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Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155☆

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Abstract

In the present study the effect of quercetin and its major metabolites quercetin-3-glucuronide (Q3G) and isorhamnetin on inflammatory gene expression was determined in murine RAW264.7 macrophages stimulated with lipopolysaccharide. Quercetin and isorhamnetin but not Q3G significantly decreased mRNA and protein levels of tumor necrosis factor alpha. Furthermore a significant decrease in mRNA levels of interleukin 1β, interleukin 6, macrophage inflammatory protein 1α and inducible nitric oxide synthase was evident in response to the quercetin treatment. However Q3G did not affect inflammatory gene expression. Anti-inflammatory properties of quercetin and isorhamnetin were accompanied by an increase in heme oxygenase 1 protein levels, a downstream target of the transcription factor Nrf2, known to antagonize chronic inflammation. Furthermore, proinflammatory microRNA-155 was down-regulated by quercetin and isorhamnetin but not by Q3G. Finally, anti-inflammatory properties of quercetin were confirmed in vivo in mice fed quercetin-enriched diets (0.1 mg quercetin/g diet) over 6 weeks.
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Keywords: Quercetin; Macrophage; Inflammation; Flavonoid metabolism; MicroRNA

1. Introduction

Flavonoids comprise a large group of secondary plant metabolites with more than 6000 substances known to date. The major representative of the flavonol subgroup is quercetin which is ubiquitously present in edible plants. Onions, apples, tea and red wine are important sources of quercetin in the human diet [1,2]. Quercetin exhibits a wide range of biological effects including inhibition of low-density lipoprotein oxidation and platelet aggregation as well as improvement of endothelial function [3–5]. Cell culture and animal studies suggest potential anti-inflammatory properties of quercetin [6–8]. Furthermore, there is evidence from epidemiological and intervention studies that dietary flavonoids may reduce cardiovascular disease risk in humans [9,10].

Dietary quercetin undergoes intensive intestinal and hepatic metabolisation (Fig. 1), resulting in glucuronidated, sulphated and methylated quercetin derivatives [11–13]. Quercetin metabolism may affect its anti-inflammatory properties [8,14–16].

Recently, microRNAs (miRNAs), which are endogenous non-coding RNAs of ~22 nucleotides length, have been identified as potential regulators of immune function and inflammation [17,18]. Mammalian miRNAs post-transcriptionally modulate the expression of multiple target genes and are thus implicated in a wide array of cellular and developmental processes [19]. miR-155 is a component of the macrophage response to different types of inflammatory mediators, such as lipopolysacharide (LPS) [17].

The aim of the present study was to compare the impact of the parent compound quercetin with its two major metabolites quercetin-3-glucuronide and isorhamnetin on the expression of pro-inflammatory markers in LPS-stimulated murine macrophages, a cellular model for testing potential anti-inflammatory properties of plant bioactives [20,21]. Furthermore, we have investigated whether miR-155, a recently identified modulator of the inflammatory response may be modulated by quercetin and its metabolites. Potential anti-inflammatory properties of dietary quercetin were additionally determined in an in vivo study in mice.

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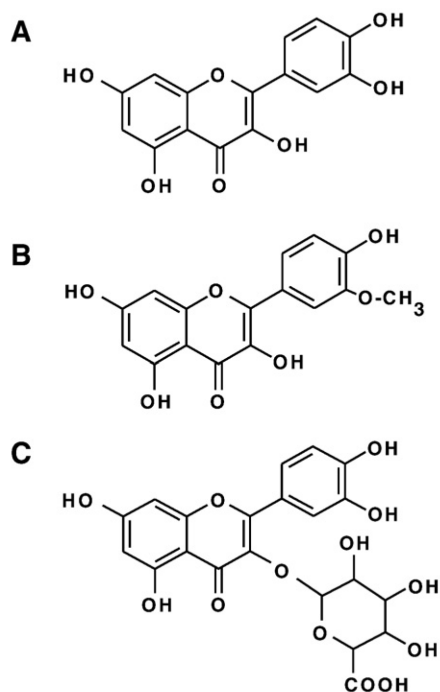

Fig. 1. Structure of quercetin (A) and its metabolites isorhamnetin (B) and quercetin-3-glucuronide (C).

2. Methods and materials

2.1. Materials

Quercetin dihydrate (>98%) and quercetin-3-glucuronide (Q3G) (>98%) were purchased from Carl Roth (Karlsruhe, Germany). Isorhamnetin (>95%) was obtained from Sigma, Deisenhofen, Germany. 100 mmol/l stock solutions of the test compounds were prepared in DMSO and stored in aliquots at −80°C. Cell culture media and supplements were from PAA (Coelbe, Germany). The murine macrophage cell line RAW264.7 was purchased from DSMZ (Braunschweig, Germany). Western blotting reagents and materials were purchased from Bio-Rad (Bio-Rad Laboratories GmbH, Muenchen, Germany). Reagents for RNA isolation and quantitative real time polymerase chain reaction (PCR) were from Bioline (Luckenwalde, Germany) and Quantace (London, UK) except the primers which were obtained from MWG Biotech (Ebersberg, Germany). Luciferase activity assay was obtained from Promega (Mannheim, Germany). TaqMan MicroRNA Assays, transcription kit and TaqMan Universal PCR master mix were obtained from Applied Biosystems (ABI, Foster City, CA, USA). Other reagents were from Sigma if not specified otherwise.

2.2. Cell culture and cytotoxicity assays

Murine RAW264.7 macrophages were cultivated in Dulbecco's modified eagle medium, supplemented with 10% foetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were grown under standard conditions in a humidified incubator at 37°C and 5% CO_2 with medium changes every second day. For experiments, cells were seeded at an initial density of 0.5×10^5 cells/cm^2.

To examine whether the test compounds may affect cell viability, RAW264.7 cells were incubated with increasing concentrations of quercetin, isorhamnetin and Q3G (0, 10–100 μmol/l) for 24 h. Cell viability was assessed by the neutral red assay [22,23] and viability calculated in per cent of solvent treated control cells. For subsequent experiments, RAW264.7 cells were incubated with the test compounds (0, 1–10 μmol/l) and stimulated with LPS (10 ng/ml; from *Salmonella enteritidis*, Sigma) for 2 up to 24 h.

2.3. Cytokine quantification

Tumor necrosis factor alpha (TNFα) was determined in cell culture supernatants (24 h) after appropriate dilution by the mouse TNFα Duo Set ELISA development kit (R and D Systems, Wiesbaden, Germany). TNFα concentration in murine plasma samples was determined by a fluorescence based Multiplex method (Bio-Rad).

2.4. RNA isolation and real time PCR

Total RNA was isolated with Trizure reagent and quantified photometrically. Real time PCR was performed as a one-step procedure using the SensiMix One-Step Kit. The Rotorgene 3000 (Corbett Life Science, Sydney, Australia) cycler was used for quantification of gene expression which was calculated by use of a standard curve. Primers for TNFα, interleukin (IL) 1β, IL6, macrophage inflammatory protein 1α (MIP1α) and inducible nitric oxide synthase (iNOS) were designed by standard tools (Spidey, Primer3, NCBI Blast) and the sequence information is given in Table 1. Expression of the target genes was normalized to the housekeeping gene β-actin.

2.5. Western Blotting

For whole cell extracts, macrophages were lysed in RIPA buffer (50 mmol/l Tris-HCl, 150 mmol/l NaCl, 0.5% deoxycholate, 0.1% sodium dodecyl sulphate (SDS), and 1% NP-40; pH 7.4 with protease inhibitor cocktail, 1:100, by incubation on ice for 30 min and subsequent centrifugation at 12,000×g (4°C, 30 min). Protein concentration was determined in the supernatants by the BCA Assay (Pierce, Illinois, USA). Protein 40 μg was separated on a 12% SDS/polyacrylamide gel and transferred onto an immunoblot polyvinylidenfluoride membrane. The membrane was blocked with 3% non-fat dried milk in Tris-buffered saline, pH 7.4, with 0.05% Tween-20 (TBS/T) for 2 h and probed with anti-HO-1 antibody (Stressgen, Biotech, Canada) and β-actin (Santa Cruz Biotechnology, Heidelberg, Germany) at 4°C overnight. Then, the membranes were incubated with secondary antibody (1:4000) conjugated with horseradish peroxidase for 45 min. Specific bands were visualized by chemiluminescence (ECL) reagent on a ChemiDoc system and quantitated densitometrically by using the program Quantity One. The predicted sizes for HO-1 and β-actin are 32 and 43 kDa, respectively, which were checked by the use of molecular weight markers.

The nuclear fraction was isolated as described in [24]. Protein content was determined in nuclear and cytoplasma extract supernatants and Western blotting was performed as described above using anti-p65 (Santa Cruz) for detection of nuclear translocated p65 protein (75 kDa).

2.6. Transcription factor activity

To study the effect of the test compounds on antioxidant response element (ARE) activity in RAW264.7 macrophages, cells were transiently transfected with a plasmid which contained the ARE sequences driving the expression of luciferase (kindly provided by J.A. Johnson, University of Wisconsin, Madison, WI, USA). Cells in 24-well plates were transfected with 0.5 μg of the vector by SuperFect Transfection Reagent (Qiagen, Hilden, Germany) for 24 h according to the manufacturer`s protocol. Then, the medium was changed and the test substances were added for another 24 h. The cells were lysed and the activity of luciferase was determined in cell lysates by chemiluminesence. Data were normalised for total cell protein.

The nuclear factor κB (NFκB)-secretory alkaline phosphatase (SEAP) (Clontech, BD Biosciences, Palo Alto, CA, USA) reporter construct was used to measure the activation of the NFκB signalling pathway. As recently described [25], cells were transiently transfected, incubated with the test compounds and stimulated with LPS (10 ng/ml) for 8 h. Chemiluminescent SEAP assay was measured in cell culture media and data were normalized to total cell protein.

2.7. miRNA determination

miR-155 levels were determined by two-step RT-PCR. Reverse transcription reaction was performed with specific miRNA primers. Real-time PCR amplification was done with a Rotorgene 3000 machine (Corbett) using standard conditions. Relative miRNA concentrations are given as the ratios between the amount of the target gene and the endogenous control snoRNA-202 (mouse).

Table 1
PCR primer sequences and annealing temperatures

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing temperature (°C)
TNFα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC	57
IL1β	CAGGCAGGCAGTATCACTCA	AGCTCATATGGGTCCGACAG	55
IL6	AGTTGCCTTCTTGGGACTGA	CAGAATTGCCATTGCACAAC	55
iNOS	GGCAGCCTGTGAGACCTTTG	GCATTGGAAGTGAAGCGTTTC	57
MIP1α	CCTCTGTCACCTGCTCAACA	GATGAATTGGCGTGGAATCT	55
β-Actin	GACAGGATGCAGAAGGAGATTACT	GACAGGATGCAGAAGGAGATTACT	55

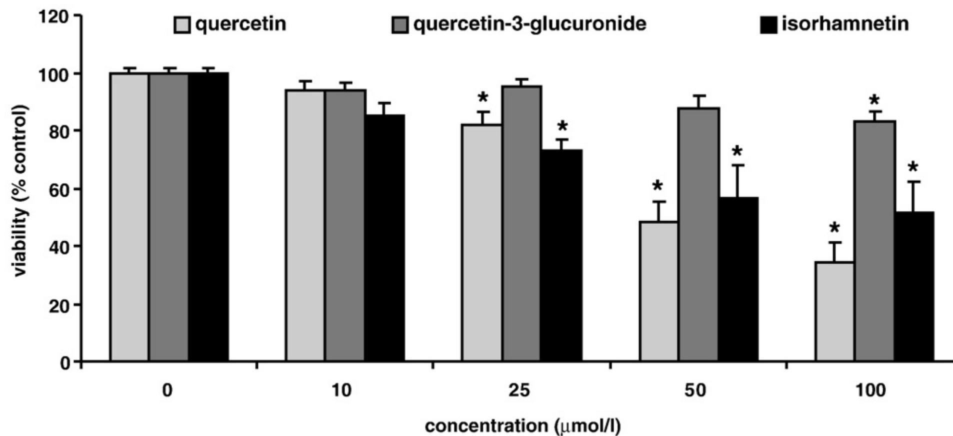

Fig. 2. Effect of quercetin, quercetin-3-glucuronide and isorhamnetin on cell viability of RAW264.7 macrophages. Cells were incubated with increasing concentrations of the test compounds and cell viability was determined by the neutral red assay. Data are mean with S.E.M. of three independent experiments performed in triplicate. Asterisk indicates significant differences from untreated control cells ($P<.05$).

2.8. Feeding study in mice

Twenty female C57BL/6 mice (Harlan Teklad, Borchen, Germany) with an initial body weight of 16.6±0.8 g were divided into two groups of 10 animals and fed quercetin-enriched diets for 6 weeks. A standard diet based on corn starch (43%), casein (24%), and 10% corn oil (ssniff, Soest, Germany) was supplemented with quercetin dihydrate to a final concentration of 0.1 mg quercetin per g diet. The control group received the standard diet only. Diets were prepared weekly and stored at 4°C until use. The animals were kept in macrolon cages under standard conditions (21–25°C, with a 12-h day/night cycle). The diets and water were provided ad libitum, and live weight was recorded weekly. Mice were kept according to the German Regulations of Animal Welfare with permission of the responsible authority. After 6 weeks, the mice were anaesthesized and decapitated. Blood was collected in EDTA-tubes, centrifuged to generate plasma (3000×*g*, 10 min, 4°C) and stored at −80°C.

2.9. Quercetin analysis in plasma samples

Plasma quercetin and isorhamnetin levels were measured according to the method of Bieger et al. [26]. Briefly, plasma samples were acidified, treated with β-glucuronidase/sulfatase type H-2 and centrifuged after acetone extraction. After evaporation of the resulting supernatant, the remainings were resolved in methanol/aqua bidest., injected into HPLC (Jasco FP 920 fluorescence detector; Gross-Umstadt, Germany) and detected at excitation 422 nm and emission 485 nm. Calibration curves for quercetin and isorhamnetin were obtained by the addition of these flavonols from methanolic stock solutions to native pig plasma with rhamnetin serving as an internal standard.

2.10. Statistical analysis

Statistical analysis was performed using SPSS Version 13.0 (Munich, Germany). Data were analysed for normality of distribution (Kolmogorow-Smirnov and Shapiro-Wilk test) and equality of variance (Levene test). Student *t* test and Mann-Whitney *U* test were used to compare data of two treatment groups. Ln-transformation was used if data were not normally distributed. Data are expressed as mean±S.E.M. Significance was accepted at $P<.05$.

3. Results

3.1. Cell viability

To assure that the test compounds may not affect cell viability, RAW264.7 cells were incubated with increasing concentrations of quercetin, Q3G and isorhamnetin (10–100 μmol/L) for 24 h and subjected to the neutral red assay. As shown in Fig. 2, quercetin decreased cell viability to 82% at a concentration of 25 μmol/l; higher concentrations of 50 and 100 μmol/l decreased cell viability to 44 and 37%, respectively. Q3G in contrast did only slightly diminish macrophage cell viability at 50 and 100 μmol/l to 90 and 83%, respectively. Similarly to quercetin, isorhamnetin dose-dependently reduced cell viability starting from 25 μmol/l (77%). Based on these findings, a non-cytotoxic concentration of 10 μmol/l for all test compounds was chosen for all further experiments.

3.2. Effect of quercetin and metabolites on TNFα mRNA and secretion levels

Murine macrophages were incubated with quercetin, Q3G and isorhamnetin (10 μmol/l) and stimulated with LPS for 24 h. Quercetin and isorhamnetin significantly decreased both TNFα mRNA levels (determined after 2 h) and TNFα secretion (determined after 24 h) (Fig. 3). However, TNFα mRNA and protein levels remained largely unchanged in response to the Q3G treatment.

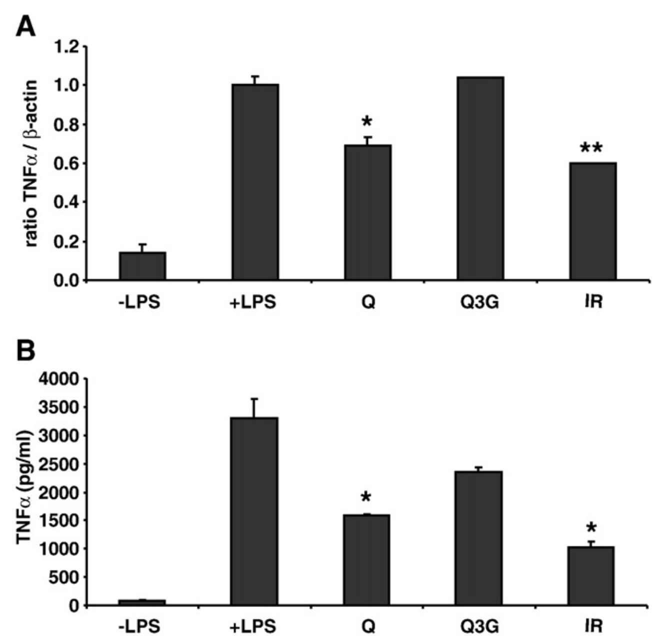

Fig. 3. Effect of quercetin (Q), Q3G and isorhamnetin (IR) on TNFα mRNA and levels and TNFα secretion. RAW264.7 macrophages were incubated with 10 μmol/l Q, Q3G or IR and stimulated with LPS (10 ng/ml) for 2 and 24 h to determine (A) TNFα mRNA levels and (B) TNFα secretion, respectively. Data are mean with S.E.M. of three independent experiments performed in duplicate. Asterisks indicate significant differences compared to LPS-stimulated control cells: *$P<.05$; **$P<.01$.

3.3. Effect of quercetin and metabolites on expression of other cytokines

Quercetin and isorhamnetin significantly decreased mRNA and protein levels of iNOS (Fig. 4) as well as IL1β, IL6 and MIP1α mRNA levels in LPS-stimulated RAW264.7 macrophages (Fig. 5). Q3G did not change mRNA levels of these pro-inflammatory markers.

3.4. Effects of test compounds on NFκB nuclear translocation

To study the translocation of the activated p65 subunit into the nucleus following stimulation of RAW264.7 macrophages with LPS in the presence and absence of quercetin, Q3G and isorhamnetin, Western blot analyses were performed using nuclear extracts. As shown in Fig. 6, p65 nuclear translocation was markedly induced by LPS and decreased in the presence of quercetin and isorhamnetin. Again, Q3G did not affect nuclear p65 translocation as compared to LPS-stimulated control cells. Similarly, quercetin and isorhamnetin but not Q3G did inhibit NFκB transactivation (data not shown).

3.5. Nrf2 activation by quercetin

The NF-E2-related factor 2 (Nrf2), a member of the basic leucine zipper transcription factor family is a potent activator of ARE-mediated gene expression. To investigate the effect of quercetin on ARE-dependent transactivation of Nrf2, macrophages were transiently transfected with an ARE plasmid and Nrf2 transactivation was measured by a luciferase reporter gene assay. As shown in Fig. 7A, quercetin dose-dependently induced Nrf2 transactivation by 1.7- and 2.7-fold as compared to controls following 5 and 10 μmol/l quercetin supplementation, respectively. In addition, protein levels of heme oxygenase (HO-1), a down stream target of Nrf2, were determined by western blotting. Quercetin elevated HO-1 protein levels in macrophages (Fig. 7B) in a concentration dependent manner.

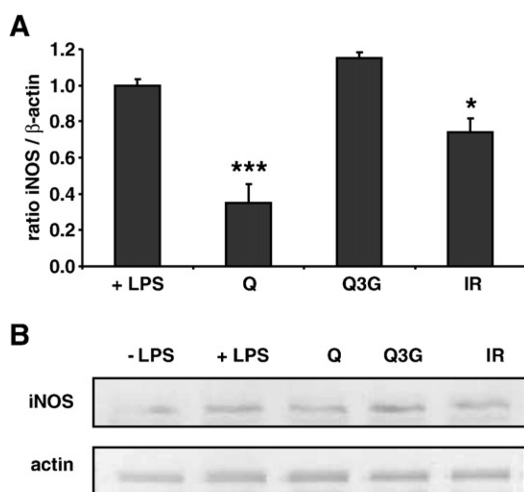

Fig. 4. Effect of Q, Q3G and IR on iNOS expression in RAW264.7 macrophages. Cells were incubated with 10 μmol/l Q, Q3G or IR and stimulated with LPS (10 ng/ml). (A) iNOS mRNA levels were determined after 6 h by quantitative real time PCR and normalized to the housekeeping gene β-actin. Data are mean with S.E.M. of three independent experiments performed in duplicate. (B) iNOS protein levels were determined via western blotting after 24 h incubation. Shown is a representative western blot. Asterisks indicate significant differences compared to LPS-stimulated control cells: *$P<.05$; ***$P<.001$.

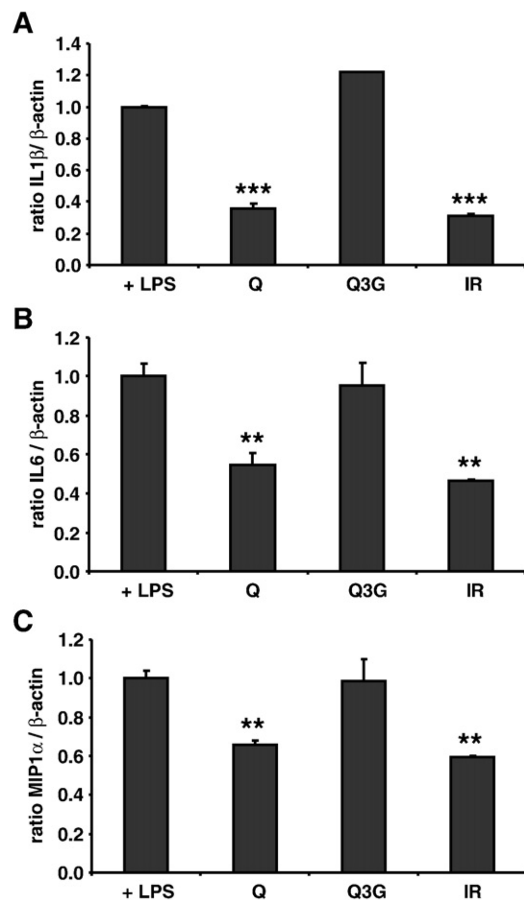

Fig. 5. Effect of Q, Q3G and IR on IL1β (A), IL6 (B) and MIP1α (C) mRNA levels in LPS-stimulated RAW264.7 macrophages (10 ng/ml). Cells were incubated with 10 μmol/L Q, Q3G or IR and stimulated with LPS (10 ng/ml) for 6 h. Target gene mRNA levels were normalized to the housekeeping gene β-actin. Data are mean with S.E.M. of three independent experiments performed in duplicate. Asterisks indicate significant differences in comparison to LPS-stimulated control cells: **$P<.01$; ***$P<.001$.

3.6. miRNA determination

Stimulation of RAW264.7 cells with LPS resulted in a 12fold increase in miR-155 as compared to unstimulated macrophages. Quercetin as well as isorhamnetin counteracted LPS induced increase in miR-155 whereas Q3G did not change miR-155 levels (Fig. 8).

3.7. Plasma quercetin and TNFα levels in mice

Feeding laboratory mice with quercetin-enriched diets over a 6-week experimental period did not affect feed intake and live weight gain (data not shown). Plasma levels of quercetin and isorhamnetin, however, significantly increased following dietary quercetin supply (Fig. 9A). Plasma TNFα levels, as measured by a fluorescent

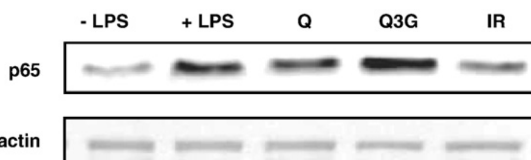

Fig. 6. Effect of Q, Q3G and IR on p65 translocation in LPS-stimulated RAW264.7 macrophages (10 ng/ml). Shown is a representative western blot.

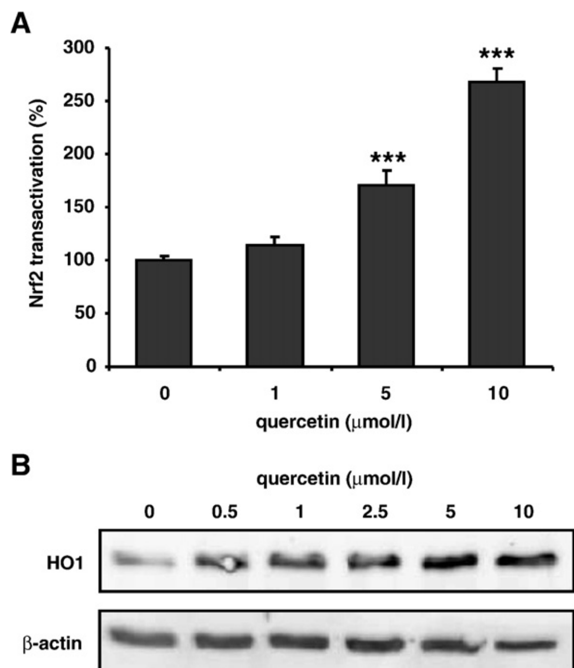

Fig. 7. Increase of Nrf2 transactivation (A) and HO-1 protein levels (B) in RAW264.7 macrophages after incubation with quercetin for 24 h. Reporter gene data are mean with S.E.M. of three independent experiments performed in duplicate. Western blot data show a representative blot out of three. Asterisks indicate significant differences compared to control cells ($P<.001$).

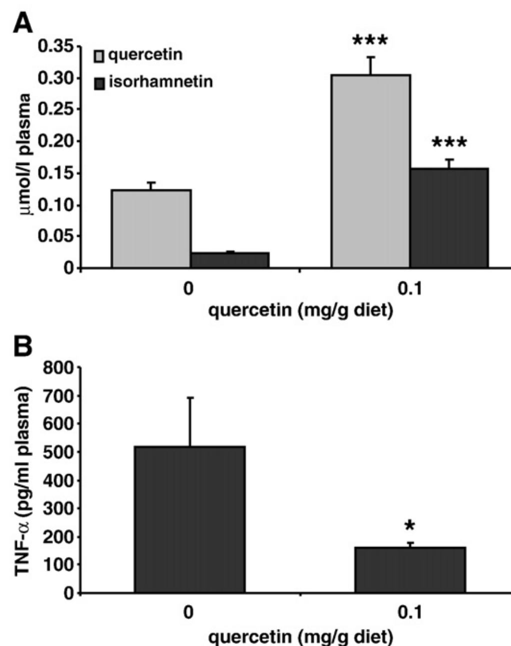

Fig. 9. Impact of a 6-week quercetin supplementation of C57BL/6 mice (0.1 mg quercetin per g diet) on (A) quercetin and isorhamnetin concentration and (B) basal TNFα levels in murine plasma samples. Asterisks indicate significant differences compared to the non-supplemented control group with $P<.05$ and $P<.0$ 1, respectively.

multiplexing method, were significantly decreased in mice fed quercetin-enriched diets ($P<.05$) as compared to controls fed the unsupplemented diets (Fig. 9B).

4. Discussion

Following absorption in the gastrointestinal tract, dietary quercetin is intensively metabolized leading to methylated derivatives as well as conjugates with glucuronic acid and sulphuric acid [11]. Conjugation of quercetin is mainly catalysed by UDP-glucuronosyltransferases and sulfotransferases, enzymes present in the gut and in the liver [27]. The resulting compounds include quercetin monoglucuronides and monosulfates, diglucuronides and disulfates, and mixed conjugates [12,28]. It is suggested that the formation of conjugates converts quercetin into more water-soluble products and may affect their cellular activity. Importantly, only a relatively small portion of the free aglykone has been detected in blood, demonstrating a high rate of conjugation [13,29]. Our cell culture data in RAW264.7 macrophages suggest that quercetin but not Q3G counteracts inflammatory gene expression. Thus glucuronidation, which masks important hydroxyl groups of the quercetin molecule, decreases its anti-inflammatory properties. Hepatic methylation of quercetin is mainly mediated by catechol-*O*-methyl-transferase [30]. Interestingly, in our RAW264.7 cells isorhamnetin (3′-methylquercetin) exhibited an anti-inflammatory activity similar to quercetin indicating that methylation unlike glucuronidation of quercetin is not associated with a loss of its biological properties. Collectively, our and literature data [14,31,32] suggest that glucuronidated but not methylated quercetin metabolites may possess biological properties different from their nonconjugated parent compound.

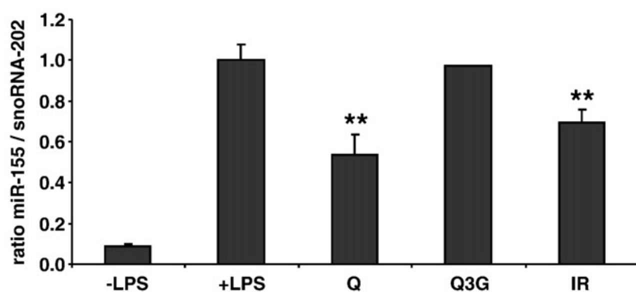

Fig. 8. Effect of Q and its metabolites Q3G and IR on LPS-induced miR-155 levels in murine RAW264.7 cells. RAW264.7 macrophages were incubated with 10 μmol/l Q, Q3G or IR and stimulated with LPS (10 ng/ml) for 6 h. Data are mean with S.E.M. of three independent experiments performed in duplicate. Asterisks indicate significant differences in comparison to LPS-stimulated control cells ($P<.001$).

The underlying molecular mechanisms by which quercetin may antagonize inflammatory gene expression in RAW264.7 cells have yet not been fully elucidated. In our study NFκB activity, as determined by p65 nuclear translocation, was inhibited by quercetin and isorhamnetin but not by Q3G. NFκB is a master switch of inflammatory gene expression and regulates the expression of IL1β, IL6, iNOS and MIP1α [33] — biomarkers of inflammation which were all down-regulated by quercetin and isorhamnetin.

There is cross-talk between the transcription factors NFκB and Nrf2 as far as inflammatory gene expression is concerned [34,35]. Overexpression of Nrf2 suppressed proinflammatory gene expression in TNFα-activated human aortic endothelial cells [36]. Furthermore the pretreatment of peritoneal macrophages with the Nrf2 inducer sulforaphane potently inhibited LPS-stimulated induction of TNFα, IL1β, cyclooxygenase 2 and iNOS [37]. Our results further indicate that quercetin induces HO-1, a downstream target of Nrf2, most likely via an Nrf2 dependent signal transduction pathway. Induction of HO-1 suppressed whereas a knockdown of HO-1 enhanced iNOS gene expression in macrophages [38,39]. Furthermore, elevated HO-1 levels may improve the cellular antioxidant status by the formation

of bilirubin [37] which in turn prevents LPS-and TNFα-induced inflammation [40]. However, in order to prove that the induction of HO-1, as observed in the present study, is directly mediating the anti-inflammatory properties of quercetin and isorhamnetin, further RNAi experiments are necessary where HO-1 is down-regulated and inflammatory gene expression is determined in the absence and presence of quercetin and isorhamnetin.

miR-155 has been identified as a positive regulator of the LPS signalling pathway. miR-155 is a common target for a broad range of inflammatory mediators through either MyD88 (Myeloid differentiation primary response gene 88) or TRIF (TIR-domain-containing adapter-inducing interferon-β)-dependent signalling pathways in monocytes and macrophages [17]. Induction of miR-155 depends on the NFκB but also on the JNK pathway [41]. It is suggested that miR-155 plays an important role in innate immunity but does also affect a large spectrum of immune reactions, ranging from cytokine production by T and B cells to antigen presentation, by dendritic cells and the germinal center B-cell response [42,43]. In macrophages, miR-155 affects (down-regulates) proteins involved in the TIR (toll-like and IL1-receptor) signalling pathway including TAK1-associated binding protein 2, Fas-associated death domain protein, IkB kinase ε and receptor interacting serine-threonine kinase 1 [17,44]. Interestingly, miR-155 enhances TNFα translation, although in general, miRNAs negatively regulate the translation of target transcripts [45]. The down-regulation of mature miR-155 levels by quercetin and isorhamnetin, which may relate to the inhibition of NFκB activation in our cell culture model, has possibly contributed to the anti-inflammatory properties of both flavonoids. The novel finding that miR-155 is regulated by dietary flavonoids provides a more detailed insight into the mechanism of flavonoid-induced attenuation of inflammatory processes. However, further experiments are warranted to identify whether the Nrf2 signalling pathway may also be directly affected by miR-155. Interestingly, there is recent evidence that Bach1, a repressor of Nrf2 signalling, is targeted by miR-155 [46].

Although plasma quercetin and isorhamnetin concentrations of our mice fed quercetin-enriched diets (0.1 mg/g diet) for a period of 6 weeks were lower than the concentrations used in our cell culture studies, we observed a significant decrease in circulating TNFα levels in response to the dietary quercetin supplementation. Thus, chronic intake of the dietary flavonol quercetin in a non-pharmacological concentration may antagonize inflammatory gene expression in vivo. Present cell culture data suggest that quercetin when conjugated with glucuronic acid does not exhibit anti-inflammatory properties. Bieger et al. [26] have recently studied the tissue distribution of quercetin in pigs after long-term dietary quercetin supplementation. Although quercetin was mainly present in the conjugated form in the plasma all tissues except the plasma contained variable amounts of deconjugated quercetin in a range of 30–100% of total flavonols. Furthermore, it has been shown that the aglycone may be locally generated from its metabolites at sites of inflammation [47] which could in turn affect the anti-inflammatory properties of quercetin in vivo. Thus, similar to our cell culture study in macrophages, quercetin per se but not its conjugated metabolites may be mainly responsible for its TNFα lowering effects in our mouse study.

In summary, our data demonstrate that quercetin and its methylated derivative isorhamnetin attenuate proinflammatory gene expression in LPS-activated murine macrophages. Besides the NFκB signal transduction cascade, we propose that the activation of the Nrf2 pathway by quercetin and isorhamnetin may mediate its anti-inflammatory properties. Moreover, we suggest that miR-155, as a further regulatory element of the inflammatory response, is targeted by quercetin and isorhamnetin (Fig. 10). Collectively, the regulation of NFκB, Nrf2 and miR-155 due to quercetin and isorhamnetin may contribute to a down-regulation of inflammatory gene expression both in cultured cells and in vivo.

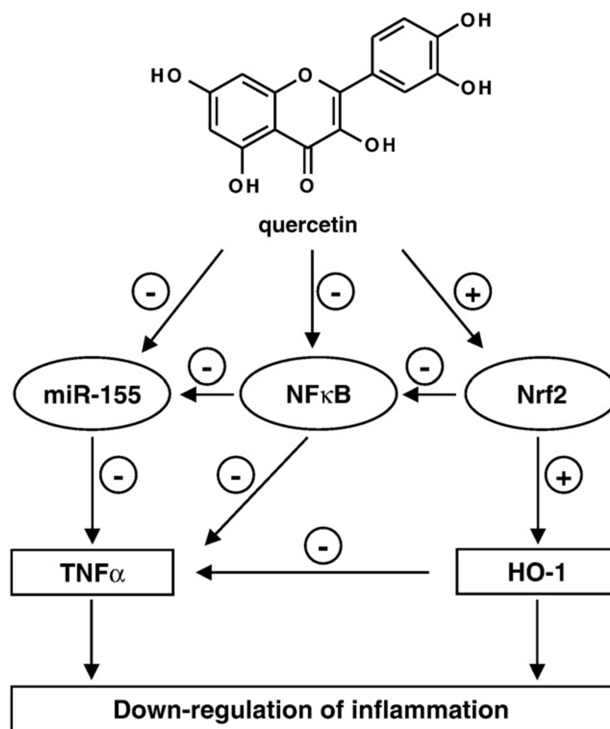

Fig. 10. Potential molecular mechanism by which quercetin may down-regulate inflammation.

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