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Differential Effects of Lipid and Vitamin D treatment on Cell Viability and CYP24A1 Expression in Hepatocytes and Hepatic Stellate Cells

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Abstract

Activated hepatic stellate cells (HSCs) are a key contributor to liver fibrosis and drive the progression to advanced disease for many liver conditions, including non-alcoholic fatty liver disease. Previous studies suggest vitamin D may reduce inflammatory and pro-fibrogenic activity of HSCs *in vitro*. However, the mechanisms underpinning the effects of vitamin D in HSCs are not fully understood. The overall aim of these experiments was to mimic a lipid loading model on immortalised HSCs to test their responses to $1\alpha,25$ -dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$). Two different human immortalised cell lines: HepG2, hepatocellular carcinoma cells, and LX-2, hepatic stellate cells; were cultured using standard methods. Cell viability in different treatment vehicles (2% DMSO and/or 0.1% ethanol) under serum free conditions was measured by MTT assay after 6 and 24 h. Cells were cultured with increasing concentrations of fatty acids (0–500 μ M, 1:1 oleic acid: palmitic acid) or vitamin D. Nile red, a neutral lipophilic fluorescent dye, was used to measure total intracellular lipid and quantified relative to vehicle. CYP24A1 mRNA expression was measured by qPCR in response to 1000 nM $1\alpha,25(OH)_2D_3$ treatment in both cell lines for 24 h using TaqMan® gene expression assays and normalised to 18S rRNA. Cell viability in response to vehicle was examined at 6 h and 24 h to determine the optimal experimental time points. Whereas, HepG2 cells remained unaffected at 24 h in response to either or both vehicles combined ($n = 4$; combined vehicles, $P = 0.3187$), LX-2 cells showed reduced viability even at 6 h ($n = 5$; combined vehicles, $P = 0.0050$). Fatty acid treatment led to intracellular lipid accumulation in both cell lines. In response to 500 μ M fatty acid treatment, intracellular lipid increased by 1.7-fold in LX-2 cells at 6 h ($n = 5$, $P = 0.00174$) and 3.9-fold in HepG2 cells after 24 h ($n = 4$, $P = 0.00184$). Notably, CYP24A1 mRNA expression was markedly induced by vitamin D treatment in LX-2 cells (136 ± 7.64 -fold, $n = 3$, $P = 0.0010$) in comparison to HepG2 cells (22 ± 0.78 -fold, $n = 3$, $P < 0.0001$). In summary, the cell viability data suggested optimal time points for both fatty acid and vitamin D treatments may be 6 h for LX-2 cells, and 24 h for HepG2 cells. While intracellular lipid accumulation differed between the cell lines in response to fatty acid treatment, both cell lines produced a dose-dependent increase in intracellular lipid. Lastly, CYP24A1 mRNA expression confirmed the responsiveness of both cell types to vitamin D treatment. Ongoing experiments are examining microRNA expression in HSCs in response to both vitamin D and lipid loading.

Conflict of Interest

There is no conflict of interest