PS 2358 Silencing of Keap1 in Macrophages Boosts Lipopolysaccharide-Induced Transcription of Interleukin 6 via IKKβ Activation.

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Interleukin-6 (IL6) is a multifunctional cytokine that regulates immune and inflammatory responses. Multiple transcription factors, including NF-κB and nuclear factor E2-related factor 2 (Nrf2), are implicated in the transcriptional regulation of Il6. Kelch-like ECH-associated protein 1 (Keap1) is a substrate adaptor protein for a Cullin 3-dependent E3 ubiquitin ligase complex, which regulates the degradation of various vital proteins, including Nrf2 and Ikkβ. In agreement with previous studies, stable knockdown of Nrf2 in RAW 264.7 mouse macrophages led to significantly attenuated antioxidant response and decreased expression of Il6 under basal and lipopolysaccharides (LPS)-treated conditions. However, Nrf2 activation alone (e.g. under tert-butylhydroquinone exposure) did not increase the expression of Il6, suggesting that Nrf2 is a necessary, but not sufficient, factor in regulating LPS-induced transactivation of Il6. In contrast, silencing of Keap1 in RAW cells and human monocyte THP1 cells markedly augmented the expression of Il6 under non-stressed and LPS-challenged conditions. The enhanced expression of Il6 in Keap1-knockdown (Keap1-KD) cells was significantly attenuated by silencing of Ikkβ, but not Nrf2, suggesting that stabilized Ikkβ resulting from Keap1 silencing is the major downstream event responsible for the transactivation of Il6. This finding was further confirmed by the enhanced protein levels of Ikkβ and subsequent increased expression and phosphorylation of NF-κB p65 in the Keap1-KD cells. Together, the present studies demonstrated that silencing of Keap1 in macrophages boosts LPS-induced transcription of Il6 via IKKβ activation. Given the importance of IL6 in inflammatory response, targeting Keap1 could be a novel approach in the treatment and prevention of inflammation and associated disorders.

PS 2359 Potent Protection against PM2.5 Diesel Exhaust Particle-Caused ROS Generation and Vasculature Permeable through Regulation of Nrf2-Induced Pathways by Triterpenoids.

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Epidemiologies suggest that an increase of PM2.5 diesel exhaust particles (DEP) in ambient air corresponds to an increase in myocardial infarctions within 48 hours. To cause such disorder, the close association of capillaries and alveoli should allow inhaled DEP to get in close proximity to capillary endothelial tubes. However, the mechanism of how DEP travel from the alveolar space into bloodstream remains unclear. Our group has suggested that DEP might upregulate Nrf2 pathway and induce vascular permeability factor VEGF-A secretion. Once VEGF-A goes up, DEP may cause cell-cell adherent junction disruption and transmigrate into the circulation. In order to minimize the level that DEP traveling in the bloodstream, two triterpenoids (oleanic acid, ursolic acid) were used as antioxidant. After DEP ± triterpenoids treatment, MTT was used to examine cell viability of 3D capillary-like endothelial cultures, Cm-H2DCFDA assays were used to determine the extent of ROS production in the model, and confocal microscopy was used to evaluate the endothelial junctional proteins in the cell-cell borders and localization of Nrf2 as well. At high dose DEP, 80% of the tube cells die within 24 hours. Cells treated with 25 μg/ml DEP plus triterpenoids not only the translocation of Nrf2 and downstream HO-1 mRNA expression was reduced, but also ROS generation was inhibited. Additionally, Z-stacks images revealed that DEP not only accumulated on the surface of capillary tubes, but also penetrated into the lumen. VE-cadherin was observed to redistribute in response to DEP. Once combine with triterpenoids, endothelial tube cells were slightly affected only at high dose DEP, injures caused by DEP-induced ROS were blocked. Our results suggest that triterpenoids might prevent DEP transmigration into bloodstream by inhibiting oxidative stress production and endothelial adherent junctions alternation.

PS 2360 Differential Responses upon Inhalation Exposure to Biodiesel versus Diesel Exhaust on Oxidative Stress, Inflammatory, and Immune Outcomes.

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Biodiesel (BD) exhaust may have reduced adverse health effects due to lower mass emissions and reduced production of hazardous compounds compared to diesel exhaust. To investigate this possibility, we compared adverse effects in lungs and liver of BALB/cJ mice after inhalation exposure (0, 50, 150 and 500 ug/m3; 4 hr/day, 5 d/wk, for 4 wk) to combustion exhaust from 100% biodiesel (B100) and diesel (D100). Compared to D100, B100 exhaust caused a significant accumulation of oxidatively modified proteins (carbonyls), increase in 4-hydroxynonenal (4-HNE), reduction of protein thiols, depletion of antioxidant - gluthatione (GSH), a dose-dependent increase in the levels of biomarkers of tissue damage (LDH) in lungs, and inflammation (myeloperoxidase, MPO) in both lungs and liver. B100 exposure also significantly enhanced expression of cytokines IL-6, and IL-12p70 (in a dose-dependent manner), along with IL-10, TNF-a and MCP-1 (increased compared to control) in both lung and liver tissues. Overall, the cytokine profiles in the lung and liver suggest that B100 and D100 exhaust elicit similar innate immune responses, predominantly involving T-cell independent pathways; however, the magnitude of inflammation was greater following B100 exhaust exposure. Interestingly, exposure to D100, but not B100 exhaust, induced a significant increase in the levels of IFN-g in the lungs, suggesting a broader engagement of Th1 component by D100 exhaust. Based on this, we hypothesize that the distinctive organic compounds and/or oxidative products formed as a result of increased oxidative stress upon B100 exposure, are capable of targeting biological/molecular pathways that are distinct from D100 exposure. (This abstract does not represent US EPA policy).

PS 2361 THP-1 and HMC-1 Cell Interaction with Epithelial Cells in a 3D Tetraculture System of the Alveolar Barrier Modulates the Response to Oxidative Stress.

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Exposure to fine and ultra-fine ambient particles is still a problem of concern in many industrialised parts of the world and the intensified use of nanotechnology may further increase exposure to small particles. Among the various mechanisms, the production of oxidative stress is considered to be one of the key mechanisms how particles affect tissues. Complex in vitro coculture systems may be valuable tools to study related processes and to further evaluate the effects of particles on the lung (Klein et al., 2011). Therefore, a system consisting of four different human cell lines that should mimic the cell response of the alveolar surface in vitro was developed in order to be used with a native aerosol exposure system (Vitrocell™ chamber). It is composed of an alveolar type-II cell line (A549), differentiated macrophage-like cells (THP-1), mast cells (HMC-1) and endothelial cells (EA.hy 926), seeded in a 3D orientation on microporous membranes.
Oxidative stress was induced by incubating the cells with 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH; 20 mM), and quantified as the oxidization of dichlorofluorescein diacetate (DCFH-DA) by measuring fluorescence. Results are reported as fold increase in ROS production relatively compared to untreated cells.
Single cell cultures of EA.hy 926 (11.8 +/- 1.4), THP-1 (11.5 +/- 1.3) and HMC-1 (14.7 +/- 2.9) showed significantly higher oxidative stress than the tetraculture (6.6 2 +/- 0.75). A549 cells alone show the lowest amount of oxidative stress (3.4 +/- 0.18) compared to other cultures. The interplay of model cell for the immune system (THP-1 and HMC-1) with A549 epithelial cells strongly influences the behaviour of our system, resulting in an alleviative effect for oxidative stress compared to the monocultures. The use of the tetraculture system may lead to a more realistic judgement about the hazard of new compounds in the future.

PS 2362 ADME Studies on Nanoparticles Are So Far of Limited Use for PBPK Modeling.

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The health hazards with nanoparticles (NP) are largely unknown, and human data are unlikely to be generated to any great extent. Previous experience with xenobiotics shows that combined use of animal ADME studies and PBPK modeling is

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