9-2017

Epigenetic biomarkers for MWCNT exposure and lung disease

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University Grant Program – Final report (M25391)

Project title: Epigenetic biomarkers for MWCNT exposure and lung disease
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Background: Multi-walled carbon nanotubes (MWCNT) are engineered nano-materials being developed and used in a wide variety of medical, engineering, and personal products with many potential benefits [1]. MWCNT have been shown to cause significant pathological changes in animal models, particularly in the airways, raising the concern that adverse human health effects will emerge with increasing use and exposure to these materials [2-4]. The potential bioactivity of MWCNT, including *in vitro* and *in vivo* toxicity and increased inflammation and pathology, has been attributed to their unique physical and chemical characteristics such as length, diameter, contaminants and rigidity [5]. However, MWCNT toxicity and/or the mechanisms of bioactivity have not been extensively studied. Moreover, a mechanistic predictive model based on physical and surface properties of MWCNT has not been established to aid in protecting human health. Given the increasingly widespread use of MWCNT and significant potential for human exposure during their lifecycle, it is imperative that we gain a better understanding of the associated disease processes. In addition, the development of biomarkers for exposure and disease development would represent an important advance in our ability to evaluate future health impacts from potential exposures.

It is becoming more evident that environmental influences can result in physiological changes through epigenetic alterations, which offers a plausible mechanistic explanation in addition to gene-environment interactions for some of the molecular events linking environmental exposures with the onset and development of disease [6]. The goal of my laboratory, with expertise in epigenetic studies, is to create a predictive model for determining inflammation and pathology of MWCNT based on physicochemical properties and epigenetic changes that can be utilized to improve overall safety. Therefore, we used a murine model to determine epigenetic changes and examine their relationship to increased inflammation and development of lung disease in response to various sized MWCNT exposures.

Methods: Adult 2-month old C57BL/6 mice were exposed via oropharyngeal instillation to a single dose (50 μg) of either dispersion media only (DM), or to one of three different diameter and length MWCNT "as-received", including "wide short" (WS), "narrow short" (NS), and "narrow long" (NL). Lung lavage fluid (LLF) and lung tissues were collected after 24 hours and 7 days post-exposure in order to examine pulmonary inflammatory responses (cell counts and differentials and cytokine production) and pathological airway thickening. Global DNA methylation and promoter methylation of inflammation, inflammasome, and fibrosis-related genes in lung tissue DNAs were measured with the luminometric methylation assay (LUMA) and pyrosequencing assay, respectively.

Results: Total inflammatory cell counts were higher overall in all MWCNT-exposed groups than those in the control group; however, this result was not statistically significant. There were no effects on the number of alveolar macrophages in any of the MWCNT-exposed groups; however, the number of neutrophils was significantly increased in LLF 24 hours post NS and WS MWCNT exposures. At 7 days post-exposure, there was significant pathological airway thickening in NL MWCNT and borderline significant thickening in NS and WS MWCNT; there appeared to be a hierarchy of airway thickening and pathology scores among the three sizes with NS < WS < NL.

Different patterns of methylation in lung tissue were observed 24 hours post exposure among the differently sized and shaped MWCNT. Notably, WS MWCNT induced statistically significant alterations in promoter region methylation of several genes implicated in the process of inflammation and lung fibrosis. After 24 hours of exposure, global DNA and pro-inflammatory cytokines (*IL-1β, CXCL-1* and *TNF-α*) were hypo-methylated and fibrosis suppressor, fibroblast *Thy-1* was hyper-methylated in WS MWCNT-exposed mice. Hypomethylation of the pro-inflammatory cytokine *IL-6* and hypermethylation of *TNF-α* were observed in NS MWCNT-exposed mice at 24 hours. NL MWCNT induced significant hypo-methylation of *IL-1β* after 24 hours of exposure and the greatest global hypomethylation among other sized MWCNT;

however, altered methylation of the selected genes did not appear to be as sensitive in group exposed to NL MWCNT as that observed in the WS MWCNT group. Furthermore, no significant methylation changes in the autophagy marker *LC3a,* autophagic regulator and macrophage secreted cytokine *HMGB1,* and inflammasome *NLRP3* were observed in any of the MWCNT treatment groups.

Global DNA methylation levels 7 days post exposure decreased in the order NS < WS < NL as shown 24 hours post exposure, and WS and NL MWCNT induced significantly more global DNA hypomethylation than that in the control group. In addition, significant hypermethylation in *CXCL-1* and *IL-1β* was observed in WS MWCNT-exposed mice. The methylation trends in the selected genes observed after 24 hours did not appear to persist 7 days post-exposure, suggesting dynamic epigenetic alterations and multi-gene mechanistic processes occurring in the acute to sub-acute immune response after MWCNT exposure.

Conclusion: Exposure to MWCNT with increased surface area showed higher/greater inflammation and lung disease (NS < WS < NL). There were significant methylation changes in global DNA and the selected genes after MWCNT exposures, and different sized/shaped MWCNT showed different patterns of methylation changes. The methylation changes induced by different shaped MWCNT observed in this study did not appear to correspond to differences in measurements of MWCNT bioactivity (cell count and differentials, and airway thickening). MWCNT size/shape appears to be an important determinant influencing epigenetic changes occurring immediately and sub-chronically after exposure; this in turn results in different inflammatory responses.

Impact of Study: The funding obtained from the UGP provided great opportunities for me to generate preliminary data for my epigenetic research. The preliminary data gathered have been used to apply for two NIH grants in 2016 and 2017, including an R01 (not funded) and R15 (in pending review). I am currently preparing a manuscript for a peer-reviewed publication.

References

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