Titanium dioxide nanoparticle ingestion alters nutrient absorption in an in vitro model of the small intestine

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• A Western diet includes $10^{12} - 10^{14}$ engineered nanoparticles per day
  • Primarily metal oxide NP used in processed foods and food packaging
  • More recent studies estimated that daily adult intake of nano TiO$_2$ is 0.2 – 1 mg/kg
    • Pediatric intake is likely even higher

**Research goal:** Determine if and how ingested metal oxide (TiO$_2$) NP alter intestinal function.

1. Cell culture model of the intestinal epithelium
2. Effects of pristine NP exposure on GI function *in vitro*
Nanoparticle dose

- Efforts made to recreate physiologically realistic doses
  - Dose calculated as the mass per unit area from published estimates
    - Density and size of 30 nm TiO$_2$ (anatase) nanoparticles converted to physiological doses
    - Also studied doses two orders of magnitude higher and lower than the physiological dose
  - Total ingested material/surface area of the human small intestine (2x10$^6$ cm$^2$)
**In vitro experimental design**

**Caco-2/HT29-MTX**

- Add TiO$_2$ nanoparticles (30 nm in low mineral MEM)
- Add 10 mM $^{58}$Fe(II)-ascorbate
- Collect apical and basolateral medium for ICP-MS
- Wash and collect cells for molecular (RNA, protein) or structural (microvilli) analysis

2 weeks

4 hours or 5 days

2 hours

**Medium dose**: ~2.5 mg per day divided by the total intestinal surface area ($2 \times 10^6$ cm$^2$)

**Low, high**: two orders of magnitude lower or higher
- $1.4 \times 10^{-4}$ mg/mL
- $1.4 \times 10^{-6}$ mg/mL
- $1.4 \times 10^{-8}$ mg/mL
Barrier function

- The effects TiO$_2$ NP on tight junctions evaluated
  - TER
- ROS is produced in response to $^{58}$Fe, $^{67}$Zn, and acute or chronic exposure to TiO$_2$
Nutrient Transport & Uptake

- Fe and Zn transport and uptake are sensitive to particle exposure at realistic doses.
- In general, NP decrease transport and uptake following acute and chronic exposure.
Nutrient Transport & Uptake

- Fatty acid uptake affected at high doses
- Intestinal alkaline phosphatase (IAP) increases in response to NP exposure
  - IAP concentrated in the BBM, cells and plays a critical role in barrier function and stress responses
Gene expression

- Nutrient transporter, pro-inflammatory genes analyzed
- Most significant increases seen in pro-inflammatory genes, likely related to ROS signaling
Microvilli density

- Acute and chronic high doses have ~25% less surface area covered by microvilli
- Similar results have been found by other groups

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Conclusions

- Metal oxide nanoparticles significantly affect Fe, Zn, and fatty acid nutrient transport and BBM enzyme activity
- Gene expression and ROS formation analysis showed NP changed the expression levels of nutrient transport proteins and induced ROS and pro-inflammatory signaling
- Metal oxide NP exposure decreased the number of intestinal microvilli, which decreased the surface area available for nutrient absorption
- Overall, the results from this study indicate that the intestinal epithelium is affected at a functional level by physiologically relevant exposure NP commonly ingested from food
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