5-FLUOROURACIL AND THE PRO-STEATOTIC PROCESS IN CELL CULTURE: HEPATOTOXICITY AND IMPACTS ON ENERGY METABOLISM

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INTRODUCTION: Fatty liver diseases also known as steatosis is a global public health concern, characterized by accumulation of fat in the liver equal or higher than 5% of liver weight. These diseases may progress to serious conditions, like fibrosis and hepatocellular carcinoma. The etiologies of steatosis are diverse, including exposure to xenobiotics, such as pharmaceuticals drugs. The chemotherapeutic drug 5-fluorouracil has an uracil analogue structure, and it is used in colorectal and gastrointestinal cancer treatment. Due to its molecular structure, it may disrupt the homeostasis of pyrimidines metabolism when incorporated into RNA, leading to health impairments through different biochemical pathways. OBJECTIVE: Evaluate the effects of 5-fluorouracil in hepatotoxicity, detailing its impact on energetic metabolism, mitochondrial activity and gene expression in hepatic coculture. MATERIALS AND METHODS: To evaluate 5-fluorouracil mechanisms of toxicity, hepatic cellular coculture model was used, using stellate liver cell line, LX-2, and hepatocytes, HepG2. The chemicals were incubated for 48 hours. For these analyses, different concentrations of 5-fluorouracil were added and cellular viability were evaluated by MTT analyses, trypan blue and LDH measurements. Next, triglyceride and glucose production were measured, and fluorescence microscopy evaluated lipid droplets. Gene expression was performed through qPCRs assays, outlining genes related to energetic metabolism, oxidative stress, cellular senescence and mitochondria activity. RESULTS AND CONCLUSION: The MTT, LDH and trypan blue assays demonstrated reduction in cell viability in coculture exposed to 5-

fluorouracil in concentrations of 10 nM, 100 nM, 1μM, 10 μM. For the next assays, concentrations of 100 nM and 1μM were chosen. Triglycerides and glucose assays demonstrated an increase of 600% in both concentrations when compared to the control group. Those results were confirmed by fluorescence microscopy, that showed lipid droplet increase up to 500% when compared to the control group. Alterations in genes related to lipid metabolism and oxidative stress, such as *PGC-1α*, *PPAR*α and *NRF2* revealed modifications in several pathways induced by 5-FU. These results highlighted metabolic changes related to the induction of pro-steatotic condition in cells exposed to 5-fluorouracil, indicating the need to understand the mechanisms involved in the induction of hepatotoxicity in cells treated with the chemotherapeutic.

Keywords: Steatosis; 5-Fluorouracil; Hepatotoxicity

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