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ISSN: 2578-8760

Journal of Nanomedicine

Open Access | Review Article

Titanium Dioxide Nanoparticles: Evidence of Damage Induced in Respiratory and Nervous System Models through Time

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Received: Dec 06, 2021

Accepted: Jan 20, 2022

Published Online: Jan 24, 2022

Journal: Journal of Nanomedicine

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abstract

Titanium Dioxide Nanoparticles (TiO_2-NPs) are one of the most used materials in the production of inputs from different industries such as food, cosmetics and many others. However, the prolonged occupational and consumption exposure to these TiO_2-NPs has been shown to have serious health consequences. One of the main entry routes to humans of these TiO_2-NPs is the respiratory route. Once inside, they can go to the lungs and enter the circulation or, come into contact with the brain through olfactory bulb. This review shows the most relevant works of damage associated with TiO_2-NPs in the respiratory and nervous systems in tests performed both *in vivo* and *in vitro*, with a vision that encompasses everything from the first findings to the most novel data.

Keywords: Titanium dioxide nanoparticles; Lungs; Neurons; Astrocytes; Respiratory system; Nervous system; In vivo; *In vitro.*

Abbreviations: TiO2-NPs: Titanium Dioxide Nanoparticles; ROS: Reactive Oxygen Species; IL: Interleukin; GSH: Glutathione; BBB: Blood Brain Barrier; TNF-α: Tumor Necrosis Factor.

Cite this article: Arizti JAP, Chafre GS, Marure RL. Titanium Dioxide Nanoparticles: Evidence of Damage Induced in Respiratory and Nervous System Models through Time. J Nanomed. 2022; 5(1): 1048.

Introduction

The first reports on the use of Nanoparticles (NPs) date back to the late 70's and describe the use of particles in nanometric size as delivery systems for anticancer drugs [1]. Almost two decades later their concept was established [2] defining NPs as particles with a diameter below 50 nm separating them from other ultrafine particles, although the current accepted size is 100 nm. In those years, we can find the first scientific reports that propose the use of NPs in other industries for example as molecular sunscreens due to their ability to reflect UV radiation [3].

One of the most used NPs are Titanium Dioxide Nanoparticles (TiO_2-NPs). These are produced with titanium isopropoxide solubilized in ethanol, which is subjected to an industrial stirring process at 0 °C and a hydrothermal treatment to produce three different crystalline forms: rutile, anatase and brookite, where rutile is the stable phase in the bulk material. In solution, reparation methods for TiO_2 generally favor the anatase structure (Figure1) [4]. The size of the TiO_2-NPs also plays a role on their structure. X-ray absorption spectra indicate that TiO_2-NPs with an average particle size of approximately 12-30 nm have an anatase structure, whereas TiO_2-NPs with an average particle size of approximately 7 nm has a structure very similar to that of the rutile phase, which generally arises only under high-pressure conditions. This difference can be attributed to size-induced radial pressure within the smaller nanoparticles [5]. Size, shape and crystalline phase are important differences between different types of TiO_2-NPs, but in this review we made a selection of principal works reported with all the TiO_2-NPs types used in the research field.

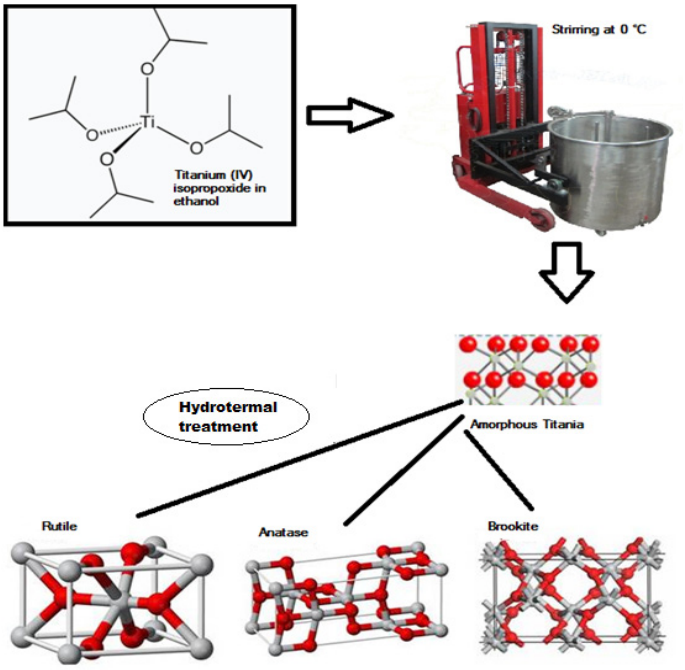

Figure 1: Schematic flow chart illustrating the steps in the synthesis of phase-pure TiO_2 nanomaterials.

Today TiO_2-NPs are widely used in many industries. For example, E171 TiO_2-NPs are used in the food industry they are a common food additive used to enhance the white color, brightness, and sometimes flavor of a variety of food products [6]. It has been used for the production of coated candy, preserved fruits, chewing gum, carbonated drinks, powdered drinks (in unsweetened dosage form or concentrated), milk and dairy products, and other food categories [7,8]. The concentration of TiO_2-NPs in food reaches as high as 0.5-9 g/kg [6,7]. TiO_2-NPs have also been widely used in biomedicine, organic pollutant treatment, materials engineering and cosmetics [9,10]. As mentioned above, one of the most important uses of TiO_2-NPs is in the sunscreen industry. P25 TiO_2-NPs (TiO_2-NPs for industrial applications) can absorb and reflect UV radiation of two wavelengths (310 and 400 nm) [11]. Modern sunscreens contain TiO_2-NPs, which are colorless, reflecting and scattering Ultraviolet Light (UV) more efficiently than larger particles [12]. In the occupational settings such as factories, employees are directly in contact with TiO_2-NPs and the primary exposure route to them internalization is the inhalation through the respiratory system [13]. Once inside the respiratory tract, TiO_2-NPs can be internalized to the lower respiratory tract, coming in contact with lungs and substructures, and reach many organs through blood circulation. Due to their nanometric size, they can also translocate to the upper respiratory tract, crossing the olfactory bulb and the blood-brain barrier entering in contact with the brain crossing the Blood-Brain Barrier (BBB) [14] (Figure 2). In addition, *in vivo* studies shown that TiO_2-NPs can reach the brain after chronic oral intake [15].

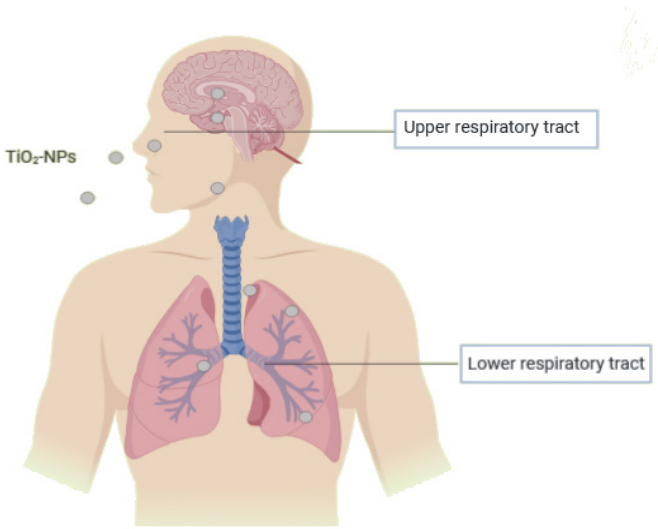

Figure 2: Schematic representation of the entry of the TiO_2-NPs through the respiratory tract.

For many years TiO_2-NPs were considered inert and safe for human health [12], but in the last decade, various studies demonstrated that TiO_2-NPs induce cell toxicity and damage both *in vitro* and *in vivo*. Due to their occupational exposure and human consumption, more studies about toxic effects induced by TiO_2-NPs are needed. In this work, a review of effects of TiO_2-NPs on the principal cells and organs from the respiratory and nervous systems both *in vivo* and *in vitro* is shown, to consider the future perspectives for large-scale manufacturing and applications of TiO_2-NPs.

TiO_2-NPs and the respiratory system

The first study that reported damage induced by TiO2-NPs was in 2004. Studies have reported an increment in the polymorphonuclear neutrophils recruitment at the bronchoalveolar lavage in rats instilled with TiO2-NPs [16]. Neutrophils function as the first line of defense against infections being responsible for the containment and elimination of pathogens. They are prevalent at sites of tissue trauma and are the hallmark of acute inflammation [17]. Inflammation induced by TiO2-NPs also increases macrophage activity, another indicator of a stimulated immunity response [18].

***In vitro* studies**

Treatments with TiO_2-NPs in epithelial cells of human lung Adenocarcinoma (A549) report an inflammatory response releasing Interleukin 18 (IL-8) [18-20] and Reactive Oxygen Species (ROS) [20] plus Glutathione (GSH) depletion [18] as oxidative stress markers with a decrease on the cell viability and proliferation [21]. In a concentration-dependent manner in A549 and L-132 lung cell lines, oxidative stress were also induced [22,23]. The DNA structure of A549 cells was affected in presence of TiO_2-NPs causing single and double-strand breaks plus oxidative lesions [24-26]. However, results from another group did not support these findings. They did not found DNA damage and ROS release after TiO_2-NPs exposition, in comparison with cobalt NPs treatment [27]. Apoptosis and other signals of cell damage like LDH release, mitochondrial injury, changes in ATP levels, and morphological alterations in this cell line have been reported after a TiO_2-NPs exposure [23,28,29]. One of the most recent studies with A549 cells treated with TiO_2-NPs showed an increase in triglycerides evaluated By Surface-Enhanced Raman Spectroscopy (SERS). In normal conditions, cholesterol levels are fairly constant and triglyceride levels can fluctuate, but under stress conditions cells can deposit more fatty acids as triglycerides reducing cholesterol levels, creating a linear increase of the triglyceride/glycogen ratio. This indicates a switch of metabolic activity to fatty acid biosynthesis due to mitochondrial dysfunction [30].

TiO_2-NPs exposure in normal human bronchial epithelial cells showed similar results to lung adenocarcinoma, such as induction of oxidative stress, pro-inflammatory responses, DNA damage and apoptosis through lysosomal membrane destabilization and lipid peroxidation [31-36]. TiO_2-NPs induced mucus hypersecretion in human bronchial epithelial cells ChaGo-K1 via a Ca (2^+) signaling mediated pathway. The increase of mucus secretion is the major clinical manifestation commonly found in Chronic Obstructive Pulmonary Disease (COPD) and asthma [37]. A proteomic investigation in BEAS-2B cells, revealed that TiO_2-NPs altered 46 proteins expression levels, which include some key proteins involved in cellular stress response, metabolism, adhesion, cytoskeletal dynamics, signaling, cell growth and death [38]. Although one study found neither genotoxic damage nor ROS liberation in these cells [39]; in the same year, another research group confirmed genotoxic damage induced by TiO_2-NPs in TK6, human endothelial and cerebral endothelial cells, hepatocytes, Kupffer cells, CoS-1, HEK293, BeWo b30 and bronchial 16HBE14o cells [40]. In the Endoplasmic Reticulum (ER) of 16HBE14o cells, NPs induced stress, disrupting the mitochondria-associated endoplasmic reticulum membranes (MAMs) and calcium ion balance, thereby increasing autophagy [13]. An epigenetic study showed global hypomethylation after 24 h of TiO_2-NPs treatment at sub cyto-genotoxic concentrations [41]. It is well known that DNA methylation is an epigenetic process involved in gene silencing.

Some studies have compared the cytotoxic effects of TiO_2-NPs in the phagocytic RAW 264.7 cells, a common lung target for NPs. TiO_2-NPs did not induce ROS production, LDH release [42] and apoptotic death [43] in comparison with carbon black NPs, and ZnO, DQ12 quartz and amorphous silica respectively. Finally, in Normal Human Bronchial Epithelial cells (NHBE) the release of IL-6, Granulocyte Colony-Stimulating Factor (G-CSF) and Vascular Endothelial Growth Factor (VEGF) was induced by TiO_2-NPs as a result of an immune response [19], while in human lung fibroblasts inhibited the intercellular communication in gap junctions, affecting juxtacrin signaling [44].

***In vivo* studies**

Since 2006, *in vivo* studies in rats instilled with TiO_2-NPs resulted in transient inflammatory responses and cell injury at 24 h post-exposure, inducing pulmonary emphysema, macrophages accumulation, extensive disruption of alveolar septa, type II pneumocyte hyperplasia, and epithelial cell apoptosis [45,46]. Another research group found differences in relation to surface properties like crystal structure in cell damage induced by TiO_2-NPs. They showed that 80/20 anatase/rutile TiO_2-NPs produced pulmonary inflammation, cytotoxicity and adverse effects in lung tissue, versus pure rutile TiO_2-NPs that induced only transient inflammation [47]. Several studies showed that TiO_2-NPs are deposited inside the lungs and can translocate from the pulmonary airways into other pulmonary compartments or the systemic circulation, and their accumulation sites are time-dependent, for example, 1 h after exposure to NPs, the connective tissue was the preferential target while after 24 h most TiO_2-NPs were located in the capillary lumen [48-49].

On the other hand, various works have shown that TiO_2-NPs can favor cancer development. Rats exposed by intratracheal instillation to TiO_2-NPs showed carcinogenic responses and lung neoplastic lesions induced by N-bis (2-hydroxypropyl) nitrosamine (DHPN) [50].

Recent observations indicate that nasal exposure to TiO_2-NPs promotes lung tumorigenesis with increased levels of tumor markers including cytokeratin 19 and carcinoembryonic antigen, as well as higher LDH, alkaline phosphatase and infiltration of inflammatory cells in Bronchoalveolar Lavage Fluid (BALF) [51].

Additionally, TiO_2-NPs effects have been associated with inflammatory processes in lungs. Mice treated with TiO_2-NPs by intratracheal instillation for 2 and 4 hours over 4 weeks, experienced chronic inflammation with increased IgE production in BALF and serum. In lung tissue, an increase of Inflammatory Proteins (MIP and MCP) and granuloma formation were also observed [52]. Neutrophilia was induced in rats by inhalation of TiO_2-NPs [53]. However, a study reported that in comparison with another metallic NPs (cerium, nickel, zinc and copper oxides, silicon dioxide and carbon black), TiO_2-NPs did not induce inflammatory responses even in high concentrations [54], in contrast to other works that support this fact and show that lung inflammation occurs in mice primarily through the NF-κB signaling pathways after intragastric administration [55]. Additionally, particle size is not important to induce a toxic effect compared with other surface characteristics such as chemical reactivity and surface area. TiO_2-NPs did not show measurable differences in toxicity induction compared with fine-sized particles (300 nm), producing an enhanced inflammatory response with particles of similar sizes but different surface areas [56].

On the other hand, TiO_2-NPs have an impact in lung physiology altering the structure and function of the pulmonary surfactant. Under TiO_2-NPs exposure, lamellar body-like structures were deformed and decreased in size; and unilamellar vesicles were formed. Particle size and surface area play a critical role in the response of pulmonary surfactant, with an increment in adsorption surface tension [57].

Recent studies in rats and mice treated by instillation and intraperitoneal injection confirmed the accumulation of TiO_2-NPs in lungs, accompanied by reduction in organ/body weight ratios and tissue damage by oxidative stress [58,59].

An investigation on the kinetics of TiO_2-NPs accumulation in many organs of rats exposed by different intake routes (intravenous injection, intratracheal instillation and oral application) to a low single dose (typically 40-400 µg/kg BW), found the presence of NPs in all organs tested by all administration ways [60-62].

The respiratory exposure to metallic NPs (including cerium oxide, silver and TiO_2-NPs) during pregnancy impaired lung development in the offspring with lasting effects in adult mice, independently of the type of NPs [63]. In an adult mouse model of Ovalbumin (OVA)-induced allergic lung disease, TiO_2-NPs exacerbated the airway hyperresponsiveness and inflammation, increasing IL-1β and IL-18 release plus NLRP3 inflammasome and caspase-1 activity in the lung [64]. Finally, in humans, studies in exhaled breath condensate of workers exposed to TiO_2-NPs found DNA, protein and lipid oxidation [65-66]. The exhaled breath condensate technique consists in collect and cools the exhaled air of people. The liquid obtained reflects the composition of the airway lining fluid [65]. Specific markers of nucleic, protein and lipid damage induced by oxidative stress were analyzed. All these markers are significantly increased in workers that were exposed to TiO_2-NPs. A summary of experiments performed *in vitro* and *in vivo* by exposure to NPs is shown Figure 3.

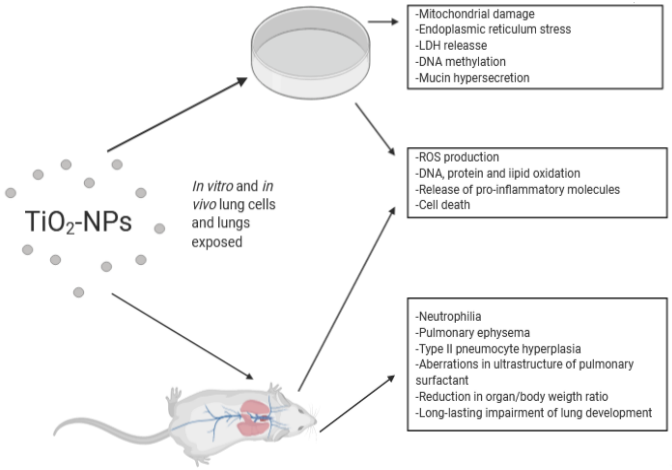

Figure 3: Abstract graphic of the alterations induced in lung cell cultures and lungs of animals exposed to TiO_2-NPs.

TiO_2-NPs and the nervous system

Since 2006, TiO_2-NPs were reported as agents that can cause brain damage [67], and one work showed induction of oxidative stress in microglia [68]. In that year also appeared one of the first reviews describing that NPs can deposit in the respiratory tract after inhalation, and the uptake of nanoparticles by the brain via the olfactory epithelium [69]. One year later, other studies showed that TiO_2-NPs could be translocated and deposited in murine brain after absorption by the nasal mucosa altering the release and metabolism of neurotransmitters in brain [70].

Fishes are one of the most used models to evaluate the toxicity of TiO_2-NPs in many organs including the brain. The mechanisms of absorption, distribution, metabolism and excretion of TiO_2-NPs have been analyzed across different NPs in these models [71]. Some reports in *Oncorhynchus mykiss* and *Cyprinus carpio* showed that TiO_2-NPs have sub-lethal toxicity leading to oxidative stress, organ pathologies, and the induction of anti-oxidant defenses such as glutathione [72,73]. In *Danio rerio* exposed to NPs, several alterations were observed in major biochemical constituents such as proteins, lipids and nucleic acids of brain tissues [74], a decrease of spatial recognition memory and levels of norepinephrine, dopamine, and 5-hydroxytryptamine, an increment in NO [75], and a lower cumulative number of viable embryos produced [76].

In vitro studies

Alzheimer´s disease is one of the most studied neurodegenerative disorders. One of the most relevant events in the development of this disease is the accumulation of amyloid deposits as extracellular plaques mediated by fibrillation of the Amyloid-β peptide (Aβ). One study showed that TiO_2-NPs can induce Aβ fibrillation by reducing the nucleation process, which is the key step of fibrillation [77]. In PC12, a cell line derived from the rat adrenal medulla used for brain neurons research, TiO_2-NPs diminished cell viability, ROS generation and apoptosis [78].

In primary cultures of olfactory bulb neurons, TiO_2-NPs caused neuronal apoptosis, and down-regulated the expression of Olfactory Marker Protein (OMP), which is associated with the mature olfactory neuronal receptor in many vertebrate species; also down-regulating Tyrosine Hydroxylase (TH) [79]. In rats treated intravenously with a single dose of TiO_2-NPs, the Ti content in the brain significantly increased at early end points followed by a subsequent decrease at 24 h, in contrast with liver, spleen and lungs where Ti persisted for a year. Ti deposits induced an increment of tight junction proteins (claudin-5 and occludin), IL-1β, Chemokine Ligand 1 (CXCL1) and γ Inducible Protein-10 (IP-10/CXCL10) in endothelial cells of the brain microvasculature. These results suggest a potential effect of TiO_2-NPs in organs distant from the brain, possibly via mediators transported by circulation [80].

There is a kind of cytoskeletal proteins that are essential in eukaryotic cells for a variety of functions such as cellular transport, cell motility and mitosis named microtubule proteins. In neurons, these proteins are used to transport substances such as neurotransmitters. In microtubule proteins isolated from sheep brains, TiO2-NPs induced a significant tubulin conformational change and disrupted tubulin polymerization [81].

In human-derived cells some studies have been performed. In endothelial cells from the brain, TiO_2-NPs were up taken and transported into the lysosomes, activating lysosomal proteases and oxidative stress, correlated with an increase in DNA strand breaks and defensive mechanisms that, ultimately induced an autophagy process in the cells [82]. Other studies focused their attention in the capacity of TiO2-NPs to cross the BBB using a model of hCMEC/D3 endothelial cells. They observed endocytosis and eventual transcytosis of these NPs [83]. In another study, Neural Stem Cells (hNSCs) were exposed to TiO_2-NPs to evaluate the effects on neurogenesis and brain function. The results showed that hNSCs formed aggregates and exhibited abnormal morphology, also affecting the expression of Nestin (stem cell marker) and Neurofilament Heavy polypeptide (NF-H, neuron marker) [84].

Some works have reported the effect of TiO_2-NPs on glial cells. These cells including the astrocytes are very important for the correct brain function and maintenance. In C6 and U373 rat and human cell lines, respectively, TiO_2-NPs induced many injuries as morphological changes that were related with a decrease in immuno-location of F-actin fibers, DNA fragmentation, apoptosis, oxidative stress induction, lipid peroxidation, increased expression of antioxidant molecules and changes

in mitochondrial membrane potential [85,86]. The last work group also proved that TiO_2-NPs were internalized shortly after exposure (30 min and 2 h in C6 and U373 cells, respectively) with formation of pseudopodia and intracellular vesicles. NPs internalization was strongly inhibited by cytochalasin-D (actin-dependent endocytosis inhibitor) in both cells and by amiloride in U373 cells, indicating that macropinocytosis is the main process of internalization in the latter [87]. In primary cultured rat astrocytes, NPs induced significant loss of glutamate uptake, indicative of a loss of vital astrocyte functions. NPs also increased ROS and mitochondrial damage evidenced as changes in morphology and decreased membrane potential. NPs at low and high concentrations altered the expression pattern of dynamin-related and apoptotic fission proteins respectively, both related with mitochondrial dynamics [88]. Finally, in microglial BV-2 cells, another kind of glial cells involved in inflammatory response, internalization of TiO_2-NPs, mitochondrial dysfunction and oxidative stress were observed [89].

Neuron-glial interactions were recently studied to elucidate how TiO_2-NPs can affect these interactions. Co-cultures of ALT astrocyte-like, BV-2 microglia and differentiated N2a neuroblastoma cells were treated with TiO2-NPs. ALT and BV-2 cells internalized more TiO_2-NPs than N2a cells resulting in lower cell viability. TiO_2-NPs also induced release of IL-1β in all cell lines and IL-6 in N2a. Glial cells were activated by pre-treatment with Lipopolysaccharide (LPS) before the TiO_2-NPs treatment, thereby mimicking NPs exposure under brain injury. LPS-activated BV-2 cells internalized more TiO_2-NPs than normal BV-2 releasing more intra/extracellular ROS, IL-1β, IL-6 and MCP-1 proteins. Although TiO_2-NPs did not directly cause loss of viability in N2a cells, when these cells were co-cultured in the transwell system with LPS-activated BV2 cells treated with NPs, late apoptosis and loss of cell viability in N2a cells were observed. However, none of the adverse effects in N2a or BV-2 cells were observed when these were co-cultured with ALT cells, demonstrating that neuronal damage can result from TiO_2-NPs-mediated ROS and/or cytokines release from microglia, but not from astrocytes [90].

***In vivo* studies**

Studies in insects, for example *Bombyx mori* (silkworm) exposed to TiO_2-NPs showed an increment in 20-hydroxyecdysone, an important protein during transition from larvae to pupae, shortening the developmental progression, and the duration of molting [91].

Many studies confirm the cytotoxicity of TiO_2-NPs in the brain of rodents. Rats and mice exposed to TiO_2-NPs showed damage by oxidative stress and lipid peroxidation [92-95]. NPs also produced a high inflammation responses associated with Tumor Necrosis Factor-α (TNF-α) and IL-1β in a time-dependent manner in sub-brain regions including olfactory bulb, cerebral cortex, hippocampus, and cerebellum [92,96]. Increased apoptosis and dopamine and norepinephrine levels in hippocampus and cerebral cortex were also reported after TiO_2-NPs exposure [94,95,97]. Wang and collaborators also reported morphological changes of hippocampal neurons and high number of GFAP-positive astrocytes in the CA4 region of hippocampus. They reported lipid and protein oxidation, catalase activity and glutamic acid plus nitric oxide release in this brain region [98]. A recent work confirmed that TiO_2-NPs downregulated acethylcholinesterase activity, increasing IL-6 release and GFAP reactivity in rat cerebral cortex [99]. TiO2-NPs exposure induced glutamate release, phosphate-activated glutaminase activity, and reductions in glutamine and glutamine synthetase expression in the hippocampus. Furthermore, TiO_2-NPs significantly inhibited the expression of N-methyl-d-aspartate receptor subunits (including NR1, NR2A, and NR2B) and metabotropic glutamate receptor 2 in this tissue [100].

One work group proposed that TiO_2-NPs induced oxidative damage in mice brain may occur via the p38-Nrf-2 signaling pathway, because NPs significantly activated p38, c-Jun N-terminal kinase, NF-κB, Nrf-2 and heme oxygenase-1 expression, increasing ROS, as well as lipid, protein and DNA peroxidation [101]. A neurotoxicity and gene-expressed profile showed significant alterations in 249 genes. This profile showed up- and down-regulation of 113 genes and 136 genes respectively, related with oxidative stress, immune response, apoptosis, memory and learning, brain development, signal transduction, metabolic processes, DNA repair, response to stimulus, and cellular processes [97]. The homeostasis of neuronal synaptic plasticity was investigated at the level hippocampal mRNA expression in mice treated with TiO_2-NPs by subchronic oral exposure. They observed that NPs caused severe pathological changes, down-regulating N-methyl-D-aspartate (NMDA), receptor subunits NR2A and NR2B, associated with the simultaneous inhibition of CaMKIV, Cyclic-AMP, Responsive Element Binding Proteins (CREB-1, CREB-2), and FosB/DFosB [102]. TiO_2-NPs also induced neuroinflammation, upregulating Toll-Like Receptors (TLR2, TLR4), TNF-α, nucleic IκB kinase, NF-κB-inducible kinase, NF-κB, NF-κB2 (p52), RelA (p65); while suppressing IκB and IL-2 in the hippocampus [103]. Recently, molecular studies focused on epigenetic patterns proposed that TiO_2-NPs-induced brain damage is associated with DNA methylation [104]. Presence of TiO2-NPs in brain was reported in mice after 60 days of dermal exposure in hairless mice [105]. In contrast, one research using dispersive X-rays found that TiO2-NPs aggregates administrated by intravenous and subcutaneous injection were not deposited in the brain [106].

Inside the Alzheimer´s disease research, one investigation found that TiO_2-NPs produce mild to moderate changes in the cytoarchitecture of the brain tissue in a time-dependent manner, and a point mutation of Presenilin 1 gene at exon 5, gene linked to inherited forms of this disease [107].

NPs can cross the placenta barrier in pregnant mice and induce neurotoxicity in their offspring. TiO_2-NPs internalization causes complications during pregnancy like smaller uteri and fetuses [108]. TiO_2-NPs affected gene expression related to development and function of the central nervous system in offspring, with changes oxidative stress-related genes in the brains of two and three-week old mice [109,110]. TiO_2-NPs also influenced the development of the central dopaminergic system in offspring increasing dopamine and its metabolites in prefrontal cortex and neostriatum [111]. The same effect was observed in cerebral cortex, olfactory bulb and some regions intimately related to this system [112]. TiO_2-NPs could affect the synaptic plasticity in offspring's hippocampal dentate gyrus area (associated with learning and memory), indicating that brain development, especially during lactation, is susceptible to TiO_2-NPs exposure [113].

TiO_2-NPs increase IL-1β, TNF-α and IL-10 in brain, and disrupt BBB leading to brain tissue necrosis, inflammation and cellular edema as shown in rat astrocytes [114]. This contrasts with an *in vitro* study reporting that TiO_2-NPs promoted the acquisition of a proinflammatory phenotype specifically in microglia and not in astrocytes [115].

Conduct, cognition and behavior studies are very important fields in neurosciences research. Rats treated by intraperitoneal injection with TiO_2-NPs were tested in elevated plus-maze. This test model is based on animal's aversion to open spaces and tendency to avoid the physical contact, where anxiety is expressed by the animal spending more time in the enclosed arms. Results obtained with this test showed that treated rats spent more time in the secured closed arms and entered the anxiogenic open arms less frequently than control, with increased brain/body ratio [116]. Additionally, spatial recognition was impaired in rats with subchronic oral exposure to TiO2-NPs [102]. Finally, central administration of TiO2-NPs by intracerebroventricular injection induced behavioral deterioration in freely moving intact rats [117].

Prenatal exposure to TiO_2-NPs have a negative impact on the offspring´s behavior: for example, TiO_2-NPs enhanced the depressive-like behaviors during adulthood in the forced swimming test. This test evaluates three variations of mobility (immobile, mobile, and highly mobile). A sucrose preference test was also performed. Results showing that TiO_2-NPs-treated mice increased immobility in the forced swimming test. Immobility is a sign of passive stress–coping strategy and depression-like behavior. Finally, treated mice showed less interest in sucrose water versus tap water. In this test, the loss of interest is a core symptom of depression [118]. TiO_2-NPs also decreased memory and learning in the offspring [119]. These results suggest that stress during fetal life induced by prenatal exposure to TiO_2-NPs could be implicated in depressive-like behaviors and the loss of memory in adulthood. A summary of experiments performed *in vitro* and *in vivo* by exposure to NPs is shown in Figure 4.

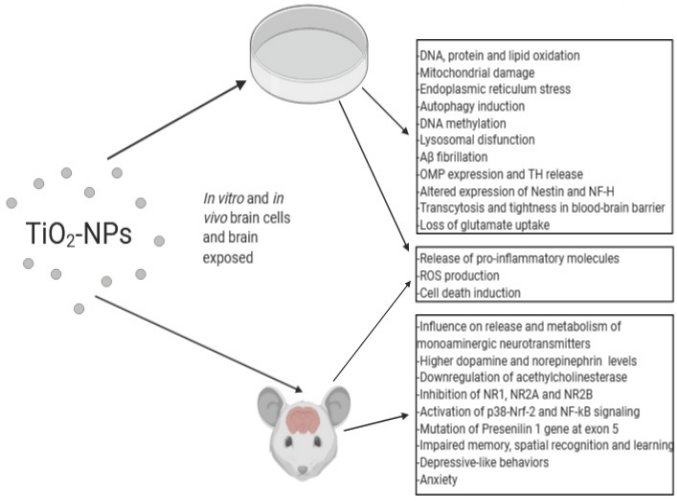

Figure 4: Abstract graphic of the alterations induced in brain cell cultures and brains of animals exposed to TiO_2-NPs.

Conclusion

In conclusion, there is significant *in vitro* and *in vivo* evidence that TiO_2-NPs exposure in cells and organs associated with respiratory and nervous system produces cell toxicity, stress, inflammatory response, damage and death. In normal life the occupational and ordinary exposure to these nanoparticles occurs mainly through the respiratory system, involving internalization and distribution inside the body followed by accumulation in lungs and brain. The presence of TiO_2-NPs may lead to chronic damage and dysfunction of these organs also triggering the development of diseases, which turns TiO_2-NPs into a serious health issue that requires immediate attention.

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