

## CO.10- TOXICOMETABOLOMIC INSIGHTS INTO THE MECHANISMS OF HEPATOTOXICITY TRIGGERED BY AMPHETAMINIC DRUGS

Ana Margarida Araújo<sup>1</sup>, Márcia Carvalho<sup>1,2,3</sup>, Eduarda Fernandes<sup>4</sup>, Maria de Lourdes Bastos<sup>1</sup>, Félix Carvalho<sup>1</sup>, Paula Guedes de Pinho<sup>1</sup>

<sup>1</sup>UCIBIO, REQUIMTE, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal. ana.margarida.c.araujo@gmail.com

<sup>2</sup>FP-ENAS, Fernando Pessoa Energy, Environment and Health Research Unit, University Fernando Pessoa, Praça Nove de Abril, 349, 4249-004, Porto, Portugal

<sup>3</sup>Faculty of Health Sciences, University Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150, Porto, Portugal

<sup>4</sup>UCIBIO, REQUIMTE, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal

Hepatic injury following the recreational use of amphetamine-like drugs has been extensively reported, but the mechanisms underlying liver damage caused by concentrations representative of common use or intoxication scenarios have not been fully elucidated. Therefore, in this work, we used a sensitive untargeted metabolomic approach, based on gas chromatography-mass spectrometry (GC-MS), to identify the early adverse events caused by human relevant concentrations of amphetamine-like drugs. Intracellular metabolome analysis was performed on primary mouse hepatocytes exposed for 24h to two subtoxic concentrations of 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxypyrovalerone (MDPV) or methylone (corresponding to LC01 and LC10, as evaluated by the MTT assay). In order to establish the value of metabolomics to reveal toxicological pathways, traditional toxicological endpoints (i.e. MTT and LDH cell viability, GSH and ATP assays) were also evaluated.



The results obtained by the multivariate analysis showed that all tested amphetaminic derivatives significantly affected the intracellular metabolome of primary hepatic cells, even at concentrations that caused a minimal decrease in cell viability. Most of the altered metabolites belong to the class of amino acids, fatty acids, carbohydrates and carboxylic acids. Although each drug revealed a characteristic metabolic signature, changes in many metabolic pathways were found to be common to all drugs tested, suggesting that these three compounds share, at least in part, the same mechanisms of toxicity. Briefly, the studied amphetaminic derivatives induce significant alterations in energy metabolism, antioxidant defenses, amino acids metabolism and pentose phosphate pathway. The combination of metabolites and their respective levels was unique for each tested drug, being therefore useful in understanding their specific toxicological outcome. Most importantly, our data provide compelling evidence that metabolomics is a suitable and more sensitive approach when compared to conventional toxicological assays, as significant alterations in metabolite levels were already found at the lowest concentration tested. To the best of our knowledge, this is the first study highlighting the potential of the intracellular metabolome analysis to assess the early adverse events of drugs of abuse, providing new insights into their mechanisms of toxicity.

### **Acknowledgements**

A.M.A. thanks Fundação para a Ciência e Tecnologia (FCT) for her PhD fellowship (SFRH/BD/107708/2015). This work was supported by the Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from FCT (UIDP/04378/2020 and UIDB/04378/2020).



JORNADAS IBÉRICAS DE TOXICOLOGIA

