Mesothelioma markers expressed in human cell line exposed to single walled carbon nanotubes

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Background

Carbon nanotubes (CN) are important industrially; however, evidence of their potential toxicity is also increasing. The fibers produced by CN in lung compare to asbestos, a known mesothelioma producer (1). Early detection methods are greatly needed.

Nanoparticles are fibers or particles with one dimension less than 100 nanometers.

- This size gives them unusual electrical and magnetic characteristics; it also means they are too small to see, except with a very high magnification (electron) microscope.
- Single wall carbon nanotubes ***(SWCNT)*** can cross intact skin and cell membranes.
- When inhaled, they create needle-like fibers in lung that cause mechanical necrotic damage by piercing cell membranes.

Mesothelioma was rare until about 30 years ago when incidence began to rise in relation to exposure to asbestos and asbestos products.

- The median survival is only about 7 months (2).
- Most mesothelioma surveillance (on asbestos exposure groups) is done by chest x-ray and pulmonary function tests (3).
- The disease is highly fatal after diagnosis, but asbestos exposure may take 20 years to produce mesothelioma (4).

Clinical Application: Serum and pleural fluid are both being tested for proteins secreted by the body when malignant mesothelioma is present.

- No marker is yet available that gives a reliable diagnosis.
- The latest "soluble marker" candidates are ***mesothelin*** and ***osteopontin*** (5,6,7).

Scientific Question/Aims

Scientific question: Do SWCNTs induce mesothelioma-like behavior in mesothelial cells?

Specific aims:

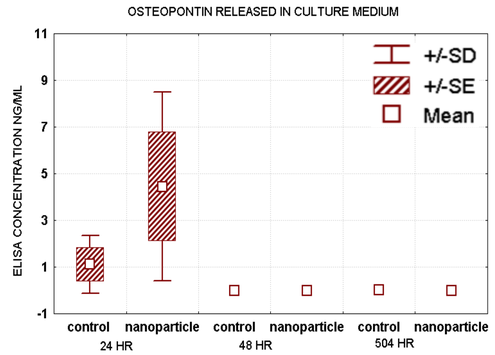
- Determine an appropriate endpoint measure for mesothelioma in human cell culture. Utilize a normal mesothelial cell line, untransformed, and, as positive control for my marker(s), a tumor (mesothelioma) cell line.
- Establish parameters of SWCNT exposure (amount, time of exposure) to non-transformed, healthy human mesothelial cells at minimal levels of cell toxicity.
- Determine if/when non-transformed human mesothelial cells, with controlled exposure to SWCNT, produce or upregulate a response shown to be secreted as an endpoint measure for mesothelioma.

Materials and Methods

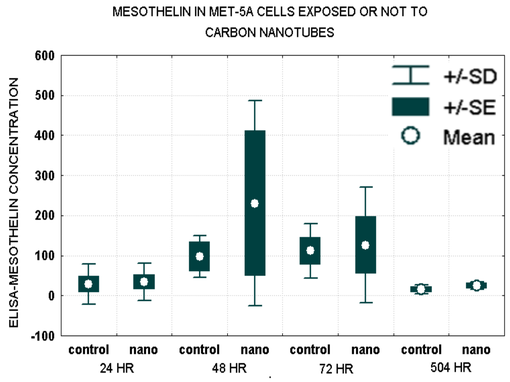
Cell lines were from the American Type Culture Collection (ATCC); Untransformed human mesothelial cells, MeT-5A which grow in KGM basal medium with additives plus 10% fetal calf serum (Lonza) and NCIH28 (CRL5820) human mesothelioma cells which grow in RPMI 1640 with 10% fetal calf serum.

Purified ***SWCNTs*** (SES Research, Houston TX) were used at 500 micrograms/mL media with 25% fetal calf serum to assist suspension and added to the cell cultures at 25 micrograms/mL of media, which has been shown to produce 80% viability in cultured cells by earlier work in our group.

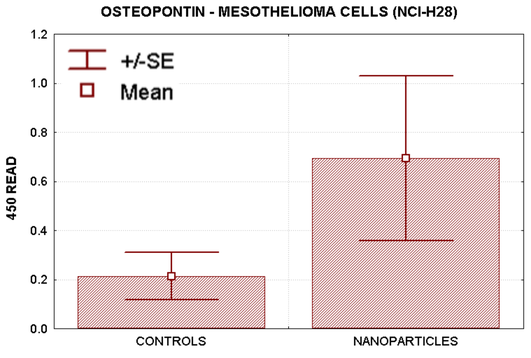
Results



Osteopontin was expressed early in the medium. It is secreted from the cell; cell homogenate did not show an increase.



Mesothelin, a clinically utilized marker of mesothelioma, is significantly elevated from untreated cell culture only at 48 hours (the elevation at 72 hr is not significant).



The positive control for these experiments, NCI-H28 human mesothelioma, responded to nanoparticles by upregulating osteopontin and mesothelin only after over night exposure.

Summary

- ***ELISAs*** on our samples showed brief expression of both mesothelin and osteopontin in the SWCNT-stressed cells but not in controls.
- ***Mesothelin*** averaged 411.7 ng/mL SWCNT exposed vs. 44.5 controls at 48 hr, $p=0.03$ ANOVA it remained elevated at 72 hr but not significantly.
- ***Osteopontin*** appeared only at 24 h: 4.44 ng/mL SWCNT-exposed cells vs. 1.1 ng/mL controls with standard deviations precluding significance.
- The ***positive control*** cells, NCI-H28 mesothelioma cells, released both mesothelin and osteopontin after overnight nanoparticle exposure, $p<0.001$ vs. controls.
- These ***data*** show acute stress produced in MeT-5A cells by a minimally toxic quantity of SWCNT (tested to equal 80% viability by MTT).
- ***Clinical relevance:*** These assays demonstrate that SWCNT-induced stress generates an array of signals and ***measurable proteins*** relating clinically and experimentally to a cancerous state.

References

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