SPANDIDOS PUBLICATIONS

MOLECULAR MEDICINE REPORTS 32: 177, 2025

Understanding the role of iron/heme metabolism in the anti-inflammatory effects of natural sulfur molecules against lipopolysaccharide-induced inflammation

DONG YOUNG KANG[1*], SE WON BAE[2*] and KYOUNG-JIN JANG[3]

[1]Department of Immunology, School of Medicine, Institute of Biomedical Science and Technology, Konkuk University, Chungju, North Chungcheong 27478, Republic of Korea; [2]Department of Chemistry and Cosmetics, Jeju National University, Jeju 63243, Republic of Korea; [3]Department of Integrative Biological Sciences and Industry, College of Life Science, Sejong University, Seoul 05006, Republic of Korea

Received October 11, 2024; Accepted March 25, 2025

DOI: 10.3892/mmr.2025.13542

Abstract. Iron transport and heme synthesis are essential processes in human metabolism, and any dysregulation in these mechanisms, such as inflammation, can have deleterious effects. Lipopolysaccharide (LPS)-induced inflammatory responses can result in a number of adverse effects, including cancer. Natural mineral sulfur, methylsulfonylmethane (MSM) and nontoxic sulfur (NTS) suppress inflammatory responses. The present study hypothesized that MSM and NTS may inhibit LPS-induced inflammatory responses in THP-1 human monocytes. Reverse transcription-quantitative PCR and western blotting assays were performed to analyze the molecular signaling pathways associated with sulfur-treated and untreated cells. A comet assay was used to evaluate DNA damage, flow cytometry was performed to analyze cell surface receptors and chromatin immunoprecipitation was used to examine molecular interactions. Notably, LPS-induced inflammation increased iron/heme metabolism, whereas MSM and NTS inhibited this effect. Furthermore, LPS treatment activated the Toll-like receptor 4/NF-κB signaling axis, which was downregulated by NTS and MSM. These sulfur compounds also suppressed the nuclear accumulation of LPS-induced NF-κB, which could induce the production of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6. Finally, MSM and NTS inhibited LPS-induced reactive oxygen species generation and DNA damage in THP-1 monocytic leukemia cells. These results suggested that natural sulfur molecules may be considered promising candidates for anti-inflammation studies.

Correspondence to: Professor Kyoung-Jin Jang, Department of Integrative Biological Sciences and Industry, College of Life Science, Sejong University, 209 Neungdong-ro, Gwangjin, Seoul 05006, Republic of Korea
E-mail: jangkj@sejong.ac.kr

[*]Contributed equally

Key words: lipopolysaccharide, oxidative stress, iron/heme metabolism, anti-inflammation, natural sulfur

Introduction

Iron is a major nutrient and an essential component of hemoglobin, which is present in red blood cells and carries oxygen from the lungs to the rest of the body. Iron is also involved in metabolism redox regulation and reactive oxygen species (ROS) production. To support these mechanisms, a comprehensive association between iron transport and homeostasis is required (1). The balance between iron as a nutritional source and a toxicant is sensitive and delicate, and iron transport and homeostasis are closely associated with inflammation (2,3). Macrophages are essential in balancing iron transport and inflammation. M1 macrophages are involved in iron uptake when iron is accumulated through ferroportin (FPN) and heme oxygenase (HO)-1 activities; this directs M2 macrophages, along with ferritin, to export excess iron and to inhibit proinflammatory cytokines (4).

Iron is a major contributor in the biosynthesis of heme, which is a porphyrin molecule containing a ferrous ion (Fe^{2+}). Notably, iron-dependent ferritin, carbon monoxide and bilirubin are formed through heme degradation (5), and heme is involved in various physiological processes, including inflammation that is induced by excess heme production. However, heme is an iron-containing complex that is essential for a number of biological processes, including oxygen transport and storage, electron transfer and protein synthesis. Therefore, heme is considered a double-edged sword because it can be toxic by mediating inflammation (6). It also participates in oxygen transport, signal transduction and mitochondrial function, and an imbalance in iron/heme metabolism may cause tumorigenesis (7). Free heme molecules and heme-containing proteins are generated under various pathological conditions, such as ischemia/reperfusion, hemorrhage and muscle injuries (8). An excess amount of heme also contributes to renal failure because of local inflammation and promotes the expression of proinflammatory cytokines, such as IL-1β and TNF-α (9).

Lipopolysaccharide (LPS) is a vital component of the outer layer of gram-negative bacteria, which induces innate immunity and initiates the inflammatory response in other cells (10). LPS is a pathogenic stimulator and can induce an inflammatory response by regulating pathogen-associated molecular patterns (11). Toll-like receptor (TLR)4 recognizes innate LPS and controls the innate immune response by neutralizing proinflammatory factors (12). LPS also activates various inflammatory signaling molecules, such as inducible nitric oxide synthase (iNOS), COX-2, NF-κB and p38-MAP kinase pathways (13). When exposed to LPS, TLRs activate innate immunity by activating NF-κB through canonical or noncanonical pathways and proinflammatory cytokines, such as TNF-α and various ILs, in response to inflammation (14).

Sulfur is an essential element in amino acids, such as cysteine and methionine, as well as in some sulfur-containing natural compounds that exert anti-inflammatory activities (15). Sulfur intake is possible through the diet, for example via the consumption of garlic, onion and duck meat (16). Methylsulfonylmethane (MSM) is a natural sulfur compound with various capabilities, such as anti-ketotic (17), antioxidant (18,19) and anticancer activities (20,21). Studies on MSM have demonstrated its anti-inflammatory activity against various inflammatory conditions (22,23). MSM is also found in cardiac cells, where it inhibits the inflammatory response by mediating TNF-α (24). In some instances, sulfur can be toxic; however, it can be coated with various nontoxic substances to enhance the effect of sulfur compounds against various conditions, including inflammation (25). Nontoxic sulfur (NTS) is a sulfur compound coated with a nontoxic substance, which when used to enrich livestock feed can enhance meat quality and boost immunity (26). In a previous study, rats orally administered NTS did not experience cell death (27). Furthermore, our previous study on NTS indicated that it can enhance the signaling of growth hormone in C2C12 mouse myoblasts (28). In addition, NTS inhibits inflammatory responses in C2C12 cells by mediating TLR4 and JAK2/STAT3 signaling to regulate IL-6 expression (29). Our previous study also demonstrated the anti-inflammatory effects of MSM and NTS against high glucose-induced inflammatory conditions by regulating NF-κB signaling in THP-1 human monocytes (30).

The present study aimed to determine the effects of NTS and MSM on LPS-induced inflammation, and to assess the role of iron/heme metabolism in the anti-inflammatory activity of these sulfur compounds. Molecular analyses of the TLR and NF-κB signaling pathways, iron homeostasis and heme biosynthesis were also performed under LPS-induced conditions.

Materials and methods

Reagents and antibodies. MSM (cat. no. PHR1346) and LPS (cat. no. L2630) were obtained from MilliporeSigma. The following primary antibodies were purchased from Santa Cruz Biotechnology, Inc.: Ferrochelatase (FECH; cat. no. sc-377377), phosphorylated (p)-IκBα (cat. no. sc-8404), TLR2 (cat. no. sc-21759), TLR4 (cat. no. sc-293072), COX-1 (cat. no. sc-19998), COX-2 (cat. no. sc-19999, IKKα/β (cat. no. sc-7607), and β-actin (cat. no. sc-47778); from Cell Signaling Technology, Inc.: IL-1β (cat. no. 12703), p-ERK (cat. no. 9101), ERK (cat. no. 9102), IκBα (cat. no. 9242), p-IKKα/β (cat. no. 2697), NF-κB (cat. no. 8242), p-p38 (cat. no. 4511), p38 (cat. no. 8690), ATR (cat. no. 2790), ATM (cat. no. 2873), Chk2 (cat. no. 2662), BRCA1 (cat. no. 9010), p53 (cat. no. 9282), p-MDM2 (cat. no. 3521), MDM2 (cat. no. 86934) and the DNA Damage Antibody Sampler Kit (cat. no. 9947; containing p-ATM, p-ATR, p-Chk2, p-BRCA1 and p-p53 antibodies); from Abcam: Divalent metal transporter (DMT)1 (cat. no. ab55735), 5'-aminolevulinate synthase (ALAS)1 (cat. no. ab84962), STEAP3 (cat. no. ab151566), HO-1 (cat. no. ab137749), transferrin receptor (TfR; cat. no. ab84036), PKCα (cat. no. ab179523), p-PKCα (cat. no. ab59411), TNF-α (cat. no. ab183218), IL-6 (cat. no. ab6672) and TATA-binding protein (TBP; cat. no. ab818); from LifeSpan BioSciences, Inc.: ABCB10 (cat. no. LS-C381841) and FLVCR1 (cat. no. LS-C750126); from LS Bio; Vector Laboratories, Inc.: FPN (cat. no. NBP1-21502) and iNOS (cat. no. NB300-605); from Novus Biologicals; Bio-Techne: Mitoferrin (MFRN; cat. no. MBS6013473); and from Boster Biological Technology: ABCB6 (cat. no. PA1723). Horseradish peroxidase (HRP)-conjugated anti-rabbit (cat. no. 7074) and anti-mouse (cat. no. 7076) secondary antibodies were purchased from Cell Signaling Technology, Inc. NTS was provided by the NARA Bioetch.

Cell culture and treatment. Human THP-1 cells were purchased from the Korean Cell Line Bank; Korean Cell Line Research Foundation and cultured in RPMI-1640 medium (cat. no. L0498; Biowest) containing 10% fetal bovine serum (cat. no. A5670801; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin at 37˚C in 5% CO_2. The cells ($1x10^6$ cells/ml) were cultured for 72 h with 10 ng/ml LPS, with or without NTS, MSM or TLR4-C34 (cat. no. S0822; Selleck Chemicals).

Fe^{2+} determination assay. The cells were stained with 5 μM FerroFarRed solution (cat. no. GC903-01; Goryo Chemical, Inc.) and incubated in a CO_2 incubator at 37˚C for 30-40 min. After staining, the cells were washed with 1 ml pre-warmed serum-free RPMI-1640 medium and used for flow cytometric analysis (FACSCalibur; BD Biosciences). FlowJo v10 software (FlowJo; BD Biosciences) was used for analysis.

Western blotting. Whole-cell lysates were prepared by incubating untreated or LPS-treated THP-1 cells on ice with radioimmunoprecipitation lysis buffer (cat. no. 20-188; MilliporeSigma) containing protease and phosphatase inhibitors. Protein concentrations were measured using the Bradford method (Thermo Fisher Scientific, Inc.). The same amounts of protein (30 μg/well) were then separated by SDS-PAGE on 6-15% gels and the separated proteins were transferred onto nitrocellulose membranes. The blots were blocked for 1 h at room temperature with 5% skim milk (BD Biosciences) in TBS-Tween-20 (TBS-T) buffer [20 mM Tris-HCl (MilliporeSigma), pH 7.6; 137 mM NaCl (Formedium Limited); 0.1X (0.1%) Tween-20 (Scientific Sales, Inc.)]. The membranes were then incubated with primary antibodies diluted in 5% skim milk (1:1,000 dilution) overnight at 4˚C with agitation. Subsequently, the membranes were washed with TBS-T and incubated for 1 h at room

temperature with HRP-conjugated secondary antibodies (1:5,000). Detection was performed using a West-Q Pico ECL Solution (cat. no. W3652-020; GenDEPOT, LLC) and a LAS-4000 imaging device (FUJIFILM Wako Pure Chemical Corporation).

Reverse transcription-quantitative PCR (RT-qPCR) assay. Total cellular RNA was extracted using an RNeasy Mini Kit (Qiagen GmbH) according to the manufacturer's protocol. The isolated RNA was quantified spectrophotometrically at 260 nm, and cDNA was synthesized at 42˚C for 1 h and 95˚C for 5 min with oligo d(T) primers and a first-strand cDNA synthesis kit (cat. no. K-2041; Bioneer Corporation). qPCR was then conducted using a thermal cycler (C1000 Thermal Cycler; Bio-Rad Laboratories, Inc.) as follows: 2 μl diluted cDNA was added to diluted forward and reverse primers (1 μl each, 100 pM) and 10 μl TB Green Advantage Premix (Takara Bio, Inc.). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min; followed by 40 cycles of denaturation at 95˚C for 40 sec, annealing at 58˚C for 40 sec and extension at 72˚C for 40 sec; and a final extension step at 72˚C for 5 min. All measurements were performed in triplicate. Relative target gene expression was normalized to GAPDH. The calculations were performed using the $2^{-\Delta\Delta Cq}$ values obtained (31). The primer sequences are provided in Table I.

Table I. Primer sequences used for reverse transcription-quantitative PCR analysis.

Gene	Sequence
TLR2	Sense: 5'-TGCAAGTACGAACTGGACTTCT-3' Antisense: 5'-CCAGGTAGGTCTTGGTGTTCATT-3'
TLR4	Sense: 5'-TAGCCATTGCTGCCAACATCAT-3' Antisense: 5'-AAGATACACCAACGGCTCTGAA-3'
iNOS	Sense: 5'-TGCTCAGCTCATCCGCTATG-3' Antisense: 5'-GATGTTCCATGGCCACCTCA-3'
NF-κB	Sense: 5'-GAAATTCCTGATCCAGACAAAAAC-3' Antisense: 5'-ATCACTTCAATGGCCTCTGTGTAG-3'
COX-1	Sense: 5'-GGCAGCAGAGTTGGAGGAAT-3' Antisense: 5'-CTTCTTCAGTGTGGCCGTCT-3'
COX-2	Sense: 5'-TTGCATTCTTTGCCCAGCAC-3' Antisense: 5'-ACCGTAGATGCTCAGGGACT-3'
IL-1β	Sense: 5'-ATTGCCTCTTCCAGCAGCTT-3' Antisense: 5'-GGCTTCACTGAGGTTGCCTT-3'
IL-6	Sense: 5'-GCTGATCCTGCCTCTGCC-3' Antisense: 5'-GACCCTCAAACCCACCCG-3'
TNF-α	Sense: 5'-TGGTGAGACAGAAAGAGCGG-3' Antisense: 5'-AGCCCTGAGGTGTCTGGT-3'
GAPDH	Sense: 5'-ACCCACTCCTCCACCTTTGA-3' Antisense: 5'-CATACCAGGAAATGAGCTTGACAA-3'

iNOS, inducible nitric oxide synthase; TLR, Toll-like receptor.

Flow cytometric analysis. After cultured cells were washed with pre-chilled PBS, cell pellets were incubated with 10% BSA (cat. no. A3311; MilliporeSigma) on ice for 20 min. PE-conjugated anti-human CD284 (TLR4) antibody (1:200; cat. no. 312806; Biolegend, Inc.) and APC-conjugated anti-human CD282 (TLR2) antibody (1:200; cat. no. 309720; Biolegend, Inc.) was used to stain the cells on ice for 30 min. Stained cells were then washed with pre-chilled PBS. Cells were also stained with CM-H2DCFDA (5 μM; cat. no. C6827; Invitrogen; Thermo Fisher Scientific, Inc.) for cellular ROS and placed in a CO_2 incubator at 37˚C for 30 min. The stained cells were washed with 1 ml prewarmed staining buffer (cat. no. 554656; BD Bioscience). Flow cytometric analysis was performed using a FACSCalibur flow cytometer. FlowJo v10 software was used for analysis.

Comet assay. The comet assay kit (cat. no. ab238544; Abcam) was used according to the manufacturer's protocol for measuring cellular DNA damage. A base layer of comet agarose was used to coat the slide, and a layer of cells (70% confluence) treated with 10 ng/ml LPS with the indicated concentrations of NTS or MSM was added, followed by another layer of 100 cells and agarose, followed by lysis. Electrophoresis was performed under neutral conditions, and cells were stained with DNA dye. Cell morphology was observed by fluorescence microscopy (IX71/DP72; Olympus Corporation).

Chromatin immunoprecipitation (ChIP) assay. A ChIP assay was performed using an Imprint Chromatin Immunoprecipitation Kit (cat. no. 17-295; MilliporeSigma) according to the manufacturer's protocol. THP-1 cells were fixed with 1% formaldehyde for 10 min at 37˚C and quenched with 1.25 M glycine at room temperature for 30 min at 37˚C. After washing with PBS, cells were suspended in a nucleus preparation buffer and sonicated in a shearing buffer under optimized conditions at 4˚C for 10 times (20 kHz, 10 sec on/30 sec off). This sheared DNA was diluted with a dilution buffer (1:1 ratio), and 5 μl diluted sample was removed as an internal control. The diluted supernatant was incubated for 5 min at room temperature in wells pre-coated with antibodies (1:50 dilution) specific for IL-1β (cat. no. sc-12742; Santa Cruz Biotechnology, Inc.), TNF-α (cat. no. 6945; Cell Signaling Technology, Inc.) or IL-6 (cat. no. sc-57315; Santa Cruz Biotechnology, Inc.) at room temperature for 90 min. Normal mouse IgG (1:10 dilution; cat. no. M8695; MilliporeSigma) and anti-RNA polymerase II (1:10 dilution; cat. no. R1530; MilliporeSigma) were used as negative and positive controls, respectively. The unbound DNA was washed off with wash buffer, and the bound DNA was collected by cross-link reversal using a DNA release buffer containing proteinase K. The released DNA and the DNA from the internal controls were purified with GenElute Binding Column G. DNA was then quantified and RT-qPCR was performed using the aforementioned reagents; the thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min; followed by 50 cycles of denaturation at 95˚C for 20 sec, annealing at 58˚C

for 20 sec and extension at 72°C for 20 sec. All measurements were performed in triplicate. Relative target gene expression was normalized to GAPDH. The calculations were performed using the $2^{-\Delta\Delta Cq}$ values obtained.

Nuclear extraction assay and NF-κB detection. Nuclear protein extracts were prepared with the Nuclear Extract Kit (cat. no. ab113474; Abcam). After harvesting the cells, the extraction buffer was prepared by adding DTT solution and a protease inhibitor cocktail at a 1:1,000 dilution. The cell pellet was treated with the prepared extraction buffer at a volume of 10 μl/10^6 cells, and then incubated on ice for 15 min, briefly vortexing for ~5 sec every 3 min for mixing. Subsequently, the mixture was centrifuged at 18,500 x g for 10 min at 4°C. The resulting supernatant was carefully transferred to a new microcentrifuge tube; this contained the nuclear protein extract. The nuclear extract was then used for western blot analysis iof NF-κB. Equal amounts of proteins (150 μg/well) were separated by SDS-PAGE on 10% gels and were then transferred onto nitrocellulose membranes. Subsequently, he blots were blocked for 1 h at room temperature with 5% skim milk. The membranes were then incubated with NF-κB and TBP antibodies diluted in 5% skim milk overnight at 4°C with agitations. The membranes were then washed with TBS-T and incubated for 1 h with HRP-conjugated secondary antibodies at room temperature. Detection was performed as aforementioned.

Statistical analyses. All experiments were performed in triplicate. Data are presented as the mean ± SEM of three independent experiments conducted in triplicate (n=3). The control group was set to 100 for RT-qPCR results. A one-way ANOVA followed by Tukey's post hoc test was used for the statistical analysis. The analyses were performed using SAS 9.3 software (SAS Institute, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sulfur compounds inhibit LPS-induced iron metabolism and heme biosynthesis in THP-1 human monocytes. We previously demonstrated that LPS-induced inflammation enhances iron metabolism through increased iron production and transport, which is responsible for iron homeostasis (5). To determine the effect of sulfur compounds on LPS-induced iron metabolism, the amount of Fe^{2+} was estimated following the treatment of THP-1 cells with NTS, MSM or TLR4-C34, a specific TLR4 inhibitor (Fig. 1A). Flow cytometry revealed increased Fe^{2+} levels after LPS treatment, whereas NTS and MSM exhibited decreased Fe^{2+} levels. A slightly different phenomenon was observed in TLR4-C34-treated cells, indicating the activity of sulfur compounds to inhibit LPS-induced inflammation by attenuating TLR4-independent iron metabolism. Next, western blot analysis was performed to identify the proteins responsible for iron transport and the molecular mechanisms associated with sulfur compound-regulated iron homeostasis. The results indicated upregulation of the TfR and FPN proteins by LPS, which was suppressed by treatment with NTS, MSM or TLR4-C34 (Fig. 1B). In addition, the expression levels of DMT1 and STEAP3, which are key factors in iron metabolism, were increased by LPS treatment. The regulation of iron metabolism may lead to heme synthesis. To confirm the induction of heme biosynthesis by LPS, the expression levels of proteins responsible for heme synthesis were examined through western blot analysis (Fig. 1C). The results indicated notable LPS-induced upregulation of HO-1, MFRN, ABCB6, ABCB10, ALAS1, FECH and FLVCR in THP-1 cells; however, these protein levels were markedly decreased following NTS or MSM treatment, but increased by TLR4-C34 treatment. These results indicated that iron/heme metabolism is pivotal in the anti-inflammatory activity of sulfur compounds, independent of TLR4.

Sulfur compounds downregulate the LPS-induced expression of TLRs in THP-1 cells. TLRs act as receptors for the inflammatory response in immune cells. Thus, the current study analyzed the interaction of sulfur compounds with TLRs upon LPS-induced inflammation. First, the expression pattern of TLRs (TLR2/4) was examined in response to two sulfur compounds during LPS-induced inflammation. The results indicated that LPS increased the expression levels of TLR2/4 proteins, which were markedly downregulated by the addition of 3 μg/ml NTS, 200 mM MSM or 40 μM TLR4-C34 (Fig. 2A). The present study also analyzed the mRNA expression levels of TLR2/4 after LPS treatment in the presence of NTS, MSM or TLR4-C34 in THP-1 cells. The results confirmed the inhibition of LPS-induced expression of TLR2/4 by NTS, MSM and the TLR4 inhibitor (Fig. 2B). Flow cytometric analysis also confirmed that TLR2/4 was downregulated by NTS, MSM or TLR4-C34 (Fig. 2C). These results suggested that the anti-inflammatory effects of natural sulfurs depend on the inhibition of TLR2/4 during LPS-induced inflammation.

Sulfur molecules decrease LPS-induced ROS generation in THP-1 cells. Generally, LPS is known to induce inflammation by generating ROS. The present study analyzed the effects of sulfur compounds on ROS generation in LPS-induced inflammation. First, the expression levels of the iNOS protein, which serves an important role in ROS generation, were measured. Western blot analysis revealed increased expression levels of iNOS in response to LPS, which were inhibited by NTS, MSM, and TLR4-C34 (Fig. 3A). These effects were also confirmed at the transcriptional level; increased iNOS mRNA expression levels were observed following LPS treatment, which were suppressed by NTS, MSM, and TLR4-C34 treatment (Fig. 3B). These results suggested that sulfur molecules exhibit similar effects to TLR4-C34, thus indicating the anti-inflammatory effects of NTS and MSM against LPS-induced inflammation. Finally, ROS generation was measured in response to treatment with sulfur compounds at the cellular and mitochondrial levels (Fig. 3C). Increased ROS levels were observed following LPS treatment, which were significantly reduced by treatment with natural sulfur compounds and a TLR4 inhibitor. These results indicated the cytoprotective nature of NTS and MSM against inflammation.

Sulfur molecules inhibit PKC-mediated inflammation and canonical NF-κB pathways against LPS-induced inflammation in THP-1 cells. It was hypothesized that NF-κB signaling

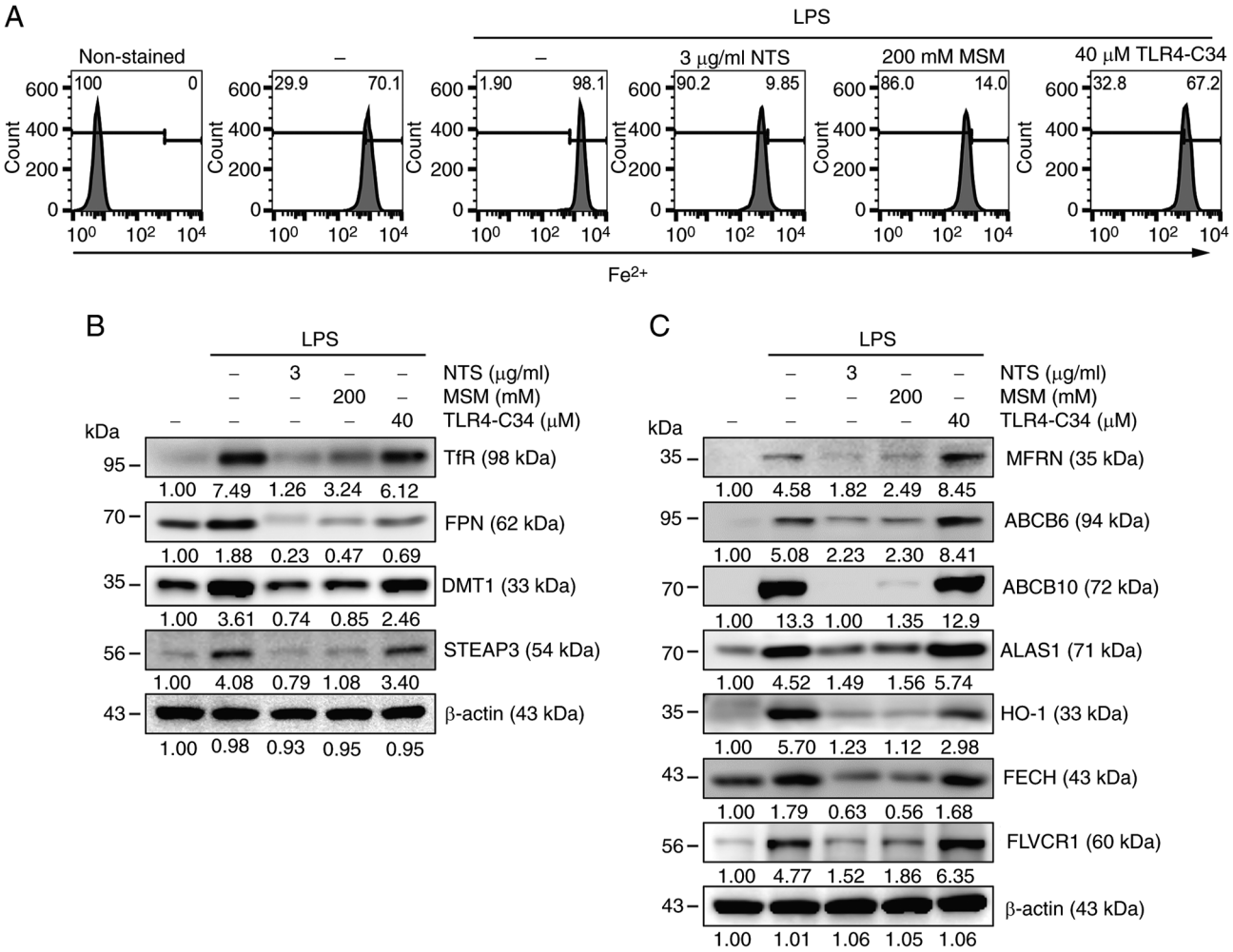

Figure 1. Sulfur compounds inhibit LPS-induced inflammation and iron/heme metabolism. (A) Flow cytometry showing Fe2+ levels in THP-1 cells following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. Western blot analysis of (B) transferrin receptor, ferroportin, DMT1 and STEAP3; and (C) MFRN, ABCB6, ABCB10, ALAS1, HO-1, FECH and FLVCR proteins in THP-1 cells following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. ALAS1, 5'-aminolevulinate synthase 1; DMT1, divalent metal transporter 1; Fe2+, ferrous ion; FECH, ferrochelatase; FPN, ferroportin; HO-1, heme oxygenase-1; LPS, lipopolysaccharide; MFRN, mitoferrin; MSM, methylsulfonylmethane; NTS, nontoxic sulfur; TfR, transferrin receptor; TLR, Toll-like receptor.

may be responsible for the anti-inflammatory response induced by NTS and MSM; therefore, the PKC-mediated upstream targets of NF-κB were examined. Increased expression levels of p-PKCα, p-ERK, and p-p38 were observed in LPS-treated THP-1 cells (Fig. 4A). By contrast, NTS, MSM and TLR4-C34 markedly reduced LPS-induced expression of PKC-mediated signaling factors, suggesting the possible involvement of NF-κB activity in the inflammatory response. Next, the present study assessed the expression levels of canonical NF-κB pathway elements. Treatment with LPS upregulated the expression levels of p-IKKα/β and p-IκBα, which were decreased by NTS, MSM, and TLR4-C34 treatment (Fig. 4B). These results indicated the involvement of NF-κB in the anti-inflammatory effects of sulfur compounds against LPS-induced inflammation.

Sulfur molecules alleviate LPS-induced DNA damage in THP-1 cells. DNA damage is a possible outcome of prolonged ROS production (32). In the present study, LPS-induced ROS generation was inhibited by treatment with sulfur compounds. Subsequently, a comet assay was performed to determine whether LPS induced DNA damage and if sulfur compounds could revert such an effect or induce a DNA damage response (DDR). The analysis of THP-1 cells by fluorescence microscopy revealed an increase in comet length and the number of comet-positive cells following LPS exposure, whereas these effects were reversed by NTS, MSM, and TLR4-C34 treatment (Fig. 5A). These results suggested the possible induction of DDR by sulfur compounds. Next, the current study analyzed the expression levels of several molecular signaling proteins responsible for DDR. LPS induced the expression of DNA damage markers, including p-ATR, p-ATM, p-Chk2, p-BRCA1 and p-p53, and DNA damage by LPS stabilized p53 and p-p53 by inhibiting MDM2-mediated degradation (Fig. 5B); however, NTS, MSM, and TLR4-C34 notably decreased their expression levels, indicating the ability of natural sulfurs to induce DDR in response to LPS-induced inflammation by regulating TLR4 expression.

Sulfur molecules suppress the expression of LPS-induced NF-κB and proinflammatory cytokines in THP-1 cells. Two sulfur compounds downregulated the upstream targets of

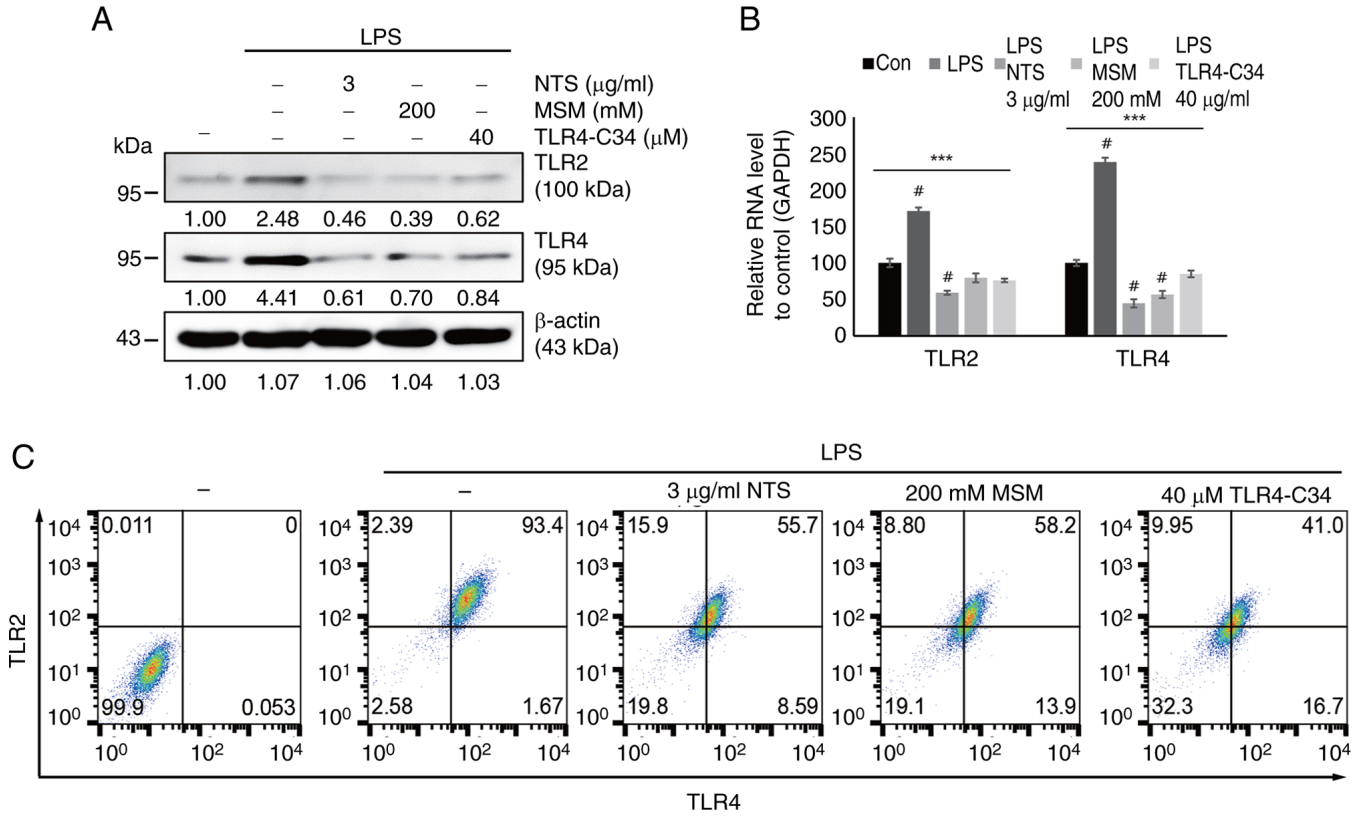

Figure 2. Sulfur compounds inhibit LPS-induced TLR expression. (A) Western blot analysis of TLR2 and TLR4 expression in THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. (B) Reverse transcription-quantitative PCR analysis of the relative expression levels of TLR2 and TLR4 normalized to GAPDH following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. ***P<0.001; #P<0.001 vs. non-treated control (one-way ANOVA and Tukey's test). (C) Flow cytometry showing the inhibition of LPS-induced TLR4 expression by 48-h treatment with NTS (3 μg/ml), MSM (200 mM) and TLR-C34 (40 μM). LPS, lipopolysaccharide; MSM, methylsulfonylmethane; NTS, nontoxic sulfur; TLR, Toll-like receptor.

NF-κB during LPS-induced inflammation; therefore, the current analyzed the regulation of NF-κB under the same conditions. The results indicated increased protein expression levels of COX-2 and NF-κB, but not COX-1, in response to LPS, whereas treatment with MSM, NTS, and TLR4-C34 markedy reduced their expression levels (Fig. 6A). The mRNA expression levels of NF-κB and COX-2 were consistent with their protein levels; sulfur molecules inhibited the LPS-induced expression of NF-κB and COX-2, but not COX-1 (Fig. 6B). The present study also analyzed the expression of proinflammatory cytokines, including IL-6, IL-1β and TNF-α, at the protein and mRNA levels (Fig. 6C and D); these were consistently upregulated by LPS and downregulated by NTS, MSM and the TLR4 inhibitor. Next, the current study evaluated the binding of NF-κB to the promoter region of proinflammatory cytokines using ChIP assay, and a significant inhibition of NF-κB binding to proinflammatory cytokines was observed by natural sulfur compounds (Fig. 6E). The inhibition of the LPS-induced nuclear translocation of NF-κB by NTS or MSM also strongly supported the inhibitory mechanism against the LPS-induced NF-κB-dependent inflammatory response (Fig. 6F). These results suggested that sulfur compounds may suppress LPS-induced inflammatory responses by inhibiting iron homeostasis and heme biosynthesis in THP-1 human monocytes (Fig. 7).

Discussion

Natural compounds can effectively ameliorate inflammatory responses as they generally lack side effects; they also exhibit anti-inflammatory properties against LPS-induced inflammation (33). MSM and NTS exert anti-inflammatory effects, and these compounds may be suitable candidates for anti-inflammatory treatment, similar to artemisinin or artesunate, if they can inhibit the effects of LPS-induced inflammation. In the present study, it was demonstrated that NTS and MSM inhibited LPS-induced inflammation in THP-1 human monocytes and that iron/heme metabolism may serve an important role in this mechanism.

Iron metabolism is important to the inflammatory process and is essential for human health. Notably, an imbalance in iron transport can cause inflammation (34). LPS-treated THP-1 cells exhibit elevated Fe^{2+} levels, suggesting an increased amount of iron that must be oxidized. A failure in converting Fe^{2+} into Fe^{3+} for oxidation results in inflammation (35). The present study observed decreased Fe^{2+} levels following NTS and MSM treatment, suggesting that these sulfur compounds can regulate iron transport and inflammation. Generally, iron balance depends on FPN and TfR, which mediate iron homeostasis through iron transport; whereas FPN exports iron, TfR takes up Fe^{3+} ion for metabolism (36). DMT1 is another membrane transporter that transports iron from the endosomal system

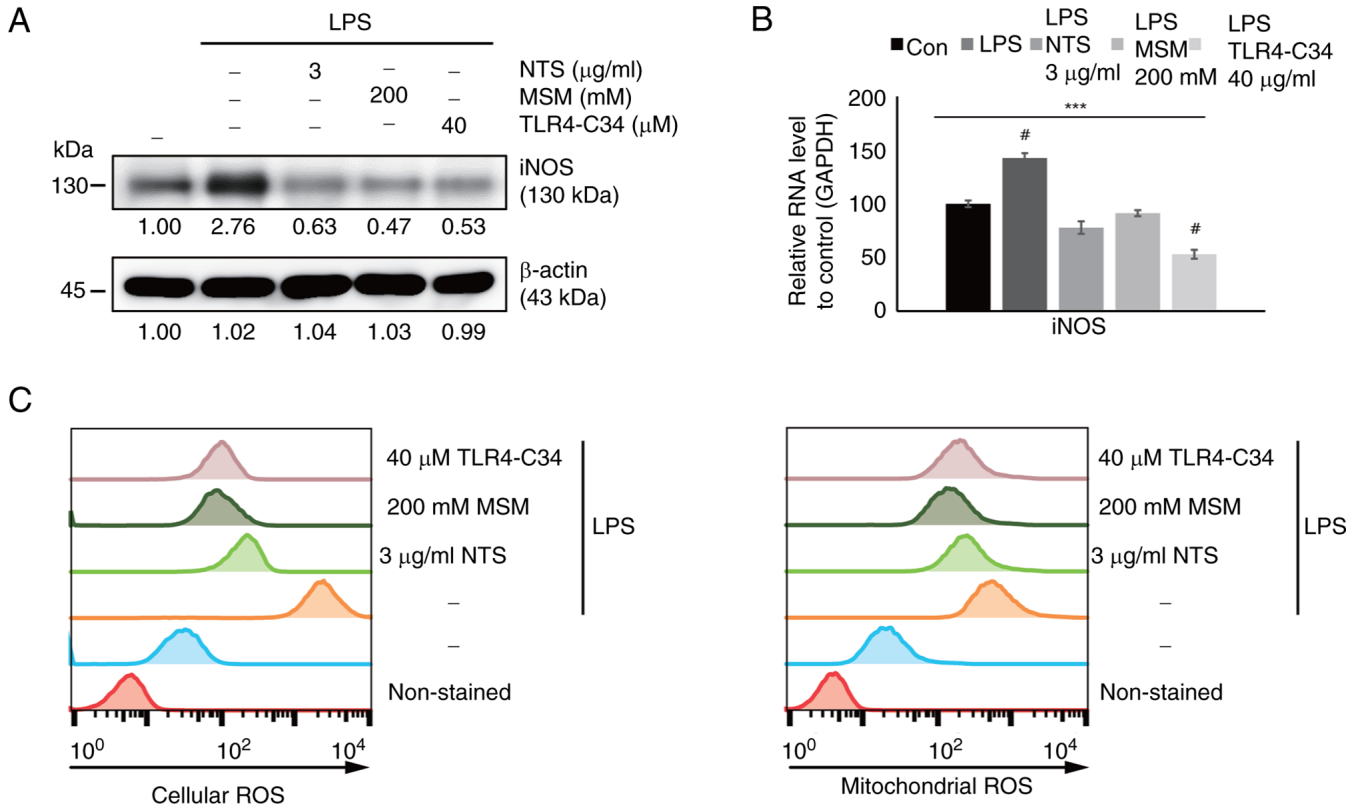

Figure 3. Sulfur compounds inhibit LPS-induced ROS. (A) Western blot analysis of iNOS expression in THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. (B) Reverse transcription-quantitative PCR analysis of iNOS mRNA expression in cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. Data are normalized to GAPDH. ***P<0.001; $^{\#}$P<0.001 vs. non-treated control (one-way ANOVA and Tukey's test). (C) Flow cytometric analysis of cellular and mitochondrial ROS in THP-1 cells following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MSM, methylsulfonylmethane; NTS, nontoxic sulfur; TLR, Toll-like receptor; ROS, reactive oxygen species.

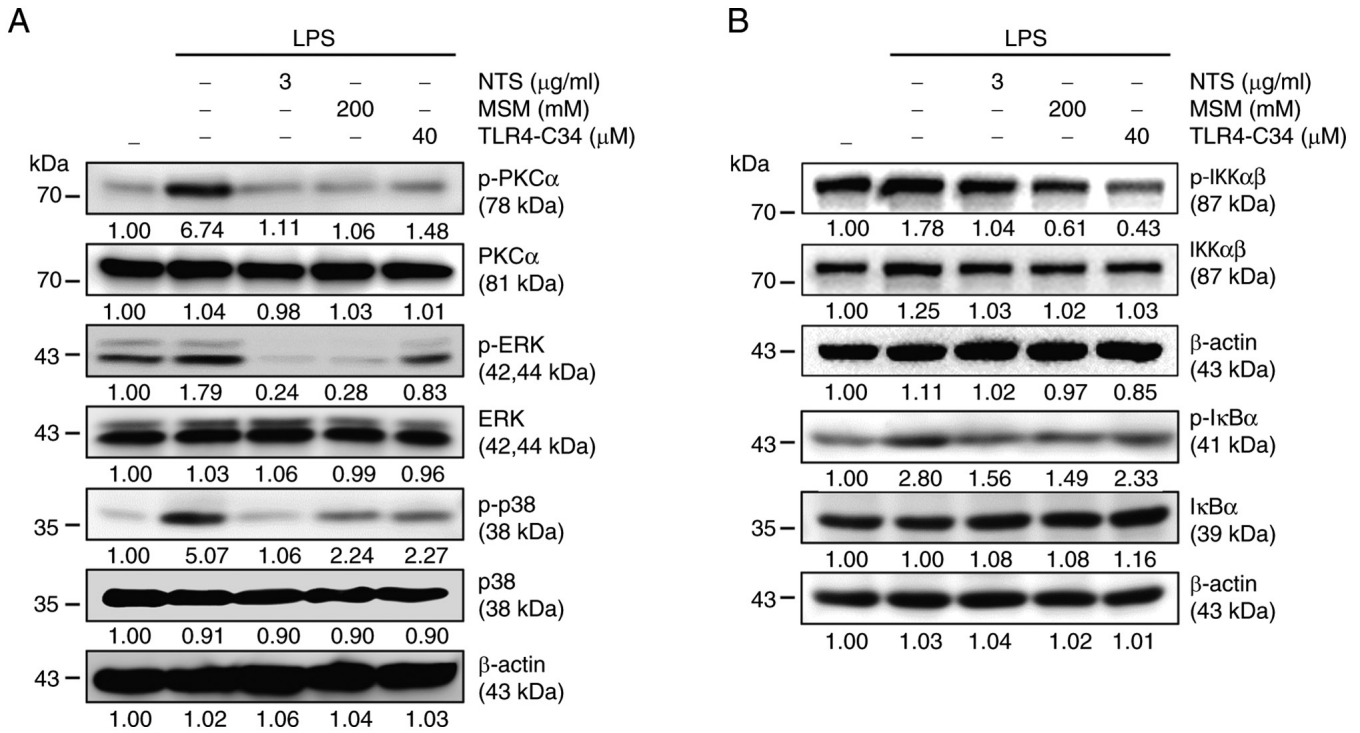

Figure 4. Sulfur compounds inhibit the LPS-induced PKC-dependent and canonical NF-κB pathways. Western blot analysis of THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h showing the inhibition of LPS-induced (A) p-PKC-α, p-ERK and p-p38 expression; and (B) p-IKKα/β and p-IκBα expression. LPS, lipopolysaccharide; MSM, methylsulfonylmethane; NTS, nontoxic sulfur; p-, phosphorylated; TLR, Toll-like receptor.

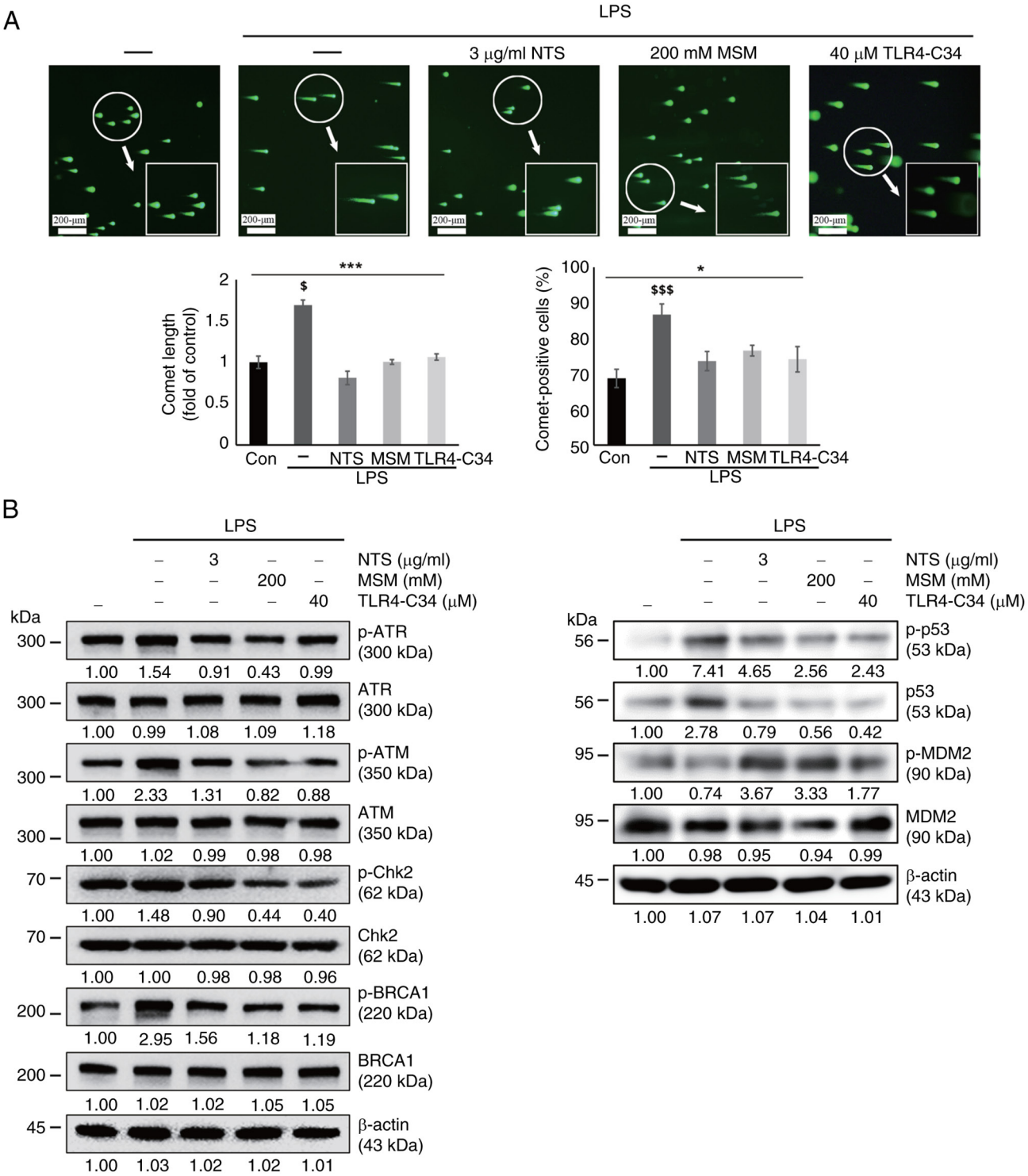

Figure 5. Sulfur compounds induce DNA damage response following LPS-induced DNA damage. (A) Images of the comet assay were captured by fluorescence microscopy at x10 and x40 magnification levels, showing the fragmented DNA migrating out of the nucleoid body, which formed a comet tail following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. *P<0.05 and ***P<0.001; $^{\$}$P<0.05 vs. non-treated control; and $^{\$\$\$}$P<0.001 vs. non-treated control (one-way ANOVA and Tukey's test). (B) Western blot analysis of THP-1 cells; NTS (3 μg/ml) and MSM (200 mM) inhibited the LPS-induced expression of p-ATM, p-ATR, p-Chk2, p-BRCA1, and p-p53. However, the expression levels of p-MDM2 were suppressed by LPS treatment, and were increased by NTS (3 μg/ml), MSM (200 mM) or TLR4-C34 (40 μM). LPS, lipopolysaccharide; MSM, methylsulfonylmethane; NTS, nontoxic sulfur; p-, phosphorylated; TLR, Toll-like receptor.

to the cytosol and functions in intestinal iron absorption by oxidizing Fe^{3+} before the translocation of Fe^{2+} (37,38). Inside the endosome, STEAP3 controls iron transport by reducing Fe^{3+} to Fe^{2+} (39). Therefore, dysregulation in the expression of these receptors may contribute to inflammation. The present study observed the increased expression of TfR, FPN, DMT1 and STEAP3 following LPS treatment, suggesting increased iron transport. NTS and MSM inhibited the expression of these receptors, which in turn, ameliorated iron transport and exerted anti-inflammatory effects.

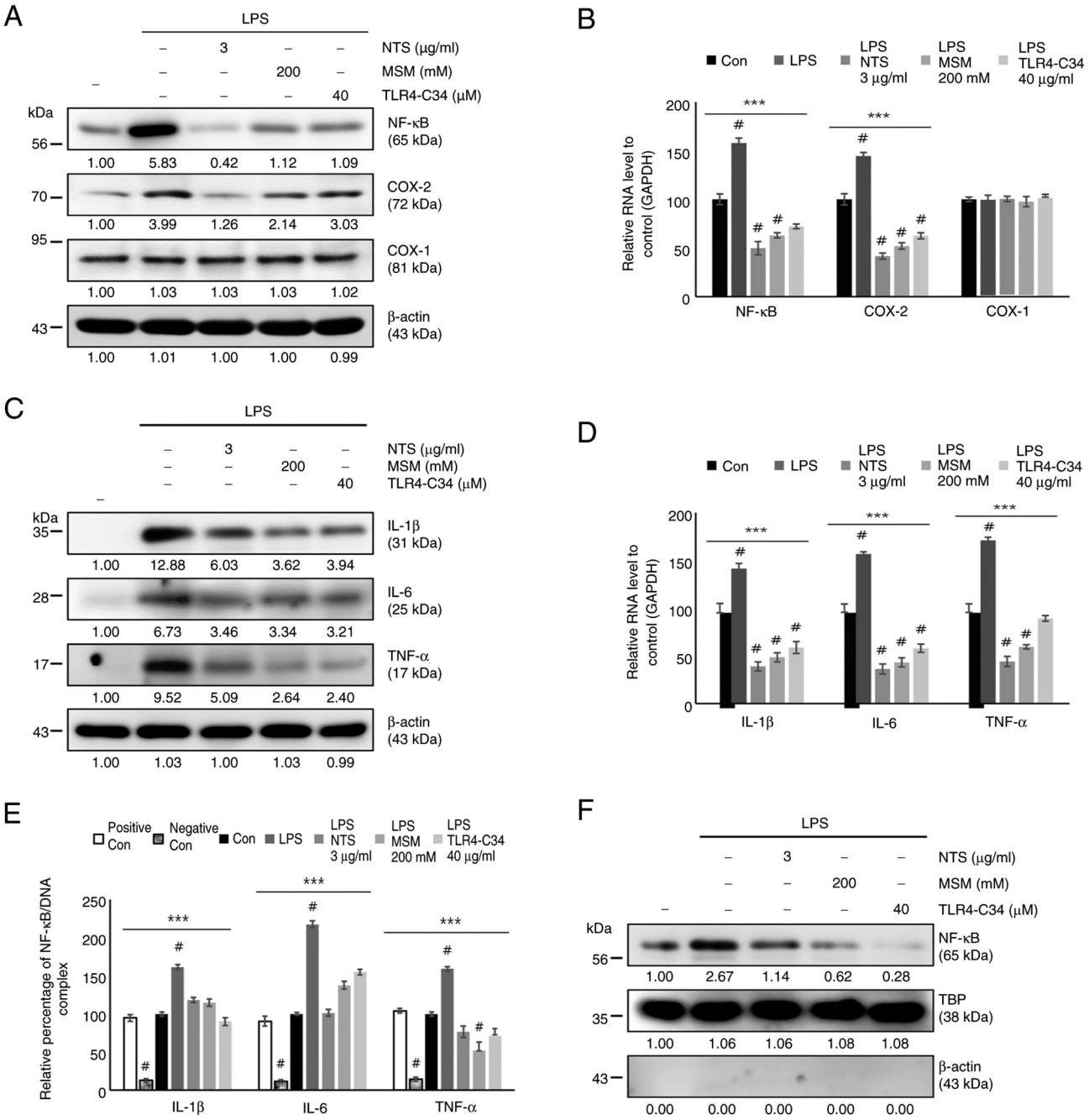

Figure 6. Sulfur compounds inhibit LPS-induced NF-κB, COX-2 and proinflammatory cytokine expression. (A) Western blot analysis of the expression levels of NF-κB, COX-1 and COX-2 in THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. (B) RT-qPCR analysis of the relative expression levels of NF-κB, COX-1 and COX-2 normalized to GAPDH following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. ***P<0.001; #P<0.001 vs. non-treated control (one-way ANOVA and Tukey's test). (C) Western blot analysis of the expression levels of IL-1β, IL-6 and TNF-α in THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. (D) RT-qPCR analysis of the relative expression levels of IL-1β, IL-6 and TNF-α normalized to GAPDH in cells following treatment with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h. ***P<0.001; #P<0.001 vs. non-treated control (one-way ANOVA and Tukey's test). (E) Chromatin immunoprecipitation assay of THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h showing the relative binding of NF-κB to the promoters of IL-1β, IL-6 and TNF-α. ***P<0.001; #P<0.001 vs. non-treated control (one-way ANOVA and Tukey's test). (F) Nuclear protein extract analysis of THP-1 cells treated with LPS (10 ng/ml) + NTS (3 μg/ml), MSM (200 mM) or TLR-C34 (40 μM) for 48 h showing the protein expression levels of nuclear NF-κB. TBP was used as the housekeeping protein for the nuclear extract and β-actin was used to show the efficacy of nuclear protein extraction. LPS, lipopolysaccharide; MSM, methylsulfonylmethane; NTS, nontoxic sulfur; RT-qPCR, reverse transcription-quantitative PCR; TBP, TATA-binding protein; TLR, Toll-like receptor.

Iron is an important molecule for heme biosynthesis and in the mitochondria, where it is essential for energy production, antioxidant defense and signal transduction (40). Generally, ALAS1 is important for heme synthesis, and combines glycine and succinyl-CoA to form aminolaevulinic acid. The FECH enzyme is also involved in heme synthesis by combining Fe^{2+} and protoporphyrin IX (41). ABCB6 is a putative promoter that contributes to the transition of ALA, and with the help of ALAS1, FECH and MFRN, promotes iron uptake for its interaction with FECH and ABCB10, which leads to heme synthesis (42,43). The resulting

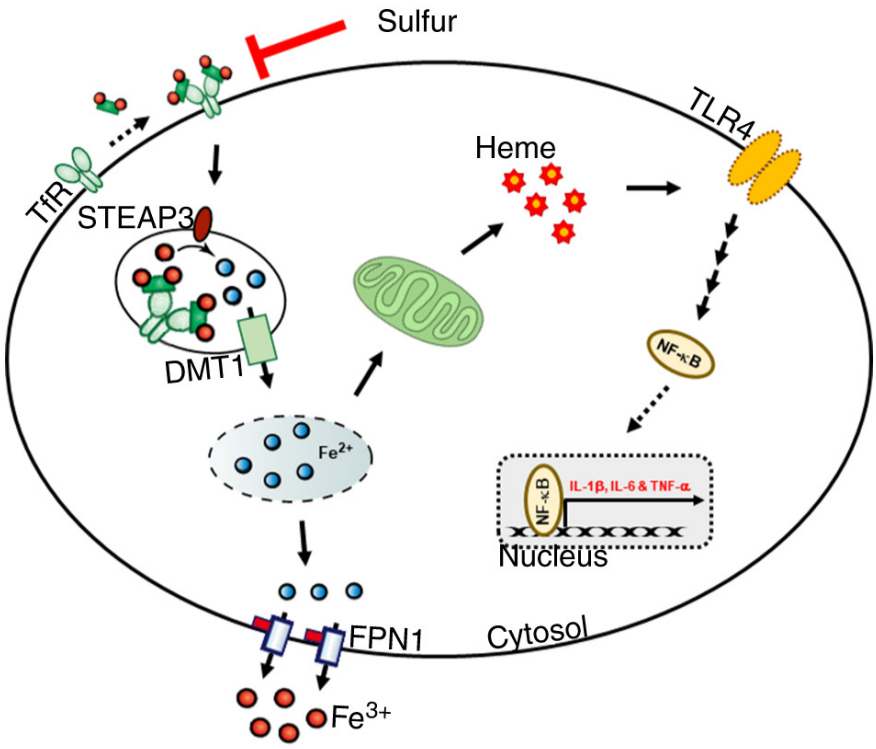

Figure 7. Molecular mechanism of LPS-induced regulation of the inflammatory response through iron/heme metabolism and TLR4/NF-κB expression through the canonical NF-κB and PKC-mediated inflammatory pathways. The anti-inflammatory activities of NTS and MSM were achieved by inhibiting iron/heme metabolism and suppressing the expression of TLR4/NF-κB signaling molecules, thus blocking the binding of NF-κB to the gene promoters of proinflammatory cytokines. DMT1, divalent metal transporter 1; Fe^{2+}, ferrous ion; FPN, ferroportin; TfR, transferrin receptor; TLR4, Toll-like receptor 4.

heme is then transferred to the cytoplasm with the help of a heme exporter, FLVCR1 (44). Similar to the present study, in a previous study, LPS has been shown to upregulate the expression of HO-1, which catalyzes the degradation of heme, and the expression of the proteins responsible for heme synthesis, including ALAS1, FECH, ABCB6, MFRN, ABCB10 and FLVCR (5). These results indicated that LPS-induced inflammation may promote heme production. NTS and MSM inhibited the expression of these proteins, which indicated an inhibition of heme synthesis by these sulfur compounds. The TLR4 inhibitor did not alter the expression of these proteins, indicating that heme synthesis may be independent of TLR4 expression.

TLR4 is a transmembrane receptor that mediates a signaling response to inflammation and represents a major part of the LPS-induced inflammatory response (45,46). TLR4 transduces signals to the major downstream target NF-κB, which is translocated into the nucleus upon activation to upregulate proinflammatory cytokines (47,48). The current study observed increased expression levels of TLR2 and TLR4 during LPS treatment, which were significantly reduced by NTS or MSM. These findings indicated that the anti-inflammatory effect of these sulfur compounds may be mediated by regulating TLR expression. A similar effect was also observed on NF-κB expression. Furthermore, the inhibition of NF-κB expression by TLR4-C34 treatment indicated that these sulfur compounds act through TLR4/NF-κB signaling.

Activation of NF-κB occurs through a canonical pathway (49) or a PKC-dependent pathway (50). The canonical pathway depends on IκBα and IKKα/β, whereas the PKC-dependent pathway signals through the ERK/p38 signaling pathway (11). Similar to the present study, in a previous study, LPS has been reported to upregulate p-IKKαβ and IκBα levels in the canonical pathway, whereas NTS or MSM downregulated the expression of these molecules without affecting the expression of total IκBα (11). In the PKC-dependent pathway, LPS-induced inflammation increased the expression of p-PKCα, p-ERK and p-p38 without altering the expression levels of their total forms. By contrast, the natural sulfur compounds NTS and MSM suppressed the LPS-induced expression of p-PKCα, p-ERK and p-p38 during TLR4-independent signaling. Upon inflammation, NF-κB is translocated into the nucleus and induces the transcription of genes encoding proinflammatory cytokines to elicit an immune response (51). The current study demonstrated that LPS-induced inflammation promoted the translocation of NF-κB into the nucleus, where it may bind to the promoters of the proinflammatory cytokines, IL-6, IL-1β and TNF-α. Notably, NTS and MSM successfully inhibited the translocation of NF-κB into the nucleus and blocked its binding to these proinflammatory cytokine gene promoters. Furthermore, the LPS-induced COX-2 expression was reversed by the two sulfur compounds without affecting the expression of COX-1, indicating the inhibition of the inflammatory response by NTS and MSM in a COX-1-independent and TLR4-dependent manner.

LPS-induced inflammation generates ROS that leads to oxidative damage (52). iNOS induction also results in ROS generation, and LPS can induce iNOS expression and ROS generation (53). The present results confirmed the increase in iNOS mRNA and protein expression, as well as cellular and mitochondrial ROS generation. Moreover, NTS and MSM suppressed the expression levels of iNOS and ROS generation at the cellular and

mitochondrial levels. These results indicated the role of oxidative stress in LPS-dependent inflammation and the effect of sulfur compounds, which markedly reduced the inflammatory response by suppressing oxidative stress. This prolonged oxidative stress can cause DNA damage, and LPS has been shown to induce DNA double-strand breaks to promote tumorigenesis (32). Anti-inflammatory drugs may inhibit DNA damage by inducing DDR. In the present study, NTS and MSM suppressed the formation of DNA strand breaks, as determined using the comet assay, suggesting the induction of DDR by these sulfur molecules. The downregulation of LPS-induced expression levels of the proteins responsible for DDR by NTS or MSM also strongly supported the anti-inflammatory effects of these sulfur molecules by inhibiting LPS-induced oxidative stress. The present study also observed that the natural sulfur molecules could decrease the expression levels of MFRN, ABCB6, ABCB10, ALAS1, FECH and FLVCR, which are associated with the iron/heme metabolism in THP1 cells, whereas TLR4-C34 (TLR4 inhibitor) did not affect these expression levels. In addition, it was confirmed that natural sulfur molecules inhibited the TLR4/NF-κB-mediated inflammatory response. Although the direct interaction between sulfurs and TLR4-C34 has not yet been assessed, it may be hypothesized that sulfur molecules decrease the inflammatory response by inhibiting iron/heme metabolism prior to suppressing the TLR4/NF-κB pathway.

In conclusion, the natural sulfur compounds NTS and MSM inhibited the LPS-induced inflammatory response in THP-1 human monocytes. Iron/heme metabolism is important to the anti-inflammatory activity of these sulfur compounds, which includes the inhibition of LPS-induced expression of TLR4 and NF-κB through canonical and PKC-dependent pathways, thus suppressing the production of the proinflammatory cytokines, COX-2, IL-1β and IL-6. In addition, NTS and MSM suppressed LPS-induced ROS production and induced DDR in THP-1 human monocytes. Therefore, NTS and MSM may have potential as therapeutic candidates for inflammatory diseases caused by LPS, similar to artemisinin or artesunate.

Acknowledgements

Not applicable.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (Ministry of Science and ICT) to KJJ (grant no. RS-2024-00450676). This work was also supported by the Basic Science Research Program to the Research Institute for Basic Sciences of Jeju National University through the NRF funded by the Ministry of Education to SWB (grant no. 2019R1A6A1A10072987). Additionally, this research was supported by a Korea Basic Science Institute (National Research Facilities and Equipment Center) grant funded by the Ministry of Education (grant no. 2023R1A6C101A045).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KJJ designed the experiments and wrote the manuscript. DYK and SWB performed all the experiments and analyzed the data. KJJ, DYK and SWB confirm the authenticity of all the raw data. All authors helped to revise the manuscript, and read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Wessling-Resnick M: Iron homeostasis and the inflammatory response. Annu Rev Nutr 30: 105-122, 2010.
2. Martins AC, Almeida JI, Lima IS, Kapitao AS and Gozzelino R: Iron metabolism and the inflammatory response. IUBMB Life 69: 442-450, 2017.
3. Alam Z, Devalaraja S, Li M, To TKJ, Folkert IW, Mitchell-Velasquez E, Dang MT, Young P, Wilbur CJ, Silverman MA, *et al*: Counter regulation of spic by NF-kappaB and STAT signaling controls inflammation and iron metabolism in macrophages. Cell Rep 31: 107825, 2020.
4. Wang L, Harrington L, Trebicka E, Shi HN, Kagan JC, Hong CC, Lin HY, Babitt JL and Cherayil BJ: Selective modulation of TLR4-activated inflammatory responses by altered iron homeostasis in mice. J Clin Invest 119: 3322-3328, 2009.
5. Kang DY, Sp N, Jo ES, Lee JM and Jang KJ: New insights into the pivotal role of iron/Heme metabolism in TLR4/NF-κB Signaling-mediated inflammatory responses in human monocytes. Cells 10: 2549, 2021.
6. Wagener FA, Volk HD, Willis D, Abraham NG, Soares MP, Adema GJ and Figdor CG: Different faces of the heme-heme oxygenase system in inflammation. Pharmacol Rev 55: 551-571, 2003.
7. Sp N, Kang DY, Jo ES, Lee JM, Bae SW and Jang KJ: Pivotal role of iron homeostasis in the induction of mitochondrial apoptosis by 6-Gingerol through PTEN regulated PD-L1 expression in embryonic cancer cells. Front Oncol 11: 781720, 2021.
8. Nath KA, Vercellotti GM, Grande JP, Miyoshi H, Paya CV, Manivel JC, Haggard JJ, Croatt AJ, Payne WD and Alam J: Heme protein-induced chronic renal inflammation: Suppressive effect of induced heme oxygenase-1. Kidney Int 59: 106-117, 2001.
9. Wagener FADTG, Eggert A, Boerman OC, Oyen WJ, Verhofstad A, Abraham NG, Adema G, van Kooyk Y, de Witte T and Figdor CG: Heme is a potent inducer of inflammation in mice and is counteracted by heme oxygenase. Blood 98: 1802-1811, 2001.
10. Yucel G, Zhao Z, El-Battrawy I, Lan H, Lang S, Li X, Buljubasic F, Zimmermann WH, Cyganek L, Utikal J, *et al*: Lipopolysaccharides induced inflammatory responses and electrophysiological dysfunctions in human-induced pluripotent stem cell derived cardiomyocytes. Sci Rep 7: 2935, 2017.
11. Sp N, Kang DY, Kim HD, Rugamba A, Jo ES, Park JC, Bae SW, Lee JM and Jang KJ: Natural sulfurs inhibit LPS-induced inflammatory responses through NF-κB signaling in CCD-986Sk skin fibroblasts. Life-Basel 11: 427, 2021.
12. Piktel E, Wnorowska U, Ciesluk M, Deptula P, Pogoda K, Misztalewska-Turkowicz I, Paprocka P, Niemirowicz-Laskowska K, Wilczewska AZ, Janmey PA and Bucki R: Inhibition of inflammatory response in human keratinocytes by magnetic nanoparticles functionalized with PBP10 peptide derived from the PIP2-binding site of human plasma gelsolin. J Nanobiotechnology 17: 22, 2019.
13. Rafi MM, Yadav PN and Rossi AO: Glucosamine inhibits LPS-induced COX-2 and iNOS expression in mouse macrophage cells (RAW 264.7) by inhibition of p38-MAP kinase and transcription factor NF-kappaB. Mol Nutr Food Res 51: 587-593, 2007.

14. Bjorkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, Means T, Halmen K, Luster AD, Golenbock DT and Freeman MW: Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med 10: 416-421, 2004.
15. van der Merwe M and Bloomer RJ: The influence of methylsulfonylmethane on inflammation-associated cytokine release before and following strenuous exercise. J Sports Med (Hindawi Publ Corp) 2016: 7498359, 2016.
16. Koh E and Surh J: Influence of sulfur fertilization on the antioxidant activities of onion juices prepared by thermal treatment. Prev Nutr Food Sci 21: 160-164, 2016.
17. Preetha NS, Kang DY and Darvin P: Induction of ketosis condition and suppression using methylsulfonylmethane by altering ANGPTL3 expression through STAT5b signaling mechanism. Anim Cells Syst 19: 30-38, 2015.
18. P NS, Kang DY, Kim BJ, Joung YH, Darvin P, Byun HJ, Kim JG, Park JU and Yang YM: Methylsulfonylmethane induces G1 arrest and mitochondrial apoptosis in YD-38 gingival cancer cells. Anticancer Res 37: 1637-1646, 2017.
19. Kang DY, Sp N, Bae SW and Jang KJ: Methylsulfonylmethane relieves cobalt chloride-induced hypoxic toxicity in C2C12 myoblasts. Life Sci 301: 120619, 2022.
20. S PN, Darvin P, Yoo YB, Joung YH, Kang DY, Kim DN, Hwang TS, Kim SY, Kim WS, Lee HK, *et al*: The combination of methylsulfonylmethane and tamoxifen inhibits the Jak2/STAT5b pathway and synergistically inhibits tumor growth and metastasis in ER-positive breast cancer xenografts. BMC Cancer 15: 474, 2015.
21. Kim DH, Nipin SP, Kang DY, Jo ES, Rugamba A, Jang KJ and Yang YM: Effect of methylsulfonylmethane on proliferation and apoptosis of A549 lung cancer cells through G/M Cell-cycle arrest and intrinsic cell death pathway. Anticancer Res 40: 1905-1913, 2020.
22. Sousa-Lima I, Park SY, Chung M, Jung HJ, Kang MC, Gaspar JM, Seo JA, Macedo MP, Park KS, Mantzoros C, *et al*: Methylsulfonylmethane (MSM), an organosulfur compound, is effective against obesity-induced metabolic disorders in mice. Metabolism 65: 1508-1521, 2016.
23. Miller L, Thompson K, Pavlenco C, Mettu VS, Haverkamp H, Skaufel S, Basit A, Prasad B and Larsen J: The effect of daily methylsulfonylmethane (MSM) consumption on High-density lipoprotein cholesterol in healthy overweight and obese adults: A randomized controlled trial. Nutrients 13: 3620, 2021.
24. Miller LE: Methylsulfonylmethane decreases inflammatory response to tumor necrosis factor-alpha in cardiac cells. Am J Cardiovasc Dis 8: 31-38, 2018.
25. Caron JM, Bannon M, Rosshirt L, Luis J, Monteagudo L, Caron JM and Sternstein GM: Methyl sulfone induces loss of metastatic properties and reemergence of normal phenotypes in a metastatic cloudman S-91 (M3) murine melanoma cell line. PLoS One 5: e11788, 2010.
26. Kim YB, Lee SH, Kim DH, Lee HG, Choi Y, Lee SD and Lee KW: Effects of dietary organic and inorganic sulfur on laying performance, egg quality, ileal morphology, and antioxidant capacity in laying hens. Animals (Basel) 12: 87, 2021.
27. Lee JS, Kwon JK, Han SH, An IJ, Kim SJ, Lee SH, Park YS, Park BK, Kim BS, Kim S, *et al*: Toxicity study of detoxication sulphur at 3 months post-treatment in rats. J Food Hygiene Safety 25: 263-268, 2010.
28. Kang DY, Sp N, Jo ES, Kim HD, Kim IH, Bae SW, Jang KJ and Yang YM: Non-toxic sulfur enhances growth hormone signaling through the JAK2/STAT5b/IGF-1 pathway in C2C12 cells. Int J Mol Med 45: 931-938, 2020.
29. Kang DY, Sp N, Jo ES, Rugamba A, Kim HD, Kim IH, Park JC, Bae SW, Jang KJ and Yang YM: Non-toxic sulfur inhibits LPS-induced inflammation by regulating TLR-4 and JAK2/STAT3 through IL-6 signaling. Mol Med Rep 24: 485, 2021.
30. Jo ES, Sp N, Kang DY, Rugamba A, Kim IH, Bae SW, Liu Q, Jang KJ and Yang YM: Sulfur compounds inhibit high Glucose-induced inflammation by regulating NF-κB signaling in human monocytes. Molecules 25: 2342, 2020.
31. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
32. Qiao W, Huang Y, Bian Z, Sun X, Wang X, Gao Q, Peng Y and Meng L: Lipopolysaccharide-induced DNA damage response activates nuclear factor κB signalling pathway via GATA4 in dental pulp cells. Int Endod J 52: 1704-1715, 2019.
33. Lee DY, Li H, Lim HJ, Lee HJ, Jeon R and Ryu JH: Anti-inflammatory activity of sulfur-containing compounds from garlic. J Med Food 15: 992-999, 2012.
34. Soares P, Silva C, Chavarria D, Silva FSG, Oliveira PJ and Borges F: Drug discovery and amyotrophic lateral sclerosis: Emerging challenges and therapeutic opportunities. Ageing Res Rev 83: 101790, 2023.
35. Osterholm EA and Georgieff MK: Chronic inflammation and iron metabolism. J Pediatr 166: 1351-1357.e1, 2015.
36. Zhang MW, Yang G, Zhou YF, Qian C, Mu MD, Ke Y and Qian ZM: Regulating ferroportin-1 and transferrin receptor-1 expression: A novel function of hydrogen sulfide. J Cell Physiol 234: 3158-3169, 2019.
37. Latunde-Dada GO, Van der Westhuizen J, Vulpe CD, Anderson GJ, Simpson RJ and McKie AT: Molecular and functional roles of duodenal cytochrome B (Dcytb) in iron metabolism. Blood Cells Mol Dis 29: 356-360, 2002.
38. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD and Andrews NC: Nramp2 is mutated in the anemic Belgrade (b) rat: Evidence of a role for Nramp2 in endosomal iron transport. Proc Natl Acad Sci USA 95: 1148-1153, 1998.
39. Byrne SL, Krishnamurthy D and Wessling-Resnick M: Pharmacology of iron transport. Annu Rev Pharmacol Toxicol 53: 17-36, 2013.
40. Richardson DR, Lane DJR, Becker EM, Huang ML, Whitnall M, Suryo Rahmanto Y, Sheftel AD and Ponka P: Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. Proc Natl Acad Sci USA 107: 10775-10782, 2010.
41. Chiabrando D, Mercurio S and Tolosano E: Heme and erythropoieis: More than a structural role. Haematologica 99: 973-983, 2014.
42. Chen W, Paradkar PN, Li LT, Pierce EL, Langer NB, Takahashi-Makise N, Hyde BB, Shirihai OS, Ward DM, Kaplan J and Paw BH: Abcb10 physically interacts with mitoferrin-1 (Slc25a37) to enhance its stability and function in the erythroid mitochondria. Proc Natl Acad Sci USA 106: 16263-16268, 2009.
43. Chen W, Dailey HA and Paw BH: Ferrochelatase forms an oligomeric complex with mitoferrin-1 and Abcb10 for erythroid heme biosynthesis. Blood 116: 628-630, 2010.
44. Chiabrando D, Marro S, Mercurio S, Giorgi C, Petrillo S, Vinchi F, Fiorito V, Fagoonee S, Camporeale A, Turco E, *et al*: The mitochondrial heme exporter FLVCR1b mediates erythroid differentiation. J Clin Invest 122: 4569-4579, 2012.
45. Yu B, Li Q and Zhou M: LPS-induced upregulation of the TLR4 signaling pathway inhibits osteogenic differentiation of human periodontal ligament stem cells under inflammatory conditions. Int J Mol Med 43: 2341-2351, 2019.
46. Ngkelo A, Meja K, Yeadon M, Adcock I and Kirkham PA: LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and Giα dependent PI-3kinase signalling. J Inflamm (Lond) 9: 1, 2012.
47. Xiang PJ, Chen T, Mou Y, Wu H, Xie P, Lu G, Gong X, Hu Q, Zhang Y and Ji H: NZ suppresses TLR4/NF-κB signalings and NLRP3 inflammasome activation in LPS-induced RAW264.7 macrophages. Inflamm Res 64: 799-808, 2015.
48. Zusso M, Lunardi V, Franceschini D, Pagetta A, Lo R, Stifani S, Frigo AC, Giusti P and Moro S: Ciprofloxacin and levofloxacin attenuate microglia inflammatory response via TLR4/NF-κB pathway. J Neuroinflammation 16: 148, 2019.
49. Sun SC: The non-canonical NF-κB pathway in immunity and inflammation. Nat Rev Immunol 17: 545-558, 2017.
50. Dasu MR, Devaraj S and Jialal I: High glucose induces IL-1beta expression in human monocytes: Mechanistic insights. Am J Physiol Endocrinol Metab 293: E337-E346, 2007.
51. Lee JS and Surh YJ: Nrf2 as a novel molecular target for chemoprevention. Cancer Lett 224: 171-184, 2005.
52. Zhang XQ, Wang CH, Shan S, Liu XY, Jiang ZM and Ren T: TLR4/ROS/miRNA-21 pathway underlies lipopolysaccharide instructed primary tumor outgrowth in lung cancer patients. Oncotarget 7: 42172-42182, 2016.
53. Lee CW, Kim SC, Kwak TW, Lee JR, Jo MJ, Ahn YT, Kim JM and An WG: Anti-Inflammatory effects of Bangpungtongsung-San, a traditional herbal prescription. Evid Based Complement Alternat Med 2012: 892943, 2012.

Copyright © 2025 Kang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.