Elucidation of the Molecular Actions of 1,25 Dihydroxyvitamin D_3 and Docosahexaenoic Acid that May Mediate Cardiovascular Health

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Abstract

Omega 3 polyunsaturated fatty acids (PUFAs), composed of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been demonstrated to be beneficial in primary and secondary cardiovascular disease (CVD) prevention. There is now evidence that vitamin D may be cardioprotective as well, but the mechanism is not fully understood. In the present research, we probed six genes identified both through literature searches and their logical association with proposed 1,25 D_3 and DHA cardioprotective mechanisms. Treating human embryonic kidney cells (HEK293) with 1,25 D_3 and DHA independently and in combination, we demonstrate significant changes in expression of three genes through quantitative real time polymerase chain reaction analysis (qRT-PCR). Nitric oxide synthase 2 (NOS2) showed a significant decrease in expression with 1,25 D_3 alone, DHA alone, and in combination (fold effect 0.84, 0.85, 0.74 respectively, and p<0.04, p<0.01, p<0.01 respectively). Serpin peptidase inhibitor (SERPINE1) exhibited a significant decrease with 1,25 D_3 alone (fold effect 0.78, p<0.01). Thrombomodulin (THBD) demonstrated a significant decrease in expression with DHA alone (fold effect 0.69, p<0.01) and with combination 1,25 D_3 and DHA (fold effect 0.75, p value 0.04). The remainder of the treatment groups for SERPINE1 and THBD, as well as all groups in VEGFA, PDGFA, and EDN1 did not show statistically significant changes in expression in the presence of any of the treatment groups. The altered expression of these genes in response to 1,25 D_3 and/or DHA may partially represent the mechanisms by which cardioprotective benefits are achieved.

Cardiovascular Benefits of DHA

Diets rich in fatty fish or those including polyunsaturated fatty acid (PUFA) supplementation have proven to be cardioprotective, particularly post myocardial infarction (MI), and have little associated side effects. Studies have demonstrated that populations with high intake of dietary PUFA have lower rates of cardiovascular disease (CVD). Other studies demonstrate PUFA supplementation in post MI patients markedly reduces the risk of recurrence and decreases overall mortality. Current guidelines recommend 2 oily fish meals per week, or 1000 mg/day of omega-3 PUFA for those with coronary artery disease. There are multiple mechanisms by which PUFAs are thought to provide these benefits, including inhibiting atherosclerotic plaque formation; inhibition of fast voltage dependent sodium and L type calcium channels, evidenced by the reduction of sudden cardiac death; reduction of serum triglyceride level; improvement in endothelial function; and reduction in platelet aggregation. Thus the role of PUFAs, including DHA, in cardiovascular health has become firmly established and will continue to be an area of active research.

Gene	Gene product and physiological role	Hypothesis when exposed to:		
		+1,25 D_3	+DHA	+1,25 D_3 +DHA
VEGFA (Vascular Endothelial Growth Factor A)	Growth factor signaling molecule Induces angiogenesis in embryos and hypoxic tissues Increases vascular permeability Promotes cellular migration Inhibits apoptosis	++	+	+++
NOS2 (Nitric Oxide Synthase)	Synthesizes nitric oxide Vasodilation Produces nitrous oxide burst as part of the immune defense system	-	-	--
EDN1 (Endothelin 1)	Protein signaling molecule Potent inducer of vasoconstriction	--	--	---
PDGFA (Platelet Derived Growth Factor A)	Growth factor signaling molecule Induces cellular differentiation, particularly in the vascular system	+	+	++
SERPINE1 (Serpin Peptidase Inhibitor, or endothelial plasminogen inhibitor)	Plasminogen activator inhibitor Promotes thrombus formation by inhibiting thrombolysis		--	---
THBD (Thrombomodulin)	Thrombin cofactor Promotes thrombolysis through indirectly activating Protein C	+	++	+++

Table 1: Genes studied, predominant gene product and physiological role, and the hypothesized result when cells are treated with 1,25-dihydroxyvitamin D_3 and/or docosahexaenoic acid (DHA); (+) increased and (-) decreased expression, with a magnitude proportional to number of plus or minus signs

Cardiovascular Benefits of Vitamin D

Vitamin D is a fat soluble hormone acquired through diet and supplementation, as well as photosynthesis in the skin. It is converted to 25-hydroxyvitamin D (25 D_3) in the liver, and finally to 1,25-dihydroxyvitamin D (1,25 D_3) in the kidney. Clinically, vitamin D status is measured via serum levels of 25 D_3. Active 1,25 D_3 exerts its effects through the activation of the nuclear vitamin D receptor (VDR) and transcriptional control of downstream genes, with the most commonly known function being serum calcium and phosphate homeostasis. However, a growing body of evidence suggests that vitamin D may play a role in cardiovascular health. Patients with end stage renal disease suffer chronically low 25 D_3 levels and have 10-20 times the CVD mortality of the general population. Supplementation with 1,25 D_3 or analogs markedly reduces this increase in CVD mortality. Epidemiological evidence correlates low 25 D_3 with increased CVD mortality, and several studies demonstrated reduction in this mortality with vitamin D supplementation. At this point in time, the evidence for cardioprotection by vitamin D is still growing and is promising.

Potential Vitamin D and DHA Synergy

Six potential vitamin D and DHA regulated genes (Table 1) known to play critical roles in the integrity of the CV system were identified through MEDLINE searches. Taking into consideration what is known about the physiological role of each gene and the hypothesis that vitamin D and DHA should prove beneficial to the CV system, individual hypotheses were created for each gene in the form of expected alterations in expression. Given the CVD benefits offered by DHA and the potential benefits of adequate 25 D_3, it is possible the combination of both agents may have a synergistic effect, defined as an effect greater with vitamin D and DHA combination treatment than with the summation of the individual treatments. Clinical studies have already addressed this possibility to some extent and demonstrated no benefit, but the studies were limited to low doses of either DHA or vitamin D. Currently, the VITAL trial (the VITamin D and OmegA 3 triaL) is underway, which uses high does vitamin D and/or DHA or placebo in patients with no CVD history. The study seeks to identify whether the combination of vitamin D and DHA, or each agent alone, reduces CVD, cancer, or stroke. Preliminary results are pending. Regardless, understanding of the cardioprotective actions of both DHA and 1,25 D_3 at the molecular level is very incomplete. This research and its potential impact may provide important clues for future studies.

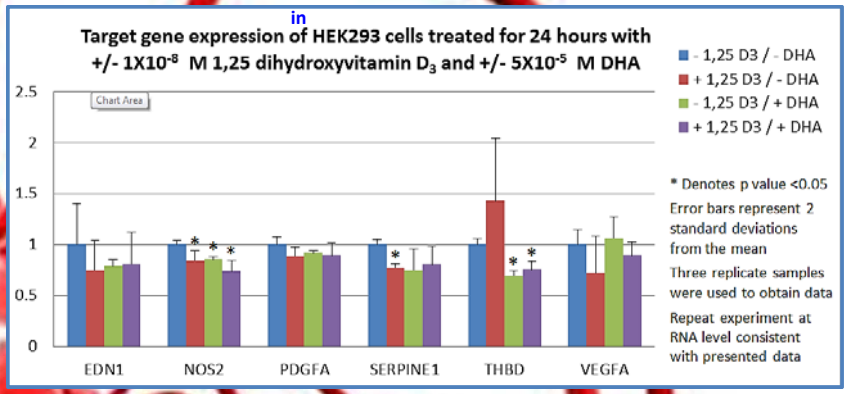

Figure 1: Human HEK293 cells were treated with either ethanol vehicle control, 1X10^{-8} M 1,25 D_3, or 5X10^{-5} M DHA independently, or in combination for a time period of 24 hours. Both reagents were suspended in ethanol of equal volumes. Reverse transcription of 2 mg total RNA was performed using the iSript cDNA Synthesis Kit according to the manufacturer's instructions in a total volume of 40 µL. Quantitative real time polymerase chain reaction (qrt-PCR) was performed using the cDNA product and a SYBR Green Quantitative PCR kit according to the manufacturer's instructions in a total reaction volume of 10 µL. Forward and reverse primers for the genes of interest were either obtained from publications or engineered using IDT Technologies online primer design tools. Expression of all genes was normalized to GAPDH mRNA levels and are reported as fold effect (or decimal fraction) of control mRNA level.

Results

Treatment with ethanol vehicle, 1,25 D_3, DHA, or the combination of both ligands for 24 hours did not appear to alter the morphology or growth rate of the HEK293 cells at any stage of the experiments. Nitric oxide synthase 2 (NOS2) showed a significant decrease in expression (Figure 1) with 1,25 D_3 alone, DHA alone, and in combination (fold effect 0.84, 0.85, 0.74 respectively, and p<0.04, p<0.01, p<0.01 respectively). Serpin peptidase inhibitor (SERPINE1) exhibited a significant decrease (Figure 1) with 1,25 D_3 alone (fold effect 0.78, p<0.01), and an insignificant decrease with DHA alone and combination treatment. Thrombomodulin (THBD) demonstrated a significant decrease in expression (Figure 1) with DHA alone (fold effect 0.69, p<0.01), an insignificant increase with 1,25 D_3 alone, and a significant decrease with combination treatment (fold effect 0.75, p value 0.04). Endothelin 1 (EDN1), platelet derived growth factor alpha (PDGFA), and vascular endothelial growth factor alpha (VEGFA) did not show statistical significance in any of the treatment groups.

Discussion

The decrease in expression of NOS2 is consistent with the hypothesis (Table 1), and is greatest in the combination treatment group, implicating an additive inhibition. NOS2 is expressed predominately in macrophages during the oxidative burst, thus it stands to reason the decreased expression in the presence of both 1,25 D_3 and DHA may attenuate the immune system, serving a protective role through local modulation of inflammatory factors. The additive effect in the combination treatment group is particularly interesting, but falls short of true synergism. Potential mechanisms for this include DHA binding to VDR or RXR resulting in transactivation of downstream genes, or possibly activation of a kinase signaling cascade or other more generalized mechanism.

Discussion - Continued

SERPINE1 showed a robust decrease of expression with the addition of 1,25 D_3, but the results with addition of DHA were not statistically significant. The natural activity of SERPINE1 is to inhibit the activator of plasminogen, thus at high levels it would augment thrombus formation. A decrease in SERPINE1 expression in the presence of 1,25 D_3 should reduce the likelihood of thrombus formation, a key component in the pathophysiology of the majority of MIs and cerebro-vascular events. Unfortunately, statistical significance was lacking in the remainder of SERPINE1 groups. THBD exhibited a significant decrease in both the DHA alone and combination 1,25 D_3 and DHA treatment groups, contrary to the hypothesized result (Table 1). While the explanation for this is not readily obvious, it could be that the demonstrated changes of THBD might be specific to HEK293 cells and may be different in tissues more physiologically similar to the cardiovascular system.

Future Directions

The current insight gained through this research is intriguing. We demonstrated at the molecular level the effects that 1,25 D_3 and DHA have on the expression of genes intimately involved with cardiovascular health. Repeat experiments to increase the statistical significance of the results may be performed in the future. Additionally, time course experiments may help identify genes that respond to treatment more or less quickly than the 24 hour treatments we tested. Finally, repeat experiments in other cell lines derived from actual cardivascular tissues could possibly strengthen the research by demonstrating changes in expression in cells more closely related to the cardiovascular system.