Dissertation Project – 2nd Cycle

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TITLE: Modulation of neuroinflammation by phenolic sulfates metabolites

BACKGROUND

Through the past decades, epidemiological studies have revealed that diets rich in fruits and vegetables promote health benefits, preventing against age-related disorders. Such pathologies are still cure-less and berries emerge amongst the most promising fruits, rich in (poly)phenols, which are increasingly regarded as effective protectors. (Poly)phenols and their metabolites must be bioavailable to exert biological effects. Taking this into consideration, a previous human intervention study was performed and different phenolic sulfate metabolites were identified in plasma samples. The most relevant metabolites were chemically synthesized and physiological concentrations circulating in plasma were determined (1). Evidences indicate that simple (poly)phenols can enter in the central nervous system in very small amounts but little is known about their effects in the brain, especially regarding neuroinflammation.

Severe neuroinflammatory alterations are frequently described in age-related disorders. Therefore, microglia, as the resident innate immune cells in the central nervous system plays a crucial role mediating and modulating this inflammatory response. These cells represent about 5–20% of all glial cells in the central nervous system. However, when chronically activated, these cells become deleterious by the uncontrolled release of diverse molecular mediators such as inflammatory cytokines. Because microglia derived from myeloid-monocytic precursor cells, they regulate inflammatory pathways in a similar way as the peripheral immune cells do. In this context, NF-kB and NFAT are crucial regulators of the inflammatory cascades and their study is imperative in order to fully understand the biological effect underlying (poly)phenol bioactivities in microglial cells.

1. Pimpão RC, Ventura V, Ferreira RB, Williamson G, Santos CN, (2015) Phenolic-sulfates as new and highly abundant metabolites in human plasma after ingestion of a mixed berry fruit puree, B J Nutrition, 113, 454-63.

OBJECTIVES

The aim of this project is to understand the neuroinflammatory modulation exhibited by the Human phenolic sulfates metabolites in murine microglia, using three different approaches: pre-treatment, co-treatment and post-treatment as preventive, therapeutic and recovery approaches, respectively.

PROJECT DESCRIPTION

TASK 1: Analysis of pro- and anti-inflammatory markers

A murine microglia cell line will be used as the model of neuroinflammation. The first step will be the optimization for the detection of diverse pro- and anti-inflammatory cytokines (or other markers). Parameters to be tested will be the kinetics and concentrations of the insults and the human phenolic sulfates metabolites.

Two types of insults will be tested, LPS for the NF-kB pathway stimulation and ATP for the NFAT pathway stimulation.

Additionally, different approaches to characterise the protective activity of phenolic sulfates metabolites will be performed: pre-treatment; co-treatment and post-treatment. Markers that will be evaluated include:

- TNF-α; MPC-1; MIP-1α; IL-1α; IL-4; IL-6; IL-10 by ELISA
- NO by Griess reaction
- superoxide and CD-40 by flow cytometry
- qPCR for some pro- and anti-inflammatory markers (TNF-α; IL-10; arginase I; TGF-β)

TASK 2: Study of the NF-kB activation profile

It will be studied the NF-kB activation profile, in the LPS insulted cells, with focus on the anti-inflammatory activity of human phenolic sulfates metabolites, for the condition (pre-treatment, co-treatment or post-treatment) more efficiently reducing the inflammatory markers (Task 1).

Changes in the activation state of NF-kB will be followed by western blot for phospho (ser536) NF-kB p65 and total NF-kB p65, for the analysis of the phosphorylation ratio. Another important player is the IkB (NF-kB inhibitor). To assess it, western blot analysis for the IkB levels will be done. Finally, nuclear translocation of NF-kB will be evaluated by immunocytochemistry for NF-kB p65.

TASK 3: Evaluation of NFAT activation

It will be investigated the NFAT activation profile, in the ATP insulted cells, with focus on the anti-inflammatory activity of human phenolic ates metabolites, for the condition (pre-treatment, co-treatment or post-treatment) more efficiently reducing the inflammatory markers (Task 1).

First, a fractionated protein extraction will be done to isolate nuclear and cytoplasmic protein. Then NFAT protein levels will be assessed for both protein fractions by western blot, in order to determine de activation ratio for this important transcription factor. Finally, because calcineurin in an upstream regulator of NFAT, the calcineurin activity will be analysed.

TIMELINE

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Thesis										