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In vitro toxicometabolomic evaluation of hepatic cell response to "forever chemicals"

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Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals widely used in industrial and consumer products [1]. Their chemical stability and resistance to biodegradation lead to environmental persistence and bioaccumulation, giving them the designation "forever chemicals" [2]. Despite growing research efforts, significant knowledge gaps remain regarding the consequences of human exposure. In this context, metabolomics emerged as a promising tool to characterize and enable early detection of PFAS-biological effects [3]. **Objective:** This study aimed to investigate *in vitro* metabolic alterations induced by perfluorooctanoic acid (PFOA) and its short-chain substitute, perfluorobutanoic acid (PFBA), in human hepatic cells applying an untargeted metabolomics approach. **Methods:** Proliferating HepaRG cells were exposed to increasing concentrations of PFOA and PFBA for 24 hours, and concentrations inducing at maximum 40% cytotoxicity were selected based on MTT viability assays (PFOA: 37.5, 75 and 150 µg/mL; PFBA: 75, 150 and 300 µg/mL). Intracellular metabolites (endometabolome) and extracellular culture medium metabolites (exometabolome) were analysed by GC-MS and NMR, enabling the detection of amino acids, organic acids, sugars, lipids, and nucleotides. Data were processed, statistically analysed, and biologically interpreted. **Results:** Multivariate analysis revealed discrimination of the HepaRG endometabolome after exposure to high PFOA, intermediate and high PFBA concentrations. These changes were characterised by a significant intracellular decrease in metabolites associated with antioxidant defence (pyroglutamate, glutamate, threonate and malate), osmotic regulation (taurine) and energy metabolism (malate). PFOA further disrupted intracellular amino acid homeostasis and altered phospholipid metabolism. Exometabolome analysis revealed discrimination across all PFAS concentrations. A dose-dependent increase in niacin excretion was observed in PFAS-exposed cells, suggesting enhanced NAD^+ biosynthesis. Additional extracellular changes were detected, but PFOA specifically affected amino acid levels. **Conclusions:** PFAS *in vitro* exposure induces a common pattern of metabolic alterations. Although these effects were more pronounced following PFOA exposure, the substitute PFBA also elicited metabolomic changes of potential toxicological relevance. These findings may serve as early metabolic indicators of PFAS-related hepatotoxicity and highlight the value of metabolomics in detecting *in vitro* chemical-induced metabolic alterations.

Keywords: per- and polyfluoroalkyl substances (PFAS); environmental exposure; metabolomics; hepatic cells

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