytes with BaP for 24 to 72 h is significantly increased (32,33).

In conclusion, the present results show that lack of the GSTM1 gene does not influence the genotoxicity or cytotoxicity of BaP in human lymphocytes in vitro. However, the question of whether the GSTM1 genotype influences the induction of cytogenetic damage by BaP in vitro is difficult to answer because the presence of other susceptibility genes may modify the effect of GSTM1 null. Further research is warranted to confirm these findings as well as to investigate the possible risk modulation by genetic polymorphisms in carcinogen-metabolizing enzymes and DNA repair genes that are relevant to the phenotype.

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