Distinct roles of HMGB1 in the regulation of P-glycoprotein expression in the liver and kidney of mice with lipopolysaccharide-induced inflammation

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Abstract. The role of high mobility group box 1 (HMGB1) in the regulation of efflux transporters in the liver and kidney remains unclear, although it has been reported that HMGB1 can increase P-glycoprotein (P-gp) expression in the brain. The present study aimed to clarify the involvement of HMGB1 in the regulation of P-gp expression in the liver and kidney of mice with lipopolysaccharide (LPS)-induced inflammation. Mice were treated with LPS or LPS + glycyrrhizin (GL); GL is as an HMGB1 inhibitor. Subsequently, the expression levels of transporters, such as P-gp, and HMGB1 receptors, such as toll-like receptor (TLR)4 and receptor for advanced glycation end-products (RAGE), were determined by quantitative PCR and LC-MS/MS-based targeted proteomics. For the *in vitro* study, HepG2 and KMRC-1 cells were used, as was a co-culture of KMRC-1 and differentiated THP-1 cells. The mRNA and protein expression levels of Mdr1a and Tlr4 in the kidneys of LPS + GL-treated mice were significantly decreased compared with those in LPS mice. The results indicated that HMGB1 had little effect on the expression of *Mdr1a* and *Tlr4* in the liver, since there was little change in of *Mdr1a* and *Mdr1b* expression between the LPS and LPS + GL-treated mice. Notably, regarding *MDR1* mRNA expression, KMRC-1 cells were more responsive to LPS than HepG2 cells, and KMRC-1 cells treated with LPS exhibited increased levels compared with control KMRC-1 cells. In differentiated THP-1 cells, LPS treatment decreased the mRNA expression levels of *TLR4*, whereas they were restored to control levels by HMGB1. In conclusion, HMGB1 in the plasma and TLR4 in macrophages may be involved in the regulation of P-gp expression in the kidneys of inflamed mice.

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Introduction

Efflux transporters, such as P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated protein (MRP/ABCC), and breast cancer resistance protein (BCRP/ABCG2), are involved in the active transport of endogenous substrates and xenobiotics. The regulatory mechanisms of transporter expression and activity for effective drug therapies must be understood as the expression and activity of transporters could be a determinant of the pharmacokinetics and efficiency of drugs. The induction of inflammation is known to affect the expression, plasma membrane localization, and transport activity of transporters (1-3). In addition to our group, other research groups have demonstrated that transporter expression is decreased and increased in the liver and kidney, respectively, in some animal models of inflammation (2,4). For example, our study revealed opposing changes in P-gp and MRP2 expression in the livers and kidneys of rats with adjuvant-induced arthritis (4). Similarly, Hartmann *et al* reported that P-gp expression in the liver and kidney of mice treated with lipopolysaccharide (LPS) decreased and increased, respectively (2). However, it is unclear why the effect of inflammation on transporter expression differs between the liver and kidney.

Non-histone protein high mobility group box 1 (HMGB1), which participates in the sustenance of chronic inflammation, is actively secreted by monocytes and macrophages, and passively released from necrotic cells (5,6). The binding of HMGB1 to Toll-like receptor (TLR)2, TLR4, and receptor for advanced glycation end-products (RAGE) leads to the activation of intracellular signaling for inflammation and NF-κB (7-9). LPS-TLR4 signaling transduction pathways contain MyD88-dependent and MyD88-independent signaling pathways (10). MyD88-dependent signaling pathway activates NF-kB via the upstream regulators such as MyD88 and TRAF6, while MyD88-independent signaling pathway activates IRF-3 via the upstream regulators such as TRAM and TRIF. Further, the interaction between TLR4 and RAGE is important for

the plasma membrane localization of TLR4, RAGE, and HMGB1-induced inflammation (11). HMGB1 complexed with LPS, interleukin-1, CXC chemokine ligand 12, or CD24, but not HMGB1 alone, has cytokine functions (12-14). Some reports have revealed that HMGB1 increases P-gp expression in the brain via the RAGE/NF-κB signaling pathway (15-17). However, the role of HMGB1 in the regulation of efflux transporters in the liver and kidney remains unclear. Elucidating the involvement of HMGB1 in the regulation of efflux transporters could help us understand the pharmacokinetics of efflux transporter substrates in inflammation.

Accordingly, we sought to clarify whether HMGB1 regulates the expression of efflux transporters in the liver and kidney during inflammation. Mice with LPS-induced inflammation were employed as an acute inflammatory animal model, while glycyrrhizin (GL) was employed as an inhibitor of HMGB1. The possible mechanisms of HMGB1 inhibition by GL include binding to HMGB1 (18), inhibiting the phosphorylation of HMGB1 (19), and decreasing HMGB1 release from cells (20,21). In the *in vitro* study, we investigated the roles of HMGB1 in the regulation of P-gp in the liver and kidney using human liver cancer HepG2 cells, human clear cell renal carcinoma KMRC-1 cells, and a co-culture system of KMRC-1 with macrophage-like cells.

Materials and methods

Chemicals and reagents. LPS from *Escherichia coli* was purchased from Sigma-Aldrich Co., LLC. Sepasol-RNA I Super G, GL, Dulbecco's modified Eagle's medium (DMEM), and phorbol 12-myristate 13-acetate (PMA) were purchased from Nacalai Tesque Inc. ReverTra Ace was purchased from Toyobo Co. Ltd. Fast SYBR Green Master Mix, Mem-PER plus membrane protein extraction kit, and BCA protein assay kit were purchased from Thermo Fisher Scientific Inc. Transaminase CII-test Wako and MS-grade porcine pancreatic trypsin were obtained from Fujifilm Wako Pure Chemical Co. Ltd. Recombinant human HMGB1 was obtained from Novus Biologicals. The Mouse/Rat HMGB1 ELISA Kit was obtained from Arigo Biolaboratories Co. Recombinant human IFN-γ was purchased from R&D Systems Inc. Oligonucleotide primers were obtained from Eurofins Genomics Inc. All other chemicals and solvents were of MS grade or higher and of commercially available purity.

Animals and treatments. Five- to six-week-old male ICR mice were purchased from Japan SLC Inc. Mice were housed in a climate-controlled room at 24±2˚C with relative humidity of 55±10% and a 12-h lighting schedule (7:00 a.m. to 7:00 p.m.), including free access to standard laboratory chow (MF; Oriental Yeast Co.). Mice were treated with LPS (5 mg/kg, i.p.) or GL (200 mg/kg, i.p.) 1 h before LPS (5 mg/kg, i.p.) (LPS + GL) (21,22) while control mice were treated with saline as a vehicle or GL in saline. Six or 24 h after LPS injection, LPS, LPS + GL, and control mice were assessed. The livers and kidneys were excised from mice euthanized by sodium pentobarbital (150 mg/kg, i.p.) as judged by cardiac and respiratory arrest. The blood was collected from the central vein of euthanized mice. The study protocol was approved by the Committee for the Care and Use of Laboratory Animals of the Faculty of Pharmacy of Kindai University (approval no. KAPS-2022-021; Higashiosaka, Japan).

Plasma AST and ALT activities. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using the transaminase CII-test Wako. Twenty microliters of plasma from control and LPS mice at 6 and 24 h, and LPS + GL mice at 24 h, was added to 500 µl AST or ALT substrates. After incubation for 5 min at 37˚C, chromogenic substrates were added to the reaction solutions. Absorbance at 540 nm was measured using an absorption spectrometer (Sunrise R, TECAN Group Ltd.). The activities of AST and ALT were estimated using standard curves.

Plasma HMGB1 concentrations. HMGB1 concentrations in the plasma of control, LPS, and LPS + GL mice were determined using the Mouse/Rat HMGB1 ELISA kit. Briefly, we added 100 µl of plasma to antibody-coated microplates. Thereafter, HRP-conjugated antibodies were added to each well. After 16 h at 4˚C, each well was washed with the wash buffer. 3,3',5,5'-tetramethylbenzidine substrate was then added, and the absorbance at 450 nm was measured using an absorption spectrometer (Sunrise R). The HMGB1 concentration was estimated using a standard curve.

mRNA levels of transporters and HMGB1 receptors in the liver and kidney. Total RNA was extracted from the liver and kidney of control, LPS, and LPS + GL mice, HepG2 cells, KMRC-1 cells, and M1 macrophages derived from THP-1 cells (M1) using Sepasol RNA I Super G, and reverse transcribed into complementary DNA using ReverTra Ace qPCR RT Master Mix. The PCR mixtures were incubated at 95˚C for 10 sec and then amplified at 95˚C for 5 sec, 57˚C for 20 sec, and 72˚C for 40 sec for 40 cycles using Fast SYBR Green Master Mix. The oligonucleotide sequences of the primers used for each mRNA target are shown in Table I. Data were analyzed using the StepOne Real-Time PCR System (Thermo Fisher Scientific, Inc.) and the multiplex comparative method. The target mRNA levels were normalized to those of β-actin.

Protein levels of P-gp and HMGB1 receptors in the kidney by LC-MS/MS-based targeted proteomics. The protein levels of P-gp, Tlr2, Tlr4, and Rage in the kidney were determined using LC-MS/MS-based targeted proteomics, as described previously (23,24). Briefly, membrane proteins were extracted from the kidneys of the control, LPS, and LPS + GL mice using a Mem-PER plus membrane protein extraction kit. After reduction and alkylation of 200 µg proteins, MS-grade porcine pancreatic trypsin was added to the reaction mixture, including proteins and 10 pmol reference AQUA peptide [(H)H(PC13N15)DASVNFSEFSK(OH), Sigma-Aldrich Co., LLC.]. The surrogate peptides for the target proteins, as shown in Table II, were measured using an LC-MS/MS system (UltiMate 3000 series, Thermo Fisher Scientific, Inc.) and a TSQ Endura Triple Quadrupole Mass Spectrometer with electrospray ionization (Thermo Fisher Scientific, Inc.). Finigan Xcalibur software (Thermo Fisher Scientific, Inc.) was used for data recording and analysis. The relative protein expression levels to control were evaluated by dividing the area of

Table I. Primer sequences used in reverse transcription-quantitative PCR.

Gene	Primer sequence, 5'-3'	Product size, bp
mMdr1a	Forward: CTTCATCATGAAACTGCCCCA	138
	Reverse: GCCTCGTCCAACAAAAGGATC	
mMdr1b	Forward: TTGAAGCCGTAAGAGGCTGAG	140
	Reverse: TTCAAACTCCATCACCACCTCAC	
mMrp2	Forward: AGACAGCGGCAAGATTGTTG	99
	Reverse: ACACTTTCAATGCCGGCTTC	
mBcrp	Forward: CTGGTCCTCTCCCTGCTTTTT	183
	Reverse: TGCCTTTCTCTGCTATGTGTAATG	
mTlr2	Forward: TAGGGGCTTCACTTCTCTGCT	144
	Reverse: CACCAAGATCCAGAAGAGCCA	
mTlr4	Forward: TCATGGCACTGTTCTTCTCCT	125
	Reverse: AGAAGGAATGTCATCAGGGACTT	
mRage	Forward: ACACTCGTGAAGGAAGAGACC	108
	Reverse: GAAGGTAGGATGGGTGGTTC	
mβ-actin	Forward: GATCAAGATCATTGCTCCTCCTG	171
	Reverse: GCAGCTCAGTAACAGTCCGC	
hMDR1	Forward: AGGCCAACATACATGCCTTCATC	163
	Reverse: GCTGACGTGGCTTCATCCAA	
hTLR2	Forward: GACTCTACCAGATGCCTCCC	135
	Reverse: AAGTTATTGCCACCAGCTTCC	
hTLR4	Forward: TGGATCAAGGACCAGAGGCA	141
	Reverse: GAGGACCGACACACCAATGA	
hRAGE	Forward: CCCTGCTCATTGGGGTCATC	139
	Reverse: GTACTACTCTCGCCTGCCTC	
hMyD88	Forward: GAGGCTGAGAAGCCTTTACAGG	129
	Reverse: GCAGATGAAGGCATCGAAACGC	
hTRAF6	Forward: CAATGCCAGCGTCCCTTCCAAA	142
	Reverse: CCAAAGGACAGTTCTGGTCATGG	
hTRIF	Forward: ACCTTCTGCGAGGATTTCCAGG	113
	Reverse: CGACAGTCGAAGTTGGAGGTGA	
hIRF3	Forward: TCTGCCCTCAACCGCAAAGAAG	151
	Reverse: TACTGCCTCCACCATTGGTGTC	
hβ-actin	Forward: CACCATTGGCAATGAGCGGTTC	135
	Reverse: AGGTCTTTGCGGATGTCCACGT	

Mdr, multidrug resistance protein; Mrp, multidrug resistance-associated protein; Bcrp, breast cancer resistance protein; Tlr, toll-like receptor; Rage, receptor for advanced glycation end-products; MyD88, myeloid differentiation factor 88; TRAF6, TNF receptor-associated factor 6; TRIF, TIR domain-containing adaptor-inducing interferon-β; IRF3, interferon regulatory factor 3.

surrogate peptide by the area of reference AQUA peptide obtained from each sample using Skyline software V.21 (MacCoss Lab at the University of Washington, USA).

Cell culture and treatments. Human liver cancer HepG2 cells and the human monocytic cell line, THP-1, were obtained from RIKEN BRC. Human clear cell renal carcinoma KMRC-1 cells were obtained from JCRB Cell Bank. HepG2 and KMRC-1 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. THP-1 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were grown at 37˚C in a humidified incubator equilibrated with 5% CO_2. HepG2 and KMRC-1 cells were subcultured every 3-4 days using 0.25% trypsin and 1 mM EDTA. THP-1 cells were sub-cultured every 3-4 days via dilution with fresh medium.

HepG2 or KMRC-1 cells were seeded at $5x10^4$ cells/cm^2 in 24-well plates (Sumitomo Bakelite Co. Ltd.) 1 d before treatment. HepG2 cells or KMRC-1 cells were treated with LPS alone (0.01-10 μg/ml).

THP-1 monocytes were differentiated into M0 macrophages (M0) after 24 h of incubation with 150 nM PMA. M0 macrophages were polarized in classically activated M1 by incubation with 20 ng/ml IFN-γ and 10 pg/ml LPS for 24 h. KMRC-1 cells co-culture with M1 (KMRC-1/M1)

Table II. Surrogate amino acid sequences for targeted proteins.

Protein	Sequence
Mdr1a	ATVSASHIIR
Mdr1b	GIYFSMVQAGAK
Tlr2	NYQSQSLK
Tlr4	DFIPGVAIAANIIQEGFHK
Rage	VYQIPGKPEIVDPASELTASVPNK

Mdr, multidrug resistance protein; Tlr, toll-like receptor; Rage, receptor for advanced glycation end-products.

were performed under 24-well plate with Falcon cell culture inserts (pore size: 0.4 μm; upper compartment: M1; lower compartment: KMRC-1 cells) in fresh DMEM at KMRC-1 cells:M1 ratio of 1:0.4 (inflamed conditions) (25).

Statistical analysis. Differences between means were analyzed by one-way analysis of variance followed by the Bonferroni test or by unpaired Student's t-test. The correlation between the *TLR2/4* or *RAGE* mRNA levels and the *MDR1* mRNA levels was evaluated by Pearson's correlation and regression analysis. GraphPad Prism 5 was used for all statistical analyses. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Plasma AST and ALT activities. To determine whether HMGB1 affects the expression of efflux transporters and HMGB1 receptors in the liver and kidney of mice with inflammation, control, LPS, and LPS + GL mice were used. Table III shows the body weight, liver weight, kidney weight, and plasma AST and ALT levels of control, LPS, and LPS + GL mice. Body, liver, and kidney weights were unchanged after treatment with LPS or LPS + GL at 6 and 24 h. Both AST and ALT levels in LPS mice were significantly higher than those in control mice. The combination of GL and LPS resulted in significant decreases in both AST and ALT levels compared to levels found in LPS mice (Table III).

mRNA and protein levels of efflux transporters. The mRNA levels of *Mdr1a*, *Mdr1b*, *Mrp2*, and *Bcrp* in the liver and kidney at 6 and 24 h after LPS treatment and the protein levels of Mdr1a and Mdr1b in the kidney 24 h after LPS treatment were determined to clarify the effects of LPS-induced inflammation on the expression of efflux transporters (Fig. 1). Six hours after treatment with vehicle or LPS, only a minor change in the mRNA levels of efflux transporters in the liver and kidney was observed, with the exception of *Mrp2* in the liver. Twenty-four hours after treatment, the mRNA levels of *Mdr1a*, *Mrp2*, and *Bcrp* in the liver of LPS mice were significantly decreased compared to those of control mice (Fig. 1A). In contrast, the mRNA levels of *Mdr1a* and *Mdr1b* in the kidneys of LPS-treated mice were significantly increased compared to those of control mice (Fig. 1B). The relative protein levels of Mdr1a, but not Mdr1b, in the kidneys of LPS-treated mice after 24 h of treatment were higher than those in control mice (Fig. 1C).

Plasma HMGB1 concentrations. Fig. 2 shows the plasma concentrations of HMGB1 of LPS or LPS + GL mice. The plasma concentrations of HMGB1 in LPS mice at 6 h after treatment were approximately twice the concentration in control mice. Further, the increased HMGB1 concentrations in LPS mice were maintained until 24 h after LPS treatment (Fig. 2). The combination of GL and LPS prevented the increase in plasma HMGB1 levels in LPS mice (Fig. 2).

Effects of GL on Mdr1a, Mdr1b, and HMGB1 receptors expression. When GL and LPS were combined, plasma levels of HMGB1 were comparable to those in the control (Fig. 2). Therefore, the effects of the combination of GL and LPS on the mRNA levels of *Mdr1a* and *Mdr1b* in the liver and kidney, and the protein levels of Mdr1a and Mdr1b in the kidney were determined to clarify the roles of HMGB1 in the regulation of P-gp expression (Fig. 3). Although the mRNA levels of *Mdr1a* and *Mdr1b* in the liver were unchanged between LPS and LPS + GL mice (Fig. 3A), both the mRNA and protein levels of Mdr1a in the kidneys of LPS + GL mice were significantly decreased compared with those of LPS mice (Fig. 3B and C). The mRNA levels of *Mdr1b* in the kidneys of LPS + GL mice were significantly lower than those of LPS mice (Fig. 3B). However, the protein levels of Mdr1b remained unchanged by the combination of GL and LPS by LC-MS/MS-based targeted proteomics (Fig. 3C). We proceeded to determine whether HMGB1 affected the mRNA levels of HMGB1 receptors, such as *Tlr2*, *Tlr4*, and *Rage*, in the liver and kidney of mice with LPS-induced inflammation (Fig. 4A and B). In the livers of LPS and LPS + GL mice, the mRNA levels of *Tlr2* and *Rage* were significantly increased and decreased, respectively, compared to those in the control. However, the mRNA levels of *Tlr4* in the liver remained unchanged after treatment with LPS or LPS + GL (Fig. 4A). The mRNA levels of *Tlr2* and *Tlr4* in the kidneys of LPS-treated mice were significantly increased compared with those of control mice and were reduced to significantly lower levels by the combination of GL and LPS (Fig. 4B). The mRNA levels of *Rage* in the kidneys of both LPS and LPS + GL mice were significantly lower than those in control mice (Fig. 4B). Fig. 4C shows the protein levels of Tlr2, Tlr4, and Rage in the kidneys of the control, LPS, and LPS + GL mice. The protein levels of Tlr4 were significantly increased by LPS treatment. Treatment with a combination of GL and LPS reversed the increase in Tlr4 protein by LPS treatment, in accordance with the changes in *Tlr4* mRNA in the kidney (Fig. 4C). These representative chromatograms of the surrogate peptides or Mdr1a, Mdr1b, Tlr2, Tlr4, and Rage were shown in Fig. S1. GL alone caused only a slight change in the AST, ALT, and HMGB1 levels and the expression of P-gp and HMGB1 receptors (data not shown).

Effects of LPS on MDR1 and TLR4 in HepG2 or KMRC-1 cells. To further investigate the role of HMGB1 in the regulation of P-gp expression, *in vitro* studies were performed using human liver cancer HepG2 cells and human clear cell renal carcinoma KMRC-1 cells. The mRNA levels of *MDR1* and *TLR4* were unchanged after the treatment of HepG2 cells with

Table III. Body weight, liver weight, kidney weight, plasma AST, and ALT levels in control, LPS, and LPS + GL mice. Mice were treated with vehicle, LPS (50 mg/kg, *i.p.*), or LPS (50 mg/kg, *i.p.*) combined with GL (200 mg/kg, *i.p.*). The organs and plasma were collected 6 h and 24 h after saline or LPS injection.

Time	Group	Body weight, g	Liver weight, g	Liver weight/ body weight	Kidney weight, g	Kidney weight/ body weight	AST, IU/l	ALT, IU/l
6 h	Control	29.8±0.45	1.96±0.05	0.066±0.002	0.56±0.03	0.019±0.001	14.2±2.8	14.5±7.4
	LPS	29.2±0.84	1.90±0.05	0.065±0.001	0.50±0.04	0.017±0.001	38.7±18.8[a]	61.3±35.3[b]
	LPS + GL	31.2±1.10	1.94±0.15	0.062±0.005	0.51±0.02	0.017±0.001	16.8±6.6[d]	23.5±3.5[d]
24 h	Control	30.0±1.41	1.80±0.09	0.060±0.005	0.49±0.07	0.016±0.002	13.3±2.8	17.0±1.1
	LPS	29.7±1.63	1.75±0.12	0.058±0.004	0.56±0.05	0.019±0.002	34.1±6.3[c]	23.8±5.1[a]
	LPS + GL	28.7±1.03	1.78±0.14	0.063±0.004	0.61±0.10	0.021±0.003	26.9±6.4	13.0±2.1[e]

The results are expressed as the mean ± standard deviation of each group (n=5-8). [a]P<0.05, [b]P<0.01 and [c]P<0.001 vs. control; [d]P<0.05 and [e]P<0.01 vs. LPS. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LPS, lipopolysaccharide; GL, glycyrrhizin.

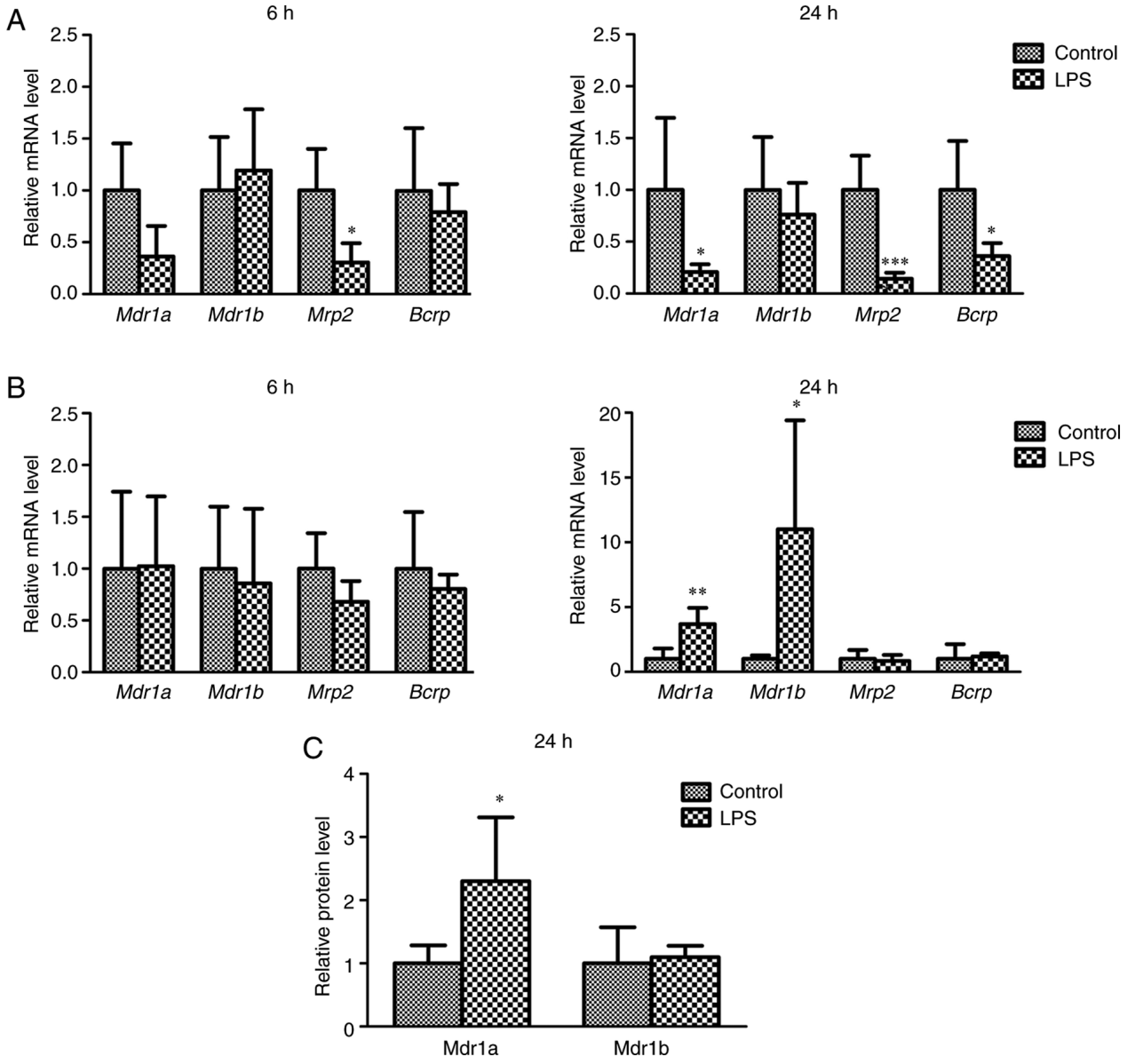

Figure 1. Relative mRNA levels of *Mdr1a*, *Mdr1b*, *Mrp2*, and *Bcrp* in the (A) liver and (B) kidney of control and LPS mice at 6 and 24 h after vehicle or LPS administration. (C) Relative protein levels of Mdr1a and Mdr1b in the kidney of control and LPS mice at 24 h after vehicle or LPS administration. The results are expressed as mean ± SD of each group (n=3-5). *P<0.05, **P<0.01 and ***P<0.001 vs. control. Mdr, multidrug resistance protein; Mrp, multidrug resistance-associated protein; Bcrp, breast cancer resistance protein; LPS, lipopolysaccharide.

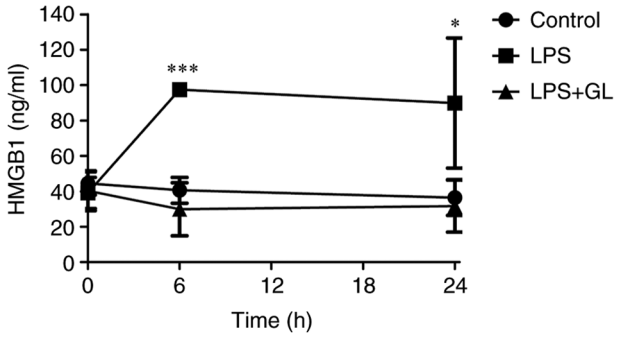

Figure 2. Time-course of the plasma concentrations of HMGB1 in control, LPS, and LPS + GL mice. The results are expressed as mean ± SD of each group (n=3–6). *P<0.05 and ***P<0.001 vs. control. HMGB1, high mobility group box 1; LPS, lipopolysaccharide; GL, glycyrrhizin.

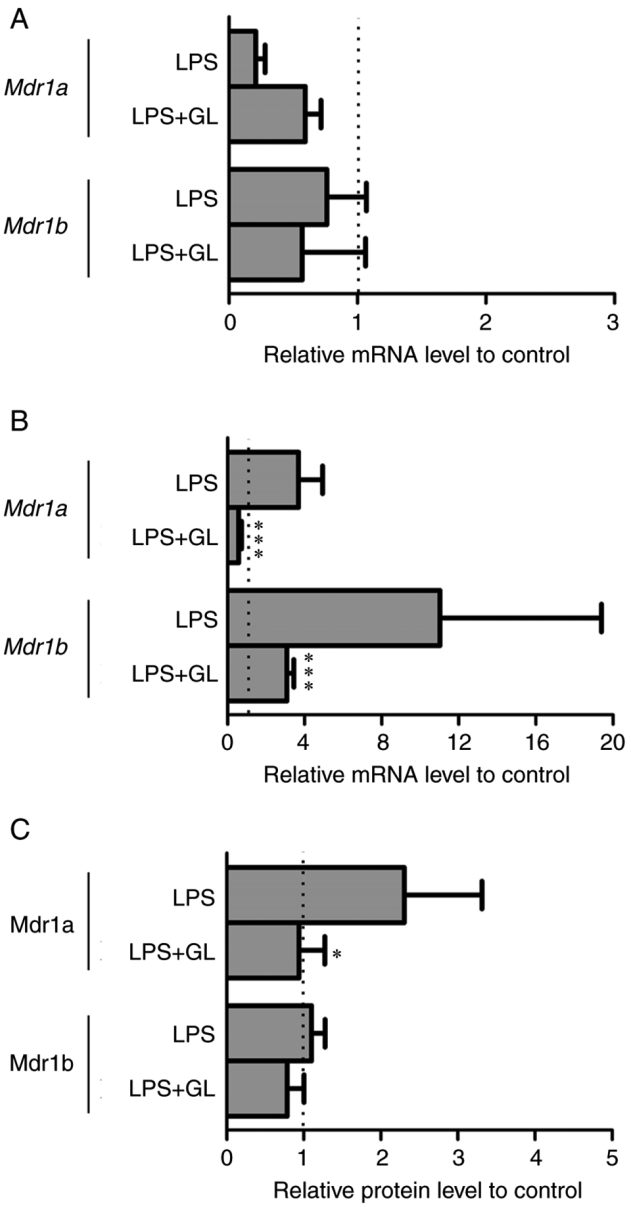

Figure 3. Relative mRNA levels of *Mdr1a* and *Mdr1b* in the (A) liver and (B) kidney, and (C) relative protein levels of Mdr1a and Mdr1b in the kidney of LPS and LPS + GL mice at 24 h after treatment. The results are expressed as mean ± SD of each group (n=5-8). *P<0.05 and ***P<0.001 vs. LPS. Dotted lines indicate the mRNA or protein levels of control mice. Mdr, multidrug resistance protein; LPS, lipopolysaccharide; GL, glycyrrhizin.

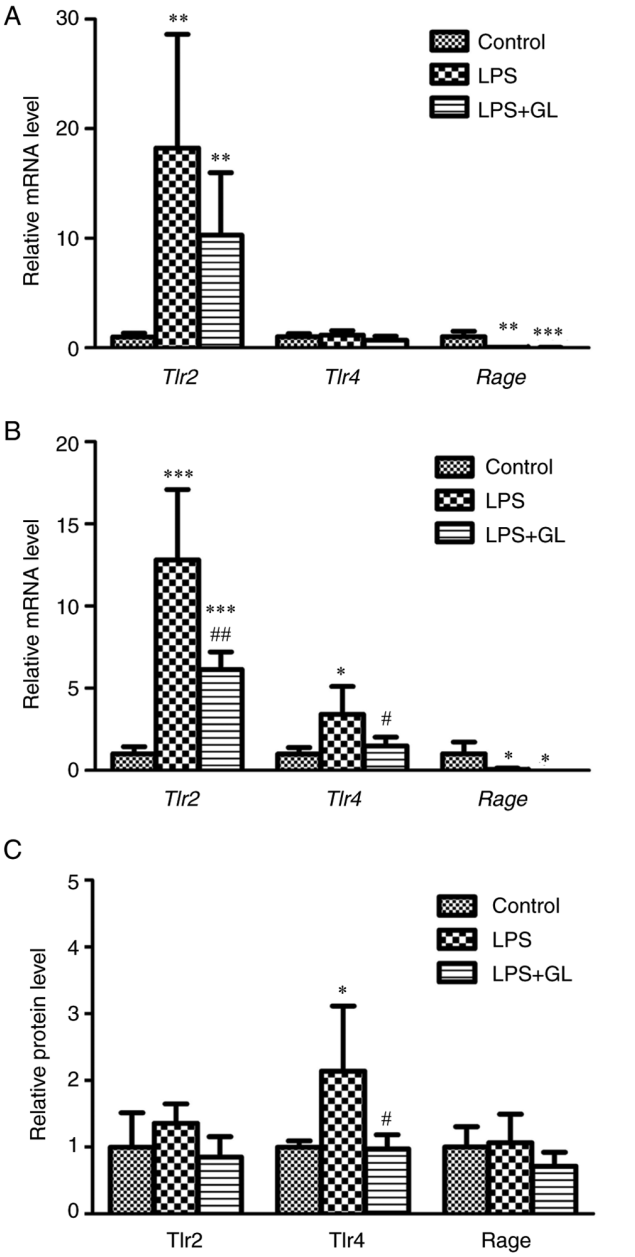

Figure 4. Relative mRNA levels of *Tlr2*, *Tlr4*, and *Rage* in the (A) liver and (B) kidney, and (C) relative protein levels of Tlr2, Tlr4 and Rage in the kidney of control, LPS, and LPS + GL mice at 24 h after treatment. The results are expressed as mean ± SD of each group (n=4–8). *P<0.05, **P<0.01 and ***P<0.001 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. LPS. Tlr, toll-like receptor; Rage, receptor for advanced glycation end-products; LPS, lipopolysaccharide; GL, glycyrrhizin.

LPS (Fig. 5A). In contrast, treatment of KMRC-1 cells with LPS (0.1, 1, and 10 μg/ml) resulted in a significant increase in *MDR1* mRNA compared with that in control cells. Only few changes occurred in the mRNA levels of *TLR4* in control and LPS-treated KMRC-1 cells (Fig. 5B).

Effects of LPS and HMGB1 on MDR1, HMGB1 receptors, and LPS-TLR4 signaling molecules in KMRC-1/M1. The co-culture of KMRC-1 cells with M1 derived from THP-1 cells was used to clarify the role of HMGB1 in the regulation of *MDR1* and *TLR4* mRNA expression (Fig. 6). The mRNA levels of *MDR1* and *TLR4* in KMRC-1 cells after treatment with LPS alone or

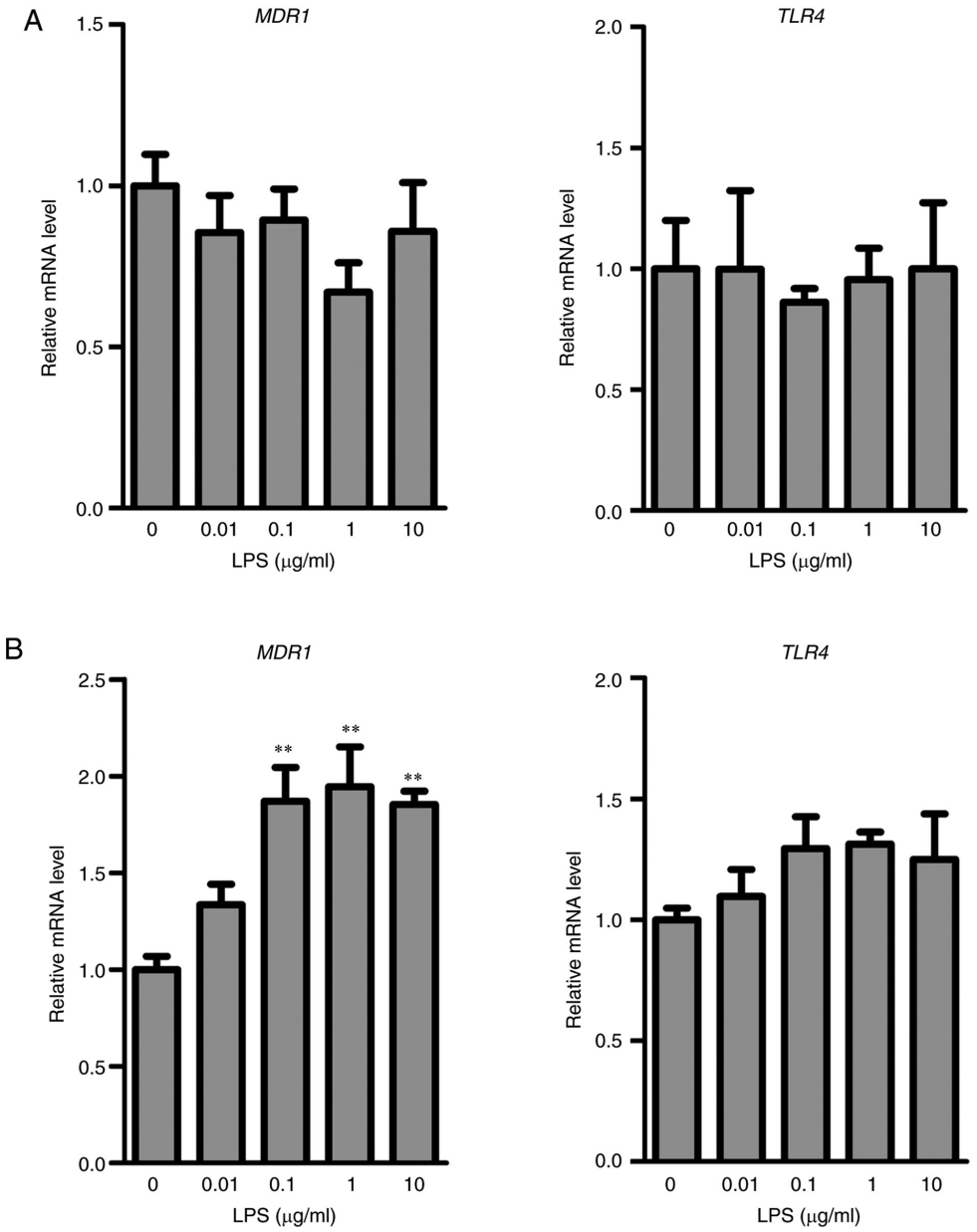

Figure 5. Relative mRNA levels of *MDR1* and *TLR4* in (A) HepG2 cells and (B) KMRC-1 cells at 24 h after treatment with vehicle or LPS (0.01-10 μg/ml). The results are expressed as mean ± standard error of each group (n=4). **P<0.01 vs. control (0 μg/ml). MDR1, multidrug resistance protein 1; TLR4, toll-like receptor 4; LPS, lipopolysaccharide.

LPS with HMGB1 were significantly increased compared to those in the control (Fig. 6A). The mRNA levels of *MyD88*, *TRAF6*, *TRIF*, and *IRF3* in KMRC-1 cells co-cultured with M1 were significantly decreased by treatments of LPS alone, or LPS with HMGB1 (Fig. 6A). In KMRC-1 alone, the mRNA levels of *MyD88*, *TRAF6*, *TRIF*, and *IRF3* were unchanged (data not shown). When HepG2 cells were co-cultured with M1 derived from THP-1 cells, the mRNA levels of *MDR1* and *TLR4* after LPS treatment were unchanged (data not shown). However, the mRNA levels of *TLR4* in M1 after treatment with LPS alone were significantly decreased compared with those of the control, and returned to control levels when treated with both HMGB1 and LPS (Fig. 6B). The mRNA levels of *TLR2* and *RAGE* in M1 remained unchanged among the three groups (Fig. 6B). The similar HMGB1 concentrations was found in the medium of the three groups at 24 h after the treatments (data not shown). The correlation between the mRNA levels of *TLR4* in KMRC-1, *TLR2*, *TLR4*, and *RAGE* in M1 and *MDR1* in KMRC-1 24 h after treatment with LPS alone (1 μg/ml) or the combination of HMGB1 (1 μg/ml) and LPS (1 μg/ml) to KMRC-1/M1 was assessed (Fig. 7). A significant positive correlation was found between the mRNA levels of TLR4 and MDR1 in KMRC-1. Further, a significant negative correlation was found between the mRNA levels of TLR4 in M1 and MDR1 in KMRC-1 cells.

Discussion

The present study revealed the roles of HMGB1 in the regulation of P-gp and HMGB1 receptors in the liver and kidney of mice with LPS-induced inflammation and the cell lines. Following treatment with LPS alone or combined with

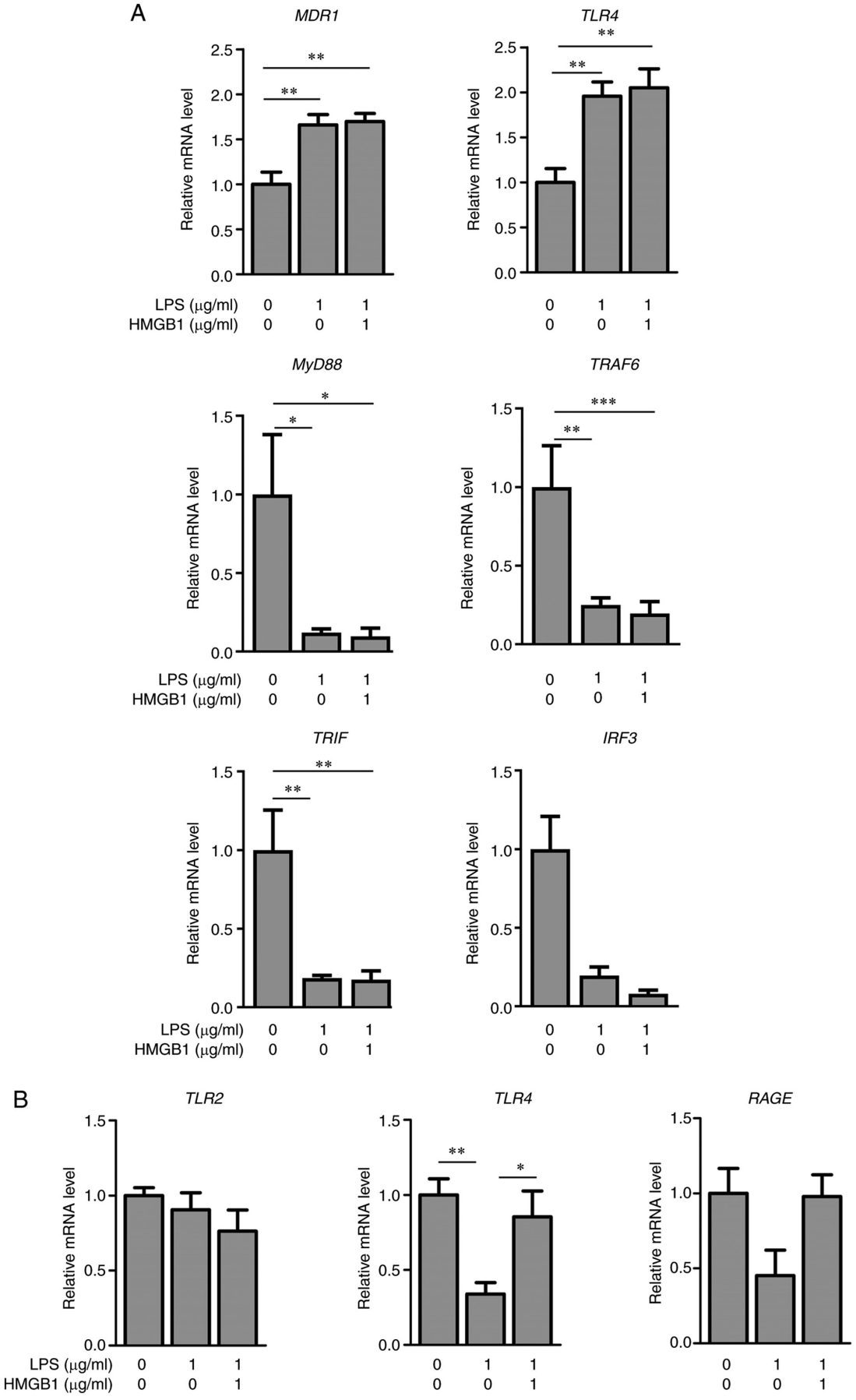

Figure 6. Effects of LPS and HMGB1 on relative mRNA levels in co-cultured KMRC-1/M1 cells. (A) Relative mRNA levels of *MDR1*, *TLR4*, *MyD88*, *TRAF6*, *TRIF* and *IRF3*, and (B) *TLR2*, *TLR4* and *RAGE* in M1 in KMRC-1/M1 24 h after treatment with vehicle, LPS alone (1 μg/ml), or the combination of HMGB1 (1 μg/ml) and LPS (1 μg/ml). The results are expressed as mean ± SE of each group (n=4-5). *P<0.05, **P<0.01 and ***P<0.001. MDR1, multidrug resistance protein 1; TLR4, toll-like receptor 4; LPS, lipopolysaccharide; HMGB1, high mobility group box 1.

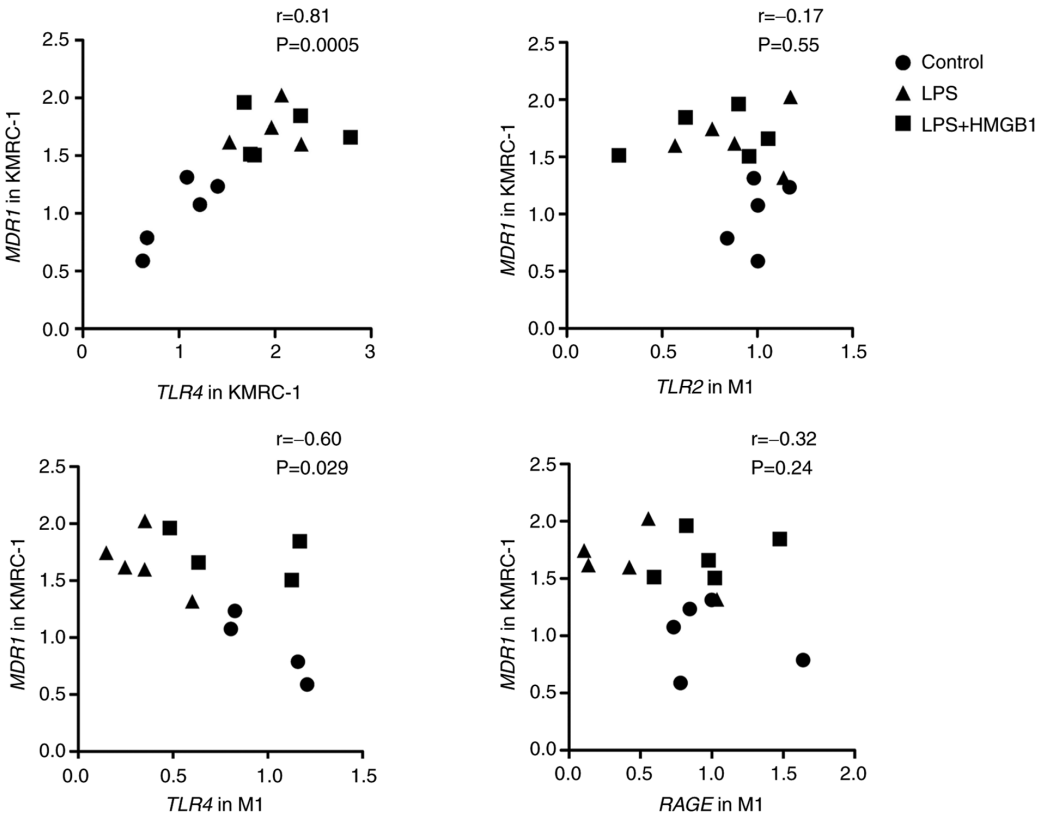

Figure 7. Correlation between the mRNA levels of *TLR4* in KMRC-1, *TLR2*, *TLR4* or *RAGE* in M1, and *MDR1* in KMRC-1 at 24 h after treatment with LPS alone (1 μg/ml) or the combination of HMGB1 (1 μg/ml) and LPS (1 μg/ml) in HepG2 cells co-cultured with M1. Pearson coefficient values (r) and P-values are shown. MDR1, multidrug resistance protein 1; TLR, toll-like receptor; RAGE, receptor for advanced glycation end-products; LPS, lipopolysaccharide; HMGB1, high mobility group box 1.

HMGB1, changes in the expression levels of P-gp and HMGB1 receptors were determined.

Based on the results, the increase in plasma HMGB1 following treatment with LPS was involved in the regulation of Mdr1a expression in the kidney, but not in the liver. *TLR4* expression levels in macrophages and HMGB1 levels in plasma may be important for the regulation of MDR1 in the kidney; several lines of evidence support this finding. First, the inhibition of HMGB1 after GL treatment masked the increase in the mRNA and protein expression of Mdr1a and Tlr4 in the kidney after LPS treatment (Figs. 3C and 4C). The addition of HMGB1 and LPS to KMRC-1/M1 suppressed the downregulation of *TLR4* mRNA expression in M1 cells after LPS stimulation (Fig. 6B). To our knowledge, this is the first study to demonstrate the roles of HMGB1 and TLR4 in the kidney, but not in the liver, of mice with inflammation.

Alterations in efflux transporters, such as Mdr1a, Mdr1b, Mrp2, and Bcrp in the liver and kidney of mice with LPS-induced inflammation were assessed. Following LPS treatment, both the mRNA and protein levels of Mdr1a in the kidneys of mice at 24 h after LPS treatment were significantly increased compared with those in control mice (Fig. 1). Hartmann *et al* demonstrated that the protein levels of P-gp in the kidneys of LPS mice were significantly higher than those in control mice (2). Our previous study also revealed opposing changes in P-gp between the liver and kidney of rats with adjuvant-induced arthritis. Further, the relative expression levels of P-gp in the kidneys of rats with adjuvant-induced arthritis were found to be higher than those in control rats (4). An increase in Mdr1a, but not Mdr1b, was observed in the kidneys of mice at 24 h after LPS treatment (Fig. 1C). Inflammation was found to have different effects on the mRNA expression between *Mdr1a* and *Mdr1b*, as revealed by previous reports (2,26,27). P-gp was selected to evaluate the roles of HMGB1 in the regulation of efflux transporters in the following studies as distinct differences were observed in the expression of P-gp in the liver and kidney of LPS-induced inflammatory mice compared with those of other efflux transporters. Previous report showed the decreases of the protein expression levels of P-gp after LPS treatments to mice (2). It has been reported that HMGB1 increases P-gp expression in the brain (15-17). The mRNA levels of *Mdr1*a and *Mdr1b* were significantly increased in the kidneys but not livers of LPS mice. GL treatment with LPS had little impacts on the *Mdr1a* and *Mdr1b* levels in the livers of inflamed mice (Fig. 3A). These results suggested that the protein expression levels of P-gp in the kidney were regulated by HMGB1 similar to the P-gp expression in brain. Therefore, we focused the changes of P-gp in the kidneys of LPS mice to clarify the roles of HMGB1 in the expression of P-gp.

The increased plasma HMGB1, AST, and ALT levels after LPS treatment returned to control levels when treated with GL. GL has been frequently used as an inhibitor of HMGB1 because it directly binds to HMGB1 and inhibits its release from cells (18,20,21). The effects of GL alone on the inflammation with mice would be excluded, because the treatment of

GL alone did not affect the AST, ALT, and HMGB1 levels and the expression of P-gp and HMGB1 receptors in control mice (data not shown). However, it is difficult to fully distinguish an effect of direct inhibition of HMGB1 by GL and an indirect effect of decreases of liver damage via HMGB1 inhibition by GL. GL does not necessarily suppress liver injury only through the pathway that inhibits HMGB1, although GL is widely used as an inhibitor for HMGB1. The further studies using anti-HMGB1 antibody are needed to clarify the direct effects of HMGB1 on the regulation of P-gp expression in inflamed kidney. If HMGB1 is involved in the P-gp regulation in inflamed kidney, the treatment anti-HMGB1 antibody with LPS results in the prevention of increase of the P-gp expression in the kidney after LPS treatment. Some reports also demonstrated that GL inhibited the liver injury via decrease of the HMGB1 secretion from Kupffer cells (28), inhibition of HMGB1-TLR4 pathway (29), and decrease of oxidative stress (30). The expression levels of P-gp in the kidneys of LPS-treated mice were similar to those in control mice (Fig. 3C), indicating that plasma HMGB1 is a determinant of P-gp expression in the kidneys of mice with LPS-induced inflammation. According to previous reports, HMGB1 promotes P-gp expression in the brain (15-17); however, whether it is involved in the regulation of P-gp expression in the kidney is unknown. The increased expression levels of Tlr4, which is a receptor for LPS and HMGB1, restored to control levels by HMGB1 inhibition (Fig. 4C). Expression levels did not necessarily exhibit corresponding changes between mRNA and protein levels due to the stability of mRNA, and post-transcriptional or post-translational regulation. However, the causes of the proportional changes between mRNA and protein levels of Tlr4 but not Tlr2 and Rage in inflamed kidney were undetermined. There is no information on the differences of regulation of Tlr2 and Tlr4 expression in the inflamed kidney. The *TLR4* mRNA levels of M1 treated with LPS and HMGB1 were significantly increased compared with those of M1 treated with LPS alone (Fig. 6B), indicating that HMGB1 participated in the upregulation of *TLR4* in M1. The lower expression of Tlr4 in the kidneys of LPS + GL mice can be partially explained by this result (Fig. 6B). TLR4 is predominantly expressed in human and rodent monocytes and immature dendritic cells, with very low expression in the liver and kidneys (31-33). Therefore, changes in TLR4 expression in monocyte-derived macrophages, as shown in Fig. 6B, in addition to plasma HMGB1, could be important for the regulation of P-gp expression in the liver and kidney of inflamed mice. Tlr4 in the kidney is reported to be involved in the induction of inflammation and tissue injury in nephrotoxicity via MyD88-dependent and MyD88-independent signaling pathways (34-36). To our knowledge, this is the first study to reveal that HMGB1 and Tlr4 expression are important factors in the induction of P-gp in the inflamed kidney. However, the expression levels of P-gp in the liver did not markedly depend on plasma HMGB1 concentrations and *Tlr4* mRNA levels in the liver. Thus, the upregulation of P-gp expression in the kidney by HMGB1 and Tlr4 could be a characterization of the organ-distinct effects of LPS-induced inflammation, differing from that of the liver. As a result, the differences in *Tlr4* expression between the liver and kidney of mice with LPS-induced inflammation should be considered. According to the *Tlr4* mRNA levels, the kidney was found to be more responsive to LPS-induced inflammation than the liver (Fig. 4). Further studies using Tlr4 deficient mice could help to elucidate the detailed roles of Tlr4 and HMGB1 in the regulation of P-gp in the kidneys of mice with inflammation.

Following the treatment of HepG2 or KMRC-1 cells with LPS, there were significant differences in the induction of *MDR1* between HepG2 and KMRC-1 cells (Fig. 5). Treatment of KMRC-1 cells, but not HepG2 cells, with LPS (0.1, 1, or 10 μg/ml) led to a significant induction of *MDR1* mRNA expression compared with that observed in control cells. However, *TLR4* mRNA expression was unchanged after LPS treatment in both KMRC-1 and HepG2 cells (Fig. 5). These results suggest that LPS stimulation of KMRC-1 cells results in an increase in *MDR1* without the upregulation of *TLR4*. Since KMRC-1 and HepG2 cells are cancer cells, some characteristics of them do not reflect the properties of normal kidney and liver. Further studies using normal proximal renal tubular cells and primary hepatocytes are needed to clarify the detailed roles of HMGB1 in the regulation of P-gp expression, although KMRC-1 and HepG2 cells are frequently used to examine the expression and function of P-gp in kidney and liver. Further, other factors, in addition to LPS stimulation, may be needed to increase TLR4 expression in KMRC-1 cells. In contrast, *TLR4* mRNA levels in KMRC-1 cells increased after LPS stimulation of KMRC-1/M1 (Fig. 6), suggesting that the presence of inflammatory macrophages participated in the upregulation of *TLR4* in KMRC-1 cells. Moreover, the mRNA levels of *MDR1* after treatment with HMGB1 and LPS were comparable to those after LPS treatment alone. Accordingly, it is difficult to determine the roles of HMGB1 and TLR4 in the regulation of MDR1 using KMRC-1 cells alone. As shown in Fig. 7, *TLR4* mRNA expression levels in KMRC-1 cells and M1 correlated with *MDR1* mRNA expression levels in KMRC-1 cells. Treatment of KMRC-1/M1 cells with LPS alone increased and decreased the expression of *TLR4* mRNA in KMRC-1 cells and M1, respectively. The addition of LPS to KMRC-1/M1 (Fig. 6), but not to KMRC-1 cells alone (Fig. 5), resulted in an increase in *TLR4* in KMRC-1 cells, which might be due to the release of inflammatory cytokines from M1. According to previous reports, TLR4 in renal parenchymal cells is an important receptor for the production of inflammatory cytokines and induction of inflammation (34,37,38). Taken together, the presence of M1 increased TLR4 mRNA expression, similar to Tlr4 expression in the kidneys of mice with LPS-induced inflammation. Owing to the combination of HMGB1 and LPS, the mRNA levels of *TLR4* were significantly higher than those with LPS alone, although similar mRNA levels of *MDR1* were observed after treatment with LPS or LPS + HMGB1. Other studies revealed the synergistic effects of HMGB1 and LPS on inflammation (39-41). HMGB1 might be involved in maintaining the regulatory levels of *TLR4* mRNA in the M1. However, the results obtained using this co-culture system were insufficient to explain the changes in P-gp expression in the kidney of LPS-induced inflamed mice. Further studies are thus needed to clarify the role of HMGB1 in the regulation of P-gp expression in the kidney.

In conclusion, to the best of our knowledge, this is the first report on the involvement of HMGB1 in the regulation of P-gp expression in the kidney, but not in the liver, of inflamed mice

similar to brain, although the interaction between HMGB1 and TLR4, inhibitory effects of GL on HMGB1, and LPS-mediated activation of HMGB1 have been reported. It was unclear whether HMGB1 regulated the transporters such as P-gp expression in the kidneys and livers of inflamed mice. These findings may provide important information for us to understand the effects of inflammation on the disposition of P-gp substrates to the kidneys and livers. The regulation of TLR4 expression in M1 by HMGB1 may contribute to P-gp expression in the kidneys. Further, the upregulation of HMGB1 and Tlr4 in the kidney could be the cause of the differing effect of inflammation on P-gp expression in the liver and kidney. Thus, altering P-gp expression in the inflammatory kidney may affect the pharmacokinetics of P-gp substrates; the involvement of HMGB1 and TLR4 should also be investigated.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AK, KI, NM, YT, HS and MI contributed to the study conception and design. AK, KI, NM and YT contributed to material preparation, data collection and analysis. AK and KI and NM confirm the authenticity of all the raw data. The first draft of the manuscript was written by AK and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Committee for the Care and Use of Laboratory Animals of the Faculty of Pharmacy of Kindai University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Vos TA, Hooiveld GJ, Koning H, Childs S, Meijer DK, Moshage H, Jansen PL and Müller M: Up-regulation of the multidrug resistance genes, Mrp1 and MdrIb, and down-regulation of the organic anion transporter, Mrp2, and the bile salt transporter, Spgp, in endotoxemic rat liver. Hepatology 28: 1637-1644, 1998.
2. Hartmann G, Vassileva V and Piquette-Miller M: Impact of endotoxin-induced changes in P-glycoprotein expression on disposition of doxorubicin in mice. Drug Metab Dispos 33: 820-828, 2005.
3. Kawase A, Nakasaka M, Bando H, Yasuda S, Shimada H and Iwaki M: Changes in radixin expression and interaction with efflux transporters in the liver of adjuvant-induced arthritic rats. Inflammation 43: 85-94, 2020.
4. Kawase A, Norikane S, Okada A, Adachi M, Kato Y and Iwaki M: Distinct alterations in ATP-binding cassette transporter expression in liver, kidney, small intestine, and brain in adjuvant-induced arthritic rats. J Pharm Sci 103: 2556-2564, 2014.
5. Rouhiainen A, Kuja-Panula J, Wilkman E, Pakkanen J, Stenfors J, Tuominen RK, Lepäntalo M, Carpén O, Parkkinen J and Rauvala H: Regulation of monocyte migration by amphoterin (HMGB1). Blood 104: 1174-1182, 2004.
6. Scaffidi P, Misteli T and Bianchi ME: Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418: 191-195, 2002.
7. Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ and Yang H: HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 26: 174-179, 2006.
8. Hreggvidsdóttir HS, Lundberg AM, Aveberger AC, Klevenvall L, Andersson U and Harris HE: High mobility group box protein 1 (HMGB1)-partner molecule complexes enhance cytokine production by signaling through the partner molecule receptor. Mol Med 18: 224-230, 2012.
9. Zhang C, Dong H, Chen F, Wang Y, Ma J and Wang G: The HMGB1-RAGE/TLR-TNF-α signaling pathway may contribute to kidney injury induced by hypoxia. Exp Ther Med 17: 17-26, 2019.
10. Godowski PJ: A smooth operator for LPS responses. Nat Immunol 6: 544-546, 2005.
11. Zhong H, Li X, Zhou S, Jiang P, Liu X, Ouyang M, Nie Y, Chen X, Zhang L, Liu Y, *et al*: Interplay between RAGE and TLR4 regulates HMGB1-induced inflammation by promoting cell surface expression of RAGE and TLR4. J Immunol 205: 767-775, 2020.
12. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A and Abraham E: Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 279: 7370-7377, 2004.
13. Lotze MT and Tracey KJ: High-mobility group box 1 protein (HMGB1): Nuclear weapon in the immune arsenal. Nat Rev Immunol 5: 331-342, 2005.
14. Chen GY, Tang J, Zheng P and Liu Y: CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. Science 323: 1722-1725, 2009.
15. Chen Y, Huang XJ, Yu N, Xie Y, Zhang K, Wen F, Liu H and Di Q: HMGB1 contributes to the expression of P-glycoprotein in mouse epileptic brain through toll-like receptor 4 and receptor for advanced glycation end products. PLoS One 10: e0140918, 2015.
16. Xie Y, Yu N, Chen Y, Zhang K, Ma HY and Di Q: HMGB1 regulates P-glycoprotein expression in status epilepticus rat brains via the RAGE/NF-κB signaling pathway. Mol Med Rep 16: 1691-1700, 2017.
17. Wang F, Ji S, Wang M, Liu L, Li Q, Jiang F, Cen J and Ji B: HMGB1 promoted P-glycoprotein at the blood-brain barrier in MCAO rats via TLR4/NF-κB signaling pathway. Eur J Pharmacol 880: 173189, 2020.
18. Mollica L, De Marchis F, Spitaleri A, Dallacosta C, Pennacchini D, Zamai M, Agresti A, Trisciuoglio L, Musco G and Bianchi ME: Glycyrrhizin binds to high-mobility group box 1 protein and inhibits its cytokine activities. Chem Biol 14: 431-441, 2007.
19. Kim SW, Jin Y, Shin JH, Kim ID, Lee HK, Park S, Han PL and Lee JK: Glycyrrhizic acid affords robust neuroprotection in the postischemic brain via anti-inflammatory effect by inhibiting HMGB1 phosphorylation and secretion. Neurobiol Dis 46: 147-156, 2012.
20. Tsoyi K, Lee TY, Lee YS, Kim HJ, Seo HG, Lee JH and Chang KC: Heme-oxygenase-1 induction and carbon monoxide-releasing molecule inhibit lipopolysaccharide (LPS)-induced high-mobility group box 1 release in vitro and improve survival of mice in LPS- and cecal ligation and puncture-induced sepsis model in vivo. Mol Pharmacol 76: 173-182, 2009.
21. Kim YM, Kim HJ and Chang KC: Glycyrrhizin reduces HMGB1 secretion in lipopolysaccharide-activated RAW 264.7 cells and endotoxemic mice by p38/Nrf2-dependent induction of HO-1. Int Immunopharmacol 26: 112-118, 2015.

22. Wong ML, Xie B, Beatini N, Phu P, Marathe S, Johns A, Gold PW, Hirsch E, Williams KJ, Licinio J and Tabas I: Acute systemic inflammation up-regulates secretory sphingomyelinase in vivo: A possible link between inflammatory cytokines and atherogenesis. Proc Natl Acad Sci USA 97: 8681-8686, 2000.
23. Kawase A, Tateishi S and Kazaoka A: Profiling of hepatic metabolizing enzymes and nuclear receptors in rats with adjuvant arthritis by targeted proteomics. Biopharm Drug Dispos 39: 308-314, 2018.
24. Kawase A, Kazaoka A, Yamamoto R, Minakata R, Shimada H and Iwaki M: Changes in transporters and metabolizing enzymes in the livers of rats with bile duct ligation. J Pharm Pharm Sci 22: 457-465, 2019.
25. Sunman JA, Hawke RL, LeCluyse EL and Kashuba ADM: Kupffer cell-mediated IL-2 suppression of CYP3A activity in human hepatocytes. Drug Metab Dispos 32: 359-363, 2004.
26. Hartmann G, Kim H and Piquette-Miller M: Regulation of the hepatic multidrug resistance gene expression by endotoxin and inflammatory cytokines in mice. Int Immunopharmacol 1: 189-199, 2001.
27. Goralski KB, Hartmann G, Piquette-Miller M and Renton KW: Downregulation of mdr1a expression in the brain and liver during CNS inflammation alters the in vivo disposition of digoxin. Br J Pharmacol 139: 35-48, 2003.
28. Ogiku M, Kono H, Hara M, Tsuchiya M and Fujii H: Glycyrrhizin prevents liver injury by inhibition of high-mobility group box 1 production by Kupffer cells after ischemia-reperfusion in rats. J Pharmacol Exp Ther 339: 93-98, 2011.
29. Shi X, Yu L, Zhang Y, Liu Z, Zhang H, Zhang Y, Liu P and Du P: Glycyrrhetinic acid alleviates hepatic inflammation injury in viral hepatitis disease via a HMGB1-TLR4 signaling pathway. Int Immunopharmacol 84: 106578, 2020.
30. Wang Y, Chen Q, Shi C, Jiao F and Gong Z: Mechanism of glycyrrhizin on ferroptosis during acute liver failure by inhibiting oxidative stress. Mol Med Rep 20: 4081-4090, 2019.
31. Ketloy C, Engering A, Srichairatanakul U, Limsalakpetch A, Yongvanitchit K, Pichyangkul S and Ruxrungtham K: Expression and function of Toll-like receptors on dendritic cells and other antigen presenting cells from non-human primates. Vet Immunol Immunopathol 125: 18-30, 2008.
32. Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdörfer B, Giese T, Endres S and Hartmann G: Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168: 4531-4537, 2002.
33. Rehli M: Of mice and men: Species variations of toll-like receptor expression. Trends Immunol 23: 375-378, 2002.
34. Zhang B, Ramesh G, Uematsu S, Akira S and Reeves WB: TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. J Am Soc Nephrol 19: 923-932, 2008.
35. West AP, Koblansky AA and Ghosh S: Recognition and signaling by toll-like receptors. Annu Rev Cell Dev Biol 22: 409-437, 2006.
36. Rothfuchs AG, Trumstedt C, Wigzell H and Rottenberg ME: Intracellular bacterial infection-induced IFN-gamma is critically but not solely dependent on Toll-like receptor 4-myeloid differentiation factor 88-IFN-alpha beta-STAT1 signaling. J Immunol 172: 6345-6353, 2004.
37. Zhang B, Ramesh G, Norbury CC and Reeves WB: Cisplatin-induced nephrotoxicity is mediated by tumor necrosis factor-alpha produced by renal parenchymal cells. Kidney Int 72: 37-44, 2007.
38. Fan HY, Qi D, Yu C, Zhao F, Liu T, Zhang ZK, Yang MY, Zhang LM, Chen DQ and Du Y: Paeonol protects endotoxin-induced acute kidney injury: Potential mechanism of inhibiting TLR4-NF-κB signal pathway. Oncotarget 7: 39497-39510, 2016.
39. Hreggvidsdottir HS, Ostberg T, Wähämaa H, Schierbeck H, Aveberger AC, Klevenvall L, Palmblad K, Ottosson L, Andersson U and Harris HE: The alarmin HMGB1 acts in synergy with endogenous and exogenous danger signals to promote inflammation. J Leukoc Biol 86: 655-662, 2009.
40. Wähämaa H, Schierbeck H, Hreggvidsdottir HS, Palmblad K, Aveberger AC, Andersson U and Harris HE: High mobility group box protein 1 in complex with lipopolysaccharide or IL-1 promotes an increased inflammatory phenotype in synovial fibroblasts. Arthritis Res Ther 13: R136, 2011.
41. He ZW, Qin YH, Wang ZW, Chen Y, Shen Q and Dai SM: HMGB1 acts in synergy with lipopolysaccharide in activating rheumatoid synovial fibroblasts via p38 MAPK and NF-κB signaling pathways. Mediators Inflamm 2013: 596716, 2013.