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RESEARCH ARTICLE

The effect of titanium dioxide nanoparticles on neuroinflammation response in rat brain

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Abstract Titanium dioxide nanoparticles (TiO_2 NPs) are widely used for their whiteness and opacity in several applications such as food colorants, drug additives, biomedical ceramic, and implanted biomaterials. Research on the neurobiological response to orally administered TiO_2 NPs is still limited. In our study, we investigate the effects of anatase TiO_2 NPs on the brain of Wistar rats after oral intake. After daily intragastric administration of anatase TiO_2 NPs (5–10 nm) at 0, 50, 100, and 200 mg/kg body weight (BW) for 60 days, the coefficient of the brain, acethylcholinesterase (AChE) activities, the level of interleukin 6 (IL-6), and the expression of glial fibrillary acidic protein (GFAP) were assessed to quantify the brain damage. The results showed that high-dose anatase TiO_2 NPs could induce a downregulated level of AChE activities and showed an increase in plasmatic IL-6 level as compared to the control group accompanied by a dose-dependent decrease inter-doses, associated to an increase in the cerebral IL-6 level as a response to a local inflammation in brain. Furthermore, we observed elevated levels of immunoreactivity to GFAP in rat cerebral cortex. We concluded that oral intake of anatase TiO_2 NPs can induce neuroinflammation and could be neurotoxic and hazardous to health.

Keywords Titanium dioxide nanoparticles · Rats · Brain · Neurochemicals · Neuroinflammation · Astrocytes

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Highlights
- TiO_2 NPs could induce a downregulated level of AChE activities.
- TiO_2 NPs provoke an increase in plasmatic IL-6 level as compared to the control group accompanied by a dose-dependent decrease inter-doses, associated to an increase in the cerebral IL-6 level as a response to a local inflammation in the brain.
- TiO_2 NPs increase GFAP expression and reflect astrocytic stimulation as an inflammatory response.

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Introduction

Nanoparticles are materials that have at least one dimension in the range of 1–100 nm in length, when particle size is reduced below this threshold, the resulting material exhibits physical and chemical properties that are significantly different from the properties of macroscale materials composed of the same substance (Duncan 2011). In recent years, titanium dioxide nanoparticles (TiO_2 NPs) have been widely used in several applications such as food colorants, drug additives, paints, paper, inks, cosmetics, sun-screens, antibacterial materials, and electronics for their whiteness and opacity (Shi et al. 2013).

Little work has been done to investigate the neurotoxicity potential of TiO_2 NPs exposed by oral administration (Cho et al. 2013, Hu et al. 2010, Hu et al. 2011,

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Wang et al. 2007). Administration routes play an important part on NPs biodistributions and neurotoxicity. Gastrointestinal absorption seems a prevalent exposure route for TiO_2 NPs through food products, drug carriers, and water and beverages. After absorption of TiO_2 NPs, the systemic circulation can distribute the particles to the important organs throughout the body (Czajka et al. 2015). After reaching the systemic circulation, TiO_2 NPs can interact with plasma-proteins, coagulation factors, platelets, and red or white blood cells (Deng et al. 2009). In our previous study, we demonstrated that TiO_2 NPs treated animals increased leucocytes level indicating the activation of the immune systems of the body (Grissa et al., 2015).

Circulating nanoparticles found in blood circulation depending on their material, size, charge, surface engineering, and others characteristics (Leite et al. 2015) can cross the blood-brain-barrier (BBB) and interact with the central nervous system (CNS) cells of the exposed animals (Hanada et al. 2014, Oberdörster et al. 2004). Different NPs such as silver NPs, gold NPs, manganese oxide NPs, iron oxide NPs, copper NPs, and TiO_2 NPs were described to cross BBB and accumulate in several CNS areas (An et al. 2012, Czajka et al. 2015, Elder et al. 2006, Skalska et al. 2015, Sousa et al. 2010, Wang et al. 2008b, Wu et al. 2013). The permeability of BBB can be altered by TiO_2 NPs, which could assist in influx of exogenous substances into the brain. As a result, NPs induced inflammation, edema, and cell injury or even cell death in brain regions (Song et al. 2015).

Local or systemic inflammation can alter BBB selectivity properties, influencing the transport and inducing physiological and mechanical changes in specialized endothelial cells of BBB comprise increased levels of cytokines secretion (Erickson et al. 2012). In fact, one of the first indicators that can initiate an inflammatory response is the activation of microglia and astrocytes. Microglia cells are generally considered the immune cells of the brain, in that, they have the ability to respond to infection or lesion in the CNS (Kim and de Vellis, 2005). Active microglia release chemokines and proinflammatory cytokines. Microglial-derived cytokines can activate astrocytes, which are characterized by hypertrophy and hyperplasia of cell bodies (Banks and Lein, 2012, Spooren et al. 2011). TiO2 NPs was demonstrated to promote an exaggerated neuroinflammatory response by enhancing microglial activation in the pre-inflamed brain of mice (Shin et al. 2010), that in turn causes release of chemokines and proinflammatory cytokines, producing further neurodegeneration and brain injury (Czajka et al. 2015).

Astrocytes, as a glial cell in the CNS, have a primordial role in sustaining the neuronal survival by controlling neuronal differentiation and maintaining environmental conditions around the neurons (Booth et al. 2000, Riethmacher et al. 1997). Neurons regulate the differentiations and functions of astrocytes by the modulation of neuritogenesis through their secretion of neurotransmitters such as acetylcholine (ACh) (Guizzetti et al. 2008). Disrupted the distributions of trace elements, disruption of signaling pathways, dysregulation of neurotransmitters levels, and synaptic plasticity may contribute to the neurotoxicity of TiO_2 NPs (Song et al. 2016).

In addition, there is a general upregulation of glial fibrillary acidic protein (GFAP) the active astrocyte marker gene that regulates the morphology of astrocytes and determines astrocyte neuronal interactions, can be used to monitor astrocyte differentiation and is expressed in differentiated astrocytes (McCall et al. 1996, Ozawa et al. 2013). It was reported that TiO_2 NPs increased the levels of GFAP positive astrocytes after nasal instillation (Wang et al. 2008a).

The increase of GFAP expression occurs through stimulation of the interleukin-6 (IL-6) autocrine system (Takanaga et al. 2004). IL-6 has dual roles in brain injury and disease. It is produced during reactive astrogliosis as a response to neuronal damage acting as a neurotrophin promoting neuronal survival, while elevated levels of IL-6 have also been adversely associated with several brain diseases (Erta et al. 2012, Ramesh et al. 2013).

In this article, we investigated the effects of intragastric subchronic anatase TiO_2 NPs exposure on the brain of rats to understand its neurotoxicity. We examine the effects of neurochemicals through the analyzing of plasmatic and cerebral acethylcholinesterase (AChE) activities. To investigate theTiO_2 NPs neuro-inflammatory potential, we measured plasmatic and cerebral IL-6 levels and we performed GFAP immunostaining as an indicator of astrocyte differentiation.

Materials and methods

Chemicals

The TiO_2 NPs used in this study were the commercial products “titanium dioxide” produced by AZ tech (Italy) and marketed in Tunisia as a paint ingredient in the ceramic sector. The CAS Registry Number for titanium dioxide is 13463–67-7. Solutions of dispersed TiO_2 particles were prepared with distilled water and then were treated by ultrasonic vibration for 30 min and mechanically vibrated for 5 min before every use. Distilled water was used as a vehicle control.

Animals and treatment

Male Wistar rats aged 4 months were obtained from SIPHAT (Pharmaceutical Industrial Society of Ben Arous, Tunisia). The animals were randomly assigned to control group (n = 6) and treatment groups (three groups, n = 6 per group) and housed at 21 ± 5 °C with a 12-h light/dark cycle and 55 % humidity. The rats were fed with a standard commercial pellets diet from Sico Sfax, (Tunisia) and with drinking water ad libitum. They were maintained during the whole experimental period in accordance with the guidelines for animals care of the "Faculty of Medicine of Monastir", Tunisia.

Dose levels

The animals were randomly divided into a control group and three experimental groups (50, 100, and 200 mg/kg BW TiO_2 NPs). They were weighed and the volume of TiO_2 NPs suspensions was calculated for each rat. Fresh TiO_2 suspensions were administered to the rats by an intragastric administration every day for 60 days. Dosing was made with volume of suspension 10 ml/kg of the rat weight. Each animal received TiO_2 NPs daily and each control animal received the same volume of distilled water.

Any symptoms, growth state, eating, drinking and activity, or mortality were observed and recorded carefully daily during the 60 days of treatment. After 60 days and 24 h after the last TiO_2 NPs dose, all rats were weighed, anesthetized with ether, and blood samples were collected by cardiac puncture in heparinized tubes. Plasma was separated from the blood cells by centrifuging the blood at 3000 g for 15 min at 4 °C and stored in aliquots at −20 °C until analysis. Rats were then euthanized and the brain from each rat was removed, weighed accurately and quickly frozen at −80 °C.

Coefficients of organs and preparation of brain

After weighting the body and the brain, the coefficients of brain to body weight were calculated as the ratio of tissues (wet weight, mg) to body weight (g).

Tissue homogenization

The cytosolic fractions of brain tissue were obtained by homogenizing tissues (10 % w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 100,000 g for 60 min at 4 °C and the resultant post-mitochondrial supernatant was stored at −80 °C to be used for IL-6 immunoassay.

Protein total in plasma and brain was measured using a KONE 30 automated analyzer (Thermoclinical Labsystems, Espoo, Finland) at "Fattouma Bourguiba University Hospital" in Monastir (Laboratory of Biochemistry).

Acetylcholine esterase activity

Acetylcholine esterase activity in brain was done using the spectrophotometric method of (Ellman et al. 1961). Homogenate (10 %) of brain was prepared in 30 mM sodium phosphate buffer, pH 8.0, containing 1 % Triton X 100 to release the membrane bound enzyme, and it was centrifuged at 12,500 g for 30 min at 4 °C. Acetylthiocholine iodide of different concentrations, 0.25–0.5 mM for cerebral cortex was used as substrate. The mercaptan formed as a result of the hydrolysis of the ester reacting with an oxidizing agent 5,5′-dithiobis (2-nitrobenzoate) read at 412 nm in Shimadzu UV1700 spectrophotometer.

IL-6 immunoassay (enzyme-linked immunosorbent assay)

Blood samples were collected under anesthesia by cardiac puncture in heparinized tubes. Plasma was separated from the blood cells by centrifuging the blood at 3000 g for 15 min at 4 °C and stored in aliquots at −20 °C until analysis. IL-6 immunoassay (enzyme-linked immunosorbent assay) levels of IL-6 were measured in the brain and plasma by Quantikine Immunoassay kit (R&D Systems, Abingdon, UK) in accordance with the manufacturer's instructions. Results were expressed in pg/mg of protein in brain and pg/ml in plasma.

Immunohistochemical analysis of GFAP expression

The immunohistochemical staining procedure was performed on formalin-fixed, paraffin-embedded cerebral cortex sections using a rabbit polyclonal anti-GFAP antibody (abcam, Paris, France; ab16997; dilution 1:100). Thus, 5-μm-thick tissue sections were cut, dried overnight at 56 °C, deparaffinized in toluene, rehydrated through a series of alcohol and washed in Tris-buffered saline (TBS; 0.05 mmol/l Tris-HCl; 1.15 mmol/l NaCl, pH 7.6). For antigen retrieval, sections were boiled in a water bath with citrate buffer (10 mmol/l, pH 6.0) for 40 min at 100 °C. Sections were then allowed to cool at room temperature for 20 min, rinsed thoroughly with water, and placed in TBS. Endogenous peroxidase activity was blocked with hydrogen peroxide/methanol for 5 min. Subsequently, sections were rinsed gently with TBS and incubated at 4 °C overnight with the primary antibody. Immunostaining was performed using the high-sensitive polymer-based En Vision system (DakoCytomation, Glostrup, Denmark) in compliance with the manufacturer's instructions. Immunoreactivity was visualized with 3,3′ -diaminobenzidine tetrahydrochloride. Sections were counterstained with Mayer hematoxylin, permanently mounted, and viewed with a standard light microscope (Belaid-Nouira et al. 2013).

Cell counts

In addition to morphological analysis, the number of stained cells was quantified in a blinded manner in the cerebral cortex. The numbers of GFAP-positive cells of each rat brain section were counted in three nonoverlapping high-power fields (HPFs, × 400 magnifications) for each section and sections of three rats per group were analyzed. The HPFs were selected from cerebral cortex areas that had a maximum of positive cells. In each field studied, only positive cells with the nucleus at the focal plane were counted. Results were given as arithmetic means of positive cell numbers per HPF and then expressed as percentage of control to make a clearer comparison between different groups.

Statistical analysis

The data was expressed as means ± SD based on the indicated number in the experiment ($n = 6$). The data was analyzed using the Statistical Package for Social Sciences (SPSS, version 21). The statistical significance has been determined using one-way analysis of variance (ANOVA) following by Tukey's post hoc comparisons. The differences were considered significant at $p \leq 0.05$.

Results

Characterization of TiO_2 NPs

Detailed characteristics including the TEM (transmission electron microscopy), the XRD (X-ray diffraction), the XPS (X-ray photoelectron spectroscopy), and the EDX (energy-dispersive X-ray) analysis of used TiO_2 NPs is published in our previous work (Grissa et al. 2015) and confirmed that it is a pure anatase NPs and had sizes in the range 5–10 nm.

Coefficient of brain to body weight

Table 1 shows the coefficients of the brain to body weight, which were expressed as milligrams (wet weight of tissues)/grams (body weight). No significant differences were found in the body weight of all groups. With increasing dosages, the coefficients of the brain to body weight were decreased gradually, and those of 100 and 200 mg/kg bw TiO_2 NPs treated groups were significantly lower than that of the control ($p < 0.05$ and $p < 0.01$), indicating that higher doses of TiO_2 NPs might cause the rat brain damages.

Acetylcholine esterase activity

The activity of acetylcholine esterase (AChE) was measured to investigate the interaction between AChE and TiO_2 NPs in plasma and brain of rats after 60 days of an intragastric administration of anatase TiO_2 NPs. As shown in Fig. 1a, AChE activity in the rat plasma from the 50,100 and 200 mg/kg bw groups were significantly lower than those in the control group ($p < 0.05$ or 0.01).

The activity of AChE in brain (Fig. 1b) showed a statistically significant decrease in 100 and 200 mg/kg bw groups.

Plasmatic and cerebral IL-6 level

As shown in Fig. 2a, the IL-6 level in plasma showed a statistically significant ($p < 0.05$ and $p < 0.01$) increase in 50, 100, and 200 mg/kg bw of anatase TiO_2 NPs compared to the control group. Whereas, we noticed a statistically significant ($p < 0.001$) decrease between the lowest and the highest doses. This decrease was approximately 1.23-fold compared to the 100 mg/kg bw group and 1.35-fold compared to the 50 mg/kg bw group. However, in the brain (Fig. 2b), the opposite was observed. The cerebral IL-6 level showed a statistically dose-dependent increase in 100 and 200 mg/kg bw of anatase TiO_2 NPs. This increase was approximately 2.23-fold compared to the 100 mg/kg bw group and 2.55-fold compared to the 50 mg/kg bw group.

Immunohistochemistry

Cerebral cortex was immunostained for GFAP to detect astrocyte activation. GFAP staining analyses showed elevated levels of immunoreactivity to GFAP in rat cerebral cortex

Table 1 The decrease of net weight and coefficient of brain of rats after intragastric administration with anatase TiO_2 NPs for consecutive 60 days

Indexes	Anatase TiO_2 NPs (mg/kg bw)			
	0	50	100	200
Net increase of bw (%)	65.67 ± 11.47	56.88 ± 11.80	44.57 ± 11.77	43.54 ± 3.31
Brain/bw (%)	0.89 ± 0.15	0.71 ± 0.06	0.68 ± 0.11*	0.53 ± 0.04**

Values represent means ± SD

*Significantly different from the control (no anatase TiO_2 NPs) at 5 %

**significantly different from the control at 1 % confidence level

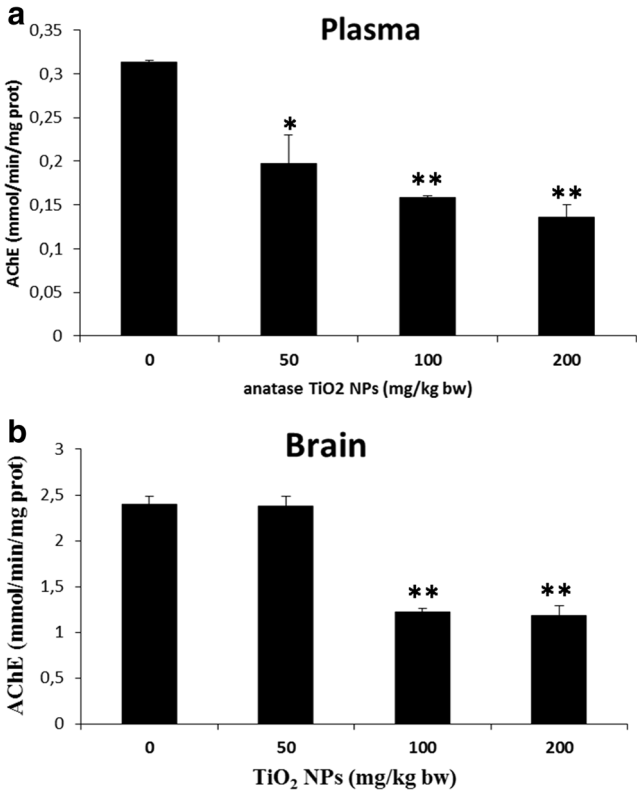

Fig. 1 AChE activity of the rats plasma (**a**) and brain (**b**) after an intragastric exposure to anatase TiO_2 NPs for 60 days. Values represent means ± SD. *Significantly different from the control (no anatase TiO_2 NPs) at 5 %, ** significantly different from the control at 1 % confidence level

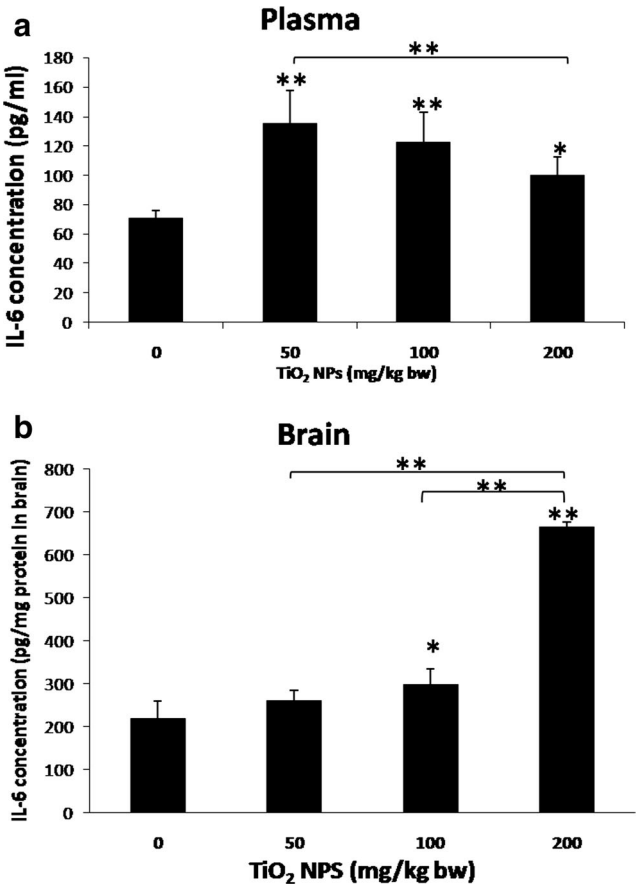

Fig. 2 IL-6 secretion in plasma and brain of rats treated with intragastric administration of anatase TiO_2 NPs for 60 days. a Plasmatic IL-6 level expressed as pg/ml and b cerebral IL-6 level expressed as pg/mg protein. Values represent means ± SD. *Significantly different from the control (no anatase TiO_2 NPs) at 5 %, **significantly different from the control at 1 % confidence level, [significantly different between groups

upon intragastric administration of 100 and 200 mg/kg bw of TiO_2 NPs, as compared to control animals (Fig. 3g, h).

Figure 3c, d) also clearly revealed an obvious decrease in the intensity of blue-stained granule cells in TiO_2 NPs treated rats.

Cell counts

In the brain of 100 and 200 mg/kg bw TiO_2 NPs treated rats, the density of GFAP positive treated cells was increased significantly (37.2 ± 2.48 and 46 ± 3.74 per HPF) compared to the control group (16.2 ± 2.71 per HPF). Except for the 50 mg/kg bw TiO_2 NPs group, the density of positive cells was almost near to that of the control group (17 ± 2.76 per HPF; Fig. 4).

Discussion

While rats that were treated with TiO_2 NPs did not significantly alter their body weight (bw), the coefficients of the brain to the body weight were indeed decreased significantly when high-dose anatase TiO_2 NPs were used. Similar results were also observed in previous the study (Hu et al. 2010, Ma et al. 2010, Ze et al. 2014).

Neurotransmitters are small and polar molecules and play critical roles in mediate signal transmission between neurons and other cells around the synapse (Bai et al. 2014). AChE is widely distributed, especially in the brain and blood, and mainly located on the extracellular surface of neurons and erythrocytes (Rotundo 2003, Stasiuk et al. 2008). Nanoparticles may bind to AChE and affect AChE activity after entering the body (Wang et al. 2009). In order to investigate the interaction between AChE and TiO_2 NPs, the AChE activity was evaluated in plasma and brain of rats after 60 days of an intragastric treatment of anatase TiO_2 NPs. The plasmatic AChE activity showed a significant decrease ($p < 0.05$ or $p < 0.01$) with the increasing anatase TiO_2 NPs doses. Treatment with higher doses of TiO_2 NPS caused a significant decrease ($p < 0.01$) in the AChE activity of the brain.

In fact, AChE can hydrolyze the neurotransmitter acetylcholine (ACh) in cholinergic synapses and is one of the most crucial enzymes for nerve response and function in higher organisms. Inhibition of AChE causes the accumulation of ACh, interfering with the function of the nervous system and eventually leads to

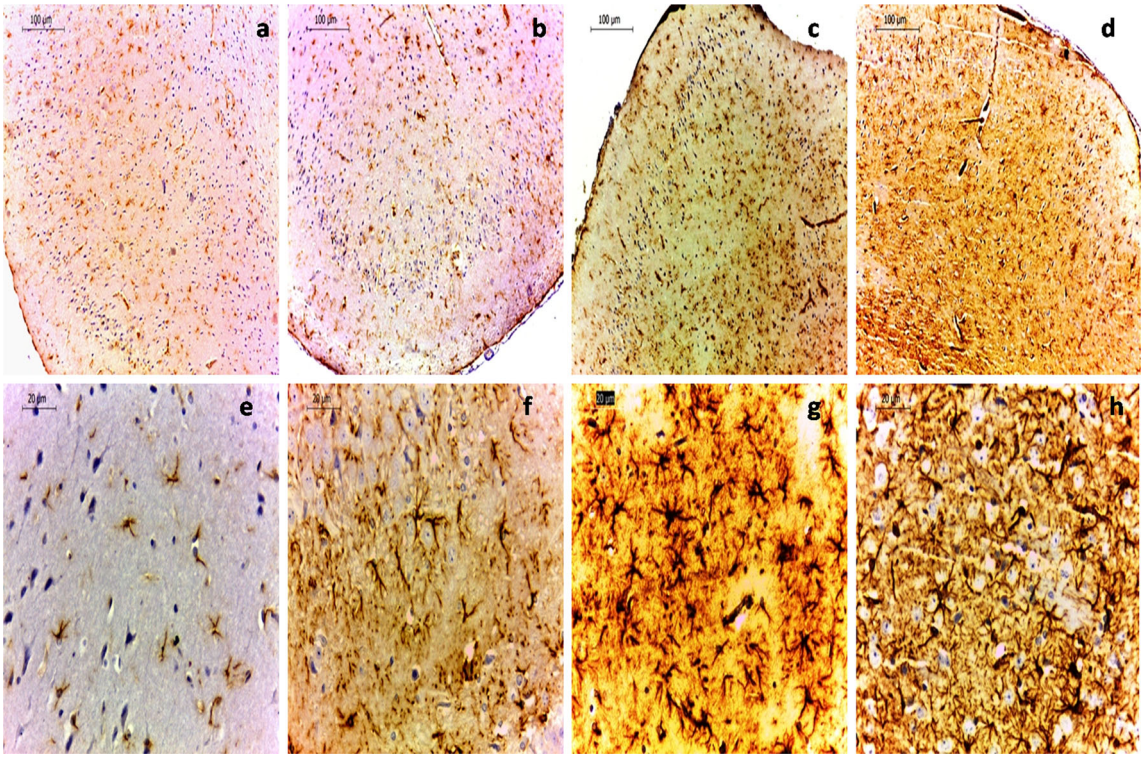

Fig. 3 GFAP-immunostained sections of rat cerebral cortex in control (**a**), 50 mg/kg bw TiO_2 NPs (**b**), 100 mg/kg bw TiO_2 NPs (**c**) and 200 mg/kg bw TiO_2 NPs (**d**) treated rats (×100). Astrocytes aspect in control (**e**), 50 mg/kg bw TiO_2 NPs (**f**), 100 mg/kg bw TiO_2 NPs (**g**) and 200 mg/kg bw TiO_2 NPs (**h**) treated rats. GFAP immunostaining (×400)

respiratory failure and death (Worek et al. 2002). Consistent with this, (Ma et al. 2010) have reported that AChE activity of the brain decreased with increasing anatase TiO_2 NPs doses, indicating that the cognitive function of the brain might be decreased.

To investigate the TiO_2 NPs neuro-inflammatory potential in vivo, we measured plasmatic and cerebral IL-6 levels. In blood, a significant increase in IL-6 level ($p < 0.05$ and $p < 0.01$) was observed with all the doses investigated in our study. But we observed that with increasing dosages, the IL-6 levels were decreased gradually and we noticed a statistically significant dose-dependent decrease inter 50 and 200 mg/kg bw groups. We supposed that TiO_2 NPs exerts a local stimulation of IL-6 production specific to the brain and that IL-6 decrease in the blood with higher doses was a result of its escape from blood to brain through BBB and its accumulation on glial cells. That is for we measured the cerebral IL-6 level. In the brain, as expected, the IL-6 increased statistically with the increasing of doses.

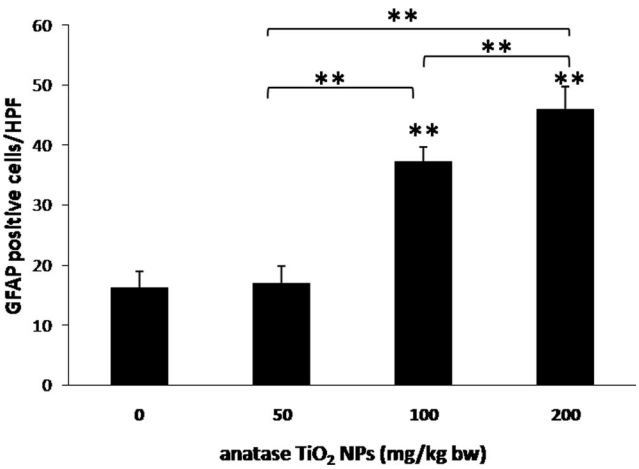

Fig. 4 Cell counts for GFAP-positive cells in the cerebral cortex of rats treated with an intragastric administration of anatase TiO_2 NPs for 60 days. Values represent means ± SD. * significantly different from the control (no anatase TiO_2 NPs) at 5 %, ** significantly different from the control at 1 % confidence level

A recent study (Chen et al. 2015) showed that IL-6 in the serum of rats treated with intaragastric administration of 10 and 50 mg/kg TiO_2 NPs for 30 days were significantly higher ($p < 0.05$) than the control group. But, when we check their results, we found that the average of the group of 50 mg/kg bw is lower than the group of 10 mg/kg bw. Nevertheless, the greatest difference between our study and the previous one (Chen et al. 2015) concerns TiO_2 time exposure. It seems that when we increased the dose and the time of exposure, the IL-6 decreased in blood and increased in the brain in a consistent dose-dependent manner as a response to a local inflammation in brain.

To understand the cause of IL-6 hyperproduction in brain after TiO_2 NPs intoxication, we performed GFAP immunostaining widely used as an indicator of astrocyte activation and

considered to be a biomarker of early biological effects including cell communication, cell structure and movement, and BBB function. It is reported that GFAP expression is increasing during neuropathology and neurodegeneration (Johnston-Wilson et al. 2000).

Because, cytokines IL-6 are generally synthesized by activated microgalia and macrophages in response to pathogens and trauma (Campbell et al. 2002). Hence, any hyperstimulation of astrocytes could lead to the hyperdisruption of IL-6 secretion.

In fact, TiO_2 NPs has been demonstrated to induce NF-κB activation, which is the major transcription factor for the induction of inflammatory molecules, in microglia (Long et al. 2007, Xue et al. 2012). Activated NF-κB-related signal cascades caused the subsequent increase in the expression of downstream proinflammatory mediators (Delgado et al. 2002) and elevated the secretion of cytokines IL-6, TNF-α, and IL-1β (Xue et al. 2012).

Immunohistochemistry analysis showed elevated levels of GFAP in rat cerebral cortex upon intragastric administration of 100 and 200 mg/kg bw of TiO_2 NPs, as compared to control animals, which are confirmed by the significant increase in 100 and 200 mg/kg bw treated animals in the quantitative analyses of GFAP-positive cells in cerebral cortex. Similarly, Wang et al. (2008a) demonstrated that intranasally instilled TiO_2 NPs increased the level of GFAP in animal brain.

The data obtained allow suggestions that TiO_2 NPs can provoke neuroinflammation within the brain by inducing IL-6 production in astrocytes.

Few studies reported the invasion and the accumulation of TiO_2 NPs into the brain after an intragastric treatment. Wang et al. (2007) showed that 2 weeks after oral administration to a single dose of 5 g/kg bw TiO_2 NPs, titanium content was significantly elevated in the brain, as compared to controls. Other studies reported a dose-dependent titanium accumulation in mice brain observed after 60 days of intragastric administration of 5, 10, and 50 mg/kg bw TiO_2 NPs (Hu et al. 2010, Hu et al. 2011). On the other hand, Cho et al. (2013) investigated the biodistribution of TiO_2 NPs after an oral administration for 13 weeks and showed minimal titanium content in the brain of treated rats.

Our present study showed an upregulated IL-6 level in circulation induced by TiO_2 NPs. Upregulated level of cytokines including IL-6 may damage the BBB and increase its permeability (Yarlagadda et al. 2009) facilitating TiO_2 NPs and/or IL-6 crossing the BBB to induce neuroinflammation. Consistent with this, Disdier et al. (2015) reported that neuroinflammation was induced by cytokines or pro-inflammatory mediators in systemic circulation and not directly by titanium accumulation in the brain. The translocation of TiO_2 NPs into the brain would be influenced by several parameters, such as the administration route, size, dosage, and physicochemical characteristic of the NPs (Song et al. 2015).

Conclusion

Our study showed that rats treated with an intragastric treatment of 100 and 200 mg/kg bw anatase TiO_2 NPs for consecutive 60 days display decreased coefficient of the brain, decreased plasmatic and cerebral AChE, and showed for the first time in this research an increase in plasmatic IL-6 level as compared to the control group accompanied by a dose-dependent decrease inter doses, this decrease was associated with an increase in the cerebral IL-6 level. We also observed elevated levels of immunoreactivity to GFAP in rat cerebral cortex. But 50 mg/kg bw anatase TiO_2 NPs dose showed only a little influence on plasmatic AChE level and showed the most significant increase in plasmatic IL-6 level compared to the other doses.

Our findings imply that the application of TiO_2 NPs as a food and drug additives could be harmful to health by inducing neuroinflammation and brain injury.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval "All procedures performed in studies involving animals were in accordance with the guidelines for animals care of the "Faculty of Medicine of Monastir", Tunisia at which the studies were conducted."

The manuscript does not contain clinical studies or patient data.

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