ISSN: 2320-1983 Print ISSN: 2320-1991 Online Volume 3 Issue 4

Applied Cell Biology

Regular Paper

ACB, 3(4), 2014 [129-138]

Expression of key circadian genes as well as protein kinase NUAK2 as sensitive markers of *in vivo* toxic action of methyl tertial butyl ether

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ABSTRACT

Circadian regulatory factors are the molecular components of biological clock, which play an important role in the regulation of most physiological and metabolic processes in the organism. We studied the effect of methyl tert-butyl etheron the expression level of PER1, ARNTL, CLOCK, and CSNK1E mRNA, which are the most important factors of the circadian clock, as well as protein kinase NUAK2. It was shown that the expression level of all these genes is significantly increased in lung and liver of rats, treated for the period of one month with the maximum permissible dose of methyl tert-butyl ether. Results of this investigation demonstrate that low dose of methyl tert-butyl ether affects the expression level of NUAK2,*CSNK1E, PER1, BMAL1*, and *CLOCK*genes. This could be important sensitive markers oftoxic action on the organism of methyl tert-butyl ether and possibly otherecologically dangerous chemical compounds.

KEYWORDS

Methyl tert-butyl ether;
Gene expressions;
CSNK1E;
NUAK2;
Circadian genes;
Lung;
Liver;
Rats.

INTRODUCTION

Methyl tert-butyl ether (2-methoxy-2-methyl-propane, MTBE) is environmentally dangerous chemical compound that is used to produce leaded gasoline. It was found that the methyl tert-butyl ether is one of the most dangerous global chemical pollutants in the environment due to its high stability, the ability to accumulate in the soil and underground water sources and a pronounced negative impact on human health[1-3]. Contamination of the environment by this compound can poison people and initiate the development of a number of pathological conditions: chronic kidney disease, liver hypertrophy, allergic and respiratory diseases, neurotoxic manifestations, including the occurrence of malignant tumors in the kidney, liver and testes as well as initiate development of leukemia[1,4,5]. Thus, the introduction of methyl tert-butyl ether into gasoline provides strong and unquestionable evidence that gasoline containing methyl tert-butyl ether is associated with human illnesses[6]. Recent studies have confirmed the carcinogenic effects of this agent and have identified additional

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sites of tumor induction (i.e., brain)[7].

In experiments on two species of fish it was shown that short-term exposure of fish in water with methyl tert-butyl ether or tert-butanol leads to deformations of the eyes and mouth and to increased mortality of larvae[8]. MTBE altering the expression of vascular endothelial growth factor-A (VEGFA) and VEGFC mRNA as well as transcript levels of genes required for general development[9,10]. The major methyl tert-butyl ether metabolites in the blood are methanol and tret-butyl alcohol, whereas in the liver MTBE transforms by cytochrome P-450 2A6 to formaldehyde and tret-butanol, but the main place of accumulation of methyl tert-butyl ether is adipose tissue and to a lesser extent, blood and brain[1,5,11-13]. Recently has been shown that exposure of Wistar rats to MTBE in ~~the~~ drinking water resulted in renal changes and in an exacerbation of chronic progressive nephropathy at the end of 1 year of exposure[14]. However, the detailed mechanisms of toxicity of methyl tert-butyl ether that initiate the development of various diseases, including cancer, are not understood yet.

We have previously found that intra-gastric administration of different doses of methyl tert-butyl ether to rats within 1-2 months leads to significant disturbances in the expression of circadian genes, protein kinases and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB). Furthermore, it affects alternative splicing of PFKFB3, PFKFB4 and vascular endothelial growth factor-A (VEGFA). There is a clear indication of high sensitivity of the protein kinase SNARK, casein kinase-1epsilon and circadian gene expressions to the toxic action of this chemical compound[15-17]. An urgent question arose about the possibility of what might be the repercussions, that have previously gone unnoticed, of exposure tothe maximum permissible dose of methyl tert-butyl ether (100 mg in 1 cubic meter), which is accepted in Ukraine and mostly relates to workers of plants that produce MTBE.

In this context, we studied the effects of MTBE existing in the air on the expression of genes which encode for key circadian factors and protein kinases, which were previously affected by intra-gastric administration MTBE in different doses. Circadian rhythms govern fundamental physiological functions in almost all organisms and are closely related to metabolic homeostasis at both the behavioral and molecular levels[18–24].

Circadian factors ARNTL and CLOCK are transcription factors that form transcriptionally active complex by heterodimerization, which is able to bind to *cis*-acting element in the promoter of various target genes, including *PER1*, *PER2*, *PER3*, *CRY1* and *CRY2*, as well as enhance transcription of *ARNTL* gene, generating positive loop of the biological clock. On the other hand, complex CRY-PER creates a negative loop interactions clock by inhibiting transcriptional activity of the heterodimer complex CLOCK/BMAL1. Another negative loop interaction of circadian clock factors generates REV-ERBA that inhibits the activity of the transcriptional complex CLOCK/BMAL in the nucleus[25–27]. Thus, the feedback mechanisms in the regulation of circadian factors in mammalian are essential for accurate and precise work of circadian clock[28–30]. Moreover, a disruption of the regulation of circadian genes and processes in the body is one of the causes of a number of pathological processes, including malignant tumors[31–33].

The expression and function of most circadian factors are under the control of protein kinases, particularly casein kinase-1epsilon (CSNK1E) and casein kinase-1delta (CSNK1D); these kinases also participate in the regulation of several other extremely important processes[34–39]. Thus, CSNK1 regulates each component of the circadian negative-feedback loop, especially PER proteins, because phosphorylation of PER1 and PER3 proteins results in their rapid degradation. Moreover, CSNKIE and CSNK1D are able to induce nuclear translocation of PER3 as well as affect the inhibitory effect of PER proteins on the transcriptional activity of ARNTL-CLOCK[37].

The sucrose-non-fermenting protein kinase (SNF1)/AMP-activated protein kinase-related kinase (SNF1/AMP-activated protein kinase; SNARK) is a member of AMPK kinases (NUAK family SNF1-like kinase 2; NUAK2). It is related to serine/threonine protein kinases[40]. This protein kinase is a molecular component of the cellular stress response and is commonly activated in response to cellular and environmental stresses and is an important regulator of whole-body metabolism[41]. Moreover, protein kinase NUAK2 is consistently localized in the nuclei and itsdeficiency contributed to the early phase of tumorigenesis via obesity-dependent and -independent mechanisms[42,43].

The aim of this study was to learn the possible ef-

fect of maximum permissible dose of environmentally hazardous chemical compound of methyl tert-butyl ether on living organisms through investigation of the expression of protein kinase NUAK2 andCSNK1E as well askey circadian genes in rat lung and liver after prolonged (a month) treatment of rats by this chemical compound existing in air.

MATERIALS AND METHODS

Animals and treatment

Experiments were performed on male Wistar rats, body weight 230 - 250 g. The animals were fed a standard rat chow diet and had *ad libitum* access to water. Eight animals were divided into two equal groups: 4 control rats and 4 rats treated with methyl tert-butyl ether. For treatment by MTBE the rats were kept in a chamber with accepted in Ukraine the maximum permissible dose of this chemical compound (100 mg in 1 cubic meter) for 4 hours a day, five times a week for four weeks.

The experiments were performed in accordance with regulations specified by The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) and the Palladin Institute of Biochemistry Protocol for Animal Studies.

Isolation of RNA

Total RNAs were isolated from rat liver and lung using Trizol reagent (Invitrogen, USA) as described previously[44] and precipitated from aqueous phase by equal volume of 2-propanol. RNA pellets washed twice with 75% ethanol, dissolved in ribonuclease free water, re-precipitated with ethanol, dissolved again in ribonuclease free water and used for the synthesis of complementary DNA (cDNA).

Gene expressions

The expression of NUAK2, CSNK1E, PER1, BMAL1 and CLOCK mRNA in rat liver and lung was investigated by polymerase chain reaction (PCR) as well as by quantitative PCR. Synthesis of cDNA was performed using QuaniTect Reverse Transcription Kit (QIAGEN, Germany) according to manufacturer's protocol, using total RNA from rat lung and liver. For amplification of NUAK2, CSNK1E, PER1, ARNTL, and CLOCK cDNA used HotStarTaq Master Mix Kit (QIAGEN, Germany) and specific for these genes primers. Quantitative polymerase chain reaction was performed using "Mx 3000P QPCR"(Stratagene, USA) and ABsolute qPCR SYBR Green Mix (Thermo Scientific, UK). The expression of beta-actin mRNA was used as control of analyzed RNA. The primers were received from the Sigma-Aldrich, USA.

Amplification of protein kinase NUAK2 (SNF1/AMP-activated protein kinase; SNARK) cDNA was performed using the following primers: direct - 5'-AAGTCTCGGCAGCGTGAATC -3 'and reverse 5'-CAGGATGCTGTCCTCACTCA -3'. The nucleotide sequences of these primers correspond to sequences of nucleotides 1544 – 1564 and 1737 – 1718, respectively, of rat NUAK2 mRNA (GenBank number NM_001007617). For amplification of casein kinase-1epsilon (CSNK1E) cDNA were used next primers: forward (5'-GACATCTACCTGGGTGCCAAC -3') and reverse (5'-TGATCATCTGGTCGGCCAGC -3'). Nucleotide residues of these primers correspond to nucleotide sequences 64 – 84 and 340 – 321 of the rat CSNK1E mRNA (GenBank number NM_031617). Amplification of cDNA circadian factor PER1 was performed using the following primers: forward - 5'-TCTCTTCTCAGAACTGGATG -3' and reverse 5'-GGAAGCCTCTCATTAGACTGC -3', nucleotide sequences which correspond to residues of nucleotides 3699 – 3718 and 3983 – 3963, respectively, of rat PER1 mRNA (GenBank number NM_001034125). For amplification of brain and muscle ARNT-like protein (ARNTL or BMAL1) cDNA were used forward (5'-TGACCCTCATGGAAGGTTAG -3 ') and reverse (5'-AATCCATCTGCTGCCCTGAG -3') primers. Nucleotide residues of these primers correspond to nucleotide residues 753 – 772 and 1042 – 1061 of rat ARNTL mRNA (GenBank number NM_024362). For amplification of circadian locomotor output cycles kaput (CLOCK) cDNA were used next primers: forward (5'-TGCACAGTCAGATGCTAGTG -3 ') and reverse (5'-TGATCCACAAGATCAGATGG -3'). Nucleotide residues of these primers correspond to nucleotide residues 264 – 283 and 455 – 436 in the sequence of rat CLOCK mRNA (GenBank number NM_021856). The amplification of beta-actin (ACTB) cDNA was performed using forward - 5'– GGACTTCGAGCAAGAGATGG –3' and reverse - 5'– AGCACTGTGTTGGCGTACAG –3' primers.

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These primers nucleotide sequences correspond to 747 – 766 and 980 – 961 of human *ACTB* cDNA (GenBank accession number NM_001101).

The same pair of primers was also used for amplification in the quantitative polymerase chain reaction. An analysis of quantitative PCR was performed using special computer program "Differential expression calculator". The values of NUAK2, CSNK1E, PER1, BMAL1, and CLOCK mRNA expressions were normalized to the expression of beta-actin mRNA and represent as percent of control 1 (100 %).

Amplification products were analyzed by electrophoresis in 2% agarose gels. Gels were analyzed in the Quantity One BioRad System (U.S.A.).

Statistical analysis

Statistical analyses were performed according to Student's *t*-test using OriginPro 7.5 software. All values are expressed as mean ± SEM from fourindependent experiments; $p < 0.05$ was considered as significant difference.

RESULTS AND DISCUSSION

We investigated the mRNA expression of key circadian genes and casein kinase-1epsilon as well as protein kinase NUAK2 in the lung and liver of rats in normal conditions and after prolonged exposure of animals to low doses of methyl tert-butyl ether in air. The data of protein kinase NUAK2 mRNA expression is presented in Figure 1. It was found that the expression level of protein kinase NUAK2 mRNA almost doubles in the lung and liver of rats under the influence of maximum permissible dose methyl tert-butyl ether. This data is consistent with that obtained earlier on the increased expression of this protein kinase under the influence of small doses of methyl tert-butyl ether under its internal gastric administration to rats[17].

NUAK2 is an important regulator of whole-body metabolismas a molecular component of the cellular stress response andcommonly activated in response to cellular and environmental stresses[41]. Moreover, the overexpression of protein kinase NUAK2 in human liver hepatoma cells results in the up-regulation (more than 2.0-fold) of 76 mRNA targets and down-regulation (more than 2.0-fold) of 32 mRNA targets, suggesting that this protein kinase can work as a stress-responsive

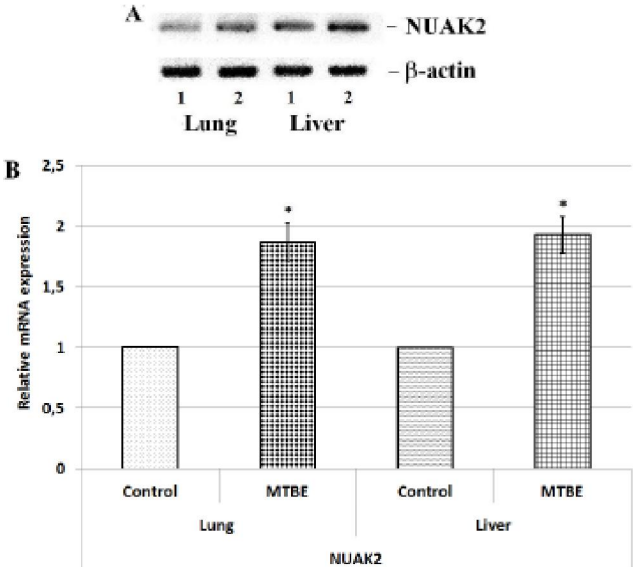

Figure 1 : The expression of SNF1/AMP-activated protein kinase (NUAK2) mRNA in the lung and liver of rats treated for the period of one month with the maximum permissible dose of MTBE measured by RT/PCR (A) and qPCR (B). 1 - Control animals; B – MTBE treated rats. The level of NUAK2 mRNA expression was normalized to beta-actin mRNA expression and represent as percent of control (100 %); mean ± SEM; *n* = 4; * – P < 0.05 as compared to control.

transcriptional modulator in the nucleus[43]. Thus, the nuclear localizing NUAK2 alters transcriptomeprofiles and a considerable part of these alterations were cancelled by the mutation of nuclear localization signal. It is important to note, that the activity of protein kinase NUAK2 changes under various stress conditions cells, but not in all cell types, and strongly depends on the levels of glucose and glutamine in the cells. It therefore represents an NF-κB-regulated anti-apoptotic gene that contributes to the tumour-promoting activity of death receptor CD95 in apoptosis-resistant tumour cells and plays an important role in cancer development and tumour progression[45].

Thus, the increased expression of protein kinase NUAK2 can be a result of stress, induced by MTBE treatment, as well as can be responsible for some negative effects of prolonged action low dose of this chemical compound. This data argues with several *in vitro* studies that metabolic stresses as well as genotoxic or environmental stresses induce NUAK2 activation; however, the physiological roles of this protein kinase remain uncertain yet[40,41]. Interestingly, NUAK2(+/-) mice exhibited mature-onset obesity and related metabolic disorders[41]. It is possible that dysregulation of this important gene expression is responsible for variable meta-

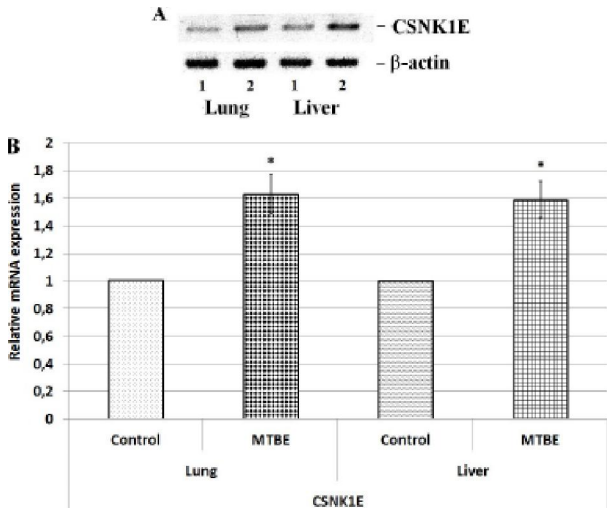

Figure 2 : The expression of casein kinase-1epsilon (CSNK1E) mRNA in the lung and liver of rats treated for the period of one month with the maximum permissible dose of MTBE measured by RT/PCR (A) and qPCR (B). 1 - Control animals; B – MTBE treated rats. The level of CSNK1E mRNA expression was normalized to beta-actin mRNA expression and represent as percent of control (100 %); mean ± SEM; *n* = 4; * – P < 0.05 as compared to control.

bolic disturbances, including circadian clock function, especially in response to the action of toxic compounds.

As seen from the data shown in Figure 2, the expression of another protein kinase, casein kinase-1epsilon, also significantly increased in the lung and liver of rats exposed to the maximum allowable dose of MTBE. Thus, quantitative PCR analysis shown that CSNK1E mRNA expression is increased in the lung and liver of MTBE treated rats by 64 % and 57 %, correspondingly, as compared to control animals. This protein kinase as well as casein kinase-1delta is very important in regulation of the expression and function of most circadian factors but these protein kinases also participate in the regulation of several other extremely important processes[34,36-39]. Thus, it was found that casein kinase-1epsilon bound to PER1, PER2 PER3 and phosphorylates them that significantly alters the function of genes that control cell division cycle (Cyclin D1, Cyclin A, MDM2, cMYC and GADD45alpha) and a number of oncogenes and well as tumor suppressor genes[37,46-49]. This protein kinase also takes part in the destabilization of β-catenin-degrading complex[48], in the functioning of TGF-β signaling cascade[50], the inactivation of protein BID by caspase cleavage of its[51], phosphorylates TP53[55], negatively regulates AKT phosphorylation through PTEN[37]. In this reason, the increased expression level of CSNK1E in the lung and liver of treated by methyl tert-butyl ether animals can participate in the developing of negative effect induced by this chemical compound.

The data of circadian transcription factor ARNTLalso known as BMAL1 (brain and muscle ARNT-like protein)mRNA expression is presented in Figure 3. It was found that the expression level of this circadian geneis increased in the lung (by 73 %) and liver (by 91 %) of rats under the influence of maximum permissible dose methyl tert-butyl ether (Figure 3B).

The investigation of the expression of another circadian transcription factor CLOCK (circadian locomotor output cycles kaput) showed that in control rats and in animals that treated with methyl tert-butyl ether

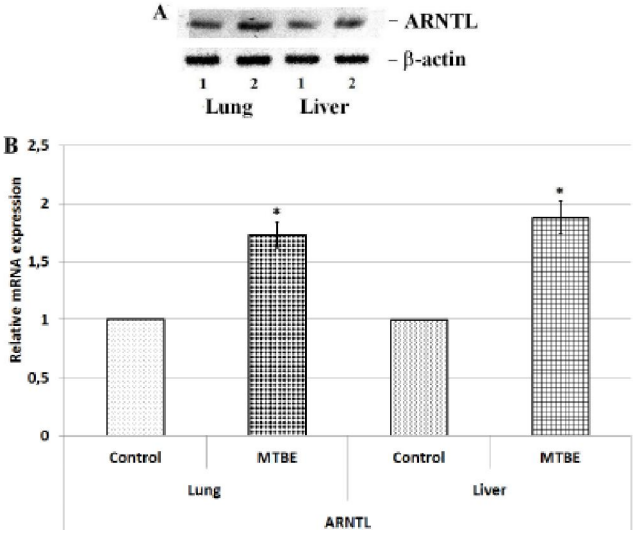

Figure 3 : The expression of brain and muscle ARNT-like protein (BMAL1 or ARNTL) mRNA in the lung and liver of rats treated for the period of one month with the maximum permissible dose of MTBE measured by RT/PCR (A) and qPCR (B). 1 - Control animals; B – MTBE treated rats. The level of ARNTL mRNA expression was normalized to beta-actin mRNA and represent as percent of control (100 %); mean ± SEM; *n* = 4; * – P < 0.05 as compared to control.

there are two isoforms of this transcription factor and that the expression level of both isoforms increases under the influence of this toxic chemical compounds both in the lung and liver (Figure 4A). Using quantitative PCR, we have shown that under the influence of maximum permissible doses of methyl tert-butyl ether the expression level of CLOCK mRNA was increased in the lung to a much greater extent than in the liver: in the lung an increase of 125%, while in liver only 62% (Figure 4B).

It is important to note that the increased level of CLOCK mRNA expression was earlier established by us under prolonged exposure to low doses of methyl

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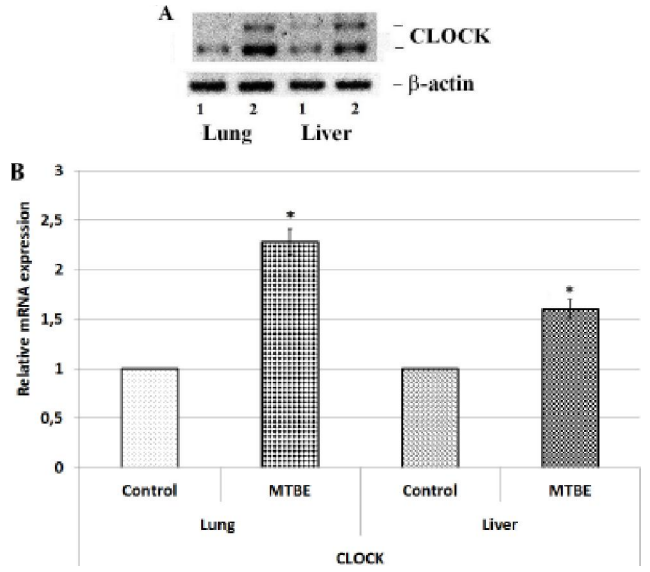

Figure 4 : The expression of brain and muscle ARNT-like protein (BMAL1orARNTL) mRNA in the lung and liver of rats treated for the period of one month with the maximum permissible dose of MTBE measured by RT/PCR (A) and qPCR (B). 1 - Control animals; B – MTBE treated rats. The level of ARNTL mRNA expression was normalized to beta-actin mRNA and represent as percent of control (100 %); mean ± SEM; *n* = 4; * – P < 0.05 as compared to control.

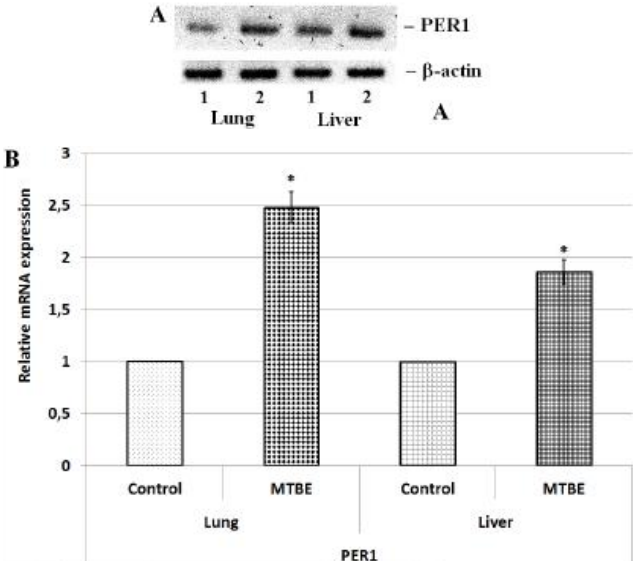

Figure 5 : The expression of circadian protein period 1 (PER1) mRNA in the lung and liver of rats treated for the period of one month with the maximum permissible dose of MTBE measured by RT/PCR (A) and qPCR (B). 1 - Control animals; B – MTBE treated rats. The level of PER1 mRNA expression was normalized to beta-actin mRNA and represent as percent of control (100 %); mean ± SEM; *n* = 4; * – P < 0.05 as compared to control.

tert-butyl ether under its internal gastric administration to rats[15]. Moreover, the intra-gastric treatment of rats with small dose of methyl tert-butyl ether leads to increase of casein-1epsilon mRNA expression level only in the liver and ARNTL – only in the lung[17].

As shown in Figure 5, the expression level of circadian factor PER1 was also increased in the level of mRNA both in the lung and liver under the action of methyl tert-butyl ether, but in the lung effect of this toxic chemical compounds was significantly higher. Thus, under the influence of maximum permissible doses of methyl tert-butyl ether the expression level of PER1 mRNA was increased in the lung by 146 %, but in the liver only by 85 %, compared with control animals.

Thus, increased expression of PER1, CLOCK, and ARNTL mRNA levels in both lung and liver of MTBE treated rats are correlated to enhanced expression of CSNK1E mRNA. There is data that phosphorylation of PER1 protein by CSNK1 results in their rapid degradation, which is dependent on the ubiquitin-proteasome pathway[37]. At the same time, MTBE treatment modified the phosphorylation of PER1 and some other proteins and its functional activity as well as stability. Moreover, CSNKIE and CSNK1D are able to affect the inhibitory effect of PER proteins on the transcriptional activity of ARNTL-CLOCK[37].

Previously, we have shown that hypoxia stabilizes HIF-1alpha protein through the blockade its ubiquitination, while significantly decreasing the expression level of its mRNA[53-55]. Therefore, for this regulatory protein we have negative correlation between mRNA and protein levels.

The above data clearly shown that the expression of protein kinase NUAK2, whichis a molecular component of the cellular stress response and commonly activated in response to environmental stresses[41], CSNK1E and key circadian factors is affected by air treatment with methyl tert-butyl ether.

A number of studies have shown that abnormalities in the regulation of circadian gene expressions occur in some diseases and may also be implicated in the emergence and progression of malignant tumors[31,45,56-65]. It has been also discovered that the expression of several circadian genes depends on hypoxia[66], which can also significantly contribute to the progression of most tumors by disrupting the function of biological clock.

Thus, the results of this work indicates a pronounced effect of the maximum permissible dose of methyl tert-butyl ether on the process of gene expression of extremely important protein kinases and circadian factors, which are responsible for the regulation of basic

metabolic processes, in the lung and liver. It is possible that changes in the expression level of NUAK2, CSNK1E, and different circadian factor mRNAs upon MTBE treatment can indicate, at least in part, the biological clock dysfunction and initiate some metabolic abnormalities in the body, which was shown by numerous studies[4,6,7,9,10,13,14].

Moreover, these results also indicate the need for further research to elucidate the detailed molecular mechanism ofpossible toxic action of methyl tert-butyl ether and find ways to neutralize their negative effects on the body as well as to reevaluate the maximum permissible dose of this compound. It is possible that the normal biological clock function dysregulation and associated signaling pathways in cells is one of the mechanisms of methyl tert-butyl ether action on the body.

CONCLUSIONS

Prolonged treatment of rats for one month with the maximum permissible dose of methyl tert-butyl ether increases the protein kinase NUAK2 and CSNK1E mRNA expression in the lung and liver. Moreover, the expression level of key circadian factors also significantly increased in the lung and liver of rats treated with methyl tert-butyl ether. This dysregulation of the circadian clockgenes as well as protein kinase genes can possibly disrupt the regulation of basic metabolic processes in the body and maybe contributes to the development of various pathological conditions. Thus, the expression of *NUAK2*, *CSNK1E*, *PER1*, *ARNTL* and *CLOCK* genes can be important sensitive markers of possible harmful effects of very low doses of MTBE and maybe many others environmental chemical pollutants. More definitive studies are needed to understand the mechanisms by which MTBE may pose health and environmental impacts. The scientific value of this study is that prolonged (a month) treatment of rats by the maximum permissible dose of MTBE existing in air affects the expression of protein kinase NUAK2 and CSNK1E as well as key circadian genes in rat lung and liver after and that this permissible dose of MTBE should be reevaluated.

REFERENCES

[1] D.E.Hutcheon, J.D.Arnold, W.Hove, J.3rdBoyle; Disposition, metabolism, and toxicity of methyl tertiary butyl ether, an oxygenate for reformulated gasoline.J.Toxicol.Environ.Health, **47**, 453–464 **(1996)**.

[2] D.McGregor; Ethyl tertiary-butyl ether: a toxicological review.Crit. Rev.Toxicol., **37**, 287–312 **(2007)**.

[3] O.H.Minchenko, O.P.Yavorovsky, Y.O.Paustovsky, D.O.Minchenko, I.V.Zavgorodny; Circadian genes as sensitive markers of bioinsecurity. Environment & Health, **48**, 10-17 **(2007)**.

[4] F.E.Ahmed; Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. Toxicol.Lett., **123**, 89-113 **(2001)**.

[5] D.Mc Gregor; Methyl tertiary-butyl ether: studies for potential human health hazards. Crit.Rev.Toxicol., **36**, 319 –358 **(2006)**.

[6] M.A.Mehlman; Dangerous and cancer-causing properties of products and chemicals in the oil-refining and petrochemical industry—Part XXII: Health hazards from exposure to gasoline containing methyl tertiary butyl ether: Study of New Jersey residents. Toxicol. Ind. Health, **12**, 613–627 **(1996)**.

[7] K.M.Burns, R.L.Melnick; MTBE: Recent carcinogenicity studies. Int.J.Occup.Environ.Health, **18**, 66–69 **(2012)**.

[8] D.Moreels, P.Lodewijks, H.Zegers, E.Rurangwa, N.Vromant, L.Bastiaens, L.Diels, D.Springael, R.Merckx, F.Ollevier; Effect of short-term exposure to methyl-tert-butyl ether and tert-butyl alcohol on the hatch rate and development of the African catfish, Clariasgariepinus. Environ. Toxicol. Chem., **25**, 514 –519 **(2006)**.

[9] J.A.Bonventre, L.A.White, K.R.Cooper; Methyl tert butyl ether targets developing vasculature in zebrafish (Daniorerio) embryos. Aquat.Toxicol., **105**, 29-40 **(2011)**.

[10] J.A.Bonventre, L.A.White, K.R.Cooper; Craniofacial abnormalities and altered wnt and mmp mRNA expression in zebrafish embryos exposed to gasoline oxygenates ETBE and TAME. Aquat.Toxicol., **120-121**, 45–53 **(2012)**.

[11] J.J.Clary; Methyl tert butyl ether systemic toxicity. Risk Anal, **17**, 661–672 **(1997)**.

[12] M.Imbriani, S.Ghittori, G.Pezzagno; Partition coefficients of methyl tert-butyl ether (MTBE). G.Ital.Med.Lav.Ergon., **19**, 63–65 **(1997)**.

[13] M.Shamsipur, A.A.MiranBeigi, M.Teymouri, T.Poursaberi, S.M.Mostafavi, P.Soleimani, F.Chitsazian, S.A.Tash; Biotransformation of me-

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thyl tert-butyl ether by human cytochrome P450 2A6. Biodegradation, **23**, 311–318 **(2012)**.

[14] E.Bermudez, G.Willson, H.Parkinson, D.Dodd; Toxicity of methyl tertiary-butyl ether (MTBE) following exposure of Wistar Rats for 13 weeks or one year via drinking water. J.Appl.Toxicol., **32**, 687–706 **(2013)**.

[15] D.O.Minchenko, O.P.Yavorovsky, I.V.Zavgorodny, Y.O.Paustovsky, K.Tsuchihara, H.Esumi, O.H.Minchenko; Experimental data on disorders of expression of circadian genes in liver and lung as a marker of methyl-tretbutyl ether toxic effect on the body. Ukr.J.Occupat.Health, **15(3)**, 20-26 **(2008)**.

[16] D.O.Minchenko, V.G.Mykhalchenko, K.Tsuchihara, S. Kanehara, O.P.Yavorovsky, I.V.Zavgorodny, Y.O.Paustovsky, S.V.Komisarenko, H.Esumi, O.H.Minchenko; Unique alternative splice variants of rat 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 mRNA. Ukr.Biokhim.Zh., **80(4)**, 66–73 **(2008)**.

[17] O.H.Minchenko, D.O.Minchenko, O.P.Yavorovsky, I.V.Zavgorodny, Y.O.Paustovsky, K.Tsuchihara, H.Esumi; Expression ofcasein kinase-1e and SNARK in rat liver, lung and myocard as marker of methyl-tertbutyl ether action on laboratory animals. Sci. Bull.Nat.BogomoletsMed.Univ., **18(2)**, 21-27 **(2008)**.

[18] J.Kovac, J.Husse, H.Oster; A time to fast, a time to feast: the crosstalk between metabolism and the circadian clock. Mol.Cells, **28**, 75-80 **(2009)**.

[19] M.H.Hastings, E.S.Maywood, A.B.Reddy; Two decades of circadian time. J.Neuroendocrinol., **20**, 812–819 **(2008)**.

[20] L.L.Karbovskyi, D.O.Minchenko, Y.A.Harmash, O.H.Minchenko; Molecular mechanisms of circadian clock function. Ukr.Biokhim.Zh., **83(1)**, 5–25 **(2010)**.

[21] M.S.Bray, M.E.Young; Regulation of fatty acid metabolism by cell autonomous circadian clocks: time to fatten up on information? J.Biol.Chem., **286**, 11883–11889 **(2011)**.

[22] C.Crumbley, T.P.Burris; Direct regulation of CLOCK expression by REV-ERB. PLoS ONE, **6**, e17290 **(2011)**.

[23] W.Huang, K.M.Ramsey, B.Marcheva, J.Bass; Circadian rhythms, sleep, and metabolism. J.Clin.Invest., **121**, 2133–2141 **(2011)**.

[24] S.Shimba, T.Ogawa, S.Hitosugi, Y.Ichihashi, Y.Nakadaira, M.Kobayashi, M.Tezuka, Y.Kosuge, K.Ishige, Y.Ito, K.Komiyama, Y.Okamatsu-Ogura, K.Kimura, M.Saito; Deficient of a clock gene, brain and muscle Arnt-like protein-1 (BMAL1), induces dyslipidemia and ectopic fat formation. PLoS One, **6**, e25231 **(2011)**.

[25] A.Bugge, D.Feng, L.J.Everett,E.R.Briggs, S.E.Mullican, F.Wang, J.Jager, M.A.Lazar; Rev-erbα and Rev-erbβ coordinately protect the circadian clock and normal metabolic function. Genes Dev., **26**, 657–667 **(2012)**.

[26] P.Emery, S.M.Reppert; A rhythmic Ror., Neuron., **43**, 443-446 **(2004)**.

[27] A.C.Liu, H.G.Tran; Redundant function of REV-ERBalpha and beta and non-essential role for Bmal1 cycling in transcriptional regulation of intracellular circadian rhythms, PLoSGenet, **4**, e1000023 **(2008)**.

[28] K.Oishi, H.Shirai, N.Ishida; CLOCK is involved in the circadian transactivation of peroxisome-proliferator-activated receptor alpha (PPARalpha) in mice. Biochem.J., **386**, 575–581 **(2005)**.

[29] T.K.Sato, R.G.Yamada, H.Ukai, J.E.Baggs, L.J.Miraglia, T.J.Kobayashi, D.K.Welsh, S.A.Kay, H.R.Ueda, J.B.Hogenesch; Feedback repression is required for mammalian circadian clock function. Nat. Genet., **38**, 312–319 **(2006)**.

[30] D.Motzkus, S.Loumi, C.Cadenas, C.Vinson, W.G.Forssmann, E.Maronde; Activation of human period-1 by PKA or CLOCK/BMAL1 is conferred by separate signal transduction pathways. Chronobiol.Int., **24**, 783–792 **(2007)**.

[31] S.T.Chen, K.B.Choo, M.F.Hou,K.T.Yeh, S.J.Kuo, J.G.Chang; Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis, **26**, 1241–1246 **(2005)**.

[32] Z.L.Zeng, M.W.Wu, J.Sun, Y.L.Sun, Y.C.Cai, Y.J.Huang, L.J.Xian; Effects of the biological clock gene Bmal1 on tumour growth and anti-cancer drug activity. J.Biochem., **148**, 319-326 **(2010)**.

[33] L.Xiao, A.K.Chang, M.X.Zang, H.Bi, S.Li, M.Wang, X.Xing, H.Wu; Induction of the CLOCK gene by E2-ERα signaling promotes the proliferation of breast cancer cells, PLoS One, **9**, e95878 **(2014)**.

[34] E.J.Eide, E.L.Vielhaber, W.A.Hinz, D.M.Virshup; The circadian regulatory proteins BMAL1 and cryptochromes are substrates of casein kinase Iepsilon. J.Biol.Chem., **277**, 17248–17254 **(2002)**.

[35] E.J.Eide, M.F.Woolf, H.Kang; Control of mammalian circadian rhythm by CKIepsilon-regulated proteasome-mediated PER2 degradation. Mol.Cell.Biol., **25**, 2795–2807 **(2005)**.

[36] G.A.Keesler, F.Camacho, Y.Guo,D.Virshup, C.Mondadori, Z.Yao; Phosphorylation and destabilization of human period I clock protein by human

casein kinase I epsilon. Neuroreport, **11**, 951–955 **(2000)**.

[37] M.Akashi, Y.Tsuchiya, T.Yoshino, E.Nishida; Control of intracellular dynamics of mammalian period proteins by casein kinase I epsilon (CKIepsilon) and CKIdelta in cultured cells. Mol.Cell.Biol., **22**, 1693–1703 **(2002)**.

[38] A.Okamura, N.Iwata, A.Tamekane,K.Yakushijin, S.Nishikawa, M.Hamaguchi, C.Fukui, K.Yamamoto, T.Matsui; Casein kinase Iepsilon down-regulates phospho-Akt via PTEN, following genotoxic stress-induced apoptosis in hematopoietic cells. Life Sci., **78**, 1624–1629 **(2006)**.

[39] T.Shirogane, J.Jin, X.L.Ang, J.W.Harper; SCFbeta-TRCP controls clock-dependent transcription via casein kinase 1-dependent degradation of the mammalian period-1 (Per1) protein. J.Biol.Chem., **280**, 26863–26872 **(2005)**.

[40] D.O.Minchenko, O.H.Minchenko; SNF1/AMP-activatedprotein kinases: genes, expression and biological role. In: Protein Kinases. Book 1, Dr. Gabriela Da Silva Xavier (Ed.). InTech., 41-62 **(2012)**.

[41] D.L.Lefebvre, C.F.Rosen; Regulation of SNARK activity in response to cellular stresses. Biochim.Biophys.Acta, **1724**, 71–85 **(2005)**.

[42] K.Tsuchihara, T.Ogura, R.Fujioka, S.Fujii, W.Kuga, M.Saito, T.Ochiya, A.Ochiai, H.Esumi; Susceptibility of Snark-deficient mice to azoxymethane-induced colorectal tumorigenesis and the formation of aberrant crypt foci. Cancer Sci., **99**, 677– 682 **(2008)**.

[43] W.Kuga, K.Tsuchihara, T.Ogura, S.Kanehara, M.Saito, A.Suzuki, H.Esumi; Nuclear localization of SNARK; its impact on gene expression. Biochem.Biophys.Res.Commun., **377**, 1062–1066 **(2008)**.

[44] O.H.Minchenko, I.L.Opentanova, D.O.Minchenko, T.Ogura, H.Esumi; Hypoxia induces transcription of 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 4 gene via hypoxia-inducible factor-1alpha activation. FEBS Lett., **576**, 14–20 **(2004)**.

[45] P.Legembre, R.Schickel, B.C.Barnhart, M.E.Peter; Identification of SNF1/AMP kinase-related kinase as an NF-kappaB-regulated anti-apoptotic kinase involved in CD95-induced motility and invasiveness. J.Biol.Chem., **279**, 46742–46747 **(2004)**.

[46] A.Gréchez-Cassiau, B.Rayet, F.Guillaumond, F.Guillaumond, M.Teboul, F.Delaunay; The circadian clock component bmal1 is a critical regulator of p21waf1/cip1 expression and hepatocyte proliferation. J.Biol.Chem., **283**, 4535-4542 **(2008)**.

[47] K.Miyazaki, T.Nagase, M. Mesaki, J.Narukawa, O.Ohara, N.Ishida; Phosphorylation of clock protein PER1 regulates its circadian degradation in normal human fibroblasts. Biochem.J., **380**, 95–103 **(2004)**.

[48] Q.Cao, S.Gery, A.Dashti, D.Yin, Y.Zhou, J.Gu, H.P.Koeffler; A role for the clock gene per1 in prostate cancer. Cancer Res., **69**, 7619–7625 **(2009)**.

[49] E.Vielhaber, E.Eide, A.Rivers, Z.H.Gao, D.M.Virshup; Nuclear entry of the circadian regulator mPER1 is controlled by mammalian casein kinase I epsilon. Mol.Cell.Biol., **20**, 4888–4899 **(2000)**.

[50] D.S.Waddell, N.T.Liberati, X.Guo, J.P.Frederick, X.F.Wang; Casein kinase Iepsilon plays a functional role in the transforming growth factor-beta signaling pathway. J.Biol.Chem., **279**, 29236–29246 **(2004)**.

[51] S.Desagher, A.Osen-Sand, S.Montessuit, E.Magnenat, F.Vilbois, A.Hochmann, L.Journot, B.Antonsson, J.C.Martinou; Phosphorylation of bid by casein kinases I and II regulates its cleavage by caspase 8. Mol. Cell, **8**, 601–611 **(2001)**.

[52] U.Knippschild, D.M.Milne, L.E.Campbell, A.J.DeMaggio, E.Christenson, M.F.Hoekstra, D.W.Meek;p53 is phosphorylated in vitro and in vivo by the delta and epsilon isoforms of casein kinase 1 and enhances the level of casein kinase1 delta in response to topoisomerase-directed drugs. Oncogene, **15**, 1727–1736 **(1997)**.

[53] A.Minchenko, J.Caro; Regulation of endothelin-1 gene expression in human microvascular endothelial cells by hypoxia and cobalt: role of hypoxia responsible element. Mol. Cell.Biochem., **208**, 53-62 **(2000)**.

[54] A.Y.Bobarykina, D.O.Minchenko, I.L.Opentanova, I.O.Kovtun, S.V.Komisarenko, H.Esumi, O.H.Minchenko; Expressionof HIF-1a, HIF-2a ?and VHL mRNAin different cell lines upon hypoxia, Ukr.Biokhim.Zh., **78**, 62-72 **(2006)**.

[55] A.Y.Bobarykina, D.O.Minchenko, I.L.Opentanova, M.Moenner, J.Caro, H.Esumi, O.H.Minchenko; Hypoxicregulationof PFKFB-3 and PFKFB-4 gene expression in gastric and pancreatic cancer cell lines and expression of PFKFB genes in gastric cancers. Acta Biochim. Pol., **53**, 789-799 **(2006)**.

[56] M.S.Bray, M.E.Young; The role of cell-specific circadian clocks in metabolism and disease. Obes. Rev., 10, Suppl., **2**, 6–13 **(2009)**.

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[57] D.A.Bechtold, J.E.Gibbs, A.S.Loudon; Circadian dysfunction in disease. Trends Pharmacol.Sci., **31**, 191–198 **(2010)**.
[58] L.Fu, H.Pelicano, J.Liu, P.Huang, C.Lee; The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell, **111**, 41–50 **(2002)**.
[59] S.Gery, A.F.Gombart, W.S.Yi, C.Koeffler, W.K.Hofmann, H.P.Koeffler; Transcription profiling of C/EBP targets identifies Per2 as a gene implicated in myeloid leukaemia. Blood, **106**, 2827–2836 **(2005)**.
[60] C.C.Lee; The circadian clock and tumor suppression by Mammalian period genes. Methods Enzymol., **393**, 852–861**(2005)**.
[61] H.C.Shih, K.B.Choo, T.J.Chang,M.Y.Yang, M.C.Shih, K.T.Yeh, T.C.Liu, S.F.Lin, J.G.Chang; Disturbance of circadian gene expression in endometrial cancer: detection by real-time quantitative RT-PCR. Oncol. Rep., **14**, 1533–1538 **(2005)**.
[62] F.W.Turek, C.Joshu, A.Kohsaka,E. Lin, G.Ivanova, E.McDearmon, A.Laposky, S.Losee-Olson, A. Easton, D.R. Jensen, R.H.Eckel, J.S.Takahashi, J.Bass; Obesity and metabolic syndrome in circadian Clock mutant mice. Science, **308**, 1043–1045 **(2005)**.
[63] S.Winter, L.Bosnoyan-Collins, D.Pinnaduwage, I.L.Andrulis; Expression of the circadian clock genes Per1 and Per2 in sporadic and familial breast tumors. Neoplasia, **9**, 797–800 **(2007)**.
[64] S.You, P.A.Wood, Y.Xiong, M.Kobayashi, J.Du-Quiton, W.J.Hrushesky; Daily coordination of cancer growth and circadian clock gene expression. Breast Cancer Res. Treat., **91**, 47–60 **(2005)**.
[65] H.Ando, M.Kumazaki, Y.Motosugi, K.Ushijima, T.Maekawa, E.Ishikawa, A.Fujimura; Impairment of peripheral circadian clocks precedes metabolic abnormalities in ob/ob mice, Endocrinology, **152**, 1347–1354 **(2011)**.
[66] D.Chilov, T.Hofer, C.Bauer, R.H.Wenger, M.Gassmann; Hypoxia affects expression of circadian genes PER1 and CLOCK in mouse brain. FASEB J., **15**, 2613–2622 **(2001)**.