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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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ABSTRACT

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.

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.

Table 1
HPLC–DAD–MS analysis of main phenols in quince peel acetonic extract as reported by Fattouch et al. [15].

Peak #	Rt[a] (min)	HPLC–DAD λ_{max} (nm)	$[M+H]^+$ m/z	Identity	Concentration (mg/100 g fw)[b]
1	6.83	279	291	(+)-Catechin	5.07 (2.15)
2	7.55	325	355	Neochlorogenic acid (3-*O*-Caffeoylquinic acid)	3.94 (0.17)
3	8.31	279	291	(−)-Catechin	0.10 (0.24)
4	11.63	324	355	Cryptochlorogenic acid (4-*O*-Caffeoylquinic acid)	0.51 (0.32)
5	12.37	326	355	Chlorogenic acid (5-*O*-caffeoylquinic acid)	12.85 (0.16)
6	17.71	278	579	Procyanidin	NQ
7	32.53	355	465	Hyperin (Quercetin-3-*O*-galactoside)	12.40 (2.37)
8	33.31	356	611	Rutin (Quercetin-3-*O*-rutinoside)	47.21 (4.56)
9	35.84	275	595	Kaempferol-3-*O*-rutinoside	3.96 (1.10)
10	36.06	354	465	Isoquercitrin (Quercetin-3-*O*-glucoside)	9.23 (2.79)
11	43.55	278	449	Kaempferol 3-*O*-glucoside	10.65 (3.81)
12	6.77	371	303	Quercetin	7.01 (2.92)
13	48.67	313	612	Quercetin glycoside acylated with *p*-coumaric acid	5.92 (1.15)
14	49.57	340	287	Kaempferol	12.60 (3.87)
Total	–	–	–	–	131.45 (25.61)

Standard deviation is reported in parentheses ($n = 3$).
NQ: not quantified.
[a] HPLC retention time.
[b] Based on the fresh weight (fw).

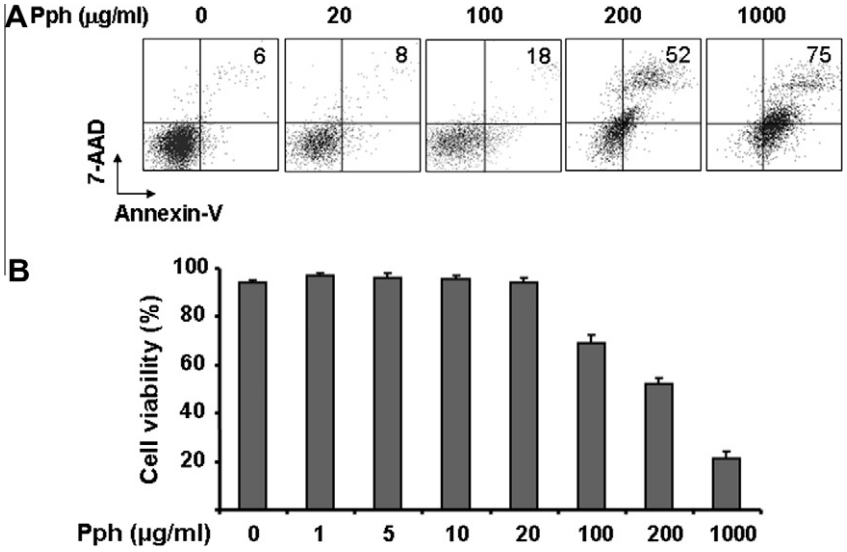

Fig. 1. Low concentrations of quince peel polyphenolic extract present no toxicity to THP-1-derived macrophages. Human THP-1-derived macrophages were incubated for three days with different concentrations of quince peel polyphenolic extract (Php) as indicated. Cell survival was then measured by annexin V-PE/7-AAD staining and flow cytometry analysis. (A) Diagrams show one experiment representative of two independent experiments. Percentages of dead and apoptotic cells (annexin V-positive) are on the top right side of each diagram; only the concentrations of 20, 100, 200 and 1000 µg/ml of polyphenols are represented. (B) Histograms representing the effect of different concentrations of quince peel polyphenols on cell viability (annexin V-negative). Results are reported as the means ± SE of two independent experiments performed in duplicate ($p < 0.05$).

3.2. Quince peel polyphenols inhibit LPS-induced secretion of TNF-α and IL-8

LPS treatment (1 µg/ml) of THP-1-derived macrophages for 24 h induced the secretion of the pro-inflammatory effector TNF-α (~1.7 ng/10^6 cells) that decreased in a dose-dependent manner when quince peel polyphenolic extract was added to the cells. A maximum inhibition of 55% was reached with a dose of 20 µg/ml of quince peel polyphenols (Fig. 2A). It is worth noting that treatment of macrophages with this extract alone did not affect the levels of any of the studied cytokines (Figs. 2 and 3). Analysis of the cell supernatants for the presence of IL-8 also showed a similar dose-dependent inhibition of LPS-induced secretion of such pro-inflammatory chemokines by the quince peel polyphenolic extract. The inhibition level reached nearly 50% with the highest dose of polyphenols (Fig. 2B). TNF-α and IL-8 have major systemic effects when produced either acutely in large amounts, as in the case of bacterial sepsis, or chronically in lesser amounts, as in the case of chronic infections and inflammatory diseases [19]. These two effectors of inflammation are also involved in both neutrophils activation and their recruitment to the inflamed tissue [20]. The modulation of the secretion of TNF-α and IL-8 is therefore of paramount importance to counteract pathologic inflammation. This is better supported by the relative success of anti-TNF therapy using neutralizing anti-TNF antibodies [21] and the fact that several chemical anti-inflammatory drugs act through the inhibition of TNF-α and IL-8 secretion [7]. However, most of these drugs are expensive and present high toxicity, and their use is frequently associated with side effects [22]. Our data show the clear inhibition of the LPS-induced secretion of both TNF-α and IL-8 by quince peel polyphenols extract, suggesting a newly discovered anti-inflammatory feature of quince polyphenols. This harmless product may then be proposed as an alternative, cost-effective product that may help in preventing and/or treating inflammatory disorders.

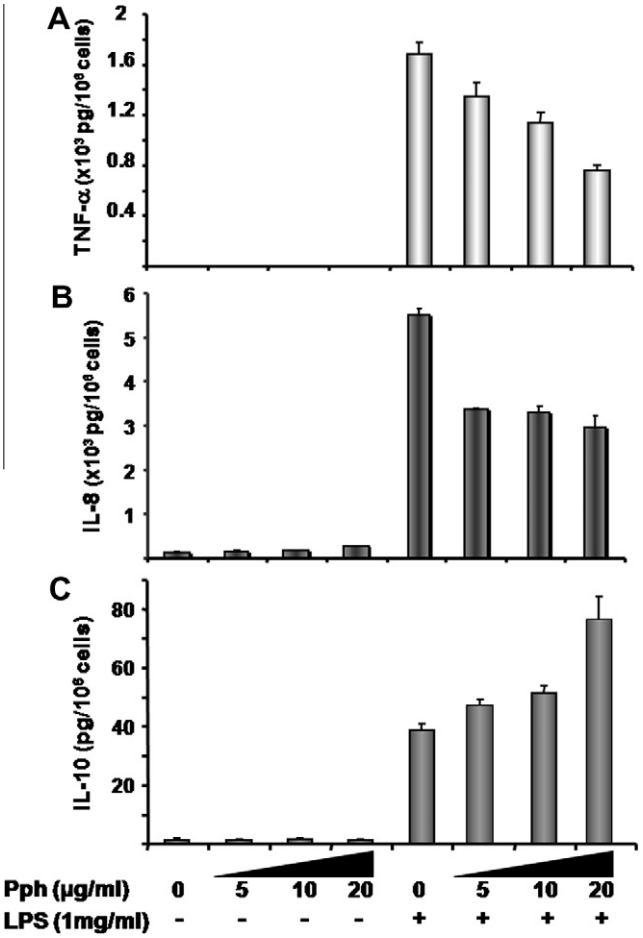

Fig. 2. Quince peel polyphenols inhibited LPS-induced secretion of (A) TNF-α and (B) IL-8 while augmenting the secretion of (C) IL-10. Human THP-1-derived macrophages were incubated for 24 h in the absence or presence of 1 μg/ml LPS with or without the indicated concentrations of quince peel polyphenols. Supernatants were then collected and analysed for cytokine content by ELISA. Results are reported as the means ± SE of three independent experiments performed in triplicate ($p < 0.05$).

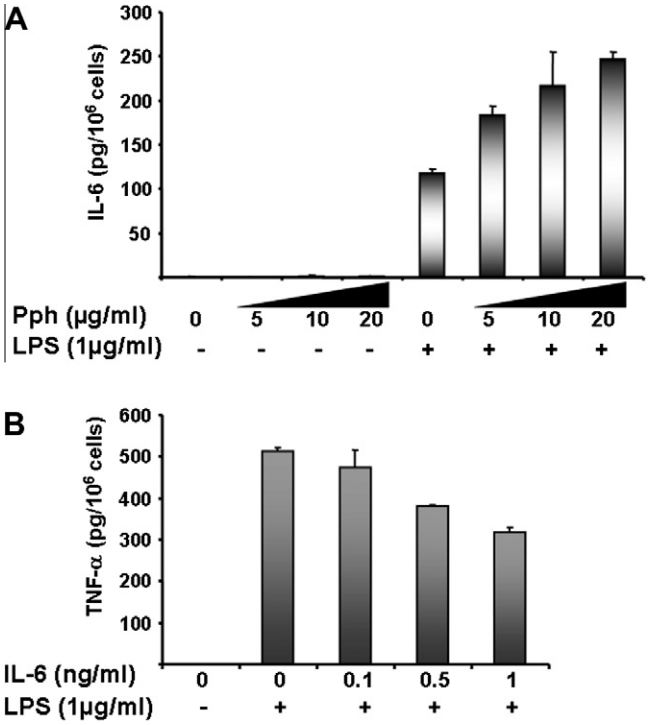

Fig. 3. Quince peel polyphenols-mediated inhibition of the LPS-induced secretion of TNF-α is partially mediated by IL-6. (A) Human THP-1-derived macrophages were incubated for 24 h in the absence or presence of 1 μg/ml LPS with or without the indicated concentrations of quince peel polyphenols. Supernatants from the treated cells were analysed for IL-6 content by ELISA. (B) Cells were treated with LPS as described above with or without the indicated concentrations of human recombinant IL-6. The data shown represent the means ± SE of three independent experiments performed in triplicate ($p < 0.05$).

3.3. Quince peel polyphenols augment LPS-induced secretion of IL-10

IL-10 is a potent immunoregulatory cytokine, the primary biological function of which seems to be the limitation and termination of inflammatory responses [23]. In order to assess the effect of quince peel polyphenol extract on IL-10 secretion by LPS-inflamed macrophages, we measured its level in the different conditions described above. LPS treatment of THP-1-derived macrophages induced the secretion of a low level of the immunomodulator cytokine IL-10 (~38 pg/10^6 cells). However, when quince peel polyphenolic extract was added to the cells, the IL-10 level increased in a dose-dependent manner and doubled with the highest concentration (20 μg/ml) of polyphenolic extract (Fig. 2C). Previous reports have clearly shown the antagonist effect of IL-10 on the secretion of both TNF-α and IL-8 [24], suggesting that quince polyphenols-mediated inhibition of the LPS-induced secretion of these pro-inflammatory effectors may pass through the induction of IL-10 secretion. Several inflammatory diseases share the dual characteristic of a very low blood level of IL-10 and a high blood level of TNF-α. Injection of the exogenous recombinant form of IL-10 caused a decrease in the blood level of TNF-α that has proven beneficial for such diseases [25,26]. However, this treatment remains too expensive for wide-scale use. The ability of quince peel polyphenolic extract to increase the level of IL-10 secreted by inflamed macrophages represents an additional argument for the suggestion that it is an alternative or a compliment that may help in the treatment and/or prevention of inflammatory diseases.

3.4. Quince peel polyphenolic extract inhibition of TNF-α is partially mediated by IL-6

Up-regulation of IL-6 production has been observed in a variety of chronic inflammatory disorders [27,28]. To determine whether quince polyphenols can also inhibit the LPS-induced secretion of IL-6, we quantified its level in the supernatant of inflamed macrophages treated as described earlier. Surprisingly, we found that quince polyphenols increased IL-6 secretion by LPS-treated macrophages in a dose-dependent manner (Fig. 3A). This increase reached a maximum of 50% when the highest concentration of polyphenols was used (20 μg/ml). Previous reports have shown the inhibitory effect of IL-6 on inflammation through the inhibition of TNF-α production, providing negative feedback to limit the acute inflammatory response [29,30]. To determine whether the polyphenols-mediated increase of IL-6 is responsible for their inhibitory effect on TNF-α secretion, we incubated the LPS-treated macrophages with different concentrations of human recombinant IL-6. We found that IL-6 partially inhibited the LPS-mediated secretion of TNF-α in a dose-dependent manner. A maximum inhibition of about 40% occurred with 1 ng/ml of recombinant IL-6 (Fig. 3B). This data suggests that quince polyphenols-mediated inhibition of TNF-α secretion, by LPS-treated macrophages, partially relies on IL-6 induction.

3.5. The anti-inflammatory effects of quince polyphenols pass through NF-κB, p38MAPK and Akt inhibition

NF-κB is an important transcription factor that regulates the expression of a large number of inflammatory-related genes, representing an ideal target to inhibit inflammation [31]. Therefore, we examined the effect of quince peel polyphenols on LPS-induced activation of NF-κB by western blot. The activation state of the NF-κB pathway can be assessed by analysing the phosphorylation statue of the upstream I kappa B kinases, IKKα and IKKβ [32]. THP-1-derived macrophages were mock-treated or actually treated with LPS in the presence or absence of 20 μg/ml of quince peel polyphenolic extract for different lengths of time. Phospho-IκK-α/IκK-β antibody revealed an LPS-induced phosphorylation/activation of NF-κB pathway, primarily at 30 and 60 min post-treatment, that was clearly inhibited by quince polyphenols extract (Fig. 4). This finding suggests that quince polyphenols-mediated inhibition of inflammation passes through NF-κB inhibition. p38 α MAPK, the fourth member of the mammalian p38 MAP kinases (α, β, γ and δ), was also recognized for its role in regulating the biosynthesis of pro-inflammatory cytokines, namely IL-1 and TNF-α, in monocytes [33]. We then assessed its phosphorylation/activation statue in the same described cell lysates. Phospho-specific antibody against p38 α MAPK revealed the LPS-mediated activation of this kinase that was inhibited by quince polyphenols (Fig. 4), suggesting an additional pathway by which quince polyphenols block LPS-mediated inflammation of human macrophages. COX-2, an important pro-inflammatory enzyme that generates ROS in inflamed tissues, has been reported to be regulated by both NF-κB and p38 α MAPK [34]. Since these two effectors were inhibited by quince polyphenols in inflamed macrophages, one could suggest that this may also lead to a reduction in COX-2-mediated ROS production, adding one more pathway by which quince polyphenols may inhibit macrophage inflammation. Nevertheless, we previously reported the potent antioxidant effect of quince peel polyphenols [15]. One can then speculate that such a feature may also contribute to their anti-inflammatory effect through the scavenging of ROS produced by inflamed tissues. NF-κB-mediated signal transduction by LPS has been reported to be induced by activated Akt kinase [2,35,36]. In order to examine whether quince polyphenols modulate the Akt pathway, we assessed its activation in LPS-treated THP-1-derived macrophages. The use of a specific antibody that recognizes the phosphorylated Serine 473 of Akt, a hallmark of its full activation, has revealed that quince polyphenols suppressed the LPS-induced phosphorylation/activation of Akt (Fig. 4). This suggests that quince polyphenols-mediated inhibition of the NF-κB pathway may rely on Akt inhibition.

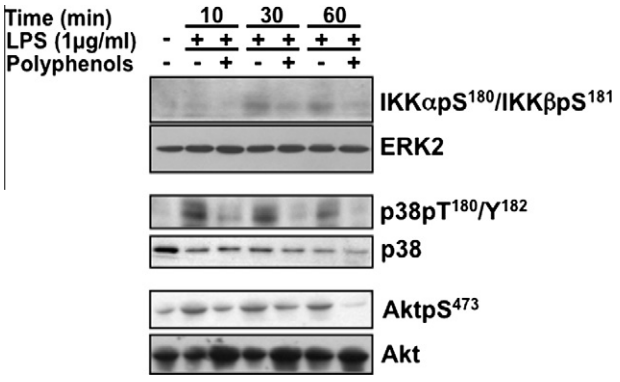

Fig. 4. Quince polyphenols block the LPS-induced activation of NF-κB, p38MAPK and Akt. Human THP-1-derived macrophages were left untreated (−) or treated (+) with LPS (1 μg/ml) with or without quince peel polyphenols extract (20 μg/ml) for the indicated time. Protein extracts (30 μg) were analysed by western blotting for the indicated proteins.

In summary, our work represents the first report of an anti-inflammatory effect of quince (*Cydonia oblonga* Miller) peel polyphenolic extract in LPS-stimulated human THP-1-derived macrophages. Quince polyphenols inhibited the secretion of pro-inflammatory cytokines by inflamed macrophages and enhanced the secretion of those with an anti-inflammatory effect. This inhibition was primarily mediated by the modulation of three major cellular effectors of inflammation, Akt kinase, p38-α MAPK kinase and NF-κB. Catechin, a phenolic molecule found in the quince peel polyphenolic extract, has been recently reported to downregulate the endotoxin-mediated activation of NF-κB, inhibiting TNF-α and ROS production [37]. Rutin, an other major component (~36%) of the peel quince polyphenolic extract, has been also reported to present a beneficial anti-inflammatory effect against inflammatory bowel diseases and induced arthritis [38,39]. Hyparin and procyanidin, two additional members of the quince peel polyphenols, have been reported to modulate the inflammatory response through p38MAPK and NF-κB inhibition [40,41]. All the remaining components of the peel quince polyphenolic extract like chlorogenic acids, kaempferol and quercetins have been also shown to have a potent anti-inflammatory effect [40,42,43]. Our data suggest that each molecule may have contributed to the anti-inflammatory effect of the quince peel polyphenolic extract. Although we still ignore the cellular target of each molecule, we propose that such phenolic components may have acted synergistically to generate the potent anti-inflammatory effect of the quince peel acetonic extract. Thus, we must underline the importance of using quince peel polyphenolic extract to benefit of its total bioactive compounds. The extract evaluated here presents the advantage of being simply prepared from naturally occurring which could be marketed as nutraceuticals, and might be industrially exploited. We therefore propose this extract, a natural food product that contains a mixture of anti-inflammatory non toxic molecules, as a potential cost-effective alternative for the treatment and/or prevention of inflammatory diseases. Deeper investigation using an *in vivo* model may prove helpful to better characterize the beneficial effect of quince peel polyphenols on inflammatory disorders.

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References

[1] T. Zarubin, J. Han, Activation and signaling of the p38MAP kinase pathway, Cell. Res. 15 (2005) 11–18.
[2] L.V. Madrid, C.Y. Wang, D.C. Guttridge, A.J.G. Schottelius, A.S. Baldwin Jr., M.W. Mayo1, Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-kappaB, Mol. Cell. Biol. 20 (2000) 1626–1638.
[3] S.W. Tas, P.H. Remans, K.A. Reedquist, P.P. Tak, Signal transduction pathways and transcription factors as therapeutic targets in inflammatory disease: towards innovative antirheumatic therapy, Curr. Pharm. Des. (2005) 581–611.
[4] K. Ito, G. Caramori, I.M. Adcock, Therapeutic potential of phosphatidylinositol 3-kinase inhibitors in inflammatory respiratory disease, J. Pharmacol. Exp. Ther. 321 (2007) 1–8.
[5] D. Mewar, A.G. Wilson, Treatment of rheumatoid arthritis with tumour necrosis factor inhibitors, Br. J. Phamacol. 162 (2011) 785–791.
[6] H.Y. Yong, M.S. Koh, A. Moon, The p38MAPK inhibitors for the treatment of inflammatory diseases and cancer, Expert. Opin. Invest. Drugs 18 (2009) 1893–1905.
[7] Y. Yamamoto, R.B. Gaynor, Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer, J. Clin. Invest. 107 (2001) 135–142.
[8] T.D. Warner, F. Giuliano, I. Vojnovic, A. Bukasa, J.A. Mitchell, J.R. Vane, Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-

oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis, Proc. Natl. Acad. Sci. USA 96 (1999) 7563–7568.
[9] C. Manach, A. Scalbert, C. Morand, C. Rémésy, L. Jiménez, Polyphenols: food sources and bioavailability, Am. J. Clin. Nutr. 79 (2004) 727–747.
[10] N.J. Kang, S.H. Shin, H.J. Lee, K.W. Lee, Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis, Pharmacol. Ther. 130 (2011) 310–324.
[11] D.O. Kim, K.W. Lee, H.J. Lee, C.Y. Lee, Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals, J. Agric. Food. Chem. 50 (2002) 3713–3717.
[12] M.V. Eberhardt, C.Y. Lee, R.H. Liu, Antioxidant activity of fresh apples, Nature 405 (2000) 903–904.
[13] M.B. Korycka-Dahl, T. Richardson, Activated oxygen species and oxidation of food constituents, CRC, Crit. Rev. Food. Sci. Nutr. 10 (1978) 209–241.
[14] V. Lavelli, S. Hippeli, C. Peri, E.F. Elstner, Evaluation of radical scavenging activity of fresh and air-dried tomatoes by three model reactions, J. Agri. Food. Chem. 47 (1999) 3826–3831.
[15] S. Fattouch, P. Caboni, V. Coroneo, C.I. Tuberoso, A. Angiono, S. Dessi, N. Marzouki, P. Cabras, Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peelpolyphenolicextracts, J. Agric. Food. Chem. 55 (2007) 963–969.
[16] D.L. Laskin, V.R. Sunil, C.R. Gardner, J.D. Laskin, Macrophages and tissue injury: agents of defense or destruction?, Annu Rev. Pharmacol. Toxicol. 51 (2011) 267–288.
[17] M. Daigneault, J.A. Preston, H.M. Mariott, M.K. Whyte, D.H. Dockrell, The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages, PLoS One 5 (2010) e8668.
[18] U. Singh, J. Tabibian, S.K. Venugopal, S. Devaraj, I. Jialal, Development of an in vitro screening assay to test the anti-inflammatory properties of dietary supplements and pharmacologic agents, Clin. Chem. 51 (2005) 2256.
[19] C.A. Feghali, T.M. Wright, Cytokines in acute and chronic inflammation, Front. Biosci. 2 (1997) d12–d26.
[20] A.R. Huber, S.L. Kundel, R.F. Todd III, Regulation of transendothelial neutrophil migration by endogenous interleukin-8, Science 254 (1991) 99–102.
[21] H. Rosenblum, H. Amital, Anti-TNF therapy: safety aspects of taking the risk, Autoimmun. Rev. 10 (2011) 563–568.
[22] T.D. Warner, F. Giualiano, I. Vojnovic, A. Bukasa, J.A. Mitchell, J.R. Vane, Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis, Proc. Natl. Acad. Sci. USA 96 (1999) 7563–7568.
[23] W. Ouyang, S. Rutz, N.K. Crellin, P.A. Valdez, S.G. Hymowitz, Regulation and functions of the IL-10 family of cytokines in inflammation and disease, Annu. Rev. Immunol. 29 (2011) 71–109.
[24] R. de Waal Malefyt, J. Abrams, B. Bennett, C.G. Figdor, J.E. de Vries, Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes, J. Ex. Med. 174 (1991) 1209–1220.
[25] M. Friedrich, W.D. Döcke, A. Klein, S. Philipp, H.D. Volk, W. Sterry, K. Asadullah, Immunomodulation by interleukin-10 therapy decreases the incidence of relapse and prolongs the relapse-free interval in Psoriasis, J. Invest. Dermatol. 118 (2002) 672–677.
[26] F.E. Buruiana, I. Solà, P. Alonso-Coello, Recombinant human interleukin-10 for induction of remission in Crohn's disease, Cochrane, Database. Syst. Rev. 11 (2010) CD005109.
[27] T. Hirano, Interleukin-6 and its relation to inflammation and disease, Clin. Immunol. Immunopathol. 62 (1992) S60–S65.
[28] P.L. Tan, S. Farmiloe, S. Yeoman, J.D. Watson, Expression of the interleukin 6 gene in rheumatoid synovial fibroblasts, J. Rheumatol. 17 (1990) 1608–1612.
[29] R. Schindler, J. Mancilla, S. Endres, R. Ghorbani, S.C. Clark, C.A. Dinarello, Correlations and interactions in the production of interleukin-6, IL-1, and tumor necrosis factor in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF, Blood 75 (1990) 40–47.
[30] R. Starkie, S.R. Ostrowiski, S. Jauffred, M. Febbraio, B.K. Pedersen, Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans, FASEB J. 17 (2003) 884–886.
[31] A.S. Baldwin, The NF-kappaB and I kappa-B proteins: new discoveries and insights, Annu. Rev. Immunol. 14 (1996) 649–683.
[32] S. Ghosh, M. Karin, Missing pieces in the NF-kappaB puzzle, Cell 109 (2002) S81–S96.
[33] J.C. Lee, J.T. Laydon, P.C. McDonnell, T.F. Gallagher, S. Kumar, D. Green, D. McNulty, M.J. Blumenthal, J.R. Heys, S.W. Landvatter, et al., A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, Nature 372 (1994) 739–746.
[34] D. Hwang, B.C. Jang, G. Yu, M. Boudreau, Expression of mitogen-inducible cyclooxygenase induced by lipopolysaccharide: mediation through both mitogen-activated protein kinase and NF-kappaB signaling pathways in macrophages, Biochem. Pharmacol. 54 (1997) 87–96.
[35] M.M. Monick, A.B. Carter, D.M. Flaherty, M.W. Peterson, G.W. Hunninghake, Protein kinase C zeta plays a central role in activation of the p42/44 mitogen-activated protein kinase by endotoxin in alveolar macrophages, J. Immunol. 165 (2000) 4632–4639.
[36] M.M. Monick, A.B. Carter, P.K. Robeff, D.M. Flaherty, M.W. Peterson, G.W. Hunninghake, Lipopolysaccharide activates Akt in human alveolar macrophages resulting in nuclear accumulation and transcriptional activity of beta-catenin, J. Immunol. 166 (2001) 4713–4720.
[37] S.A. Koul, K. Chopra, P. Rishi, Catechin suppresses an array of signalling molecules and modulates alcohol-induced endotoxin mediated liver injury in a rat model, PLoS One 6 (2011) e20635.
[38] K. Nones, Y.E. Dommels, S. Martell, et al., The effects of dietary curcumin and rutin on colonic inflammation and gene expression in multidrug resistance gene-deficient (mdr1a-/-) mice, a model of inflammatory bowel diseases, Br. J. Nutr. 101 (2009) 169–181.
[39] Y. Han, Rutin has therapeutic effect on septic *arthritis caused by Candida albicans*, Int. Immunopharmacol. 9 (2009) 207–211.
[40] S. Lee, H.S. Park, Y. Notsu, et al., Effects of hyperin, isoquercitrin and quercetin on lipopolysaccharide-induced nitrite production in rat peritoneal macrophages, Phytother. Res. 22 (2008) 1552–1556.
[41] X. Terra, P. Palozza, J. Fernandez-Larrea, et al., Procyanidin dimer B1 and trimer C1 impair inflammatory response signalling in human monocytes, Free Radic. Res. 45 (2011) 611–619.
[42] D.A. Chagas-Paula, R.B. Oliveira, V.C. da Silva, et al., Chlorogenic acids from *Tithonia diversifolia* demonstrate better anti-inflammatory effect than indomethacin and its sesquiterpene lactones, J. Ethnopharmacol. 136 (2011) 355–362.
[43] J.H. Gong, D. Shin, S.Y. Han, J.L. Kim, Y.H. Kang, Kaempferol suppresses eosinophil infiltration and airway inflammation in airway epithelial cells and in mice with allergic asthma, J. Nutr. 142 (2012) 47–56.