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Biopersistence and translocation to extrapulmonary organs of titanium dioxide nanoparticles after subacute inhalation exposure to aerosol in adult and elderly rats

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HIGHLIGHTS

- Lung uptake and clearance of titanium (Ti) in young adults and elderly rats.
- TiO_2 nanoparticles translocation to spleen, lymphnodes and liver.
- Delay in translocation and higher amount of Ti in spleen, liver in the elderly group.

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ABSTRACT

The increasing industrial use of nanoparticles (NPs) has raised concerns about their impact on human health. Since aging and exposure to environmental factors are linked to the risk for developing pathologies, we address the question of TiO_2 NPs toxicokinetics in the context of a realistic occupational exposure. We report the biodistribution of titanium in healthy young adults (12–13-week-old) and in elderly rats (19-month-old) exposed to 10 mg/m^3 of a TiO_2 nanostructured aerosol 6 h/day, 5 days/week for 4 weeks. We measured Ti content in major organs using inductively coupled plasma mass spectrometry immediately and up to 180 days after the end of exposure. Large amounts of titanium were initially found in lung which were slowly cleared during the post-exposure period. From day 28, a small increase of Ti was found in the spleen and liver of exposed young adult rats. Such an increase was however never found in their blood, kidneys or brain. In the elderly group, translocation to extra-pulmonary organs was significant at day 90. Ti recovered from the spleen and liver of exposed elderly rats was higher than in exposed young adults. These data suggest that TiO_2 NPs may translocate from the lung to extra-pulmonary organs where they could possibly promote systemic health effects.

1. Introduction

Due to their remarkable properties, nanoparticles (NPs) are currently used in a large array of applications, from electronics to medicine, and thus are present in a growing number of commercial products. According to the project on emerging nanotechnologies, the number of nanotechnology-based products increased nearly 30 times from 2005 to 2015, reaching a total of 1814 items produced by companies located in 32 countries (Vance et al., 2015). Titanium dioxide NPs, the second most widely used NPs, are present in one-tenth of the abovementioned manufactured goods (Chen and Mao, 2007), including cosmetics and food products. For decades, TiO_2 was considered as an inert, non-toxic substance but recently the International Agency for Research on Cancer (IARC) has reassessed

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the toxicity of this material and decided to classify it as a possible carcinogen (group 2B) for humans (Baan, 2007; IARC, 2010). The reason for that arose from a study reporting that the inhalation of ultrafine (<100 nm) TiO_2 dusts starting at a concentration of 10 mg/m^3 was associated with cancer of the respiratory tract in rats (Dankovic et al., 2007); however an increased risk of cancer in workers exposed to such poorly soluble particles has not been reported so far (Warheit and Donner, 2015). Recent *in vivo* studies also pointed out that inhalation of TiO_2 nanostructured aerosol, both in occupational and environmental conditions, may cause extra-pulmonary adverse health effects, such as cardiovascular disorders or neurodegenerative diseases (Baisch et al., 2014; Christensen et al., 2011; Landsiedel et al., 2014).

Due to their small size, NPs may present a different biokinetic profile compared to bulk material. Consequently, in case of a facilitated absorption or/and slower elimination of NPs, the potential risk following exposure is expected to increase. This is supported by several inhalation studies in rodents showing the distribution of various types of NPs in different organs outside the respiratory system. For example, it was reported that 30 min after a 6-h inhalation of 133 μg/m^3 silver NPs (4–10 nm), a small amount of silver was recovered from the liver, spleen, brain, heart, and blood of rats (Takenaka et al., 2001); a similar study highlighted the biodistribution of silver into liver starting 24 h after the end of a 4-day inhalation period of 179 μg/m^3 silver NPs (15 nm) (Braakhuis et al., 2014). It was also observed that gold was found in the heart, liver, pancreas, spleen, kidney, brain and testis of rats exposed to 2.10^6 gold NPs/cm^3 (approximately 9 mg/m^3) by inhalation during 5 consecutive days (Yu et al., 2007). Geraets et al. highlighted the translocation to extrapulmonary organs of cerium oxide NPs (5–10 nm) 6 h after the end of a 28 days exposure from 10 to 55 mg/m^3 (Geraets et al., 2012). In these studies, the translocation to extra-pulmonary organs was described after a short recovery period. Furthermore, another study reported long-term NP biokinetics in secondary target organs over six months after a single short-term inhalation of iridium NPs (15–20 nm) (Semmler et al., 2004). Taken together, these findings suggest that inhalation of various types of NPs can result in their translocation across the lung air-blood barrier.

For TiO_2NPs, the assessment of their extrapulmonary translocation is not as straightforward. Even though few studies have shown such a process after intratracheal or intranasal instillation in rodents (Huang et al., 2015; Husain et al., 2015; Shinohara et al., 2015, 2014), discordant results were obtained following inhalation. Indeed, in a study where the authors used TiO_2 NPs labeled with radioactive ^{48}V, it was shown that following a 2-h inhalation exposure to 3–6 × 10^6 NPs/cm^3 only 2% of the lung deposited dose reached secondary organs (Kreyling et al., 2010). Nonetheless, in experiments following inhalation of 2 to 50 mg/m^3 of TiO_2 NPs 6 h per day during 5 consecutive days, authors did not observe any translocation to extrapulmonary organs (Ma-Hock et al., 2009). It is worth noticing that in these reports, which were not dedicated to particles toxicokinetics, titanium tissue content was assessed by a non-optimized method which also was not as sensitive as the one using radiolabelled nanoparticles.

In addition, studies regarding the nano-toxic effects on susceptible populations (such as pregnant, neonate, unhealthy, and aged populations) are still scarce (Geiser et al., 2014; Gustafsson et al., 2014; Li et al., 2014; Semmler-Behnke et al., 2012). Nevertheless, It has been observed an age-dependent pulmonary inflammation, cardiovascular injuries, and an increase of serum biomarkers after the exposure of old (20 months), adult (8 weeks) and young (3 weeks) rats to silica NPs by inhalation for a period of 4 weeks (24.1 mg/m^3 40 min/day) (Chen et al., 2008). The induction of pulmonary inflammation and cardiovascular alterations was higher in the older rats than in the younger age groups. Such results emphasize the differences in response to inhaled NPs between the two populations. In addition, even though it is expected that pulmonary clearance of particles from the lung through the mucociliary elevator is impaired in aged lung (Lowery et al., 2013) little is known about the differences in terms of lung deposition as well as extrapulmonary translocation of inhaled nanoparticles between adult and elderly rats.

In this context, the present study is the first designed to compare the biokinetics (over 3–6 months) of TiO_2 NPs after a subacute nose-only inhalation exposure (at 10 mg/m^3) in elderly (19-month-old) and young adult (12–13-week-old) Fischer 344 rats.

2. Methods

2.1. Animals

The animal experiments were performed according to European (Directive 2010/63/EC) and French (Décret n°2013-118) legislations regarding the protection of animals used for scientific purposes. The INRS animal facility has full accreditation (authorization n° D54-547-10) from the French Ministry of Agriculture. This study was approved by the regional ethical committee appointed by the Ministry of Higher Education and Research (Authorization n°00692.01).

Male Fisher F344 rats (from Charles River Laboratories, France), 12–13-week old and weighing 300–320 g referred as young adults and 19 months old, weighing 400–425 g referred as elderly group (Sengupta, 2013), were housed in standard environmental conditions (room humidity 55 ± 10% and temperature 22 ± 2 °C; with a 12:12 h light-dark cycle) and maintained with free access to water and standard laboratory diet. The aging rats were fed with free access to A04 diet (Safe diet) from 1 month of age to 7 months then with A05 diet (Safe diet) adapted to long term studies.

2.2. Generation and monitoring of TiO_2 nanostructured aerosol

TiO_2 P25 NPs (Aeroxide® P25, 75% anatase 25% rutile, Evonik®) were from Sigma Aldrich (Saint-Quentin Fallavier, France). Their full physical and chemical characteristics have been previously published (Disdier et al., 2015) (Table 1).

The inhalation system described previously (Cosnier et al., 2016) is mainly composed of an aerosol generation system and inhalation towers for nose-only exposure. Exposure capability is around 100 rats: 50 NP-exposed rats (in 6 × 9 ports manifold) and 50 control rats (in 2 × 27 ports manifold). Controls were exposed to filtered air. During the 6-h exposure, rats did not have access to

Table 1
Titanium dioxide particle and aerosol main characteristics.

Particle size (nm)	Specific surface area (m^2/g)	Aerosol mass concentration (mg/m^3)	Aerosol number concentration (particle/cm^3)	CMAD (nm) (ELPI)	MMAD (nm) (SIOUTAS)
21.5 ± 7.2	51	10.17 ± 3.29 (young) 10.42 ± 1.80 (elderly)	24000 ± 6400	269 (GSD: 2.22)	905 (GSD: 2.19)

CMAD: Count Median Aerodynamic Diameter; MMAD: Mass Median Aerodynamic Diameter; GSD: Geometric Standard Deviation. Full nanoparticle and aerosol characteristics have already been published (Cosnier et al., 2016; Disdier et al., 2015).