Development of Analytical Methods for Identifying and Quantifying Oxidized Phospholipids in Ferroptosis

Tarik Daniel de Santana Kissuani[1], Sayuri Miyamoto[1], Larissa Diniz [1], Thais Satie Iijima[1]

[1.] IQ - USP, Chemistry Institute, University of São Paulo, Av. Prof. Lineu Prestes, 748 - Butantã, São Paulo - SP, 05508-900;

The cell membrane in mammals is primarily composed of polyunsaturated fatty acids (PUFAs), which, due to their high degree of unsaturation, are highly susceptible to oxidation, whether mediated by enzymes or reactive oxygen species. The oxidative products of these PUFAs play a role in physiological and pathological processes, including inflammation, neurodegenerative diseases, and cell death signaling. These characteristics make them important biological markers, which can only be identified using highly sensitive and specific analytical methods such as mass spectrometry. This project aims to develop a method to identify and quantify oxidized phospholipid species in cells undergoing ferroptosis. The approach utilizes phospholipid hydroperoxides and hydroxides, synthesized through photooxidation, introducing significant isomer variability. The chosen standards include hydroperoxide derivatives of phosphatidylethanolamine (PE-OOH) and phosphatidylglycerol (PG-OOH). PE was selected because it is the most abundant phospholipid in the ferroptosis process, followed by PC. PG is included due to its relevance in neuroblastoma cell-induced ferroptosis. Purification and quantification methods were developed using HPLC and UHPLC techniques, respectively. Due to its greater stability hydroperoxides were reduced to hydroxides using sodium borohydride. We successfully optimized PE-OOH and PG-OOH separation in HPLC to achieve effective purification conditions. Additionally, we accomplished the quantification of hydroperoxide standards and the synthesis of hydroxides, producing reproducible results. Although mass spectrometry analysis could not be performed due to the instability of hydroperoxides, which degraded immediately after purification despite careful temperature control and clean containers, the concentration was sufficient to validate the quantification method and hydroxide synthesis.

Agradecimentos: FAPESP CEPID–Redoxoma, CNPq, CAPES and PRPUSP.