ELECTROPHYSIOLOGICAL ALTERATIONS AND GENERAL TOXIC SIGNS OBTAINED BY SUBACUTE ADMINISTRATION OF TITANIUM DIOXIDE NANOPARTICLES TO THE AIRWAYS OF RATS

Tamara HORVÁTH[1], András PAPP[1], Dávid KOVÁCS[2], Ildikó KÁLOMISTA[3], Gábor KOZMA[4], Tünde VEZÉR[1]

[1]Department of Public Health, University of Szeged Faculty of Medicine, Szeged
[2]Department of Biochemistry and Molecular Biology, University of Szeged Faculty of Science and Informatics, Szeged
[3]Department of Inorganic and Analytical Chemistry, University of Szeged Faculty of Science and Informatics, Szeged
[4]Department of Applied and Environmental Chemistry, University of Szeged Faculty of Science and Informatics, Szeged

PRINT | English | http://dx.doi.org/10.18071/isz.70.0001 | www.elitmed.hu

Introduction and aims – Particles of titanium dioxide (TiO_2) with typical size below 100 nm have gained a broad range of application by now, partly involving direct human exposure. Their known properties – high specific surface, mobility within the organism, induction of oxidative stress, release of inflammation mediators etc. – raise the possibility of nervous system damage but the available data regarding this are scarce and contradictory. Based on that, and the experiences with other metal oxide nanoparticles, the aim of the present study was to investigate certain general end nervous system toxic effects of TiO_2 nanoparticles applied in the airways of rats.

Materials and methods – Young adult Wistar rats (5 groups of 10 rats each) received, daily for 28 days, intratracheal instillations of titanium dioxide nanoparticles of ca. 10 nm diameter, suspended in 1% hydroxyethyl cellulose dissolved in phosphate-buffered saline, in the doses of 1, 3, and 10 mg/kg b. w. Vehicle controls received the suspension medium and there was also an untreated control group. During treatment, the rats' body weight was measured, and their clinical state observed, daily. After the 28 days, spontaneous cortical activity, sensory evoked potentials and tail nerve action potential was recorded in urethane anesthesia, then the rats were dissected and tissue samples were taken for Ti level determination and biochemical measurements of some oxidative stress indicators.

TITÁN-DIOXID NANORÉSZECSKÉK SZUBAKUT LÉGÚTI ADAGOLÁSÁVAL KIVÁLTOTT ELEKTROFIZIOLÓGIAI ELTÉRÉSEK ÉS ÁLTALÁNOS TOXICITÁS PATKÁNYBAN

Horváth T, MD; Papp A, MD; Kovács D, MD; Kálomista I, MD; Kozma G MD; Vezér T, MD

Ideggyogy Sz 2017;70(1–2):000–000.

Bevezetés és célkitűzés – A titán-dioxid (TiO_2) nanoméretű, jellemzően 100 nm-nél kisebb, részecskéi ma már számos, részben közvetlen emberi expozícióval járó alkalmazásban megjelentek. Ismert tulajdonságaik – nagy fajlagos felület, szervezeten belüli mozgékonyság, oxidatív stressz keltése, gyulladásmediátorok felszabadítása stb. – alapján idegrendszeri károsodást okozhatnak, azonban erre vonatkozóan csak kevés és ellentmondó adat áll rendelkezésre. Erre, és más fémoxid-nanorészecskékkel szerzett tapasztalatokra alapozva a jelen munkában azt vizsgáltuk, milyen általános és idegrendszeri toxikus hatás idézhető elő TiO_2-nanorészecskék patkányok légutaiba való adagolásával.

Anyagok és módszerek – Fiatal felnőtt hím Wistar-patkányokat (öt csoport, 10-10 állat) kezeltünk naponta, 28 napig, 1% hidroxietil-cellulózt tartalmazó foszfátpufferelt fiziológiás oldatban szuszpendált, körülbelül 10 nm átmérőjű TiO_2-nanorészecskék intratrachealis instillációjával, 1, 3 és 10 mg/ttkg dózisban. A vivőanyagos kontrollcsoport a szuszpendáló közeget kapta instillálva, míg a kezeletlen kontrollok semmit sem. A kezelés során naponta mértük a testtömeget és megfigyeltük az állatok általános klinikai állapotát. Az expozíciós periódust követően uretánaltatásban kérgi alapaktivitást, szenzoros kiváltott potenciálokat, és a farokideg akciós potenciálját regisztráltuk; végül az állatokat felboncoltuk és szövetmintákat vettünk fémszint-meg-

Correspondent: András PAPP MD, Department of Public Health, University of Szeged Faculty of Medicine; 6720 Szeged, Dóm tér 10. Phone: +36-62-545-119, Fax: +36-62-545-120, E-mail: papp.andras@med.u-szeged.hu

Érkezett: 2016. november 2. Elfogadva: 2017. január 5.

Results – The two higher doses reduced the rate of body weight gain significantly. Sensory evoked potentials and tail nerve action potential were significantly slowed, but the change in the spectrum of spontaneous cortical activity was not significant. Correlation of moderate strength was found between certain evoked potential parameters and brain Ti level and oxidative stress data.
Conclusion – Our results underlined the possible neurotoxicity of TiO_2 NPs but also the need for further investigations.

Keywords: *nanoparticles, titanium dioxide, neurotoxicity, oxidative stress*

Eredmények – A két nagyobb dózisú csoportban a testtömeg-gyarapodás üteme szignifikánsan csökkent. A szenzoros kiváltott potenciálok és a farokideg akciós potenciálja szignifikánsan lassabbak lettek, a spontán kérgi aktivitás spektrumának változása azonban nem mutatott szignifikáns eltérést. A kiváltott potenciálok egyes paraméterei, illetve az agykéreg titánszintje és oxidatívstressz-jellemzője között közepes mértékű korreláció mutatkozott.
Következtetés – Az eredmények rámutatnak a TiO_2-nanorészecskék lehetséges neurotoxicitására, de egyben a feltételezett hatásmechanizmus alátámasztása céljából végzendő további vizsgálatok szükségességére is.

Kulcsszavak: *nanorészecskék, titán-dioxid, neurotoxicitás, oxidatív stressz*

Nano-titanium, more exactly particles of TiO_2 with typical size below 100 nm, has gained by now a broad range of application, first of all as white pigment; in paints and coatings but also in food, personal care and pharmaceutical products[1], possibly leading to direct exposure of humans. Primary production (yearly a few thousand tons worldwide) and processing to final products involves the risk of workplace exposure by inhalation[2]; the actuality of the health risk is indicated by the occupational exposure limit of 0.3 mg/m^3 for ultrafine (<100nm diameter) TiO_2, set by National Institute of Occupational safety and Health[3].

On inhalation (the most typical way of exposure) nanoparticles (NPs) are either deposited in the nasopharynx or get down to the alveoli; then reach distant body parts by migrating along the olfactory and other nerves, or by crossing the alveolar and capillary wall and entering systemic circulation. The high surface-to-volume ratio of NPs results in intense surface-dependent reactions, among others generation of reactive oxygen species. NPs were shown also to target mitochondria directly and disrupt oxidative phosphorylation, also resulting in ROS[4].

Oxidative stress of any source can especially affect the nervous system because of its high sensitivity, due to highly active mitochondrial energy production to cover the neurons' energy demand, to abundance of (unsaturated) structural lipids, and to low antioxidant defence capacity[5]. The nervous system consequences seen in animals treated experimentally with TiO_2 NPs have been up to now rather variable. In some experiments, access of TiO_2 NPs to the rat brain after application to the airways was verified, together with damage to the blood-brain barrier[6]; the same authors also reported dose-and time-dependent toxicity of the identical TiO_2 NPs on rat astrocytes in vitro. Others, however, found that most of the nano-TiO_2 remained in the lungs[7].

Functional alterations, possibly resulting from access of TiO_2 NPs to the brain, have been described at the level of electrophysiological changes only a few times up to now. Using murine frontal cortex neuronal networks cultured *in vitro*, uptake of nano-TiO_2 by the cells and massive depression of spike activity was found[8]. In mice, 60 days oral nano-TiO_2 treatment caused neuronal degeneration and dose-dependently reduced hippocampal long-term potentiation *in vivo*[9].

Human cultured neuronal (SH-SY5Y) and glial (D384) cells also showed TiO_2 NP internalization, followed by mitochondrial and membrane damage[10]; and neurological symptoms were detected in humans exposed to airborne particles of a titanium-based pigment at the workplace[11].

Based on the multitude of applications of nano-TiO_2 mentioned above, and the experiences with other metal oxide nanoparticles applied intratracheally[12], the aim of the present study was to investigate certain general and (electrophysiologically detectable) neurotoxic effects of TiO_2 NPs suspension applied in the airways of rats.

Methods

ANIMALS AND TREATMENT

Young adult male SPF Wistar rats (Crl:WIBr; 6 weeks old, 170±20 g body weight) were used, obtained from Toxi-Coop Ltd. (Budapest, Hun-

gary). The total number of animals was 50, sufficient for p=0.8 in power analysis. The rats were kept, two in a cage, in a GLP-rated animal house (12-12 hour light/dark cycle with light on at 06:00; temperature 22±3 °C, 30-70% relative humidity); and could consume unlimited amount (20-30 g/day/animal) of standard rodent food (Ssniff R/M-Z+H, also from Toxi-Coop Ltd., Budapest, Hungary) and water. After one week of acclimation, the rats were randomized to 5 treatment groups of 10 rats each, on the basis of their body weight and spontaneous exploratory activity. The groups and corresponding treatments are shown in **Table 1**.

Table 1. *Groups and treatment*

Groups	Code	Treatment
Untreated control	*C*	–
Vehicle control	*VC*	PBS-HEC, 1 ml/kg b. w.
Treated, low dose	*LD*	TiO_2 NPs in PBS-HEC, 1 mg/kg b. w.
Treated, medium dose	*MD*	TiO_2 NPs in PBS-HEC, 3 mg/kg b. w.
Treated, high dose	*HD*	TiO_2 NPs in PBS-HEC, 10 mg/kg b. w.

The TiO_2 NPs used to treat the rats were synthesized as follows: Titanium isopropoxide (TTIP; 7.32 g) was added to 50 ml ethanol (absolute) and stirred for ten minutes. Simultaneously, 20 ml ethanol was mixed in 165.5 ml distilled water and stirred for the same duration. Then, the ethanol-water mix was added dropwise (slowly: one drop per 5 seconds) to the TTIP solution which was continuously stirred at high speed (1200 rpm), and stirring was continued for 30 min after adding all the ethanol-water mix. The TiO_2 nanoparticles generated were collected from the suspension by centrifuging and were dried for 36 hours at 80°C in air. Diameter of the NPs produced, determined by transmission electron microscopy, was ca. 10 nm. The size histograms, in dry and freshly suspended state, are given in **Figure 1**.

Treatment of the rats was done daily for 28 days (five treatment days/week), always between 8:00 and 10:00 am. The TiO_2 NPs were suspended to the doses given in **Table 1** in PBS (pH 7.4) with 1% hydroxyethyl cellulose (efficient dispersion was aided by sonication using a UP200HT instrument) and were applied to the rats by intratracheal instillation (1 ml/kg b. w. volume) in brief anesthesia (see[12] for details of the technique). Rats in the vehicle control group *(VC)* underwent the same daily procedure of anaesthesia and instillation while the untreated controls *(C)* had nothing beyond daily animal care.

Urethane and the chemicals for the NP synthesis were from Sigma-Aldrich. Hydroxyethyl cellulose was obtained at the pharmacy of the Medical Faculty.

GENERAL TOXICOLOGICAL AND CHEMICAL MEASUREMENTS

The rats' body weight was measured every morning, before treatment, to determine the exact daily doses to be instilled and to see the effect on body weight gain.

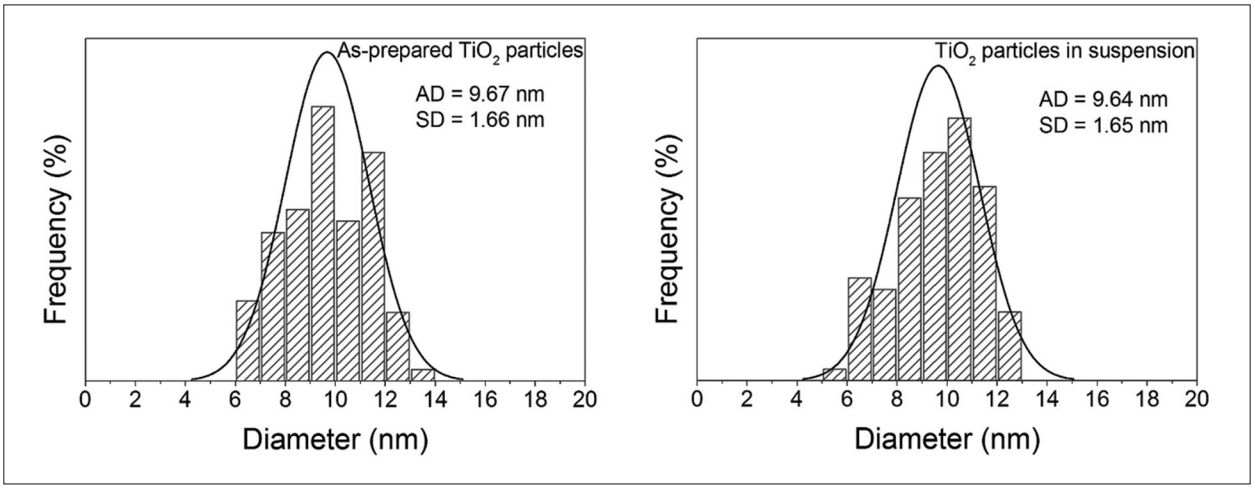

Figure 1. *Particle size histograms of the TiO_2 nanoparticles in dry (as-prepared, left graph) and freshly suspended (right graph) state*

AD: average diameter; SD: standard deviation.

After electrophysiological recording at the end of the experiment, the rats were sacrificed by an overdose of urethane (twice the anaesthetic dose mentioned below) and were dissected. First, 2-3 ml blood was taken from the abdominal vein of each rat for Ti level and some ROS parameters measurement. Three rats from each group, chosen randomly, were then transcardially perfused with 300 ml saline of 4°C temperature to remove blood from the organs, and were then dissected. The organs heart, kidneys, adrenals, liver, lungs, spleen and thymus were weighed, and relative organ weights (to 1/100 body weight) were calculated. Brain, liver and lung samples were stored at –20°C for Ti level measurement. In another three rats per group, also chosen randomly, dissection was done rapidly right after sacrifice and blood taking. Having the organs weighed, brain, liver and lung samples were shock-frozen in liquid nitrogen and stored at –20°C for subsequent measuring lipid peroxidation, as an indicator of oxidative stress induced by the TiO_2 NPs, using the thiobarbiturate reaction. Ti level from the perfused rats' samples was determined by ICP-MS. For that, the samples were dried to constant weight at 80°C, and were digested as follows: 3 ml cc. HCl/g wet tissue for 90 min at 90°C, then an equal volume of cc. HNO_3 was added and digested for further 90 min. This procedure proved to be necessary to reliably dissolve all TiO_2. The resulting liquid was filtered on 0.45 mm hydrophilic membrane filter and diluted to 100 ml final volume.

ELECTROPHYSIOLOGICAL RECORDING

Electrophysiological recording was done on the day following the last TiO_2 NP administration. In urethane anaesthesia (1000 mg/kg ip.) the left hemisphere was exposed by a sagittal cut in the head skin, blunt removal of connective tissues, then drilling around and removing the left parietal bone. Following recovery (at least 30 min), recording electrodes were placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas. The areas were identified by means of a somatotopic map, and fine positioning of the electrodes was done by searching for the punctum maximum of the evoked responses. A stainless steel clamp, attached to the cut skin edge, was used as indifferent electrode. With the cortical electrode positions finalized, electrocorticogram (ECoG) was recorded for 6 min. Analysis of the ECoG records provided the power spectrum based on standard human EEG bands.

Then, trains of sensory stimuli (SS: electric shocks [3-4 V, 0.05 ms] to the contralateral whisker pad, VIS: flashes [0.2 ms] from a high-luminance white LED, AUD: clicks [70 dB] through the hollow ear bar of the stereotaxic frame) were applied, and cortical evoked potentials (EPs) recorded from the same sites. One train of 50 stimuli was given with 1 Hz frequency. SS stimulation was repeated with 2 and 10 Hz to see any frequency effect. On the EPs, latency and duration of the main waves was measured manually after averaging.

From the tail nerve, compound action potential (CAP) was recorded by inserting a pair of needle electrodes at the base of the tail for stimulation (3-4 V, 0.05 ms shocks) and another pair 50 mm distally for recording. Conduction velocity was calculated from this distance and the onset latency of the CAP. Relative refractory period was measured by double stimuli with inter-stimulus interval decreased from 10 to 1 ms, based on the extra delay of the second potential. From the data of the SS EPs and the CAPs, it was possible to calculate the ratio of identical parameters (latency or amplitude) of the last and first five potentials from a series of 50, and use this as an indicator of fatigue (as outlined in[13]).

Stimulation, recording and analysis was done using the NEUROSYS 1.11 software (Experimetria Ltd., Budapest, Hungary). For further details of electrophysiological recording and analysis, see[13].

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed. The methods used in this work were licensed by the authority competent in animal welfare issues under No. XXI./151/2013.

DATA PROCESSING

From the individual rats' data, group mean and standard deviation was obtained. Depending on the normality of data distribution, checked by Shapiro-Wilk test. Body/organ weight data, of sufficiently normal distribution, were analysed by one-way ANOVA and post hoc Tukey test while for electrophysiological and chemical-biochemical data non-parametric Kruskal-Wallis ANOVA and post hoc Mann-Whitney U-test was used, with $p<0.05$ as limit of significance for both. The software used was SPSS version 22.0 (IBM Corp., USA).

The possible linear correlation between data sets was tested by the "linear fit" function of MS Excel. This function uses the least squares method to fit a straight line to the measurement data, and examines the strength of relationship with Fisher's F test.

Results

EFFECT ON BODY AND ORGAN WEIGHT GAIN

The effect of treating the rats with TiO_2 NPs on the body weight gain is shown in **Figure 2**. From the 3rd week on, a significant deficit in body weight gain was observed in the groups *MD* and *HD*, compared to the untreated controls *(C)*. A clear difference was also present between the weight gain of the untreated *(C)* and vehicle treated *(VC)* controls, showing that the treatment procedure itself had some effect, but that was not significant. It is also of interest that the strongest effect was seen with the medium, and not the high, dose. In the overall weight gains (*C*: 213.65±34.98 g; *VC*: 177.38±43.39 g; *LD*: 187.66±37.19 g; *MD*: 142.15±16.49 g; *HD*: 165.16±26.61 g) also *MD* vs. *VC* was significant at p<0.05.

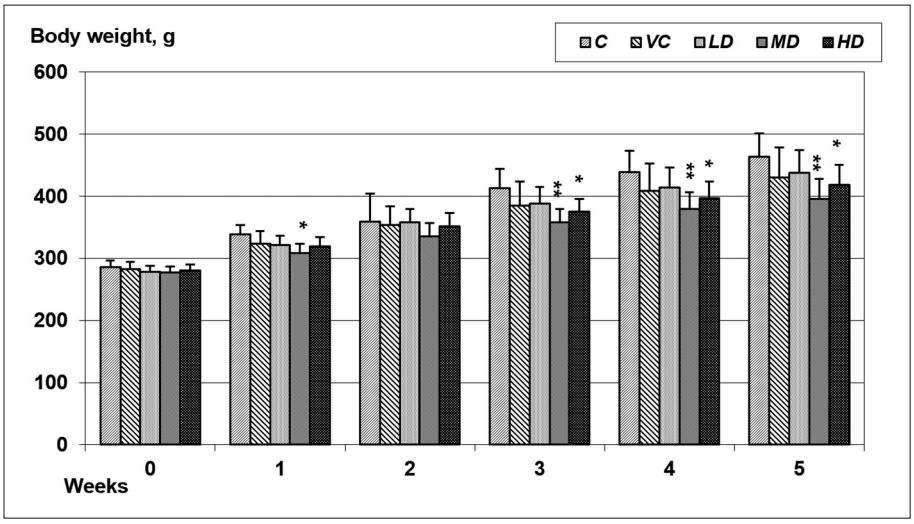

Figure 2. *Time course of body weight in the control and treated rats over the weeks of nano-Ti administration. The data shown were measured on Friday of each week*

Mean+SD, n=10. *, **: p<0.05, 0.01 vs. C.

Table 2. *Relative organ weights (to 1/100 body weight) in the control and treated rats at the end of treatment*

Groups	Relative organ weights			
	Brain	Lungs	Kidneys	Liver
C	0.443±0.03	0.303±0.02	0.584±0.02	3.221±0.321
VC	0.483±0.06**	0.559±0.04***	0.632±0.09***	3.153±0.213
LD	0.508±0.07***	0.565±0.09***	0.691±0.09***	3.180±0.183
MD	0.510±0.02***	0.656±0.10***#	0.669±0.09***#	3.183±0.290
HD	0.498±0.04	0.642±0.06***#	0.640±0.07*	3.223±0.361

Mean+SD, n=10. *, **, ***: p<0.05, 0.01, 0.001 vs. C; #: p<0.01 vs. VC.

The relative weight of the lungs was significantly increased in the groups *MD* and *HD*, both vs. *C* and *VC* **(Table 2)**. The increase of the relative brain and kidney weight was significant only vs. *C*. Again, the effect of the medium dose was the strongest. Other organ weights showed no marked alteration.

ELECTROPHYSIOLOGICAL CHANGES

On the cortical EPs, lengthening of the latency was observed in the treated rats, first of all with the two higher doses. Significant lengthening of the SS EP latency, vs. both *C* and *VC* **(Figure 3)** was seen in group *MD* and *HD*. In these groups, also the frequency-dependent extra lengthening of latency was more marked than in *LD* and both controls. The effect of nano-TiO_2 exposure on the VIS and AUD EPs was similar **(Figure 2)**.

The [last 5 / first 5] ratio of the SS EP latency **(Figure 4)** suggested increased fatigability of the peripheral and central structures involved in the generation of evoked potentials, and again showed the non-monotonous dose dependence seen with body weight gain and EP latency.

Conduction velocity of the tail nerve decreased significantly in the *MD* and *HD* rats vs. *C* and *VC* **(Figure 5)**. Some increase of the relative refractory period was seen but proved to be non-significant. The [last 5 / first 5] ratio of the tail nerve CAP latencies **(Figure 6)** was similar to that seen with the SS EPs. The change in the ECoG spectrum, a shift to higher frequencies in groups *MD* and *HD* was, in contrast to the evoked activity forms, insignificant (not shown).

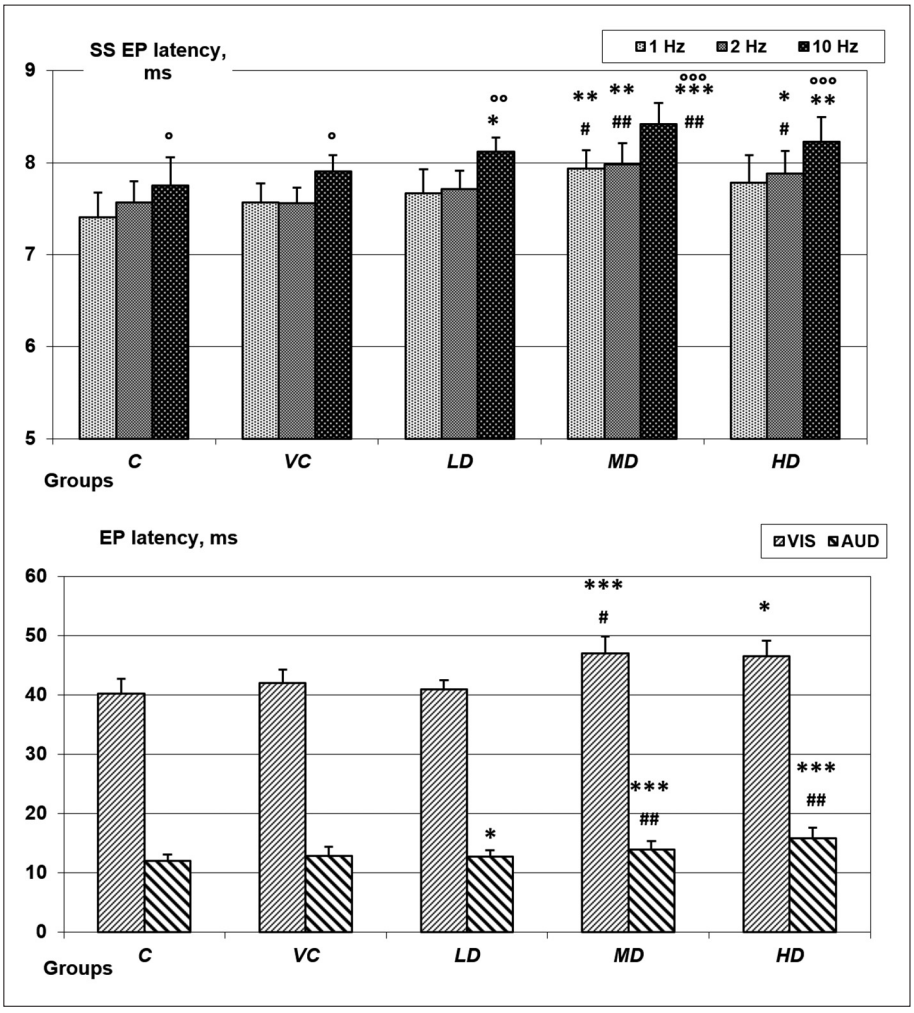

Figure 3. *Latency of the somatosensory (upper graph) and visual and auditory (lower graph) cortical evoked potentials*

Mean+SD, n=10.
*, **, ***: p<0.05, 0.01, 0.001 vs. C; #, ##: p<0.05, 0.01 vs. VC; °, °°, °°°: p<0.05, 0.01, 0.001 vs. 1 Hz stimulation within the same group.

TISSUE METAL LEVELS AND BIOCHEMICAL ALTERATIONS

The measured levels of tissue Ti content and lipid peroxidation are shown – only for groups with significant electrophysiological effect, that is, in *MD* and *HD* – in **Table 3**. Most of the Ti administered to the rats remained in the lungs but there was a measurable dose-dependent deposition of Ti also in the brain mass, and, with less clarity, in the liver. The low number of measured samples (see Methods) mostly precluded the detection of significant differences but the proportionality of inner Ti levels and lipid peroxidation to the doses applied was clear.

The relationship suggested by the data of internal Ti load, oxidative stress, and functional alterations was tested by examining the possible linear correlations. A seen in **Figure 7**, correlation of moderate strength was found between the latency of SS EP at 10 Hz stimulation (exerting the maximal strain on the somatosensory pathway in this experimental scheme) and the level of Ti and TBARS in the brain samples (in case of the latter, in spite of apparent no-effect at group level). The level of correlation between VIS EP latency and the same chemical parameters was similar (not shown).

Discussion

The results showed that the doses and way of application of TiO_2 NPs to rats, as performed in the present experiment, were effective in creating internal load and inducing signs of general and nervous system toxicity. According to the measured tissue Ti levels, most of the amount applied remained in the lungs but the load measured in the brain and other organ samples was also considerably higher than in the rats without nano-TiO_2 exposure. The high degree of deposition of TiO_2 NPs in the lungs was in similar to the observation in[7] that a single amount instilled in the trachea of rats was completely retained in the lungs for 7 days.

The adequacy of the model is determined, however, also by the relationship of the experimentally generated exposure to that described in exposed humans and to the relevant limit values. There are not many data available on occupational airborne Ti exposure. Those published in[14] show that in Europe and North America there were no workplace levels above 1 mg/m^3 (for the whole respirable fraction) in the last 20 years. Recommended exposure limits of NIOSH[3] from 2011 are 2.4 mg/m^3 for suspended

TiO_2 dust and 0.3 mg/m^3 for ultrafine dust (that is, for TiO_2 NPs) in a 4×10 hours per week exposure scheme. Calculating with the daily ventilation volume of rats[15], the dose of TiO_2 NP applied in the rats' trachea in the present study would be equal to atmospheric concentrations of ca. 5, 15 and 50 mg/m^3. These are about one degree of magnitude higher than the limit recommended by NIOSH, but the length of exposure, 28 days, is a relatively short fraction (ca. 1/30) of the expectable life span of rats and would thus correspond to ca. 2.5 years in humans (a relatively short part of a job career).

Due to the tendency of NPs to induce oxidative stress in living organisms, and the especial sensitivity of the nervous system to that (mentioned in Introduction), ROS generation by TiO_2 NPs in various experimental settings both *in vitro* and *in vivo*[1] is an important aspect of possible CNS effects. *In vitro* studies indicated mitochondrial damage and ROS generation on exposure to TiO_2 NPs in human neuronal and glial cell lines[10]. The tendency of NPs to migrate to the mitochondria and interfere with oxidative phosphorylation[4] might have contributed to that.

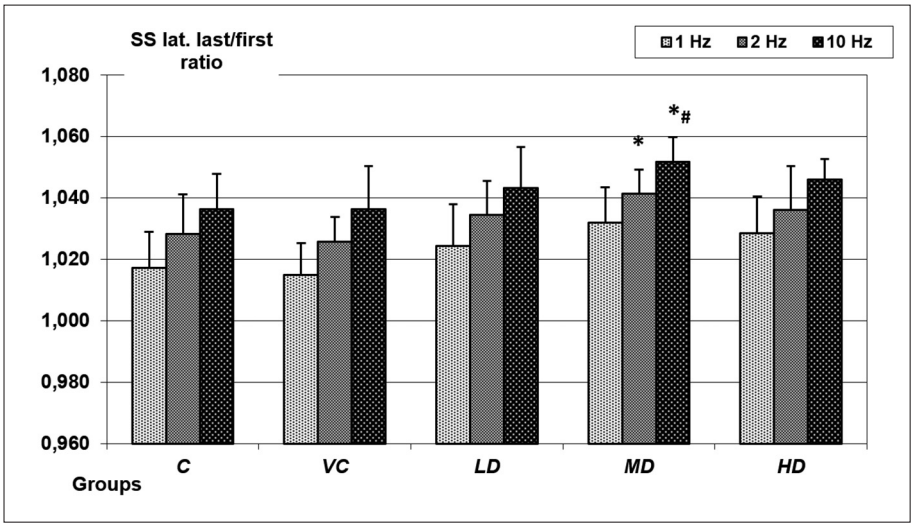

Figure 4. *Fatigue of the SS EPs during a series of 50 stimuli, quantified by the ratio of the last and first EPs' latencies (see Methods for details)*

Mean+SD, n=10.
*: p<0.05 vs. C.

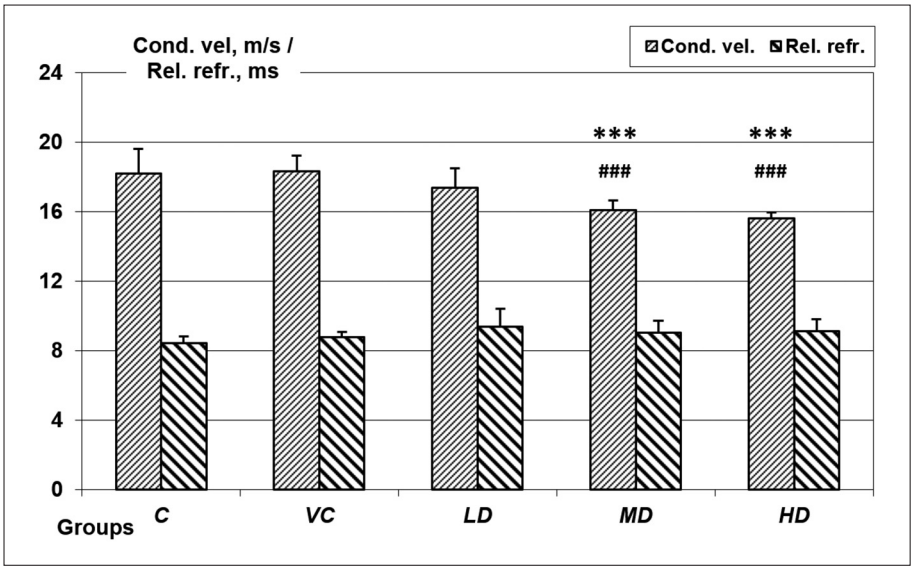

Figure 5. *Conduction velocity and relative refractory period of the tail nerve in the control and treated rats*

Mean+SD, n=10.
***: p<0.001 vs. C; ###: p<0.001 vs. VC.

Oxidative damage to membrane lipids is likely to result in changes of membrane fluidity and this way in alterations of pulse propagation and synaptic transmission[16]. The causal chain from inner exposure through oxidative stress up to functional alteration was suggested by the results of the present work in the relationship of SS EP latency and the level of Ti and TBRAS in the brain **(Figure 6)**. The similar pattern of changes of EP latency and tail nerve conduction velocity suggested that the effect of TiO_2 NPs on axonal conduction and synaptic transmission were both involved in the mechanism of the observed changes. In[8], spike rate in a cultured neuronal network (including both neurons and glial cells) decreased and the number of ROS-positive cells increased in parallel. It was also found, however, that neuronal activity was first impaired at a much lower TiO_2 NP dose than where ROS production started to increase, suggesting con-

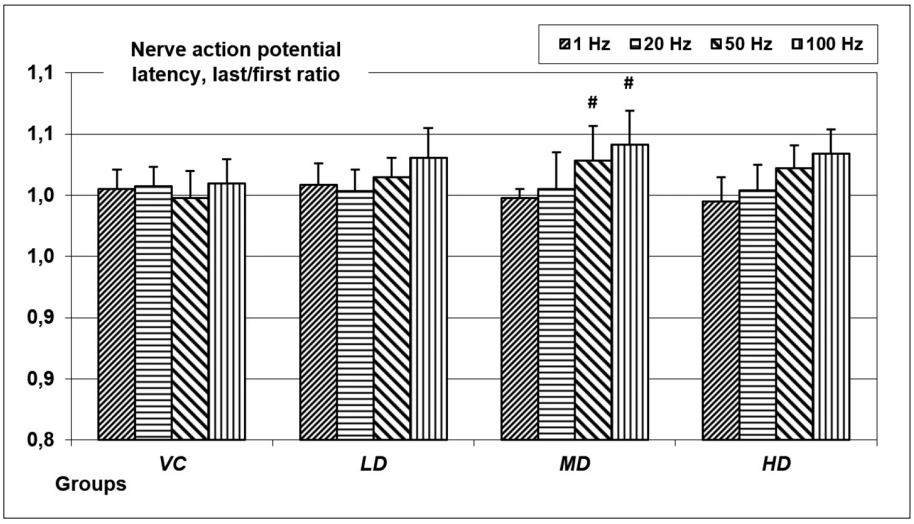

Figure 6. *Fatigue of the tail nerve action potential during a series of 50 stimuli, quantified by the ratio of the last and first CAPs' latencies (see Methods for details)*

Mean+SD, n=10.
#: p<0.05 vs. VC.

tribution of another, not oxidative, mechanism such as abnormal transmitter turnover, i.e., impaired removal by glial cells[6] and/or disturbed metabolism in the liver[17]. The non-monotonous relationship, between cortical and peripheral electrophysiological changes and brain TBARS levels on one hand and applied TiO_2 NP doses and measured tissue Ti levels on the other, was a peculiar phenomenon. Its possible background was that TiO_2 NPs in the *HD* treatment suspension had, in spite of

Table 3. *Results of Ti level and TBARS determinations in the tissue samples from 3 randomly chosen rats per group of groups VC, MD and HD*

Measured parameters	Tissue sample	Groups VC	MD	HD
Tissue Ti level (μg/kg)	brain	1739.58±639.05	3703.62±1161.03	4785.98±1316.65
	liver	651.30±568.52	771.78±91.90	2267.24±631.31#
	lungs	353.96±611.34	492642.37±214699.00##	551847.60±65797.20##
TBARS reaction (nM MDA/ mg tissue)	brain	13.68±0.85	14.72±1.56	13.73±0.20
	liver	16.22±1.48	24.11±1.58	25.49±5.88
	lungs	34.62±6.61	46.40±7.80	49.72±2.96

#: p<0.05; ##: p<0.01 vs. VC, n=3.

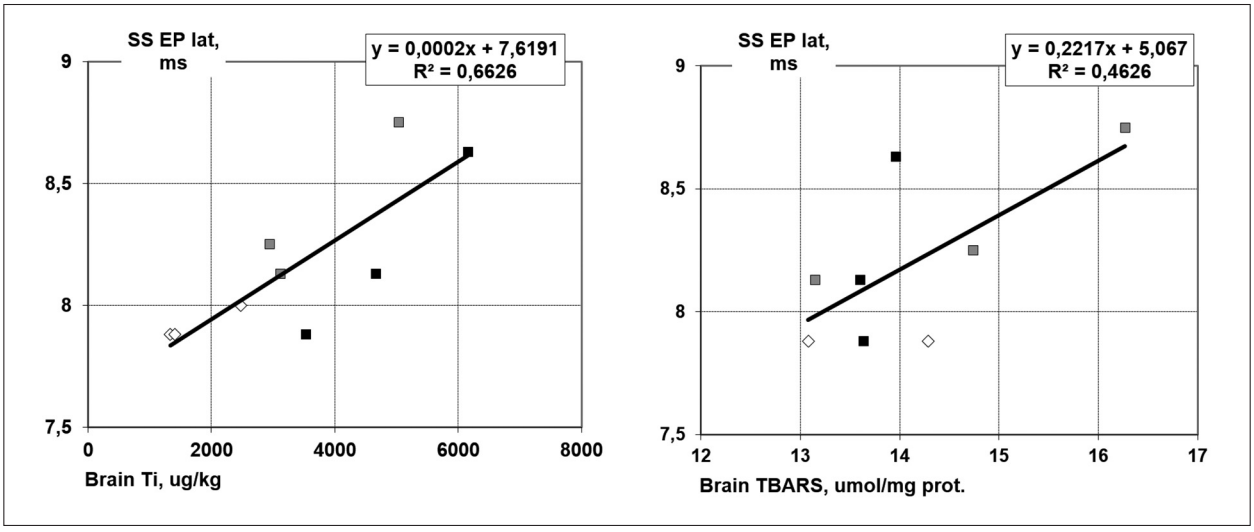

Figure 7. *Correlation diagrams showing the strength of relationship of SS EP latency obtained with 10 Hz stimulation to brain Ti levels (left graph) and brain lipid peroxidation (right graph). Each point represents the data pairs of one animal (diamonds, VC; grey squares, MD, black squares, HD)*

the stabilizing agent HEC and sonication, an increased tendency to aggregate, resulting in decreased relative surface, diminishing both surface reactions and dissolution of ionic Ti.

Lipid peroxidation was considerable in both brain and liver samples of the treated rats **(Table 3)** but, as judged from the relative organ weights **(Table 2)**, the resulting damage in these organs was not gross. In the liver, however, the turnover of monoaminergic transmitters could be disturbed, as mentioned in[17], possibly contributing to the neurofunctional alterations. The metabolic disturbance suggested by increased presence of free radicals in the liver could also provide explanation of the reduced body weight gain seen in groups *MD* and *HD* of the treated rats[18]. Increased relative kidney weight **(Table 2)** was another indication of systemic effect of TiO_2 NPs and (as stated in[1]) ROS-induced damage.

The results of the present study underlined the possible neurotoxicity of TiO_2 NPs, but left some questions open. The possible role of Ti dissolved from the NPs, as well as the histological changes possibly evolving in parallel to the observed neurofunctional and biochemical ones, need further investigations.

REFERENCES

1. *Shi H, Magaye R, Castranova V, Zhao J.* Titanium dioxide nanoparticles: a review of current toxicological data. Part Fibre Toxicol 2013;10:15. http://dx.doi.org/10.1186/1743-8977-10-15
2. *Warheit DB, Donner EM.* Risk assessment strategies for nanoscale and fine-sized titanium dioxide particles: Recognizing hazard and exposure issues. Food Chem Toxicol 2015;85:138-47. http://dx.doi.org/10.1016/j.fct.2015.07.001.
3. *NIOSH.* Occupational Exposure to Titanium Dioxide. Current Intelligence Bulletin 63. Department of Health and Human Services (National Institute of Occupational Safety and Health) Publication No. 2011-160. http://www.cdc.gov/niosh/docs/2011-160/
4. *Oberdörster G, Oberdörster E, Oberdörster J.* Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113: 823-39. http://dx.doi.org/10.1289/ehp.7339
5. *Guerra-Araiza C, Álvarez-Mejía AL, Sánchez-Torres S, Farfan-García E, Mondragón-Lozano R, Pinto-Almazán R, et al.* Effect of natural exogenous antioxidants on aging and on neurodegenerative diseases. Free Rad Res 2013;47:451-62. http://dx.doi.org/10.3109/10715762.2013.795649
6. *Liu Y, Xu Z, Li X.* Cytotoxicity of titanium dioxide nanoparticles in rat neuroglia cells, Brain Injury 2013;27:934-9. http://dx.doi.org/10.3109/02699052.2013.793401
7. *Baisch BL, Corson NM, Wade-Mercer P, Gelein R, Kennell AJ, Oberdörster G, Elder A.* Equivalent titanium dioxide nanoparticle deposition by intratracheal instillation and whole body inhalation: the effect of dose rate on acute respiratory tract inflammation. Part Fibre Toxicol 2014;11:5. http://dx.doi.org/10.1186/1743-8977-11-5
8. *Gramowski A, Flossdorf J, Bhattacharya K, Jonas L, Lantow M, Rahman Q, et al.* Nanoparticles induce changes of the electrical activity of neuronal networks on microelectrode array neurochips. Environ Health Perspect 2010; 118:1363.1369. http://dx.doi.org/10.1289/ehp.0901661
9. *Ze Y, Sheng L, Zhao X, Ze X, Wang X, Zhou Q, et al.* Neurotoxic characteristics of spatial recognition damage of the hippocampus in mice following subchronic peroral exposure to TiO_2 nanoparticles. J Hazard Mat 2014;264: 219-29. http://dx.doi.org/10.1016/j.jhazmat.2013.10.072
10. *Coccini T, Grandi S, Lonati D, Locatelli C, De Simone U.* Comparative cellular toxicity of titanium dioxide nanoparticles on human astrocyte and neuronal cells after acute and prolonged exposure. NeuroToxicology 2015;48:77-89. http://dx.doi.org/10.1016/j.neuro.2015.03.006
11. *Oleru UG.* Respiratory and nonrespiratory morbidity in a titanium oxide paint factory in Nigeria. Am J Ind Med 1987;12:173-80. http://dx.doi.org/10.1002/ajim.4700120206
12. *Oszlánczi G, Horváth E, Szabó A, Horváth E, Sápi A, Kozma G, et al.* Subacute exposure of rats by metal oxide nanoparticles through the airways: general toxicity and neuro-functional effects. Acta Biol Szeged 2010;54:165-70.
13. *Papp A, Pecze L, Vezér T.* Dynamics of central and peripheral evoked electrical activity in the nervous system of rats exposed to xenobiotics. Centr Eur J Occup Environ Med 2004;10:52-9.
14. *Hext PM, Tomenson JA, Thompson P.* Titanium dioxide: Inhalation toxicology and epidemiology. Ann Occup Hyg 2005;49:461-72. http://dx.doi.org/10.1093/annhyg/mei012
15. *Strohl KP, Thomas AJ, St.Jean P, Schlanker EH, Koletsky RJ, Schork NJ.* Ventilation and metabolism among rat strains. J Appl Physiol 1997;82:317-23.
16. *Coyle JT, Puttfarcken P.* Oxidative stress, glutamate and neurodegenerative disorders. Science 1993;262:689-95.
17. *Yurdaydin C, Hörtnagl H, Steindl P, Zimmermann C, Pifl C, Singer EA, et al.* Increased serotoninergic and noradrenergic activity in hepatic encephalopathy in rats with thioacetamide-induced acute liver failure. Hepatology 1990; 12:695-700.
18. *Merry BJ* Molecular mechanisms linking calorie restriction and longevity. Int J Biochem Cell Biol 2002;34:1340-54.