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Pharmacological evaluation of the effects of enzymatically liberated fish oil on eosinophilic inflammation in animal models

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Abstract

The inappropriate activation of eosinophils is a well-recognized driver of various human inflammatory diseases including asthma, chronic rhinitis, and various gastrointestinal diseases, including eosinophilic esophagitis. Steroids, both topical and systemic, remain a cornerstone of treatment and can be highly effective. However, some individuals suffer side effects, unresolved symptoms, or both. OmeGo, an enzymatically liberated fish oil, has demonstrated anti-inflammatory and antioxidant properties as well the reduction of the activation, migration, and survival of eosinophils. Two animal models of eosinophilic inflammation were used to further assess OmeGo's profile. A house dust mite model of induced asthma showed a significant reduction in eosinophilic lung inflammation compared to the negative control, linoleic acid. The CRTH2 antagonist fevipiprant showed a similar eosinophilic inhibitory profile to OmeGo. In contrast, cod liver oil had no impact on any measure of inflammation. A guinea pig model of mild intraperitoneal eosinophilia showed a significant reduction in eosinophil activity by OmeGo, assessed by chemotaxis and chemokinesis. Apolipoprotein A-IV, an endogenous human protein with anti-inflammatory actions, showed a similar but numerically lower effect. OmeGo therefore combines a consistent antieosinophilic action with the known anti-inflammatory effects of polyunsaturated fatty acids. Proof-of-concept studies in asthma are warranted.

KEYWORDS
allergy, asthma, eosinophils, immune health, marine nutrition, natural therapeutics

Abbreviations: ANOVA, analysis of variance; APOA-IV, apolipoprotein A-IV; BAL, broncho-alveolar lavage; CPSCEA, committee for the purpose of control and supervision of experiments on animals; DHA, docosahexanoic acid; DPA, docosapentaenoic acid; EDTA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; FBS, fetal bovine serum; GLP, good laboratory practice; HED, human equivalent dose; HDM, house dust mite; LA, linoleic acid; oxLDL, oxidized low-density lipoprotein; PBS, phosphate-buffered saline; PUFA, polyunsaturated fatty acids; SPMs, specialized pro-resolving mediators of inflammation.

1 | INTRODUCTION

Increased numbers of eosinophils are found in a range of human inflammatory diseases, including asthma, chronic rhinosinusitis with nasal polyps, eosinophilic granulomatosis with polyangiitis, hypereosinophilic syndrome, and eosinophilic gastrointestinal diseases. In these diseases, eosinophils secrete proteins that promote inflammation and tissue remodeling. Different therapeutic strategies, such as monoclonal antibodies directed against the interleukin-5 (IL-5; a cytokine involved in eosinophil recruitment and activation) pathway have been developed to treat diseases characterized by eosinophilic inflammation.

Regular consumption of fish has numerous health benefits driven by the anti-inflammatory and antioxidant effects of the polyunsaturated fatty acids (PUFAs) contained in the oil fraction of the fish, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[1] Fish oil is now commonly used to garner similar benefits to eating whole fish. However, epidemiological data and clinical trials with fish oil supplementation have shown inconsistent cardiovascular outcomes. Most of these trials have focused on the effects of EPA and DHA either alone or together. However, whole fish contains a broad array of PUFAs including DPA[2]. PUFAs are metabolized into specialized proresolving mediators (SPMs), each with specific, nonredundant, inflammation-resolving effects.[3] Supplementation with whole fish oil may therefore hold the potential to provide broader health benefits, particularly if minimally processed to limit any degradation of the individual PUFAs.

Reactive oxygen species directly damage cell membranes and tissues, thereby generating the release of proinflammatory mediators.[4] One consequence of oxidative stress is the production of oxidized LDL (oxLDL), which is a highly inflammatory blood lipid and an independent risk factor for cardiovascular disease.[5] Oxidized LDL also appears to have adverse consequences on respiratory health and is associated with levels of airway inflammation in asthma.[6,7] OmeGo is an enzymatically liberated salmon oil, that is, a whole fish oil. In contrast to processed omega-3 oil and algae oil, OmeGo produced marked reductions in oxLDL in healthy human subjects[8]; these findings support the concept that whole fish oil supplementation may have greater health benefits compared to using individual components.

In vitro work using peripheral eosinophils from human subjects with allergy demonstrated attenuation of eosinophil effector function by OmeGo, with a reduction in eosinophilic shape change, integrin expression, and increased eosinophil apoptosis.[9] In contrast, processed omega-3 oil and krill oil showed very limited attenuation of the eosinophilic function. This paper describes studies designed to further evaluate the potential for OmeGo to target eosinophilic inflammation. We investigated the immunomodulatory effect of OmeGo on eosinophilic inflammation in a house dust mite (HDM) induced asthma model in mice. We also studied the effect of OmeGo on the chemotaxis ability of eosinophils obtained from guinea pigs following the induction of mild eosinophilia.[10–13]

2 | MATERIALS AND METHODS

2.1 | Study design

Both studies assessed the potential for eosinophilic modulation by OmeGo. The studies were conducted according to GLP guidelines and in accordance with the laws and regulations of India, where the studies were performed. The studies were approved by the Institutional Animal Ethics Committee before the start of each study. Health status was assessed by a veterinarian on receipt of the animals. All were found to be in good health and were acclimatized to test conditions for 5 days prior to the start of the trial. Test group allocation was determined by a manual randomization method. Individual body weights of allocated animals were within 20% of the group means, and the body weights of the animals were analyzed statistically to rule out significant differences between the groups. Following allocation to the study, each animal was assigned with an individual cage card, labeled with a study number, study type, test system, sex, dose, group, temporary animal number, permanent animal number, date of dosing/treatment, experiment start, and end dates.

The HDM study involved 20 healthy female adult mice. Randomization was performed 1 day prior to the first day of treatment with the animals randomized into four groups, with five mice per group. The guinea pig study involved 21 healthy male animals randomized into seven groups.

The mice were housed in experimental groups of five animals per individually ventilated cage, and the guinea pigs were housed in groups of three per individual cage. The room temperature was maintained between 21 to 24.4°C and relative humidity at 47.9–66.2% during the entire study period. Artificial light was set to give a cycle of 12 h light and 12 h dark. The animals were offered a conventional laboratory rodent diet ad libitum supplied by Nutrivet Life Sciences and Aquaguard filtered drinking water was provided ad libitum.

2.2 | HDM study: Experimental design and procedures

On day 1, all 20 mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) via intraperitoneal injection and sensitized intranasally with 1 μg HDM protein in 40 mcL phosphate-buffered saline (PBS). Following this, from day 7 to day 11, the mice were challenged daily with 10 μg HDM protein in 40 mcL PBS intranasally. From day 7 to 14, the mice were treated with either vehicle control PBS test item, OmeGo (dosed at 20 and 60 μg) or positive control apolipoprotein A-IV (dosed at 5 μg), all given intraperitoneally. On day 15, all mice were anesthetized, their trachea was exposed and 3 mL of PBS containing 1 mM EDTA was instilled into the lungs, and bronchoalveolar lavage (BAL) fluid was then collected and stored on ice.

The BAL fluid was centrifuged (400*g* at 4°C for 7 min) and the cell pellet was collected and stored at −20°C. The collected pellet was analyzed for eosinophilia as well as differential cell count which consisted of eosinophil, alveolar macrophage, lymphocyte, monocyte, and neutrophil levels. This was achieved by subjecting the collected cell pellets to the following staining protocol. Erythrocytes were lysed using ammonium chloride solution. After a washing step, nonspecific binding sites were masked using TruStain fcX antibody (BioLegend, San Diego, CA) at a 1:1000 ratio for 15 min on ice, followed by incubation with specific anti-mouse antibodies: Siglec F (1:100), CD11b (1:200), CD11c (1:200), all from Sigma Aldrich, USA, Ly6G (1:500) and MHC-II (1:200), both from BioLegend, for 30 min at 4°C. Cells were washed, fixed, and analyzed using a BD FACS Canto II flow cytometer as follows. After doublet cells had been excluded, lymphocytes were identified as FCS/SSC low CD11b negative/CD11c negative cells. The remaining cells were further gated as CD11c negative/Siglec F positive eosinophils and CD11c positive/Siglec F positive alveolar macrophages. CD11c/Siglec F double negative cells were characterized as CD11b positive/Ly6G positive neutrophils and Ly6G negative/Ly6C positive monocytes.

The spleen from each animal was collected and frozen and eosinophil levels were measured. BAL fluid and spleen cellularity were assessed by flow cytometry (cell staining protocol as above). All samples, from BAL fluid and spleen, were assayed in duplicate.

All animals were observed twice daily throughout the treatment period for morbidity and mortality and were observed for clinical signs once daily during the acclimatization period and twice daily during the treatment period. The body weight of each mouse was measured during randomization, on day 1, day 7, and day 15 of the study. Percent body weight change for each test group of mice was calculated by comparing the first day body weight. Mean change in body weight in the treatment groups was also assessed versus those of the vehicle control group.

Necropsy at end of treatment according to the guidelines of the CPSCEA committee.

2.3 | Eosinophil chemotaxis and chemokinesis study: Experimental design and procedures

The guinea pigs were sensitized to create mild eosinophilia by the intraperitoneal injection of polymyxin B (1 mg/animal) once a week for 6 weeks. Polymyxin B was dissolved in 0.9% saline solution, and each animal was injected with 0.5 mL of the 0.9% saline solution containing 1 mg of polymyxin B. In week 6, in addition to polymyxin B, the animals were injected with the test articles individually. The test groups were OmeGo dosed at 30 mg/kg; OmeGo dosed at 300 mg/kg; sea cod (cod liver oil/omega 3) dosed at 30 mg/kg; sea cod dosed at 300 mg/kg; fevipiprant dosed at 5 mg/kg; fevipiprant dosed at 20 mg/kg; linoleic acid (LA/omega 6) dosed at 300 mg/kg.

In the sixth week, animals were anesthetized with isoflurane and intraperitoneal fluid collected by injecting 50 mL of saline into the peritoneal cavity. After massaging the peritoneum for 15 s, the fluid was drained and collected in centrifuge tubes. The fluid was centrifuged for 10 min (400*g* and 4°C) and resuspended in FBS (fetal bovine serum) medium. The cell pellet was again resuspended in 2 mL of Percoll solution (1.070 g/mL density) that contained deoxyribonuclease I (30 tg/mL) and 5% FCS. The suspension was layered onto discontinuous Percoll gradients of 2-mL steps of varying densities (1.080–1.100 g/mL), and the discontinuous gradients were centrifuged for 25 min (1600*g*, 10°C). Eosinophils with a normal density were recovered from the 1.090/1.095/1.100 g/mL interfaces. The cell suspension was centrifuged for 8 min (400*g*, 4°C), washed with distilled water (4°C) for 30 s followed by 2× FCS medium. The purity of the eosinophil population was ~92% as determined by Randolph's phloxine B-methylene blue staining and analyzed for eosinophil chemotactic and chemokinetic responses to leukotriene B4 by a modified Boyden technique using a 96-well micro chemotaxis test chamber. Chemokinesis is defined as random cell motility and chemotaxis as cell motility directed towards increased concentrations of chemical attractants. Here we assessed eosinophilic chemotaxis by adding 0.1 μm leukotriene B4 in the lower compartment of the chamber only, whereas chemokinetic activity was examined by placing the same concentration of leukotriene B4 in both compartments of the chamber which contained a framed polycarbonate filter with a 5-μm pore place between the two compartments. The viability of the eosinophils was determined by the

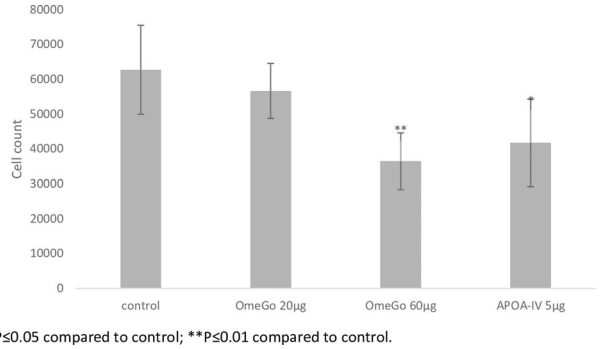

FIG 1 Mean total cell count (± standard deviation) bronchoalveolar lavage fluid

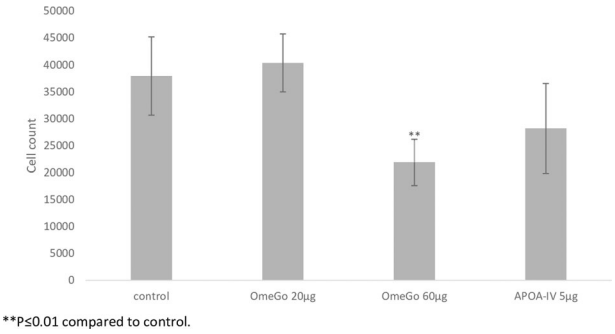

FIG 2 Mean eosinophil count (± standard deviation) bronchoalveolar lavage fluid

Trypan blue dye exclusion test after incubation for 90 min. All samples were assayed in duplicate.

2.4 | Statistical analysis

All raw data from this study were analyzed using "Sigma Plot" v14 statistical software. In the HDM study, all continuous data were checked for normality using the Shapiro–Wilk test. Data were analyzed using analysis of variance (ANOVA), with Dunnett's *t*-test or Bonferroni's multiple comparison test. p values ≤ 0.05 were deemed statistically significant. In the guinea pig study, Kruskal–Wallis (nonparametric ANOVA) followed by Dunn's multiple comparison test was used to compare each treatment against the control (LA).

3 | RESULTS

No treatment-related mortality/morbidity was noted in any of the animals throughout the study period, and there were no treatment-related clinical observations.

No statistically significant differences in mean body weight were observed between the treatment groups and the vehicle control group throughout the study period. No significant percent body weight change was observed in any of the treatment groups compared to day 1.

3.1 | House dust mite model

The total cell count in the BAL fluid was significantly reduced by OmeGo (60 μg dose; $p \leq 0.01$) and APOA-IV (5 μg dose; $p \leq 0.05$) by 42% and 34%, respectively, compared to the control group (Figure 1). Treatment with OmeGo 60 μg also provided a 42% significant reduction in the eosinophil count in the BAL fluid ($p \leq 0.01$) (Figure 2). No other groups showed a significant reduction in eosinophil count compared to control including APOA-IV, which showed a 32% nonsignificant reduction (Figure 1). The analysis of spleen cellularity showed a significant reduction in the percentage of eosinophils in mice treated with OmeGo 60 μg ($p \leq 0.01$) and APOA-IV ($p \leq 0.001$), 38% and 30%, respectively, compared to the control group (Figure 3).

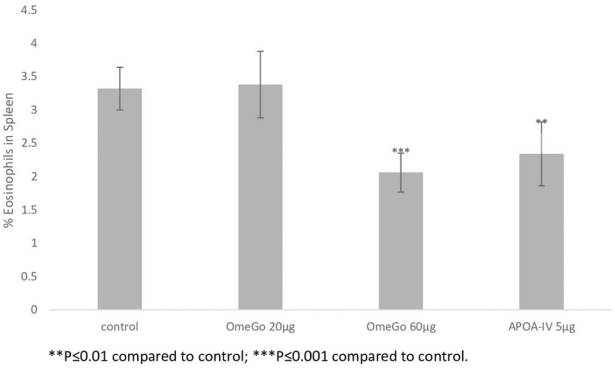

FIG 3 Percent mean eosinophils (± standard deviation) in spleen tissue

3.2 | Eosinophil chemotaxis model

Eosinophils obtained from guinea pigs exposed to polymyxin B were obtained. Eosinophils from animals treated with OmeGo 300 mg/kg showed a significant reduction of eosinophil chemotaxis (Figure 4) and chemokinesis (Figure 5), with 50.7% ($p = 0.02$) and 55.7% ($p = 0.005$) inhibition, respectively, compared to LA control. The reduction with 30 mg/kg was nonsignificant at 39.7% ($p = 0.45$) and 31% ($p = 0.19$) for chemotaxis and chemokinesis, respectively. Neither pretreatment with cod liver oil nor linoleic acid produced any inhibition of eosinophil chemotaxis or chemokinesis. Pretreatment with the positive control fevipiprant 20 mg/kg also significantly inhibited both measures of eosinophil activity,

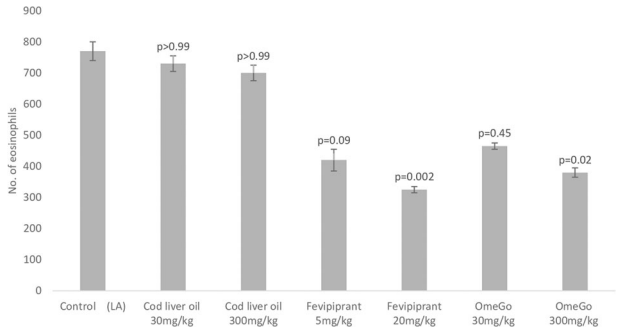

FIG 4 Impact of pretreatment on chemotaxis of eosinophils exposed to leukotriene B4 (error bars = range)

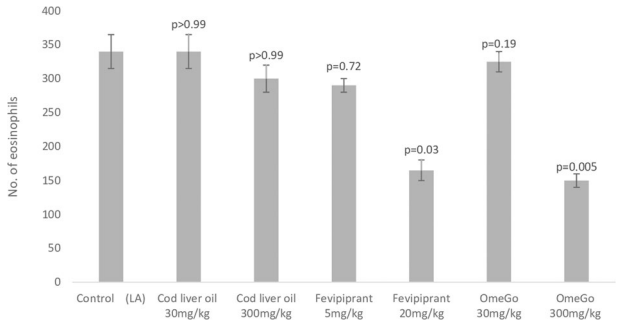

FIG 5 Impact of pretreatment on chemokinesis of eosinophils exposed to leukotriene B4 (error bars = range)

with 68% inhibition of chemotaxis ($p = 0.002$) and 51% reduction in chemokinesis ($p = 0.033$). The reductions with the lower dose (5 mg/kg) were nonsignificant at 45.5% ($p = 0.09$) 14.8% ($p = 0.72$), respectively. After a 90-min incubation with leukotriene B4, eosinophil viability was greater than 96% in all treatment groups.

4 | DISCUSSION

We investigated the effect of OmeGo on the modulation of eosinophilic inflammation using two animal models: an HDM-induced asthma mouse model and an intraperitoneal eosinophilia model in guinea pigs. OmeGo, an enzymatically liberated whole fish oil, significantly reduced both lung and splenic eosinophilia in the HDM model, and chemotaxis and chemokinesis of eosinophils obtained from the guinea pigs. The inhibition of guinea pig eosinophil chemotaxis with OmeGo was not observed with other fish oils including krill oil and processed DHA/EPA concentrated fish oil. This suggests that OmeGo contains a biologically active fraction, which is not present in other oils; here, we demonstrate the potential for this fraction to modulate eosinophilic inflammation. These results complement previous in vitro work on eosinophils derived from human subjects with allergies, where eosinophil shape change and CD11b expression (a marker of activation) after exposure to the chemokine CCL11 were attenuated by OmeGo.[9]

In the HDM-induced asthma model, OmeGo (at the 60 μg dose) reduced lung inflammation: significant reductions in lung eosinophilia and total cell count were demonstrated in the BAL fluid. Splenic eosinophil levels were also significantly reduced. The positive control, APOA-IV, showed a broadly similar effect to OmeGo but of a slightly lower magnitude.

The HDM model is commonly used as a model of human allergic asthma, as lung eosinophilia and inflammatory mediators associated with type 2 inflammation are induced.[14,15] APOA-IV is an endogenous protein with anti-inflammatory actions, and its levels are reduced in allergic individuals and HDM-challenged mice.[16] Previous work with APOA-IV in a mouse HDM model demonstrated a 42% reduction in BAL eosinophil count combined with a reduction of 70% in methacholine-induced airway resistance.[16] Here, OmeGo (60 μg) showed a 42% reduction in BAL fluid eosinophils compared to control and APOA-IV at 5 μg/mouse showed a 32% nonsignificant reduction.

The guinea pig study induced mild intraperitoneal eosinophilia, with a standard peritoneal aspiration technique to assess modulation of eosinophil function ex vivo. OmeGo 300 mg/kg demonstrated a significant reduction in eosinophil chemotaxis and chemokinesis compared to control. The fish oil concentrate from the cod liver (omega-3) failed to show any modulation of eosinophil function as did linoleic acid (omega-6). Omega-3 has been associated with reducing the risk of asthma and improving symptoms, whereas omega-6 has been associated with worsening bronchial hyperactivity although the mechanism has yet to be elucidated.[17] In our study, neither agent had an impact on eosinophil activity.

OmeGo's reduction of eosinophil function was similar to that of positive control, the CRTH2 antagonist fevipiprant, dosed at 20 mg/kg. The CRTH2 receptor is expressed on eosinophils and is involved in eosinophil activation and allergic inflammation.[18,19] Fevipiprant was dosed at between 150 and 450 mg/day in late-stage clinical development[20,21] and this study, the human equivalent doses (HED) of fevipiprant were 65 and 260 mg. In contrast to fevipiprant, OmeGo holds broad anti-inflammatory properties in addition to modulating eosinophil function.

Eosinophils are a significant component of lung inflammation in many patients with asthma, and there is growing evidence of involvement in a subset of COPD patients.[22] Monoclonal antibodies targeting IL-4, -5. and -13 have advanced the treatment of severe eosinophilic asthma.[23] In mild to moderate asthma characterized by increased eosinophilic inflammation, there is often a need for additional anti-inflammatory treatment in addition to

inhaled corticosteroids[24]; the results here suggest the potential for OmeGo to be used as a practical, orally administered treatment for eosinophilic asthma.

Our study results are consistent with epidemiological data which suggest that fish PUFAs can reduce asthma symptoms and improve lung function.[25] This effect results from the metabolism of the various PUFAs into SPMs of inflammation. Several murine studies have demonstrated PUFAs to reduce airway eosinophilic inflammation, hyperresponsiveness, and mucus production.[25] The gentle enzymatic liberation of OmeGo avoids damage to the various PUFA elements and thereby retains the significant anti-inflammatory effects associated with the consumption of fresh fish.

Using standard conversion metrics, we calculate the HDM study dose to be a HED of 12 mg/day of OmeGo.[26] As a lipophilic agent, OmeGo would be anticipated to have a high volume of distribution and oral dosing work will be required to elucidate the most effective human dose. Fish oil capsules have shown therapeutic benefits for cardiovascular disease when dosed at 2 and 4 g per day.[27,28] It appears feasible that a similar dose range for OmeGo could be used to treat eosinophilic inflammation in humans. In the guinea pig study, the efficacious dose of 300 mg/kg equates to a HED of 4 g.

In summary, OmeGo significantly reduced lung and systemic eosinophilia in an HDM-induced asthma study and significantly reduced ex vivo eosinophil chemotaxis and chemokinesis in a guinea pig model of induced eosinophilia. OmeGo has the potential for broad anti-inflammatory effects, and here we demonstrate attenuation of eosinophilic activity that is relevant to diseases such as asthma. These data support the case for proof-of-concept studies in asthma.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Calder, P. C. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015;1851:469-484.
2. Drouin, G., Rioux, V., Legrand, P. The n-3 docosapentaenoic acid (DPA): a new player in the n-3 long chain polyunsaturated fatty acid family. Biochimie. 2019;159:36-48.
3. Regidor, P. A., Mueller, A., Sailer, M., Gonzalez Santos, F., Rizo, J. M., Egea, F. M. Chronic inflammation in PCOS: the potential benefits of specialized pro-resolving lipid mediators (SPMs) in the improvement of the resolutive response. Int J Mol Sci 2020;22:384
4. Sedgwick, J. B., Hwang, Y. S., Gerbyshak, H. A., Kita, H., Busse, W. W. Oxidized low-density lipoprotein activates migration and degranulation of human granulocytes. Am J Respir Cell Mol Biol. 2003;29:702-709.
5. Rhoads, J. P., Major, A. S. How oxidized low-density lipoprotein activates inflammatory responses. Crit Rev Immunol. 2018;38:333-342.
6. Can, U., Yerlikaya, F. H., Yosunkaya, S. Role of oxidative stress and serum lipid levels in stable chronic obstructive pulmonary disease. J Chin Med Assoc. 2015;78:702-708.
7. Shen, Y., Yang, T., Guo, S., Li, X., Chen, L., Wang, T., Wen, F. Increased serum ox-LDL levels correlated with lung function, inflammation, and oxidative stress in COPD. Mediators Inflamm. 2013;2013:972347.
8. Sen N., Bomi F. The effect of dietary oil capsules on reducing serum concentrations of oxidized low density lipoprotein- β2-glycoprotein-i complex. J Nutr Food Sci. 2013;3:5.
9. Framroze, B., Heggdal, H. An in vitro study to explore the modulation of eosinophil effector function in human allergic peripheral blood eosinophils using enzymatically extracted salmonid oil. Funct Foods Health Dis. 2020;10:357.
10. Bafadhel, M., Pavord, I. D., Russell, R. E. K. Eosinophils in COPD: just another biomarker? Lancet Respir Med. 2017;5:747-759.
11. Emanuele, E., Falcone, C., D'Angelo, A., Minoretti, P., Buzzi, M. P., Bertona, M., Geroldi, D. Association of plasma eotaxin levels with the presence and extent of angiographic coronary artery disease. Atherosclerosis. 2006;186:140-145.
12. Qin, M., Wang, L., Li, F., Yang, M., Song, L., Tian, F., Yukht, A., Shah, P. K., Rothenberg, M. E., Sharifi, B. G. Oxidized LDL activated eosinophil polarize macrophage phenotype from M2 to M1 through activation of CD36 scavenger receptor. Atherosclerosis. 2017;263:82-91.
13. Singh, D., Kolsum, U., Brightling, C. E., Locantore, N., Agusti, A., Tal-Singer, R. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. Eur Respir J. 2014;44:1697-1700.
14. Gregory, L. G., Lloyd, C. M. Orchestrating house dust mite-associated allergy in the lung. Trends Immunol. 2011;32:402-411.
15. Plantinga, M., Guilliams, M., Vanheerswynghels, M., Deswarte, K., Branco-Madeira, F., Toussaint, W., Vanhoutte, L., Neyt, K., Killeen, N., Malissen, B., Hammad, H., Lambrecht, B. N. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. Immunity. 2013;38:322-335.
16. Roula, D., Theiler, A., Luschnig, P., Sturm, G. J., Tomazic, P. V., Marsche, G., Heinemann, A., Sturm, E. M. Apolipoprotein A-IV acts as an endogenous anti-inflammatory protein and is reduced in treatment-naïve allergic patients and allergen-challenged mice. Allergy. 2020;75:392-402.

17. Adams, S., Lopata, A. L., Smuts, C. M., Baatjies, R., Jeebhay, M. F. Relationship between serum omega-3 fatty acid and asthma endpoints. Int J Environ Res Public Health. 2018;16:43.
18. Kupczyk, M., Kuna, P. Targeting the PGD(2)/CRTH2/DP1 signaling pathway in asthma and allergic disease: current status and future perspectives. Drugs. 2017;77:1281-1294.
19. Singh, D., Ravi, A., Southworth, T. CRTH2 antagonists in asthma: current perspectives. Clin Pharmacol. 2017;9: 165-173.
20. Castro, M., Kerwin, E., Miller, D., Pedinoff, A., Sher, L., Cardenas, P., Knorr, B., Lawrence, D., Ossa, D., Wang, W., Maspero, J. F. Efficacy and safety of fevipiprant in patients with uncontrolled asthma: Two replicate, phase 3, randomised, double-blind, placebo-controlled trials (ZEAL-1 and ZEAL-2). EClinicalMedicine. 2021;35:100847.
21. Brightling, C. E., Gaga, M., Inoue, H., Li, J., Maspero, J., Wenzel, S., Maitra, S., Lawrence, D., Brockhaus, F., Lehmann, T., Brindicci, C., Knorr, B., Bleecker, E. R. Effectiveness of fevipiprant in reducing exacerbations in patients with severe asthma (LUSTER-1 and LUSTER-2): two phase 3 randomised controlled trials. Lancet Respir Med. 2021;9:43-56.
22. Higham, A., Beech, A., Wolosianka, S., Jackson, N., Long, G., Kolsum, U., Southworth, T., Pham, T. H., Sridhar, S., McCrae, C., Newbold, P., Singh, D. Type 2 inflammation in eosinophilic chronic obstructive pulmonary disease. Allergy. 2021;76:1861-1864.
23. McGregor, M. C., Krings, J. G., Nair, P., Castro, M. Role of biologics in asthma. Am J Respir Crit Care Med. 2019;199:433-445.
24. Busse, W. W., Fang, J., Marvel, J., Tian, H., Altman, P., Cao, H. Uncontrolled asthma across GINA treatment steps 2–5 in a large US patient cohort. J Asthma. 2021. 10.1080/02770903.2021.1897834.
25. Miyata, J., Arita, M. Role of omega-3 fatty acids and their metabolites in asthma and allergic diseases. Allergol Int. 2015;64:27-34.
26. Nair, A. B., Jacob, S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7;27-31.
27. Bhatt, D. L., Steg, P. G., Miller, M., Brinton, E. A., Jacobson, T. A., Ketchum, S. B., Doyle, R. T., Jr., Juliano, R. A., Jiao, L., Granowitz, C., Tardif, J. C., Ballantyne, C. M. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med 2019;380:11-22.
28. Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito, Y., Ishikawa, Y., Oikawa, S., Sasaki, J., Hishida, H., Itakura, H., Kita, T., Kitabatake, A., Nakaya, N., Sakata, T., Shimada, K., Shirato, K. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet. 2007;369:1090-1098.

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