3431 Polystyrene Microplastics Impair the Growth, Survival, and Macrophage Differentiation of Human THP-1 Monocytes

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Background and Purpose: Microplastics (MPs) are pervasive environmental pollutants with little existing toxicological information. Human exposures to MPs occur through a variety of routes, including the consumption of contaminated drinking water. Despite a growing awareness of the potential health implications associated with MP exposures, their effects on the human immune system remain largely uncharacterized. The purpose of this study was to evaluate the *in vitro*immunotoxicity of polystyrene MPs exposures using the human monocytic cell line, THP-1. **Methods:** The growth and viability of THP-1 monocytes was assessed in the presence of increasing concentrations (0, 1, 10 and 100 µg/mL) of 1 µm or 5 µm polystyrene MPs. In addition, the effects of MPs on the phorbol 12-myristate 13-acetate (PMA)-induced macrophage differentiation of THP-1 cells was assessed based on *Cd14* mRNA expression using qPCR. Macrophage differentiation of M1 and M2 phenotypes will be also assessed in the presence of 1 µm or 5 µm polystyrene MPs based on cytokine secretion of TNF-α and IL-1β, respectively. **Results:** THP-1 cell growth was significantly suppressed following exposure to high-doses of 1 µm or 5 µm MPs. No substantial effects on cell viability were observed at low MP concentrations, but subtle reductions were observed following exposure to 100 µg/mL of 1 µm and 5 µm MPs. A significant suppression of *Cd14* mRNA expression was also observed following exposure to 100 µg/mL of 1 µm and 5 µm MPs. **Conclusions:** This suggests that exposure to MPs may impact the development and function of human THP-1-derived macrophages. Results from the present study provide important information for understanding the immunotoxicity of MPs and will serve as a foundation for future mechanistic investigations.

3432 Evaluating the Gene Expression of Cannabinoid receptors 1 and 2 in Monocytes

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Background and Purpose: With increased legalization of cannabis across the United States, usage has increase 13.2% between 2011 and 2021 for adults aged 19-30 years. With this increase there is a need to better understand the health effects of cannabis. When ingested or inhaled, some of the bioactive constituents of *Cannabis sativa*, termed cannabinoids, bind cannabinoid receptors (CB). Cannabis contains over 110 structurally related phytocannabinoids. Δ9-Tetrahydrocannabinol (THC) is the main psychotropic cannabinoid and can bind to both CB1 and CB2 receptors. Published studies suggest that when THC binds CB2 it can mediate immunomodulatory activity on immune cells, such as monocytes, to produce anti-inflammatory effects. Monocytes produce inflammatory cytokine, including interleukin-1β (IL-1β), upon toll-like receptor (TLR) activation. THC suppresses the production of IL-1β in a concentration dependent manner in TLR activated monocytes. The objective of these studies was to test the hypothesis: *Monocyte activation increases CB2 expression, the primary cannabinoid receptor expressed by immunocompetent cells, upon cellular activation via TLRs*. The rationale for testing this hypothesis was to begin to elucidate whether CB2 or CB1 is the primary cannabinoid receptor by which cannabinoids like THC mediate their anti-inflammatory activity in resting and after *in vitro* activation of primary human monocytes. **Methods:** Peripheral blood mononuclear cells (PBMC) were isolated from leukocyte enriched blood packs through density gradient centrifugation. The cells were then magnetically isolated for primary human pan monocytes, CD16+ monocytes and CD16- monocytes. To determine basal (resting) expression of our endpoints some cells were lysed at t=0 hours. For TLR activation experiments, cells were either left untreated or treated with TLR7 agonist, Imiquimod (10ug/mL), TLR4 agonist, lipopolysaccharide (LPS, 10ng/mL) or TLR8 agonist, ssRNA40 (0.5 ug/mL). At t=6 hours cells were lysed and lysates were used for RNA isolation before undergoing polymerase chain reaction (PCR) to evaluate CB1 and CB2 mRNA levels. Supernatants were saved from all TLR experiments for quantification of IL-1β by enzyme-linked immunosorbent assay (ELISA). **Results:** Our studies showed CB2 is 31 times more highly expressed by resting monocytes than CB1 ($p > 0.05$). Resting monocyte expression of CB2 had little variation between the three isolated cell populations. Furthermore, CB2 expression was significantly decreased after TLR7 activation in CD16+ and CD16- monocytes by 57% and 45%, respectively, when compared to unstimulated (resting) cells ($p > 0.05$). By contrast, TLR4 and TLR8 activation did not influence pan monocytes, CD16+ monocytes or CD16- monocytes. CB1 expression was not able to be detected through PCR analysis of these monocyte populations. **Conclusions:** These results suggest that CB2 may play a larger role in the anti-inflammatory properties associated with monocytes suppression by THC than previously postulated. The results of the TLR activation studies also suggest that the anti-inflammatory effects observed with THC treatment may be dependent on the context of the inflammatory pathway triggering the inflammatory event in a given individual.

3433 Δ9-Tetrahydrocannabinol (THC) and cannabidiol (CBD) suppress toll-like receptor (TLR)- 7 and 8 induced production of inflammatory factors from CD16+ human monocytes

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Background and Purpose: Over recent years, cannabis has been legalized on the state level in nearly half of the US. Recent studies from the Kaminski laboratory have shown that major cannabinoids present in *Cannabis sativa*, Δ9-Tetrahydrocannabinol (THC) and cannabidiol (CBD), possess immune modulating properties, specifically on pan monocytes. A subset of monocytes, identified by expression of CD16 ($CD16^+$ monocytes), are of particular interest as they are implicated in contributing to neuroinflammation in HIV-associated neurocognitive disorder. Interestingly, HIV is capable of infecting $CD16^+$ monocytes, as well as activation via viral ssRNA sensing toll-like receptors (TLR) 7 and 8. These monocytes have the capacity to cross the blood brain barrier where they can contribute to neuroinflammation even when the individual is successfully on combined anti-retroviral therapy. Many individuals living with HIV often utilize cannabis to self-medicate with little knowledge of its immunomodulatory properties. Therefore, the purpose of this study was to determine the effects of cannabinoids, THC and CBD, on inflammatory cytokine production by TLR7 and 8 activated $CD16^+$ monocytes. **Methods:** Primary human peripheral blood mononuclear cells (PBMCs) were isolated from enriched human leukocyte blood packs via density gradient centrifugation. Pan human monocytes were isolated from PBMCs utilizing negative magnetic selection. For studies utilizing $CD16^+$ monocytes, pan monocytes underwent positive magnetic isolation, wherein labeled cells consisted of $CD16^+$ monocytes and unlabeled cells consisted of $CD16^-$ cells. Monocytes were pre-treated with cannabinoids THC, CBD, or JWH-015 (0.5, 1, 5, 10 µM) for 30 minutes prior to activation with TLR7 or 8 agonists, R837 (10µg/mL) and ssRNA40 (0.5µg/mL), respectively. Cell supernatants were collected after 22 hours of culture and quantified for cytokines via 13-plex LEGENDplex HU Essential Immune Response Panel (quantifies IL-4, IL-2, CXCL10, IL-1β, TNF-α, CCL2, IL-17A, IL-6, IL-10, IFN-γ, IL-12p70, CXCL8, and TGF-β) or IL-1ß, IL-6, and TNF-α ELISAmax assays. For RT-qPCR studies, cells were lysed at 6 hours post activation and quantified for IL-1ß mRNA levels. **Results:** LEGENDplex assay was utilized to determine whether $CD16^+$ and $CD16^-$ monocytes exhibit differential cytokine secretory profiles in response to TLR7 or 8 stimulations. Results show that both cell populations primarily produce IL-1ß, IL-6, and TNF-α in response to TLR activations, wherein the capacity to produce both IL-1ß and IL-6 are comparable between $CD16^+$ and $CD16^-$ monocytes. Interestingly, $CD16^+$ monocytes have a greater capacity to produce TNF-α compared to $CD16^-$ monocytes. All other cytokines in the LEGENDplex panel were produced at negligible levels. For this reason, only IL-1ß, IL-6, and TNF-α ELISA assays were conducted for remaining cytokine quantification studies to determine the effect of cannabinoid treatment on secretion profiles. These studies demonstrated that THC significantly suppressed TLR7 and 8-induced IL-1ß production in a concentration-dependent manner by both cell populations (IC50 > 5µM). Furthermore, treatment with JWH-015 did not suppress IL-1ß production. Though CBD treatment suppressed IL-1ß production in TLR7-activated $CD16^+$ monocytes and $CD16^-$ monocytes, as well as TLR8 stimulated $CD16^+$ cells, the observed reduction was not to the extent of THC. Additionally, IL-6 and TNF-α secretion was minimally affected by cannabinoid treatments. RT-qPCR was conducted on pan monocytes to determine whether IL-1ß production may be affected by cannabinoids at the level of mRNA transcription. Results from this study demonstrate that monocyte IL-1ß mRNA levels were not affected by cannabinoid treatment. **Conclusions:** Results from this study show that THC is superior to CBD in its ability to suppress inflammatory cytokine IL-1ß by mimicking viral stimulation of monocytes. Furthermore, JWH-015 treatment and PCR studies suggest that THC does not mediate this anti-inflammatory effect via CB2 ligation, nor through suppression of IL1ß mRNA levels. This study is of importance due to the wide use of cannabis as a result of legalization, as it provides evidence of the anti-inflammatory properties of the main psychotropic cannabinoid, THC, on monocytes in the context of viral infection. (Supported in part by NIH R01 DA047180).

3434 Synergistic effect of World Trade Center (WTC) dust exposure and chronic intermittent hypoxia on oxidative stress, inflammation, and proliferation

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Background and Purpose: The collapse of the World Trade Center (WTC) led to a large dust cloud of particles consisting of highly alkaline crushed concrete, gypsum, and synthetic fibers that were inhaled and deposited in the conducting airways. In earlier studies, we reported a 75% prevalence of obstructive sleep apnea (OSA) leading to chronic intermittent hypoxia (CIH) in WTC responders. In rodents, WTC

dust exposure leads to upregulation of genes and proteins related to inflammation and oxidative stress, along with abnormal pulmonary structure and function. We hypothesized that CIH due to OSA, in combination with WTC dust exposure, would exacerbate oxidative stress, inflammation, and lung injury. To assess this, we analyzed the effects of CIH following WTC dust exposure on the composition of pulmonary cells and markers of oxidative stress, inflammation, and proliferation. **Methods:** C57BL/6J mice (male, 6-8 wk) were treated intratracheally with 1 mg WTC dust suspended in 50 µl saline or 50 µl saline control. Mice were then exposed to CIH (90s of hypoxia to reach a nadir oxygen saturation of 5% followed by 90s of re-oxygenation for 8h/day) or room air (19-21% O2) for 5d, 14d, or 28d. The effects of WTC dust and CIH on pulmonary cells were determined by flow cytometry and cytology. Oxidative stress, inflammation, and proliferation markers were evaluated by immunohistochemistry (IHC) for expression of HO-1, CD11b, and PCNA, respectively. **Results:** Following WTC dust exposure, HO-1 expression significantly increased after 28d (35.2±13.1 v 14.1±6.2), while CD11b significantly increased at all post-exposure time points (25.8±0.6 v 17.0±4.4 (5d); 11.9±1.1 v 8.7±2.0 (14d); 17.0±1.4 v 12.1±3.0 (28d)), and PCNA expression significantly increased in the 5d but decreased in the 28d compared to control (7.3±1.1 v 5.5±0.6; 17.9±0.6 v 19.7±0.5, respectively). CIH caused an increase in expression of HO-1 in both 5d and 28d (37.6±5.4 v 21.2±3.1; 30.9±7.0 v 14.1±6.2, respectively), an increase in CD11b expression at 14d and 28d (23.8±2.7 v 8.7±2.0; 33.3±2.4 v 12.1±3.0, respectively), and an increase in PCNA staining at 5d (6.8±1.0 v 5.5±0.6). The combination of WTC dust and CIH resulted in a significant increase in neutrophils and lymphocytes at 14d (1.0±0.5 v 10.8±3.5; 2.0±0.6 v 20.6±4.4, respectively), an increase in HO-1 and CD11b expression at all time points (HO-1: 34.6±9.4 v 21.2±3.1 (5d); 94.1±5.0 v 66.0±17.1 (14d); 53.4±15.2 v 14.1±6.2 (28d)) (CD11b: 26.2±2.4 v 17.0±4.4 (5d); 12.7±1.0 v 8.7±2.0 (14d); 19.7±1.4 v 12.1±3.0 (28d)), and an increase in the percentage of PCNA stained cells at 5d and 28d (7.3±0.6 v 5.5±0.6; 23.2±3.0 v 19.7±0.5, respectively). There were no observed changes in the phenotype of the cells as determined by flow cytometry. **Conclusions:** The combination of WTC dust and CIH exacerbates oxidative stress in pulmonary epithelial cells and inflammatory cells. This was associated with upregulation of a pro-fibrotic marker at 5d and 28d, and a heightened immune response at 14d. The two-hit effect of CIH and dust exposure does reveal a novel injury model. Supported by NIOSH Grant 5 U01 OH012072-02 and ES05033.

3435 Direct enhancement of Mitogen-activated Protein Kinase phosphorylation in dendritic cells by exposure to mycotoxin Nivalenol, leads to significant exacerbation of the development of atopic dermatitis in a mouse model

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Background and Purpose: Nivalenol (NIV) is a type B trichothecene mycotoxin produced by *Fusarium* spp. that cause *Gibberella zeae*. Contamination of the grains such as wheat, barley and corn with NIV can induce considerable adverse effects on human and animal health. High contamination of NIV is observed in East Asian countries such as Korea and Japan. Therefore, dentification of toxicological properties of NIV is quite important as well as development of the analytical method. Previous study demonstrated that oral exposure to Lowest Observed Adverse Effect Level (LOAEL) of NIV (0.4 mg/kg) caused lymphopenia, however, concrete effect particularly against the immune system is still unclear. The aim of this study is to investigate the detailed immunotoxic effects of NIV *in vitro* and *in vivo* using the antigen-presenting cell and a mouse model of atopic dermatitis (AD). **Methods:** *In vitro* experiments were conducted using mouse dendritic cell line (DC 2.4). After cells (1×10^5 cells) were exposed to NIV (0.19 ~ 5.00 µmol/L) for 24 h, the concentration of TNFα in the supernatant was quantified using ELISA. To further investigate the inflammatory cytokine production pathway, possible involvement of mitogen-activated protein kinase (MAPK) pathway such as ERK1/2, p-38 and JNK with NIV exposure was analyzed using MAPK-inhibitors and phosphorylation analysis. In addition, pro-inflammatory effects of oral exposure to NIV at low concentration (1 ppm or 5 ppm) were evaluated by a NC/nga mouse model of hapten (toluene-2,4-diisocyanate)-induced AD. Ear and back skin thickness were monitored once weekly during the experimental period. The back skin, auricular lymph node (LN), and serum were collected from each mouse four weeks after the first sensitization. The collected samples were processed for histology, cytokine analysis, IgE detection, RNA analysis, and cell differentiation. **Results:** *In vitro* experiment with DC 2.4 demonstrated that exposure to NIV significantly enhanced the production of TNFα (0 µmol/L: 11.4 ± 5.6 pg/mL, 1.67 µmol/L: 343.0 ± 54.0 pg/mL). Exposure to NIV also directly induced the phosphorylation of MAPK, which was corroborated by the inhibition of TNFα production by pretreatment with MAPK inhibitors. *In vivo* experiment with a mouse model of AD indicated that exposure to NIV significantly exacerbated the symptom of AD as compared to the vehicle control group, in a dose-dependent manner. Number of helper T cells and IgE-produced B cells in auricular lymph nodes and pro-inflammatory cytokine secretion such as IL-4 (Control: 18.9 ± 3.9 pg/mL, 5 ppm: 57.6 ± 3.2 pg/mL), IL-5 and IL-13 were also significantly increased by the NIV exposure compared to the vehicle control group. In addition, oral exposure to NIV significantly increased ear skin thickness, which corroborated by histological features such as significant induction of inflammatory cell infiltration. **Conclusions:** Our *in vitro* findings implied that exposure to NIV directly enhanced the phosphorylation of MAPK resulted in the significant increase of TNFα production in antigen presenting cells which is closely related to the development of atopic dermatitis. Significant aggravation of pro-inflammatory responses by NIV exposure in a mouse model of AD supported the *in vitro* evidence.

3436 Immunomodulatory effect of 6-pentadecyl salicylic anacardic acid on the human NK-92 MI cell line

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Background and Purpose: 6-Pentadecylsalicylic acid (6SA) is an efficient antineoplastic agent that induces immunostimulation in different models. There is evidence that 6SA increases the Natural Killer (NK) cell population in *in vivo* models, as well as the phosphorylation of different mitogen-activated protein kinases such as p38 and JNK, which could be involved in the activation of these cells. NK cells belong to the innate immune system as the first line of defense against pathogens, transformed or autoimmune cells. NKs do not require antigen recognition to activate; they are activated in the absence of recognition of their markers (Human Leukocyte Antigen; HLA). After activation, they release the contents of their cytolytic granules, enzymes such as granzymes, and perforins that induce cell death of target cells. Thus, this study aimed to evaluate if 6SA has a direct effect on the proliferation and activation of NK cells by increasing their cytolytic capacity. **Methods:** Human NK-92 MI and K-562 cells were cultured in flasks with RPMI medium supplemented with 10% fetal bovine serum, non-essential amino acid solution, L-glutamine, sodium pyruvate, and antibiotic-antimycotic in a humidified atmosphere of 5% CO_2 at 37 °C. NK-92 MI cells were exposed to 6SA (5-100 µM) dissolved in DMSO for 15, 24, or 48 h, as appropriate. The cell viability of NK-92 MI cells exposed to 6SA was determined using the neutral red (NR) uptake assay. The cells were exposed for 24 and 48 h to 6SA and 4 h before the end of the treatment time, NR was added. Subsequently, the cells were washed twice with PBS. The absorbance at 540 nm was quantified with a spectrophotometer. The stages of cell death were determined using the Annexin V/propidium iodide (PI) assay. After the treatments, the cells were washed with PBS and stained with annexin V and PI for 5 min. Data were collected from 10,000 events by flow cytometry. Proliferation of NK-92 MI was determined by the tritiated thymidine (^{3}[H]T) incorporation assay. Cells were exposed to 6SA and 20 h before finishing the treatments, ^{3}[H]T was added. Cells were collected with a cell harvester on glass fiber filters. The counts per minute of tritium were recorded using a liquid scintillation counter. To evaluate the cytolytic activity of NK-92 MI they were treated with 6SA overnight and washed with PBS. Separately, K562 cells were incubated with CFSE for 10 min in the dark. Subsequently, NK cells were added to K562 cell cultures in polystyrene tubes for 4 h at different effector (E; NK cells) and target (T; K562 cells) ratios (0:1, 1:1, 2:1, 5:1, 10:1, 20:1 and 50:1), placing 6 x 10^5 target cells in each tube. After 4 h, the cells were stained with PI, and 10,000 events were acquired by flow cytometry. The supernatants were used to evaluate the secretion of perforin, granzyme B, TNF-α, IFN-γ, IL-10, and GM-CSF by ELISA. **Results:** Inhibitory concentration at 50% (IC_{50}) of 6SA on NK-92 MI cells From the NR uptake assay, it was determined that the 6SA IC_{50} at 24 and 48 h on NK cells was 40.93 and 39.11 µM, respectively. The cell death assay with Annexin V and PI indicated that 6SA induces apoptosis in the cells and the IC_{50} at 24 and 48 h was 73.79 and 45.07 µM, respectively. These results indicate that cellular activities, such as endocytosis or excretion vesicle formation, could be affected previously by the induction of death by apoptosis in the cells, suggesting also, that the apoptotic mechanism of 6SA on NK cells could be related to the endoplasmic reticulum stress. Effect of 6SA on NK-92 MI Cell Proliferation The proliferation results indicate differences in non-cytotoxic concentrations between 24 and 48 h of exposure; when compared with the viability, they showed an increase in proliferation at non-cytotoxic concentrations. This may indicate a probable immunostimulatory effect of 6SA on NK-92 MI. Effects of 6SA on the cytolytic activity of NK-92 MI against K562 Because the main participation of NK cells in an immune response is to induce the death of target cells, the cytolytic activity of NK-92 MI cells treated with non-cytotoxic concentrations of 6SA was evaluated. The co-cultures of marked K562 cells with 6SA-preincubated NK-92 MI cells at different E: T ratios for 4 and 24 h are under evaluation. These experiments are currently being conducted, as well as the determination of granzyme B, perforine, and cytokines release. **Conclusions:** The preliminary data indicates that human NK-92 MI cells are susceptible to the cytotoxic effects of 6SA at concentrations close to those of other tumoral cell types. The exposure to 6SA at concentrations below its IC_{50} increases cell proliferation in viable cells over time. Acknowledgments NK-92 MI cells were donated by Dr. Erika Rendón from the Medicine Faculty of the National Autonomous University of Mexico. Funding was provided by CONAHCyT's grant number 21067.

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