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Down-regulatory effect of quercitrin gallate on nuclear factor-κB-dependent inducible nitric oxide synthase expression in lipopolysaccharide-stimulated macrophages RAW 264.7

Byung Hak Kim [a], Sung Min Cho [a], Alavala Matta Reddy [a], Yeong Shik Kim [b], Kyung Rak Min [a], Youngsoo Kim [a,*]

[a] *College of Pharmacy and Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763, Republic of Korea*
[b] *Natural Products Research Institute, Seoul National University, Seoul 110-460, Republic of Korea*

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Abstract

Quercetin 3-*O*-β-(2″-galloyl)-rhamnopyranoside (QGR) is a naturally occurring quercitrin gallate, a polyphenolic compound isolated from *Persicaria lapathifolia* (Polygonaceae). In the present study, QGR compound was discovered to have inhibitory effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated macrophages RAW 264.7. QGR compound attenuated LPS-induced synthesis of both mRNA and protein of inducible nitric oxide synthase (iNOS), in parallel, and inhibited LPS-induced luciferase expression as a reporter of iNOS promoter activity in the macrophages. As a mechanism of the anti-inflammatory action shown by QGR compound, suppression of nuclear factor (NF)-κB activation has been documented. QGR compound exhibited inhibitory effect on LPS-mediated NF-κB transcriptional activity in macrophages RAW 264.7. Furthermore, the compound inhibited LPS-mediated nuclear translocation of NF-κB p65 and DNA binding activity of NF-κB complex, in parallel, but did not influence LPS-mediated IκBα degradation. Taken together, QGR compound suppressed LPS-mediated NF-κB activation, specifically to nuclear localization step of NF-κB p65, which was attributable to its down-regulatory action on LPS-induced NO production and iNOS expression.

Keywords: Quercitrin gallate; Nitric oxide; Inducible nitric oxide synthase; Nuclear factor-κB; Anti-inflammation; Macrophages

1. Introduction

NO is a free radical gas and can be produced with citrulline from L-arginine by catalytic reaction of NO synthase [1]. Macrophages have shown to produce iNOS in response to bacterial LPS and pro-inflammatory cytokines [2]. Most interests have focused on the mechanism of iNOS expression in macrophages and the role of NO production in their immune responses, including the fact that NO can react with macrophages-derived superoxide to generate highly cytotoxic peroxynitrite for destroying the invading microorganisms [3,4]. However, it is also clear that iNOS induction takes place only in a variety of other cell types including vascular smooth muscle and neuronal cells, but also that high-output NO may provoke deleterious consequences such as septic shock, neurotoxicity, and inflammatory diseases [5–7].

Expression of iNOS gene in macrophages is under control of several transcription factors including NF-κB [8–10]. NF-κB is functional as hetero- or homo-dimeric form of Rel family proteins such as RelA (p65), RelB, cRel, p50 and p52 and is sequestered in the cytoplasm by binding to IκB proteins such as IκBα, IκBβ, IκBε, p105 and p100 [11,12]. LPS is a major component of the outer membranes in Gram-negative bacteria that can trigger a variety of inflammatory reactions by binding to its specific receptor, TLR4 [13,14]. Signaling components downstream the receptor can activate IKK complex [15,16]. Activation of IKK complex results in phosphorylation of IκB, which marks for ubiquitination and then followed by proteasome-mediated degradation [17,18]. IκB degrada-

Abbreviations: IκB, inhibitory κB; IKK, inhibitory κB kinase; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; NO, nitric oxide; PDTC, pyrrolidine dithiocarbamate; QGR, quercetin 3-*O*-β-(2″-galloyl)-rhamnopyranoside; SEAP, secretory alkaline phosphatase; TLR4, Toll-like receptor 4

* Corresponding author. Tel.: +82 43 261 2823; fax: +82 43 268 2732.
E-mail address: youngsoo@chungbuk.ac.kr (Y. Kim).

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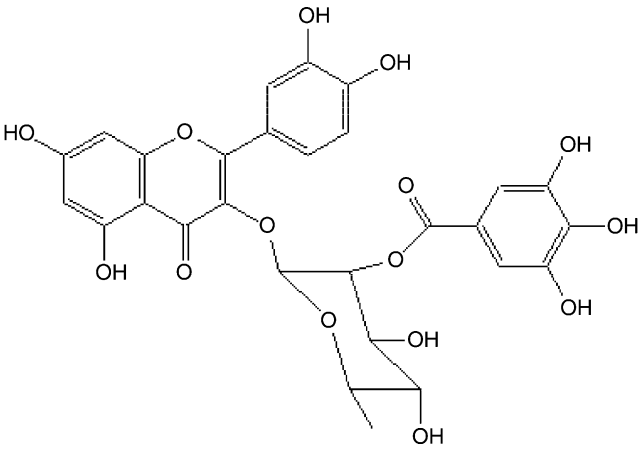

Fig. 1. Chemical structure of quercetin 3-*O*-β-(2″-galloyl)-rhamnopyranoside (QGR).

tion unmasks the nuclear localization signal motif of NF-κB, allowing the transcription factor to move into the nucleus, and then NF-κB binds to the promoter region of immune and inflammatory genes including iNOS for transcriptional regulation [19,20].

Quercetin 3-*O*-β-(2″-galloyl)-rhamnopyranoside (QGR) is a naturally occurring quercitrin gallate (Fig. 1). The polyphenolic compound was previously isolated as an inhibitor of NADPH oxidase complex-mediated superoxide production from *Persicaria lapathifolia* (Polygonacease) [21]. In the present study, QGR compound was found to inhibit NO production in LPS-stimulated macrophages RAW 264.7. The compound showed down-regulatory effect on LPS-induced iNOS expression at the transcription level. As a mechanism of the anti-inflammatory action shown by QGR compound, suppression of LPS-mediated NF-κB activation has been documented in this study.

2. Materials and methods

2.1. Materials

LPS (*E. coli* 055:B5) and PDTC were purchased from Sigma–Aldrich, and fetal bovine serum (FBS) from Invitrogen. Antibodies against iNOS, NF-κB p65 or IκBα were obtained from Santa Cruz Biotech, and antibody against phospho-IκBα (Ser-32/36) from Cell Signaling Tech. QGR compound (purity, >98%) was isolated from *P. lapathifolia* (Polygonaceae) as described in our previous work [21].

2.2. Cell culture

Macrophages RAW 264.7 were obtained from American Type Culture Collection. The cells were cultured in DMEM (13.4 mg/ml Dulbecco's modified Eagle's medium, 24 mM $NaHCO_3$, 10 mM HEPES, 143 U/ml benzylpenicillin potassium, 100 μg/ml streptomycin sulfate, pH 7.1) containing 10% FBS and maintained at 37°C with 5% CO_2. The RAW 264.7 cells harboring pNF-κB-SEAP-NPT reporter construct [22] were cultured under the same conditions except supplement of 500 μg/ml geneticin to the media.

2.3. Measurement of NO in cell-free culture media

Macrophages RAW 264.7 were treated with QGR compound plus LPS (1 μg/ml) for 24 h. Amounts of NO end-product nitrite in the cell-free culture media were measured using Griess reaction [23]. Briefly, cell-free culture media (100 μl) was reacted with 1:1 mixture (100 μl) of 1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-(1-naphthyl)ethylenediamine in distilled water, and then absorbance at 540 nm was measured.

2.4. Western immunoblot analysis

Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS (1 μg/ml) for 5 min (phospho-IκBα), 10-60 min (IκBα), 1 h (NF-κB p65) or 18 h (iNOS). Cytoplasmic or nuclear extracts of the cells were resolved on SDS-acrylamide gel by electrophoresis, and transferred to polyvinylidene difluoride membranes. Membranes were incubated with anti-phospho-IκBα antibody (1:1000), anti-IκBα antibody (1:300), anti-NF-κB p65 antibody (1:300) or anti-iNOS antibody (1:2000) at room temperature for 16 h. After incubation with horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:2500) at room temperature for 3 h, the blots were treated with ECL reagents (Amersham-Pharmacia) and then exposed to X-ray films.

2.5. Semi-quantitative RT-PCR

Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS (1 μg/ml) for 4 h. Total RNA of the cells was subjected to semi-quantitative RT-PCR using an RNA PCR kit (Bioneer). Primers used for quantification of iNOS and β-actin transcripts were constructed as described previously [24]. Briefly, total cellular RNA (1 μg) was reverse transcribed into cDNA by incubation at 42 °C for 1 h. The resulting cDNA samples were heated at 94 °C for 5 min and subjected to 30 cycles of PCR with 30 s denaturation at 94 °C, 30 s annealing at 56 °C and 90 s extension at 72 °C, and then followed by an additional 5 min extension at 72 °C. Amplified cDNA products were resolved on 1.5% agarose gel by electrophoresis and then stained with ethidium bromide.

2.6. Transient transfection and measurement of iNOS promoter activity

Both iNOS-luciferase reporter plasmid [8] and pSV-β-galactosidase control vector (Promega) were transiently transfected to macrophages RAW 264.7 using Lipofect-

AMINE (Invitrogen). The transfected RAW 264.7 cells were pre-treated with QGR compound for 2 h and stimulated with LPS (1 μg/ml) for 16 h. Lysates of the cells were subjected to luciferase assay using Luciferase Reporter Assay System (Promega) and to β-galactosidase assay using β-Galactosidase Enzyme Assay System (Promega).

2.7. Measurement of NF-κB transcriptional activity

Macrophages RAW 264.7 harboring pNF-κB-SEAP-NPT reporter construct [22] were pre-treated with QGR compound for 2 h and stimulated with LPS (1 μg/ml) for 16 h. Aliquots of the cell-free culture media were heated at 65 °C for 5 min, reacted with SEAP assay buffer (500 μM 4-methylumbelliferyl phosphate, 2 M diethanolamine, 1 mM $MgCl_2$) in the dark at room temperature for 1 h, and then measured the fluorescence in terms of relative units with emission 449 nm and excitation 360 nm.

2.8. Electrophoretic mobility shift assay (EMSA)

Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS (1 μg/ml) for 1 h. Nuclear extracts of the cells were reacted with NF-κB-specific ^{32}P-labeled oligonucleotide, and resolved on non-denaturing 6% acrylamide gel by electrophoresis. The gels were dried and exposed to X-ray film.

2.9. Cytotoxicity measurement

Macrophages RAW 264.7 were incubated with various concentrations of QGR compound for 24 h. The cells were treated with WST-1 solution (Dojindo) and then absorbance at 450 nm was measured as described elsewhere [25].

2.10. Statistical analysis

Results are expressed as the mean ± S.E.M. Data were analyzed by ANOVA followed by the Student's *t*-test. A value of $P < 0.01$ was considered significant.

3. Results

3.1. Effect of QGR compound on LPS-induced NO production

Macrophages RAW 264.7 in resting state released 7.1 ± 3.3 μM of nitrite, a stable metabolite of NO, during incubation for 24 h, whereas the cells markedly increased NO production up to 48.5 ± 4.1 μM of nitrite by stimulation with LPS alone (Fig. 2A). No significant difference in the NO production was found between resting RAW 264.7 cells and the cells treated with QGR compound (100 μM) alone (Fig. 2A). QGR compound inhibited LPS-induced

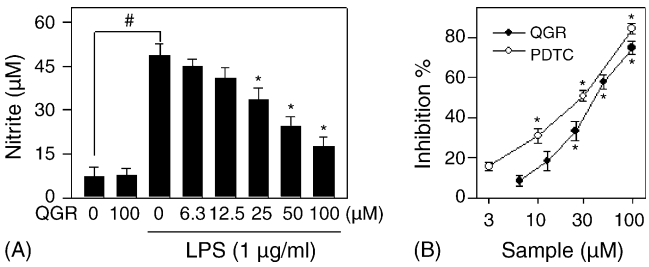

Fig. 2. LPS-induced NO production. Macrophages RAW 264.7 were treated with LPS plus QGR compound for 24 h. Amount of nitrite, a stable metabolite of NO, was measured with the cell-free culture media (A). Effects of QGR compound (solid circle) and PDTC (open circle) on LPS-induced NO production are represented as inhibition % (B). Values are mean ± S.E.M. ($n = 5$). $^{\#}P < 0.01$ vs. media alone-treated group. $^{*}P < 0.01$ vs. LPS alone-treated group.

NO production in a dose-dependent manner, corresponding to 33.3 ± 5.0% inhibition at 25 μM, 57.9 ± 3.6% at 50 μM and 74.7 ± 3.3% at 100 μM, showing an IC_{50} value of 40.6 μM (Fig. 2A and B). As a positive control, PDTC also inhibited LPS-induced NO production in a dose-dependent manner with an IC_{50} value of 29.3 μM (Fig. 2B). QGR compound at below 200 μM did not show significant cytotoxic effects to the RAW 264.7 cells (Fig. 3), indicating that inhibitory effect of the compound on LPS-induced NO production was not attributable to its non-specific cell toxicity.

3.2. Effect of QGR compound on LPS-induced iNOS synthesis

To examine whether inhibitory effect of QGR compound on LPS-induced NO production was attributable to its influence on iNOS synthesis, Western immunoblot analysis was carried out. iNOS protein was hardly detectable in resting macrophages RAW 264.7, but pronounced amount of iNOS protein was induced upon exposure to LPS alone (Fig. 4A). However, synthesis of housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was not affected by LPS and QGR compound (Fig. 4A). Treatment of QGR compound to the RAW 264.7 cells decreased LPS-

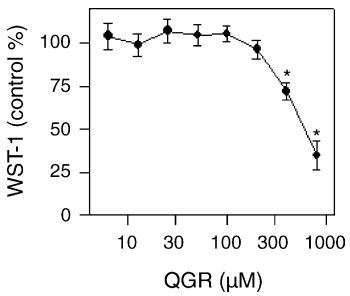

Fig. 3. Cytotoxicity of QGR compound. Macrophages RAW 264.7 were treated with various concentrations of QGR compound for 24 h. Proliferation of the cells was analyzed using WST-1 method, and is represented as control %, compared with that of media alone-treated group. Values are mean ± S.E.M. ($n = 5$). $^{*}P < 0.01$ vs. media alone-treated group.

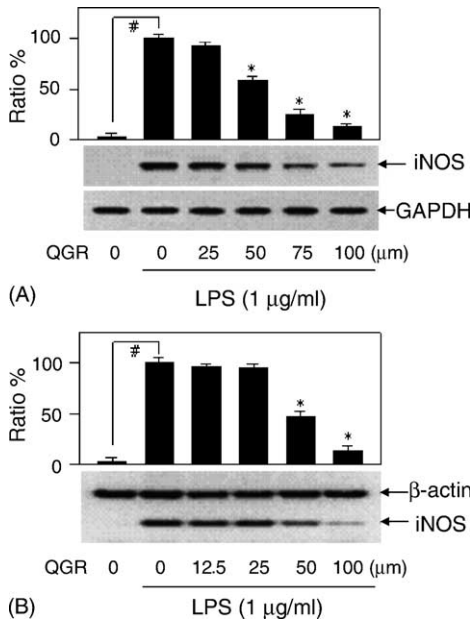

Fig. 4. LPS-induced iNOS synthesis. Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS for 18 h. Lysates of the cells were subjected to Western immunoblot analysis with anti-iNOS antibody. One of similar results is represented and relative ratio % is also shown, where iNOS signal was normalized to GAPDH signal (A). The cells were pre-treated with QGR compound for 2 h and stimulated with LPS for 4 h. Total RNA of the cells was subjected to semi-quantitative RT-PCR. One of similar results is represented and relative ratio % is also shown, where iNOS signal was normalized to β-actin signal (B). Values are mean ± S.E.M. ($n = 3$). $^{\#}P < 0.01$ vs. media alone-treated group. $^{*}P < 0.01$ vs. LPS alone-treated group.

induced synthesis of iNOS protein in a dose-dependent manner, corresponding to 41.4 ± 3.5% inhibition at 50 μM, 75.5 ± 5.0% at 75 μM and 87.3 ± 3.1% at 100 μM (Fig. 4A). Semi-quantitative RT-PCR was also carried out to understand whether QGR compound could influence LPS-induced synthesis of iNOS transcript. Amount of iNOS transcript in steady state was markedly increased by treatment of macrophages RAW 264.7 to LPS alone (Fig. 4B). QGR compound inhibited LPS-induced synthesis of iNOS transcript in a dose-dependent manner, corresponding to 53.2 ± 4.7% inhibition at 50 μM and 86.1 ± 5.0% at 100 μM (Fig. 4B). However, synthesis of housekeeping β-actin transcript was not affected by LPS and QGR compound (Fig. 4B).

3.3. Effect of QGR compound on LPS-induced iNOS expression at the transcription level

Transcriptional regulation of iNOS expression by QGR compound was documented using macrophages RAW 264.7 transfected transiently with iNOS-luciferase construct containing murine iNOS promoter (–1592/+183) fused to luciferase gene as a reporter [8]. Upon exposure to LPS alone, the transfected cells increased luciferase expression up to 17-fold over the basal level (Fig. 5). No significant difference in luciferase expression was found between

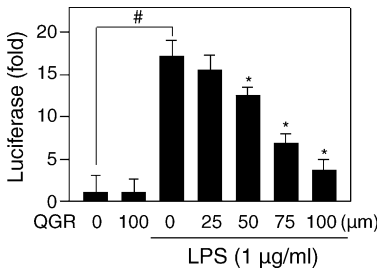

Fig. 5. LPS-induced iNOS promoter activity. Macrophages RAW 264.7 transfected transiently with iNOS-luciferase reporter plasmid and pSV-β-galactosidase control vector were pre-treated with QGR compound for 2 h and stimulated with LPS for 16 h. Luciferase and β-galactosidase activities were measured with lysates of the cells. Luciferase expression as a reporter of iNOS promoter activity is represented as relative fold, where luciferase activity was normalized to β-galactosidase activity. Values are mean ± S.E.M. ($n = 3$). $^{\#}P < 0.01$ vs. media alone-treated group. $^{*}P < 0.01$ vs. LPS alone-treated group.

resting RAW 264.7 cells and the cells treated with QGR compound (100 μM) alone (Fig. 5). QGR compound inhibited LPS-induced luciferase expression in a dose-dependent manner, corresponding to 29.2 ± 1.9% inhibition at 50 μM, 64.2 ± 2.2% at 75 μM and 83.3 ± 2.4% at 100 μM (Fig. 5).

3.4. Effect of QGR compound on LPS-mediated NF-κB transcriptional activity

NF-κB transcription factor has been evidenced to play a key role in LPS-induced iNOS expression in macrophages [8,9]. To investigate a molecular mechanism of the anti-inflammatory action shown by QGR compound, NF-κB transcriptional activity was monitored using macrophages RAW 264.7 harboring pNF-κB-SEAP-NPT construct that contains four copies of κB sequence fused to SEAP gene as a reporter [22]. Upon exposure to LPS alone, the transfected cells increased SEAP expression to about three-fold over the basal level, indicating that cellular NF-κB is transcriptionally functional (Fig. 6A). No significant difference in the SEAP expression was found between

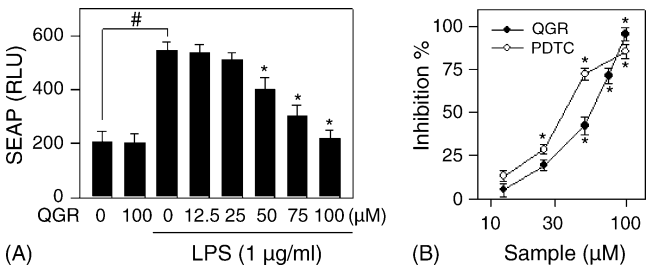

Fig. 6. LPS-mediated NF-κB transcriptional activity. Macrophages RAW 264.7 harboring pNF-κB-SEAP-NPT reporter construct were pre-treated with QGR compound for 2 h and stimulated with LPS for 16 h. SEAP activity as a reporter of NF-κB transcriptional activity was measured with the cell-free culture media, and is represented as relative fluorescence units (RFU) (A). Effects of QGR compound (solid circle) and PDTC (open circle) on LPS-mediated NF-κB transcriptional activity are represented as inhibition % (B). Values are mean ± S.E.M. ($n = 5$). $^{\#}P < 0.01$ vs. media alone-treated group. $^{*}P < 0.01$ vs. LPS alone-treated group.

RAW 264.7 cells and the cells treated with QGR compound (100 μM) alone (Fig. 6A). QGR compound inhibited LPS-mediated SEAP expression in a dose-dependent manner, corresponding to 42.3 ± 4.9% inhibition at 50 μM, 71.4 ± 4.6% at 75 μM and 95.6 ± 3.5% at 100 μM, showing an IC_{50} value of 56.6 μM (Fig. 6A and B). As a positive control, PDTC also inhibited LPS-mediated SEAP expression in a dose-dependent manner with an IC_{50} value of 37.5 μM (Fig. 6B).

3.5. Effect of QGR compound on LPS-mediated DNA binding activity of NF-κB complex and nuclear translocation of NF-κB p65

To elucidate inhibitory mechanism on NF-κB activation, we next determined whether QGR compound could affect DNA binding activity of NF-κB in LPS-stimulated macrophages RAW 264.7, which was analyzed by EMSA with NF-κB-specific ^{32}P-labeled oligonucleotide. The RAW 264.7 cells markedly increased DNA binding activity of NF-κB complex, p65/50 and p50/p50, upon exposure to LPS alone for 1 h (Fig. 7). QGR compound decreased LPS-mediated DNA binding activity of NF-κB complex in a dose-dependent manner (Fig. 7). To further investigate whether QGR compound could affect nuclear translocation of NF-κB, Western immunoblot analysis for NF-κB p65 was carried out with nuclear extracts of LPS-stimulated macrophages RAW 264.7. Amount of NF-κB p65 in the nucleus was markedly increased upon exposure to LPS alone (Fig. 8). QGR compound inhibited LPS-mediated nuclear translocation of NF-κB p65 in a dose-dependent manner, corresponding to 29.6 ± 3.5% inhibition at 50 μM, 74.6 ± 4.2% at 75 μM and 99.3 ± 6.0% at 100 μM (Fig. 8).

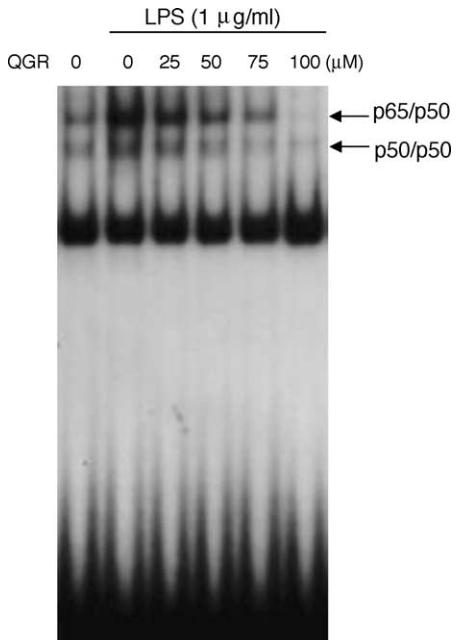

Fig. 7. LPS-mediated DNA binding activity of NF-κB complex. Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS for 1 h. Nuclear extracts of the cells were reacted with NF-κB-specific ^{32}P-labeled oligonucleotide and then resolved on non-denaturing 6% acrylamide gel by electrophoresis. NF-κB complex, p65/p50 and p50/p50, are indicated by an arrow.

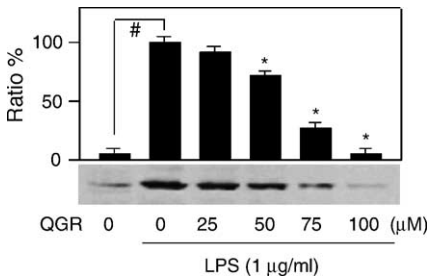

Fig. 8. LPS-mediated nuclear translocation of NF-κB p65. Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS for 1 h. Nuclear extracts of the cells were subjected to Western immunoblot analysis with anti-NF-κB p65 antibody. One of similar results is represented and relative ratio % is also shown. Values are mean ± S.E.M. ($n = 3$). $^{\#}P < 0.01$ vs. media alone-treated group. $^{*}P < 0.01$ vs. LPS alone-treated group.

3.6. Effect of QGR compound on LPS-mediated degradation and phosphorylation of IκBα

Another immunoblot analysis was carried out with cytoplasmic extracts of LPS-stimulated macrophages

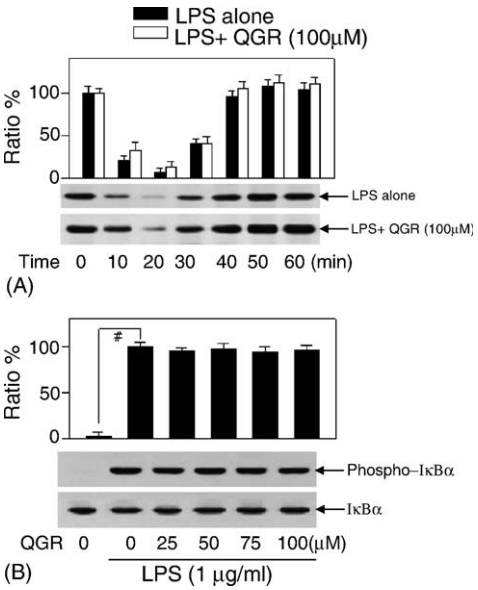

Fig. 9. LPS-mediated degradation and phosphorylation of IκBα. Macrophages RAW 264.7 were pre-treated with QGR compound for 2 h and stimulated with LPS (1 μg/ml) for indicated times (10–60 min). Cytoplasmic extracts of the cells were subjected to Western immunoblot analysis with anti-IκBα antibody to measure IκBα degradation. One of similar results is represented and amounts of IκBα in densitometry are also shown as relative ratio % for LPS alone (solid box) and LPS plus QGR compound (open box) (A). The cells were pre-treated with QGR compound for 2 h and stimulated with LPS for 5 min. Cytoplasmic extracts of the cells were subjected to Western immunoblot analysis with anti-phospho-IκBα (Ser-32/36) antibody or anti-IκBα antibody to measure IκBα phosphorylation. One of similar results is represented and relative ratio % is also shown, where phospho-IκBα signal was normalized to IκBα signal (B). Values are mean ± S.E.M. ($n = 3$). $^{\#}P < 0.01$ vs. media alone-treated.

RAW 264.7 to understand whether QGR compound could affect IκBα degradation. Upon exposure to LPS alone, IκBα degradation was dramatically happened within 20 min, and amount of IκBα in the cytoplasm was recovered to the normal level by 50 min after LPS stimulation (Fig. 9A). QGR compound (100 μM) did not show significant inhibitory effect on LPS-mediated IκBα degradation, but also IκBα recovery in the time course study (Fig. 9A). IκBα can be phosphorylated at its Ser-32 and -36 residues by IKK complex, which marks for ubiquitin-dependent IκBα degradation [15]. IκBα phosphorylation was also documented by Western immunoblot analysis. Upon exposure to LPS alone for 5 min, IκBα phosphorylation was markedly caused but IκBα degradation was not started yet (Fig. 9B). However, phosphorylated forms of IκBα were hardly detectable in resting macrophages RAW264.7 (Fig. 9B). At the 5 min time point after LPS challenge, QGR compound (25–100 μM) did not inhibit LPS-mediated IκBα phosphorylation, at all (Fig. 9B).

4. Discussion

In the present study, QGR compound was discovered to have a dose-dependent inhibitory effect with an IC_{50} value of 40.6 μM on LPS-induced NO production in macrophages RAW 264.7 (Fig. 2). Furthermore, QGR compound attenuated LPS-induced synthesis of both mRNA and protein of iNOS, in parallel (Fig. 4) and inhibited LPS-induced iNOS promoter activity (Fig. 5). These results indicate that QGR compound could down-regulate LPS-induced iNOS expression at the transcription level.

As a molecular mechanism for the anti-inflammatory action shown by QGR compound, suppression of NF-κB activation has been demonstrated. QGR compound inhibited LPS-mediated NF-κB transcriptional activity in a dose-dependent manner with an IC_{50} value of 56.6 μM (Fig. 6). Furthermore, QGR compound inhibited LPS-mediated DNA binding activity of NF-κB complex and nuclear translocation of NF-κB p65, in parallel (Figs. 7 and 8). However, QGR compound influenced neither LPS-mediated IκBα degradation nor IκBα phosphorylation (Fig. 9). These results indicate that QGR compound could suppress LPS-mediated NF-κB activation, specifically to nuclear translocation step of NF-κB p65 without affecting IκBα degradation.

Quercetin is an aglycone of QGR compound and was reported to inhibit LPS-dependent production of iNOS mRNA and to decrease NO release in macrophages RAW 264.7 [26]. As the anti-inflammatory mechanism, quercetin was described to affect IKK complex as a mixed type of inhibitor, suggesting that a binding site of the flavonoid would likely overlap with both ATP and IκBα binding pockets on the enzyme [27]. However, QGR compound did not inhibit LPS-mediated IκBα phosphorylation dependent upon IKK complex (Fig. 9B). Therefore, QGR compound exhibited different inhibitory mechanism on LPS-induced NF-κB activation from its aglycone, quercetin.

In addition to the TLR4 pathway, reactive oxygen species-mediated signaling pathway was also involved in the expression of interleukin-1 and tumor necrosis factor after LPS challenge in macrophage cell lines [28,29]. Using macrophages RAW 264.7 transfected with small GTP-binding protein Rac1 which activates NADPH oxidase complex and produces superoxide, Sanlioglu et al. demonstrated that LPS-induced Rac1 activation for NF-κB transcriptional activity could be independent but amplify TLR4-mediated inflammatory responsiveness [29]. Consequently, from a therapeutic standpoint, dual inhibition of both TLR4 and Rac1-dependent pathways could provide the most efficacious strategy for inhibiting LPS-induced inflammatory mediator production. As a positive control, PDTC inhibited LPS-induced NO production but also NF-κB transcriptional activity in this study (Figs. 2B and 6B), which would be attributable to its antioxidant activity to block reactive oxygen species-mediated signaling pathway after LPS challenge [29,30]. QGR compound was previously reported to inhibit NADPH oxidase complex-mediated superoxide production in unopsonized zymosan-stimulated human monocytes with its very weak scavenging effect of oxygen/nitrogen radical species such as superoxide and NO [21]. In the present study, QGR compound was discovered to inhibit NO production and iNOS expression in LPS-stimulated macrophages RAW 264.7, which was attributable to its suppression of nuclear translocation event of NF-κB p65. Taken together, QGR compound could provide an invaluable tool to investigate NF-κB-dependent iNOS expression, in addition to its therapeutic potential in inflammatory diseases.

Acknowledgements

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