
Supplementary Materials

Titanium dioxide nanoparticles suppress the systemic immune response in mice after seven-week inhalation

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Table S1. In vivo inhalation studies investigated the adverse effects of TiO₂ NPs on systemic immune response and oxidative stress

Model / Animal	Administration mode	Particulate size	Dose and duration of treatment	Outcomes	Authors	Ref. No.
mice	whole-body inhalation chambers	29.6 nm	0.00167 and 0.1308 mg TiO₂/m³, continuously for 7 weeks i.e. 0.012 and 0.958 mg/kg b.w.	- decreased percentage of T lymphocytes in spleen in both dose groups - decreased percentages of T-helper lymphocytes in spleen at the low dose - stimulated phagocytic activity of monocytes in blood at the low dose - decreased levels of IL-4 and IL-18 in spleen at the high dose - increased levels of GSH and GSH/GSSG ratio	Our study	
mice	whole-body inhalation chambers	20 nm (anatase)	6.34 ± 0.22 mg TiO₂/m³, 8 hours/day, for 3 weeks	- decreased white blood cell count - increased platelet and reticulocyte counts	Yin et al. 2014	[7]
mice	whole-body inhalation exposure system	30 nm (anatase)	2, 8 or 32 mg/m³ of TiO₂ NPs, 6 hours/day, 5 days/week for 26 weeks	- decreased red blood cell count and hematocrit value in males - increased MCV and MCH - decreased white blood cell count in females	Yamano et al. 2021	[53]
mice	intratracheal instillation	20 nm	5, 20, and 50 mg/kg b.w., single dose, animals sacrificed at 1, 7, 14 days after treatment	- induced pro-inflammatory cytokines, Th1-type cytokines and Th2-type cytokines followed by B cell proliferation, - dose-dependently elevated levels of the pro-inflammatory cytokines IL-1, TNF- α , and IL-6 - dose-dependently elevated levels of the Th1 cytokines IL-12 and IFN- γ , and the Th2 cytokines IL4, IL-5 and IL-10 - increased numbers of B lymphocytes in both spleen and blood	Park et al. 2009	[35]
mice	intratracheal instillation	10.5 x 50-60 nm (nanorods) (rutile)	0.162 mg TiO₂/kg b.w., single dose	- no effect on hematological parameters	Modrzynska et al. 2021	[76]
mice	intranasal instillation	80 nm (rutile)	500 μg TiO₂ NP suspension, every other day for 30 days	- no effect on GSH content, GPx, GST, and SOD activities in brain - increased catalase activity in brain	Wang et al. 2008	[73]

mice	intranasal instillation	80 nm (rutile)	500 µg TiO₂ NP suspension, applied every other day, for 2, 10, 20 and 30 days	- increased activities of GPx, GST and SOD and GSH level in brain at 10 days postexposure - no effect on activities of GPx, GST and SOD and GSH level in brain at 20 and 30 days postexposure - increased MDA in brain at 30 days postexposure	Wang et al. 2008	[74]
rats	intratracheal instillation	4-6 nm nanorods (primary diameter) (rutile)	1 and 5 mg/kg b.w., single dose	- increased number of monocytes and granulocytes in the blood in a dose-dependent manner - decreased number of platelets - no effect on the number of lymphocytes	Nemmar et al. 2008	[33]
rats	intratracheal instillation	21 nm (80% anatase, 20% rutile)	0.5, 4, and 32 mg/kg b.w., twice a week for 4 weeks	- increased proliferation of T cells and B cells following mitogen stimulation - enhanced natural killer (NK) cell killing activity in spleen - increased number of B cells in blood - no significant changes of Th1-type cytokines (IL-2 and INF-γ) and Th2-type cytokines (TNF-α and IL-6)	Fu et al. 2014	[8]
rats	intratracheal instillation	23 nm (anatase, rutile)	3.5 and 17.5 mg/kg b.w. once every 2 days for 5 weeks	Blood: - increased MDA, NO, NEUT, and IL-1 - decreased GSH, WBC, IFN-γ, and TNF-α Spleen: - increased MDA Liver: - decreased GSH level and SOD activity - increased MDA Thymus: - dose-dependently decreased SOD activity	Liu et al. 2015	[62]
rats	intratracheal instillation	23 nm (anatase, rutile)	0.5, 2.5 and 10 mg/kg b.w., subdivided into 3 instillations at 4-day intervals, animals sacrificed 2 hours and 35 days after 3 treatments	- no effect on total glutathione content in plasma - oxidized glutathione levels in blood were below the detection range	Relier et al. 2017	[72]
rats	intranasal instillation	7 x 80 nm Fe-TiO ₂ nanorods (rutile)	1 and 5 mg/kg b.w., single dose, animals sacrificed 24 hours treatment	- increased plasma levels IL-6 - dose-dependently decreased plasma SOD and GSH activities - decreased number of platelets	Nemmar et al. 2011	[77]

rats	intranasal instillation	21 nm (75% anatase, 25% rutile)	5 mg TiO₂/kg b.w., single dose, animals sacrificed at 1, 2, 8, 16, 30, and 90 days after treatment	- NP-induced immunoactivating and proinflammatory activity in blood: Days 1–2 post-exposure, - elevated levels of IL-2, IL-4, IL-6, IL-10, and IFN- γ Days 2–8 post-exposure, - increased CINC-1 levels Day 16 post-exposure, - increased TNF- α	Gustafsson et al. 2011	[34]
rats	nose-only inhalation	21 nm (80% anatase, 20% rutile)	10 mg/m³ of TiO₂ NPs, for 21 days, 6 h/day, animals sacrificed at 3, 28, and 90 days after treatment	Day 3 post-exposure: - no significant changes in hematology. Day 28 post-exposure: - reduced white blood cell and lymphocyte counts Day 90 post-exposure: - reduced white blood cell and lymphocyte counts - reduced number of segmented neutrophils	Eydner et al. 2012	[78]

Abbreviations

GSH: reduced form of glutathione

GSSG: oxidized form of glutathione

GST: glutathione S-transferase

GPx: glutathione peroxidase

IFN- γ : interferon- γ

IL: interleukin

MCH: mean corpuscular haemoglobin

MCV: mean corpuscular volume (as red blood cell indices)

MDA: malondialdehyde

NEUT: neutrophils

SOD: superoxide dismutase

TiO₂ NPs: titanium dioxide nanoparticles

TNF- α : tumor necrosis factor α

WBC: white blood cells

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