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Research Paper

New insights into the effects of onion consumption on lipid mediators using a diet-induced model of hypercholesterolemia

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ABSTRACT

The levels and roles of lipid mediators can be modified in response to nutritional stimuli. The aim of this study was to investigate shifts in oxylipin and sphingolipid profiles stimulated by a hypercholesterolemic (HC) diet along with the modulating effects of onion introduced as an antioxidant functional ingredient characterized in the diet (HCO). Oxylipin and sphingolipid profiles were determined in plasma and tissues from Wistar rats using LC-MS/MS. Plasma ω-3 and ω-6 PUFA-derived oxylipins decreased in rats after 7 weeks of HC feeding, but did not evidence a further shift with HCO diet. Onion ingredient supplementation modulated the hepatic concentrations of prostaglandins and enhanced ω-3 oxylipins in the liver of HCO-fed rats relative to the HC group. The HC diet induced shifts in plasma sphingolipids, increasing sphingoid bases, dihydroceramides and ceramides, whilst the sphingomyelin, hexosylceramide and lactosylceramide families decreased. The HCO diet modified some HC diet-induced changes in sphingolipids in liver and spleen tissue. Onion supplementation effected changes in lipid mediator levels in diet-induced hypercholesterolemic Wistar rats. The potential of onion as regulator of pro-inflammatory mediators, and possible enhancer of pro-resolution pathways, warrants further study of the interaction of functional ingredients with bioactive lipid mediators and their potential impact on inflammation, oxidative stress and organ dysfunction.

1. Introduction

Specific dietary patterns have significant implications in the development of multiple alterations directly associated with inflammatory events [1,2]. Inflammation, both acute and chronic, is triggered as a response to different disorders, including autoimmune, metabolic, intestinal or homeostatic imbalances that can be found in a large variety of diseases [3–5]. Several factors, including the genetic component as well as the microbial and environmental conditions, have been suggested to influence its initiation, progression and resolution [5]. In addition, there is a growing interest in dietary components as key antioxidants and inflammatory regulators [6–10]. However, the difficulty to define the direct effect of individual dietary components on certain pathologies and their translation on human health is well recognized [11–14].

Hypercholesterolemia and inflammation are known to be closely linked processes, and they are clearly implicated in non-alcoholic fatty liver disease and atherosclerosis development [15]. Dietary cholesterol intake is known to induce an elevation of lipids and stimulate oxidative stress, which lead or promote the action of pro-inflammatory signaling cascades [16–18]. On the other hand, the bioactive nature of some foods like onion (*Allium cepa* L.), which is an important dietary source of antioxidant and anti-inflammatory compounds, has been related to the modulation of such cascades and could reduce the production of pro-inflammatory mediators. The discoveries about the inhibitory effect of garlic and onion exerted on the conversion of arachidonic acid (AA) into eicosanoids metabolism were summarized by Ali et al. [19]. More recently, another study also evidenced the potential activity of some compounds found in small yellow onion on the modulation of cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LOX) activity [20]. In a recent report, Suleria et al. [21] stated that onion-derived phenolic compounds like flavonols and organosulfur compounds (specially thiosulfinates) play relevant anti-inflammatory effects, showing interactions to the inhibition of arachidonic acid metabolic pathways.

Oxylipins in general have attracted great interest as oxidation products of fatty acids such as arachidonic acid (AA), linoleic acid (LA), α-linolenic acid (α-LA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This process is mainly initiated *via* three

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enzymatic pathways: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP). It is well accepted that the levels of oxylipins in the open circulation and tissues may give insight into their role in the response to different physiological abnormalities, including a disequilibrium of the lipid levels, and different metabolic stresses accompanied by inflammation [22]. Likewise, sphingolipids are an important class of structural components and signaling molecules within the cell, whose metabolism can also be modified by constituents of the diet, such as cholesterol, with consequences for cell regulation and disease [23]. The biochemical synthesis of sphingolipids and their regulation are involved in the response processes of pathogenesis of metabolic and cardiovascular dysfunctions (*e.g.*, type 2 diabetes, insulin resistance, obesity, metabolic syndrome, atherosclerosis and cardiomyopathy) [24]. Moreover, sphingolipids participate in multiple cellular signaling pathways such as the responses to cytokines and stress [25].

In addition, the presence and abundance of certain lipids in a specific tissue may give insight about the impact of pathological damage. This may lead to the discovery of new markers of injury and possible diagnosis when released and detected in blood [26,27]. However, there are not many studies about the effect of functional food ingredients on the synthesis and actions of oxylipins and sphingolipids.

Recent findings by our group have shown that the consumption of onion processed for use as a functional ingredient, induced changes in the circulating fatty acids [28] and the recovery from the oxidative damage caused by a cholesterol overload in the antioxidant and vascular status of hypercholesterolemic Wistar rats [29]. Nonetheless, the functionality of this onion product as a powdered dietary ingredient on the modulation of signaling bioactive lipid mediators and its possible connection with findings addressing vascular benefits still remains unknown. Therefore, the aim of the present study was to determine the impact of diet enrichment with onion in plasma and tissue oxylipin and sphingolipid levels of hypercholesterolemic Wistar rats using ultra performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) targeted approaches.

2. Materials and methods

2.1. Onion ingredient preparation

Onions (Allium cepa L. var *cepa*, 'Recas') supplied by Cebacat (Asociación Catalana de Productores-Comercializadores de Cebolla) were harvested in Lleida (Spain) and stored at 4 °C until processing. Onions free from external damages were hand-peeled, cut into 10 mm diced-pieces, packaged in bags with very low gas permeability (Doypack®, Polyskin XL, Amcor Flexibles Hispania, S.L., Granollers, Barcelona, Spain) and treated with high-pressure to obtain a stable functional ingredient as previously described [30]. Briefly, the high-pressure treatment (400 MPa, 5 min, 25 °C) was applied in a High Pressure Iso-Lab System [High Pressure Iso-Lab System (model FPG7100:9/2C, Stansted Fluid Power Ltd., Essex, UK)]. The onion processed was directly frozen with liquid nitrogen and freeze-dried using a lyophilizer (model Lyoalfa, Telstar, S.A., Barcelona, Spain). A subsequent pulverization of the lyophilized diced onion was carried out with an ultra centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany) obtaining a particle size ≤250 μm. The obtained onion powder was stored at −20 ± 0.5 °C until dosage for the formulation of the diets. The nutritional and phytochemical composition, and antioxidant activity of the onion ingredient is presented in Supplementary Table S1.

2.2. Experimental design

The present study was performed in compliance with the Directive 2010/63/UE regarding the protection of animals used for scientific purposes and was approved by the Spanish Ministry of Science and Innovation Advisory Committee [project AGL2010-15910 (subprogram ALI)] and by an Ethics Committee of the Complutense University of Madrid (Spain).

Eight weeks-old male Wistar rats (n=24) with a weight of 245 ± 5 g were supplied by Harlan Laboratories Models (Harlan, SL, Barcelona, Spain) and transferred to the Faculty of Medicine in the Complutense University of Madrid (Spain) to conduct the experiments. Animals were acclimatized for 1 week and then randomly assigned to 3 experimental groups in individual metabolic cages placed under controlled conditions of light (12 h light/dark), and temperature (22 ± 1 °C). Each group (n=8) received water *ad libitum* and had free access to one of the following three diets: control (C) diet, composed of a homogeneous mixture of 100% rodent diet (based on the AIN-93M diet); high-cholesterol (HC) diet, composed of control diet with 2% cholesterol and 0.5% cholic acid and high-cholesterol enriched with onion (HCO) diet was identical to the HC diet, but with 10% onion powder, balancing the dietary fibre with cellulose powder. The amount of maize starch in the HC and HCO diets was adjusted to compensate for the addition of cholesterol and cholic acid in the HC diet, and onion powder in the HCO diet. The exact composition of each diet can be found in Supplementary Table S2.

Animal feeding and water was daily replaced to monitor a normal rate of consumption, deposition of feces and urine throughout the 7 weeks of experimental feeding. Body weight was weekly evaluated ensuring the correct growth of all animals until euthanasia.

2.3. Blood and tissues sampling

Animals in fasting conditions were anaesthetized and euthanized by extracting blood by cardiac puncture until exsanguination, taking randomly one animal at a time, of each one of three groups. Blood collection was carried out in BD Vacutainer® tubes containing EDTA as anticoagulant. Plasma was obtained from each sample by centrifuging at 1,500*g* for 15 min at 4 °C and stored at −80 °C in aliquots.

Organ collection of heart, liver and spleen was conducted in aseptic conditions just after exsanguination. The organs of each animal were carefully collected and fat was removed. Dissected parts were individually frozen in liquid nitrogen and immediately stored at −80 °C.

Homogenates from laminar samples of the heart, the central lobe of liver and the spleen were prepared for extraction and analysis. Approximately, 50 mg and 20 mg of tissue (for oxylipins or sphingolipids, respectively) were weighed and homogenized in 200 μL of methanol with a Bullet Blender®. Homogenization was performed using 0.5 mm Zirconium Oxide Beads (liver and spleen tissues) or Stainless Steel Beads 0.9–2.0 mm (heart tissues). In order to avoid inter assay variations that could affect the comparison of data from different groups, all samples of the same type of tissue were manipulated on dry ice, weighed in a controlled room temperature (4 °C) and kept in Eppendorf® tubes at −80 °C the same day. Then, each set of samples was extracted separately and analyzed on consecutive days.

2.4. UPLC-MS/MS determination of oxylipins

Extraction of oxylipins from plasma and liver homogenate was carried out as previously described [31] with minor modifications. Briefly, 10 μL of internal standards solution were spiked to 250 μL of plasma or 50 mg of liver tissue homogenate. After dilution of samples with 2.5 mL of 0.1% acetic acid in water, solid phase extraction was performed using Oasis HBL 60 mg cartridge columns (Waters, Milford, MA) [31] and dry extracts were stored at −80 °C for a maximum of two days. On the day of analysis, extracts were reconstituted in 100 μL of MeOH and filtered using 0.1 μm membrane spin filters (Merck Millipore, Darmstadt, Germany). The chromatographic separation was performed on an Acquity UPLC separation module (Waters) equipped with a 2.1x150 mm BEH C18 column with a 1.7 μm particle size (Waters). Oxylipins were determined using a Xevo TQ-S mass