LP-68 Subtoxic doses of polystyrene nanoplastics and microcystin-LR affect the bioenergetic status of Caco-2 and HepG2 cells

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risk of developing chronic kidney disease (CKD). However, there is a lack of understanding on how PM2.5 deteriorates kidney function prematurely. Acute kidney injury (AKI) is an important risk factor for progression to CKD. The role of PM2.5 in the AKI-to-CKD transition has still to be described.

Methods: In temperature/humidity-controlled chambers within the Harvard ambient particle concentrator, wild-type C57BL/6 male mice (4 weeks old) were exposed to a real-world concentrated PM2.5 stream (PM2.5) or to high-efficiency filtered air (FA). These experiments were performed at the Faculty of Medicine of University of São Paulo. After 12 weeks of exposure, mice were subjected to bilateral renal ischemia/reperfusion injury (IRI), a preclinical model of AKI, and sacrificed 48 hours after surgery. Sham mice underwent the same procedure except for the clamping of the renal arteries. Kidneys were processed for histology, RNA isolation, and protein determination.

Results: Mice exposed to PM2.5 + IRI showed increased renal damage determined by a significant decrease in creatinine clearance and increased tubular injury assessed by pathological score. *Kim-1* and *Ngal* transcript, markers of tubular damage were also increased in PM2.5 + IRI group as compared to mice exposed to FA + IRI. Infiltration of inflammatory cells in renal parenchyma, evaluated by immunohistochemistry (IHC), revealed a significant increase in Ly6G+ granulocytes and F480+ macrophages in the PM2.5+IRI group. Additionally, these animals showed enhanced expression of p21+ senescent cells detected by IHC and increased expression of senescence-associated secretory phenotype (SASP) components (*Tgfb1, Ctgf,* and *Serpine1*). The antiaging factor Klotho, measured by IHC, was also significantly decreased in the PM2.5 + IRI group, compared to FA+IRI. Finally, increased inflammation and senescence were associated with enhanced expression α-smooth muscle actin+ interstitial myofibroblast and vimentin expression, markers of fibrosis.

Conclusion: Exposure to PM2.5 contributes to AKI-to-CKD progression by inducing premature aging. PM2.5 exposure affects different renal cellular and molecular processes, leading to innate immune activation, senescence and fibrosis. Reducing air pollution might represent an unconventional (i.e. non-pharmacological) strategy to prevent CKD and achieve healthy renal aging.

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LP-65
Interstrain variation of cellular dose response traits in mouse pluripotent stem cells establishes feasibility for population-based studies of genetic susceptibility to triphenyl phosphate exposure

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Genetic variation impacts an individual's biological response to chemical and environmental exposures, contributing to differences in toxicant resistance or susceptibility. Forward genetic screens of genetically diverse cell populations can be used to identify the precise genetic variants that drive response variation, but such screens are limited by the lack of genetic diversity in human-derived cell panels. Cell lines from laboratory mouse genetic reference populations like the Diversity Outbred (DO) population are genetically diverse and powered for genetic mapping, but also offer the significant advantage of *in vitro/in vivo* trait correlation and *in vivo* validation using genetically matched individuals. We have created a panel of induced pluripotent stem cells (iPSCs) from more than 300 DO mice, as well as the 8 inbred founder strains that were used to create the population. To identify cellular endpoints associated with developmental exposure risk, I have exposed a subset of these lines to triphenyl phosphate (TPP), an organophosphate flame retardant that has been recently linked to adverse developmental effects. To identify dose response traits that could be useful for *in vitro*, forward genetic screening and mapping, I have used high content imaging to quantify cellular morphological traits that exhibit a dose response relationship following TPP exposure. I have also found that some of these cellular dose response traits, including colony morphology, nuclear count, and cell death show significant interstrain variation in cell lines derived from WSB/EiJ and NZO/HILtJ strain backgrounds. I propose a potential cellular mechanism of TPP induced effects on pluripotent stem cells, where TPP causes altered cell cycle and proliferation, in addition to increased cell death. Moving forward, these heritable, cellular dose response traits and molecular traits from gene expression will be used for quantitative trait mapping to reveal the genetic underpinnings of TPP susceptibility and to use this information to further understand the molecular and biological pathways that modulate these gene by environment interactions. Ultimately, our laboratory's goal is to use these data to make and test genetic predictions of adverse outcomes observed *in vivo*, and to better inform risk assessments for diverse populations.

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LP-68
Subtoxic doses of polystyrene nanoplastics and microcystin-LR affect the bioenergetic status of Caco-2 and HepG2 cells

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Nanoplastic particles (NPs) and the cyanobacterial toxin microcystin-LR (MC-LR) are emerging contaminants that may (co-)occur in (sea) food and water [1,2]. In addition, NPs have been found to stimulate MC-LR synthesis and release from its producing cyanobacteria [3]. The available data on MC-LR quantification in human plasma is ranging from 0.1 to 1.8 ng/mL [4,5], while human exposure to NPs is still largely unknown. The current work aimed at investigating the (combined) effect of polystyrene NPs and MC-LR on the bioenergetic status of the intestinal Caco-2 and Hepatic HepG2 cell lines, as early marker of cell dysfunctionality which may lead to chronic disorders. Caco-2 or HepG2 cells (20,000 cells/well of 96-well plates) cultured in DMEM media were exposed to commercial polystyrene NPs spheres of 60 nm (1–50 μg/mL) and/or MC-LR toxin (0.1–250 ng/mL) for 24 h. The tetrazolium-based colorimetric (MTT) assay was conducted to determine the IC50 values within the applied range. Afterwards, subtoxic concentrations were selected to study the solo effect of NPs and MC-LR on the energy metabolism using Agilent Seahorse XFe96 Analyzer. Cells were seeded, at the same density for the MTT assay, in XF96 cell culture microplates and exposed to polystyrene NPs or MC-LR for 24 h. Next, Real-Time ATP Rate Assay kit and Cell Mito Stress Test kit were used to quantify the rate of adenosine triphosphate (ATP) production from glycolysis and mitochondrial respiration and to assess the mitochondrial function, respectively. An optimization for the cell culture density of Caco-2 and HepG2 and concentrations of the stressors/modulators of cellular respiration (oligomycin and FCCP) was performed before running the experiments. For data analysis, the cloud-based Agilent Seahorse Analytics application was used. After examining the ATP level and several mitochondrial parameters, the combined effect on mitochondrial function was assessed by applying different concentrations of both polystyrene NPs (1, 5, 10 μg/mL) and MC-LR (1,10, 100 ng/mL).

The results show that short-term exposure to polystyrene NPs (2.5–10 μg/mL) inhibited the mitochondrial respiration in Caco-2 cells, but not in HepG2. The inhibitory effect was observed in all the mitochondrial parameters (basal respiration, ATP-linked respiration, proton leak, maximal respiration, spare respiratory capacity, and non-mitochondrial respiration). Interestingly, the glycolytic ATP rate was increased, thereby leading to a less efficient energy production. The applied concentrations of MC-LR neither caused cytotoxicity nor affected the respiration in both cell lines. However, the co-exposure of polystyrene NPs and MC-LR increases the hepatotoxicity in a dose-dependent manner. As MC-LR is a potent tumour promoter, long-term of exposures in combination with several types of NPs need to be further investigated to assess the health impact in real life.

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LP-70
Toxicity of graphene nanomaterials in HEK 293T cells – effects of surface modifications by thermal treatment

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Graphene family nanomaterials have attracted great scientific interest in biomedicine. The biocompatibility of these innovative materials with human cell lines is still to be clarified. The surface functionality of nanomaterials plays an important role in the change of their physical and chemical properties. Therefore, the aim of this study was to bring new cytotoxic and genotoxic data concerning the impacts of *in vitro* exposure of the human embryonic kidney cells (HEK 293T) to highly oxidized graphenes. Cell cultures were treated by two graphene oxides in three concentrations (2.5 μg/ml, 5 μg/ml, 10 μg/ml). For each graphene oxide, separate cultures were treated with either untreated graphene nanomaterial or after its prolonged mild thermal treatment. Prolonged mild thermal treatment provide sufficient energy and time for the aggregation of oxygen functional groups, which is in accordance with Fourier-transform infrared spectroscopy (FTIR) and Raman spectra of all materials, as well as with their redox behavior. Effects of graphene oxides were measured by MTT assay after 24 h exposure. MTT and formazan adsorption isotherms were determined for both materials, for quantitative determination of interference caused by adsorption of the dyes on applied graphene materials. DNA damage was evaluated by tail intensity (TI) in the alkaline comet assay, following 3 h of exposure. Corresponding to the size of tested graphene materials, results show association between cytotoxic and genotoxic effects. Thermal modifications have no impact on the cytotoxicity of graphenes while variously affecting their genotoxicity. These findings should be considered in design of reliable and safe graphene derivatives for bioapplications.

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LP-71
Metabolism of benzo[*a*]pyrene in colon epithelial cell models and its modulation by bacterial products

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Benzo[a]pyrene (BaP) is a genotoxin and dietary carcinogen that can contribute, together with other dietary mutagens, to the development of colon carcinoma. Its metabolism and bioactivation in intestinal epithelial cells are primarily under control of the aryl hydrocarbon receptor (AhR), a transcription factor regulating the expression and activity of cytochrome P450 family 1 (CYP1) enzymes. Recent data indicate that within the intestinal microenvironment, the expression of CYP1 enzymes may depend on the presence of various types of bacterial products, including products of bacterial tryptophan metabolism. In the present work, we evaluated interactive effects of a selected set of tryptophan-derived indoles with short-chain fatty acids (SCFAs) produced in the colon via fiber fermentation, including butyrate, propionate and acetate. The first two significantly promoted the indole-induced expression of CYP1 enzymes, as well as CYP1 enzymatic activity, in cell models derived from colon epithelial tumor cells. Pre-incubation of cells with SCFAs and indoles significantly increased formation of phenolic, diol and tetrol metabolites of BaP, as well as formation of covalent DNA adducts. Taken together, the present results indicate that interactive effects of indoles activating AhR and SCFAs acting as histone deacetylase inhibitors, substantially impact metabolism and bioactivation of BaP in various models of colon epithelial cells. The combined effects of these bacterial products derived from gut microbiota may thus significantly alter the capacity of intestinal epithelium to metabolize dietary carcinogens via CYP1-dependent metabolic pathways.

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