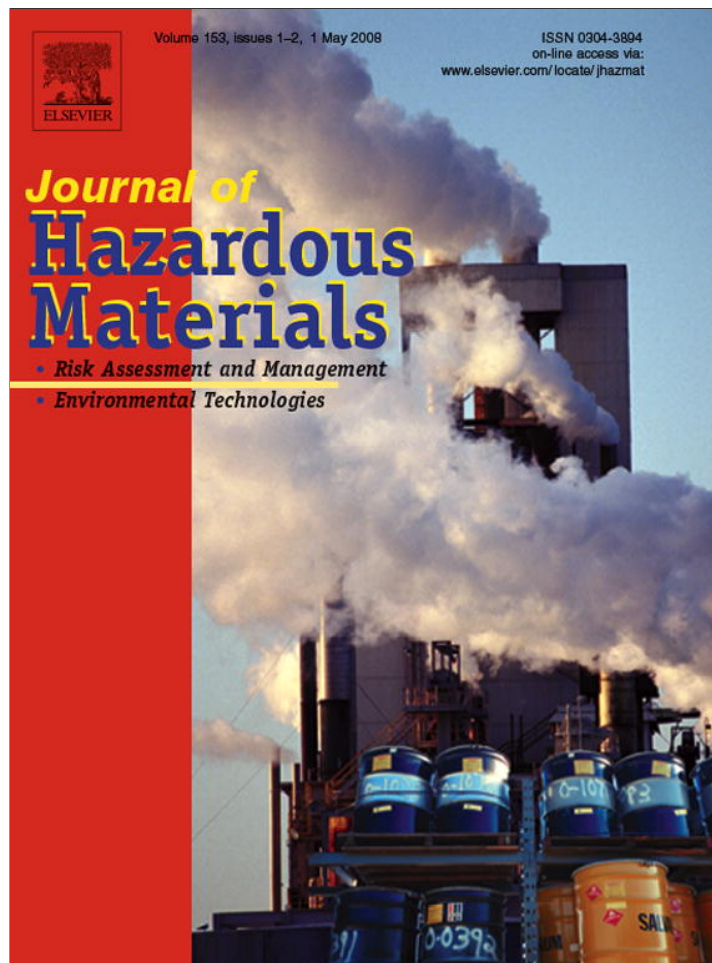


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Assessment of genotoxicity of methyl-*tert*-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay

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Abstract

Methyl-*tert*-butyl ether (MTBE) is a gasoline oxygenate and antiknock additive substituting for lead alkyls currently in use worldwide. Benzene, toluene, ethylbenzene, and xylene (BTEX) are volatile monoaromatic hydrocarbons which are commonly found together in crude petroleum and petroleum products such as gasoline. The aim of this study is to evaluate the genotoxic effects of these tested chemicals in human lymphocytes. Using the alkaline comet assay, we showed that all of the tested chemicals induce DNA damage in isolated human lymphocytes. This effect could follow from the induction of DNA strands breaks. The neutral version of the test revealed that MTBE, benzene, and xylenes induce DNA double-strand breaks at 200 μ M. Apart from MTBE, the spin traps, 5,5-dimethyl-pyrroline-*N*-oxide (DMPO) and *N*-*tert*-butyl- α -phenylnitron (PBN) can decrease the level of DNA damage in BTEX at 200 μ M. This indicated that DNA damage could result from the participation of free radicals in DNA-damaging effect, which was further supported by the fact that post-treatment of formamidopyrimidine-DNA glycosylase (Fpg), enzyme recognizing oxidized DNA purines, gave rise to a significant increase in the extent of DNA damage in cells treated with benzene, and xylene at 200 μ M. The results obtained suggested that MTBE and BTEX could induce a variety type of DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and oxidative base modification.

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Keywords: Spin traps; Lymphocyte; DNA strands breaks; MTBE; BTEX

1. Introduction

Motor fuels are complex organic mixtures comprised of hundreds of specific compounds. Indicator compounds are usually defined as those compounds which can be considered the most toxic and, the most mobile in soil and groundwater. For these reasons, many cleanup standard or guidelines focus on benzene, toluene, ethylbenzene, and xylenes, commonly known as “BTEX.” The BTEX chemicals are volatile monoaromatic hydrocarbons which are commonly found together in crude petroleum and petroleum products such as gasoline. The BTEX compounds represent some of the most hazardous components of gasoline [1]. They are also produced on the scale of megatons per year as bulk chemicals for industrial use as solvents and starting materials for the manufacture of pesticides, plastics, and synthetic fibers [2].

Organic compounds can be a major pollution problem in groundwater. Their presence in water can create a hazard to public health and the environment. The practice of adding methyl-*tert*-butyl ether, MTBE, to gasoline started in the late 1970s and increased dramatically in the 1990s in an effort to increase combustion efficiency and reduce air pollution. Like other gasoline components, MTBE is released into the environment during the production, distribution, storage and use of MTBE-blended fuels. Following an accidental release of MTBE-blended gasoline, MTBE is typically the first compound to be detected in groundwater due to its high solubility and low sorption potential [3]. One of the most common sources for BTEX and MTBE contamination of soil and groundwater are spills involving the release of petroleum products such as oxygenated gasoline from leaking oil tanks. Because of the relatively high water solubility and low K_{ow} (i.e., octanol–water partition coefficient) values, these compounds will tend to be dissolved in the water phase or evaporated into the air spaces of the soil. Because of their relative hydrophilic nature, they are not attenuated very much by the soil particles or constituents

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and can be transported rather long distances if the right conditions are there. In some sites, some BTEX and MTBE are found several kilometres downstream the source. The reason why the BTEX entering our soil and groundwater system are considered such a serious problem is that they all have some acute and long-term toxic effects. While MTBE is likely to dominate at leading edges of gasoline-impacted groundwater plumes, it can be expected to coexist with the monoaromatic components of gasoline near contaminant sources and at lagging edges of contaminant plumes. The major aromatic constituents of gasoline, collectively known as BTEX compounds, have high water solubility relative to the aliphatic constituents of gasoline. However, given more time and sufficient distance from point sources, dissolved plumes of MTBE are expected to migrate beyond BTEX plumes.

In rodents and humans, MTBE is metabolized via oxidative demethylation to the known weak carcinogens, *tert*-butanol (TBA), and formaldehyde [4], both of which induce the renal tubular tumors in male and female Fischer 344 rats [5], hepatic neoplasms in CD-1 mice [6], and adducts with DNA in mouse lung, liver, and kidney. The latter is induced in a log–linear dose–response relationship [7]. Benzene has been found to be mutagenic and carcinogenic in various animal experiments and epidemiological studies, which is especially associated to bone marrow toxicity and leukemia [8,9]. Cok et al. [10] suggested that the DNA damage in lymphocytes of glue sniffers is caused by toluene; however, further *in vitro* experiments with toluene still needed be investigated, as other components of the glue may also play a role. The genotoxicity of three isomers of xylene in human lymphocyte determined with the comet assay has not been reported. Ethylbenzene (EB) was reported to induce a non-genotoxic mode of action responsible for the lung and liver tumors observed in mice following 2 years inhalation exposure to EB [11].

In the present work, we investigated the DNA-damaging potential of MTBE and BTEX in human lymphocytes using the comet assay. We employed the comet assay at two versions: the alkaline version revealing single-strand breaks (SSBs) and double-strand breaks (DSBs), and alkali-labile sites, and the neutral version revealing only DSB [12]. Furthermore, in order to assess whether oxidative DNA damage may contribute to MTBE and BTEX-induced lesions, we used formamidopyrimidine-DNA glycosylase (Fpg) to convert oxidized purines into strand breaks, which can be detected by the comet assay [12]. In addition, the involvement of free radicals in the genotoxicity of MTBE and BTEX was evaluated by pre-treating cells with 5,5'-dimethyl-pyrroline-*N*-oxide (DMPO) or *N*-*tert*-butyl- α -phenylnitron (PBN), two scavengers of free radicals

2. Materials and methods

2.1. Chemicals

MTBE and BTEX were purchased from Fisher Scientific and Tedia in U.S.A. L-Glutamine, phosphate buffered saline (PBS; Ca²⁺, Mg²⁺ free), RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco-Invitrogen (Carlsbad,

CA), while low melting-point agarose, normal melting-point agarose, Fpg, Tris buffer, ethidium bromide, trypsin, antibiotic solution, phytohemagglutinin, and trypan blue were obtained from Sigma (St. Louis, MO). 5,5'-dimethyl-pyrroline-*N*-oxide and *N*-*tert*-butyl- α -phenylnitron were from TCI (Japan) and ACROS (U.S.A.). All of these chemicals were of analytical reagent grade.

Tested chemicals were freshly prepared by dissolving in DMSO and were kept in the dark. The final concentration of DMSO was less than 1% of the reaction mixtures.

2.2. Cultures human peripheral blood lymphocytes treated with tested chemicals

Lymphocytes cultures were performed according to the procedures of Feng et al. [13]. Blood withdrawn from a female donor (health and non-smoker, aged 25) was collected into Ficoll-Hypaque. The samples were then centrifuged at 200 \times *g* at 25 °C for 20 min. The formed lymphocyte forming a layer was directly above the Ficoll-Hypaque. The isolated lymphocytes (0.3 ml) were cultured in 4.7 ml RPMI 1640 medium including 20% heat-inactivated fetal calf serum, 2% phytohemagglutinin (PHA), 100 IU/ml of penicillin, 100 μ g of streptomycin, and 2 mM of L-glutamine at 37 °C under 5% CO₂ atmosphere.

The cells were diluted down to a concentration of 2.5 \times 10⁵ cells/ml prior to use. Lymphocytes were incubated with different concentrations of the tested chemicals (50, 100, and 200 μ M) dissolved in DMSO (1% as a final concentration) at 37 °C for 1 h in a dark incubator. Subsequently, the cells were centrifuged at 200 \times *g* for 3 min at 4 °C and then were mixed with low melting point agar for the comet assay as described below. In spin trapping experiments, the incubation of cells with MTBE and BTEX was preceded by incubation with a spin trap, either DMPO or PBN, at a final concentration of 100 μ M for a few seconds, which was according to the procedures of Wozniak and Blasiak [12].

2.3. Cell viability analysis

The procedures were conducted following the procedures in Chen et al. [14]. A volume of 0.49 ml cell suspension treated with each tested chemical at the doses ranging from 0 to 200 μ M was mixed with 10 μ l of 0.4% trypan blue solution. Its viability was determined after 5 min of reaction. The cells were analyzed through microscopic observation to determine the percentage of viable cells.

2.4. Comet assay

The comet assay was performed under alkaline conditions following the method of previous study [14]. Conventional microscope slides were dipped with a solution of 85 μ l 0.5% of normal melting point agarose (NMPA) and 0.5% low melting point agarose (LMPA) in PBS (pH 7.4), and allowed to dry on a flat surface at room temperature. Ten microliters of cell suspension (2.5 \times 10⁵ cells/ml) was gently mixed with 75 μ l of 0.5% (w/v) of LMPA in PBS (pH 7.4). Seventy-five micro-

liters of this suspension was rapidly layered onto the slides pre-coated with the mixtures of 0.5% NMPA and 0.5% LMPA, and covered with a cover glass. The slides were maintained at 4 °C for 5 min, the cover glass was removed, and cells were immersed in a freshly made lysis solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris and 1% (v/v) Triton X-100 at pH 10) at 4 °C for 1 h. The Slides were then placed in a double row in a 260 mm wide horizontal electrophoresis tank containing 0.3 M NaOH and 1 mM Na₂EDTA for 10 min. Thereafter, the electrophoresis (1 V/cm, 300 mA) was conducted for 15 min at 4 °C. After the electrophoresis, the slides were then soaked in a cold neutralizing buffer (400 mM Tris buffer, pH 7.5) at 4 °C for 10 min. Slides were dried in 100% methanol for 5 min, and stored in a low humidity environment before staining with 40 µl PI (2.5 µg/ml).

The neutral comet assay was performed the same as the alkaline comet assay, except that the cells were lysed in neutral lysis buffer (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris–HCl, 1% sodium *N*-laurylsarcosine, 1% Triton X-100, 10% DMSO, pH 8.5) for 1 h [15], and electrophoresed in a freshly neutral buffer (0.3 M sodium acetate, 0.1 M Tris–HCl, at pH 8.5) at 1 V/cm, 300 mA for 15 min.

2.5. Quantification of the comet assay

One hundreds comets on each slide were scored visually according to the relative intensity of the tail. An intensity score from class 0 (undamaged) to class 4 (severely damaged) was assigned to each cell. Thus, the total score for the 100 comets could range from 0 to 400 because the 100 cells were observed individually in each comet assay. The extent of DNA damage was analyzed and then scored by the same experienced person, using a specific pattern when moving along the slide. The method of the observation was barred in a blind way during which the observer had no knowledge of the identity of the slide.

2.6. DNA repair enzyme treatment

For examining the levels of oxidized purines in lymphocytes, the slides after cell lysis were washed three times (each for 5 min at 4 °C) in an enzyme buffer containing 40 mM HEPES–KOH, 0.1 M KCl, 0.5 mM EDTA, and 0.2 mg/ml bovine serum albumin at pH 8.0. The slides were then drained. Aliquots of 30 µL of Fpg at 1 µg/ml in the buffer were applied to the agarose on slides, and were incubated for 30 min at 37 °C [12]. The control received only the buffer. To check the ability of the enzymes to recognize oxidized purines in our experimental conditions, lymphocytes were incubated with H₂O₂ and then were post-treated with the Fpg enzyme.

2.7. 2.7. Statistical analysis

Images from 300 random cells (100 from triplicate slide) were analyzed for each experiment. The experiment (and not the cell) was used as the experiment unit. We followed the statistical method of Baipayee et al. [16]. The homogeneity of variance between treatment groups was ascertained prior to the

statistical analysis of the Comet assay data. The mean Comet data (DNA damage scores) were analyzed using one-way analysis of variance (ANOVA), with DNA damage as the dependant variable and concentrations of the tested compounds as the independent variable. If a significant *F*-value was obtained, then Dunnett's multiple comparison tests were conducted. *P* < 0.05 was considered to be significant.

For the spin trapping and DNA repair experiments, the differences between control group and tested group was analyzed using Students paired *t*-test. *P* < 0.05 was considered to be significant.

3. Results

3.1. DNA damage

The viability of lymphocytes cells treated with MTBE and BTEX (50–200 µM) for 1 h was evaluated. It was observed that the cell viability after the treatment of these compounds was at least >95% using trypan blue dye assays (data not shown). Furthermore, at the doses over 200 µM, the solubility of these tested chemicals dissolved in 1% DMSO as the solvent was limited. Thus, MTBE and BTEX at doses below 200 µM were used separately for the determination of genotoxicity using the comet assay. Table 1 summarized the results of DNA damage in human lymphocytes treated with varying concentrations of tested chemicals at 37 °C for 1 h, as measure by the alkaline comet assay. Results indicated that the positive group (cells pre-treated with 50 µM H₂O₂) showed maximum levels of DNA damage score, while the negative control (0.5% DMSO as solvent for each tested chemical) revealed very low DNA damage score. At a concentration of 100 µM, the tested chemicals, except for EB, exhibited significant DNA damage when compared to the negative control group (*p* < 0.05). All seven tested chemicals revealed the genotoxicity to lymphocytes. In the neutral comet assay (Table 2), except for toluene and EB, other six compounds at the dose of 200 µM showed the genotoxicity to human lymphocytes, as compared to the control group (0.5% DMSO) (*p* < 0.05). This result indicates that these DNA-damaging chemicals can induce DNA double-strand breaks in lymphocytes.

3.2. Effect of spin traps pre-treatment on DNA damage

To check the ability of spin traps to scavenge free radical in our experimental conditions, incubation of the lymphocytes with H₂O₂ at concentration of 50 µM for 1 h at 37 °C in the presence or in the absence of DMPO or PBN was performed following the procedures of Wang et al. [12]. A significant decrease (*p* < 0.05) of DNA damage of the cells incubated with H₂O₂ and DMPO or PBN in comparison with cells not treated with the traps was observed. The decrease was from 354 ± 16 to 311 ± 11 for DMPO and to 328 ± 12 for PBN (Table 3). Results also show that the presence of spin traps did evoke the decrease in the levels of DNA damage (*p* < 0.05) in lymphocytes treated with six tested compounds at 200 µM for 1 h, except for MTBE.

Table 1
DNA damage in human lymphocytes exposed to different doses of MTBE and BTEX (benzene, ethylbenzene, toluene, and xylene) using the alkaline comet assay

Chemicals	Dose (μM)	DNA damage (mean \pm S.D., arbitrary units) ^a
MTBE	0	92 \pm 7
	50	112 \pm 5 ^b
	100	121 \pm 5 ^b
	200	124 \pm 10 ^c
H ₂ O ₂	50	327 \pm 8 ^c
Benzene	0	87 \pm 9
	50	141 \pm 21 ^b
	100	151 \pm 12 ^c
	200	168 \pm 7 ^c
H ₂ O ₂	50	325 \pm 10 ^c
Ethylbenzene	0	84 \pm 6
	50	101 \pm 6
	100	123 \pm 12 ^b
	200	129 \pm 7 ^b
H ₂ O ₂	50	330 \pm 5
<i>m</i> -Xylene	0	78 \pm 8
	50	87 \pm 6
	100	106 \pm 8 ^b
	200	116 \pm 10 ^c
H ₂ O ₂	50	353 \pm 8 ^c
<i>o</i> -Xylene	0	88 \pm 6
	50	96 \pm 5
	100	112 \pm 12 ^b
	200	136 \pm 22 ^c
H ₂ O ₂	50	361 \pm 15 ^c
<i>p</i> -Xylene	0	82 \pm 4
	50	105 \pm 9 ^b
	100	148 \pm 15 ^c
	200	157 \pm 13 ^c
H ₂ O ₂	50	354 \pm 12 ^c
Toluene	0	91 \pm 7
	50	151 \pm 25 ^b
	100	196 \pm 14 ^c
	200	276 \pm 11 ^c
H ₂ O ₂	50	316 \pm 13 ^c

^a The scores of DNA damage were calculated from the respective values of at least three treatment (100 cells/slide, duplicate slides/treatment).

^b Represented $P < 0.05$.

^c $P < 0.01$, respectively.

3.3. Detection of DNA damage by DNA repair enzymes

Table 4 shows the DNA damage score of lymphocytes exposed for 1 h at 37 °C to MTBE and BTEX at 200 μM with post-treatment with Fpg, compared with the cells without treatment with the enzyme. Except for toluene and ethylbenzene, other five compounds showed the increase in DNA damage score in lymphocytes ($p < 0.05$), as compared to the cells without treatment with the enzyme.

4. Discussion

Genotoxins can elicit a variety of types of DNA damage, including base modification, DNA adduction, single-strand

Table 2
DNA damage in human lymphocytes exposed to 200 μM MTBE and BTEX using the neutral comet assay

Chemicals ^a	Dose (μM)	DNA damage (mean \pm S.D., arbitrary units) ^b
Control		31 \pm 5
H ₂ O ₂	50	136 \pm 28**
MTBE	50	58 \pm 8*
	100	88 \pm 7**
	200	142 \pm 23**
Benzene	50	76 \pm 7*
	100	84 \pm 13**
	200	145 \pm 20**
Ethylbenzene	200	35 \pm 4
	Toluene	200
<i>m</i> -Xylene	50	90 \pm 8**
	100	92 \pm 1**
	200	102 \pm 13**
<i>o</i> -Xylene	50	65 \pm 6*
	100	101 \pm 4**
	200	107 \pm 8**
<i>p</i> -Xylene	50	57 \pm 4*
	100	80 \pm 3**
	200	140 \pm 22**

^a All chemicals were dissolved in 0.5% DMSO. DMSO was used as the control group.

^b DNA damage scores were calculated from the respective values of at least three treatment (100 cells/slide, duplicate slides/treatment), * and ** represented $P < 0.05$ and $P < 0.01$, respectively.

breaks, double-strand breaks, intra- or inter-strand cross-links, among which DSBs are regarded as the most severe type of damage [17]. Since the comet assay is a sensitive, reliable, and rapid method for the detection of DNA double- and single-strand breaks, alkaline-labile sites and delayed repair-site detection in eukaryotic individual cells [18], the alkaline and neutral comet assay were applied to detect the genotoxicity of MTBE and BTEX in this study. Furthermore, the information for the genotoxicity of cells treated with these parent chemicals for a short time is still unclear. Thus, the aim of this study is to focus on the determination of the genotoxicity of human lymphocytes exposed to MTBE and BTEX for 1 h.

Using alkaline comet assay (Table 1), we found that MTBE and BTEX cause an increase in DNA damage score, suggesting that these compounds are genotoxins. Our research further showed that five compounds can elicit double DNA strand breaks, except for toluene and ethylbenzene at 200 μM (Table 2). These results indicated that these tested chemicals can cause severe DNA damage. To elucidate whether MTBE and BTEX induced reactive oxygen species, we pre-incubated the lymphocytes with spin traps (DMPO and PBN). Spin traps can form adducts with free radicals and in this way reduce the extent of DNA damage. We chose DMPO and PBN because they can form complex with a lot of reactive free radicals [12]. In the presence of DMPO and PBN, a significant decrease in DNA damage caused by BTEX, except for MTBE, was observed (Table 3). This result indicated that BTEX might induce free radicals in

Table 3

DNA damage of human lymphocytes incubated with 200 μM MTBE or BTEX in the presence of DMPO or PBN at 100 μM compared with the control group (without DMPO or PBN treatment)

Chemical	DNA damage score (mean \pm S.D., arbitrary units) ^a			
	–DMPO	+DMPO	–PBN	+PBN
MTBE	158 \pm 10	151 \pm 8	158 \pm 10	146 \pm 9
Benzene	165 \pm 17	117 \pm 8 ^b	141 \pm 19	97 \pm 10 ^b
Ethylbenzene	161 \pm 16	114 \pm 9 ^b	155 \pm 11	132 \pm 7 ^b
Toluene	183 \pm 14	143 \pm 11 ^b	176 \pm 19	131 \pm 10 ^b
<i>m</i> -Xylene	141 \pm 8	123 \pm 9 ^b	135 \pm 13	104 \pm 7 ^b
<i>o</i> -Xylene	146 \pm 11	114 \pm 12 ^b	145 \pm 9	113 \pm 14 ^b
<i>p</i> -Xylene	182 \pm 10	138 \pm 14 ^b	177 \pm 18	134 \pm 6 ^b
Control (0.5% DMSO)	86 \pm 7	103 \pm 10	79 \pm 10	85 \pm 9
H ₂ O ₂	354 \pm 16	311 \pm 11 ^b	356 \pm 11	328 \pm 12 ^b

^a DNA damage scores were calculated from the respective values of at least three treatment (100 cells/slide, duplicate slides/treatment).

^b Represented $P < 0.05$.

the lymphocytes. Based on the results of Tables 1–3, we suggested that single and double DNA strand breaks induced by some BTEX at 200 μM could result from free radical action.

Under our experimental conditions, we found that significant increase in DNA damage after post-treatment of DNA exposed to benzene, *m*-xylene, *o*-xylene, and *p*-xylene at 200 μM with Fpg, the DNA repair enzyme, compared with the control group (without Fpg treatment) (Table 4). Fpg has been reported to excise a variety of modified bases from DNA and enhance the DNA-damaging effects of alkylating agents [19,20,21]. But the main substrate of Fpg seems to be 8-oxoguanine (8-oxoG), which probably is the most abundant base-oxidation product to be found in DNA, and also the most commonly used biomarker for oxidative DNA damage [19,21,22,23,24]. Thus, we suggested that these chemicals would generate oxidative damage in human lymphocytes

Table 5 summaries a type of DNA damage caused by MTBE and BTEX. It is worth noting that MTBE was reported to be non-genotoxic using some genotoxic tests including the *Salmonella* micro-suspension assay and bone marrow micronucleus test [25], although it appears to be positive in animal carcinogenicity testes for a variety of species and target organ [4]. Kado et al. [25] fur-

Table 4

DNA damage in human lymphocytes incubated with 200 μM MTEX and BTEX and post-treated with Fpg compared with the untreated cells

Chemical	DNA strands breaks (arbitrary units) ^a	
	–Fpg	+Fpg
MTBE	116 \pm 12	139 \pm 7 ^b
Benzene	121 \pm 11	159 \pm 11 ^b
Ethylbenzene	111 \pm 14	118 \pm 15
Toluene	109 \pm 10	118 \pm 25
<i>m</i> -Xylene	114 \pm 7	134 \pm 9 ^b
<i>o</i> -Xylene	121 \pm 10	145 \pm 9 ^b
<i>p</i> -Xylene	164 \pm 11	190 \pm 9 ^b
Control (0.5% DMSO)	78 \pm 79	90 \pm 17
H ₂ O ₂ (50 μM)	135 \pm 16	187 \pm 8 ^c

^a DNA damage scores were calculated from the respective values of at least three treatment (100 cells/slide, duplicate slides/treatment).

^b Represent $P < 0.05$.

^c Represent $P < 0.01$.

ther pointed out that MTBE can still evoke the positive reaction in the mouse lymphomas assay, and other genotoxicity studies are necessary to further evaluate the toxicological mechanisms. To our knowledge, this is the first report on a type of DNA damage, SSB and DSB, with MTBE in vitro. Benzene cause DNA strand breaks in B-lymphocytes from the workers in a printing company [26], and act as a mutagen via an indirect mechanism, leading to oxidative DNA damage through the formation of hydroxyl radicals via hydrogen peroxide [27]. The biomarker of oxidative DNA damage, 8-OH-dG, was proved to be detected in lymphocytes of human exposed to benzene [28]. Furthermore, the metabolites of benzene bind to DNA and benzene-derived quinone metabolites can generate reactive oxygen species (ROS) through redox cycling, which can lead to oxidative DNA damage [29]. These results supported for the fact that the metabolites of benzene are genotoxins. In addition, benzene itself at the higher dose (12 mM) but not at the lower dose (2.4 mM) had been reported to cause DNA damage in human lymphocytes after the treatment of this chemical for 1 h using the comet assay [30], whereas we can detect the genotoxicity of benzene itself at the even low dose (50 μM) in this study.

As for the detection of free radical-induced genotoxicity of benzene in this study, a decrease in DNA damage in lymphocytes pre-treated with DMPO and PBN, and an increase in DNA damage in cells post-treated with Fpg suggested that benzene could cause oxidative DNA damage in human lymphocytes. In

Table 5

The summary of the results of tables 1–4

Chemical	SSB ^a	DSB ^b	DMPO	PBN	Fpg
MTBE	+ ^c	+	– ^d	–	+
Benzene	+	+	+	+	+
Ethylbenzene	+	–	+	+	–
Toluene	+	–	+	+	–
<i>m</i> -Xylene	+	+	+	+	+
<i>o</i> -Xylene	+	+	+	+	+
<i>p</i> -Xylene	+	+	+	+	+

^a SSB represented single-strand breaks.

^b DSB represented double-strands breaks.

^c The result is significant, as compared to the corresponding group.

^d The result is insignificant, as compared to the corresponding group.

presence of DMPO and PBN, the DNA damage in cells treated with toluene or EB was decreased, suggesting that the participation of free radical in DNA damage in the treated cells. However, the fact that Fpg did not enhance the DNA damaging effect of both compounds would indicate that these chemicals mediate oxidation of pyrimidine rather than purines. On the other hand, the enhancement of DNA damage of cells caused by MTBE in the presence of Fpg, and no effects of a decrease in DNA damage in these cells pre-treated with DMPO and PBN suggested that the participation of free radicals in DNA-damaging potential of MTBE is not important, since Fpg was recently reported to also recognize alkylation DNA damage [31], in addition to oxidative DNA damage.

5. Conclusion

In this study, we have been able to detect a type of DNA damage such as single-, double-strand breaks in DNA, and the oxidative DNA bases modification, in benzene, *m*-xylene, *o*-xylene, or *p*-xylene- treated with lymphocytes. Apart from MTBE, BTEX could produce oxidative DNA damage in human lymphocytes. Overall, a variety type of DNA damage was observed in human lymphocytes treated with MTBE and BTEX.

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