Nanosized TiO$_2$ Exposure Resulted in Neurotoxicity via Impairing NMDA Receptor-mediated Postsynaptic Signaling Cascade in Mice

Lei Sheng$^*$, Ling Wang$^*$, Yuguang Ze$^*$, Xiaoyang Zhao$^*$, Xiaohong Yu$^*$, Jie Hong$^*$, Dong Liu$^*$, Bingqing Xu$^*$, Xiaoyu Pan$^*$, Anan Lin$^*$, Yue Zhao$^*$, Chi Zhang$^*$, Yunting Zhu$^*$, Yi Long$^*$ and Fashui Hong$^*$

$^*$Medical College of Soochow University, Suzhou 215123, China
$^*$Library of Soochow University, Suzhou 215021, China
$^*$equally to this work

Abstract

The central nervous system (CNS) toxicity induced by exposure to nano-sized particles is of great concern, but the mechanism of how this toxicity may be incurred has yet to be elucidated. Here, we examined how N-methyl-D-aspartate (NMDA) receptor-mediated postsynaptic signalling cascade may be affected by titanium dioxide particles (TiO$_2$ NPs) exposure for six consecutive months to contribute to the observed neurotoxicity. The results suggest that long-term exposure to TiO$_2$ NPs led to titanium accumulation and iron reduction in the blood and hippocampus tissues, and significant hippocampal injury as well as reduction of learning and memory in mice. The CNS injuries following long-term TiO$_2$ NP exposure were closely associated with significant reductions in NR1, NR2A, NR2B, calcium/calmodulin-dependent protein kinase II, postsynaptic density protein 95, nuclear activated extracellular-signal regulated kinase (ERK1/2), Dextras1, CAPON, peripheral benzodiazepine receptor-associated protein, and divalent metal transporter 1β expression and enhanced nuclear factor-κB (NF-κB) binding activity by increasing microglial activation in the pre-inflamed brain of mice, and led to an exaggerated neuroinflammatory response. Our numerous studies suggested that exposure to TiO$_2$ NPs resulted in excessive species reactive oxygen (ROS) production and decreased antioxidant capacity, calcium overload, proliferation of glial cells, and altered contents trace elements neurotransmitters, led to hippocampal apoptosis via mitochondrial or the intrinsic pathway and a reduction in spatial recognition memory in mice. Furthermore, TiO$_2$ NP-induced oxidative damage in the mouse brain was demonstrated to be via the p38-Nrf-2 signaling pathway, and TiO$_2$ NP-induced neuroinflammation was associated with activation of the TLRs/TNF-α/NF-κB pathway. However, the mechanisms of how this neurotoxicity are not understood.

Keywords: Titanium dioxide nanoparticles; Hippocampus; N-methyl-D-aspartate receptors; Postsynaptic signalling proteins; Neurotoxicity

Introduction

Titanium dioxide nanoparticles (TiO$_2$ NPs) have been used in various areas, including pigment [1], paints [2], medicine [3], sunscreens [4], cosmetics [5], food additives and food packaging [6,7], and in environmental decontamination systems [8,9]. However, numerous studies demonstrated that TiO$_2$ NP exposure can conduct the damages of central nervous system (CNS) [10-17]. For instance, Wang et al. [12] indicated that TiO$_2$ NPs damaged CA1 region of the hippocampus and caused high inflammatory responses by elevating TNF-α and IL-1β levels, oxidative stress in the exposed mice [13]. Shin et al. [18] demonstrated that TiO$_2$ NPs induced TNF-α and IL-1β expression and enhanced nuclear factor-kB (NF-kB) binding activity by increasing microglial activation in the pre-inflamed brain of mice, and led to an exaggerated neuroinflammatory response. Our numerous studies suggested that exposure to TiO$_2$ NPs resulted in excessive species reactive oxygen (ROS) production and decreased antioxidant capacity, calcium overload, proliferation of glial cells, and altered contents trace elements neurotransmitters, led to hippocampal apoptosis via mitochondrial or the intrinsic pathway and a reduction in spatial recognition memory in mice [16,19]. Furthermore, TiO$_2$ NP-induced oxidative damage in the mouse brain was demonstrated to be via the p38-Nrf-2 signaling pathway [20], and TiO$_2$ NP-induced neuroinflammation was associated with activation of the TLRs/TNF-α/NF-kB pathway [21]. However, the mechanisms of how this neurotoxicity are not understood.

N-methyl-D-aspartate receptors (NMDARs), which are glutamate-gated ion channel receptors, are widely expressed in the CNS and play pivotal roles in excitatory synaptic transmission, synaptic plasticity, learning and memory of mammalian brain [22]. NMDARs include different subunits within a repertoire of three subtypes: NR1, NR2 (NR2A-D) and NR3 (NR3A and NR3B) [23]; and NR1 and either NR2B or NR2A are most widely expressed [22]. Exposure to TiO$_2$ NPs was demonstrated to increase glutamate release [19], and to inhibit NR2A and NR2B expression as well as to impair long-term potentiation (LTP) in rat or mouse hippocampus [24,25]. Therefore, we hypothesize that these changes mentioned above may further lead to the impairment of postsynaptic signalling cascade in animals.

In excitatory synapses of the brain, specific receptors in the postsynaptic membrane can rapidly respond to the release of glutamate from the presynaptic terminal. Upon stimulation, these glutamate receptors activate postsynaptic signaling pathways that transduce signals into the postsynaptic neuron [26]. NMDAR activation can result in either LTP or long-term depression (LTD) of synaptic strength. NMDARs are embedded in the postsynaptic density (PSD), which involved in the postsynaptic membrane that contains a variety of scaffolding and signaling proteins. Many of the prominent proteins in the PSD fraction bind directly or indirectly to the NMDA receptor. Thus, the PSD fraction contains a core NMDA receptor-signaling complex, and serves as the signaling scaffold to bridge NMDARs to the intracellular signaling complexes [27-29] and is required to sustain the molecular organization of the postsynaptic density [30]. PSD-95 can also interact with a host of cytoplasmic signaling molecules, such as neuronal nitric oxide synthase (nNOS) and SynGAP, thereby connecting NMDARs to divergent signal transduction pathways [26]. Its overexpression can inhibit LTP and decrease LTD induction.

*Corresponding author: Fashui Hong, Medical College of Soochow University, Suzhou 215123, China, Tel: +86-60512-61117563; Fax: +8660512-65880103; E-mail: hongfsh_cn@sina.com

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receptor-mediated postsynaptic signaling cascade caused by TiO2 NPs. However, the NMDA overproduction [15,19], and reduced iron content in mouse brain NR2B, calcium/calmodulin-dependent protein kinase IV (CaMKIV), cascade in the hippocampus caused by TiO2 NP exposure. Other chemicals were purchased from Shanghai Chemical Co. assay (ELISA) commercial kits were purchased from R&D Systems from Sigma-Aldrich Company. Cell Lysis Kits were purchased from ACIDP 2020M+C instrument (Micromeritics Co., USA). The average particle size of the ζ potential after 24 h incubation was 9.28 mV [16].

Animals and treatment

One hundred and sixty CD-1 (ICR) female mice (24 ± 2 g) were purchased from the Animal Center of Soochow University (China). The mice were housed in stainless steel cages in a ventilated animal room. The room temperature in the housing facility was maintained at 24 ± 2°C, with a relative humidity of 60 ± 10% and a 12 h light/dark cycle. Distilled water and sterilized food were available ad libitum. Before treatment, the mice were acclimated to this environment for five days. All the animals were handled in accordance with the guidelines and protocols approved by the Care and Use of Animals Committee of Soochow University (China).

For the experiment, the mice were randomly divided into four groups (N=40 in each group), including a control group (treated with 0.5% w/v HPMC) and three experimental groups (1.25, 2.5, or 5 mg/kg BW TiO2 NPs). The mice were weighed, volume of TiO2 NP suspensions was calculated for each mouse, and the fresh TiO2 NP suspensions were administered to the mice by nasal administration every day for 6 months. Any symptom or mortality was observed and recorded carefully everyday during the 6 months. In addition, the mice were regularly handled and weighed before the behavioral experiments.

Behavioral experiment

Following the 6 months of TiO2 NP administration, the acquisition of spatial recognition memory was determined using the Y-maze in mice (N=10 in each group). In order to avoid any stress-related interference with the learning procedure, mice were not handled by the experimenter but were allowed to voluntarily enter the maze. To assess spatial recognition memory, the Y-maze test consisted of two trials separated by an intertrial interval (ITI). The Y-maze was consisted of three arms and was randomly designated: Start arm, in which the mouse started to explore (always open), Novel arm, which was blocked during the 1st trial, but open during the 2nd trial, and other arm (always open). The maze was placed in a sound attenuated room with dim illumination. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze, and the observer was always in the same position at least 3 m from the maze. Assay of acquisition of spatial recognition memory in mice was described in previous reports [42,43]. To measure spatial recognition memory, the number of entries and time spent in each arm of the maze by each mouse was recorded and novelty versus familiarity was analyzed by comparing behavior in all three arms. The number of arms visited was taken as an indicator of locomotor and exploratory activity.

Preparation of hippocampus

After behavioral detection, mice were weighed. Blood samples were collected from the eye vein by rapidly removing the eyeball. The hippocampi from all animals were quickly dissected from brains and placed in ice-cold dish.

Analysis of titanium and iron content

The hippocampi were thawed and approximately 0.1 g samples were weighed, then these tissues and 5 ml blood were digested, and analyzed for titanium, and iron content (N=5 in each group). Briefly, 43x441]receptor-mediated postsynaptic signaling cascade caused by TiO2 NPs. However, the NMDA overproduction [15,19], and reduced iron content in mouse brain NR2B, calcium/calmodulin-dependent protein kinase IV (CaMKIV), cascade in the hippocampus caused by TiO2 NP exposure. Other chemicals were purchased from Shanghai Chemical Co. assay (ELISA) commercial kits were purchased from R&D Systems from Sigma-Aldrich Company. Cell Lysis Kits were purchased from ACIDP 2020M+C instrument (Micromeritics Co., USA). The average particle size of the ζ potential after 24 h incubation was 9.28 mV [16].

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prior to elemental analysis, the blood and hippocampal tissues were digested overnight with nitric acid (ultrapure grade). After adding 0.5 mL of H₂O₂, the mixed solutions were incubated at 160°C in high pressure reaction containers in an oven until the samples were completely digested. Then, the solutions were incubated at 120°C to remove any remaining nitric acid until the solutions were colorless and clear. Finally, the remaining solutions were diluted to 3 mL with 2% nitric acid. Inductively coupled plasma-mass spectrometry (Thermo Elemental X7; Thermo Electron Co., Waltham, MA, USA) was used to determine the titanium, and iron concentration in the samples. Indium of 20 ng/mL was chosen as an internal standard element. The detection limit of titanium, and iron was 0.089 ng/mL, and 0.062 ng/mL, respectively.

**Histopathological examination**

For pathologic studies, all histopathologic examinations were performed using standard laboratory procedures. Briefly, hippocampi (N=5 in each group) were embedded in paraffin blocks and placed onto glass slides. After hematoxylin–eosin staining, the stained sections were evaluated by a histopathologist unaware of the treatments, using an optical microscope (Nikon U-III Multi-point Sensor System, Japan).

**Assay of gene and protein expression**

The levels of mRNA expression of NR1, NR2A, NR2B, CaMKII, PSD-95, SynGAP, ERK1/2, Dexras1, CAPON, PAP7, DMT1, and nNOS in the hippocampi were determined using real-time quantitative RT polymerase chain reaction (RT-qPCR) (N=5 in each group) [44-46]. Synthesized cDNA was used for the real-time PCR. Primers were designed using Primer Express Software according to the software guidelines (Table 1). Total RNA was extracted from individual hippocampi using from the homogenates was isolated using Tripure Isolation Reagent (Roche, USA) according to the manufacturer's instructions. The RT reagent (Shinegene Co., China) of 30 µl was prepared by mixing 15 µl of 2×RT buffer, 1 l random primer in a concentration of 100 pmol·µl⁻¹, 1µl of RTase, 5 µl RNA, and 8 µl DEPC water together. The reaction condition was 25°C for 10 min, 40°C for 60 min, and 70°C for 10 min. The internal reference gene was actin3. qRT-PCR was performed using the 7500 Real-time PCR System (ABI) with SYBR Premix Ex Taq” (Takara) according to the manufacturer’s instructions. The RT-qPCR data were processed with the sequence detection software version 1.3.1 following the method of Schefe et al. [47], analyzed based on the standard curve using the threshold cycle (Ct) model for relative quantification [45] and the expression levels of mRNA of all genes were normalized by the contents of actins mRNAs.

To determine protein levels of NR1, NR2A, NR2B, CaMKII, PSD-95, SynGAP, ERK1/2, Dexras1, CAPON, PAP7, DMT1, and nNOS in the hippocampi, total protein from the frozen hippocampal tissues (N=5 in each group) from experimental and control mice was extracted using Cell Lysis Kits (GENMED SCIENTIFICS INC.USA) and quantified using BCA protein assay kits (GENMED SCIENTIFICS INC.USA). ELISA was performed using commercial kits that were selective for each respective protein (R&D Systems, USA), following the manufacturer’s instructions. The absorbance was measured on a microplate reader at 450 nm (Varioskan Flash, Thermo Electron, Finland), and the concentrations of NR1, NR2A, NR2B, CaMKII, PSD-95, SynGAP, ERK1/2, Dexras1, CAPON, PAP7, DMT1, and nNOS were calculated from a standard curve for each sample.

**Statistical analysis**

All results are expressed as means ± SD. The Kolmogorov-Smirnov test with Dunn’s post test was used to compare control and treated groups using SPSS 19 software (SPSS, Inc., Chicago, IL, USA). A P-value<0.05 was considered statistically significant.

| Gene name | Description | Primer sequence | Primer size (bp) |
|-----------|-------------|-----------------|-----------------|
| Refer-actin | macdin-F | 5'-GAGACCTTCAACACACCCACGC-3' | 263 |
| | macdin-R | 5'-ATGTCAGCAAGATTTCCCTC-3' | |
| NR1 | mNR1-F | 5'-CAGTGGCAGAAGTTCTCC-3' | 166 |
| | mNR1-R | 5'-CCTTCCTACTCTTCCTGC-3' | |
| NR2A | mNR2A-F | ATGAAAGCCGACTGACCTAAG | 246 |
| | mNR2A-R | GGCTCCGCTGCTTGATGA | |
| NR2B | mNR2B-F | AATGTTGAGTTGGAGATAG | 255 |
| | mNR2B-R | ATTACCTCGGCTTGGGACT | |
| CaMKII | mCaMKII-F | 5'-ATCCAGGTCGAGGCTTCG-3' | 175 |
| | mCaMKII-R | 5'-GGGTCAGACATCTCTCAG-3' | 191 |
| PSD--95 | mPSD-95-F | 5'-GTTCCTCCAGAAAGTTTGAG-3' | 100 |
| | mPSD-95-R | 5'-CCGCCAGAAGCTGGACG-3' | |
| SynGAP | mSynGAP-F | 5'-ATCAACGCTTAACCCACA-3' | 144 |
| | mSynGAP-R | 5'-CTCATAGCTTGACCCTTCG-3' | 137 |
| ERK1/2 (Mapk1) | mERK1/2-F | 5'-GCAACGTGAGGATCTCAAGGC-3' | 146 |
| | mERK1/2-R | 5'-TGCGCAGCCACCAAGAAA-3' | 138 |
| | mDexras1-F | 5'-GACTGAGGAGCTTCACCAG-3' | 144 |
| | mDexras1-R | 5'-GCTGAAACCAAGATGAAGACG-3' | |
| CAPON | mCAPON-F | 5'-ACGAGAATCTACGCCTCGG-3' | |
| | mCAPON-R | 5'-TCTCGAGGGGTCGGTGGAG-3' | 263 |
| PAP7 | mPAP7-F | 5'-GAGAAGTCTCACACTCCG-3' | |
| | mPAP7-R | 5'-AAATAACCCCAAATTGACTC-3' | |
| nNOS | mnNOS-F | 5'-CGGTCGTCAACCCTCCTGCT-3' | 144 |
| | mnNOS-R | 5'-TGAGCAGGAGGAGACAC-3' | |
| DMT1 | mDMT1-F | 5'-TCACATGAGGAGACTTTTTG-3' | |
| | mDMT1-R | 5'-GACAGGAGCGACGAACAT-3' | 174 |

Table 1: Real time PCR primer pairs. PCR primers used in the gene expression analysis.
Results

Spatial recognition memory and locomotor activity

Table 2 exhibits effects of TiO2 NPs on the spatial recognition memory of mice. It can be observed that the percentage duration in the novel arm in control mice was significantly higher than that in the start and other arms, whereas the percentage duration in the novel arm in 1.25, 2.5, or 5 mg/kg BW TiO2 NP-exposed mice was significantly decreased as compared to the control mice throughout the experiment (P<0.05), respectively, suggesting that long-term exposure to TiO2 NPs reduced learning and memory of mice. To confirm effects of TiO2 NPs on locomotor activity of mice, number of arm visits was also examined and are presented in Figure 1. With increased TiO2 NP dose, the number of arm entries markedly decreased (P<0.05).

Titanium and iron contents

Figure 2 presents titanium and iron contents in the blood and hippocampus caused by TiO2 NP exposure. With increased TiO2 NP dose, there were significant increases of titanium levels, whereas iron levels were markedly reduced in the blood and hippocampus (Figure 2, P<0.01). Titanium content in the control mice was negligible (Figure 2). The increased titanium and decreased iron may lead to hippocampal injury and impairment of hippocampal function, which were confirmed by the assays of NMDA receptor and postsynaptic signalling factors as well as histopathological observations of mouse hippocampus.

Hippocampal histopathological observations

Following long-term exposure to 1.25, 2.5, or 5 mg/kg BW TiO2 NPs, histopathological changes from hippocampal CA region were observed (Figure 3B-3D), which suggested significant edema of glial cells, disperative replication of neuron cells, decreased size of cell volume, nuclear irregularity, and necrosis or abscission of neuron cells.

Expression of NMDA receptor subunit and postsynaptic signaling factor

In the present study, actin3 was chosen as the endogenous control gene. The expression level of the actin3 gene was constant, with an

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### Table 2: Effect of TiO2 NPs on the spatial recognition memory of mice in Y-maze after nasal administration of TiO2 NPs for six consecutive months.

|          | Control | 1.25 mg/kg | 2.5 mg/kg | 5 mg/kg |
|----------|---------|------------|-----------|---------|
| Novel arm| 44 ± 5.5| 30 ± 4.2** | 16 ± 2.8**| 6 ± 1.7***|
| Start arm| 26.5 ± 2.9| 32 ± 4.1* | 38 ± 5.7* | 42 ± 5.9**|
| Other arm| 29.5 ± 3.3| 38 ± 5.1* | 46 ± 6.6**| 52 ± 7.8**|

*p < 0.05, and **p<0.01. Values represent means ± SD (N=10).
ELISA. As the dose of TiO₂ NPs increased, there were significant decreases of iron contents in the blood and hippocampus (Figure 2). Our previous study has been confirmed by the markedly increased titanium levels in the blood and hippocampus (Figure 2). Our previous study has been confirmed by the markedly increased titanium levels in the blood and hippocampus (Figure 2). Our previous study has been confirmed by the markedly increased titanium levels in the blood and hippocampus (Figure 2). Our previous study has been confirmed by the markedly increased titanium levels in the blood and hippocampus (Figure 2).

### Discussion

In the current study, the effects of long-term exposure to TiO₂ NPs on the expression of NMDA receptor and postsynaptic signaling factors in mouse hippocampus were evaluated. The TiO₂ NP accumulation and the levels of the 12 NMDA receptor subunit and/or postsynaptic signaling factor genes were evaluated and compared following exposure to TiO₂ NPs for six consecutive months (Table 3).

Long-term exposure to TiO₂ NPs resulted in a dose-dependent marked decrease in the mRNA and protein expression of NMDA receptor subunits, including NR1, NR2A and NR2B in the hippocampus (Tables 3 and 4), suggesting reductions of 16.67%, 64.71% and 75.49%; 15.63%, 62.41% and 77.11% for NR2B, respectively.

In the current study, the effects of long-term exposure to TiO₂ NPs on the expression of NMDA receptor and postsynaptic signaling factors in mouse hippocampus were evaluated. The TiO₂ NP accumulation was confirmed by the markedly increased titanium levels in the blood and hippocampus (Figure 2), suggesting that TiO₂ NPs can easily cross the blood–brain barrier into the hippocampus, depositing TiO₂ NPs in the hippocampus (Figure 2) and damaging hippocampus (Figure 3). In addition, TiO₂ NP exposure resulted in reductions of iron contents in the blood and hippocampus (Figure 2). Our previous study has been demonstrated that exposed mice to TiO₂ NPs presented low iron content [20]. Numerous studies demonstrated that TiO₂ NP accumulation and iron deficiency in mouse brain resulted in excessive production of species reactive oxygen (ROS), and increased peroxidation levels [15-17,19-50], which may damage hippocampus. The Y maze is regarded as one of common behavioral tasks to evaluate cognitive abilities of rodents. Hippocampus-dependent spatial learning and memory are frequently investigated by observing the behavioral performance of animals in the Y maze. The results of this study indicated that long-term exposure to TiO₂ NPs resulted in decreases in spatial recognition memory (Table 2), for example, the time spent in the unfamiliar novel arm in the TiO₂ NP-exposed mice was lower than unexposed mice (Table 2).

Locomotor activity acts as a function of the excitability level of CNS neurons [51]. The present study shows that TiO₂ NP expression for six consecutive months decreased locomotor activity in mice (Figure 1), which is consistent with our previous reports [15-17,19-21,25]. Decreased spatial cognition of mice caused by TiO₂ NP exposure may be closely associated with the accumulation of TiO₂ NPs, reduction of iron uptake and the damaged hippocampus. Furthermore, decreased spatial cognition of mice may be triggered through NMDA receptor-mediated postsynaptic signaling cascade in the hippocampus.

The present study shows that long-term exposure to TiO₂ NPs significantly decreased NR1, NR2A and NR2B in the hippocampus.
(Tables 3 and 4), which is consistent with our previously reported results [25]. This finding supports our assumed alteration of NMDA receptor in the TiO2 NP-exposed mice. Numerous important NMDA receptor properties are influenced by the subunits composing the receptor assembly [52]. It was reported that LTP in the hippocampus is specifically related to NR2A-containing NMDARs [53]. TiO2 NP exposure was suggested to markedly inhibit the induction and establishment of LTP in rats and mice [24,25]. Alteration of NMDA receptor expression may affect the expression of post-synaptic signaling factors. Upon NMDA receptor stimulation, CaMKII is essentially induced and is essential for NMDAR-dependent LTP [54]. CaMKII expression has been demonstrated to play an important role in learning, memory, and synaptic plasticity [55]. Toscano et al. [56] demonstrated that Pb2+ exposure could decrease CaMKII activity and expression in rats. In current study, reduced NR1, NR2A and NR2B and CaMKII expression were found in the TiO2 NP-exposed mice, suggesting that TiO2 NPs may disrupt the normal NMDA receptor assembly and the function of CaMKII. Our previous finding also indicated that TiO2 NP exposure led to reductions of CaMKIV activity and expression, spatial cognition, and synaptic plasticity in mice [25]. As a signaling scaffold, PSD-95 brings the Ca2+ influx to the specific downstream signaling events [29]. Our data suggest that with increased TiO2 NP dose, decreased PSD-95 expression in the hippocampus was significantly observed (Tables 3 and 4), which would impair the molecular organization of the postsynaptic density, synaptic strength and plasticity [30]. SynGAP had been demonstrated to be a negative regulator of Ras at excitatory synapses [33], and to be inhibited by CaMKII phosphorylation [57]. Furthermore, ERK activation had been suggested to play an important role in the consolidation and reconsolidation of recognition memory [58]. In the present study, our data show that TiO2 NP exposure significantly reduced CaMKII expression and increased SynGAP expression, leading the inhibition of ERK1/2 expression in mouse hippocampus (Tables 3 and 4).

Nitric oxide (NO) may not freely diffuse to reach its physiological targets but may be conveyed to these sites by interactions of NOs with other proteins [40]. As shown by reports, nNOS can bind to the PSD-95/93, which in turn binds to NMDA receptors [59,60]. This ternary complex enables NO to S-nitrosylate NMDA receptors and alters their signaling [61]. Therefore, we presume that increased nNOS expression and decreased PSD-95 expression caused by TiO2 NPs may influence NO to S-nitrosylate NMDA receptors and interfere their signaling in the hippocampus.

CAPON was identified to be a 55 kDa protein that contains a C-terminal domain that binds to the PDZ domain of nNOS and an N-terminal phosphotyrosine binding (PTB) domain [38], and interacts with Dcxras1 [40,62,63]. While Dcxras1 shares about 35% homology with the Ras subfamily of proteins and contains all of the conserved domains of typical GTPases, and has also been designated an activator of G protein signaling 1 (AGS1) or RASD1 [40,64], activating extracellular signal-regulated kinases 1, 2 (ERK1, 2) [65-67], PAP7 is proved to bind to DMT1, the only known physiological import channel for iron, activation of NMDA receptor stimulates nNOS, resulting in S-nitrosylation and activation of Dcxras1, which induces iron uptake via interactions with PAP7 and DMT1. Glutamate, acting via NMDA receptors, activates nNOS to form NO [37], which leads to protein S-nitrosylation [68]. This modification activates Dcxras1, which, by its link to PAP7, augments both Tf-mediated and NTBI uptake. From Figure 3, we observed a marked reduction of the Fe content in the TiO2 NP-exposed hippocampus. The roles of intraneuronal iron are involved in synthesis, packaging of neurotransmitters, uptake as well as degradation of the neurotransmitters into other iron-containing proteins that may directly or indirectly alter brain function through peroxide reduction, amino acid metabolism and fat desaturation, thus changing post synaptic membrane functioning [69].

In the present study, long-term exposure to TiO2 NPs significantly decreased levels of ERK1/2, Dcxras1, CAPON, PAP7, and DMT1 expressions and elevated nNOS level (Tables 3 and 4), which may be associated with reduction of iron uptake (Figure 2), thus impairing NMDA-NO-Dcxras1-PAP7-DMT1-iron uptake post synaptic signaling cascade in the hippocampus [70].

Conclusion

Mice were exposed to TiO2 NPs for six consecutive months, titanium accumulation and iron reduction in the blood and hippocampus tissues were observed, which in turn resulted in significant hippocampal injury and reduction of spatial cognition in mice. The CNS injuries following long-term TiO2 NP exposure may be closely associated with NMDA receptor-mediated postsynaptic signaling cascade, marked by significant reductions in NR1, NR2A, NR2B, CaMKII, PSD-95, ERK1/2, Dcxras1, CAPON, PAP7, and DMT1 expressions as well as elevations of SynGAP and nNOS expressions in the hippocampus. Therefore, the application of TiO2 NPs should be carried out cautiously, especially in humans.

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