Evidence that Marine-derived, Multi-mineral, Aquamin Inhibits the NF-κB Signaling Pathway
In Vitro

INTRODUCTION

Nuclear factor kappa B (NF-κB) is the master regulator of the immune response, its dysregulation has been shown to contribute to the pathogenesis of a wide range of autoimmune and inflammatory diseases (D’Acquisto et al., 2002). Recent reports suggest that NF-κB is a promising target for the anti-inflammatory activity of many natural compounds including flavonoids, curcumin, green tea polyphenols, resveratrol and grape seed procyanidins (Luqman and Pezzuto, 2010). NF-κB also controls the expression of a number of key pro-inflammatory genes including cyclo-oxygenase-2 (COX2). COX2 is a well-established target for non-steroidal anti-inflammatory drugs (NSAIDs) frequently employed to provide relief from inflammation and pain.

Growing evidence suggests that mineral-rich seaweed extracts may play an important role in the regulation of inflammation (Smit, 2004). The food supplement Aquamin is a natural, seaweed-derived, multi-mineral rich in calcium, magnesium and 72 other trace minerals. Aquamin has been shown previously to provide relief from the symptoms of osteoarthritis (Frestedt et al., 2008, 2009) and to be of benefit in bone (Aslam et al., 2010a) and digestive health (Aslam et al., 2010b). It has also been shown recently that Aquamin attenuates lipopolysaccharide (LPS)-induced tumour necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β) secretion in vitro (Ryan et al., 2011).

This study aims to elucidate whether Aquamin can inhibit the NFκB signalling pathway and the expression of the downstream pro-inflammatory gene, COX2, following LPS-stimulation in macrophages.

MATERIALS AND METHODS

Aquamin (GRAS 000028) is a natural product obtained from the skeletal remains of the red marine algae Lithothamnion corallioides harvested under approved licence off the coasts of Ireland and Iceland and prepared under ISO and HACCP certification. Aquamin contains 12% calcium, 1% magnesium and measurable levels of 72 other trace minerals as previously reported (Ryan et al., 2011). Approximately 0.5 mg/mL of Aquamin is equivalent to a physiological level of extracellular Ca²⁺.

Lipopolysaccharide from E. coli (O55:B5) was purchased from Sigma (St Louis, MO). The murine macrophage cell line RAW 264.7 was cultured in Dulbeco’s modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS). The NF-κB luciferase reporter plasmid and the pRLTK plasmid were purchased from Promega (Madison, WI). The RAW cells were transfected with reporter constructs using Turbofect (Fermentas). The luciferase activities of the whole cell lysates were analysed using a dual-luciferase reporter assay system as described previously (Dyer et al., 2000). Co-transfection of the Renilla luciferase expression vector pRL-TK (Promega) was used as an internal control for all reporter assays. For all samples, the data were normalized for transfection efficiency by dividing firefly luciferase activity by that of the Renilla luciferase.
To prepare whole cell lysates, the cells were lysed in RIPA buffer containing 50 mM Tris-HCl pH 7.4, 1% NP40, 0.25% deoxycholate, 1.50 mM NaCl, 1 mM EDTA, 1 mM sodium orthovanadate and 1x Complete Protease Inhibitors (Roche). Equal quantities of whole cell lysates were resolved by electrophoresis on a denaturing SDS polyacrylamide gel and transferred to a nitrocellulose membrane. Following immunoblotting with DyLight conjugated secondary antibodies (Pierce), the membrane was analysed using an Odyssey infra red scanner (LiCor). The antibodies used for immunoblotting were anti-IκBα, anti-phospho-IκBα (Cell Signalling Technologies) and anti-β-actin antibody (Sigma).

For real time PCR analysis cells were lysed in Side-step lysis buffer (Stratagene) and cDNA synthesized using the Transcriptor cDNA synthesis kit (Roche). Real time PCR was performed using primers for COX2 and the Universal Probe Library system (Roche) and analysed on a Light Cycler 480 (Roche).

RESULTS AND DISCUSSION

The effect of Aquamin on NF-κB transcriptional activity was analysed using a NF-κB luciferase reporter plasmid in the RAW 264.7 macrophage cell line. NF-κB transcriptional activity was induced by stimulating cells with LPS, a well characterized trigger of inflammation and activator of NF-κB. As demonstrated in Fig. 1A, the RAW264.7 cells exhibited a robust NF-κB transcriptional response when compared with unstimulated control cells. Cells that were pretreated with Aquamin (0.5 mg/mL) for 1 h prior to LPS stimulation exhibited a clear reduction in the level of NF-κB transcriptional activity when compared with untreated cells (p < 0.05). This demonstrates that Aquamin is an inhibitor of NF-κB activity in LPS treated macrophages.

NF-κB transcriptional activity is inducible, and under normal conditions is tightly regulated. In unstimulated cells NF-κB is maintained in the cytoplasm through interaction with the inhibitory protein, inhibitor of kappa B alpha (IκBα). In stimulated cells, IκBα is phosphorylated by the upstream IκB kinase (IKK) kinase complex. Phosphorylation of IκBα triggers its ubiquitination and proteasomal degradation. In the absence of IkBα, NF-κB is free to translocate to the nucleus where it may bind its cognate binding sites in the promoter regions of target genes and activate transcription. Thus, the phosphorylation and degradation of IκBα is a key event in the activation of NF-κB transcriptional activity. This paper analysed the effect of Aquamin treatment on the ability of LPS to induce the phosphorylation and degradation of IκBα to gain further insight into the mechanism of NF-κB inhibition. As Fig. 1B shows, pretreatment of cells with Aquamin (0.5 mg/mL) for 1 h prior to LPS stimulation resulted in a significant inhibition in the phosphorylation and degradation of IκBα when compared with the untreated cells. This indicates that Aquamin is acting at or upstream of the IKK kinase complex in the NF-κB pathway.

The study next sought to determine the effect of Aquamin on the expression of COX2 in LPS stimulated RAW 264.7 macrophages to further characterize its anti-inflammatory properties. Real time PCR analysis of the LPS-stimulated cells demonstrated a significant inhibition of COX2 expression in cells pretreated with Aquamin (0.5 mg/mL) for 1 h prior to stimulation relative to the untreated cells (Fig. 2). These data suggest that the previously observed anti-inflammatory properties of Aquamin may result from reduced NF-κB activation and transcription of target genes such as COX2.

The naturally occurring food supplement, Aquamin, is rich in calcium and magnesium as well as trace amounts of other minerals. Many of these minerals including magnesium, copper, manganese, selenium and zinc are now recognized as important regulators of inflammation. Magnesium may influence inflammation through reducing the serum level of the pro-inflammatory protein C-reactive protein. Copper, zinc and manganese are essential cofactors of the antioxidant enzyme superoxide dismutase. Selenium is a vital constituent of glutathione peroxidase. Consequently, many of the antioxidant-related minerals that compose Aquamin may be anti-inflammatory in nature.

This study has elucidated a role for Aquamin in regulating NF-κB and COX2 activity in LPS-stimulated murine macrophages. These findings suggest that in
addition to Aquamin-mediated inhibition of IL-1β and TNFα (Ryan et al., 2011), inhibition of NF-κB and COX2 may be an additional mechanism by which Aquamin exerts its anti-inflammatory effects. Further studies are needed in animals and humans to confirm these in vitro findings.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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