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Peripheral blood mononuclear cells from rat model of pleurisy: The effects of hesperidin on ectoenzymes activity, apoptosis, cell cycle and reactive oxygen species production

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ABSTRACT

The present study investigates the effect of hesperidin; a flavonone commonly found in citrus fruits, on the ectoenzymes (ectonucleotidase and ecto-adenosine deaminase) activity, cell viability, apoptosis, cell cycle arrest and reactive oxygen species production in peripheral blood mononuclear cells (PBMCs) from rat model of pleurisy. Wistar rats were pretreated with either saline or hesperidin (80 mg/kg) by oral gavage for 21 days and injected intrapleurally with 2% carrageenan or saline on the 22nd day. PBMCs were subsequently prepared after 4 h of carrageenan induction. The results revealed that hesperidin may exhibit its anti-inflammatory effects through possible modulation of ectonucleotidase (E-NTPDase) and ecto-adenosine deaminase (E-ADA) activities, reduction of intracellular reactive oxygen species, prevention of DNA damage and modulation of apoptosis as well as activation of cell cycle arrest. This study suggests some possible underlying anti-inflammatory mechanisms of hesperidin on PBMCs in acute inflammatory condition. Furthermore, hesperidin may minimize oxidative injury mediated pleurisy in rat.

1. Introduction

Acute inflammation represents a rapid, nonspecific and spontaneous response of a tissue to injury [1]. It serves as the first line of defense against tissue injuries; consisting of both local and systemic coordination as well as mobilization of immunological, endocrinological and neurological mediators [2]. Pleurisy induced with carrageenan has been reported to be a veritable experimental model for assessing acute inflammation in rats [3–5]. It has been reported that pleurisy can activate immune system and elicit inflammatory responses [6,7]. These inflammatory responses are characterized by elevation in blood vessels permeability, exudation, migration and activation of polymorphonuclear neutrophils (PMNs), production of prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor, and interleukins [8–10].

Reactive oxygen species (ROS) produced from PMNs and other cell sources, may play a crucial role in the destruction of tissues and organs that are associated with the inflammatory process [11]. Perhaps, if the oxidative injury or insult persists, the inflammatory response may become chronic and may no longer be a physiological event but rather pathological [7,12]. Several cell types, including neutrophils, macrophages, monocytes and lymphocytes, have been implicated in the incidence of acute inflammation [7]. Lymphocytes (B and T cells), are present in lung parenchyma and along the airways [6]. Cells may respond to stress in a number of ways including the activation of pathways that permit survival and promotion of apoptosis (programmed cell death) as well as elimination of damaged cells [13].

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Extracellular nucleotides and nucleosides, including adenine nucleotides and nucleoside derivatives, are purinergic signaling molecules that perform crucial roles in immunological and inflammatory reactions [14]. Ectoenzymes are group of membrane bound enzymes that are often expressed in immune cells during the purinergic signaling [15]. These ectoenzymes include ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase; CD39; EC 3.6.1.5) which catalyses the spontaneous breakdown of adenosine 5′ triphosphate (ATP) to adenosine 5′ diphosphate (ADP) and adenosine 5′ monophosphate (AMP), ecto-5′-nucleotidase (EC 3.1.3.5; CD73) which catalyses the hydrolysis of AMP to adenosine and ecto-adenosine deaminase (E-ADA; EC 3.5.4.4) catalyses the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine [14,16,17]. Adenosine, its receptor and ectoenzymes are critical players in the regulation of immune responses [15]. When adenosine is present in the extracellular environment, it protects the cells and tissues from excessive inflammation and immune-mediated damage. During inflammation and initiation of primary immune responses, ATP is released into the extracellular environment following cell damage [14]. ATP; a damage associated molecule and a potent activator of the immune response, increases the production of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α and IL-2 [14–16]. ATP also increases the potency of oxidative stress response of activated macrophages [14].

Flavonoids are group of polyphenolic compounds, synthesized by plants and have been known for their health-promoting properties [18]. These properties have been linked to the high antioxidant capacity of flavonoids in both *in vivo* and *in vitro* systems as a result of their functional hydroxyl groups [18,19]. Citrus fruits and their byproducts are rich sources of hesperidin [20]. Hesperidin is a flavanone glycoside containing hesperetin and rutinose [21]. Hesperidin derivatives have been reported for their anti-inflammatory activity *in vivo* and *ex vivo* [22,23]. It has been shown to exhibit antimicrobial [24], antioxidative [25], anticancer [26], cardioprotective [27], hepatoprotective [28], nephroprotective [29], neuroprotective [30] and antiarthritic [31] effects.

Considering the possible role of ectoenzymes, apoptotic and antioxidant signaling in immunomodulation of different animals or human cell types with acute inflammation, we hypothesize that hesperidin may act as an immunosuppressant and modulate cells involved in immune response. Thus, this study is aimed at investigating the effect of hesperidin on ectoenzymes activity, cell cycle, apoptosis and reactive oxygen production in peripheral blood mononuclear cells (PBMCs) of rats intrapleurally induced with carrageenan.

2. Materials and methods

2.1. Instruments and reagents

Sigma Laboratory centrifuge 4K15 (DJB Labcare Limited, Buckinghamshire, England), BD Accuri™ C6 flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA, microplate reader UV/Visible spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). Hesperidin, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine, trizma base, coomassie brilliant blue G, bovine serum albumin, Ficoll-Histopaque, RPMI 1640, 3-[4–5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 5-(and-6)-carboxy-2′,7′-dichloro-dihydro-fluorescein diacetate, λ-carrageenan and propidium iodide were obtained from Sigma Chemical Co (St. Louis, MO, USA) and Alexa Fluor 488-conjugated annexin V kit from Invitrogen (ThermoFisher Scientific, Waltham, MA USA). Dipotassium phosphate was bought from Reagen (Colombo, PR, Brazil). All the other chemicals used in this experiment were of the highest purity.

2.2. Animals

Twenty-four (24) Adult female Wistar rats (6 months old, weighing 350–450 g) were used in this experiment. The animals were maintained at constant temperature (23 ± 1 °C) on a 12 h light/dark cycle with free access to food and water. All animals were maintained in accordance with the guidelines on the use of animals, approved by the Ethics Committee of the Federal University of Santa Maria, Brazil (protocol number: 1071171215)

2.3. Experimental groups & carrageenan induction

The rats were pretreated with either sterile saline (0.9% NaCl) or hesperidin (80 mg/kg) by oral gavage for twenty-one (21) days. In this study, the hesperidin dose was chosen based on earlier report by Guardia et al. [32]. On the twenty-second (22nd) day, all the animals were intraperitoneally injected with anesthesia (ketamine and chillaxin, 3: 1 v/v) about 10 min prior to the intrapleural (i. pl.) injection of carrageenan in inflammation group and saline solution in control group. The pleurisy was induced by injection of 0.2 mL of sterile saline solution (NaCl 0.9%) containing λ-carrageenan (2%) into the right pleural space of animals under anesthesia according to the method described by Petronilho et al. [4]. The rats were divided into four (4) groups of 6 animals each namely;

- Group I: rats were administered saline solution only (CTR group);
- Group II: rats were administered saline solution (0.9% NaCl) orally and injected intrapleurally with carrageenan (INF group);
- Group III: rats were administered 80 mg/kg of hesperidin only (HESP group);
- Group IV: rats were administered 80 mg/kg hesperidin orally and injected intrapleurally with carrageenan (INF + HESP group);

Prior to euthanasia, rats were anaesthetized with isoflurane. Subsequently, the chest was carefully opened and about 5 mL of blood was collected by cardiac puncture into ethylenediaminetetraacetic acid (EDTA) bottles for the isolation of peripheral blood mononuclear cells (PBMCs).

2.4. Isolation of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were isolated from rat blood collected into bottle containing 7.2 mg dipotassium EDTA as anticoagulant and separated on Ficoll-Histopaque density gradients as described by Böyum [33]. After centrifugation, the cell pellet was washed with phosphate-buffered saline (PBS), pH 7.4 and suspended in Roswell Park Memorial Institute (RPMI) 1460 medium (Sigma–Aldrich, St Louis, USA) supplemented with 10% Fetal Bovine Serum (Vitrocell, Campinas, Brazil), 100 U/mL penicillin and 100 μg/mL streptomycin Complete Growth Medium (CGM). Cells were counted in the Neubauer chamber and adjusted to 1×10^6 cells/mL. The isolated PBMCs were predominantly lymphocytes as reported by Jaques et al. [34].

2.5. Histopathological examination of the lungs

Lungs (right side) were excised, collected and fixed in 10% formalin solution and then dehydrated and embedded in paraffin, followed by sectioning and histopathological staining with haematoxylin and eosin. The slides were observed in optical