
Plan Overview

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Title: Evaluation of secretory Phospholipase A2 associated with PLA2 lipoproteins (PLA2-Lp) from *Danio rerio* as a biomarker of emerging pollutants and estimation of oxidative stress

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Project abstract:

Several studies show that xenobiotic agents can modulate the inflammatory response that involves the mobilization of arachidonic acid (AA) via the action of cytosolic PLA2 enzymes (cPLA2) and its subsequent metabolization by COX-2 and cytochrome P-450 enzymes leading to increased cellular, physiological oxidative stress and several pro-inflammatory cytokines such as IL-6. Omics analyses performed in *D. rerio* and other fish show two major groups of Phospholipase A2 (PLA2): cytosolic PLA2 (cPLA2), which is an already described and characterized enzyme and lipoprotein-associated secretory PLA2 (sPLA2-Lp), which is still poorly studied and both enzymes have an 80% identity similarity to human cPLA2 and sPLA2-Lp. Lipoprotein-associated phospholipase A2 (Lp-PLA2), a unique member of the phospholipase A2 superfamily, is secreted in active form by T lymphocytes, macrophages, and monocyte-derived mast cells. PLA2-Lp is mainly bound to circulating low-density lipoprotein (LDL) by hydrolyzing the oxidized phosphatidylcholine component of oxidized LDL (ox-LDL), Lp-PLA2 generates two potent pro-inflammatory and pro-atherogenic mediators, oxidized free polyunsaturated fatty acids and lysophospholipids. Studies show that there is a relationship between increased PLA2-Lp enzymatic activity and increased plasma lipoprotein concentrations HDL and LDL, which can aggregate and potentiate increased cellular oxidative stress resulting from increased macrophage- and lymphocyte-mediated defense processes. Cytosolic and lipoprotein-associated PLA2 are two key enzymes for most mammals and

modulate several events that encompass inflammatory, metabolic, and developmental processes. The main product of this process is arachidonic acid which is a key element in several fundamental processes. In this project we intend to develop a protocol for monitoring the enzymatic activity of PLA2-Lp associated with the quantification of LDL and HDL, pro-inflammatory interleukins and oxidative stress markers to validate the use of PLA2-Lp monitoring as a new biomarker of plasma oxidative stress and physiological health in fish. Using pro-inflammatory agents and agents inhibiting cytosolic and lipoprotein-associated phospholipase A2 enzyme activity we will seek to evaluate teratogenic changes in zebrafish embryos and zebrafish adults. Finally, using in silico modeling techniques of the zebrafish cPLA2 and PLA2-Lp proteins as well as molecular docking, we intend to generate a dataset capable of providing or understanding the action of pollutants.

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Evaluation of secretory Phospholipase A2 associated with PLA2 lipoproteins (PLA2-Lp) from *Danio rerio* as a biomarker of emerging pollutants and estimation of oxidative stress

Data Collection

What data will you collect or create?

These generated data can be crucial for two potential areas, one related to the extensive breeding and management of fish in captivity. On the other hand, the use of a new indicator parameter for the physiological and metabolic status of teleost fish. Since the increase in secretory PLA2 enzymatic activity in mammals is closely related to an adaptation of the cellular immune system and mainly by the action of microorganisms or foreign agents that lead to the activation of phagocytic cells and that in turn induces an increase in PLA2 associated with lipoproteins as a response to oxidative changes on oxidized phospholipids transported in lipoproteins, which are degraded and released from particles and deposited in the arteries.

The data to be collected will be data from biochemical, enzymatic analysis, and other in vitro and in vivo activities.

In addition to these data, histological and morphological image analysis data will also be collected.

Film and photo analysis will be performed to evaluate the results of the zebrafish experiments

To evaluate and support the results of the experimental part in relation to PLA2 we will evaluate the results in silico.

Besides statistical treatments

How will the data be collected or created?

By assays using 96-well end point ELISA measurement techniques, to measure the enzymatic activities of the different kits, before and after the administration of the drugs or the chemical compounds present in pollutants or the micro plastics, which are the materials that will be used in the project. All the procedures for histological and histopathological analysis will also be done, and a camera will also be used to collect the images. Finally, a very strong computer part will be used to create models of the secretory PLA2 associated with lipoproteins and cytosolic PLA2 that will be used to evaluate by molecular modeling analysis the interaction of the compounds with these two proteins and thus establish and help explain the experimental data.

Documentation and Metadata

What documentation and metadata will accompany the data?

Data packages and statistical analysis.

Ethics and Legal Compliance

How will you manage any ethical issues?

Ethics and Legal Compliance

All experiments to be performed in vivo and tissue processing will be carried out after approval by the animal ethics committee.

How will you manage copyright and Intellectual Property Rights (IPR) issues?

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Storage and Backup

How will the data be stored and backed up during the research?

Results of the research will be made available in digital form as PDFs, spreadsheet tables, tab-delimited files, and image and/or video files. Images will be saved in standard image formats such as JPEG, TIFF, or PNG. The resulting manuscripts will appear as PDFs and contain text, calculations, drawings, plots, and images. In addition, the creators of the project also commit to keeping copies of the work on home pages created and maintained by the authors of the project. In addition, the papers will also be deposited in other open-access databases such as ResearchGate and in reprint journals.

How will you manage access and security?

The access will be free, in the open-access system and all interested parties will be able to access the research results, the maintenance will be done by the service providers, in the case of papers published in the Electronic Scientific Library Online (Scielo) system, or other recognized and non-predatory publishers or other copies stored in the UNESP Institutional Repository, the access will be regulated according to the responsible institution, all open access. On the other hand, all our papers will be deposited on our home page on Instagram and access, done by registration and this is important for our group as feedback. In addition, we will create our own central office to store our data physically and in the cloud.

Selection and Preservation

Which data are of long-term value and should be retained, shared, and/or preserved?

Data from this research will be stored in the official repository of the Repositório Institucional UNESP and will get a specific DOI generated as a unique and persistent identifier. They will be retained dependent upon storage capacity.

What is the long-term preservation plan for the dataset?

Data will also be stored in personal computers and external hard drives of the researchers involved in the present study. In this case, storage will last up to 5 years after the end of the study.

Data Sharing

How will you share the data?

All of them are open or maintained with publishers with an open access policy, by the DOI of the article to be accessed at UNESP's digital library or by request for copies that are stored on our computer.

Are any restrictions on data sharing required?

Only previous identification of the user who is interested in accessing the article, creation of a registration.

Responsibilities and Resources

Who will be responsible for data management?

For digital libraries, open access journals, or the institutional repository it will be done by the managers themselves, and in our case, it will be done by the project manager.

What resources will you require to deliver your plan?

Technical reserve of the person in charge, specific calls from the UNESP Pro Rectory, and the productivity scholarship.