Evaluating Synergism Between Vitamin D Metabolites and ω-3 Fatty Acids in Ovarian Cancer Cells

Introduction

Ovarian cancer is one of the most lethal cancers affecting women, with an estimated 22,000 newly diagnosed each year. Of those diagnosed, only 15% are detected within the early stages, when the cancer is most treatable. Both preventative and therapeutic measures need to be taken to increase the survival rate of this cancer. Research suggests vitamin D and ω-3 fatty acids have a positive effect on the prevention of multiple cancers, including ovarian cancer. Studies in breast cancer cell lines indicate these molecules act synergistically to inhibit growth of cells. Our research project seeks to elucidate the effects of vitamin D metabolites (Calcidiol and Calcitriol) and the ω-3 fatty acid Docosahexaenoic acid (DHA) alone and in combination on the growth and gene expression of ovarian cancer cells.

Materials & Methods

The effect of these molecules on the growth of the ovarian cancer cell line OVCAR4 was measured by a Sulforhodamine B (SRB) assay. OVCAR4 cells were cultured in RPMI 1640 media supplemented with 10% FBS (Fetal bovine serum), 2mM L-glutamine, and 100 U/ml of penicillin/streptomycin. Cells were stored at 37°C in a humidified incubator containing 5% CO₂.

Experimental Methods

Day 1: OVCAR4 cells were plated a density between 2-5x10⁵ cells/well in 96 well plates.

Day 2: Cells were treated with varying levels of DHA (docosahexaenoic acid), Calcidiol and Calcitriol alone and in combination.

Day 5: Following 72 hour incubation, cells were washed with 1X PBS then fixed to the plate with 20% trichloro acetic acid.

Day 6: Cells were washed with deionized water four times and allowed to air dry.

Day 7: Cells were stained with 0.04% SRB reagent for 30 minutes. After staining, the cells were washed 4 times with 1% acetic acid and allowed to dry.

Day 8: Cells were resuspended in 200 µL of 10 mM Tris buffer at pH 7.6 for 30 minutes. The absorbance of these plates were measured at 505 nm.

Results

Figure 1: Treatment of OVCAR4 cells with DHA demonstrates a dose dependent decrease in relative cell growth. * indicates a significant difference from control treatment (0.1% ethanol) determined by 1-way ANOVA (25 µM DHA P<0.0149 n=9, 50 µM DHA P< 0.0036 n=5, 100 µM DHA P< 0.0001 n=2).

Figure 2: Treatment of OVCAR4 cells with Calcidiol or Calcitriol confers a dose dependent decrease in relative cell growth. * indicates significant difference from control treatment (0.1% ethanol) determined by 1-way ANOVA (2.5 µM Calcidiol P=0.001 n=8, 25 µM Calcidiol P<0.003 n=8, 25 µM Calcitriol P<0.003 n=7).

Figure 3: Cotreatment of OVCAR4 cells with DHA and Calcitriol shows no difference in relative growth compared to control or individual treated cells (n=2).

Figure 4: Cotreatment of OVCAR4 cells with DHA and Calcitriol shows a slight, albeit non-significant decrease in relative growth compared to control and individually treated cells (n=4).

Conclusions

- Individual treatments of DHA, Calcidiol and Calcitriol conferred a dose dependent decrease in relative cell growth of OVCAR4 cells.
- Treatment of OVCAR4 with both DHA and Calcitriol revealed no significant effect on relative cell growth at the concentrations tested.
- Treatment of OVCAR4 with both DHA and Calcidiol demonstrated a potential synergistic effect, although the results were not significant.
- Future studies will investigate the effects of single and combination treatments on gene regulation of downstream targets of vitamin D and DHA signaling pathways using qRT-PCR and Western blotting.
- Future studies will also include testing additional ovarian cancer cell lines to validate the observations.

Literature Cited


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