Effects of Titanium Dioxide Nanoparticles (TiO_2) on Behavioral Parameters of Rats

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ABSTRACT

In general, *in vivo* investigations that evaluate the toxicity of TiO_2 particles concentrate on a particular route of exposure, which is driven by the anticipated application of TiO_2, for example the utilisation of TiO_2 containing sunscreens has prompted investigations into the dermal toxicity of TiO_2. However, the effects of TiO_2 NPs on the central nervous system are still unclear. In the present study, we investigated the effects of sub-acute exposure to TiO_2 NPs on emotional behavior in adult Wistar rats. Animals were injected intraperitoneally (ip) with TiO_2 NPs (25 mg/kg body weight) every two days for 20 days and then tested in the elevated plus-maze and in an open field. The elevated plus-maze test showed that sub-acute TiO_2 NPs treatment increased significantly the anxious index (AI) which is expressed [AI= Closed arm entries / (Closed arm entries + Open arm entries)*100]. TiO_2 NPs treatment decreased the frequency of entries in the central part of the open field (session 1). Central part entries also became more frequent from day to day with no significant difference between days 1, 2 and 3. The immobility time of TiO_2 NPs injected rats increased significantly. However, no significant effect was observed in the number of contacts with objects between treated and control groups. Titanium content assessed in the homogenate brain remained unchanged in the TiO_2 NPs treated group.

In conclusion, analyses of open-field data confirm the plus maze results indicating that TiO_2 NPs could altered the neurobehavioral performance of adult Wistar rats.

Keywords: TiO_2 Nanoparticles, Emotional behavior, Elevated plus-maze, Open field, Rats.

1 INTRODUCTION

Nanosized titanium dioxide (TiO_2) is used widely in various everyday products and can be applied to the medical field for diagnostic or therapeutic tools [1]. Because of its whitening and photocatalytic effects, is used in the production of everyday products such as paper, cosmetics and food [1]. Indeed, the International Life Sciences Institute Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Work Group has recently discussed the importance of studying nanoparticles for their potential toxic effects. TiO_2 NPs are toxic to human organs, and cause oxidative stress [2]. So nanoparticles can enter into organisms via nasal, oral, intraocular, intratracheal, injection and other routes [3]. Recent studies have unequivocally showed that exposure to TiO_2 NPs could be translocated into the central nervous system via the olfactory pathway and damage brain neurocyte and tissue *in vitro* and *in vivo*[4]. Moreover, TiO_2 NPs with oral gavage caused a slight brain lesion of mice, such as vacuoles of neurons and fatty degeneration of hippocampus [4]. Nanoparticles can cross the blood–brain barrier and enter (in low numbers) the central nervous system (CNS) of the exposed animals [5].

In the present work we evaluated the sub-acute toxicity of TiO_2 nanoparticles in central nervous system. For this purpose, we have studied the effects of the particle exposure on neurobehavioral performance of rats.

2 MATERIALS AND METHODS

2.1 Animals

Experiments were performed in male rats (weighing 100–110 g at the beginning of the experiment). Rats were acclimatized for 1 week before the experiments in an animal room under a 12 h light/dark cycle at 25±2°C and 60±10% relative humidity. Distilled water and sterilized food for rats were available ad libitum. All procedures used in this experiment were compliant with the local ethics committee.

2.2 Treatment

Animals received Rats were divided into two groups (n=12), control and experimental groups. Treated animals received 10 ip injections with moderate dose of TiO_2 NPs (20 mg /Kg) every two days and control group received a

dose of 9‰ sodium chloride. Then they were tested in the elevated plus-maze and in an open field.

2.3 Behavioral testing

Elevated plus maze test

The aim of this test was to identify the effect of titanium dioxide nanoparticles on anxiety behaviours at adult rats [6].

The plus maze was made of clear painted wood. The arm was elevated 60 cm above floor level with two open arms (50 x 10cm) and two closed arms with 50cm high walls [7]. Arms of the same type were located opposite from each other. Individual rats were placed on the center of the maze facing an open arm, and the time spent in open and closed arms as well as the path length were recorded during a 5min period using the videotracking system.

The number of entries into an arm, and the time spent in the open and closed arms were recorded. Percentage of entries into open arms, time spent in open arms (s) and number of total entries were analyzed. In addition, the anxious index (AI) which is expressed [AI= Closed arm entries / (Closed arm entries + Open arm entries)*100] was calculated.

Open field test

Locomotor activity and anxiety were measured using an open field test [7]. So it's often used to assess anxiety by including additional measures of defecation, time spent in the center of the field, and the first five minutes of activity.

Each rat was placed in the starting box of the open field apparatus (a circular enclosure steel, 1 meter in diameter and 50cm high).The apparatus was divided by black lines into one central and six peripheral parts of equal surface and three identical objects (whiteglass incubation tray) were placed in the field. The experiment took place in the same room as the plus maze experiment and, therefore, under very similar conditions (intensity of light). At the beginning of the test, the rat was placed in the peripheral part of the open field. Number of entries into the central part of the field were measured and considered as an approach-avoidance measure and an index of anxiety since , time spent in the center area, frequency of contact with an object and total number of faecal boli (defecation).

3 STATISTICAL ANALYSIS

The data from are expressed as mean (±SEM) or as percentages. Multiple comparisons were evaluated by the analysis of variance (ANOVA) for repeated measurement followed by post hoc Tukey's HSD tests using the statistical software program (Statistica.8) and Microsoft Office Excel 2007. Differences were considered significant at $P<0.05$.

4 RESULTS

X-ray diffraction measurements (Figure 1) show that TiO_2 NPs exhibit the anatase structure, and the average grain size calculated from the broadening of the XRD peak of anatase was roughly 30nm using Scherer's equation.

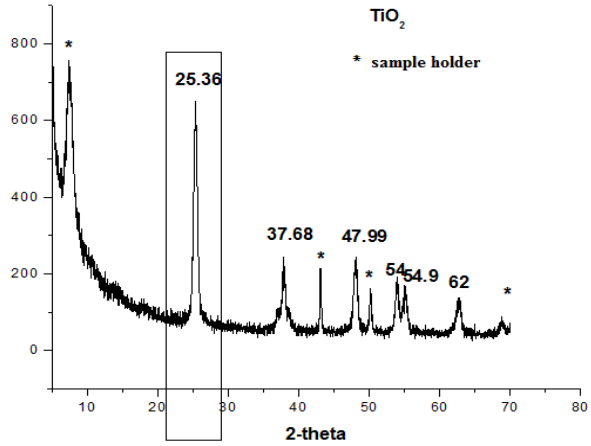

Figure 1: XRD pattern of nano-sized TiO_2 particles.

The elevated plus-maze test showed that sub-acute TiO_2 NPs treatment decreased both the percentage of time spent in the open arms and the number of visits to the open arms (Figure 2). The anxious index (AI) was significantly increased **(**Figure 3**)**. TiO_2 NPs treatment decreased the frequency of entries in the central part of the open field (session 1). Central part entries also became more frequent from day to day with no significant difference between days 1, 2 and 3. The immobility time of TiO_2 NPs injected rats increased significantly. However, no significant effect was observed in the number of contacts with objects between treated and control groups (Figure 4, 5).

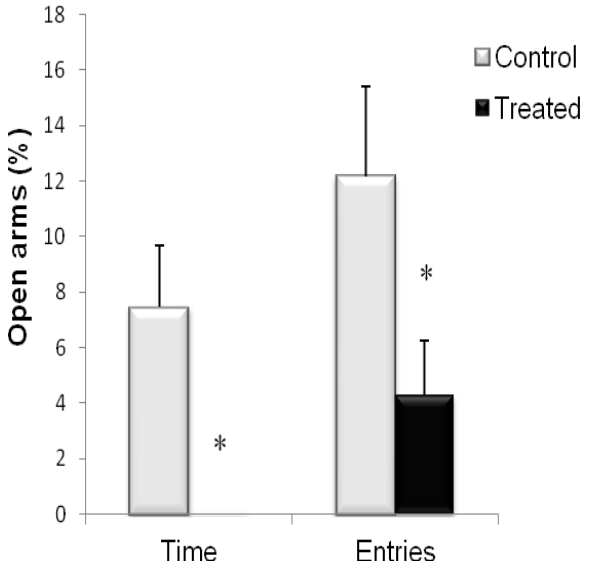

Figure 2: Percentage of time spent in the open arms and number of visits to the open arms of an elevated plus-maze of control and (ip) injected rats with TiO_2 NPs. (*) P<0.05 vs. control; n = 12; (test *t* Student). Values represent mean ± SEM.

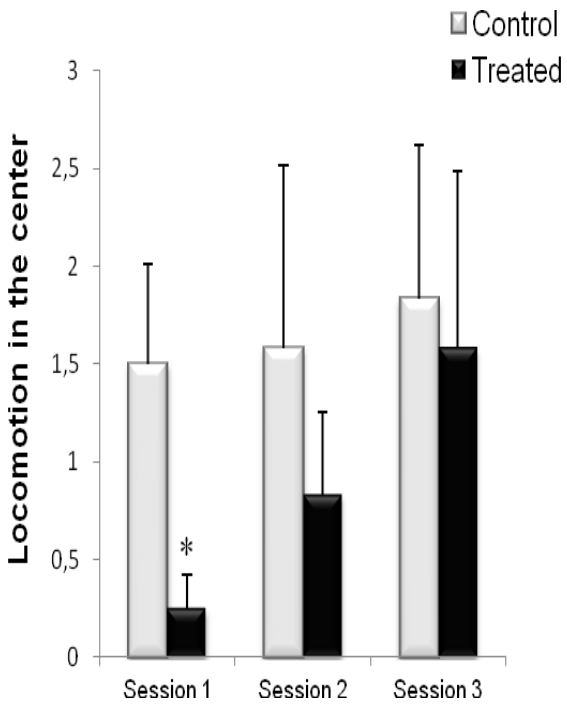

Figure 4: The frequency of the central entries of control and (ip) injected rats with TiO_2 NPs in three sessions of open field test. (*) P<0.05 vs. control; n = 12; (ANOVA test). Values represent mean ± SEM.

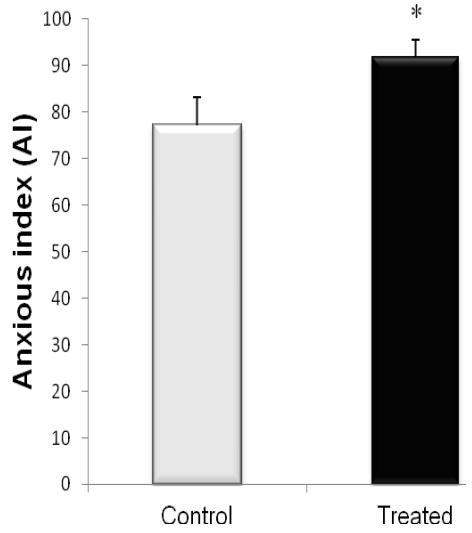

Figure 3: Anxious index (AI) of control and (ip) injected rats with TiO_2 NPs. (*) P<0.05 vs. control; n = 12; (test *t* Student). Values represent mean ± SEM.

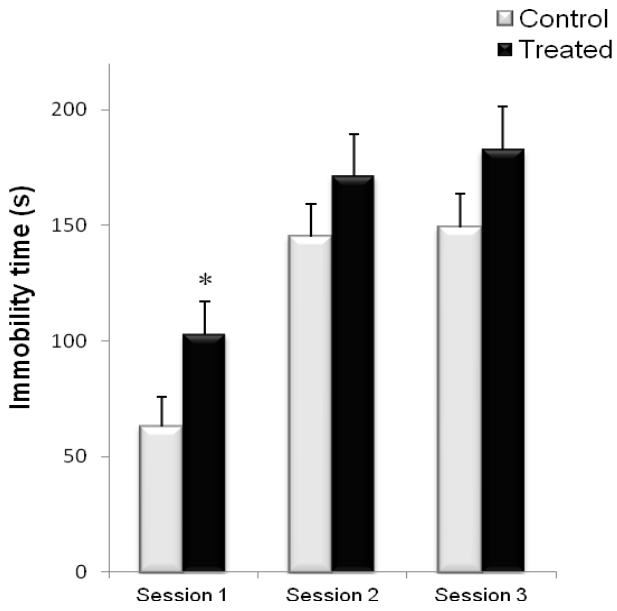

Figure 5: The frequency of the The immobility time of control and (ip) injected rats with TiO_2 NPs in three sessions of open field test. (*) P<0.05 vs. control; n = 12; (ANOVA test). Values represent mean ± SEM.

5 DISCUSSION

Previous reports indicated that nano-sized component of particulate matters can reach the brain and may be associated with neurodegenerative diseases [8] [9]. Whereas, little has been known about nano-TiO_2 neurotoxicity *in vivo*. In the present study, we investigated the effects of nano-TiO_2 on emotional behavior in adult Wistar rats. In our study, results from the elevated plus maze session showed that TiO_2 nanoparticles-injected group entered less frequently the open arms than control group. Moreover, sub-acute TiO_2 NPs treatment increased significantly the anxious index (AI). This effect was associated with the poor performance of animals in the first session of the open field test. TiO_2 nanoparticles can induce oxidative stress leading to the generation of free radicals that could disrupt the neurobehavioral performance of rats. TiO_2 nanoparticles were shown to stimulate ROS generation in the brain microglia and cause neuron damages *in vitro* [10]. The brain is highly vulnerable to oxidative stress due to its high metabolic rate, the reduced capacity for cellular regeneration (low levels of endogenous scavengers, e.g., vitamin C, catalase, superoxide dismutase), and numerous cellular oxidative stress targets (i.e., lipids, nucleic acids, and proteins) [11]. However, the oxidative toxicity of TiO2 nanoparticles in the brain has not been well studied *in vivo* to date

Interestingly, the study performed by Hu et al. [4] reported that neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO_2 nanoparticles could be related to the disruption of trace elements homeostasis, neurotransmitters content and enzymes in brain. TiO_2 NPs are able also to induce an inflammatory effect in brain of treated mice and could affect synaptic plasticity [1] [12].

This study reveals the possible toxicity of TiO_2 NPs in the central nervous system, which indicates that TiO_2 NPs altered the neurobehavioral performance of adult Wistar rats.

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