Short Communication

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Preliminary Insights into the Inflammation-Resolving Effect of OmeGo, An Enzymatically Liberated Fish Oil, Compared to Diclofenac in A Rat Paw Edema Injection Model

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ABSTRACT

Whilst inflammation is a natural response to injury and wear and tear on the body the associated pain and swelling are clearly problematic for the sufferer. Marin polyunsaturated fatty acids, including omega-3, are known to have inflammation-resolving effects and our previous work has shown the minimally processed whole fish oil, OmeGo, to significantly reduce type 2, allergic inflammation. As a next step, this preliminary study used a standard carrageenan-induced rat paw swelling model of (type 1) acute inflammation to assess whether OmeGo could alleviate the paw swelling. Over the 24-hour period of the study, OmeGo showed a similar impact on paw swelling as active control, the non-steroidal anti-inflammatory drug, diclofenac. Diclofenac also markedly reduced the inflammatory cytokines IL-1 and IL-6 whereas OmeGo did not. This suggests that OmeGo's effects occur through different pathways compared to NSIAD therapy. Further work is planned to further characterise OmeGo's modulation of inflammatory pathways and its potential as a pragmatic approach to help resolve acute inflammation.

Introduction

Inflammation is a natural response to tissue injury to initiate tissue repair and regeneration [1]. However, inflammation has adverse consequences, including pain and swelling, and if not resolved in a timely manner, potential negative consequences to health [2-4]. Fish oil and Omega-3 fatty acids have been shown to help the body resolve inflammation via their metabolism into specialised pro-resolving mediators (SPMs) [5,6]. These mediators have been noted to provide clinically important reductions in inflammation in several conditions, including cardiovascular and inflammatory lung disease, as well as gynecological disease [7-12]. Cortico-steroids and Non-Steroidal Anti-Inflammatory Drugs (NSAID) are used extensively in the management of inflammatory conditions and pain. However, their prolonged is associated with significant side effects, including osteoporosis, diabetes, gastrointestinal ulceration and bleeding and increased cardiovascular risk [13,14]. OmeGo is an enzymatically liberated salmon oil which contains all 21 omegas and fatty acids present in the oil fraction of whole fish. Our previous work has shown OmeGo to have antioxidant and anti-inflammatory properties enabling the reduction in oxidised LDL in healthy human subjects and the attenuation of allergic inflammation in preclinical models [15-18]. The antioxidant action of the oil alone could reasonably be expected to also reduce type 1 inflammation. We therefore undertook a preliminary rat paw injection model to assess OmeGo's impact on the resulting inflammation compared to diclofenac.

Materials and Methods

The study was conducted according to GLP guidelines and in accordance with the rules and regulations of India where the study was performed. All the experimental procedures were done following the guidelines of the Institutional Animals Ethics Committee (IEAC) and the study was approved by the IEAC prior to the start of the study. Twenty male Wistar rats were used in the study and their health status was assessed by a veterinarian on receipt of the animals. All animals were found to be in good health and were acclimatised to laboratory conditions prior to the start of the study. They were fed with a standard pellet diet and water ad libitum. The animals were divided into four groups of five animals. The negative control group received 500µL of distilled water. The other three groups received either 10mg OmeGo (50 mg/kg rat bodyweight), 50mg OmeGo (250 mg/kg rat bodyweight) or 30mg (150mg/kg rat body weight) diclofenac sodium, suspended or dissolved in 500µL distilled water.

Carrageenan-Induced Acute Inflammatory Model

The anti-inflammatory activity of OmeGo compared to diclofenac was assessed using the carrageenan-induced rat paw edema assay. Edema was induced by a sub-plantar injection of 100µl of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat. 30 minutes prior to the carrageenan injection, animals were treated with a single sub-plantar dose of distilled water, OmeGo or diclofenac, respectively (doses as above). Paw thickness (volumetric) was measured just before the carrageenan injection, designated "0 hour" and then at 1, 2, 4, and 24 hours after the carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at "0 hour" compared to paw thickness at the respective time point. The measurements were summed, and values given as the mean value for each group.

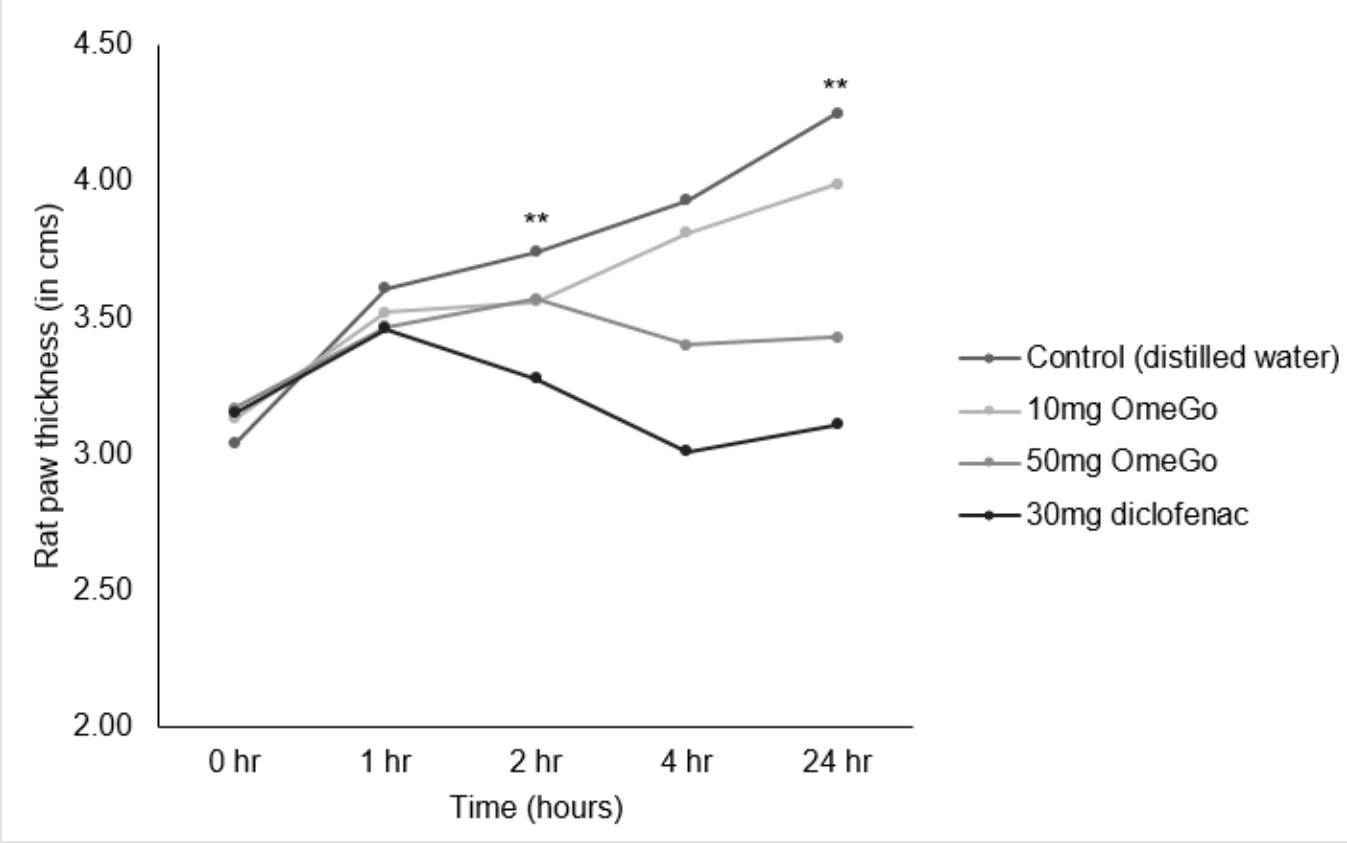
Serum Sample Collection of Cytokines

After 24 hours, all animals were sacrificed with cardiovascular bleeding with the help of diethyl ether according to the guidelines of CPSCEA committee. Blood was collected for cytokine assay at "0 hour", "1 hour", "4 hour" and "24 hour". Blood samples were left to coagulate at room temperature for 60 minutes and then centrifuged at 1500g for 15 minutes and crude serum was stored in new tubes at-700C until used. On the account of small volumes, blood samples from each individual rat in the same group were pooled to enable the blood assays to be performed.

Results

Carrageenan-Induced Acute Inflammatory Model

Injection of carrageenan into the hind paw induced a progressive edema in the negative control group with an almost 40% increase in paw thickness from baseline (3.04 ± 0.2 cm) to 24 hours (4.25 ± 0.3 cm). The increase at each time point was significant ($p<0.01$). A similar profile of a progressive increase in swelling was seen in the low dose OmeGo group with a 27% increase in paw thickness (3.13 ± 0.2 cm at baseline and 3.99 ± 0.2 cm at 24 hours). In contrast to the progressive edema noted in the negative control and low dose OmeGo groups, the high dose OmeGo group progressive paw swelling only occurred over the first two hours of the study, changing from 3.17 ± 0.2 cm to 3.57 ± 0.1 cm, equivalent to an increase of 9.5%. Thereafter, the swelling remained stable and was measured at 3.43 ± 0.2 cm at 24 hours. In the diclofenac treated group an initial increase in paw thickness was observed over the first hour, from 3.15± 0.1 cm at baseline to 3.46 ± 0.2 cm: an increase of 10%. Over the following 23 hours paw volume steadily declined and was fully resolved by the end of the study period in the diclofenac group (Figure 1).



Note: **denotes p<0.01.

Figure 1: Change in paw thickness (cms) over 24-hour study period with 10mg OmeGo, 50mg OmeGo and 30mg diclofenac compared to control (distilled water). Each group contained 5 rats.

Serum Sample Collection of Cytokines

Pooled serum from each group of rats was analyzed for cytokines IL-1 and IL-6 and IL-4, IL-5 and IL-13. Marked IL-1 elevations were seen in the negative control and OmeGo groups and at 24 hours IL-1 levels remained 4-5 times higher than baseline levels. In contrast, only small increases were seen in the diclofenac group and at 24 hours IL-1 had returned close to baseline levels. Consistent with diclofenac's moderation of IL-1 levels, serum IL-6 returned to baseline levels in the diclofenac-treated group over the study period. In the negative control and OmeGo groups IL-6 levels remained markedly elevated with a 4-fold increase compared to baseline. No clear impact was noted on IL-4, IL-5 & IL-13 over the duration of the study.

Discussion

In this preliminary study, OmeGo (at 250mg/kg per rat) demonstrated the potential to help resolve inflammation in a standard carrageenan-induced acute inflammatory paw edema model compared to negative control. The reduction in paw volume was similar to that observed in the diclofenac-treated group. The carrageenan-induced edema model is a well validated preclinical test to evaluate anti-inflammatory drugs, both steroidal and non-steroidal [19]. Carrageenan is highly irritant to subcutaneous tissues and produces a rapid inflammatory response with associated swelling. The initial phase is driven by histamine, serotonin and bradykinin followed by prostaglandins and a variety of cytokines including TNF, IL-1 and IL-6, resulting in a neutrophil dominated infiltrate [20]. In this study, serum IL-1 levels in the diclofenac group returned towards baseline levels after 24 hours and that IL-6 elevations returned fully to baseline levels. NSAIDs, including diclofenac, inhibit cyclooxygenase and thereby decrease prostaglandin production with a decline in inflammatory drivers including IL-1, IL-6 and TNF. These results are therefore consistent with the know mode of action of NSAIDs. OmeGo did not impact serum IL-1 or IL-6 levels. The positive impact of higher dose OmeGo on paw swelling must have been driven by other actions not assessed in this exploratory trial. Further, this cytokine result looks to fit with prior research indicating that fish oil helps the body resolve inflammation rather than suppressing it [21]. Previous research with the OmeGo has demonstrated the reduction of

inflammatory mediators associated with type 2 inflammation [16-18]. This would be expected to moderate the initial inflammatory response to carrageenan-induced inflammation. However, OmeGo must presumably have other inflammatory-modulating effects to enable the sustained reduction in soft tissue swelling seen over the duration of the study. We have previously demonstrated that the oil has significant antioxidant effects.

The release of reactive oxygen species (ROS) from activated neutrophils and macrophages in acute inflammation is a further driver of tissue damage and a reduction in ROS has been shown to reduce tissue swelling [22]. However, in this exploratory study we did not formally assess ROS levels or other potential anti-inflammatory effects of the oil. The absence of effect on the cytokine analysis suggests that OmeGo does not significantly inhibit cyclooxygenase and prostaglandin production, in contrast to NSAIDs. Prostaglandins have a role in protecting the gastric mucosa and maintaining renal perfusion. NSAID use has been noted to increase the risk of stomach ulceration and negatively impact kidney function in at risk populations. They also inhibit prostacyclin production, shifting the coagulation balance within the body towards thrombosis and a signal of increased risk of cardiovascular events has been seen with sustained NSAID use. In contrast, marine omega-3 supplementation has been linked to a reduction in cardiovascular events [23,24] with important underlying inflammation-resolving effects [25,26]. In this present study, we calculate the human equivalent dose at 1.5g [27], well within the recommended daily intake for fish oil supplementation. Our study has limitations, which would be expected of preliminary work. It was planned to give overall insights into inflammation-resolution and formal statistical analysis was not planned or performed apart from assessing whether the paw swelling in the control group showed a significant increase. The low volume of available serum samples limited the extent of cytokine analysis that could be performed. Nevertheless, the overall effect of diclofenac was consistent with its known anti-inflammatory properties confirming the robustness of positive control and the read-through for the impact of OmeGo in a standard model of induced inflammation. In summary, in this carrageenan-induced paw edema model the enzymatically liberated fish oil OmeGo had an impact on inflammation as a commonly used NSAID, diclofenac. The accompanying cytokine analysis indicates that OmeGo achieves this through different pathways compared to NSAIDs. Further research is planned to elucidate the underlying actions of the oil and its application to support human and animal health including the potential to help the repair and resolution of tissue injury.

Conflicts of Interest

The authors are all employees of Hofseth BioCare, the sponsor of the study.

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