Exposure to manufactured nanoparticles during gestation: impact on the respiratory tract of the offspring in a mouse model

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Pulmonary exposure to nanoparticles (NP)

Consequences in adults

- **Lung remodelling:**
  - Granuloma (CNT and CeO₂)
    Lam *et al.* 2004; Aalapati *et al.* 2014
  - Fibrosis (CNT, CeO₂, TiO₂ and SiO₂)
    Park *et al.* 2011; Ma *et al.* 2012; Morimoto *et al.* 2013; Ma *et al.* 2014
  - Emphysema (TiO₂)
    Chen *et al.* 2006

- **Underlying biological mechanism:**
  - Inflammation (TiO₂, Ag and CeO₂)
    Moon *et al.* 2010; Ma *et al.* 2011; Haberl *et al.* 2013; Mishra *et al.* 2016
  - Oxidative stress (TiO₂, Ag, CeO₂ and SiO₂)
    Park *et al.* 2008; Ha Ryong *et al.* 2011; Srinivas *et al.* 2011; Pen *et al.* 2014; Petrache Voicu *et al.* 2015
  - Genotoxicity (Ag and TiO₂)
    Chen *et al.* 2006; Armand *et al.* 2016
  - Apoptosis (TiO₂, CB and CeO₂)
    Hussain *et al.* 2010, Rice *et al.* 2015

Consequences in the offspring

- **Reproduction alterations:** Decreased sperm production (TiO₂, CB)
  Yoshida *et al.* 2010; Jackson *et al.* 2011; Hougaard *et al.* 2010

- **Pregnancy complications:**
  - Decreased pregnancy rate (Cd)
    Blum *et al.* 2012
  - Decreased fetal development (Cd)
    Fujitani *et al.* 2012
  - Decreased fetal weight (MWCNT)
    Hougaard *et al.* 2010 & 2013; Jackson *et al.* 2011

- **Alteration of brain development** (CB, MWCNT and TiO₂)
  Hougaard *et al.* 2010 & 2013; Jackson *et al.* 2011

**Respiratory effects of maternal exposure during pregnancy?**
Hypothesis

Pulmonary exposure to NPs during pregnancy induces lung alterations in pups

Aims

- To assess the impact of exposure to 3 NPs during pregnancy on lung development
- To decipher the underlying mechanisms involved in lung alterations (direct or indirect?)
Non-surgical intratracheal instillation of pregnant mice (once a week)
Saline or NPs (100µg): TiO$_2$ (insoluble), CeO$_2$ (insoluble) or Ag (soluble), all spherical, diameter: 10-20 nm

Mating
1 male with 2 multiparous females

Gestation: 19.5 days

Delivery

C57/Bl6J

Lung (ARN/Protein)

GD 2.5
GD 9.5
GD 16.5

Alveolization

Lung (ARN/Protein/Histology)

PD 14.5

Lung maturity

Lung (Histology)

PD 49.5

GD: Gestational day
PD: Post delivery day
Results: Fetotoxicity

• **Effect of NPs on fetuses at GD 17.5:**

![Fetotoxicity graphs and images](image)

**Significant increase of fetal death after AgNPs treatment**

**Significant decrease of fetal weight after maternal exposure to NPs**
Results: impairment of lung development

- Effect of NPs on lung development at PD 14.5:

Significant decrease in the alveolization of the offspring at PD 14.5 whatever the NPs treatment.
Results: impairment of lung development

- Effect of NPs in lung development at PD 49.5:

Significant decrease in the alveolization of the offspring at PD 49.5 whatever the NPs treatment

Pulmonary exposure to NP during pregnancy induces an impairment of lung development with a persistent effect at adult age
Which possible mechanisms?

1. Inflammatory response

Nanoparticles

Impaired lung development of the offspring
Results: Inflammatory response

- Cytokines and chemokines in the amniotic fluid (*Luminex®*) and fetal lung (*qPCR, Luminex®*):

  G-CSF, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, KC, MCP-1, MIP-1α, MIP-1β, MIP-2, TNF-α and VEGF

  No inflammation in the amniotic fluid or the fetal lung

  Inflammation might not be the mechanism involved in the lung impairment of the offspring
Which possible mechanisms?

1. Inflammatory response
2. Placental alterations (Translocation of NPs)

Impaired lung development of the offspring
Results: 2 Placental alteration

- Placental alteration at GD 17.5:

- NPs exposure increases placental weight
- NPs exposure decreases placental efficiency
- NPs exposure induces placental vascular oedema

NPs exposure during pregnancy induces placental alterations

Direct toxic effect of NPs?
Results: Placental alteration

- NPs translocation into the placenta:

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Ti (µg/g)</th>
<th>Ce (µg/g)</th>
<th>Ag (µg/g)</th>
</tr>
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<tbody>
<tr>
<td>Placenta</td>
<td></td>
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<tr>
<td>Saline</td>
<td>0.611 (0.256-1.431)</td>
<td>0.001 (0.001-0.001)</td>
<td>0.001 (0.001-0.003)</td>
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<tr>
<td>TiO₂</td>
<td>5.092 (1.624-5.513)**</td>
<td>0.004 (0.001-0.007)**</td>
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<tr>
<td>CeO₂</td>
<td>0.001 (0.001-0.115)</td>
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<tr>
<td>Ag</td>
<td>5.484 (2.872-7.136)**</td>
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<tr>
<td>Fetal</td>
<td>2.298 (2.013-3.082)</td>
<td>0.005 (0.001-0.026)</td>
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<tr>
<td>Saline</td>
<td>1.477 (0.127-3.259)</td>
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</tr>
<tr>
<td>CeO₂</td>
<td>0.001 (0.001-0.115)</td>
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<tr>
<td>Ag</td>
<td>0.054 (0.036-0.066)**</td>
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</tbody>
</table>

Ti, Ce and Ag elements are found in the placenta

Ag is detected in the chorionic plate of the placenta, mostly in the nano form (measured by XANES)

NPs are found in the placenta and may be involved in the placental alterations
Which possible mechanisms?

1. Inflammatory response

2. Placental alterations (Translocation of NPs)

3. Altered expression of genes involved in lung development

Impaired lung development of the offspring
Results: 3 Effect of NPs on VEGF expression?

- **VEGF expression:**

**Gestational Day 17.5**

**Post-delivery Day 14.5**

VEGF expression decreases significantly at different stages of lung development.
Conclusion

- **Pulmonary exposure to NPs during pregnancy induces:**
  - Fetotoxicity (fetal death, decreasing weight of fetuses)
  - Lung impairment with decreased alveolization, with a persistent effect at adult age

- **At least 2 mechanisms could be involved in lung impairment:**
  - Placental alterations (efficiency, oedema ➔ link with presence of NP?)
  - Downregulation of the expression of VEGF (FGF-18, MMP-9?)
THANK YOU

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Respiratory effects of environmental contaminants

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