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Quince (*Cydonia oblonga* Miller) peel polyphenols modulate LPS-induced inflammation in human THP-1-derived macrophages through NF-κB, p38MAPK and Akt inhibition

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Chronic inflammation is a hallmark of several pathologies, such as rheumatoid arthritis, gastritis, inflammatory bowel disease, atherosclerosis and cancer. A wide range of anti-inflammatory chemicals have been used to treat such diseases while presenting high toxicity and numerous side effects. Here, we report the anti-inflammatory effect of a non-toxic, cost-effective natural agent, polyphenolic extract from the Tunisian quince *Cydonia oblonga* Miller. Lipopolysaccharide (LPS) treatment of human THP-1-derived macrophages induced the secretion of high levels of the pro-inflammatory cytokine TNF-α and the chemokine IL-8, which was inhibited by quince peel polyphenolic extract in a dose-dependent manner. Concomitantly, quince polyphenols enhanced the level of the anti-inflammatory cytokine IL-10 secreted by LPS-treated macrophages. We further demonstrated that the unexpected increase in IL-6 secretion that occurred when quince polyphenols were associated with LPS treatment was partially responsible for the polyphenols-mediated inhibition of TNF-α secretion. Biochemical analysis showed that quince polyphenols extract inhibited the LPS-mediated activation of three major cellular pro-inflammatory effectors, nuclear factor-kappa B (NF-κB), p38MAPK and Akt. Overall, our data indicate that quince peel polyphenolic extract induces a potent anti-inflammatory effect that may prove useful for the treatment of inflammatory diseases and that a quince-rich regimen may help to prevent and improve the treatment of such diseases.

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1. Introduction

Acute inflammation is a protective response of the host against irritation, injury and infection. Its primary function is to induce the secretion of several pro-inflammatory gene products such as TNF-α, IL-8 and IL-6, while low levels of products with anti-inflammatory effects such as IL-10 and TGF-β are produced by the insulted tissue. However, when it becomes chronic, inflammation can lead to cancer, diabetes, and pulmonary, cardiovascular and autoimmune diseases. Nuclear factor-kappaB (NF-κB) and p38mitogen-activated protein kinase (MAPK) have been reported to be the major effectors of inflammation through the induction of several cytokines (IL-1β, TNF-α and IL-6) and enzymes such as cyclooxygenase 2 (COX-2) [1]. In mammals, the phosphatidylinositol-3-kinase (PI-3K)/Akt pathway, has also been reported to control monocyte inflammation [2]. While inhibitors of Akt and p38MAPK are still being studied with extensive assays for possible use in the treatment of inflammatory diseases [3–6], many anti-inflammatory drugs targeting NF-κB/COX-2 have been used in the last decades [7]. However, these drugs are highly toxic, and their use is frequently associated with side effects, ranging from dyspeptic symptoms to life-threatening bleeding or perforation of gastroduodenal ulcers [8]. Plants polyphenols are a group of chemicals that have more than one phenol ring per molecule. They are found in grapes, berries, tea, coffee, soybean and other fruits and vegetables that represent important parts of the human diet [9]. Several studies have shown that the high consumption of polyphenols has protective effects against cancer and inflammatory diseases [10]. The anti-inflammatory effects of polyphenols have been attributed primarily to their antioxidant activity because they were known to scavenge and prevent the formation of reactive oxygen (ROS) and nitrogen (RNS) species [11–14], which are important hallmarks of inflammation. Nevertheless, the anti-inflammatory effects of

Abbreviations: 7-AAD, 7-amino-actinomycin D; LPS, lipopolysaccharides.

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polyphenols are also thought to rely on their ability to inhibit the activation of several pro-inflammatory pathways, such as the MAPK, the PI3-K/Akt and the NF-κB pathways [11]. We previously reported the potent antioxidant activity of polyphenolic extract from the Tunisian quince (*Cydonia oblonga* Miller) peel [15]. However, its potential anti-inflammatory effect has never been assessed. In this study, we investigated the effect of this extract on LPS-induced inflammation of human THP-1-derived macrophages.

2. Materials and methods

2.1. Quince peel polyphenols extraction

Quince peel polyphenols preparations along with the assessment of their antioxidant activity were performed as previously reported by our team [15]. Each compound was identified and quantified using high-performance liquid chromatography with diode-array detection (HPLC–DAD) coupled on line to a mass spectrometer (MS) [15].

2.2. Cell culture and induction of inflammation

Human myelomonocytic cell line THP-1 (American Type Culture Collection TIB-202) was maintained in RPMI 1640/Glutamax-I media (Invitrogen) supplemented with 10% heat-inactivated foetal calf serum (Sigma) plus penicillin G (100 U/ml) and streptomycin (100 μg/ml), subsequently named 'complete media'. THP-1 cells were differentiated to macrophages as previously described with a few modifications [17]. Cells were treated with 20 ng/ml phorbolmyristate acetate (PMA) (Sigma, St. Louis, MO, USA) for 48 h at 37 °C, 5% CO_2. Differentiated cells were then washed three times with RPMI 1640, seeded in 24-well tissue culture plates at 5×10^5 viable cells per well in complete media and incubated for one more day at 37 °C, 5% CO_2. Cells were treated with LPS (1 μg/ml) in the presence or absence of different concentrations of quince peel polyphenols. The cells were counted 24 h later, and their viability was assessed with a trypan blue exclusion assay. The supernatants were then collected and stored at −80 °C until they were assayed for cytokine release as detailed below.

2.3. Assessment of polyphenols cytotoxicity

Polyphenols cytotoxicity was evaluated using the annexin V/7-amino-actinomycin D (7-AAD) apoptosis detection kit (BD Bioscience) according to the manufacturer's protocol. THP-1-derived macrophages were incubated in the presence or absence of different concentrations of quince peel polyphenols for three days. Cells were stained with annexinV-PE/7-AAD, and viability was assessed on a Becton Dickinson FACScantoII flow cytometer and further analysed with BD FACSDiva6 software (Becton Dickinson). Cell death was quantitatively evaluated by measuring the proportion of annexin V-positive cells, regardless of their staining for 7-AAD in order to include both apoptotic and necrotic cell death.

2.4. Cytokine quantification

Harvested supernatants were quantified for their TNF-α, IL-8, IL-6 and IL-10 contents by sandwich enzyme-linked immunosorbent assay (ELISA) kit, the OptEIA from BD Biosciences. The dosages of cytokines were determined according to the manufacturer's instructions. Cytokine levels were normalized to the cell number.

2.5. Statistical analysis

Data from individual experiments are expressed as means ± S.E. Differences between means were evaluated using an unpaired, two-sided Student's *t*-test. Differences with *p*-values of less than 0.05 were considered statistically significant.

2.6. Western blotting

THP-1-derived macrophages were mock-treated or stimulated with 1 μg/ml LPS for 10, 30 or 60 min in the presence or absence of 20 μg/ml of peel quince polyphenolic extract. Cells were washed twice with ice-cold PBS to arrest the stimulation and then lysed at room temperature with 100 μl 1× Laemmli buffer per 5×10^5 cells. Whole-cell lysates (30 μg/lane) were then separated by sodium dodecylsulfate polyacrylamide gel electrophoresis, transferred to polyvinyl difluoride membrane and probed by immunoblotting with primary and HRP-conjugated secondary antibodies followed by enhanced chemiluminescence (Amersham, GE Healthcare). Rabbit monoclonal anti-phospho-p38 α MAPK (Thr180/Tyr182), rabbit polyclonal anti-p38 α MAPK, rabbit monoclonal anti-phospho-Akt ($Serine^{473}$) and rabbit polyclonal anti-phospho-IκK α (Ser180)/IκKβ (Ser181) primary antibodies along with the HRP-conjugated secondary antibodies were purchased from Cell Signalling Technology. Goat polyclonal anti-Akt1 and rabbit anti-ERK2 antibodies were from Santa Cruz Biotechnology.

3. Results and discussion

Macrophages are the major players of the innate immune response that promote inflammation via production of various key biomediators, including cytokines such as TNF-α and IL-6 and chemokines such as IL-8. However, these same cells can adapt an anti-inflammatory behaviour in order to stop the acute inflammation and retrieve a steady state through the secretion of immuno-modulating agents such as TGF-β and IL-10 [16]. The human THP-1 cell line and THP-1-derived macrophages have been reported to be useful cellular models for both host/pathogen interaction studies and anti-inflammatory drug screening [17,18]. Here, we used THP-1-derived macrophages to assess the effect of quince peel polyphenolic extract on LPS-induced inflammation.

3.1. Phenolic profiles analysis of peel quince extract

About 130 mg of polyphenols per 100 g of quince peel were purified as detailed in Section 2. The phenolic composition of such peel acetonic extract along with the quantification of each molecule were previously performed by our team based on a combination of retention time and spectral matching [15]. According to Table 1, the major polyphenols in the Tunisian quince peel extract are hydroxycinnamic acids, principally the chlorogenic acid that represented 13% with rutin as the major polyphenol (36%). Flavonols are present as mixture of different aglycone and glycosylated quercetin and kaempferol. Flavanols are essentially catechins and procyanidins. The cell toxicity of the polyphenolic extract was assessed by counting viable cells after three days treatment with different concentrations of quince peel polyphenols. The annexinV/7-AAD binding assay revealed that this extract did not affect cell viability below a dose of 200 μg/ml (50% cell viability) as shown in Fig. 1. As a result, we chose not to exceed a dose of 20 μg/ml to study the effect of quince peel polyphenols on LPS-induced inflammation of THP-1-derived macrophages over a 24-h period.

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