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Involvement of neurotrophins and related signaling genes in TiO_2 nanoparticle–induced inflammation in the hippocampus of mouse

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Abstract

Background: Titanium dioxide particles (TiO_2 NPs) have been widely used in industries and daily life; their potential neurotoxic effects are of great concern. The aim of present study is to determine whether TiO_2 NPs exposure mediated neurotrophins and related receptor expression in the mouse hippocampus under TiO_2 NPs-induced neuroinflammation.

Methods: Mice were nasally administered by 0.25, 0.5, or 1 mg/kg body weight TiO_2 NPs for nine months, how neurotrophin-related factors and signaling pathway might be affected by TiO_2 NPs exposure for nine months, using real-time PCR and ELISA methods was investigated.

Results: The results suggest that exposure to TiO_2 NPs caused TiO_2 NPs deposition, excessive proliferation of all glial cells and tissue necrosis in the hippocampus. The hippocampal injury due to TiO_2 NPs exposure were closely associated with significant reduction in nerve growth factor, brain-derived neurotrophic factor, tyrosine kinases (Trk A, B), calcium/calmodulin-dependent protein kinases (CaMKII, CaMKIV), cyclic-AMP responsive element binding proteins (CREB-1, CREB-2), dopamine receptors (D1, D2), and Tyrosine hydroxylase expression in the hippocampus.

Conclusions: The findings imply that long-term exposure to TiO_2 NPs may induce neuroinflammation via impairing neurotrophin-mediated signaling pathway in animals.

1. Introduction

Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in various areas such as plastics, toothpaste, paper and skin care products, food, medicine, and environment due to their high stability, anti-corrosiveness, and photo-catalytic properties. Because of these versatile benefits, the toxicol properties of TiO_2 NPs have been elucidated during recent years, [1-3] especially TiO_2 NPs have been demonstrated neurotoxicant, central nervous system (CNS) is a primary target in both acute and chronic exposure of TiO_2 NPs-toxicity. [4-13] Past data from invivo and invitro animal studies strongly support that hippocampus is target for TiO_2 NPs. [12-17] However, little is known about the effects of TiO_2 NPs on neurotrophin-related factor in the damaged hippocampus. TiO_2 NPs exposure may cause alteration in the hippocampal function of individuals with underlying stressful conditions. [13, 14-17] Therefore, in the present study we used TiO_2 NPs as a stressor to investigate whether exposure to low level and longer period of TiO_2 NPs might also affect hippocampal neurotrophin-related factor expression.

As we know, neurotrophins belong to be a group of structurally related polypeptides, they support the survival, differentiation, and maintenance of neuronal population that express appropriate high-affinity neurotrophin receptors. Neurons in the hippocampus are maintained by neurotrophins including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and the tyrosine kinase (Trk) family of neurotrophin receptors. Neurotrophins and their related receptors have been suggested as neurotoxicant targets and are known to play a role in bidirectional signaling between cells of the immune and nervous system. Numerous studies

demonstrated the function of neurotrophic factors and their receptors in the brain, because these factors and receptors may be possible target for neurotoxicants like lead, [18] chlorpyrifos,[19] and toluene. [20] While the neurobehavioral and neurotoxic effect of TiO_2 NPs have been thoroughly examined, the mechanism by which TiO_2 NPs exert their effect in the brain is not fully understood. Exposure to TiO_2 NPs led to persistent deficit in spatial learning and memory in mouse brain. [10, 13-15] From a dosimetric analysis of behavioral effects of subchronic TiO_2 NPs exposure in mouse, cognitive function depend on TiO_2 NPs dose and duration of exposure. [10, 13-16] TiO_2 NPs also affect the neurotransmitter system in the mouse brain. Wang et al. indicated that norepinephrine, Dopamine and its metabolite 3, 4-dihydroxyphenylacetic acid, 5-hydroxytryptamine and its metabolite 5-hydrooxyidoleaacetic acid were reduced, however, acetylcholine, glutamate, and nitric oxide were elevated in TiO_2 NPs-exposed mouse brain. [7] Expecially, recent study showed that TiO_2 NPs exposure cause neuroinflammation in mouse hippocampus, which involved in activation of the expression of Toll-like receptors, tumor necrosis factor-α, nucleic IκB kinase, NF-κB-inducible kinase, nucleic factor–κB, NF-κB2(p52), RelA(p65), and suppression of IκB and interleukin-2 expression.[13] However, whether the neuroinflammation is triggered by neurotrophin-related factors or signaling pathway is unclear.

We hypothesized that brain inflammation due to TiO_2 NPs exposure may be via the modulation of neurotrophin-related factors and signaling pathway affecting the function of the hippocampus in mice. The present study designed to investigate

whether TiO_2 NPs exposure mediated neurotrophins and related receptor expression in the mouse hippocampus and examine the effect of TiO_2 NPs exposure on neurogenesis-related markers in the hippocampus. Moreover, the study would be helpful to understand the toxic effects of TiO_2 NPs exposure on the CNS in animals and human.

2. Materials and Methods

2.1 Chemicals

The preparation, characteristics of TiO_2 NPs including the anatase structure, size, surface area, mean hydrodynamic diameter and ζ potential have been described in our previous work.[14, 21] Briefly, the average particle size of powdered TiO_2 NPs suspended in 0.5% w/v hydroxypropylmethylcellulose (HPMC) K4M solvent after 24 h (5 mg/mL) incubation ranged from 5 to 6 nm, and the surface area was 174.8 m^2/g. The mean hydrodynamic diameter of TiO_2 NPs in HPMC solvent (5 mg/mL) ranging from 208 to 330 nm (mainly 294 nm), and the ζ potential after 24 h incubation was 9.28 mV.[14] The anatase structure, size, surface area, mean hydrodynamic diameter and ζ potential have been described in supplementary file.

2.2 Ethics Statement

All experiments were conducted during the light phase and were approved by the Animal Experimental Committee of Soochow University (Grant 2111270) and in accordance with the National Institute of Health Guidelines for the Care and Use of

Laboratory Animals (NIH Guidelines).

2.3 Animals and treatment

One hundred CD-1 (ICR) 4-week old 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, provided with distilled water and sterilized food *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*=25 in each group), including a control group (treated with 0.5% w/v HPMC) and three experimental groups (0.25, 0.5, or 1 mg/kg BW TiO_2 NPs). The mice were weighed, volume of TiO_2 NPs suspensions was calculated for each mouse, and the fresh TiO_2 NPs suspensions were nasally administered to the mice that were lightly anesthetized with ether and sloped to 45° by a flat head syringe (10 μl) every day for nine months. Any symptom of morbidity, or any mortality was observed and recorded carefully everyday during nine month period

2.4 Preparation of hippocampus

After nine months, mice were weighed and killed under mild ether anesthesia. The hippocampi from all animals were quickly dissected from brains and placed in

ice-cold dish. Hippocampal indices (coefficients of hippocampus, *N*=10 each) were calculated as the ratio of hippocampus (wet weight in mg) to body weight (g).

2.5 Analysis of titanium

The hippocampi were thawed and approximately 0.1 g samples were weighed, then these tissues were digested, and analyzed for titanium content (*N*=5 in each group). Briefly, prior to elemental analysis, the hippocampal tissues were digested overnight with nitric acid (ultrapure grade). After adding 0.5 mL of H_2O_2, 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 in the samples. Indium of 20 ng/mL was chosen as an internal standard element. The detection limit of titanium was 0.089 ng/mL.

2.6 Histopathological examination

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

2.7 Assay of gene and protein expression

Total RNA was extracted from individual hippocampus from homogenates was isolated using Tripure Isolation Reagent (Roche, USA) according to the manufacturer's instructions (*N*=5 in each group). Probes and cycling condition were optimized in accordance with MIQE guidelines for PCR.[22] Synthesized cDNA was used for the real-time PCR by employing primers designed using Primer Express Software according to the software guidelines. PCR primers used in the gene expression analysis are listed in Table 1. Gene expression level was calculated as a ratio to the expression of the reference gene, GAPDH and data were analyzed using the ΔΔCt method. The probes for neurotrophin-related genes (i.e., *NGF*, *BDNF*, *TrkA*, *TrkB, calcium/calmodulin-dependent protein kinases (CaMKII, CaMKIV)*, *cyclic-AMP responsive element binding proteins (CREB-1*, *CREB-2)*, *dopamine receptors (D1*, *D2)*, and *Tyrosine hydroxylase (TH)* were designed by the manufacturer and purchased from Shinegene Company (Shanghai, China). The RT-qPCR data were processed with the sequence detection software version 1.3.1 following the method of Schefe et al.[23]

To determine protein levels of NGF, BDNF, TrkA, TrkB, CaMKIV, CREB1, CREB2, D1, D2, and TH 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). Enzyme linked immunosorbent assay (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 concentration of NGF, BDNF, TrkA, TrkB, CaMKIV, CREB1, CREB2, D1, D2, and TH was calculated from a standard curve for each sample.

2.8 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.

3. Results

3.1 Body weight, hippocampal indices and titanium content

Table 2 lists net increase of body weight, hippocampal indices and titanium accumulation in the hippocampus. It can be seen that TiO_2 NPs exposure resulted in significant reduction of body weight and hippocampal indices, and obvious increase of titanium accumulation ($p<0.05$). The decreased hippocampal indices caused by TiO_2 NPs exposure may be related to tissue injury and TiO_2 NPs deposition in the mouse hippocampus which are confirmed by the further histopathological observations of hippocampus of mice.

3.2 Histopathological observations

Figure 1 shows the histopathological changes in the mouse hippocampus. In control

mice, the hippocampal tissue showed normal brain architecture (Fig. 1), suggesting that the control had no abnormal pathological changes in the hippocampus. However, in mice exposed to 0.25, 0.5, or 1 mg/kg BW TiO_2 NPs, excessive proliferation of all glial cells, and tissue necrosis were observed (Fig. 1), which may be related to expressions of neurotrophin-related factors and signaling pathway.

3.3 Neurotrophins and related receptors

Figure 2 shows alteration of neurotrophins and related receptor expression in the TiO_2 NPs-exposed hippocampus in mice. NGF mRNA and its protein expression in the hippocampus were obviously decreased with increasing dose of TiO_2 NPs ($P < 0.05$). The mRNA and protein expression of TrkA, a receptor for NGF also significantly lowered in mice exposed to TiO_2 NPs ($P < 0.05$). BDNF mRNA and its protein expressions in the hippocampus were greatly reduced in mice exposed to TiO_2 NPs ($P < 0.05$). The mRNA and protein expression of TrkB, the receptor for BDNF, were also remarkably inhibited in mice due to TiO_2 NPs exposure ($P < 0.05$).

3.4 Transcription factor

As we know, multiple protein kinases have been demonstrated to translocate to the nucleus to activate transcriptional activator CREB in NGF signal transduction pathways. Two of the kinases are CaMKII and CaMKIV, which phosphorylates CREB, we assayed the gene and protein expressions of both CaMKII and CaMKIV, and observed significant reductions of both CaMKII and CaMKIV expression in the TiO_2 NPs-exposed hippocampus of mice ($P < 0.05$, Fig.3). We also examined the expression of NGF-related transcriptional factors, CREB1 and CREB2 expressions in

the hippocampus, suggesting marked reductions of gene and protein expression of both CREB1 and CREB2 due to TiO_2 NPs exposure ($P < 0.05$, Fig.3).

3.5 Dopamine markers

The dopamine receptor signaling pathway may be related to cross-talk with neurotrophin signaling pathways following exposure to TiO_2 NPs. To confirm the signaling pathway mediating the effect of TiO_2 NPs in the hippocampus, we examined the gene and protein expression of D1 and D2, the results are shown in Fig. 4. Here, we obsereved significant reductions of D1 and D2 expression in mice exposed to TiO_2 NPs. Tyrosine hydroxylase (TH) is involved in dopamine receptor activity. Therefore, we also detected the expression of TH gene and its protein in the hippocampus, indicating lower expressions due to TiO_2 NPs exposure (Fig. 4).

4. Discussion

Numerous studies have demonstrated that TiO_2 NPs could cross the blood–brain barrier and translocate into the CNS of exposed mice, [6, 7, 9, 10, 13, 16] especially inhaled TiO_2 NPs had been demonstrated to enter the olfactory nerve layer, granular cell layer of olfactory bulb, and olfactory ventricle and further the hippocampus, thalamus, and CA3 area of the brain through the olfactory nervous system,[24] and led to neuroinflammation.[10, 12, 16] In the present study, TiO_2 NPs exposure for longer duration and the lower dose resulted in reduction in body weight and hippocampal indices, TiO_2 NPs deposition and over proliferation of all glial cells, and necrosis in mouse hippocampus. As we know, excessive proliferation of all glial cells is an important

event in CNS damage. Glial cells are required for the development, formation and repair of neurons; and the axonal regeneration process is guided by these cells. In the present study, excessive proliferation of all glial cells due to TiO_2 NPs exposure suggested that inflammatory/immune responses occurred in the hippocampus in addition to hippocampal injury. The neuroinflammation of mice was triggered by TiO_2 NPs, as evidenced by altered expression levels of the genes and their proteins involved in neurotrophin-mediated signaling pathway. Previous report show that the NF-κB–mediated inflammatory cascade and histopathological changes in the mouse brain or hippocampus observed following exposure TiO_2 NPs.[11,16] However to our knowledge, there are no published values(data) on the effects of nanomaterial particles on the neurotrophin-mediated signaling pathway in neuroinflammation in animals.

As we know, NGF can regulate inflammation as well as neural hyper-responsiveness, [25, 26] suggesting that NGF is associated with neural and immune sensitization. Among the neurotrophins, NGF, BDNF and Trk play crucial roles in the survival and development of specific peripheral and brain neurons. [27-29] They are synthesized and released by a variety of cells localized in the central and peripheral nervous system and by cells of the immune and endocrine systems.[30, 31] The major findings of our present study are that TiO_2 NPs exposure for longer duration and the lower dose can down-regulate the expressions of neurotrophins and their related receptors in the mouse hippocampus. Our data suggested that TiO_2 NPs exposure with increasing dose significantly inhibited the mRNA and protein expression of NGF,

BDNF, TrkA, and TrkB in the hippocampus of mice. One possibility is that TiO_2 NPs contribute to neural dysfunction related to sensory stimulation; and these reductions of neurotrophin expression and homeostatic mechanisms were involved in toxicant-induced changes. Neurotrophins play critical roles in neurogenic inflammation by modulating the activity of sensory neurons and decreasing the synthesis and release of neuropeptides.[32] Another possibility is that in TiO_2 NPs-exposed mice, immune system might be impaired. Our previous studies have demonstrated that TiO_2 NPs exposure could decrease immune capacity in mice.[33-39] In addition, the decreased NGF and its receptor TrkA expression may promote cell or tissue damages and may decrease cell repair and remodeling of damaged tissue in the mouse hippocampus due to TiO_2 NPs exposure.[40] Taken together, alteration of brain NGF expression may indicate a link between nervous, endocrine and immune systems and may translate environmental messages into pathophysiological responses. NGF and BDNF take part in the fine tuning of learning and memory performances and in some behavioral processes associated with stress conditions.[27, 40] In the present study, we observed significantly reduction of BDNF and its receptor TrkB expressions in the TiO_2 NPs-exposed hippocampus of mice. This finding is consistent with NGF and TrkA levels in the hippocampus.

Our past data show that significant down-regulation of N-methyl D-aspartate (NMDA) receptor subunits (such as NR2A and NR2B) expression was associated with reduction of CaMKIV, CREB1 and CREB2 expression in the hippocampus of mice due to TiO_2 NPs exposure.[15] In the present study, exposure to 125, 2.5, and 5

mg/kg BW TiO_2 NPs for nine months also significantly decreased the expression of CaMKII, CaMKIV, CREB1, and CREB2 in the hippocampus of mice, which is consistent with suppression of neurotrophins and related receptor expression. The findings showed that these transcription factors may mediate expressions of neurotrophins and related receptors in the hippocampus.

Dopamine D1/D5 receptors have been demonstrated to be a novel neuromodulatory role in modulating hippocampal synaptic plasticity, i.e., time-dependent reversal of NMDAR-dependent long-term depression. [41] Yang et al. also demonstrated that ciliary neurotrophic factor mediates dopaminergic innervation and D2 receptor induced neurogenesis in the adult brain. [42] Furthermore, Win-Shwe et al. indicated that NGF expression is related to dopaminergic system in the mouse hippocampus.[20] To the best of our knowledge, however, the effects of long-term TiO_2 NPs exposure on the expressions of dopamine receptors in animal brain have been not investigated. In this study, therefore, we also examined the dopamine receptors D1, D2 and related enzyme TH expressions in the hippocampus, suggesting significant decrease of D1, D2 and TH expressions due to TiO_2 NPs exposure. It implied that NGF my mediate mechanism to induce neurogenesis in the hippocampus dependent of dopaminergic system in the mouse hippocampus.

5. Conclusion

Mice exposed to low dose TiO_2 NPs for nine months, titanium accumulation in the hippocampus were observed, which in turn resulted in significant reduction of body

weight and hippocampus indices, neuroinflammation in the hippocampus. The neuroinflammation due to TiO_2 NPs exposure may be closely associated with neurotrophin-mediated signaling pathway, marked by significant reduction in NGF, BDNF, TrkA, TrkB, CaMKII, CaMKIV, CREB1, CREB2, D1, D2 and TH expression in the hippocampus. Further studies are needed to evaluate the existence of a positive relation between NGF–TrkA-signal transduction pathway and neurobehavioral alteration following TiO_2 NPs exposure.

Declaration

The submission complies with the Helsinki Declaration with no information that has been published elsewhere. There is also no diagnostic testing and copyrighted material.

Disclosure and Acknowledgment

Mingyu Su, Xiaoyang Zhao, and Ling Wang contributed to the design of the whole study; Jie Hong, Xiaohong Yu contributed to the experiments of animal treatment, histopathological examination and assay of gene and protein expression; Bingqing Xu, Dong Liu contributed to the biodistribution of TiO_2 NP in mice; Yuguan Ze and Fashui Hong contributed to the proof reading of the paper. We thank for the financial support from National Natural Science Foundation of China (grant No. 81273036, 81473007, 30901218), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, and the National Bringing New Ideas Foundation of Student of Soochow University (grant No. 201310285040Z, 201410285040Z).

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

Gene name	Description	Primer sequence	Primer size (bp)
Refer-GAPDH	mGAPDH F	5′- TGTGTCCGTCGTGGATCTGA -3′	
	mGAPDH R	5′- TTGCTGTTGAAGTCGCAGGAG -3′	150
CREB1	mCREB1F	5′-TACGGATGGGGTACAGGGC-3′	
	mCREB1R	5′-CAATGGTGCTCGTGGGTG-3′	197
CREB2	mCREB2F	5′-GCCAGCCACCTCCACTACA-3′	
	mCREB2R	5′-CTCAGCAGGGTGACTTCGC-3′	255
BDNF	mBDNF F	5′-GCCCAACGAAGAAAACCATAA-3′	
	mBDNF R	5′-ACACGCTCAGCTCCCCAC-3′	188
NGF	mNGFF	5′-CAGATAGCAATGTCCCAGAAGG-3′	
	mNGFR	5′-AGTGATGTTGCGGGTCTGC-3′	149
TrkA	mTrkAF	5′-TGAATCTGTCCTCCAATGCG-3′	
	mTrkAR	5′-GCCCTTCCTGCTCCCAAC-3′	141
TrkB	mTrkB F	5′-AGTTTTCCTTGCCGAGTGCTA-3′	
	mTrkB R	5′-CTTGTTGAGGTCCCCGTGC-3′	229
D1	mD1F	5′-GGCTCCCTTTCTTCATTTCG-3′	
	mD1R	5′-CCCGTTGTTGTTGATGCTTAC-3′	248
D2	mD2F	5′-GCCTTCGTGGTCTACTCCTCC-3′	
	mD2R	5′-AGTTGCCCTTGAGTGGTGTCT-3′	168
TH	mTHF	5′-CTCCTCAGTTCTGTGCGTCG-3′	
	mTHR	5′-GCTTGTATTGGAAGGCAATCTC-3′	214
CaMK□	mCaMK Ⅱ F	5′-AGTCCAGTTCCAGCGTTCAGT-3′	
	mCaMK Ⅱ R	5′-GGGTCGCACATCTTCGTGTA-3′	166
CamkIV	mCamk4F	5′-ATTTCAAGCCCTCCAACACC-3′	
	mCamk4R	5′-CTTGGATGCTGGTGTGGCTA-3′	179

Table 2 Body weight, hippocampal indices and titanium content in hippocampus of mice after nasal administration of TiO_2 NPs for nine months

Index	TiO_2 NPs (mg/kg BW)			
	Control	0.25	0.5	1
Net increase of body weight (g)	25±1.83	22±0.72	18±0.51*	12±0.5**
Hippocampal indices (mg/g)	18.71±1.35	15.25±1.12*	11.71±1.06**	9.72±0.1.06**
Ti contents (ng/g tissure)	Didn't detect	49±3.58	86±6.27**	138±9.96***

*p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (*N*=10 for body and hippocampus weight, *N*=5 for Ti contents).

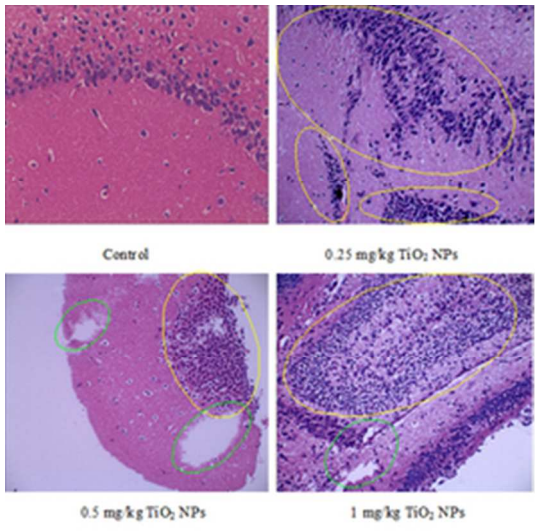
Figure legends

Fig. 1 Histopathology of the hippocampus in mice following nasal administration of TiO_2 NPs for nine months. Yellow circle shows significant proliferation of all glial cells; green circle shows tissue necrosis.

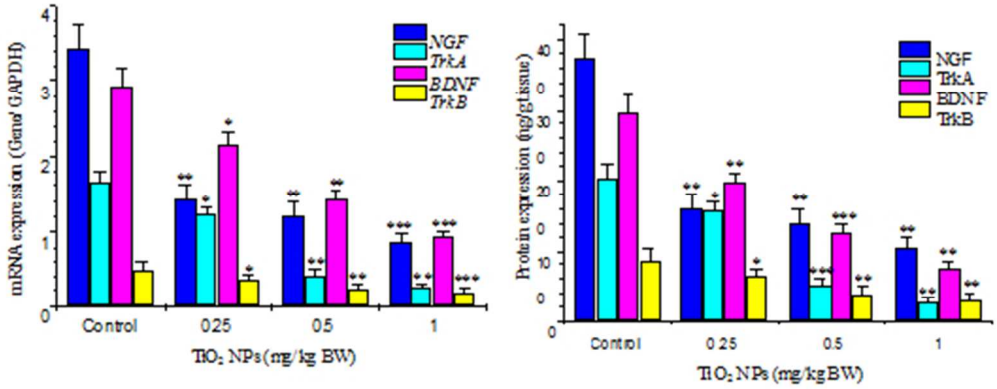
Fig. 2 Alteration of neurotrophins and related receptor expression in the hippocampus in mice following nasal administration of TiO_2 NPs for nine months. *p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (*N*=5).

Fig. 3 Alteration of transcription factor expression in the hippocampus in mice following nasal administration of TiO_2 NPs for nine months. *p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (*N*=5).

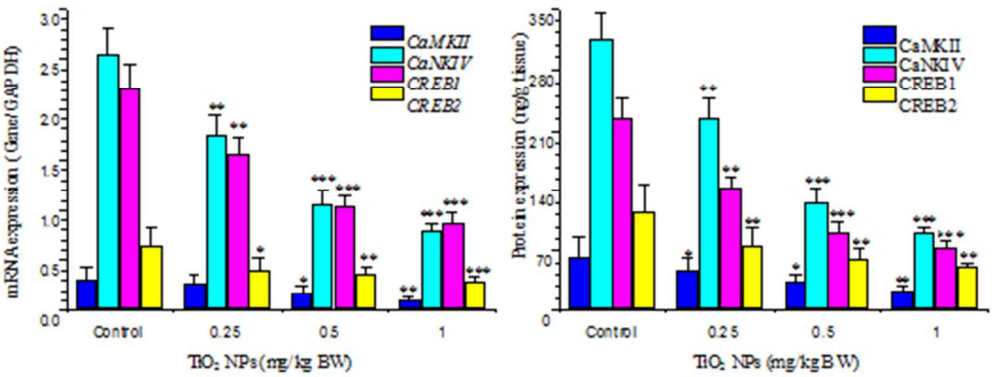
Fig. 4 Alteration of Dopamine marker expression in the hippocampus in mice following nasal administration of TiO_2 NPs for nine months. *p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (*N*=5).



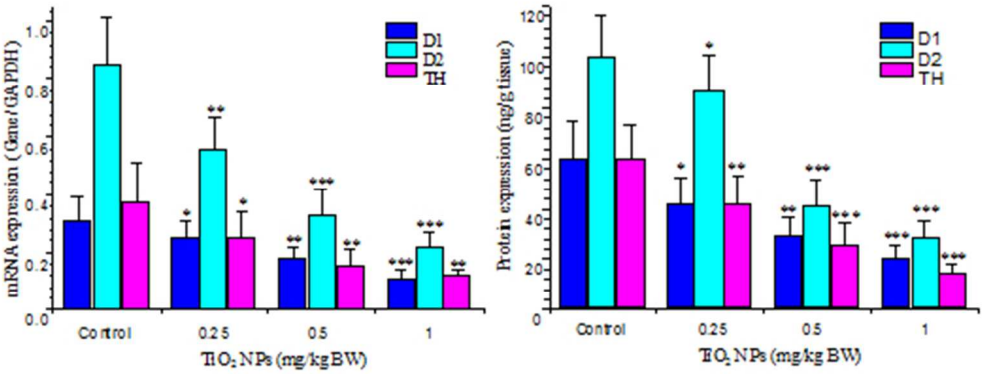
Histopathology of the hippocampus in mice following nasal administration of TiO2 NPs for nine months. Yellow circle shows significant proliferation of all glial cells; green circle shows tissue necrosis.
22x22mm (300 x 300 DPI)



Alteration of neurotrophins and related receptor expression in the hippocampus in mice following nasal administration of TiO2 NPs for nine months. *p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (N=5).
44x18mm (300 x 300 DPI)



Alteration of transcription factor expression in the hippocampus in mice following nasal administration of TiO2 NPs for nine months. *p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (N=5).
44x18mm (300 x 300 DPI)



Alteration of Dopamine marker expression in the hippocampus in mice following nasal administration of TiO2 NPs for nine months. *p<0.05, **p<0.01, and *** p<0.001. Values represent means ± SD (N=5).
44x18mm (300 x 300 DPI)