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Inhibitory effects of honokiol on lipopolysaccharide-induced cellular responses and signaling events in human renal mesangial cells

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ABSTRACT

Honokiol has been shown to possess a lot of pharmacologic benefits, including antioxidative, antiangiogenic and antineoplastic effects. In the present study, we investigated the anti-inflammatory effects of honokiol and the signaling mechanisms involved in lipopolysaccharide (LPS)-induced conditions in human renal mesangial cells (HRMCs). Honokiol did not significantly change HRMC viability when used at a concentration of <20 μmol/l but markedly altered cell viability at concentrations of >40 μmol/l. In this study, LPS treatment led to a marked upregulation of the levels of IL-1β, IL-18, TNF-α, TGF-β1, CCL2, CCL3, and CCL5 in HRMCs. The expression of COX-2, iNOS, and their products PGE_2 and NO also increased. The upregulation of these molecules was significantly abolished by honokiol in a dose-dependent manner. Moreover, honokiol almost completely reversed IL-1β, CCL3, and NO expression at 10 μmol/l, and IL-18, TNF-α, TGF-β1, and COX-2 expression at 20 μmol/l. In addition, phospho-NF-κB p65 at Ser536, phospho-Akt, and phospho-p42/44 were dramatically suppressed by honokiol in LPS-treated HRMCs. These results indicate that honokiol can inhibit the LPS-induced expression of inflammatory cytokines and mediators in HRMCs. The anti-inflammatory mechanisms of honokiol are partly due to the suppression of the phospho-NF-κB p65, phospho-Akt and phospho-p42/44 pathways.

1. Introduction

Renal damage is associated with overexpression of a large variety of growth factors and cytokines that influence the progression of kidney diseases (Sánchez-López et al., 2008). Mesangial cells within the glomerulus contribute to the regulation of glomerular filtration, phagocytosis of immune complexes, and production of extracellular matrix. When activated by immunological or inflammatory stimulation, mesangial cells generate cytokines, chemokines, and high-output nitric oxide (NO) (Yu et al., 2002). Lipopolysaccharide (LPS) or other stimulations, such as polymeric immunoglobulin A1 (pIgA1) and hyperglycemia, have been shown to activate mesangial cells to produce proinflammatory cytokines, including IL-6, transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), and NO (Leung et al., 2008; Lee et al., 2009 and Wu et al., 2007). In addition, several lines of evidence support an important role for many chemokines, such as the chemokine (c-c motif) ligand 2 (CCL2), CCL3, and CCL5, in the pathogenesis of experimental kidney disease models and human diseases (Teramoto et al., 2008 and Campbell et al., 2006).

Current clinical approaches to the treatment of inflammation focus not only on the inhibition of proinflammatory mediator production but also on the suppression of initiation of the inflammatory response (i.e., suppression of positive signaling pathways of proinflammatory cytokines). Honokiol is an active component purified from Magnolia, a plant used in traditional Chinese and Japanese medicine (Sheu et al., 2008). Honokiol has been shown to possess a number of pharmacologic benefits, including antioxidative, antiinflammatory, anxiolytic, antimicrobial, antineoplastic, and antiangiogenic effects (Sheu et al., 2008). The molecular mechanisms involved in honokiol pharmacology are believed to be managed by modulation of a signaling cascade in response to LPS or TNF-α stimulation (Lee et al., 2009). Several research groups have reported that honokiol strongly inhibits NF-κB translocation, a critical step in early inflammatory events and tumor angiogenesis (Kim et al., 2008). On the other hand, phosphorylation of p42/44 mitogen-activated protein kinase (MAPK) or inflammatory cell infiltration is known to be involved in the pathophysiological changes of renal fibrosis (Peng et al., 2007). Another finding suggest that honokiol may act as a potent antiinflammatory agent with diverse activities, owing to its inhibitory effect on the Akt pathway (Kim et al., 2008). Because of their many potential roles in various disease onsets, cytokines are regarded as therapeutic target proteins in developing antiinflammatory drugs (Kim et al., 2008 and Peng et al., 2007).

Although various cellular responses to honokiol treatment have been evaluated, whether honokiol is able to directly diminish the effect induced by LPS in cultured human mesangial cells is not completely clear. Therefore, this study examined the cytoprotective

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effects of honokiol on mesangial cells in the conditions induced by LPS. The signaling mechanisms involved were also studied.

2. Materials and methods

2.1. Reagents

LPS was obtained from Sigma Chemical Co., USA. Honokiol (purity, 98.7%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China. The Cell Titer 96 AQueous cell viability assay kit was procured from Promega Co., USA. All other chemicals were of reagent grade. ELISA kits for IL-1β, IL-18, TNF-α, CCL2, CCL3, CCL5, and TGF-β1, as well as PGE_2 and NO assay kits were purchased from R&D Systems (USA). Anti-p42/44, anti-Akt, anti-NF-κB p65, and their phosphorylated antibodies were all obtained from Cell Signalling, USA. Rabbit anti-β-actin, anti-cyclooxygenase-2 (COX-2), and anti-inducible nitric oxide syntase (iNOS) antibodies were obtained from Santa Cruz Biotechnology, Inc., USA.

2.2. Cell culture

Human renal mesangial cells (HRMCs) were purchased from ScienCell Research Laboratories, USA. Cells from passages 3 to 5 after recovery were used throughout these studies. HRMCs were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA), 5.6 mmol/l glucose, glutamine, and antibiotics (penicillin and streptomycin) at 37 °C under 5% CO_2.

2.3. Viability study

HRMCs (1×10^4 cells/well in 96-well plate) were treated with honokiol (0, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 μmol/l) or LPS (0, 0.1, 0.2, 0.5, 1, 2, 5, 10 μg/ml) for 24 h, and cell viability was evaluated through the 3,4-(5-dimethylthiazol-2-yl)-5- (3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt (MTS) assay. The tetrazolium compound MTS and an electron coupling reagent, phenazine methosulfate (PMS), were used in the MTS assay. Viable cells reduced MTS to formazan, which was measured by determining absorbance at 490 nm using a spectrophotometer. Formazan production is time dependent and proportional to the number of viable cells. The cultures were seeded with 1×10^4 cells/well and incubated overnight to allow cell attachment. After incubation for the indicated time in the appropriate medium, 20 μl MTS/PMS mixture was added to each well. Then, the cells were incubated for 1 h and absorbance was measured at 490 nm. The background absorbance from the control wells was subtracted from the actual absorbance value. Three duplicate studies were performed for each experimental condition.

2.4. Determination of cytokine production

The inhibitory effect of honokiol on IL-1β, IL-18, TNF-α, CCL2, CCL3, CCL5, and TGF-β1 production was determined with the ELISA assay. HRMCs (3×10^5/well in 24-well plate) were pretreated with honokiol (0, 1.25, 2.5, 5, 10, and 20 μmol/l) for 30 min and further incubated in the presence or absence of LPS for 24 h. The supernatants were then assayed for the levels of the previously-mentioned cytokines using ELISA kits, according to the manufacturer's instructions (R&D Systems).

2.5. Assay for PGE_2 production

The inhibitory effect of honokiol on PGE_2 production in LPS-treated HRMCs was determined by performing competitive ELISA according to the manufacturer's instructions (R&D Systems). The lower limit of PGE_2 detection was 41.4 pg/ml.

2.6. NO assay

HRMCs were preincubated with or without honokiol for 30 min and then continuously activated with LPS (1 μg/ml) for 24 h. Nitrite determination was conducted using the Griess reagent. The absorbance of the product dye was measured at 540 nm using a flow-through spectrophotometer. The sensitivity of NO assay was less than 0.78 μmol/l.

2.7. Western blotting

HRMCs (5×10^6/10 cm dish) were pretreated with honokiol for 30 min and further incubated in the presence or absence of LPS (1 μg/mL) for 24 h. Cells were collected and lysed with a lysis buffer [20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 1% NP-40, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF)] for 30 min at 4 °C. The concentration of protein in each cell lysate was determined using a BCA protein assay kit (Pierce, IL, USA). Twenty micrograms of cell protein lysates per sample were mixed with 2×SDS loading buffer containing DTT and heated at 100 °C for 10 min before resolving by SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% nonfat dry milk in PBS with 0.02% v/v Tween 20. The membrane was incubated for 16 h with primary antibodies in PBS-Tween. The membrane was washed and incubated for 2 h at room temperature with a peroxidase-labeled second antibody (Dako). After further washing, the membrane was detected with ECL chemiluminescence. For detection of β-actin as loading control, membranes were stripped with 100 mmol/l 2-ME, 62.5 mmol/l Tris–HCl (pH 6.7), and 2% SDS for 30 min at 55 °C, and reprobed with anti-β-actin antibody for 1 h at 25 °C, which was detected with an HRP-conjugated secondary antibody.

2.8. Statistical analysis

All experiments were performed in triplicate. Student's *t* test and 1-way ANOVA were used to determine the statistical significance of differences between the experimental and control groups. *P* values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effects of LPS on HRMCs viability and the expression of TNF-α and CCL2

Prior to the present study, a preliminary experiment had been carried out to determine the optimum concentration of LPS which would be used in this study. The viability and the supernatant levels of TNF-α and CCL2 of HRMCs incubated with different concentration of LPS for 24 h were determined by using the MTS and ELISA assays. The results that at concentrations greater than 2 μg/ml, LPS could inhibit cell viability were shown in Fig. 1. Further, LPS concentrations of 1–2 μg/ml could significantly elevate the expression levels of TNF-α and CCL2. Consequently, we selected a concentration of 1 μg/ml for our study.

3.2. Effect of honokiol on HRMCs viability

Determination of the cytotoxic effect of honokiol was imperative before further studies were carried out. The viability of HRMCs following incubation with different honokiol preparations for 24 h is determined by the MTS assay. Fig. 2 demonstrates that HRMCs retained almost the same viability when exposed to honokiol concentrations of 0–20 μmol/l under our incubation conditions, while more than 40 μmol/l concentrations markedly altered cell viability. Therefore, less than 20 μmol/l concentrations of honokiol were suitable for the selective pharmacological action of the drug without any interference with normal cell function.

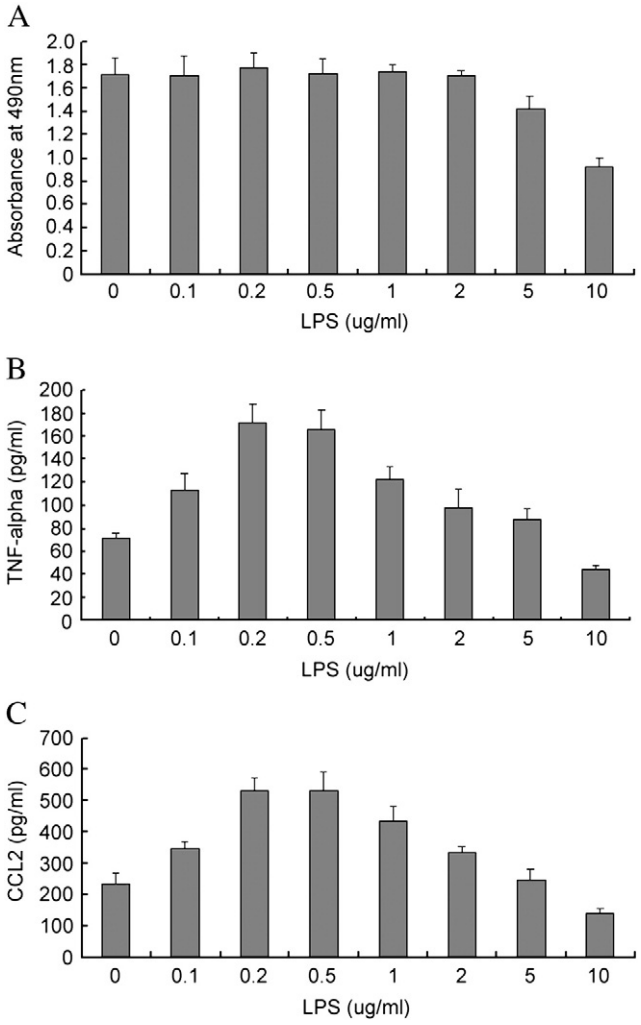

Fig. 1. Effects of LPS on HRMCs viability and the expression of TNF-α and CCL2. The viability and the supernatant levels of TNF-α and CCL2 of HRMCs incubated with different concentration of LPS for 24 h were determined by using the MTS and ELISA assays. Data represent mean ± S.E.M. ($n = 3$).

3.3. Inhibitory effect of honokiol on the LPS-induced upregulation of inflammatory factor production in HRMCs

To evaluate the inhibitory effect of honokiol on LPS-mediated production of inflammatory cytokines, the levels of IL-1β, IL-18, TNF-α, CCL2, CCL3, CCL5, TGF-β1, PGE_2, and NO in the supernatant of HRMCs were determined by ELISA and Griess assays. Cells were pretreated with various concentrations of honokiol in the presence or absence of LPS (1 μg/ml) for 24 h (Table 1). Honokiol significantly diminished the LPS-induced upregulation of cytokine production (Table 1), indicating that honokiol may affect the LPS-mediated proinflammatory responses of HRMCs. Furthermore, the inhibitory effect was in a dose-dependent manner. Indeed, honokiol almost completely reversed the IL-1β, CCL3, and NO expression at 10 μmol/l, and TNF-α, IL-18, TGF-β1, and PGE_2 at 20 μmol/l (Table 1).

3.4. Mechanisms of honokiol inhibition of LPS-induced inflammatory responses in HRMCs

To understand the molecular mechanism by which honokiol blocks various inflammatory processes, the effects of honokiol on LPS-induced signaling were investigated. NF-κB plays a key role in the initiation of inflammation. Suppression of NF-κB could be an effective method to treat inflammatory diseases (Hanada and Yoshimura, 2002). Since the p65 subunit is responsible for the transcriptional activity of NF-κB (Tse et al., 2005), phosphorylation of the p65 at Ser536 was determined using western blot analysis in this study. Moreover, the p42/p44 MAPK and Akt signaling pathways and iNOS and COX-2 expression in HRMCs were also examined. Fig. 3 shows that LPS stimulation triggered the activation of the above-mentioned signaling molecules, as assessed by their phosphorylation levels determined using phosphospecific antibodies. Interestingly, honokiol strongly blocked the phosphorylation of Akt, p42/p44 MAPK and NF-κB p65 at Ser536 without altering the total levels of these signaling molecules. Furthermore, honokiol also remarkably suppressed iNOS and COX-2 expressions induced by LPS, which are known to induce the phosphorylation of Akt (Fig. 3B). This suggests that these signal pathways could be considered target events.

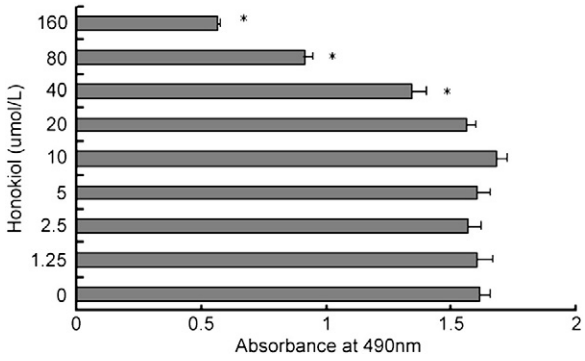

Fig. 2. Effect of honokiol on the viability of HRMCs. HRMCs (1×10^4 cells/well) were incubated with honokiol of different concentrations for 24 h. Cell viability was determined by the MTS assay. Data represent mean ± S.E.M. ($n = 3$). *, $P < 0.05$ vs normal group.

4. Discussion

The mesangium occupies a central anatomical position in the glomerulus and plays an important regulatory role in immune-mediated glomerular diseases, through active participation in the response to local inflammation. In general, mesangial cell responses to pathological stimulation are associated with the main events of glomerular injury (Gómez-Guerrero et al., 2005). Endotoxin, such as LPS from gram-negative bacteria, is a complicating factor that can exacerbate and accelerate nephritis (Shui et al., 2007). Honokiol is known to block the LPS-induced cytotoxicity of macrophages and monocytes in a dose-dependent manner in vitro. And honokiol appears to block the production of cytotoxic cytokines such as IL-1β, TNF-α, NO, and reactive oxygen species (Lee et al., 2009). Furthermore, it has also been used without noticeable side effects for many years in traditional Asian medicine (Maruyama et al., 1998). In vivo, for example, honokiol administered at a dose of 2.5 mg/kg reduced glomerular CCL2 and intracellular adhesion molecule-1, similar to the reduction in type I (alpha1) collagen and fibronectin mRNA levels in nephritic rats treated with anti-Thy1. These results indicate that honokiol may have therapeutic potential in mesangial proliferative glomerulonephritis in rats (Chiang et al., 2006). However, its role in LPS-induced mesangial cell injury is still unreported. In this study, the antiinflammatory effect of honokiol and its regulating mechanisms in human mesangial cells treated with LPS were explored.

The cytotoxic effect of LPS or honokiol was evaluated by the MTS assay 24 h after treatment of HRMCs with variable LPS or honokiol concentrations. And the effect on the expression of TNF-α, CCL2 which was caused by different LPS concentrations was determined by ELISA assay. Fig. 1 shows that at the concentration of 1 μg/ml, LPS didn't inhibit cell viability, meanwhile it fully elevated the expression levels of TNF-α and CCL2. Ultimately, the concentration of 1 μg/ml was selected for our study. Although no cytotoxicity was evident at honokiol concentrations less than 20 μmol/l, there was a dose-dependent significant decrease in

Table 1
Inhibitory effect of honokiol on the LPS-induced upregulation of inflammatory factor production. HRMCs were pretreated with honokiol (0–20 μmol/l) for 30 min and further incubated in the presence or absence of LPS (1 μg/ml) for 24 h. Levels of TNF-α, IL-1β, IL-18, TGF-β1, CCL2, CCL3, CCL5, PGE2, and NO were determined by ELISA and Griess assays. Data represent mean ± S.E.M. ($n = 3$). [a], $P<0.05$ vs normal group 1; [b], $P<0.05$ vs LPS alone (group 2).

Conditions/ groups/cytokines	1	2	3	4	5	6	7
LPS(μg/ml)	0	1	1	1	1	1	1
HNK(μmol/l)	0	0	1.25	2.5	5	10	20
TNF-α (pg/ml)	37.33 ± 4.73 [b]	161.12 ± 11.79 [a]	147.67 ± 9.02 [a]	101.24 ± 13.75 [a,b]	79.01 ± 6.24 [a,b]	55.33 ± 4.51 [a,b]	43.11 ± 7.03 [b]
TGF-β1 (pg/ml)	1282.67 ± 141.12 [b]	4405.33 ± 242.18 [a]	3895.33 ± 222.13 [a,b]	2900.04 ± 158.19 [a,b]	2153.13 ± 175.45 [a,b]	1727.23 ± 40.82 [a,b]	1554.04 ± 71.69 [b]
IL-18 (pg/ml)	43.04 ± 13.08 [b]	263.33 ± 33.02 [a]	240.06 ± 19.47 [a]	223.01 ± 16.82 [a,b]	165.33 ± 15.63 [a,b]	96.32 ± 15.61 [a,b]	67.67 ± 8.14 [b]
IL-1β (pg/ml)	71.33 ± 8.74 [b]	303.67 ± 33.08 [a]	259.33 ± 23.54 [a,b]	211.67 ± 13.05 [a,b]	151.12 ± 16.09 [a,b]	102.07 ± 8.72 [b]	87.02 ± 13.32 [b]
CCL2 (pg/ml)	116.33 ± 15.95 [b]	513.67 ± 24.44 [a]	399.67 ± 15.5 [a,b]	324.67 ± 15.63 [a,b]	217.67 ± 27.15 [a,b]	153.04 ± 14.53 [a,b]	136.03 ± 6.56 [b]
CCL3 (pg/ml)	365.67 ± 100.83 [b]	1804.67 ± 173.79 [a]	1623.06 ± 166.47 [a]	1238.03 ± 61.29 [a,b]	713.33 ± 74.65 [a,b]	506.67 ± 75.86 [b]	447.22 ± 109.78 [b]
CCL5 (ng/ml)	0.48 ± 0.16 [b]	4.81 ± 0.26 [a]	4.53 ± 0.12 [a,b]	3.38 ± 0.27 [a,b]	2.36 ± 0.23 [a,b]	1.23 ± 0.087 [a,b]	0.93 ± 0.12 [a,b]
PGE_2 (ng/ml)	0.17 ± 0.06 [b]	3.31 ± 0.18 [a]	3.16 ± 0.12 [a]	2.81 ± 0.12 [a,b]	1.81 ± 0.09 [a,b]	1.12 ± 0.17 [a,b]	0.47 ± 0.07 [a,b]
NO (μmol/l)	29.82 ± 6.77 [b]	258.47 ± 13.82 [a]	194.83 ± 6.53 [a,b]	94.93 ± 11.12 [a,b]	52.83 ± 5.86 [a,b]	35.17 ± 5.07 [b]	33.47 ± 8.34 [b]

[a] $P<0.05$ vs normal group(group 1).
[b] $P<0.05$ vs LPS alone (group 2).

cell number with higher concentrations above 40 μmol/l, such that 160 μmol/l resulted in more than 65% loss in cell viability compared to the basal concentration (Fig. 2). The final concentrations of honokiol used in our experiments were 1.25, 2.5, 5, 10, and 20 μmol/l.

One local effect of LPS on glomeruli is to stimulate mesangial cells to produce growth factors and cytokines that modulate renal cell survival and glomerular inflammation (Shui et al., 2007). Among these factors, TNF-α is a primary mediator in the inflammatory process and has strong immunomodulating properties. During renal injury, other proinflammatory cytokines, such as IL-1β (Sánchez-López et al., 2008), IL-18 (Shui et al., 2007), and TGF-β1 (Schnaper et al., 2003), released by infiltrating inflammatory and intrinsic mesangial cells, may be simultaneously present in the glomerular environment. These factors can activate mesangial cells to increase ECM production and therefore contribute to progression of renal damage (Sánchez-López et al., 2008). In this study, LPS treatment led to a marked increase in the levels of TNF-α, IL-1β, IL-18, and TGF-β1 in the supernatant of HRMCs (Table 1). However, the increase of these molecules was significantly abolished by honokiol in a dose-dependent manner. In fact, honokiol almost completely reversed IL-1β expression at 10 μmol/l, and IL-18, TNF-α, and TGF-β1 at 20 μmol/l.

Chemokines are a family of small related proteins that play an important role in the selective recruitment of different leukocyte populations to the sites of inflammation. Human glomerular mesangial cells are potent producers of a variety of chemokines (Schwarz et al., 1997). In this study, the inhibitory effect of honokiol on LPS-induced mesangial cell chemokine expression was examined, with focus on CCL2, CCL3 and CCL5. As shown in Table 1, honokiol significantly suppressed CCL2, CCL3 and CCL5 production by up to about 74%, 75%, and 80%, respectively, at 20 μmol/l. These results together suggest that honokiol is capable of blocking a common pathway involved in the production of inflammatory cytokines and chemokines.

On the other hand, the glomerular filtration rate is elevated in the early phase of some forms of nephropathy, such as diabetic nephropathy (Hostetter, 2001). In this respect, the vasodilatory prostaglandins, including PGE_2, have been implicated in the glomerular hyperfiltration seen in diabetic patients (Viberti et al., 1989), as well as in experimental models of diabetes (DeRubertis et al., 1993). COX-2 is considered a rate-limiting enzyme in PGE_2 biosynthesis (Uriuhara et al., 2007). In our study, LPS-induced PGE_2 overproduction was concomitant with the increased COX-2 protein expression in HRMCs. Furthermore, NS-398, a selective COX-2 inhibitor, could inhibit methylglyoxal-induced PGE_2 production in a dose-dependent manner in rat mesangial cells (Uriuhara et al., 2007). NO also serves an important role in glomerular function, and excessive NO production has been linked to several forms of glomerular injury. NO production is generally known to be governed by the activity of 3 NOS isoforms. iNOS is quiescent in most tissues until it is transcriptionally activated by immune stimulation like LPS to produce large amounts of NO (Yu et al., 2002). Honokiol is known to suppress NO production in macrophages (Matsuda et al., 2001). As previously reported (Lee et al., 2009), honokiol suppressed LPS-induced NO production in a dose-dependent manner (Table 1). Consequently, honokiol also strongly suppressed the LPS-induced increase in the iNOS protein levels.

Finally, the signal transduction pathways involved in the cytoprotective effect of honokiol in HRMCs treated with LPS were preliminary

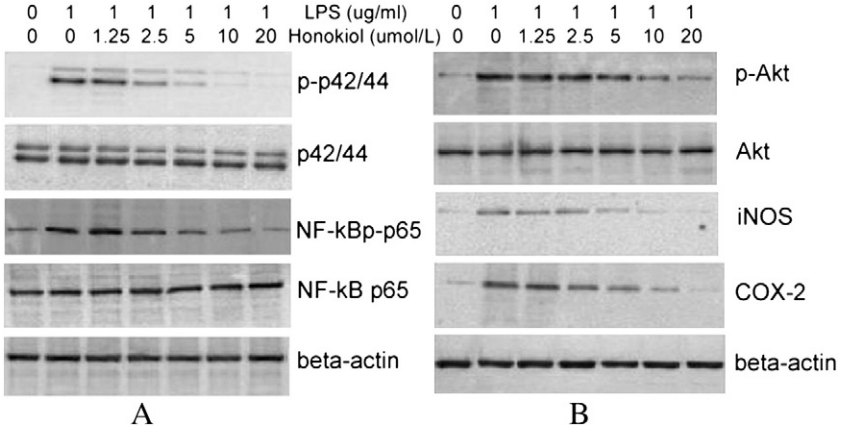

Fig. 3. Effect of honokiol on LPS-induced early intracellular signaling events. Cells were pretreated with various concentrations of honokiol in the presence or absence of LPS (1 μg/ml) for 24 h. After immunoblotting, the phosphorylation or the total levels of NF-κB p65, p42/p44 MAPK, Akt, iNOS, and COX-2 were identified through their phosphospecific or nonphosphospecific antibodies.

explored. While it is well known that activation of the NF-κB protein plays a central role in inflammation through the regulation of genes encoding proinflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as COX2 and iNOS, these pathways are activated upon appropriate extracellular stimulation, most often by stress or proinflammatory cytokines, including LPS and TNF-α (Hanada et al., 2002). Phosphorylation of the p65 subunit by a variety of kinases is known to lead to the modification in NF-κB transcriptional activity (Vermeulen et al., 2002), thus, phosphorylation of the p65 at Ser536 was determined using western blot analysis. As shown in Fig. 3A, honokiol inhibited the levels of phospho-p65 at Ser536 in the cells with LPS treatment, and the inhibitory effects were found to be dose-dependent. These results are in line with observations mentioned above that honokiol interferes with increase in LPS-mediated cytokines, chemokines, and growth factors (Table 1). Moreover, a concentration of 20 μmol/l honokiol almost completely blocked LPS-mediated p65 phosphorylation at Ser536 in whole cell extracts. Although honokiol inhibited NF-κB p65 phosphorylation at Ser536, it remained unclear that how this phosporylation affected NF-κB p65's function.

However, LPS induction of the phosphorylation of Akt, an upstream kinase of IKK that is responsible for NF-κB activation, was strongly suppressed by honokiol exposure, suggesting that the real target of honokiol may not be IKK only but also its upstream kinase (Kim et al., 2008). Fig. 3B shows that LPS stimulation triggered the activation of the upstream signaling molecule Akt, as shown by its phosphorylation level assessed using phosphospecific antibodies. This suggests that the Akt pathway may be critical in honokiol-mediated cytoprotective effects. Furthermore, MAPKs, including the p42/44 signaling pathways, which has recently been postulated to mediate COX-2 activation in a variety of cells (Uriuhara et al., 2007), are postulated to be involved in renal fibrosis (Liu et al., 2005; Xie et al., 2004 and Crean et al., 2002). Fig. 2A demonstrates that honokiol also dramatically suppressed the LPS-induced phosphorylation of p42/44, whereas total p42/44 is not changed. These data imply that p42/44 could play a role in the inhibitory effect of honokiol on the increased production of cytokines induced by LPS in HRMCs. Nevertheless, all the effects observed in the current study are not cause and effect relationship and could simply be side effects and not causal linked to the effects of honokiol.

Our data shows that honokiol dose dependently blocks the LPS-induced production of many inflammatory factors in HRMCs. Since exploring the direct targets (inflammatory factors, iNOS and NF-κB, Akt, and p42/44 pathways) of honokiol will greatly improve our understanding of its pharmacological actions, our next experiments will involve detailed molecular studies using direct kinase assays. In conclusion, honokiol may be considered a promising agent that has potent antiinflammatory activity in addition to strong anticancer, antiangiogenic, and antineurodegenerative effects.

Acknowledgements

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