EVALUATING CELLULAR EFFECTS OF BROMACIL ON THE ESTABLISHMENT OF AN PRO-STEATOTIC ENVIROMENT

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INTRODUCTION: Fatty liver diseases are considered serious global public health issue, characterized by excessive fat accumulation in the liver in percentages higher than 5% of liver weight. Those pathologies present different etiologies and may progress to more severe clinical conditions. Worldwide, use of environmental contaminants such as the pesticides used in agriculture affect non-target animals and may cause serious metabolic disruptions. Thus, clarification of details of pesticides effects in metabolic processes of fatty liver are fundamental for biotechnological innovations. OBJECTIVES: The aim of this study is to evaluate metabolic effects of the long-lasting pesticide bromacil in co-culture of hepatic cells to address its potential effects on pro-steatotic environment. MATERIALS AND METHODS: To perform the analyses coculture of hepatic cells (HepG2 and LX-2) were used and they were incubated with different concentrations of bromacil for 48 hours. Cellular viability was evaluated by MTT, trypan blue analyses and LDH measurements. To verify the metabolic effect of the pesticides on cells, biochemical analyses were performed to measure triglyceride (TG), glucose, reactive oxygen species (ROS) and mitochondrial membrane potential ($\Delta\psi$). Gene expression pathways of metabolism were evaluated using qPCRs. RESULTS: Concentrations of 5 nM, 5 μM, and 50 μM bromacil were selected for further analyses based on the initial viability rates measurements by MTT, trypan blue and lactate dehydrogenase assay. Those pesticides concentrations presented viability rates above 80%. Considering the biochemical analyses, 50 μM, TG increased ~ 6.5-fold compared to the levels of metabolite found in control culture, while glucose showed a 166% increase. At the same bromacil concentration, ROS did not present expressive changes in hepatic cells; however, expressive reduction in $\Delta\psi$ in all evaluated concentrations of bromacil were verified, when compared to control. Moreover, gene expression analysis demonstrated frustrated attempts of the cells in the activation of the β-oxidation process considering the increased expression of *ACSL4*, *ACSL5* and *PPAR-α* and reduced expression of *CPT1* and *CPT2*. CONCLUSION: Combined, the results suggested that bromacil induces metabolic changes in hepatic cells, disrupting cellular metabolism, which may cause deleterious environments in cells, favoring the establishment of a pro-steatotic environment in a dose-dependent manner.

Keywords; Alternative cellular model; Fatty liver disease; Metabolism disruption; Pesticide