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ORIGINAL ARTICLE

Study of liver function and expression of some detoxification genes in the male rats exposed to methyl-tertiary butyl ether

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Abstract *Background and purpose:* Methyl-tertiary butyl ether (MTBE) is an additive solvent that was adopted in reformulated gasoline to reduce environmental pollutants. It is still used in Middle East countries. It is suggested that the toxicity of MTBE may be attributed to induction of oxidative stress. Study of alteration of end organ markers and mRNA due to MTBE exposure is potentially important for public health programs. In this study we investigate the effect(s) of MTBE on liver function indices and expression of some genes involved in cellular detoxification process.

Materials and methods: A total of 25 adult Wistar male rats were randomly divided into five equal experimental groups after acclimation period (10 days). They received 0, 400, 800 and 1600 mg/kg/day MTBE in peanut oil by gavages for 30 consecutive days. The final group received no MTBE and peanut oil. After that the rats were euthanized and blood samples were collected for the assay of liver function indicators. Livers were immediately removed to determine the mRNA levels of three genes belonging to glutathione S-transferase family (*Gstt1*, *Gstm1*, and *Gstp1*).

Results: Statistical analysis showed that in the MTBE treated groups, serum albumin ($P = 0.007$) and total protein ($P = 0.002$) significantly increased, compared with the control groups. The other liver function indices and the mRNA levels of the examined genes did not show significant alteration in MTBE treated rats.

Conclusion: The present study revealed that exposure to MTBE has significant effect on the increasing of serum albumin and total protein, and it has no effect on the mRNA levels of the *Gstt1*, *Gstm1*, and *Gstp1* genes.

1. Introduction

Methyl-tertiary butyl ether (MTBE), a well known gasoline oxygenate, is added to gasoline in order to reduce the production of carbon monoxide and other pollutants in motor vehicle exhaust. MTBE is introduced in the early 1970s. Although it is

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banned in the USA, it is still used in Middle East countries. MTBE is rapidly and readily absorbed via inhalation and oral exposure, but at a modest rate through dermal exposure [1,2]. Most human exposure to MTBE occurs through air and drinking water [3]. Several studies indicate a relationship between MTBE and malignancies in animals [3–5], therefore, it has been listed as a potential human carcinogen.

After rats exposed to MTBE, it is distributed rapidly to all tissues with the largest percentage of initial body burden detected in the liver [3]. MTBE is metabolized in the liver by two cytochrome P-450 (CYP) isoenzymes, CYP2A6 and CYP2E1 [3,6]. It is well established that oxidative stress due to high production of reactive oxygen species (ROS) is involved in the etiology of toxicities of many xenobiotics. Based on the several studies, it is suggested that the toxicity of MTBE may be attributed to induction of oxidative stress [7–9].

Previous experiments mainly have been focused to investigate the effects of MTBE on liver biochemistry indicators such as hepatic enzymes and serum total protein; however, the results were not consistent [6,9,10]. The effects of MTBE on activity of phase II metabolic enzymes were examined. The studies also showed inconsistent results [6,11].

Alterations of several end organ markers such as liver function test indices, hematological indices and sex hormones in filling station workers and in residences of Masjid-i-Sulaiman (Khuzestan province, south-west Iran) who are living in contaminating areas have been reported [12–17]. It should be noted that alterations in the above mentioned indices, might be modulated by genetic polymorphisms of *GSTT1* and *GSTM1* [12,14,16,18]. Therefore it has been concluded that xenobiotics present in gasoline and natural sour gas might be metabolized by glutathione S-transferase gene family. Based on knowledge, there are no published data on effect(s) of exposure to MTBE and alteration in mRNA levels of antioxidant genes. For countries such as our country, where MTBE is added to gasoline, study of alteration of end organ markers and mRNA alterations due to MTBE exposure is potentially important for public health programs. Therefore, the present experiment study was carried out. Here we are going to investigate the alteration(s) of liver function indices and the mRNA levels of three members of GST gene family (*Gstm1*, *Gstp1*, *Gstt1*) in male Wistar rats treated with MTBE.

2. Materials and methods

2.1. Experimental design

A total 25 adult Wistar male rats (180–200 g) were purchased from the animal house of Shiraz University of Medical Sciences (Iran). Animals were housed in plastic cages under standard animal house conditions with a 12 h light/dark cycle and a temperature of 25 ± 2 °C, received standard pellet food, and tap water was available *ad libitum*. The experimental animals were randomly divided into five equal experimental groups after acclimation period (10 days). They received 0, 400, 800 and 1600 mg/kg/day MTBE in peanut oil (groundnut oil) by gavages (in total volume 500 µl) for 30 consecutive days. The final group received no MTBE and peanut oil. Body weights were measured every two days. MTBE CAS No. 1634-04-4 was obtained from Shiraz Oil Refinery (Iran) with 98.8% purity. None of the control or test-group animals died during the treatment. This study was approved by Ethics committee of Shiraz University. This work is carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving animal experiments.

2.2. Measurements

At the end of the exposure period, animals were anesthetized with ether and blood samples were obtained from heart. Livers were immediately removed and weighted and then were stored at −80 °C until use for determining gene expression. For measurements of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum total protein (TP) and serum albumin (ALB), blood samples were collected in 2 ml micro-centrifuge tubes and serum was separated by centrifugation at 3000 rpm for 10 min and stored at −20 °C until use. All measurements for hematological parameters (white blood count, red blood count, hematocrit, hemoglobin, platelets) were performed in one central laboratory according to standard hematological methods, by Coulter S (Biomedical).

2.3. RNA extraction and real-time PCR

Total RNA was extracted from liver by the TRIzol method using RNX plus (CinnaGen, Iran). To obtain suitable integrity of RNA, RNA concentration was measured using spectrophotometer. For cDNA synthesis briefly, 500 ng of RNA was reversely transcribed into cDNA according to the cDNA synthesis kit (Takara, Japan) in a final reaction volume of 10 µl using Oligo dT, 1.5× PrimeScript Buffer, Random hexamer and reverse transcriptase enzyme. Finally cDNA was stored at −80 °C for gene expression study.

Real-time quantitative PCR (qPCR) was done to detect the gene expression assay of *Gstm1*, *Gstp1* and *Gstt1* using SYBR Green Master Mix (Amplicon, Germany) on rotor gene 6000 detection system (Corbett Life Science, Germany). The cycling parameters for qPCR reaction of *Gstm1* and *Gstt1* were as follows: holding at 95 °C for 15 min, denaturation at 95 °C for 15 s, annealing and extension (two steps) at 57 °C for 45 s. The cycling parameters of *Gstp1* were as follows: holding at 95 °C for 15 min, denaturation at 95 °C for 20 s, annealing at 60 °C for 15 s and extension (three steps) at 72 °C for 20 s. The B_2m was used as internal control. The primers were designed for targeted genes using Allele ID software (version 7.5) and are listed in Table 1. Obtained results of gene expression were analyzed using the $2^{-\Delta\Delta ct}$ method.

2.4. Statistical analysis

Data were expressed as the mean ± standard error (SE). The significance of the difference between two control sets (not receiving peanut oil and receiving peanut oil) was evaluated with independent two samples *t*-test. Effects of MTBE on mean of measured variables were assessed using linear regression analysis. Statistical analysis was performed using SPSS statistical software package (version 11.5) for windows (SPSS Inc., Chicago, IL, USA). A two-tailed P value < 0.05 is considered to be statistically significant.

Table 1 The primers used for real-time PCR.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)
Gstm1	AATTGAGAAGACCACAGCG	AATTAAGTAGGGCAGATTGGG
Gstp1	CTGAGATACTTCATCGTCCAC	CATAAAGCCCTAAAGAGCGA
Gstt1	TGTGGCATAAGGTGATGTTC	GACGCCCTTCAAAGACTG
B_2m	CGTGCTTGCCATTCAGAAA	ATATACATCGGTCTCGGTGG

Table 2 Effects of MTBE on liver function indicators and GSTs mRNA levels.

Parameters	Control	Peanut oil	MTBE concentration (mg/kg/day)		
			400	800	1600
LFT indices					
ALT (U/L)	68.4 ± 6.8	77.6 ± 4.2	127.2 ± 58.0	114.6 ± 37.4	73.2 ± 5.5
AST (U/L)	195.4 ± 15.2	179.8 ± 7.9	305.5 ± 131.2	287.8 ± 11.1	174.4 ± 23.3
ALP (U/L)	924.0 ± 102.6	756.0 ± 141.3	522.5 ± 41.2	601.6 ± 58.0	519.0 ± 32.1
TP (g/dL)	5.20 ± 0.09	6.10 ± 0.12	7.02 ± 0.10	6.96 ± 0.15	6.94 ± 0.10
ALB (g/dL)	3.40 ± 0.04	2.82 ± 0.06	3.55 ± 0.06	3.48 ± 0.09	3.50 ± 0.05
mRNA levels					
Gstm1	1.0	0.91 ± 0.11	0.92 ± 0.15	0.78 ± 0.11	0.84 ± 0.11
Gstt1	1.0	0.82 ± 0.04	0.87 ± 0.07	0.80 ± 0.08	0.89 ± 0.05
Gstp1	1.0	0.89 ± 0.12	0.78 ± 0.12	0.83 ± 0.15	1.02 ± 0.11

3. Results

Table 2 shows the mean ± SE of liver function indices and mRNA levels of *Gstt1*, *Gstm1*, and *Gstp1* in the study groups. As mentioned in materials and methods section, in the experiments we used two groups as controls, one of them did not receive peanut oil and MTBE and the other one only received peanut oil. There were significant differences between the two control groups. In the group who received peanut oil, serum ALB ($t = 7.25$, df = 8, $P < 0.001$), and mRNA level of *Gstt1* ($t = 4.10$, df = 8, $P = 0.003$) were significantly decreased and TP ($t = 5.80$, df = 8, $P < 0.001$) was significantly increased in comparison with the other control group.

In order to control the possible confounding effect(s) of peanut oil on the study indices, we used liner regression analysis, using dosage of MTBE (at three levels) and consumption peanut oil (at two levels) as two independent variables. Table 3 shows the results of linear regression analysis. Statistical analysis revealed that in the MTBE treated groups, serum ALB ($P = 0.007$) and TP ($P = 0.002$) were significantly increased, compared with the control groups. The other liver function indices did not show significant differences between MTBE treated groups and the control groups. There was no significant correlation between treatment of MTBE and mRNA levels of the examined genes (Table 3).

Finally it should be noted that no adverse effects of treatment were reflected in liver weight, water consumption or food consumption (data not shown). Also there were no significant alterations in hematological parameters among different treatments (data not shown).

4. Discussion

Studies on alterations of liver function indices (including ALT, AST, ALP, TP, and ALB) after exposure to MTBE in animal models are limited and show inconsistent results [6,10,19–21]. Our present data indicate that serum ALB and TP were significantly increased in MTBE treated rats. Elevation of TP after exposure to MTBE was reported [20], which is confirmed by the present study. It should be noted that in filling station workers, which were occupationally exposed to gasoline, serum ALB and TP were significantly decreased [15]. We know that gasoline contain several components, whereas in the present study we used pure MTBE. The mentioned difference at least in part might be interpreted by this point. Very interestingly, here we found that peanut oil had effects on ALP, TP, ALB and mRNA level of *Gstt1* (Table 3). It is reported that peanut oil has effect on liver function indices [22,23]. It should be mentioned that researches used different oils for MTBE gavages. It is hypothesized that the confounding effects of used oil on the alterations of measured variables are one of the most important sources of heterogeneity between studies.

Similar to the present study, Zhou et al. (1999) reported that MTBE had no significant effect on the expression of *Gstp1* [24]. Our present study shows that there is no significant alteration in mRNA levels of *Gstt1* and *Gstm1* of the MTBE treated rats.

Earlier observations on the alterations of sex hormones, liver function indices, and hematologic parameters in people living in contaminated areas of Masjid-i-Sulaiman and gasoline filling station workers, might be modulated by genetic polymorphisms of *GSTT1* and *GSTM1* [12,15,16,18,25]. However, the present findings do not confirm the above-mentioned reports.

In conclusion, the present study finds that in rats exposed to MTBE, the serum total protein and serum albumin significantly were decreased. However, the MTBE treatment has no significant effect on mRNA levels of *Gstt1*, *Gstm1*, and *Gstp1* and also levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Other experiments should be carried out in order to explain the possible effect(s) of MTBE on end organ markers and also in expression levels of antioxidant genes.

Table 3 Standardized coefficients of independent variables (peanut oil and MTBE) on liver function indices and GSTs mRNA levels.

Parameters	Peanut oil			MTBE		
	Standardized coefficient	*t*	*P*	Standardized coefficient	*t*	*P*
LFT indices						
ALT	0.242	0.99	0.330	−0.411	−0.41	0.685
AST	0.140	0.57	0.575	−0.080	−0.32	0.747
ALP	−0.409	−2.10	0.048	−0.311	−1.59	0.125
TP	0.670	5.81	< 0.001	0.342	2.96	0.007
ALB	−0.427	−2.18	0.041	0.690	3.52	0.002
mRNA levels						
Gstm1	−0.159	−0.68	0.502	−0.144	−0.61	0.543
Gstt1	−0.499	−2.31	0.030	0.146	0.677	0.505
Gstp1	−0.299	−1.29	0.210	0.247	1.06	0.297

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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