Input of proteomics in nanoparticles toxicology: the example of macrophages responses to mineral nanoparticles

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Illustrations
The challenge of transposing lab. toxicology results into real life

Cross-toxicities
- synergistic toxicities without direct interactions

Gold standard: lab animals (healthy life)

Combinatorial explosion of interfering factors (lifestyle, prof. etc...)

Response mechanisms => vulnerability points => sorting cross toxicities

Role of high content in vitro approaches
Macrophages: first line sentinels, immunity effectors and final scavengers

Macrophages

- Phagocytosis, destruction of pathogens and abnormal cells
- Antigen presentation
- Cytokinetic signalling
  - Scavenging of toxic particles (e.g. altered LDL)
  - Inflammation
  - Tissue healing
The first nanoparticle investigated: ZnO

ZnO (30,000 tons/year ww) used in sunscreens, biocidal, UV protection

Parameters:
- primary particle size <50nm
- agglomerate size in culture medium ca. 200-250 nm
- moderate toxicity (LD20 ca. 10 µg/ml)

ZnO: causative agent of the metal fume fever (at doses >50mg/m³ air)

50mg/m³ air => 10 ppm in our culture system
Proteomic analysis of J774 cells in response to ZnO nanoparticles
### Global analysis of proteomic results

#### Pathway analysis (DAVID)

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<th>Term</th>
<th>Count</th>
<th>%</th>
<th>PValue</th>
<th>FDR (%)</th>
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#### False positive rate

![False positive rate graph](image)
Changes in the actin cytoskeleton

Control cells

ZnO treated

RhoGDI1

RhoGDI2

ctl Zn++ ZnO ZrO₂
Zinc genotoxicity: the genotoxicity of a non-Fenton metal
The activities are expressed in units/mg protein, the unit being defined as 1µmole of substrate converted per minute.
The methylglyoxal pathway in zinc toxicity

=> an indirect and composite genotoxic mechanism (DNA Pol \( \tau \) and \( \kappa \))

=> toward a proteomics-driven study of nanoparticles cross-toxic effects
The second nanoparticle investigated: amorphous silica

SiO₂ (100,000 tons/year FR) used in abrasives, moulding, tyres etc...

Parameters:
- primary particle size <20nm
- agglomerate size in culture medium ca. 100-200 nm
- selective toxicity for macrophages (LD20 ca. 20 µg/ml)

crystalline silica: causative agent of silicosis. Amorphous silica less toxic
The mammalian body immune cells: a wide range of killers

RAW264.7

MPC11
selective silica toxicity toward macrophages

correlation with phagocytic capacity
Different proteomic responses, even at equal effect doses
(12/99 in common between the two cell lines)

myd88: TLR signalling ; in35: interferon signalling ; cab39 PKA signalling (+ reg.)
Detoxification proteins

glyoxalase activity: µmol/min/mg prot

<table>
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<tr>
<th></th>
<th>Ctrl</th>
<th>Silica 10µg/ml</th>
<th>Silica 20µg/ml</th>
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<tr>
<td>Glyox. I</td>
<td>364</td>
<td>331 (p = 0.01)</td>
<td>295 (p = 0.04)</td>
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</table>

Notes:
- DNP H1 (D3)
- Glyox. I (H9)
Cross-toxic effects

RAW 264.7

MPC11

methyglyoxal (glyox I)

stylene oxide (dnph 1)
Conclusion: proteomics can do the job

- Proteomics underscores biologically relevant responses at non toxic doses

- Proteomics can sort different responses even if tox. parameters are similar

- Proteomics is able to underscore possible cross-toxicities

Full exploitation of proteomics data require functional validation