

The Marine-derived, Multi-mineral formula, AquaPT Reduces TNF- α Levels in Osteoarthritis Patients

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Received: March 19, 2014; Accepted: May 26, 2014; Published: May 28, 2014

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Abstract

Osteoarthritis (OA) is a common degenerative disease of the joints. Current anti-inflammatory treatment strategies for OA, such as non-steroidal anti-inflammatory drugs (NSAIDs), are effective for symptom relief but are associated with adverse side effects including gastrointestinal and cardiovascular complications during long term use. Thus, alternative treatments for the disease are necessary to supplement current treatment options. The nutraceutical, Aquamin, is a seaweed-derived, multi-mineral supplement that has proven promising in ameliorating the symptoms of moderate to severe OA, potentially through blockade of pro-inflammatory signalling pathways and cytokines. The aim of this study was to evaluate the effect of Aquamin or Aquamin supplemented with green tea and pine bark extract (AquaPT) on inflammatory biomarker levels in the blood of OA patients. OA subjects received Aquamin or AquaPT for 6 weeks. The impact of the treatment on Western Ontario and McMaster Universities (WOMAC) OA index scores and serum tumour necrosis factor-alpha (TNF- α) levels were investigated. While no significant differences in WOMAC scores were evident post treatment, the AquaPT-treated subjects had reduced serum TNF- α levels. These data suggest that the addition of green tea and pine bark extract to Aquamin gives the seaweed-derived supplement a detectable anti-inflammatory effect.

Keywords: Osteoarthritis; Immune system; Humans; Dietary supplement; Seaweed

Background

Osteoarthritis (OA) results from the breakdown of cartilage in joints over time, leading to joint pain and damage. It is the leading cause of chronic disability in the United States and affects about eight million people in the United Kingdom. Symptoms and effects include joint stiffness, swelling and pain, loss or restriction of joint mobility and joint deformity. Treatment for OA generally involves a combination of exercise, lifestyle modification and analgesics. Current anti-inflammatory treatments, while providing some relief from symptoms, are suboptimal and the side effects of these treatments, in particular the COX-2 specific

non-steroidal anti-inflammatory drugs (NSAIDs), are becoming increasingly recognized [1,2]. Thus, use of alternative treatments and complementary medicines is gaining popularity among OA sufferers.

Aquamin is a multi-mineral nutritional supplement derived from the *Lithothamnion* species of red algae and is rich in calcium, magnesium and 72 other trace minerals including zinc, iron and selenium [3]. Previously, Aquamin treatment reduced the symptoms of moderate to severe OA of the knee, significantly improving walking distances and Western Ontario and McMaster Universities (WOMAC) scores for pain, stiffness and activity over the course of a 12-week treatment [4]. In a similar study, Aquamin improved range of motion and walking distances in OA subjects who had reduced their intake of NSAIDs by 50% [3]. Recent *in vitro* studies suggest that the beneficial effect of Aquamin occurs through regulation of the inflammatory cytokines tumour necrosis factor alpha (TNF- α) and interleukin 1 beta (IL-1 β) [5] and the NF- κ B/COX2 signaling pathways [6]. The objective of the present study was to determine whether Aquamin, on its own and in a formulation supplemented with the green tea extract Suphenon and the pine bark extract Enzogenol (AquaPT), both of which have proven health benefits [7,8], could modulate inflammatory biomarkers in the blood of osteoarthritic and healthy subjects.

Materials and Methods

Study design

This was a randomized double-blinded within subject study with two treatment groups: OA + Aquamin (n=12) and OA + AquaPT (n=12). A total of 24 female subjects with moderate to severe OA (aged between 18 and 65) were enrolled. The study was conducted in accordance with the ICH Guidelines on Good Clinical Practice and the declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Treatment

For 6 consecutive weeks, the subjects with OA received either Aquamin (Food and Drug Administration (FDA) Generally recognized as safe (GRAS) 000028) (Marigot Ltd, Cork, Ireland) or Aquamin supplemented with 2% Enzogenol (Enzo Nutraceuticals, New Zealand), a pine bark extract, and 8% Sunphenon (Taiyo Kagaku Co. Japan), a green tea extract (AquaPT). The daily dose of 4 capsules of Aquamin provided 800mg calcium, (the European Union (EU) recommended dietary allowance (RDA) for calcium) and 74 mg of Magnesium (EU RDA 375mg). The daily dose of 4 capsules of AquaPT provided 720mg calcium, 200mg green tea (polyphenols) and 50mg pine bark extract. Study participation involved 3 study visits over a period of 8 weeks.

Study measurements and statistical analyses

At the first visit, subjects were screened for symptoms and effects of OA, such as joint swelling, joint tenderness and decreased range of motion in joints, visible joint damage (i.e bony growths) and crepitus. Once deemed eligible, upon review of a number of inclusion and exclusion criteria, subjects returned for a second visit (WK 0) and received a six-week supply of Aquamin or AquaPT along with food diaries. They were required to come off all NSAIDs, steroids, probiotics and vitamin supplements for 2 weeks prior to study entry and for the 6 week duration of the study and returned for their final visit at week 6 (WK 6).

At each visit, WOMAC scores for pain, stiffness and difficulty in performing daily activities were assessed. In addition, the subjects were queried about changes in their medications and any adverse events were recorded. One subject from the OA + Aquamin group was removed from the study due to taking medications prohibited in the study criteria. These effects were not treatment-related.

On WK 0 and WK 6, blood from subjects was collected in EDTA vacutainers, centrifuged @ 1200 *g* for 10 min and the serum was aspirated and stored at -80°C. Serum TNF- α levels were measured using a Meso Scale Discovery (MSD) assay kit and the electrochemiluminescent multiplex system Sector 2400 imager (Meso Scale Discovery, Gaithersburg, MD, USA), as per the manufacturer's instructions. All statistical tests were performed using commercially available statistic software (GraphPad Software, San Diego, CA, USA). Data are represented by mean \pm standard error of the mean (SEM). P values of < 0.05 were considered significant.

Results and Discussion

The data presented here demonstrate that treatment with the green tea and pine bark extract-supplemented formulation of Aquamin (AquaPT) significantly reduced basal serum levels of the pro-inflammatory cytokine TNF- α in patients with moderate to severe OA ($P < 0.05$) (Figure 1). Serum TNF- α levels in those that received Aquamin on its own were decreased by 23.6%, but this reduction, whilst trending towards significance was not significant (Figure 1). Both *in vitro* and *in vivo* data from OA studies support the role of TNF- α in disrupting the normal

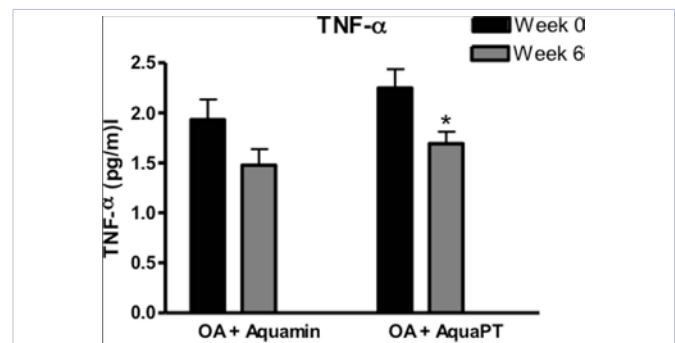


Figure 1: Effect of six week treatments with Aquamin or AquaPT on serum TNF-alpha levels in patients with OA. Data are expressed as mean \pm the standard error of the mean for 11-12 patients per group. The significance of differences between week 6 and week 0 was determined by two way anova with repeated measures and post hoc analysis (* $p < 0.05$).

biochemical metabolism of synovial joint tissues [9]. In addition, evidence from a canine model of OA suggests that both TNF- α and its receptors play a role in early development of the disease [10]. Two studies have demonstrated the anti-inflammatory effects of Aquamin *in vitro* [5, 6]. However, the present study is the first to demonstrate such an effect in human subjects with OA. Of note, the levels of TNF- α detectable in the sera of OA patients were overall very low. This phenomenon has been previously reported in a study comparing rheumatoid arthritis and OA, where cytokines were only detectable in the synovial fluid of OA patients [11]. Despite trending towards significance, no significant differences in WOMAC scores were evident post treatment, seven of the eleven OA subjects that received Aquamin and seven of the twelve that received AquaPT had improved WOMAC composite scores for pain, stiffness and activity post treatment (Figure 2). More specifically, out of the eleven subjects that received Aquamin; eight felt less pain, six felt less stiff and six felt an improvement in performing daily activities (Figure 2). Similarly, out of the twelve subjects that received AquaPT; nine felt less pain, six felt less stiff and eight felt an improvement in performing daily activities (Figure 2). These results are in agreement with previous reports that Aquamin reduces the symptoms of OA in humans [4] and reduces TNF- α levels *in vitro* [5]. Additionally, this is the first time that both of these parameters have been investigated in a single study. The potential limitations of this study include the relatively small sample size and short duration of treatment (6 weeks), however similar effects in terms of WOMAC scores have been observed in previous studies with Aquamin conducted over a 12-week period. However, it is important to note the difficulty in recruiting OA sufferers willing to come off all analgesics for a pro-longed period of time. The 'active ingredient' for Aquamin is not known since a number of the minerals present in the complex may be having anti-inflammatory effects. The prominent molecule calcium (daily dosage providing 98-100% RDA) ameliorated pain symptoms in OA patients [12]. Furthermore with regards AquaPT, Sunphenon prevented disease in a rat model of colorectal cancer [7], while Enzogenol reduced a number of risk factors associated with cardiovascular disease [8].

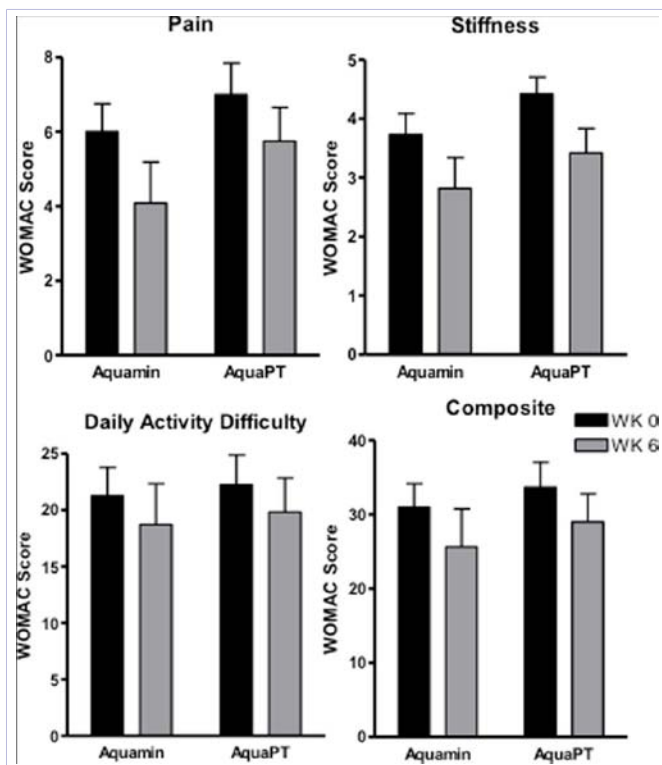


Figure 2: Effect of six week treatments with Aquamin or AquaPT on WOMAC scores in patients with OA. Reduction in WOMAC scores trended towards significance in patients with OA following six weeks treatment with AquaCal and AquaPT. Data are expressed as mean \pm the standard error of the mean for 11-12 patients per group.

Conclusion

This study provides initial evidence for the potential anti-inflammatory effects of AquaPT *in vivo* and confirms previous findings showing trends of improved pain, stiffness and activity scores with increased supply of minerals from Aquamin in patients with moderate to severe OA. These data suggest that supplementation of the seaweed-derived formula Aquamin with green tea and pine bark extract enhances the nutraceutical's beneficial and anti-inflammatory effect in OA. Finding alternative treatments for OA are merited due to the undesirable effects associated with current therapies for the disease. Our findings warrant further mechanistic studies to identify the specific immunomodulatory effects of AquaPT and further appropriately controlled clinical studies with larger subject groups conducted over longer time periods to support the interesting observations made in this study.

Acknowledgement

Materials for this project were funded by Marigot Ltd. The authors received no financial contribution and report no conflicts of interest. The authors wish to thank Matt Frevel, of Enzo Nutraceuticals Ltd. for helpful discussions and suggestions regarding this study. The authors thank Ann O'Neill, Research Nurse, Alimentary Pharmabiotic Centre, without whose help the study could not have been completed.

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