PHYTOTHERAPY RESEARCH
Phytother. Res. **25**: 765–767 (2011)
Published online 26 October 2010 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.3309

SHORT COMMUNICATION

Evidence that the Marine-derived Multi-mineral Aquamin has Anti-inflammatory Effects on Cortical Glial-enriched Cultures

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It is well established that neuroinflammation contributes to brain aging, and that cortical cells are particularly vulnerable. Lipopolysaccharide stimulates the release of the pro-inflammatory cytokines, tumor necrosis factor-alpha and interleukin-1beta from glial cells which consequently induces an impairment in neuronal cell function. The food supplement, Aquamin, is a natural, multi-mineral derived from the red algae *Lithothamnion corallioides*, rich in calcium, magnesium and 72 other trace minerals. The aim of this study was to evaluate the anti-inflammatory potential of Aquamin in lipopolysaccharide-stimulated, glial-enriched primary cultures of rat cortex. It is reported that Aquamin prevented lipopolysaccharide-induced secretion of tumor necrosis factor-alpha and interleukin-1beta from cortical glia. These data suggest that nutritional supplements such as Aquamin may play an important role in impeding the detrimental effects of excessive inflammation in the brain.

Keywords: inflammation; Aquamin; glia; TNFα; IL-1β; brain.

INTRODUCTION

Minerals such as magnesium, copper, zinc, manganese and selenium are now recognized as important regulators of inflammation, and growing evidence suggests that mineral-rich seaweed extracts may play an important role in the regulation of inflammation (Granert *et al*., 1999; Jung *et al*., 2007). The food supplement, Aquamin, is a natural seaweed-derived multi-mineral from the red algae *Lithothamnion corallioides* which is rich in calcium, magnesium and trace amounts of other minerals (Table 1). It has recently been shown to provide relief from the symptoms of osteoarthritis (Frestedt *et al*., 2009) and to be of benefit in digestive and bone health (Aslam *et al*., 2010a, 2010b).

It is well established that inflammation contributes to cortical neuronal dysfunction in age-related neurological diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis and Parkinson's disease (Minghetti, 2005). Microglia are considered to be the resident immune cells of the brain and, along with astrocytes, constitute the major glial cell types. Under conditions such as neurodegenerative disease, injury, exposure to environmental toxins, infection or age, microglia become activated to release large numbers of mediators such as cytokines and free radicals. Microglial-secreted pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) can influence neurons and their ability to process information and can ultimately contribute to neuronal cell death. Astrocytes provide structural and metabolic support to neurons and also play a role in brain inflammation through antigen presentation and cytokine secretion. It has previously been reported that exposure to the endotoxin lipopolysaccharide (LPS) induces release of IL-1β and TNFα from cortical glia to contribute to neuronal degeneration (Long-Smith *et al*., 2010). The present study was designed to investigate the anti-inflammatory potential of Aquamin in LPS-stimulated, glial-enriched rat cortical cultures to determine if nutritional supplements, such as Aquamin, may play a role in impeding the detrimental effects of excessive inflammation in the brain.

MATERIALS AND METHODS

Aquamin (Food and Drug Administration (FDA) GRAS 000028) was prepared from the mineral-rich red marine algae, *Lithothamnion corallioides*, harvested off the Atlantic coasts of Ireland and Iceland under approved licenses. The calcified seaweed was separated from extraneous materials, sterilized, dried and milled under ISO and HACCP certification. Approximately 0.5 mg/mL of Aquamin is equivalent to a physiological level of extracellular Ca^{2+}. The concentrations of Aquamin were determined from previous experiments using low passage human dermal fibroblasts, calcium-sensitive and calcium-resistant colon carcinoma cell lines (CBS, Moser, Fet, HCT-116 and SW480) (Aslam *et al*., 2009), the murine macrophage cell line RAW 264.7 and the pre-osteoblastic cell line MC3T3-E1 (unpublished data). Aquamin demonstrated no signs of

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Received 03 June 2010
Revised 18 August 2010
Accepted 07 September 2010

Table 1. Typical mineral composition of Aquamin

Mineral	Dry salt weight (ppm)
Calcium	141 200
Magnesium	18 580
Phosphorus	436
Potassium	81.5
Sulphur	3 620
Iron	234
Boron	8.45
Sodium	1 780
Manganese	9.71
Cobalt	<0.05
Copper	0.191
Zinc	10.7
Selenium	1.75

toxicity at any of the concentrations used in the current study. Glial-enriched cultures were prepared from cortical tissue isolated from seven separate postnatal day 2 Sprague-Dawley rat pups (Biological Services Unit, University College Cork) as described previously (Long-Smith *et al.*, 2010). All scientific procedures were performed under a licence issued by the Department of Health and Children (Ireland) and in accordance with the European Communities Council Directive (86/609/EEC). After 7–10 days *in vitro* (DIV), glia were incubated in the presence or absence of LPS (50 ng/mL) and Aquamin (0.05, 0.1, 0.5, 1 and 2 mg/mL). Twenty-four hours later, supernatant was removed for analysis of TNFα and IL-1β by ELISA (R&D Systems, UK). Untreated culture media was used as a control. The cells were assessed for morphological signs of apoptosis and necrosis (loss of cell membrane asymmetry and attachment, cell shrinkage, cell blebbing, nuclear fragmentation and chromatin condensation) prior to, during and after 24 h incubation with Aquamin. All cells were considered viable throughout the experiment. ANOVA with *post hoc* Student Newman-Keuls was used to determine which conditions were significantly different from each other. The results were expressed as means with standard error of the mean (SEM) and deemed significant when $p < 0.05$.

RESULTS AND DISCUSSION

The data presented demonstrate that LPS induced a significant increase in TNFα (Fig. 1A) and IL-1β (Fig. 1B) secretion from glial-enriched cortical cultures 24 h after LPS treatment ($p < 0.001$). Treatment with all doses of Aquamin significantly attenuated the LPS-induced increase in TNFα and IL-1β secretion ($p < 0.01$). Analysis of extracellular concentrations of TNFα revealed that Aquamin prevented its release from LPS-stimulated cortical glial cells in a dose-dependent manner, and that all doses of Aquamin significantly attenuated the LPS-induced release of IL-1β. These data suggest that the anti-inflammatory effects of Aquamin may be reliant on the blockade of the pro-inflammatory effects of both TNFα and IL-1β.

Seaweed extracts have been reported previously to inhibit pro-inflammatory cytokine release. Fucoidans

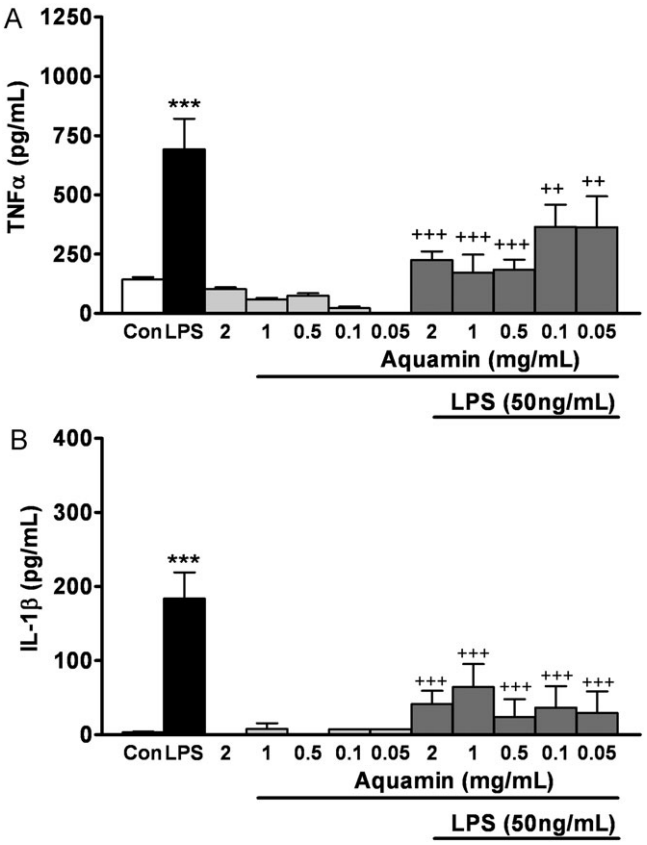

Figure 1. Aquamin prevents LPS-induced release of TNFα and IL-1β. TNFα (A) and IL-1β (B) levels from LPS-stimulated glial-enriched cortical cultures. Data are expressed mean ± SEM of seven independent experiments, each performed in triplicate. *** $p < 0.001$ vs control; ++ $p < 0.01$; +++ $p < 0.001$ vs LPS (ANOVA).

from brown algae have been shown to inhibit TNF-α and IL-1 production in cerebrospinal fluid in a rabbit meningitis model (Granert *et al.*, 1999), and an extract from the seaweed *Hizikia fusiform* reduced TNFα production in LPS-stimulated murine microglial cells (Jung *et al.*, 2007). Aquamin is a seaweed-derived multi-mineral rich in calcium and magnesium. It has been shown that IL-1β reduced Ca^{2+} channel activity and influx in rat cortical neurons (MacManus *et al.*, 2000) suggesting that Ca^{2+} supplementation may be beneficial in situations of excessive IL-1β concentration in the brain such as occurs with aging. Increased magnesium in the diet may influence inflammation through reducing the serum level of the pro-inflammatory C-reactive protein. Copper, zinc and manganese are essential cofactors of the antioxidant enzyme superoxide dismutase, and selenium is a vital constituent of the antioxidant glutathione peroxidase. A compromised antioxidant defense has been observed in the cortex of aged rats in parallel with increased IL-1β concentration (McGahon *et al.*, 1999). Accumulation of reactive oxygen species in the rat cortex and hippocampus has been demonstrated after LPS treatment (Nolan *et al.*, 2003) while an antioxidant-enriched diet has been shown to reverse age-related and inflammatory-induced neuronal deficits (McGahon *et al.*, 1999). Consequently, many of the antioxidant-related minerals that compose Aquamin

may be anti-inflammatory and may directly or indirectly have neuroprotective properties.

The current study provides clear evidence that Aquamin exerts anti-inflammatory effects by preventing LPS-induced TNFα and IL-1β release from rat cortical glia. This evidence for an anti-neuroinflammatory activity of the food supplement Aquamin may have significant implications for brain health.

Acknowledgement

This work was funded by Marigot Ltd.

Conflict of Interest

The authors have declared that there are no conflicts of interest.

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