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 24h. 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 24h. 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.

Affix

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