Clearing the Hurdles for Nanotechnology: in vivo inhalation effects

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Background

As nanotechnology develops, nanoparticles of many types have been created for industrial and medical applications.

Among these nanoparticles, single-wall carbon nanotubes (CNT) are important for their chemical properties and wide application in electronics, instrumentation, drug delivery

With the rapid expansion in CNT-based new technologies, we must understand their safety and risks for human exposure

C. Poland (*Nature Nanotechnology 3:423, 2008)* raised concerns that carbon nanotubes compare to asbestos, and long term effects of exposure may occur.

We hypothesize that CNTs trigger acute and even chronic lung cell stress; With knowledge of the subcellular mechanisms involved, safe nanoparticles may be designed

Materials & Methods

Sample Preparation

CNT in Surfactant* (30 sec. sonication, 30 Watts)
(LAL test negative < 0.03EU)

*Survanta

Animal Treatment

Sprague-Dawley Rats (~0.35 Kg),
it single dose, 0.5 mg CNT or 50 µl surfactant

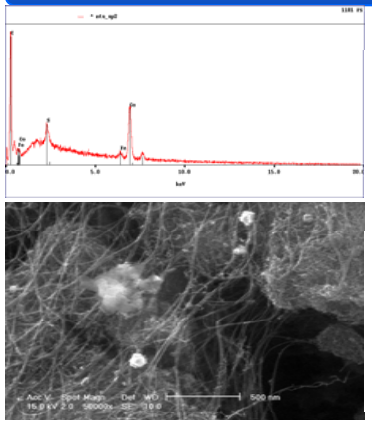
20 min | 3 h | 24h | 1 week | 2 wk | 4 weeks

Samples collected at each time point: serum, BAL, Pleural fluid, tissue homogenate, fixed tissues, lung cell isolation

Data Collection

Histopathology of fixed tissue; **ELISA** for HSP70 and HMGB1 (candidate cell stress markers); **Cytospin cell counts**, BAL & Pleural Fluid, **Western blot** of lung tissue for receptors, **ELISA** for inflammatory cytokines; **Flow cytometry** on lung cell subtypes

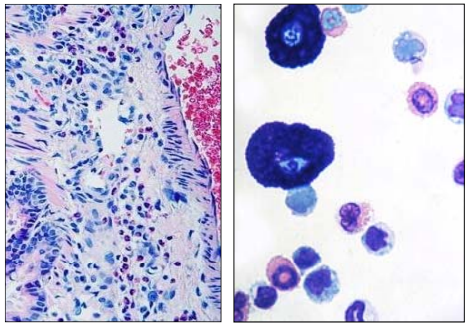
Nanoparticles



Short CNT with iron (Fe) and cobalt (Co) impurities determined by Energy Dispersive Spectrometry (EDS)

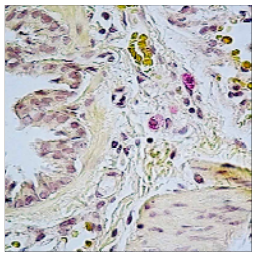
SEM image short CNT

Effects & Results

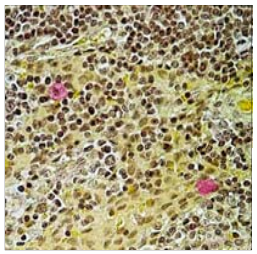


Tissue eosinophilia at 20-30 min after CNT exposure and pleural fluid eosinophilia at 1 week

To trace nanoparticle movement with light microscopy, lung and pleura sections were stained for mucin, released on particle inhalation- mΦ trap mucin & CNT



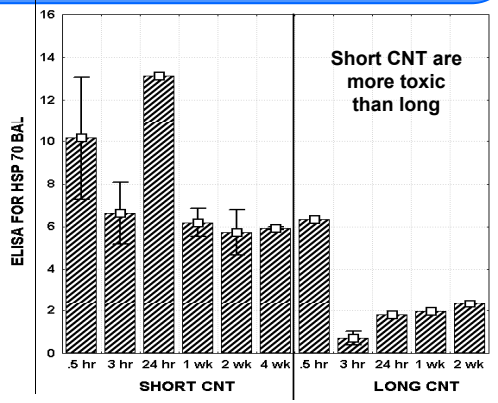
Lung tissue 1 wk



Lymph node 2 wk

TIME	HSP70	HMGB1
0.5 HR	10.17 ± 4.11	31.63 ± 9.82
3 HR	6.62 ± 2.05	10.6 ± 5.79
24 HR	9.8 ± 4.7	53.11 ±47.8
1 WK	6.2 ± 0.92	60.27 ± 84.2
2 WK	5.74 ± 1.51	19.21 ± 17.87
4 WK	5.93 ± 0.9	31.89 ± 29.17

Alarm proteins in BAL after CNT exposure. Appear to be the attractant for eosinophils. Alarm proteins are released in minutes, a signal of necrotic cell death.



Summary & Conclusions

- Unfunctionalized carbon nanoparticles induce cellular inflammation and lung cell necrosis within 20 minutes with a sterile, 0.5 milligram dose to rats.
- Smaller size particles yields larger production of "alarm" proteins in bronchial wash
- Upregulation of tissue and macrophage receptors and production of TNFα have been measured following CNT
- The increasing use of nanoparticles in manufacturing can greatly increase human exposure
- Detoxification of the particles appears to be required
  - Purify; eliminate metal contamination
  - Add functional groups to decrease cellular interaction

References

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