ACTA ABP BIOCHIMICA POLONICA

Vol. 64, No 4/2017
635–639
https://doi.org/10.18388/abp.2017_1607

Regular paper

Selenite restores *Pax6* expression in neuronal cells of chronically arsenic-exposed Golden Syrian hamsters

Alain Aguirre-Vázquez[1], Adriana Sampayo-Reyes[1], Laura González-Escalante[1], Alba Hernández[2], Ricard Marcos[2], Fabiola Castorena-Torres[3], Gerardo Lozano-Garza[4], Reyes Taméz-Guerra[1] and Mario Bermúdez de León[4,5]✉

[1]Universidad Autónoma de Nuevo León, UANL, Fac. De Biología, San Nicolás delos Garza, Nuevo León, Mexico; [2]Grup de Mutagènesi, Departament de Genèticai de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain CIBER Epidemiologíay Salud Pública, ISCIII, Madrid, Spain; [3]Tecnológico de Monterrey, Escuela de Medicina, Dirección de Investigación e Innovación. Monterrey, Nuevo León, México; [4]Department of Molecular Biology, Centro de Investigación Biomédica del Noreste,Instituto Mexicano del Seguro Social, Monterrey, Nuevo León, Mexico; [5]Departamento de Ciencias Básicas, Vicerrectoría de Ciencias de la Salud, Universidad de Monterrey, San Pedro Garza García, Nuevo León, Mexico

Arsenic is a worldwide environmental pollutant that generates public health concerns. Various types of cancers and other diseases, including neurological disorders, have been associated with human consumption of arsenic in drinking water. At the molecular level, arsenic and its metabolites have the capacity to provoke genome instability, causing altered expression of genes. One such target of arsenic is the *Pax6* gene that encodes a transcription factor in neuronal cells. The aim of this study was to evaluate the effect of two antioxidants, α-tocopheryl succinate (α-TOS) and sodium selenite, on *Pax6* gene expression levels in the forebrain and cerebellum of Golden Syrian hamsters chronically exposed to arsenic in drinking water. Animals were divided into six groups. Using quantitative real-time reverse transcriptase (RT)-PCR analysis, we confirmed that arsenic downregulates *Pax6* expression in nervous tissues by 53±21% and 32±7% in the forebrain and cerebellum, respectively. In the presence of arsenic, treatment with α-TOS did not modify *Pax6* expression in nervous tissues; however, sodium selenite completely restored *Pax6* expression in the arsenic-exposed hamster forebrain, but not the cerebellum. Although our results suggest the use of selenite to restore the expression of a neuronal gene in arsenic-exposed animals, its use and efficacy in the human population require further studies.

Key words: arsenic, selenite, α-tocopherol; neuronal cells, hamster

Received: 05 May, 2017; revised: 14 August, 2017; accepted: 23 August, 2017; available on-line: 05 December, 2017

✉e-mail: mario.bermudez@imss.gob.mx

Abbreviations: α-TOS, α-Tocopheryl succinate; ANOVA, analysis of variance; cDNA, complementary DNA; DTT, dithiothreitol; M-MLV, Moloney murine leukemia virus; PCR, polymerase chain reaction; RT, reverse transcriptase; S.D., standard deviation

INTRODUCTION

Arsenic in drinking water is one of the major threats to public health worldwide. In several countries, the arsenic concentration in water used for human consumption exceeds the maximum permitted limits (WHO, 2010). Furthermore, arsenic exposure has been associated with various types of cancers; dermal effects; cardiovascular, renal, and hepatic diseases; and neurological disorders (Jomova *et al.*, 2011). All the mechanisms of action by which arsenic induces toxicity and cancer have not yet been clarified; however, results of numerous *in vitro* and *in vivo* studies attempting to elucidate these mechanisms have been reported. Some authors attribute arsenic toxicity to the induction of oxidative stress in cells, leading to increased production of reactive oxygen species and subsequent DNA damage (Bach *et al.*, 2014). This increase in free radicals generates instability in the whole genome, which is evidenced by deregulation of the expression of various genes, including the *Pax6* gene (Bhattacharjee *et al.*, 2013; Tyler & Allan, 2014).

The *Pax6* gene is a member of the paired-box family that encodes a series of transcription factors highly conserved among species. PAX6 protein is essential for the development of the central nervous system and pancreatic cells (Blake & Ziman, 2014). PAX6 also has a crucial role in maintaining stability of genes involved in the self-renewal and neurogenesis of stem cells (Zhang *et al.*, 2010). The up- and downregulation of *Pax6* expression in neural tissues provokes abnormalities through different mechanisms (Sansom *et al.*, 2009). Reports have shown that the *Pax6* gene is a target of arsenic (Tyler & Allan, 2013). Therefore, it is important to understand the effects of arsenic on *Pax6* expression.

One possible approach to reverse the toxic effects of arsenic in exposed individuals is the use of antioxidants to control the production of free radicals resulting from arsenic metabolism (Kaur *et al.*, 2009). In particular, α-tocopheryl succinate (α-TOS), a vitamin E analog, has proapoptotic properties as well as antioxidant/anti-inflammatory activities (Neuzil *et al.*, 2006). Other essential nutrients, such as selenium, have the ability to sequester arsenic (Wang *et al.*, 2013) and inhibit its metabolism, thereby preventing its incorporation into the cell (Gailer, 2009). Therefore, the aim of this study was to evaluate the effect of two antioxidants, α-TOS and selenium, on *Pax6* expression in neural tissues of hamsters chronically exposed to arsenic.

MATERIALS AND METHODS

Chemicals. Sodium arsenite, sodium selenite, and α-TOS were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Ethical approval. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Procedures performed in all studies involving animals were reviewed and approved by the

Table 1. Scheme of treatments for each group of hamsters

Group	N	Treatment
Control	3	Tap water
Arsenic	5	100 ppm arsenic/L
α-TOS	3	6 mg α-TOS/kg/day
Selenite	3	8.5 mg selenite/kg/day
α-TOS/arsenic	3	100 ppm arsenic/L plus 6 mg α-TOS/kg/day
Selenite/arsenic	3	100 ppm arsenic/L plus 8.5 mg selenite/kg/day

Local Committee of Research and Ethics in Health No. 1902 at the Instituto Mexicano del Seguro Social and were in full compliance with the Official Mexican Standard NOM-062-ZOO-1999 for the production, care, and use of laboratory animals for scientific purposes.

Animals and treatments. Twenty adult male Golden Syrian hamsters (*Mesocricetus auratus*), 8 weeks old and weighing 90–110 g, were used. Food and water were provided *ad libitum*. The hamsters were randomly divided into six groups (n=3–5 per group), and each group received the respective treatment regimen for 7 months (Table 1). All treatments were administered in drinking water, contained in 200 mL bottles, every two days. The dose of sodium arsenite (100 ppm/L) used in this study was based on previous studies (Tripathi *et al.*, 1997; Flora, 1999; Flora *et al.*, 2005) as well as our own experience in determining levels of arsenic that cause measurable organ damage. The doses of α-TOS (6 mg/kg/day) and of sodium selenite (8.5 mg/kg/day) were selected based on studies that showed no toxicity in animals (Norppa *et al.*, 1980; Hathcock *et al.*, 2005). Animals were sacrificed in a carbon dioxide chamber. After cessation of breathing, the whole forebrains and cerebellums were removed and immediately placed in 15 mL tubes containing 2–3 mL of RNA later reagent to maintain RNA integrity. The tissues were then refrigerated (4°C) overnight, and on the next day they were frozen at –20°C until use. Forebrains and cerebellums from at least three untreated hamsters (controls) were processed in order to determine baseline *Pax6* expression levels.

Sequence alignments and primer/probe design. An *in silico* analysis was conducted with the UCSC Genome Browser (Genome Bioinformatics Group, University of California at Santa Cruz, USA) for *Pax6* primers and probe design. The Basic Local Alignment Search Tool (National Center for Biotechnology Information, Bethesda, MD, USA) was used to find orthologous sequences of the *Mus musculus* (mouse) *Pax6* gene in *M. auratus*, *Crisetulus griseus* (Chinese hamster), and *Rattus norvegicus* (rat). Using the MEGA6 program (Tamura *et al.*, 2013), evolutionarily conserved regions of the *M. musculus Pax6* gene among the afore mentioned four species were identified, and multiple alignment of these conserved regions was subsequently performed to delimit the exons. File Builder 3.1 Software (Thermo Fisher Scientific-Applied Biosystems, Grand Island, NY, USA) was used to create the base file for designing the TaqMan probe and primers. The probe was designed to span the exon 2-exon 3 junction in order to accurately evaluate the levels of *M. auratus Pax6* gene expression.

RNA extraction. Total RNA extraction was performed using TRIzol reagent (Thermo Fisher Scientific-Invitrogen) according to the manufacturer's instructions. Briefly, a sample of each forebrain and cerebellum was taken and frozen in liquid nitrogen. The frozen tissue was then pulverized avoiding thawing and was added to a microtube containing 1 mL of TRIzol reagent. Chloroform (0.2 mL) was then added and the microtube content stirred for 15 s. Samples were incubated at room temperature for 2–3 min and then centrifuged at 12 000 × g for 15 min at 4°C. The aqueous phase was removed and placed in a new microtube. Total RNA was precipitated with 0.5 mL of absolute isopropanol and centrifuged at 12 000 × g for 10 min at 4°C, followed by an ethanol wash.

Finally, the RNA pellet was resuspended in 30 μL of nuclease-free water (Invitrogen). The purity and concentration of total RNA were estimated spectrophotometrically at 260 and 280 nm. RNA integrity was assessed by electrophoresis in 1% agarose gel stained with GelRed.

Quantitative RT-PCR assays. cDNA synthesis was carried out using M-MLV reverse transcriptase and random primers according to the manufacturer's instructions. Briefly, 1 μg of total RNA plus random primers and deoxynucleosidetriphosphates were used to prepare mix1. Mix1 was incubated at 65°C for 5 min and then cooled on ice. Separately, Mix2 was prepared with first-strand buffer, DTT, and RNaseOUT (Invitrogen). Mix2 was added to Mix1 and incubated at 37°C for 2 min. M-MLV reverse transcriptase was then added to the reaction tube, and the PCR took place under the following conditions: 25°C for 10 min, 37°C for 50 min, and finally enzyme inactivation at 70°C for15 min. The functionality of the cDNA was confirmed by end-point PCR amplification of the constitutive *r18S* gene, which encodes the ribosomal RNA18S subunit (Table 2), and visualization on 1% agarose gel stained with GelRed. Quantitative PCR was performed using the 7500 Fast Real Time PCR System with primers and the TaqMan probe for the *Pax6* gene (Table 2). The PCR reaction was carried out in a 20 μL volume containing 10 μL of TaqMan Universal PCR Master Mix, 1 μL primer/probe, 3 μL of cDNA template and 6 μL of nuclease-free water. Amplification was performed in the standard mode under the following reaction conditions: initial denaturation at 50°C for 2 min; 40 cycles of denaturation at 95°C for 10 min, annealing and extension at 95°C for 15 s, 60°C for 1 min. The dynamic range curve was established using triplicate samples assayed in a 96-well format, and the 1:128 dilution was chosen. Negative template controls were included for all assays. As an endogenous control, analysis of *r18S* gene expression was performed in par-

Table 2. Primers and probe sequences for PCR assays

Gene	Primers(5´-3´)	Probe(FAM5´- 3´NFQ)
Pax6	F-GCT TGGGAAATC CGAGACAGAT R-CCAGGTTGC GAAGAACTC TGT TT	CCC AGTGTGTCA TCA AT
r18S -End-point PCR	F-GTT AATTCC AGCTCC AATAGCGTA R-GAACTA CGACGGTAT CTG ATC GTC	Not applicable
r18S-Real-time PCR	Cat. 4310893E	(Applied Biosystems)

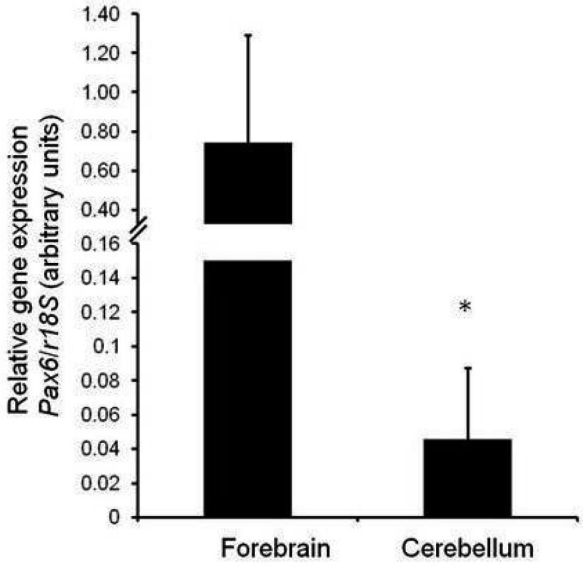

Figure 1. Relative *Pax6* expression in forebrain and cerebellum of *M. auratus*.
Each bar represents the mean ±S.D. of 3 independent experiments. Results were normalized to *r18S* gene expression. **P*<0.001.

allel (Table 2). Threshold (Fraga *et al.*, 2005) values were analyzed by the comparative $2^{-\Delta Ct}$ method described by Livak and Schmittgen (Livak Schmittgen, 2001).

Statistical analysis. The Kolmogorov-Smirnov test was performed to evaluate the normal distribution of data. Results for quantitative PCR were analyzed using one-way analysis of variance (ANOVA). Dunnett's test and Tukey's test for multiple comparisons were performed using SPSS statistical software package (version 23.0; IBM Corp, Armonk, NY, USA). Student's *t*-test was used to compare relative expression data between forebrain and cerebellum. In all cases, the criterion for significance was set at $P<0.05$. Data are shown as the mean ± standard deviation (S.D.).

RESULTS

As depicted in Fig. 1, comparison of *Pax6* baseline expression between tissues revealed a mean level of PAX6 mRNA in the forebrain that was 18.5-fold higher than that in the cerebellum (0.74 ± 0.55 *vs.* 0.04 ± 0.04). When all forebrain and cerebellum samples were compared, the significance of this difference was much greater ($P<0.001$) regardless of the treatments (Fig. 2).

Similarly, the differences in the magnitude of *Pax6* expression between tissues were maintained despite the arsenic exposure (Fig. 2A and 2B). Kolmogorov-Smirnov analysis revealed a normal distribution of the data ($P>0.05$). Although ANOVA did not show any significant differences, mean PAX6 mRNA levels in both forebrain and cerebellum of arsenic-treated hamsters were decreased by 53 ± 21% and 32 ± 7%, respectively, in comparison to the untreated group.

In addition, the effect of α-TOS and selenite on *Pax6* expression revealed unexpected phenomena.

In both forebrain and cerebellum, α-TOS reduced the mean *Pax6* expression level to 30% of that in the untreated group. Also, selenite decreased the mean PAX6 mRNA level in the forebrain and cerebellum to 49% and 43% of that in untreated hamsters, respectively (Fig. 2C and 2D).

In arsenic-exposed hamsters treated with α-TOS, the downregulation of *Pax6* expression in the forebrain and cerebellum was maintained: mean PAX6 mRNA levels were similar to those observed in the group treated only with α-TOS (Fig. 3). Interestingly, treatment of arsenic-

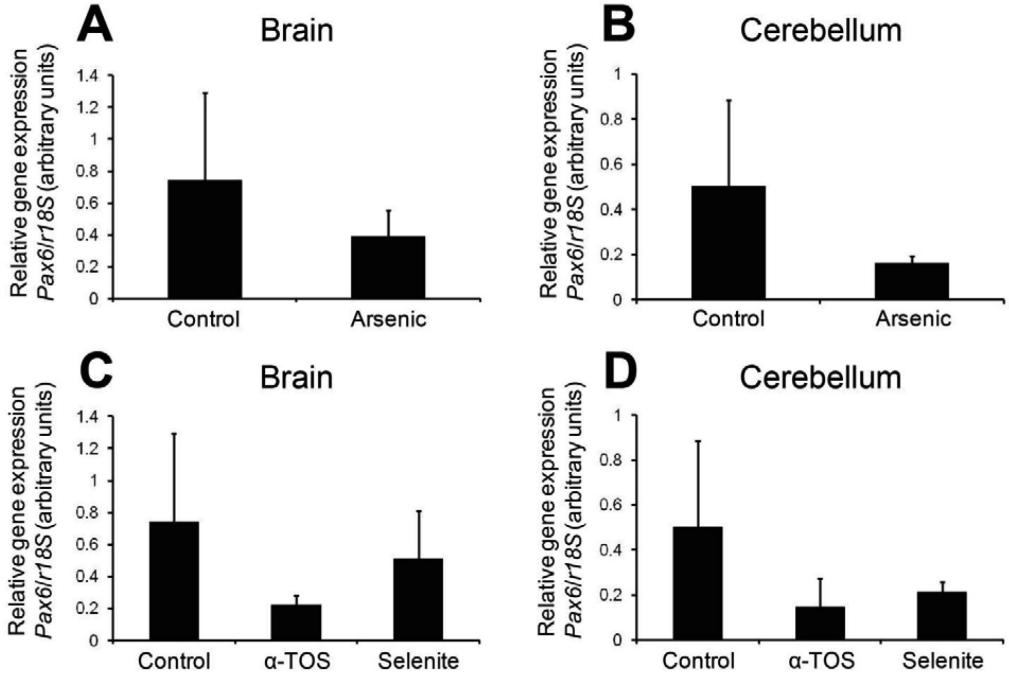

Figure 2. Effect of arsenic, α-TOS, and selenite on *Pax6* expression in forebrain and cerebellum of *M. auratus*.
(**A**) *Pax6* expression in forebrain of arsenic-exposed animals. (**B**) *Pax6* expression in cerebellum of arsenic-exposed animals. (**C**) *Pax6* expression in forebrain of α-TOS-and selenite-treated animals. (**D**) *Pax6* expression in cerebellum of α-TOS- and selenite-treated animals. Each bar represents the mean ±S.D. of 3 or 4 replicates from 3 independent experiments. Results were normalized to *r18S* gene expression. $P>0.05$.

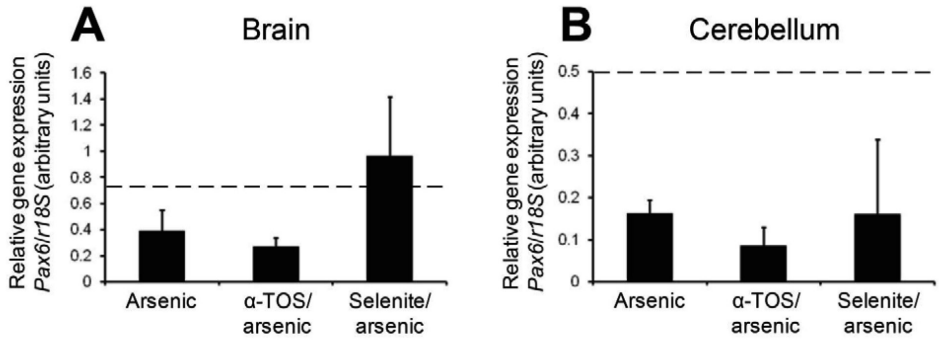

Figure 3. Effect of α-TOS and selenite on *Pax6* expression in arsenic-exposed *M. auratus*.
(**A**) *Pax6* expression in forebrain of arsenic-exposed animals treated with α-TOS and with selenite. (**B**) *Pax6* expression in cerebellum of arsenic-exposed animals treated with α-TOS and with selenite. Each bar represents the mean ±S.D. of 3 or 4 replicates from 3 independent experiments. Results were normalized to *r18S* gene expression. Dashed lines represent the mean level of *Pax6* expression in the untreated group. $P>0.05$.

exposed hamsters with selenite restored PAX6 mRNA to the control levels in the forebrain ($P=0.394$) but not in the cerebellum.

DISCUSSION

Our gene expression analysis by quantitative PCR revealed that PAX6 mRNA was more abundant in forebrain than in cerebellum, regardless of arsenic exposure or treatment with antioxidants. Although higher expression of *Pax6* has been reported in the cerebellum during adulthood (Stoykova & Gruss, 1994; Duan *et al.*, 2013), the granulosa cells, in which *Pax6* is expressed, are more susceptible to oxidative stress compared to other neural cells (Fujimura & Usuki, 2014).Considering that in our study we extracted total RNA from the forebrain and cerebellum, the oxidative response may vary in different structures of the brain, which may explain the observed differences in *Pax6* expression (Vaz *et al.*, 2011; Medeiros *et al.*, 2012).

Our expression assays further revealed that hamsters exposed to arsenic showed decreased *Pax6* expression levels in both forebrain and cerebellum. This finding is consistent with a previous report of *Pax6* down regulation in adult mice treated with arsenic during the embryonic stage (Tyler & Allan, 2013). Other studies of nervous tissue have shown altered gene expression in response to arsenic exposure (Wlodraczyk *et al.*, 1996; Luo *et al.*, 2012; Zhang *et al.*, 2014). Although PAX6 protein appears to be crucial for regulating genes involved in self-renewal and stem cell neurogenesis (Sansom *et al.*, 2009), our findings suggest that the downregulation of *Pax6* in neural tissues following arsenic exposure may trigger changes in essential cellular processes that lead to neurologic abnormalities.

In our study, the expression of *Pax6* was dramatically reduced by α-TOS treatment. This decrease in gene expression triggered by α-TOS has been previously reported by Changand coworkers (2003). Likewise, treatment with selenite resulted in a decrease in *Pax6* expression, but the magnitude of this decrease was much smaller compared to that of the α-TOS group. This reduced effect of selenite on decreasing *Pax6* expression may be related to increased biosynthesis of the cytosolic selenoprotein, glutathione peroxidase, which is believed to protect cells against peroxide damage (Bermingham *et al.*, 2014).

Treatment of arsenic-exposed hamsters with α-TOS did not show a positive effect on *Pax6* regulation neither in the forebrain nor cerebellum. Although other studies have demonstrated a protective effect of α-TOS against oxidizing agents (Prasad *et al.*, 2003; Stankov *et al.*, 2007; Bellezza *et al.*, 2014), it is important to consider that, by oral administration, some characteristic properties (i.e., proapoptotic activity) of α-TOS could be lost (Neuzil & Massa, 2005). In addition, it has been reported that α-tocopheryl is protective against arsenic (Chung *etal.*, 2011); however, high doses of α-tocopheryl may increase the toxic effect of arsenic (Rocha *et al.*, 2011). Therefore, studies using different doses of α-TOS must be done to determine the threshold level of cellular toxicity and to characterize its interactions with oxidizing agents (e.g., arsenic). By contrast, treatment of arsenic-exposed hamsters with selenite resulted in restoration of *Pax6* expression to control levels in the forebrain. Similarly, Li and coworkers (2013) demonstrated the capacity of selenite to protect against lead neurotoxicity. These results suggest that selenite has the ability to bind arsenic and form a complex with glutathione, thereby preventing arsenic from entering the cells (Gailer, 2009).

Finally, we observed a large degree of variability in forebrain and cerebellum *Pax6* expression levels among the individual animals (as denoted by a markedly increased S.D.) in the control and arsenic-exposed/selenite-treated groups. These expression variations suggest that the genetic background of the animals plays the key role in gene regulation. Another explanation for these *Pax6* expression differences in our study is the fact that the treated animals were not from the same litter, and thus there were differences in genetic load.

In conclusion, exposure to arsenic leads to decreased *Pax6* gene expression in nervous tissues of the Golden Syrian hamster, and this effect can be reversed by selenite. Although our results suggest that selenite can be used to restore the expression of a neuronal gene in arsenic-exposed animals, its use and efficacy in the human population must be evaluated in future studies.

Acknowledgements

The technical assistance in support of animal care of Q.C.B. Erika Daniela Pérez Esquivel and pQ.B.P. Estefany Guadalupe de la Rosa Cuellar is gratefully acknowledged. The authors thank Mrs. Ana María Garza Sánchez for her secretarial assistance. We thank Lic. Israel Benavides Páramo for his administrative work.

Authors' contributions: AAV conducted the lab activities, analyzed the data and co-wrote the final version of the manuscript; ASR, GLG and AH designed the strat-

egy of animal experiments, supervised the lab work, co-authored the manuscript and contributed to the financial support. LGE and FCT performed the statistical analyses. RM and RTG revised the final version of the manuscript. MBL designed the strategy of molecular experiments, supervised the lab work, contributed to the financial support and co-wrote the final version of the manuscript.

Disclosure of potential conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements of financial support

This work was supported by Instituto Mexicano del Seguro Social (FIS/IMSS/PROT/G11/956).

REFERENCES

Bach J, Sampayo-Reyes A, Marcos R, Hernandez A (2014) Ogg1 genetic background determines the genotoxic potential of environmentally relevant arsenic exposures. *Arch Toxicol* **88**: 585–596. 10.1007/s00204-013-1151-0

Bellezza I, Grottelli S, Gatticchi L, Mierla AL, Minelli A (2014) alpha-Tocopheryl succinate pre-treatment attenuates quinonetoxicityin prostate cancer PC3 cells. *Gene* **539**: 1–7. 10.1016/j.gene.2014.02.009

Bermingham EN, Hesketh JE, Sinclair BR, Koolaard JP, Roy NC (2014) Selenium-enriched foods are more effective at increasing glutathione peroxidase (GPx) activity compared with selenomethionine: ameta-analysis. *Nutrients* **6**: 4002–4031. 10.3390/nu6104002

Bhattacharjee P, Banerjee M, Giri AK (2013) Role ofgenomicinstabilityin arsenic-induced carcinogenicity. A review. *Environ Int* **53**: 29–40. 10.1016/j.envint.2012.12.004

Blake JA, Ziman MR (2014) Paxgenes: regulators of lineage specification and progenitor cell maintenance. *Development* **141**: 737–751. 10.1242/dev.091785

Chang TI, Horal M, Jain SK, Wang F, Patel R, Loeken MR (2003) Oxidant regulation of gene expression and neural tube development: Insights gained from diabetic pregnancy on molecular causes of neural tube defects. *Diabetologia* **46**: 538–545. 10.1007/s00125-003-1063-2

Chung CJ, Pu YS, Chen YT, Su CT, Wu CC, Shiue HS, Huang CY, Hsueh YM (2011) Protective effects of plasma alpha-tocopherols onthe risk of inorganicarsenic-related urothelial carcinoma. *Sci Total Environt* **409**: 1039–1045. 10.1016/j.scitotenv.2010.11.037

Duan D, Fu Y, Paxinos G, Watson C (2013) Spatio temporal expression patterns of *Pax6* in the brain of embryonic, newborn, and adultmice. *Brain Struct Funct* **218**: 353–372. 10.1007/s00429-012-0397-2

Flora SJ (1999) Arsenic-induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid in rats. *Clin Exp Pharmacol Physiol* **26**: 865–869

Flora SJ, Bhadauria S, Pant SC, Dhaked RK (2005) Arsenic induced blood and brain oxidative stress and its response tosome thiol chelators in rats. *Life Sci* **77**: 2324–2337. 10.1016/j.lfs.2005.04.016

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M (2005) Epigenetic differences arise during the life time of monozygotic twins. *Proc Natl Acad Sci U S A* **102**: 10604–10609. 10.1073/pnas.0500398102

Fujimura M, Usuki F (2014) Low *in situ* expression of antioxidative enzymes in rat cerebellar granular cells susceptible to methylmercury. *Arch Toxicol* **88**: 109–113. 10.1007/s00204-013-1089-2

Gailer J (2009) Chronic toxicity of As(III) in mammals: the role of (GS)(2)AsSe(-). *Biochimie* **91**: 1268–1272. 10.1016/j.biochi.2009.06.004

Hathcock JN, Azzi A, Blumberg J, Bray T, Dickinson A, Frei B, Jialal I, Johnston CS, Kelly FJ, Kraemer K, Packer L, Parthasarathy S, Sies H, Traber MG (2005) Vitamins E and C are safe across abroad range of intakes. *Am J Clin Nutr* **81**: 736–745

Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ, Valko M (2011) Arsenic: toxicity, oxidative stress and human disease. *J Appl Toxicol* **31**: 95–107. 10.1002/jat.1649

Kaur H, Mishra D, Bhatnagar P, Kaushik P, Flora SJS (2009) Co-administration of α-lipoic acid and vitamin c protects liver and brain oxidative stress in mice exposed to arsenic contaminated water. *Water Qual Expo Heal* **1**: 135–144. 10.1007/s12403-009-0013-8

Li WH, Shi YC, Tseng IL, Liao VH (2013) Protective efficacy of selenite against lead-induced neurotoxicity in *Caenorhabditis elegans*. *PloS One* **8**: e62387. 10.1371/journal.pone.0062387

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DeltaDeltaC(T)) method. *Methods* **25**: 402–408. 10.1006/meth.2001.1262

Luo JH, Qiu ZQ, Zhang L, Shu WQ (2012) Arsenite exposure altered the expression of NMDA receptor and post synaptic signaling proteins in rat hippocampus. *Toxicol Lett* **211**: 39–44. 10.1016/j.toxlet.2012.02.021

Medeiros MC, Mello A, Gemelli T, Teixeira C, de Almeida M, de Andrade RB, Wannmacher CM, Guerra RB, Gomez R, Funchal C (2012) Effect of chronic administration of the vinylchalcogenide 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-oneon oxidative stress in different brain areas of rats. *Neurochemical Res* **37**: 928–934. 10.1007/s11064-011-0685-x

Neuzil J, Massa H (2005) Hepatic processing determines dual activity of alpha-tocopheryl succinate: a novel paradigm for a shift in biological activity due to pro-vitamin-to-vitamin conversion. *Biochem Biophysic Res Commun* **327**: 1024–1027. 10.1016/j.bbrc.2004.12.115

Neuzil J, Wang XF, Zhao Y, Wu K (2006) *Vitamin E Analogs as Anticancer Agents*. Press Taylor & Francis Group.

Norppa H, Westermarck T, Knuutila S (1980) Chromosomal effects of sodium selenite *in vivo*. III. Aberrations and sister chromatid exchanges in Chinese hamster bonemarrow. *Hereditas* **93**: 101–105

Prasad KN, Kumar B, Yan XD, Hanson AJ, Cole WC (2003) Alpha-tocopheryl succinate, the most effective form of vitamin E for adjuvant cancer treatment: a review. *J Am College Nutrit* **22**: 108–117

Rocha RA, Gimeno-Alcaniz JV, Martin-Ibanez R, Canals JM, Velez D, Devesa V (2011) Arsenic and fluoride induce neural progenitor cell apoptosis. *Toxicol Lett* **203**: 237–244. 10.1016/j.toxlet.2011.03.023

Sansom SN, Griffiths DS, Faedo A, Kleinjan DJ, Ruan Y, Smith J, van Heyningen V, Rubenstein JL, Livesey FJ (2009) The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis. *PLoS Genetics* **5**: e1000511. 10.1371/journal.pgen.1000511

Stankov K, Bajin-Katic K, Stanimirov B, Karadzic D, Kovacevic Z (2007) Alpha-tocopheryl succinate (α-TOS) influences cell vitality and enzyme activity in Ehrlich ascites carcinoma cells. *Arch Oncol* **15**: 65–68. 10.2298/AOO0704065S

Stoykova A, Gruss P (1994) Roles of *Pax*-genes in developing and adult brain as suggested by expression patterns. *Journal of Neuroscience: the Official Journal of the Society for Neuroscience* **14**: 1395–1412

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**: 2725–2729. 10.1093/molbev/mst197

Tripathi N, Kannan GM, Pant BP, Jaiswal DK, Malhotra PR, Flora SJ (1997) Arsenic-induced changes in certain neurotransmitter levels and their recoveries following chelation in rat whole brain. *Toxicol Lett* **92**: 201–208

Tyler CR, Allan AM (2013) Adult hippocampal neurogenesis and mRNA expression are altered by perinatal arsenic exposure in mice and restored by brief exposure to enrichment. *PloS One* **8**: e73720. 10.1371/journal.pone.0073720

Tyler CR, Allan AM (2014) The effects of arsenic exposureon neurological and cognitive dysfunction in human and rodent studies: a review. *Curr Environ Health Rep* **1**: 132–147. 10.1007/s40572-014-0012-1

Vaz AR, Silva SL, Barateiro A, Falcao AS, Fernandes A, Brito MA, Brites D (2011) Selective vulnerability of rat brain regions to unconjugated bilirubin. *Mol Cell Neurosci* **48**: 82–93. 10.1016/j.mcn.2011.06.008

Wang X, Zhang J, Zhao L, Hu S, Piao F (2013) Effect of subchronic exposure to arsenic on levels of essential trace elements in micebrain and its gender difference. *Biometals: an International Journal on the Role of Metalions in Biology, Biochemistry, and Medicine* **26**: 123–131. 10.1007/s10534-012-9599-6

WHO (2010) Exposure to Arsenic: A Major Public Health Concern. In Organization WH ed. http://www.who.int/ipcs/features/arsenic.pdf

Wlodraczyk B, Bennett GD, Calvin JA, Craig JC, Finnell RH (1996) Arsenic-induced alterations in embryonic transcription factor gene expression: implications for abnormal neural development. *Develop Genetics* **18**: 306–315. 10.1002/(SICI)1520-6408(1996)18:4<306::AID-DVG4>3.0.CO;2-D

Zhang C, Li S, Sun Y, Dong W, Piao F, Piao Y, Liu S, Guan H, Yu S (2014) Arsenic down regulates gene expression at the post synaptic density in mouse cerebellum, including genes responsible for long- term potentiation and depression. *Toxicol Lett* **228**: 260–269. 10.1016/j.toxlet.2014.05.007

Zhang X, Huang CT, Chen J, Pankratz MT, Xi J, Li J, Yang Y, Lavaute TM, Li XJ, Ayala M, Bondarenko GI, Du ZW, Jin Y, Golos TG, Zhang SC (2010) Pax6 is a human neuroectoderm cell fate determinant. *Cell Stem Cell* **7**: 90–100. 10.1016/j.stem.2010.04.017