Acute and chronic histopathological alterations related to the administration of TiO₂ nanoparticles in rats

Xavier Valenti ¹, Pascaline Rugira ¹, Raphaël Conotte ², Annica Frau ¹ and Denis Nonclercq ¹

¹ Department of Histology, ² Department of Human Biology and Toxicology, Health Institute, Faculty of Medicine and Pharmacy, University of Mons, Belgium

Introduction
Titanium dioxide (TiO₂) nanoparticles (NPs) are produced abundantly and are widely used because of their high stability, catalytic properties and low cost. These particles are frequently used as a white pigment for paints, paper, toothpaste and plastics. They are also widely incorporated in sunscreens due to their capacity to reflect ultraviolet sunrays and are used in pharmaceutical excipients. Despite the wide ranges of applications, there is a lack of information on the impact of NPs on human health. A recent study has demonstrated that the smallest particles show the most widespread organ distribution including blood, heart, lungs, liver, spleen, kidneys, thymus, brain and reproductive organs.

Material and methods
- Suspension preparation: sonotrod (3 times of 30’ with vortex sessions (3’) between each cycle).
- Animal treatment: intraperitoneal (IP) injection of TiO₂ NPs at 0, 500, 1000 mg/kg BW.
- Cell proliferation: IP injection of 5-Bromo-2’-deoxyuridine (BrdU) 1 hour before sacrifice detected by immunohistochemistry.
- Oxidative stress: detection of lipid peroxidation using an anti-4-hydroxynonenal antibody.
- Liver and kidney functions: blood samples were collected, centrifuged to obtain serum analyzed with reactive test strips.

Results

![Liver cell proliferation (A)](image)

![Testicle cell proliferation (B)](image)

![Kidney cell proliferation (C)](image)

Figure 1. Masson trichrome staining of liver (A1) with accumulation of TiO₂ NP’s into phagolysosomes (circle), kidney (B1), testicle (C1). Immunodetection of BrdU positive cells (arrow) in liver (A2), kidney (B2), testicle (C2). Immunodetection of lipid peroxidation in liver (A3) and kidney (B3).

![Biochemical hepatic function (A)](image)

![Biochemical renal function (B)](image)

Figure 2. BrdU positive cells were counted in liver (A), testicle (B) and kidney (C) using a reticule of 0.084 mm². Number of BrdU positive cells increased significantly in interstitial cells in liver 4 days and 2 months after TiO₂ injection (both doses). No change in testicle proliferation. In kidney, a significant increase is observed 4 days after NPs injection (1g/kg) in cortex, OSOM and ISOM (* indicates significant values p < 0.05; student t-test).

Figure 3. Evaluation of hepatic (A) and renal (B) function. No important modification is observed. However, we note a significant increase of AST in serum 4 days (1g/kg) and 2 months (0.5g/kg and 1g/kg) after NPs injection. Renal function is not affected except BUN that increase significantly 4 days after NPs injection (0.5g/kg) (* indicates significant values p < 0.05; student t-test).

Conclusion
The acute and sub-chronic toxicity of TiO₂ nanoparticles injected intraperitoneally was investigated in our study. Results suggest a potential toxicity of TiO₂ nanoparticles in vivo. Kupffer cells in the liver of the treated animals showed numerous TiO₂ inclusions. Examination of cell proliferation reveals a significant increase of cells in S-phase in the liver, kidneys of animals exposed to TiO₂. Hepatic and renal cell proliferation gets higher in the acute phase of exposure to TiO₂ and reflects repair process of cell damages induced by oxidative stress consecutive to nanoparticles exposure. These morphological alterations were accompanied by a significant modification of some biochemical parameters of kidney and liver function such as BUN and AST.

Nowadays, more than one thousand products contain TiO₂ nanoparticles. This is an anachronistic use of these nano-objects because there is no specific regulation. Maybe, the toxicological study of these nanoparticles will lead to the emergence of laws regulating their use to avoid a health scandal like asbestos.

References