Toward the identification of a gene signature predictive of (nano)material-induced lung adverse outcome

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BACKGROUND & OBJECTIVES

The industrial use of nanomaterials is expanding while the knowledge of their toxicological properties is still insufficient. Since the major route of occupational exposure to particles is inhalation, it is essential to investigate their potential effects on lungs. Gene expression profiling is a promising approach to study the toxicological effects of nanomaterials. The aim of this work is to study the gene expression profiles induced by two nanomaterials widely used: titanium dioxide (TiO_2, p25) and carbon black (Printex 90, P90). We compared these profiles to that induced by a crystalline silica (DQ-12), known for its ability to induce pulmonary fibrosis. Modifications in gene expression was interpreted in the light of physio-pathological effects of such exposure. While exposure to the three particles induced lung inflammation, only high doses P90 and DQ-12 led to pulmonary adverse outcome observed in histopathological analysis and at the phospholipid levels. We identified a list of genes specifically deregulated in conditions that induced lung adverse effect, genes that could be used as predictive molecular markers.

METHODS

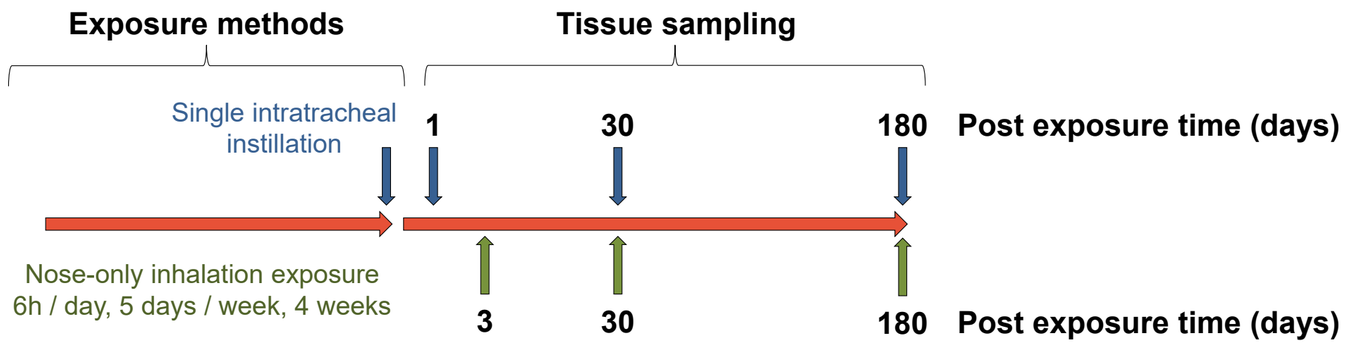


Fig 1. Experimental protocol.
Sprague Dawley rats were exposed to DQ-12 (10 mg/rat) by a single intratracheal instillation and tissues were collected 1, 30 and 180 days post-exposure; or to aerosols of TiO_2 (1.5, 5, 15 mg/m^3) or Printex 90 (5, 15, 50 mg/m^3) in nose-only inhalation chambers, 6h per day, 5 days per week for four weeks. Tissues were collected 3, 30 and 180 days post-exposure.

RESULTS

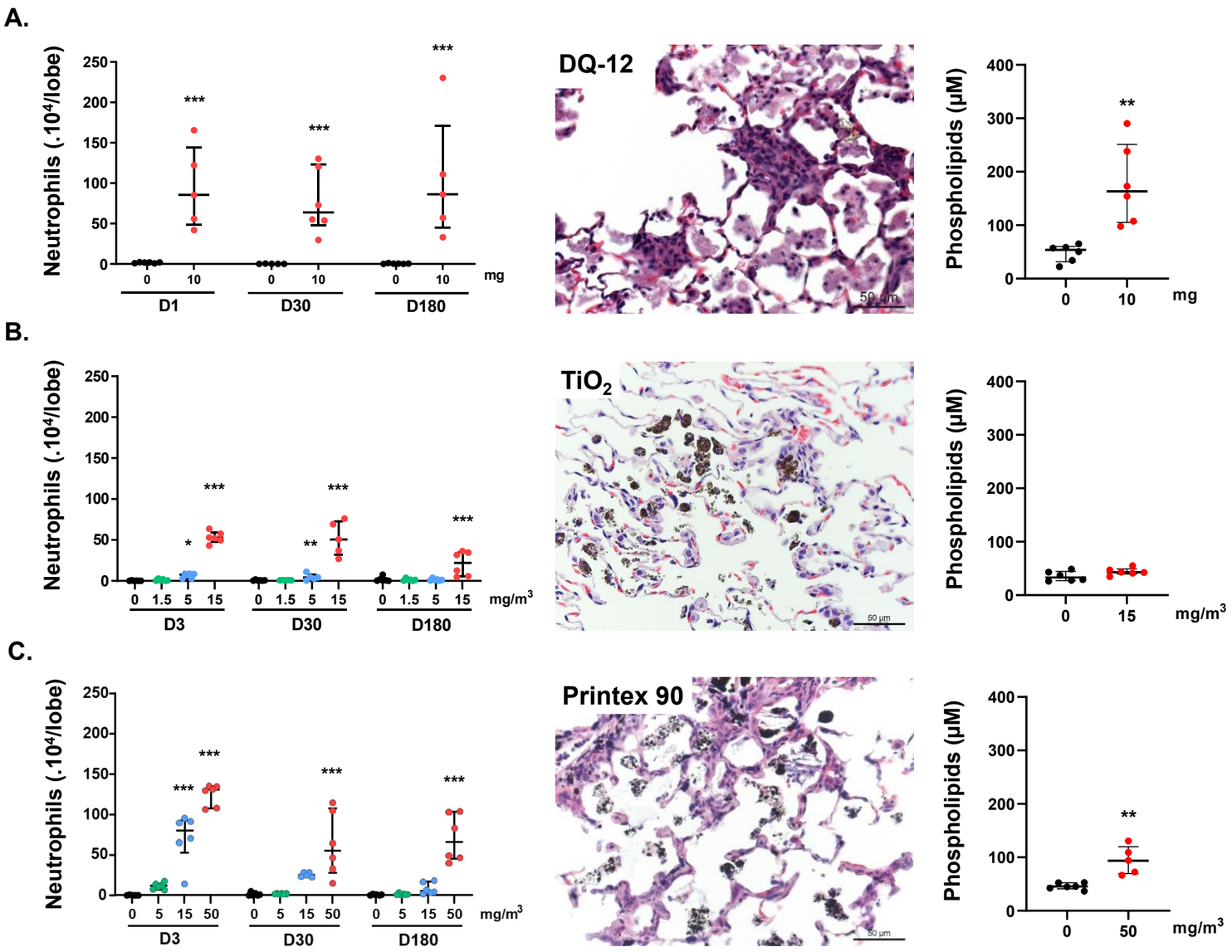
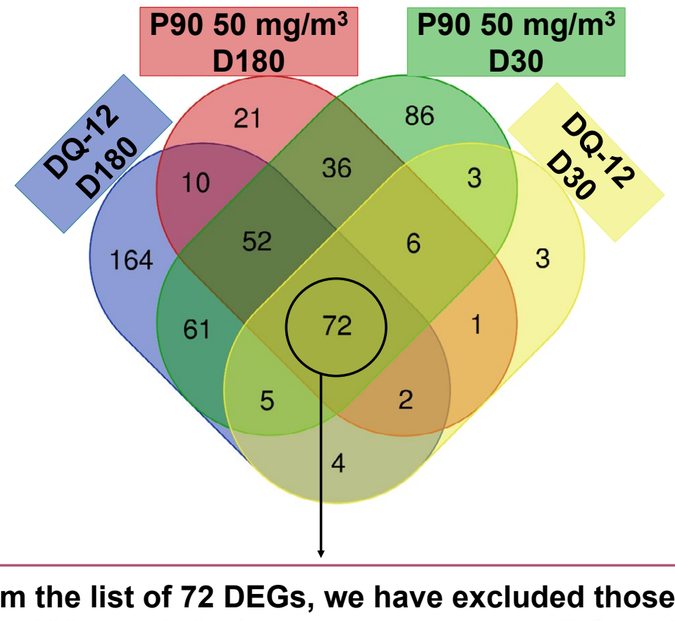


Fig 2. Effect of DQ-12, TiO_2 and Printex 90 on lung.
At the three time points following exposure to DQ-12 (A), TiO_2 (B) and Printex 90 (C) neutrophils in broncho-alveolar lavage fluids were counted (left panel), histopathology of lungs were performed (middle panel) and phospholipids were measured on day 180 in broncho-alveolar lavage fluids (right panel). The three particles induced lung inflammation, DQ-12 and Printex 90 (50 mg/m^3) induced lung adverse outcome. * $p<0.05$, ** $p<0.01$, *** $p<0.005$.



From the list of 72 DEGs, we have excluded those present on D30 and D180 following exposure to TiO_2 (1.5, 5, 15 mg/m^3) and to Printex 90 (5, 15 mg/m^3)

15 genes involved in:

- Inflammation: *Ccl12*
- Phagocytosis: *Marco*, *Msr1*, *Fcgr2b*
- Transcription factor: *Batf*
- Cell-cell adhesion: *Capg*
- Oxidative stress: *Hp*, *Slc7a11*, *Duox*
- Regulation of peptidase activity: *Fetub*, *Itih1*
- Coagulation: *Kng2*
- Ion transport: *Scn10a*
- Others: *LOC689757*, *Gsg1*

Fig 3. DEGs in conditions inducing lung adverse outcome.
Transcriptomics analysis was performed at the three time points following exposure to DQ-12, TiO_2 and Printex 90. FC>2, p value <0.05. 15 genes are commonly differentially expressed on D30 and D180 following exposure to DQ-12 and 50 mg/m^3 Printex 90, these genes were not differentially expressed on D30 and D180 following exposure to TiO_2 (1.5, 5, 15 mg/m^3) and to Printex 90 (5, 15 mg/m^3). DEGs: differentially expressed genes

CONCLUSION

- Evaluation of the broncho-alveolar lavage fluid following exposure to the three particles, DQ-12, TiO_2 and Printex 90, have demonstrated a dose-dependent induction of pulmonary inflammation, through an increase of granulocyte neutrophils. This influx was persistent up to 180 days post-exposure for the highest concentration.
- Histopathological analysis showed lipoproteinosis and fibrosis development in lung from exposed animals to DQ-12. Following TiO_2 and Printex 90 exposure, the presence of particle-laden macrophages which aggregated overtime were observed. Inhalation of the highest concentration of Printex 90 also induced interstitial thickening.
- On day 180, we observed an increase of phospholipid content in broncho-alveolar lavage fluid following exposure to DQ-12 and the highest concentration of Printex 90.
- The transcriptomics study was performed on all post-exposure time-points. Using fold change >2 and p value <0.05, we compared the differentially expressed genes (DEGs). First, we selected DEGs that were common to doses that induced adverse effects (i.e. DQ-12 and 50 mg/m^3 of Printex 90) on days 30 and 180 : 72 genes. Secondly, among these genes we excluded those that were deregulated with doses with no significant adverse effects (i.e. three concentrations of TiO_2 and 5 and 15 mg/m^3 of Printex 90) on days 30 and 180 : 15 genes. These genes were identified as specifically deregulated when animals were exposed to a material that induced lung adverse effect compared to particle/concentration which do not.

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