*Proceedings of the Nutrition Society* (2016), **75 (OCE3)**, E130 doi:10.1017/S0029665116001452

*Summer Meeting, 11–14 July 2016, New technology in nutrition research and practice*

# Vitamin D reduces hepatic stellate cell proliferation *in vitro*

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Activated hepatic stellate cells (HSCs) are a key contributor to liver fibrosis[(1)] and drive the progression to advanced disease for many liver conditions, including non-alcoholic fatty liver disease. Vitamin D has been shown to have anti-proliferative effects on colorectal cancer cells[(2)]; however less is known about its effects on hepatic stellate cells. The aim of these experiments were to determine *in vitro*: (i) basal protein expression of the vitamin D receptor (VDR); (ii) confirmation of 1α,25$(OH)_2D_3$ (1,25-$VD_3$) phenotypic effect; (iii) the time and dose response to 1,25-$VD_3$ treatment.

Three human immortalised cell lines: HT29, colorectal adenocarcinoma; HepG2, hepatocellular carcinoma, and LX-2, hepatic stellate cells, were cultured using standard methods. Several sources of 1,25-$VD_3$ were assessed. VDR protein expression was analysed by immunoblotting. VDR and CYP24A1 mRNA expression was measured in LX-2 and HepG2 cells at 0, 1, 2, 4, 6, 8, 10, 12 and 24 hour time points after treatment with 10nM of 1,25-$VD_3$. LX-2, HepG2 and HT29 were treated with a range of 1,25-$VD_3$ concentrations (1μg-1 ng) and cell proliferation was measured by clonogenic assay using crystal violet staining.

Untreated LX-2 cells had a higher abundance of VDR protein than HepG2 cells (Figure 1A). Correspondingly, basal VDR mRNA expression was higher in LX-2 in comparison to HepG2 cells ($P < 0{\cdot}0001$). However, mRNA expression for CYP24A1 was much lower in LX-2 compared to HepG2 cells ($P < 0{\cdot}0001$). Treatment with 1,25-$VD_3$ dramatically reduced hepatic stellate cell proliferation; a dose-dependent response was observed with 1μM and 100nM of 1,25-$VD_3$ eliciting a significant reduction in cell colonies ($P = 0{\cdot}0005$ and $P = 0{\cdot}016$ respectively; $n$ 4; Figure 1B) corresponding to 95 % and 82 % fewer colonies. The same trend was seen in HT29 cells ($P = 0{\cdot}053$; $n$ 3; Figure 1C).

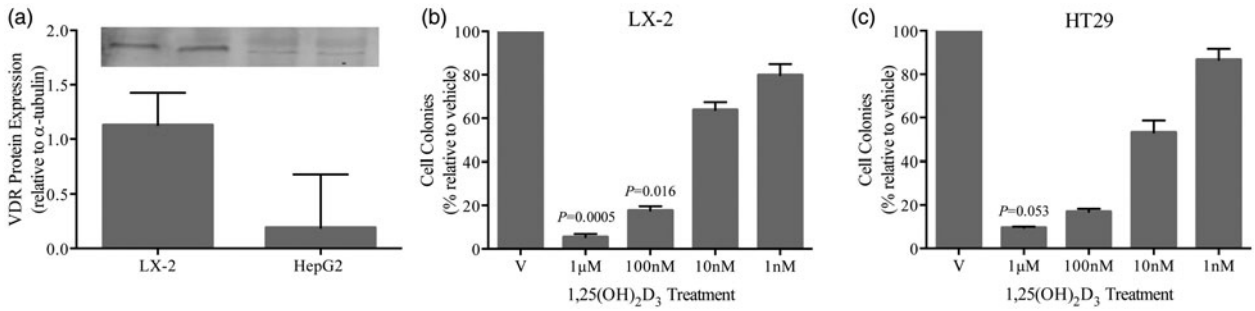


**Fig. 1.** Basal levels of VDR in LX-2 and HepG2 cells (A). Cell proliferation of LX-2 (B) and HT29 (C) cells in response to 1,25-$VD_3$. Data presented as mean + SEM. V: vehicle; VDR: vitamin D receptor.

Unexpectedly, our initial 1,25-$VD_3$ treatment appeared to have no effect on VDR and CYP24A1 mRNA expression. After an alternative vitamin D was sourced, a clear reduction in cell proliferation in response to 1,25-$VD_3$ was observed in both LX-2 and HT29 cells. Future experiments will determine the associated transcriptional response in co-treatment with lipid loading.

1. Moreira RK (2007) *Arch Pathol Lab Med* **131**: 1728–1734
2. Alvarez-Diaz S, Valle N, Ferrer-Mayorga G *et al.* (2012) *Hum Mol Genet* **21** (20): 2157–2165