Effects of Single-Wall Carbon Nanoparticles on the p53 Tumor Suppressor Gene

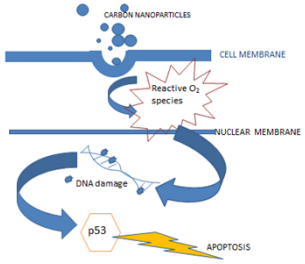
UMKC School of Medicine

Julia Frisenda and Betty Herndon
UMKC-School of Medicine

BACKGROUND

- Tumor suppressors are key genes that ensure correct functioning of cell defenses
- p53 is one of these suppressors that controls apoptosis pathways arising from cells that have received DNA damage
- Carbon nanoparticles (CN) are common in consumer products but there are literature reports documenting cytotoxic effects
- There has been concern that carbon nanoparticles may share the carcinogenic mechanisms postulated for asbestos
- To deeper understand the toxicity of CN, I asked a fundamental question: Is the cellular damage pathway through tumor suppressor p53 changed by CN?
- This study evaluates the role that p53 plays in carbon nanoparticle induced DNA damage

Interaction between Nanoparticles and p53

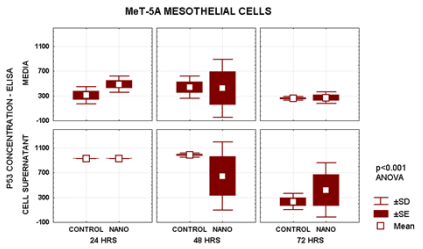


METHODS

- Normal human mesothelial cells were obtained from ATCC (Met-5A) grown in Medium 199 with Earle's salts and 10% fetal bovine serum. Positive control: ATCC human mesothelioma NCI-H28
- Cells were grown in flasks and divided into 6-well plates for controls and nanoparticles (NT) (27 µg NT/mL growth medium = cell stress and 20% cell death). All tests were in triplicate
- MeT-5A and NCI-H28 Cells were harvested at 24, 48, and 72 hr. medium and cell homogenate were frozen separately for later testing
- P53 ELISA (Roche) that reacted with human and rat was used by manufacturer's directions; plate was read on a PowerWave BioTek instrument with KC4 software
- Human samples: mesothelial cells and mesothelioma cells in culture, exposed or not to nanoparticles (in vitro p53 assays)
- Rat samples: lung homogenates from nanoparticle dosed or control rats (ex vivo p53 assays)

RESULTS

- This project looked at the effect of nanoparticles on a pathway important to carcinogenesis: p53 protein
- It was measured in healthy human pulmonary mesothelial cells, the site most likely to transform to mesothelioma with asbestos
- **At 48 hrs, culture MeT-5A cells (triplicate) with nanoparticles added to the culture showed significant decrease in p53 compared to unexposed MeT-5A wells on the same plate**
- Human mesothelioma cells in culture (positive control) produced very little p53, a fact corroborated by the literature, and did not change significantly with nanoparticle addition
- Also measured, was p53 in lungs of rats exposed intratracheally to nanoparticles 1 month or less after exposure. There was no significant difference in p53, nanoparticle exposed vs control



CONCLUSIONS

- CA cells express little p53 activity
- Healthy mesothelial cells express less p53 when exposed to nanoparticles (48 hr)
- Lungs of rats exposed to nanoparticles in vivo hours to weeks before do not upregulate p53 when compared to saline

Strengths and Weaknesses

- Strong conclusions will require molecular determination of the fate of the p53 gene in normal mesothelial cells exposed to nanoparticles. This project measured the protein product of cellular genetic change
- Compared to published literature, our selected dose was below average; other assays used 50 and 100 micrograms vs our 27.
- Measurement of apoptosis, a product of p53 effect would strengthen conclusions
- The mesothelioma cells produced no significant change in p53 with exposure to a nanoparticle dose that changed healthy cell p53 expression. We interpret this finding as showing that the activity of p53 gene is in tumorgenesis or pre-cancerous activity; these cells have gone beyond p53 control.
- The in vivo measurements (showing no p53 difference in rat lungs receiving nanoparticles vs controls) were very short term (1 month or less)