Investigating the Anti-inflammatory Potential of Phospholipids Esterified to Omega-3 and Omega-6 Fatty Acids on RAW 264.7 Macrophages-Mediated Immune Response

T.Maurício, Aveiro/PT[1,2,3], B.Neves, Aveiro/PT[2], R.Domingues, Aveiro/PT[3], P. Domingues, Aveiro/PT[1]

[1] Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro

[2] Department of Medical Sciences and Institute of Biomedicine—iBiMED

[3] CESAM—Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro

Exacerbated inflammation is a common cause of cellular and tissue damage, which triggers or exacerbates conditions with an inflammatory component. Monocytes and macrophages play essential roles in the activation and resolution phases of the inflammatory response [1]. Recent evidence suggests that complex lipids esterified with omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) can act as immunomodulators of immune cells, regulating the immune response [2,3]. However, the molecular mechanisms underlying the immunomodulatory effects of these bioactive lipids and the specific roles of polar lipid species in this activity are not well characterized. Therefore, this study aims to investigate the influence of several phospholipids (PL) from different classes, such as phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylserine (PS), esterified with omega-3 or omega-6 PUFA, on the activation and orchestration of the immune response mediated by macrophages using an *in vitro* model of inflammation induced by lipopolysaccharides (LPS). The first step of our work was to assess the viability of murine macrophages (RAW 264.7) using the resazurin assay, as well as the anti-inflammatory activity of phospholipids using the Griess method after exposure to each pure phospholipid. The results indicated that phospholipids affect differently the cell viability and nitric oxide (NO) production by LPS-stimulated macrophages. The PC standards with higher unsaturation degree (PC 18:0/20:4 and PC 18:0/22:6), lyso-PC (LPC 18:0) and PS 18:0/18:2 significantly reduced cell viability when compared to control cells. Furthermore, PL standards esterified with 20:4 (arachidonic acid, AA) and 22:6 (docosahexanoeic acid, DHA) PUFA showed the highest anti-inflammatory potential, by significantly reducing NO levels compared to LPS-stimulated macrophages alone. Thus, our findings suggest that each PL may induce different effects on immune cells, requiring further investigation to fully understand the distinct functions carried out by specific polar lipid species.

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References:

1. Fujiwara, N.; Kobayashi, K. Macrophages in Inflammation. *Curr. Drug Target -Inflamm. Allergy* **2005**, *4*, 281–286, doi:10.2174/1568010054022024.
2. Leuti, A.; Fazio, D.; Fava, M.; Piccoli, A.; Oddi, S.; Maccarrone, M. Bioactive Lipids, Inflammation and Chronic Diseases. *Adv. Drug Deliv. Rev.* **2020**, *159*, 133–169, doi:https://doi.org/10.1016/j.addr.2020.06.028.
3. Maurício, T.; Aveiro, S.; Guedes, S.; Lopes, D.; Melo, T.; Neves, B.M.; Domingues, R.; Domingues, P. Multi-Omic Profiling of Macrophages Treated with Phospholipids Containing Omega-3 and Omega-6 Fatty Acids Reveals Complex Immunomodulatory Adaptations at Protein, Lipid and Metabolic Levels. *Int. J. Mol. Sci.* **2022**, *23*, doi:10.3390/ijms23042139.