Background - Long-term hyperglycemia leads to macro and microvascular diseases. One of these microvascular diseases is diabetic retinopathy (DR) which results in blindness in patients during working age. Retina has highest O₂ uptake and glucose oxidation. Prolonged high glucose concentration provokes other alternative glucose pathways leading to accumulation of several metabolic end-products which induce oxidative stress in retina causing cellular changes of the expression of pro and inflammatory proteins. These pro and inflammatory proteins include the expression of interleukins 1-β, 6, 8, 18 and 33 and their cognate receptors and other proteins such as tumor necrosis factor-α (TNF-α). Recent studies have shown that the active form of vitamin D 1, α-25(OH)D₃, calcitriol, can protect pancreatic beta cells, improve insulin peripheral sensitivity, modulating immunologic response and protect cells from oxidative stress and apoptosis. Thus, this study aims to investigate the potential effect of calcitriol on immunological responses during the progression of DR and underlying antioxidant mechanism(s) during increased oxidative events in diabetes.

Method - ARPE-19 cells were treated with 750 µM H₂O₂, 5 and 25 mM glucose and 50 nM calcitriol or normal cell culture media or 0.1% ethanol for 48 hours in standard cell line culture conditions.

Results

Discussion

Gene expression analysis using QPCR of the selected candidate cytokines followed induced oxidative stress in ARPE-19 cell line challenged with 750 µM H₂O₂ and 5 and 25 mM glucose for 48 hours have showed that IL-33 mRNA expression was significantly suppressed (figure 1) compared to untreated cells and cells treated with 5 mM glucose. However, the IL-33 mRNA expression was markedly increased after combined treatment with 50 nM calcitriol for 48 hours compared to cells treated with either 50 nM calcitriol or 0.1% ethanol. Expression of ST2 receptor was increased significantly following H₂O₂ and 25 mM glucose for 48 hours compared to control cells. The ST2 mRNA level was significantly elevated after 50 nM calcitriol treatment compared to cells treated with 0.1% ethanol or 50 nM calcitriol alone; yet calcitriol has no effect on ST2 expression alone compared to vehicle. Although recent studies have proposed that IL-33 has potential regulatory functions in inflammatory response during aged-related macular degeneration (AMD); the mechanism in which IL-33 modulates such process remain unclear. It has been suggested that the up-regulation of IL-33 expression in AMD could be through the activation of extracellular-signal-regulated kinases (ERK1/2) and P38 MAPK. Current data show that IL-33 mRNA expression was highly induced after calcitriol treatment which may propose regulatory effect of calcitriol on MEK/ERK1/2 and P38 MAPK pathways or may suggest other up-regulatory pathway of IL-33 expression during inflammatory response.

Several studies have revealed that chronic hyperglycemia can potentially increase the level of some pro and inflammatory cytokines in diabetic patients. Present data, as shown in (figures 3, 4, 5, 6, 7 and 7), demonstrate similar finding when ARPE-19 cells treated with high concentration of glucose. Such increase in these molecules provokes inflammatory events in retina and pigmented epithelial cells which result in disrupting blood retina barrier (BRB) leading to retina detachment. Our data have shown that calcitriol can effectively suppress the expression of interleukins 1-β, 6, 8, 18 and TNF-α in ARPE-19 cells treated with H₂O₂ and 25 mM glucose. This finding is concuring with other studies suggesting protective and antioxidant mechanism of vitamin D during oxidative stress.

Conclusion

1. The expression of IL-33 and ST2 during oxidative stress events in ARPE-19 cells suggest protective mechanism of IL-33 in hyperglycemic environment.
2. Increasing in the IL-33 and ST2 mRNA levels after calcitriol treatment proposed a regulatory effect of the active form of vitamin D in IL-33 pathway during hyperglycemic oxidative stress
3. Suppression of TNF-α, IL-1β, IL-6, IL-8 and IL-18 after calcitriol treatment propose a potential therapeutic function of 1-α-25-dihydroxyvitamin D₃

Future WORK

1. Effect of 1, 25(OH)D₃ treatment on the expression of inflammatory proteins in DR using different MAPKs pathways inhibitors (MEK/ERK1/2, ERK5, P38 MAPK and c-JNK)
2. Possible effect of vitamin D on the expression antioxidant enzymes and molecules (CAT and SOD1&2 and GPX1) during DR

References

4. Liu, X.C. X.P Liu, C.X Han, C.J Li and S.H He. 2012. IL-33 is induced by anisyl-β-glucosaminide and regulates inflammatory cytokine production in retinal pigmented epithelial cell.

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The Regulatory Effect of 1, α, 25-dihydroxyvitamin D₃ on the Expression of Inflammatory Cytokines in Diabetic Retinopathy

Ali M Tohari*, John A Craft and Xinhua Shu

Department of Life Sciences, Glasgow Caledonian University, Glasgow G4 0BA UK

ali.tohari@gcu.ac.uk

P < 0.05 **, P < 0.01 ***, P < 0.001 ***