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Effect of TiO_2 nanoparticles on emotional behavior and biochemical parameters in adult Wistar rats

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Abstract. The rapidly developing field of nanotechnology is becoming a potential source for human exposure to nanoparticles. Titanium dioxide nanoparticles (TiO_2-NPs) have been widely produced in industrial processes for several years. The aim of this study was to investigate the effects of TiO_2-NPs on plasmatic biochemical parameters and the emotional behavior in adult Wistar rats. Rats were treated by intraperitoneal injection of TiO_2-NPs (20–30 nm) at a dose of 25 mg/kg. For toxicity evaluation of nanoparticles sample, body weight, organ coefficient, blood biochemistry panel assay (AST, ALT, LDH, uric acid, creatinine, and glucose content) and emotional behavior parameters were determined. Sub-acute TiO_2-NPs treatment decreased the body weight, but increased the relative brain weight. Biochemical assessment in plasma samples showed that TiO_2-NPs injection increased uric acid concentration and AST activity in rats. However, the same treatment decreased the creatinine level, but had no effect on glucose concentration, ALT and LDH activity. The emotional behavior of control and treated rats was tested in elevated plus-maze. Interestingly, our results showed that TiO_2-treated rats spent more time in the secured closed arms and entered the anxiogenic open arms less frequently than control.

Our results suggest that TiO_2-NPs intoxication could altered biochemical parameters related to changes in organ function and leads to emotional behavior impairment of rats.

Key words: TiO_2 nanoparticles — Emotional behavior — Elevated plus maze — Biochemical parameters — Rats

Introduction

With the rapid development of nanotechnology, there is a growing interest in the application of nanoparticles in various fields such as photonics, catalysis, magnetics, and biotechnology including cosmetics, pharmaceutics and medicines (Yu-Lan and Jian-Qing 2010). In fact, compared to the recent increase in applications of titanium dioxide nanoparticles (TiO_2-NPs, the health effects on human exposure have not been systematically investigated (Lavicoli et al. 2011). Fine TiO_2-NPs have been considered safe and pose little risk to humans, suggesting that exposure to this material is relatively harmless. However, available data showed that TiO_2-NPs can cause several adverse effects on mammalian cells such as an increase of reactive oxygen species (ROS) production and cytokines levels, reduction of cell viability and proliferation, induction of apoptosis and genotoxicity (Lavicoli et al. 2011). L´azou et al. (2008) investigated the *in vitro* effects of TiO_2-NPs on renal cells and reported that these nanoparticles are cytotoxic for tubular cells. Few authors have investigated the toxicity of TiO_2-NPs on hepatic cells. It seems that these nanoparticles do not exert direct effects, with the exception of the increase production of malonaldehyde (MDA) and ROS (Hussain et al. 2005; Shi et al. 2010). Recent studies have indicated that TiO_2-NPs are toxic on lung, liver, spleen, kidney and gill of animals (Liu et al. 2009, 2010; Ma et al. 2009). Many studies have unequivocally showed

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that exposure to TiO_2-NPs could be translocated into the central nervous system (CNS) *via* the olfactory pathway and damage brain neurocyte and tissue *in vitro* and *in vivo* (Long et al. 2006, 2007; Wang et al. 2008; Shin et al. 2010). TiO_2-NPs were accumulated and induced oxidative stress in the mouse brain (Renping et al. 2010). Nanosized TiO_2 could impair the spatial recognition memory ability and might decrease the cognitive function (Renping et al. 2010).

Past reports showed that the TiO_2-NPs can enter into the human body through different routes such as inhalation, ingestion, dermal penetration and injection. However, little is known about their potential toxicity to human health. Owing to their special properties, nanoparticles have the capacity to by-pass the blood-brain barrier (BBB). However, their toxic effects on the CNS are still lacking. Thus, neurotoxicity induced by nanoparticles is still a new topic that requires more attention (Yu-Lan and Jian-Qing 2010). In the present study, we investigated the effects of sub-acute TiO_2-NPs exposure on plasmatic biochemical parameters and neurobehavioral performance of adult Wistar rats.

Materials and Methods

TiO_2-NPs preparation

TiO_2-NPs were provided by the Laboratory of Physics of Materials and Nanomaterials applied to environment, College of Sciences in Gabes (Amlouk et al. 2006). The TiO_2-NPs were in the size range, between 20 and 30 nm. The crystalline data were obtained by X-ray diffractometry (XRD; Bruker D8 Advance; 40 KV, 30 mA). The synthesized products were characterized using transmission electron microscopy (TEM)) Tecnai G2-200KV with microanalysis.

Animals and treatment

Male Wistar rats (Siphat, Tunisia), weighing 140–150 g at the beginning of the experiment were randomly assigned to a control or TiO_2-NPs treated rats ($n = 6$ in each group). Animals were housed in cages at 25°C, under a 12:12 light/dark cycle (lights on at 07:00), with free access to food and water.

TiO_2 suspension was prepared using physiological saline solution (9‰ sodium chloride). The powdered TiO_2-NPs were dispersed in the fresh sterilized physiological saline solution, and the suspension was ultrasonicated for 10 min to disperse completely as well as possible (Ultrasonic Liquid Processor, Sonicator 4000). TiO_2 suspension was vortexed for 1 min before injection. Rats were treated twice with moderate doses of TiO_2-NPs (25 mg/kg) by intraperitoneal injection, once on day 0, once on day 3 and once on day 6. Control group received equivalent doses of 9‰ sodium chloride. 24 hours after the later injection treated and control groups were used to evaluate anxiety behaviours in elevated plus-maze. Animals were cared for in compliance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

Biochemistry panel analysis

All animals were sacrificed at the same time. Blood samples were taken *in heparinized tubes* and then centrifuged. In the present study, we have choosen plasmatic biochemical parameters related to liver and kidney function. We determined the glucose content, uric acid, creatinine and levels of various enzymes such as aspartate aminotransferase (AST), alanine aminotrasferase (ALT) and lactate dehydrogenase (LDH). These enzymes were examined by routine colorimetric methods using commercial kits. Organs of control and treated groups were harvested immediatly, rinsed with ice-cold deionized water and dried with filter paper. After weighing the body and tissues, the coefficients of liver, kidneys, testis and brain to body weight were calculated as tissues weight/body weight ratio.

Emotional behavior testing

The elevated plus maze test was used accordingly to previously published methodology (Pellow et al. 1985; Frye et al. 2000; Roy and Chapillon 2004; Maaroufi et al. 2009). The maze was made of clear painted wood. The arms were 50 cm long and 10 cm wide and the apparatus was elevated at a height of 60 cm. The closed arms were surrounded by a 50 cm wall while open arms had 0.5 cm edges in order to maximize open arms entries (Treit et al. 1993). The test was 5 min long and began with the placement of the rat in the centre of the maze, with its head facing an open arm. The time spent in the different parts of the maze (i.e. open arms, closed arms and central part) was recorded along with the number of entries into closed and open arms (Pellow and File 1986; Rodgers and Dalvi 1997). In addition, total activity into the maze was found to be different among groups, a ratio for open arm entries and open arm time was also calculated. The maze was cleaned with a 10% alcohol solution between each animal.

Data presentation and statistical analysis

Data were analysed using Stat View 512+ software (Abacus Concept Inc.). Means were given with ± SEM and were subjected to the unpaired Student's *t*-test. The level of significance was set at $p < 0.05$.

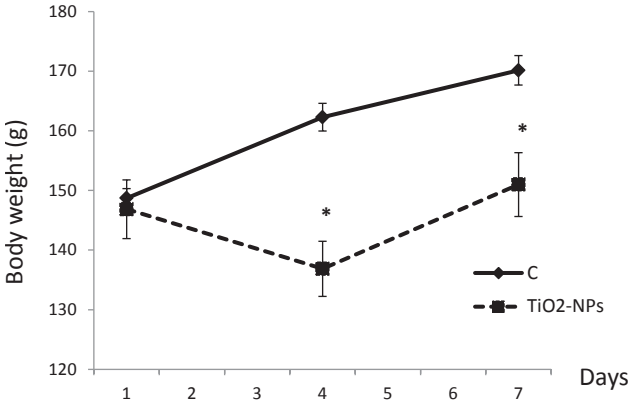

Figure 1. Body weight changes for rats treated with TiO_2-NPs at doses of 25 mg/kg (20–30 nm). Data represent the means ± SEM of 6 animals *per* group. * $p < 0.05$ compared to control (C).

Results

Coefficients of organs

TiO_2 -NPs treatment decreased significantly the body weight in day 4 (136.85 ± 4.61 *vs.* 162.28 ± 2.32; $p < 0.05$) and day 7 (151 ± 5.34 *vs.* 170.14 ± 2.46; $p < 0.05$) (Fig. 1). Table 1 showed the coefficients of liver, kidneys, testis and brain to body weight expressed as mg (wet weight of tissues)/g (body weight). No obvious significant differences were observed in the coefficients of liver (41.819 ± 0.86 *vs.* 43.308 ± 0.69; $p > 0.05$), kidney (8.83 ± 0.11 *vs.* 8.82 ± 0.13; $p > 0.05$) and testis (12.23 ± 0.54 *vs.* 11.66 ± 0.58; $p > 0.05$), but, the coefficient of brain was significantly higher in TiO_2-NPs-exposed rats than the control group (10.91 ± 0.39 *vs.* 9.61 ± 0.08; $p < 0.05$).

Biochemical measurements

Table 2 showed the changes of plasmatic biochemical parameters induced by TiO_2-NPs administration. Experimental group treated by intraperitoneal injection of TiO_2-NPs showed a significant increase of AST (72.1 ± 6.29 *vs.* 37 ± 97; $p < 0.05$) and uric acid (85.87 ± 8.11 *vs.* 36.52 ± 9.30; $p < 0.05$) but decreased creatinine levels (38.37 ± 3.28 *vs.* 59.91 ± 4.19; $p < 0.05$) compared with the control group. In contrast, glucose concentration (1.31 ± 0.03 *vs.* 1.35 ± 0.08; $p > 0.05$) and ALT (23.27 ± 5.44 *vs.* 18.37 ± 4.12; $p > 0.05$) and LDH (469.51 ± 47.20 *vs.* 461.41 ± 102.45; $p > 0.05$) activities remained unchanged.

Emotional behavior

Results from the elevated plus maze session are shown in Table 3 and Fig. 2. TiO_2-treated rats spent less time into the open arms than control group (5.65 ± 3.41 *vs.* 56 ± 11.62; $p < 0.05$). However, the centre time (31.6 ± 10.20 *vs.* 13.9 ± 6.24; $p > 0.05$) and closed arm time (2.66 ± 0.42 *vs.* 2.83 ± 0.79; $p > 0.05$) were slightly elevated in rats treated by TiO_2-NPs, with no significant differences. Moreover, behavioral experiments showed that TiO_2-NPs-injected group entered less frequently the open arms than control group. Sub-acute TiO_2-NPs treatment increased significantly the anxious index (AI) which is expressed (AI = Closed arm entries × 100/ Closed arm entries + Open arm entries) compared to control group (81.94 ± 5.85 *vs.* 45.83 ± 5.23; $p < 0.05$) (Fig. 2).

Table 1. Effect of TiO_2-NPs treatment on the tissue weight/body weight (mg/g) ratio in liver, kidney, testis and brain

Tissue	Control	TiO_2-NPs
Liver	43.308 ± 0.69	41.819 ± 0.86
Kidney	8.82 ± 0.13	8.83 ± 0.11
Testis	11.66 ± 0.58	12.23 ± 0.54
Brain	9.61 ± 0.08	10.91 ± 0.39*

Data represent the means ± SEM of 6 animals *per* group. * $p < 0.05$, compared to control.

Discussion

The potential of nanoparticles or nanomaterials to react with biological systems has been recognized in recent years and a number of toxicity studies of these emerging pollutants have appeared (Renping et al. 2010; Lavicoli et al. 2011). One of the reasons for the large amount of toxicity data

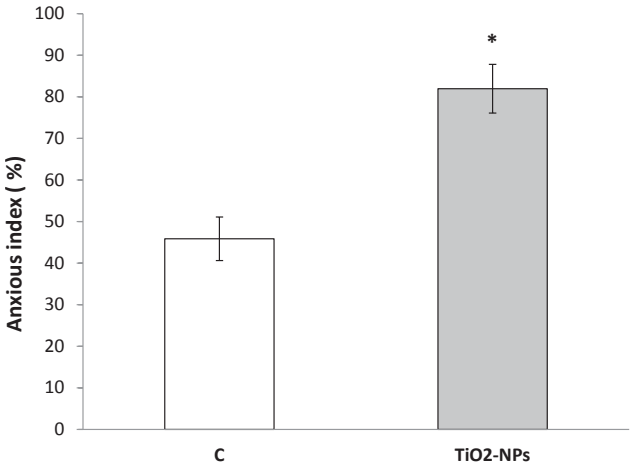

Figure 2. Graphical representation of anxious index from pulse mice assay of control and TiO_2-NPs treated rats. Data represent the means ± SEM of 6 animals *per* group. * $p < 0.05$ compared to control (C).

Table 2. Plasmatic biochemical parameters of control and TiO_2-NPs-treated rats

	Control	TiO_2-NPs
AST (UI/l)	37 ± 97	72.1 ± 6.29*
ALT (UI/l)	18.37 ± 4.12	23.27 ± 5.44
LDH (UI/l)	461.41 ± 102.45	469.51 ± 47.20
Uric acid (mg/l)	36.52 ± 9.30	85.87 ± 8.11*
Creatinine (μmol/l)	59.91 ± 4.19	38.37 ± 3.28*
Glucose (g/l)	1.35 ± 0.08	1.31 ± 0.03

Data represent the means ± SEM of 6 animals *per* group. * $p < 0.05$, compared to control. AST, aspartate aminotransferase; ALT, alanine aminotrasferase; LDH, lactate dehydrogenase.

on nanosized TiO_2 is the adoption of this nanomaterial by a variety of industries. Nanosized TiO_2 was among the first nanomaterials made readily commercially available to a wide variety of research activities (Menard et al. 2011). In the present study, emotional behavior was studied in particular since the relation between this behavior and TiO_2-NPs overload is not well-documented in the literature. To examine the effects of TiO_2-NPs on emotional behavior of adult rats, Wistar rats were treated by intraperitoneal injection of TiO_2-NPs suspension (20–30 nm) at a dose of 25 mg/kg and then tested in the elevated plus maze. Moreover, an extended set of biochemical parameters were measured in plasma to evaluate potential toxicological effects after exposure to TiO_2-NPs. Regarding to the effect of TiO_2-NPs on body weight, our results revealed that sub-acute exposure to TiO_2 decreased body weight. This result is consistent with those observed by Wang et al. (2007) who found that acute oral administration of a single higher dose of TiO_2 decreased body weight of mice. By contrast, coefficients of the brain are significantly higher than the control group and no significant difference in the liver and kidney coefficients was observed between the control and experimental groups. After respiratory exposure to ultrafine TiO_2 aerosols, rats exhibited significantly increased lung weight compared with clean-air control animals (Heinrich et al. 1995). This effect could be related to the serious pathological change disturbance in different metabolic systems which resulted probably in deficiency and interaction with intestinal absorption. For toxicity evaluation, liver function was investigated with plasmatic levels of ALT and AST. Nephrotoxicity was determined by uric acid and blood creatinine concentration. Our results showed a serious pathological change in kidney of treated rats resulting in a high plasmatic uric acid level and a decrease of creatinine content. TiO_2-NPs administration increased plasmatic AST activity, while ALT was slightly higher than control group. Our results are in agreement with the recent work reported by Unnithan et al. (2011) who mentioned that TiO_2-NPs induced biochemical perturbations and exhibited hepatic and renal toxicity in rats. Similarly, a single oral gavage of 5 g/kg TiO_2 particles induced also liver and kidneys damage, with hepatomegalia, hepatocyte necrosis, and swollen renal glomerulus and proteinic liquid accumulation in the renal tubules (Wang et al. 2007). Furthermore, it can be seen that LDH activity after intraperitoneal nanoparticles injection was slightly increased. By contrast, acute cardiotoxicity, in terms of higher serum LDH enzymes, was demonstrated after a single oral gavage of TiO_2-NPs to mice (Wang et al. 2007). The mechanism underlying the effects of $TiO_{2\text{-NPs}}$ on LDH was clarifed by Yanmei et al. (2010) using fluorescence spectral assays. TiO_2 was determined to be directly bound to LDH altering its structure and function. This effect could be related to the ultrafine structure of nanoparticulate TiO_2 (5 nm). Our results showed that glucose concentration remained unchanged in TiO_2-NPs-treated rats. However, Wang et al. (2009) reported a relative increase of glucose content in intratracheally instilled rats with TiO_2-NPs. An increasing number of studies showed that TiO_2-NPs may have negative effects on metabolic circle systems of organisms, while very few studies focused on the brain central nervous system (Liu et al. 2010; Gao et al. 2011; Menard et al. 2011). Thus, in the second part of the study, we investigated the effects of sub-acute exposure to TiO_2-NPs at a dose of 25 mg/kg on neurobehavioral performance of rats. Our results showed that TiO_2-NPs overload had serious effects on the emotional behavior. In the plus maze, TiO_2-NPs treatment increased the anxious index of animals. In fact, TiO_2-treated rats spent less time into the open arms and entered less frequently the open arms than control group. These results could be also interpreted in terms of reduced activity and exploratory drive instead of anxiety modifications. It has been shown that nanoparticles from the blood circulation may influence endothelial cell membrane integrity and/or disrupt the BBB *and may induce vesicular transport to gain access into the CNS* (Myrtill and Mats-Olof 2010). Moreover, it seems to be accepted that nanoparticles can induce oxidative stress leading to the generation of free radicals that could disrupt the BBB and cause certain dysfunc-

Table 3. Behavior of control and TiO_2-NPs-treated rats in the plus maze

Variables	Control	TiO_2-NPs
Time in the centre (s)	13.9 ± 6.24	31.6 ± 10.20*
Time in the open arms (s)	56 ± 11.62	5.65 ± 3.41*
Time in the close arms (s)	230.1 ± 15.34	259.5 ± 11.17
Open arm entries	3 ± 0.44	0.83 ± 0.307*
Closed arm entries	2.83 ± 0.79	2.66 ± 0.42

Data represent the means ± SEM of 6 animals *per* group. * $p < 0.05$, compared to control.

tions (Myrtill and Mats-Olof 2010). Another interesting explanation could relate the neurobehavioral performance deficits to the impairment in monoamine systems caused by nanoparticles, particularly in the dopamine neurotransmission known to be involved in learning, motivational and emotional processes (Win-Shwe and Fujimaki 2011). Hu et al. (2010) showed that TiO_2-NPs-exposure decreased the contents of some monoamines neurotransmitters such as norepinephrine, dopamine and its metabolite. These abnormalities in the monoaminergic systems may be associated with psychiatric diseases such as schizophrenia, depression anxiety and attention-deficit hyperactivity disorder (Ressler and Nemeroff 2000; Tamminga 2006).

Finally, this study showed that TiO_2-NPs intoxication could altere the biochemical parameters related to changes in organ function and lead to emotional behavior impairment of rats. Further investigation is needed to clarify the effect of TiO_2-NPs on neurotransmitter levels and functional alterations of the CNS.

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