Hydrogen rich water ameliorate Trimethyltin induced spatial learning and memory impairment by regulation of Siah-1

Tailai Zhou
chongqing medical university
Minghao Zhang
chongqing medical university
Hang Du
Chongqing municipal hospital for prevention control of occupational disease
Arzu Ablimit
Chongqing Medical University
Renyi Ye
Chongqing Medical University
Mingqi Lǚ
chongqing medical university
Xiaojuan Chang
Chongqing municipal and environmental sanitaion mornitoring department
Qi Zhao
Chongqing center for disease control and prevention
Yan Wang
Sichuan eletric power hospital
Qizhong Qin (qqizhong@cqmu.edu.cn)
Chongqing Medical University https://orcid.org/0000-0002-5857-4053

Research article

Keywords: Toxicity mechanisms, Neurotoxicity, Trimethyltin, Synaptophysin, Siah-1, Hydrogen rich water, Neuroprotection

Posted Date: February 12th, 2020

DOI: https://doi.org/10.21203/rs.2.23352/v1

Abstract

Background: As an important member of organic tin compounds, Trimethyltin (TMT) exists widely in industrial and agricultural productions. TMT can induce significant neurodegeneration in the limbic system, particular in hippocampus. However, the molecular mechanisms in TMT-induced learning and memory impairment are not fully clear. Thus, this research was to explore the role of Synaptophysin (SYP) and Ubiquitin-ligating enzyme Siah-1 in TMT-induced nerve impairment and the protective effect of hydrogen rich water (HRW).

Methods: Male BALB/c mice were randomly divided into 4 groups: control group (saline, 4 ml/d, i.p.for seven days), HRW group (4 ml/d, i.p.for seven days) , TMT group (2.25mg/kg, i.p. at the seventh day), and HRW plus TMT group. The spatial learning and memory was tested by Morris water maze, the protein level of SYP and Siah-1were determined by western blot& immunocytochemical analysis, and the tin contents were detected by ICP-MS.

Results: The TMT-treated mice showed significant learning and memory disability. Moreover, TMT increased the level of Siah-1, reduced the expression of SYP in hippocampus and cortex. After 7 days of continuous pretreatment with HRW, the mice showed better memory ability, the expression of Siah-1 and tin content decreased accompanied with the increase of SYP.

Conclusions: These results showed that HRW ameliorated the decrease of SYP and the nerve impairment induced by TMT, reduced tin content and up-regulated the expressions of Siah-1, and it might be an important role in the protection of neurodegenerative diseases induced by TMT.

Background

Trimethyltin (TMT) is a colorless crystal with odor of carrion grass under normal temperature and widely exists in paint, synthetic rubber, rodenticide, etc. It also can be completely absorbed by humans through the respiratory, skin, digestive tract and so on [1–3]. Besides, TMT has good lipophilicity and high affinity with hemoglobin in blood, and can be accumulated in human bodies [2]. After TMT treatment, mice can show agitation, aggressive behavior, learning and memory dysfunction [4–7]. According to reports, TMT can selectively damage the limbic system, especially in the prefrontal cortex, hippocampus and other brain regions and lead to the dysfunction and apoptosis in these regions, which are closely related to learning and memory function, suggesting that TMT-induced learning and memory dysfunction have a special correlation with such selective effect [8–9].

The mechanisms of nervous system damage induced by TMT include apoptosis, inflammatory response, oxidative damage, glutamate excitability, etc.[6, 10–11]. However, the exact mechanisms of TMT-induced widely nervous system damage are not fully clear. Synaptophysin (SYP) is a glycoprotein abundant in the membrane of synaptic vesicles; it exists in almost all neurons and neuroendocrine cells. Moreover, SYP is closely related to the release of neurotransmitters as well as the density and plasticity of synapses. Therefore, the immune activity of SYP can reflect the change in the number of the synaptic vesicles, and can be regarded as a marker of the synaptic density [12]. Our previous studies had demonstrated the connection between the decrease of SYP in the prefrontal cortex and hippocampus area and TMT-induced learning and memory impairment [13]. Above all, we believed that it was of great significance to explore how TMT reduced the expression of SYP in the prefrontal cortex and hippocampus area, thus causing the learning and memory impairment.

Ubiquitin Proteasome System (UPS) is the main pathway for protein degradation in cells [14]. As a number of ubiquitin proteasome, Siah-1 can recognize and bind the target proteins, and degrade them through 26S proteasome pathway. In Anderson VE's report, theytreated hippocampal neurons with TMT and found that the immune activity of ubiquitin in hippocampal cell was enhanced, suggesting that TMT can affect the UPS function and enhance the degradation of related proteins [15]. Currently, some researchers have used TMT to induce hippocampal neuron damage to study the occurrence of neurodegenerative diseases [16]. Moreover, researchers have found that SYP has a binding domain of Siah-1, and over expression of Siah-1 can promote the degradation of SYP by the UPS [17–18].

Hydrogen is the simplest and most widely spread element in the nature, in recent years, the anti-oxidation effect and the neuroprotective effect of hydrogen has been proved [19]. The dissolved hydrogen in water can selectively neutralized OH^- and $ONOO^-$ which cause the oxidative damage to cells [20]. Moreover, hydrogen has therapeutic effects on brain injury after cerebral ischemia and hypoxia, Parkinson's disease, Alzheimer's disease and so on [21–26]. Therefore, in this study, we aim to explore whether Siah-1 involves in the TMT-induced neurodegeneration disease by reducing SYP, and the protective effect of HRW in this approach.

Ethical Statement

This experiment was approved by the Institutional Animal care and use Committee of the Chongqing Medical University. In this experiment, the research design has scientific basis and the selected animal species, quantity and specification are appropriate. In this experiment, the animals were treated with anesthesia and analgesia and this experiment did not bring corresponding harm to the environment.

Study Design

Male BALB/c mice were randomly divided into 4 groups: control group (saline, 4 ml/d, i.p.for seven days), HRW group (4 ml/d, i.p.for seven days), TMT group (2.25mg/kg, i.p. at the seventh day), and HRW plus TMT group. The spatial learning and memory was tested by Morris water maze, the protein level of SYP and Siah-1 were determined by western blot and immunocytochemical analysis, and the tin contents were detected by ICP-MS. The specific experimental design steps please refer to the Graphical Abstract.

Methods

Reagents and experimental drugs

Trimethyltin was purchased from Sigma, USA. Primary antibodies (SYP&Siah-1, β-actin) were respectively purchased from Abcam, England and Sigma, USA. The secondary antibodies were purchased from LI-COR Abcam, England. Materials for Western blot, such as enhanced chemiluminescent (ECL) and Polyvinylidene difluoride (PVDF, 0.2μm) membrane were purchased from Elabscience Biotechnology, Wuhan, China. Zinc (Beyotime Biotechnology, Shanghai, China) reacts with dilute sulfuric acid to form hydrogen, passed the hydrogen into normal saline water placed in ice water for four to six hours to prepare for hydrogen rich water. All other chemical reagents and medicines are purchased through regular channels and are available.

Animals and experimental design

Forty-eight male BALB/c mice weighing 25-35 g were purchased from the animal center of the Chongqing Medical University（license numbers: SCXK(Yu)2012-001）, Chongqing, China. Every four mice were kept in one cage, the temperature of the animal room was kept at 21 ± 2°C, and the mice were fed once a day with 12 hours under light (8:00 AM to 8:00 PM). All the animal feeding and raising were carried out according to the Guidelines of the Care and Use of Laboratory Animals of the Chongqing Medical University. All procedures and detection methods adopted in this study were approved and supervised by the Institutional Animal care and use Committee of the Chongqing Medical University.

Animals in control group received normal saline (4 ml/d, i.p.2 ml at 9:00 AM and 5:00 PM respectively) for seven days as vehicle. The HRW group was given hydrogen rich water intraperitoneally (4 ml/d, i.p.2ml at 9:00 AM and 5:00 PM respectively) for seven days which served as HRW control. The TMT group was administrated with TMT single i.p. injection (based as control group, 2.25 mg/kg, i.p. at 12:00 AM) at the seventh day at 12:00 AM. The dose of TMT and used in this study was based on our previous study[13]. In this process, the mice did not show any significant toxicity, including tremor, fighting and apparent damage. The TMT + HRW group animals was injected with TMT on the basis of the pretreatment of HRW (based as HRW group, treated TMT <2.25mg/kg, i.p.> at 12:00 AM at the seventh day), the processing method of this group was similar to the HRW group and TMT group, respectively. All the treatments were performed gently and all efforts were made to minimize animal suffering [27].

Morris water maze task

The Morris water maze task was designed to detect the spatial learning and memory impairment induced by TMT exposure and the protective effect of HRW. The tank (Tai Meng Technology, Chengdu, China) was divided into four equal quadrants, and this tank was 120 cm in diameter, 50 cm in height, the platform was 10 cm in diameter, the temperature of the water was about 24 to 26 ℃.

Before the task, the water was added into some mike powder to make it opaque, and the platform was placed in the center of any quadrant and immersed in water for about 1-2 centimeters. The day before the task, the mice could swim freely in the tank without the platform for one minute, ten times in total, to make them familiar with the Morris water maze. In the second day, every mouse was tested eight times consecutively [28]. The mice had eight chances to search the hidden platform and each chance for 60 seconds, the time of searching would be recorded. If the mice could not find the platform for themselves within 60s, then they were gently guided to

the platform and the searching time would be recorded as sixty seconds. After the mice fond the platform, they could stay on the platform for 15 seconds to get familiar with the position of the platform. Completing all the tasks, the mice were dried and put back into the cage.

Immunohistochemical

At the end of the last Morris water maze task, about 0.4ml of 20% Urethane was intraperitoneally injected to anesthetize the mice, and then decapitated, and an appropriate amount of colorless formalin was infused through the cardia. After killing the mice, the brain tissue was quickly removed and fixed in 4% paraformaldehyde for use, or dehydrated by 75%-100% graded ethanol, cleared in xylene and embedded in paraffin. The thickness of the paraffin section was 5μm; HE staining was performed on every five sections.

Before dewaxing, the paraffin sections were placed at room temperature for 60 minutes, then the sections were immersed in xylene for 10 minutes and dehydrated in 100% - 75% graded ethanol. About 40 - 50ul polyclonal antibodies against SYP (1:1000) and Siah-1 (1:1000) were added to the tissue sample and placed them overnight at 4 °C. After placing overnight, rinsed the tissue sample three times with PBS, then added about 40 - 50ul secondary antibody at room temperature for 1 hour. After completing all the processes mentioned above, rinsed with PBS three times again.

SYP and Siah-1 were separately detected by DAB Horseradish Peroxides Color Development Ki. The images of tissue sample were taken by the Leica Microsystems (TYPE DM LB 2, Austria). By photographing the hippocampus and cortex of mice (DG, CA1, CA3), we could obtain the images of positive cells expressing SYP and Siah-1 proteins in the hippocampus and cortex.

Western blot

The cortex and hippocampus of mice were separated and frozen at - 80 ℃ for reserve,

tissue proteins were decomposed by RIPA solution (50 mM TrisHCL(PH7.5),150 mM Nacl, 1% NP-40, 0.5% Triton X-100, 0.1%SDS) and the total protein concentration was measured. The protein was separated by 10% SDS-PAGE and transferred to PVDF/NC membrane, then blocked the membranes with 5% skimmed milk powder. After blocking, added the primary antibodies (mice monoclonal anti-SYP antibody (1:1000), mice monoclonal anti-Siah-1 antibody (1:1000) and mice monoclonal anti-β-actin antibody (1:1000)) to incubate the membranes at 4°C overnight. After that, the membranes were rinsed in TBST (150mM NaCl, 50mM Tris(PH7.0), 0.05% Tween 20) three times. The Donkey anti-Mice IRDye800CW secondary anti-bodies (1:5000) for SYP, Siah-1 and β-actin were added to incubate the membranes at room temperature for one hour, and then rinsed in TBST three times again. Densitometry analysis of target protein expression would be measured by Odyssey Infrared Imaging System (LI-COR).

ICP-MS analysis

The mice were executed and the blood was removed by rinsing the tissues of the mice with ultra-pure water, 0.2mL blood and tissues samples were placed in Teflon digestion tank, 5.0mL digestion solution was added, and the temperature on the heating plate was kept at about 180℃ until the decomposition was colorless and transparent. When the solution is cooled, the nitric acid solution was added to 10mL. NexION 300X (PerkinElmer Company, USA) was used to measure the samples. The sample solution was introduced into the instrument for determination, the standard curve was drawn and the concentration of the target elements in the samples was calculated according to the regression equation. Specific machine parameters refer to other research reports [29].

Statistical analysis

The average escape latency of mice in Morris water maze was compared by repeated measurements of variance analysis. Western Blot results analysis: the gray values of total protein Siah-1 and SYP were compared with the gray values of β-actin, respectively. The rest of the data were analyzed by independent sample T test. All the data were processed by SPSS 22.0 software, expressed as mean±standard deviation, and $P<0.05$ suggesting that the difference was significant.

Results

TMT exposure induces the learning and memory impairment We used Morris water maze to show mean group latencies to reach to hidden platform for all groups, which could reflect the learning and memory dysfunction induced by TMT and the protective effect of

HRW. (Fig.1. A) The results showed TMT group had a worse performance, and TMT group mice spent a significant longer time in searching than Control group, especially from the fourth acquisition test. In the subsequent several experiences, the TMT group mice were still unable to locate the hidden platform, and their locating ability did not improve with the number of experiences. After the HRW treatment, the time for mice to search platform gradually reduced and the locating ability enhanced with the experiments, and there was no significant difference between TMT group and HRW group. Moreover, it was obvious that the searching strategies of TMT group mice were more disordered than other groups and spent more time to reach the previous platform region before reaching the target quadrant. Other three group mice adopted a relatively simple linear searching strategy, and the location was more accurate. TMT exposure increases tin content in organs. ICP-MS analysis allowed us to get the tin content in the organs of mice after the treatment of TMT. According to the Tab.1, the tin content in kidney reduced most obviously after the pretreatment of HRW compared to TMT group. Meanwhile, tin content in each brain region also reduced: left cortex was reduced to 0.57, right cortex to 0.61, left hippocampus to 0.76, right hippocampus to 0.64, Cerebellum to 0.87. In brain tissue, the tin content in left cortex decreased most significantly ($P<0.05$), while cerebellum had the most tin residues in the brain region. TMT exposure affects the distribution of Siah-1 and SYP in cortex and hippocampus In order to evaluate the effects of TMT on the expressions of Siah-1 and SYP, we used Immunohistochemical method for detection. (Fig.2. A) In the TMT group, the expressions of Siah-1 in CA3 and Cortex region obviously increased, especially in the CA3 region, but the DG and CA-1 regions did not change significantly compared to the Control group. At the meantime, the expressions of SYP in CA1, CA3, cortex regions also decreased obviously. (Fig.2. B).After a further observation on the CA3 region, the distribution of SYP had an obvious increase and the expression of Siah-1 was similar to the control group after the pretreatment of HRW. TMT exposure affects the protein levels of Siah-1 and SYP in hippocampus. In the Western Blot analysis, we respectively detected the expressions of Siah-1 and synaptophysin in hippocampus (Fig.3. A, B). In TMT group mice, the expressions of Siah-1 increased than other three groups, meanwhile, the expression of SYP also deduced, however, there were not statistical difference in the expression of Siah-1 and SYP between TMT+HRW group and HRW group. Therefore, it was obviously that the enhancement of Siah-1 and the decline of SYP were synchronized in the TMT-induced neurodegenerative disease.

Discussion

The neurotoxin effects of TMT have been widely proved by the researchers [30], but these studies have not well clarified the underlying molecular mechanisms of TMT-induced SYP decline and correlated the toxicity of TMT with tin content and distribution in the brain tissue. Previously, we had reported that TMT could lead to the impairment of spatial memory in mice, and induced the decline of SYP but not GAP-43 in brain which rutin could effectively antagonize [13]. Therefore, in this study, we focused on the spatial impairment of TMT following a single i.p. does of TMT and speculated that TMT could induce the increase of Siah-1 which leading to the decrease of SYP, and finally caused the occurrence of a variety of neurodegenerative diseases. At the meantime, we also proved that hydrogen rich water could protect against TMT induced learning and memory impaired via Siah-1.

Our study illustrated that TMT could simultaneously cause the increase of Siah-1 and the decrease of SYP in TMT-induced neurodegenerative diseases, which suggested that TMT could induce the increase of Siah-1 to promote the ubiquitination degradation of SYP. In our present study, we mainly adopted four methods to explore the neural injury induced by TMT in mice. Firstly, we confirmed the impairment of spatial memory of TMT to mice through Morris water maze task, and the final result was consistent with previous conclusions [13]. In this study, TMT group mice showed the decline in localization ability and search ability, which was inflected by much longer time spent in TMT group compared to Control group. Moreover, the platform searching strategies adopted by TMT group were much more discarded. Especially since the fourth acquisition test, this difference was gradually obvious. However, in Hyun's report, the mice's searching ability has decreased in the first acquisition task; the difference might be caused by the injection dose and the type of mice.

Secondly, we observed the specific expressions and distributions of Siah-1 and SYP in the hippocampus and cortex of mice by Immunohistochemical analysis. The immune activity of SYP can reflect changes in the number of synaptic vesicles, which is regarded as a marker of synaptic density. While over-expression Siah-1 can degrade SYP leading to neurodegenerative diseases. The result of our present study showed that the expression and distribution of Siah-1 in the brain of TMT group mice had an obvious increase and the expression of SYP decreased as expected, moreover, such synchronous increase and decrease was most significant in the CA3 regions.

Besides, we adopted Western blotting to conduct quantitative analysis of two target protein in the mice hippocampus and cortex, the results also illustrated that the increase of Siah-1 always accompanied with the decrease of SYP. In Yanxin Zhao’s report, they found

that, under the combined action of high glucose and hypoxia, the transcriptional SYP could be down-regulated, while the expression of ERK phosphorylation and siah-1 could be increased, and the over expression of Siah-1could promote the ubiquitination and degradation of SYP [20]. While Wheeler TC' report also suggested that Siah-1 had the combined region of SYP which further proved our experimental conclusion [17].

Finally, we detected the tin content in the organs of TMT group mice through ICP-MAS analysis. In the past, it has been reported that tin exists in cerebrospinal fluid of nonneoplastic neurological diseases patients [31] . Therefore, we speculated that the tin content in the brain also played an important toxic role in TMT-induced neurodegenerative diseases. Our results confirmed that the tin content of many organs of mice in TMT group was higher than TMT+HRW group, and the left cortex was the most obvious. However, the underlying metabolic mechanisms still need to be further explored.

The hydrogen in the HRW has the selective antioxidant and anti-inflammatory effects [19], in our previous study we also demonstrated the antioxidant damage effect of HRW in nonalcoholic fatty liver disease. Meanwhile, hydrogen was successively reported to have a therapeutic effect for Parkinson's, Alzheimer's disease and nerve damage caused by stress [21-25] . Therefore, we used the pretreatment (4ml/d, 7d) of hydrogen rich water to investigate the protective effect of HRW in TMT-induced neurodegenerative diseases. Furthermore, the final result showed that after the treatment of HRW for one weeks, the impairment of learning and memory ability of mice was significantly reduced, and the contents of Siah-1 and SYP had not a significant difference between the brain of TMT group mice with that of Control group mice, while the HRW also can effectively reduce the accumulation of tin in organs of mice. As a result, we put forward the view that HRW can antagonize the TMT-induced neurodegenerative diseases. Fortunately, there have been corresponding reports on the treatment of some brain diseases with HRW [32] . They treated hypoxic ischemic encephalopathy disease by giving newborns hydrogen rich water to drink. It is hoped HRW can play a more important role in the clinical treatment of TMT poisoning in the future.

Conclusion

This study expounded that TMT can induce the dysfunction of UPS, leading to the rise of Ub-ligating enzyme-Siah-1 accompanied with the degradation of SYP and spatial learning and memory impairment. This result suggested that the increase of Siah-1 was related with the TMT-induced neurodegenerative diseases, and HRW could antagonize the neuron damage and decrease the accumulation of tin in the organs. However, thisstudy only focuses on the protective effect of HRW on the acute TMT poisoning, and the relationship between the increase of tin content inducing neurological dysfunction and the protective mechanism of HRW in this process still need further study.

Abbreviations

TMT: Trimethyltin; HRW: hydrogen rich water; SYP: Synaptophysin; ICP-MS:Inductively Coupled Plasma Mass Spectrometry

Declarations

Ethics approval and consent to participate

This experiment was approved by the Institutional Animal care and use Committee of the Chongqing Medical University. In this experiment, the research design has scientific basis and the selected animal species, quantity and specification are appropriate. In this experiment, the animals were treated with anesthesia and analgesia and this experiment did not bring corresponding harm to the environment.

Consent for publication

All authors have read and agreed to submit this version for publication, and it is not currently submitted to other journals for consideration

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Funding

This research was supported by Education Commission of Chongqing Municipality (KJ1500211); and Project of Tutorial System of Medical Undergraduate in Lab Teaching & Management Center of Chongqing Medical University (LTMCMTS201804).These funding sponsored the experimental reagents, animals, data collection and analysis in this work.

Authors' contributions

TZ and MZ Planning and execution of research work, and wrote the first draft of the manuscript. HD contributed to detect the tin contents in brain tissue. AA, RY and ML contributed to measure the spatial learning and memory. XC carried out the Western blot. QZ contributed to analysis of data. YW assisted in the research work and revised the manuscript. QQ Supervision and interpretation. All authors read and approved the final manuscript.

Acknowledgements

We thank Dr Cheng-Zhi Chen for his comments and language help in writing this manuscript.

References

1. Brown A W, Aldridge W N, Street B W, Verschoyle RD. The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat. Am J Pathol.1979; 97(1): 59–82.
2. Huang J H, Matzner E. Biogeochemistry of organotin compounds and tin in a forested catchment in Germany. Sci Total Environ. 2004; 332(1-3):231-241.
3. Graceli J B, Sena G C, Lopes P F I, Zamprogno GC, da Costa MB, Godoi AF, Dos Santos DM, de Marchi MR, Dos Santos Fernandez MA. Organotins: A review of their reproductive toxicity, biochemistry, and environmental fate. Reprod Toxicol. 2013 ;36:40-52
4. Tang X, Yang X, Lai G, Guo J, Xia L, Wu B, Xie Y, Huang M, Chen J, Ruan X, Sui G, Ge Y, Zuo W, Zhao N, Zhu G, Zhang J, Li L, Zhou W. Mechanism underlying hypokalemia induced by trimethyltin chloride: Inhibition of H+/K+-ATPase in renal intercalated cells. Toxicology. 2010 ;271(1-2):45-50
5. Woodruff M L, Baisden R H. Trimethyltin Neurotoxicity in the Rat as an Analogous Model of Alzheimer's disease. Toxin-Induced Models of Neurological Disorders. Springer US, 1994; 319-335.
6. Xi Y, Liu M, Xu S, Hong H, Chen M, Tian L, Xie J, Deng P, Zhou C, Zhang L, He M, Chen C, Lu Y, Reiter RJ, Yu Z, Pi H, Zhou Z. Inhibition of SERPINA3N-dependent neuroinflammation is essential for melatonin to ameliorate trimethyltin chloride-induced neurotoxicity . J. Pineal Res. 2019; 67 (3):e12596.
7. Lycopene protects against trimethyltin-induced neurotoxicity in primary cultured rat hippocampal neurons by inhibiting the mitochondrial apoptotic pathway. Neurochemistry International, 2011; 59(8):1095-1103.
8. Choi SJ, Oh SS, Kim CR, Kwon YK, Suh SH, Kim JK, Park GG, Son SY, Shin DH. Perilla frutescens Extract Ameliorates Acetylcholinesterase and Trimethyltin Chloride-Induced Neurotoxicity. J Med Food, 2016; 19(3):281-289.
9. Sandström J, Kratschmar DV, Broyer A, Poirot O, Marbet P, Chantong B, Zufferey F, Dos Santos T, Boccard J, Chrast R, Odermatt A, Monnet-Tschudi F.In vitro models to study insulin and glucocorticoids modulation of trimethyltin(TMT)-induced neuroinflammation and neurodegeneration, and in vivo validation in db/db mice. Arch. Toxicol. 2019;93 (6):1649-1664
10. Kim J, Kim CY, Oh H, Ryu B, Kim U, Lee JM, Jung CR, Park JH. Trimethyltin chloride induces reactive oxygen species-mediated apoptosis in retinal cells during zebrafish eye development. Sci. Total Environ. 2019; 653:36-44
11. Geloso M C, Corvino V, Michetti F. Trimethyltin-induced hippocampal degeneration as a tool to investigate neurodegenerative processes. Neurochem Int.2011; 58(7):729-738.

12. Little AR, Miller DB, Li S, Kashon ML, O'Callaghan JP. Trimethyltin-induced neurotoxicity: Gene expression pathway analysis, q-RT-PCR and immunoblotting reveal early effects associated with hippocampal damage and gliosis. Neurotoxicol Teratol. 2012 ;34(1):72-82
13. Kaur S, Sharma N, Nehru B. Anti-inflammatory effects of Ginkgo biloba extract against trimethyltin-induced hippocampal neuronal injury. Inflammopharmacology. 2018; 26(1):87-104.
14. Ferraz da Silva I, Freitas-Lima LC, Graceli JB, Rodrigues LCM. Organotins in NeuronalDamage, Brain Function,and Behavior: A Short Review. Front Endocrinol (Lausanne).2018 ;8:366
15. Loncarević-Vasiljković N, Pesić V, Tanić N, Milanović D, Popić J, Kanazir S, Ruzdijić S. Changes in markers of neuronal and glial plasticity after cortical injury induced by food restriction. Exp Neurol. 2009; 220(1):198-206.
16. Zhang L , Zhao Q , Chen C H, Qin QZ, Zhou Z, Yu ZP. Synaptophysin and the dopaminergic system in hippocampus are involved in the protective effect of rutin against trimethyltin-induced learning and memory impairment. Nutr Neurosci.2014;17(5):222-229
17. Sulistio YA, Heese K. The Ubiquitin-Proteasome System and Molecular Chaperone Deregulation in Alzheimer's disease. Mol Neurobiol.2016;53(2):905-931
18. Anderson VE, Hajimohammadreza I, Gallo JM, Anderton BH, Uney J, Brown AW,Nolan CC,Cavanagh JB,Leigh PN. Ubiquitin, PGP 9.5 and dense body formation in trimethyltinin toxication: differential neuronal responses to chemically induced cell damage.Neuropathol Appl Neurobiol.1992;18(4):360-375.
19. Lee S, Yang M, Kim J, Kang S, Kim J, Kim JC, Jung C, Shin T, Kim SH, Moon C. Trimethyltin-induced hippocampal neurodegeneration: A mechanism-based review. Brain Res Bull.2016; 125:187-99.
20. Wheeler T C, Chin L S, Li Y, Roudabush FL, Li L. Regulation of synaptophysin degradation by mammalian homologues of seven in absentia.J Biol Chem. 2002 ;277(12):10273-10282
21. Zhao Y, Li Q, Jin A, Cui M, Liu X. E3 ubiquitin ligase Siah-1 downregulates synaptophysin expression under high glucose and hypoxia. Am J Transl Res. 2015;7(1):15-27
22. Ohsawa I,Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals.Nat Med. 2007; 13(6):688-94.
23. Fukuda K1, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S. Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress.Biochem Biophys Res Commun. 2007; 361(3):670-674.
24. Chen C, Chen Q, Mao Y, Xu S, Xia C, Shi X, Zhang JH, Yuan H, Sun X. Hydrogen-Rich Saline Protects Against Spinal Cord Injury in Rats. Neurochem Res. 2010;35(7):1111-1118
25. Sun Q, Kang Z, Cai J, Liu W, Liu Y, Zhang JH, Denoble PJ, Tao H, Sun X. Hydrogen-Rich Saline Protects Myocardium Against Ischemia/Reperfusion Injury in Rats. Exp Biol Med (Maywood). 2009 ;234(10):1212-1219
26. Chen H, Sun YP, Li Y, Liu WW, Xiang HG, Fan LY, Sun Q, Xu XY, Cai JM, Ruan CP, Su N, Yan RL, Sun XJ, Wang Q. Hydrogen-rich saline ameliorates the severity of l-arginine-induced acute pancreatitis in rats. Biochem Biophys Res Commun. 2010 ;393(2):308-313
27. Jiang X, Tang Q, Zhang J, Wang H, Bai L, Meng P, Qin X, Xu G, Bose DD, Wang B, Chen C, Zou Z. Autophagy-dependent release of zinc ions is critical for acute lung injury triggered by zinc oxide nanoparticles. Nanotoxicology.2018;12(9):1068-1091
28. Halladay AK, Wilson DT, Wagner GC, Reuhl KR.Trimethyltin-induced alterations in behavior are linked to changes in PSA-NCAM expression.Neurotoxicology. 2006 ;27(2):137-146
29. Gui W, Tian C, Sun Q, Li S, Zhang W, Tang J, Zhu G . Simultaneous determination of organotin pesticides by HPLC-ICP-MS and their sorption, desorption, and transformation in freshwater sediments.Water Res. 2016;95:185-194
30. Ogita K, Nishiyama N, Sugiyama C, Higuchi K, Yoneyama M, Yoneda Y. Regeneration of granule neurons after lesioning of hippocampal dentate gyrus: evaluation using adult mice treated with trimethyltin chloride as a model. J Neurosci Res. 2005;82(5)609–21.
31. el-Yazigi A, Martin CR, Siqueira EB.Concentrations of chromium, cesium, and tin in cerebrospinal fluid of patients with brain neoplasms, leukemia or other noncerebral malignancies, and neurological diseases.Clinical Chemistry. 1988;34(6):1084-1086.
32. Shen M, He J, Cai J, Sun Q, Sun X, Huo Z. Hydrogen as a novel and effective treatment of acute carbon monoxide poisoni Med Hypotheses. 2010; 75(2):235-237.