EVALUATION OF BROMACIL EFFECTS ON LIPID METABOLISM ASSOCIATED WITH FATTY LIVER DISEASES USING 3D CELL CULTURE MODEL AS ALTERNATIVE METHODOLOGY FOR TOXICOLOGICAL APPLICATIONS

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INTRODUCTION: The increasing prevalence of fatty liver diseases also named steatosis, represents a global health challenge. One relevant characteristic is the accumulation of fat in the liver at proportions higher than 5% of liver weight and is attributed to various causes, including pesticide exposure. However, the understanding of the underlying mechanisms remains limited. OBJECTIVE: In this study, we aimed to investigate the deleterious effects of persistent pesticide bromacil on steatosis establishment and progression as metabolic dysfunction and epigenetic mechanisms on a 3D cellular model. MATERIALS AND METHODS: Co-culture of hepatocytes (HepG2) and hepatic stellate cells (LX-2) lineages were seeded at the proportional rates of 7:3 cells on the top of an alginate and agar substrate. The pesticide bromacil was subsequently added at different concentrations, based on the acceptable daily intake (ADI). Cellular viability was assessed by trypan blue and LDH assays, along with biochemical and molecular analyses to quantify energetic metabolites and reactive oxygen species. Moreover, gene expressions were evaluated by qRT-PCR. RESULTS: Different concentrations of Bromacil (5 nM, 5 µM, and 50 µM) revealed effects on energetic metabolism, and general metabolism stress, when compared to the control culture condition. More pronounced results were observed at high doses (50 µM), which suggest more toxic effect if the pesticides are used under no legislation respect. Moreover, the 3D co-culture model proved to be especially sensitive for toxicological analyses related to adverse signaling pathways, whose gene expression of relevant markers of this metabolism as PXR, FXR, AhR, among others, increased significantly compared to the control. Moreover, changes in global DNA methylation profile were also observed, especially at 5µM, with a 36% increase, indicating the importance of epigenetics in modulating cellular homeostasis induced by pesticides. CONCLUSION: The findings suggested the toxic effects of bromacil in establishing pro-steatotic conditions. Moreover, the analyses also demonstrated the efficiency of the 3D model in evaluating the metabolism dysfunction induced by pesticides, which could be useful as predictive toxicological tool for further analyses.

Key Words: 3D cell culture; pesticide energetic and epigenetic mechanisms; predictive toxicological analyses; pro-steatotic cellular model.

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