Immunopharmacology and inflammation

Coptisine from *Coptis chinensis* inhibits production of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 murine macrophage cells

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**A B S T R A C T**

*Coptis chinensis* has been used for the treatment of inflammatory diseases in China and other Asian countries for centuries. However, the chemical constituents and mechanism underlying the anti-inflammatory activity of this medicinal plant are poorly understood. Here, coptisine, the main constituent of *C. chinensis*, was shown to potently inhibit the production of nitric oxide (NO) by suppressing the protein and mRNA expressions of inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Coptisine also inhibited the production of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) by suppressing expression of cytokine mRNA. Coptisine suppressed the degradation of inhibitor of nuclear factor κB (IkBα) and phosphorylation of extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase/Akt (PI3K/Akt). Coptisine had no effect on the expression of toll-like receptor 4 (TLR-4) and myeloid differentiation factor 88 (MyD88) as well as LPS binding to TLR-4. Coptisine also inhibited carrageenan-elicited rat paw edema and reduced the release of TNFα and NO in rat inflamed tissue. These results suggest that coptisine inhibits LPS-stimulated inflammation by blocking nuclear factor-kappa B, MAPK, and PI3K/Akt activation in macrophages, and can be used as an agent for the prevention and treatment of inflammatory diseases.

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1. Introduction

Inflammation is a physiological response to protect normal function in the host, and this can be initiated by microbial infection and tissue injury. It is characterized by abundant production of pro-inflammatory mediators such as nitric oxide (NO), tumor necrosis factor (TNF-α), interleukin (IL)-1β and IL-6, which are considered important targets for the development of anti-inflammatory agents (MacMicking et al., 1997; Nathan, 2002). In response to the stimulation of lipopolysaccharide (LPS), a component of gram-negative bacteria cell wall, macrophages actively participate in inflammatory responses by releasing various pro-inflammatory mediators (Mogensen, 2009). Thus, inhibition of macrophage function is an option in attenuating excessive inflammatory responses.

Nuclear factor-kappa B (NF-κB) is a transcription factor that has a critical role in the onset of inflammation and tumor progression (Karin and Greten, 2005). It is present in the cytosol, and binds to the inhibitor protein I kappa B (IkB). The proteasome-mediated degradation of IkB proteins results in the activation of NF-κB, which is followed by its translocation into the nucleus, where it induces expression of pro-inflammatory genes (Karin, 1999; Saccani et al., 2001). Intracellular signaling pathways such as mitogen-activated protein kinases (MAPKs) and phosphoinositide 3-kinase/Akt (PI3K/Akt) may also participate in NF-κB activation. MAPKs, including extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), regulate the synthesis of inflammatory mediators at the transcription and translation levels through NF-κB activation (Chan and Riches, 2001; Kaminska,
was purchased from Hyclone Thermo Sciences (RPMI)-1640 was from GE Healthcare (Logan, Utah, USA). Fetal bovine serum (FBS) (DMEM) was from Invitrogen (Carlsbad, CA, USA). Roswell Park Memorial Institute Assay (ELISA) kits were purchased from Invitrogen. IL-1 were purchased from Beyotime Institute of Biotechnology (Haimen, China). NO

2. Materials and methods

2.1. Materials

Berberine, epiberberine, cotisine, jatrorrhizine and palmatine are considered to be the active constituents of C. chinensis (Chen et al., 2008). The anti-inflammatory effect of C. chinensis collected at different times correlates positively with these five alkaloids contents (Li et al., 2015). Berberine plays a key role in the modulation of inflammatory responses through inhibition of NF-κB and AP-1 binding (Kuo, et al., 2004; Jiang et al., 2011). Palmatine has been shown to exhibit anti-inflammatory activity in a serotonin-induced model of paw edema and attenuate v-galactosamine/LPS-induced fulminant hepatic failure in mice (Kupeli et al., 2002; Lee et al., 2010a). The contribution of the other alkaloids to the anti-inflammatory property of C. chinensis, which is important for the quality control and clinical use, is not known. Therefore, we evaluated these five alkaloids from C. chinensis for their anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages and carrageenan-elicited rat paw edema.

2.2. Cell culture

Murine macrophage RAW 264.7 cells were purchased from the Center of Cellular Resources, Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Human monocyctic THP-1 cells were a kind gift from West China Center of Medical Sciences, Sichuan University, and were cultured in RPMI 1640 with additional 10% FBS, 1 mM sodium pyruvate and 1% penicillin-streptomycin. All cells were cultured at 37 °C in 5% CO₂ humidified air.

2.3. Animals

Male Sprague Dawley rats (150–180 g) were obtained from Sichuan Academy of Medical Sciences (Chengdu, China), and conducted according to a protocol approved by the Institute of Laboratory Animals, Sichuan Academy of Medical Sciences. All animals were housed under a 12 h: 12 h light/dark circle in standard polypropylene cages at 21–24 °C and were allowed free access to food and water.

2.4. Measurement of the production of NO, IL-1β and IL-6 in cell culture supernatant

Culture media were collected for NO, IL-1β and IL-6 assays. NO was measured by detection of its stable oxidative metabolite, nitrite, as previously described (Lin et al., 2013a). In brief, 50 μl of the culture media was mixed with equal volume of Griess reagent (Beyotime) and incubated for 30 min at 37 °C. Absorbance was measured at 540 nm. Nitrite concentration was determined using a calibration curve using standard solutions of sodium nitrite. Concentrations of IL-1β and IL-6 in cell-culture supernatants were measured using a Mouse IL-1β ELISA kit and Mouse IL-6 ELISA kit, respectively, following manufacturer protocols.

2.5. Cell proliferation assay

RAW 264.7 cells were plated at 4 × 10⁵ cells/ml in 96-well plates incubated at 37 °C in 5% CO₂ overnight (Hu et al., 2014). Compounds were added into each well and treated for 24 h. Alamar-Blue reagent was added into each well, and fluorescence intensity measured (excitation, 544 nm; emission, 590 nm) using a Varioskan Flash Multimode Reader (Thermo Scientific). Percent inhibition of cellular proliferation was defined as the ratio of the optical density (OD) value in test wells compared with those in control wells.

2.6. Quantitative real-time-polymerase chain reaction (qRT-PCR)

RAW 264.7 cells were plated at 1 × 10⁵ cells/ml in 6-well plates overnight, and pretreated with or without different concentrations of compounds for 1 h, followed...
by treatment with LPS (1 μg/ml) for the indicated time. Total RNA from RAW 264.7 cells was prepared using TRIzol Reagent (Invitrogen) according to manufacturer instructions. The concentration and purity of RNA were measured using a UV-1800 Spectrophotometer (Mapada, Shanghai, China). Total RNA (2 μg) was reverse-transcribed into cDNA using a SuperScript™ III First-Strand Synthesis system (Invitrogen) in a total volume of 20 μl. qRT-PCR was undertaken using a Chromo4 Real-Time PCR system (Bio-Rad, Hercules, CA, USA) with SsoFast™ EvaGreen® Supermix (Bio-Rad). The following primer sequences (forward and reverse, respectively) were used: iNOS: 5'-GGATCTTCCCAGGCAACCA-3' and 5'-ATTCATGGAGTAGAACATGTT-3'; IL-1β: 5'-GGCAATCTGGGCTCAAAGGAAATC-3' and 5'-GGAAGACACAGATTCCATGGTGAAG-3'; IL-6: 5'-TGGAGTCACAGAAGGAGTGGCTAAG-3' and 5'-TCTGACCACAGTGAGGAATGTCCAC-3'; GAPDH, 5'-TGCACCACCAACTGTGGCTTAGC-3' and 5'-GGCATGGACTGTGGTCATGAG-3'. Conditions were: 95°C for 5 min, and then 39 cycles of 95°C for 15 s, and 60°C for 30 s. Relative expression levels of target genes was calculated using the 2⁻△△Ct method according to manufacturer instructions with GAPDH as the housekeeping gene.

**Fig. 2.** Effect of coptisine on production of NO, IL-1β and IL-6. (A) RAW 264.7 cells were seeded onto 96-well plates and treated with coptisine at the indicated concentrations for 24 h. Cell proliferation was estimated by the Alamar-Blue assay and expressed relative to the control (DMSO). **P < 0.01 compared with the control group. (B–D) RAW 264.7 cells seeded in 24-well plates overnight were pretreated with coptisine at the indicated concentrations or BAY 11-7082 (10 μM) for 1 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h, and then stimulated with LPS (1 μg/ml) for 24 h. The concentration of IL-1β and IL-6 in the medium was measured by ELISA (C and D). The assay was conducted thrice in triplicate. *P < 0.05, **P < 0.01 compared with the LPS group (B–D), the IFN-γ group (E) or IFN-γ plus LPS group (F).
2.7. Western blotting

Following treatment, the cells were rinsed with cold phosphate-buffered saline (PBS) and lysed in RIPA buffer supplemented with a cocktail of protease and phosphatase inhibitors (Pierce, Rockford, IL, USA). Protein concentrations of each sample were determined using a Bicinchoninic Acid Protein Assay kit (Pierce). Equal amounts of denatured protein were subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and then blocked with 5% bovine serum albumin or non-fat dry milk in TBST. After blocking, membranes were incubated overnight at 4°C with antibodies against iNOS, IκBα, phospho-ERK, ERK, phospho-JNK, JNK, phospho-p38 MAPK, p38 MAPK, phospho-Akt, Akt, TLR-4, MyD88, and GAPDH respectively (all at 1:1000 dilution). Blots were incubated with HRP-conjugated goat anti-rabbit or goat anti-mouse antibodies for 2 h at room temperature and detected using a chemiluminescence detection system according to manufacturer instructions (Amersham, Berkshire, UK). Band intensity of each signal was quantified by densitometric analyses (Quantity One; Bio-Rad).

2.8. Confocal microscopy

LPS-TLR4 binding assay was conducted as described previously (Jang et al., 2013). Briefly, RAW264.7 cells were incubated with 10 μg/ml of alexa fluor 488-conjugated LPS in the presence and absence of coptisine or berberine for 20 min. Then the cells were fixed with 4% formaldehyde for 20 min. Thereafter, cells were incubated with rabbit anti-TLR-4 polyclonal antibody for 2 h at 4°C, followed by incubation with alexa fluor 555-conjugated goat anti-rabbit antibody for 1 h. Cells were imaged by laser scanning confocal microscopy (Leica Microsystems TCS-SP8, Wetzlar, Germany).

2.9. Carrageenan-induced paw edema

Coptisine injection was prepared as previously described (Mackenzie et al., 1997). Rats (n = 36) were randomly divided in six groups as follows: blank, model, dexamethasone (Dex) (20 mg/kg) and coptisine (0.93 mg/kg, 1.97 mg/kg and 3.87 mg/kg). The rat paw edema was induced by subcutaneous injection of 0.1 ml 1% carrageenan into the left hind paw in all rats except those in the blank group (Zekai et al., 2007). The measurement of basal paw volumes were determined by using a plethysmometer and changes in the paw volumes were monitored at 0.5 h intervals for 4 h after intravenous injection of testing compounds. The results were presented as the paw volume variation in relation to the basal values. The anti-inflammatory potency of all treatments was determined (%) relative to the animals that received only 1% carrageenan solution (model group).

2.10. Determination of hind paw skin temperature

All animals' paws were shaved from toes up to ankle joint and then marked. The temperature of skin was measured with an infrared thermometer (Tecman,
Hongkong, China) prior to the drug intravenous injection and monitored at 0.5 h intervals for 4 h (Giraudel et al., 2005). The data were recorded thrice at each time point and the difference between control paw and inflamed paw was analyzed.

2.11. Measurement of the NO and TNF-α levels in hand paw

4 h after the carrageenan administration, rats were euthanized by urethane, and subcutaneous tissue of each animal was removed and homogenized as previously reported (Hossein et al., 2011). The supernatants were collected and NO and TNF-α concentrations were measured following the instructions of TNF-α ELISA kit and NO reagent, respectively.

2.12. Statistical analyses

All statistical calculations were carried out using GraphPad Prism v5.01 (GraphPad, Avenida, CA, USA). Data were expressed as the mean ± standard error of mean of three independent experiments. Data were analyzed using one-way analysis of variance followed by Dunnett’s post hoc test. *P < 0.05, **P < 0.01 compared with the LPS group.

3. Results

3.1. Effects of alkaloids from C. chinensis on NO production in LPS-stimulated RAW 264.7 macrophages

To assess the anti-inflammatory activity of the five key alkaloids from C. chinensis (berberine, epiberberine, coptisine, jatrorrhizine and palmatine), we screened their effects on NO production in LPS-stimulated RAW 264.7 macrophages. The chemical structures of these alkaloids are illustrated in Fig. 1A. LPS treatment significantly stimulated NO production, and this effect was significantly suppressed by an IκB inhibitor, BAY 11-7082 (Fig. 1B). Berberine, coptisine and palmatine at 30 μM also exhibited significant inhibitory effects on NO production. The anti-inflammatory effect of coptisine is poorly understood, therefore, in the present research, its mechanism of action was further characterized.

3.2. Effect of coptisine on production of NO, IL-1β and IL-6

Coptisine (0.1–30 μM) did not affect the viability of RAW 264.7 cells, but caused cytotoxicity at high concentration (50 μM) (Fig. 2A). Hence, further study of Fig. 5. Effect of coptisine on degradation of IκBα protein. RAW 264.7 cells were pretreated with different concentrations of coptisine or BAY 11-7082 (10 μM) for 12 h before treatment with 1 μg/ml LPS for 15 min. Cell lysates were immunoblotted with an anti-IκBα antibody. GAPDH staining is shown as a loading control. Quantitative results are depicted. *P < 0.05, **P < 0.01 compared with the LPS group.

Fig. 6. Effect of coptisine on MAPK signaling. (A) RAW 264.7 cells were pretreated with different concentrations of coptisine and PD98059 (10 μM) for 12 h before addition of 1 μg/ml LPS for 10 min. (B) and (C) RAW 264.7 cells were pretreated with different concentrations of coptisine and SB203580 (10 μM) or SP600125 (20 μM) for 4 h before addition of 1 μg/ml LPS for 15 min. Cell lysates were immunoblotted with the indicated antibody. Quantitative results are depicted. *P < 0.05, **P < 0.01 compared with the LPS group.
3.4. Effects of coptisine on iNOS expression in RAW 264.7 cells

Coptisine was conducted at non-toxic concentrations (1–30 μM). Coptisine markedly suppressed the production of NO (Fig. 2B), IL-1β (Fig. 2C) and IL-6 (Fig. 2D) in a concentration-dependent manner in LPS-stimulated RAW 264.7 cells. IFN-γ alone or IFN-γ plus LPS stimulated the production of NO in phospholipid-13-acylate (PMA)-differentiated human THP-1 macrophage cells. Coptisine (30 μM) suppressed the production of NO induced by IFN-γ alone (Fig. 2E), while at 10 and 30 μM showed significant inhibitory effect on NO release induced by IFN-γ plus LPS (Fig. 2F). These results suggest that coptisine inhibits the production of pro-inflammatory factors in macrophages.

3.5. Effects of coptisine on expression of IL-1β and IL-6 mRNA

To ascertain if the effect of coptisine on the pro-inflammatory response was at the transcriptional level, we examined expressions of IL-1β mRNA and IL-6 mRNA in LPS-stimulated RAW 264.7 cells. At 1–30 μM, coptisine significantly decreased the mRNA expression of IL-1β, as did Bay 11-7082 (Fig. 3A). Similarly, coptisine (1–30 μM) significantly suppressed the mRNA expression of IL-6 (Fig. 3B). These results suggest that coptisine inhibits production of pro-inflammatory cytokines at the transcriptional level.

3.6. Effect of coptisine on LPS-induced MAP kinases activation

To ascertain if coptisine interferes with LPS-stimulated activation of MAPK signaling, we examined the phosphorylation of key MAPK signaling proteins. LPS significantly enhanced phosphorylation of ERK1/2, and this was inhibited by the specific ERK inhibitor PD98059 (Fig. 6A). Similarly, coptisine (1–30 μM) significantly inhibited LPS-stimulated ERK phosphorylation. LPS also significantly enhanced phosphorylation of p38, which was inhibited by SB203582 (specific p38 inhibitor) and coptisine (1–30 μM) (Fig. 6B). LPS-stimulation of JNK phosphorylation was potently inhibited by SP600125 (specific JNK inhibitor) and coptisine (1–30 μM) in a concentration-dependent manner (Fig. 6C). These results suggest that coptisine exerts its anti-inflammatory effect (at least in part) by inhibition of LPS-stimulated MAPK activation.

3.7. Effect of coptisine on LPS-induced NF-κB activation

To evaluate the effect of coptisine on LPS-stimulated activation of p385K, we examined the phosphorylation of p35K by coptisine and BAY 11-7082 at non-toxic concentrations (1–30 μM). Coptisine markedly increased phosphorylation of Akt in RAW 264.7 cells, which was potently inhibited by 1 μM (specific p35K inhibitor) and coptisine (1–30 μM) (Fig. 7). This result suggests that coptisine also exerts its anti-inflammatory effect (at least in part) by inhibition of LPS-stimulated p35K activation.

3.8. Effect of coptisine on LPS-induced TLR-4 signaling

To examine whether coptisine interferes with LPS-stimulated TLR-4 pathway signaling, we examined the effect of coptisine on the expression of TLR-4 and Myd88. LPS significantly up-regulated the expression of both TLR-4 and Myd88, while coptisine (1–30 μM) had no effect on expression of TLR-4 and Myd88 induced by LPS (Fig. 8A and B). Next, we examined whether coptisine could block the interaction between LPS and TLR-4. As shown in Fig. 8C, basal auto-fluorescence was determined in untreated RAW264.7 cells. Other samples were treated with Alexa Fluor 488-conjugated LPS alone or with different concentrations of coptisine or berberine plus Alexa Fluor 488-conjugated LPS. Co-localization of LPS with TLR-4 was observed at 20 min after LPS treatment. Coptisine did not affect LPS binding to the macrophage, but this was blocked by berberine and this result was similar to previous studies (Jeong et al., 2014). These results suggest that coptisine does not interfere with TLR-4 signaling in exerting its anti-inflammatory effect.

3.9. Effect of coptisine on carrageenan-induced paw edema

We employed the carrageenan-induced rat paw edema model to examine the anti-inflammatory activity of coptisine in vivo. After carrageenan was injected into the subcutaneous tissue, the skin temperature of each group was elevated except the blank group (Fig. 9A). Administration of coptisine at doses of 1.93 mg/kg and 3.87 mg/kg significantly inhibited the development of paw edema after the induction of inflammation as compared to the model group only induced by carrageenan treatment (Fig. 9B). As expected, the reference drug, dexamethasone (20 mg/kg), caused a significant inhibition of post-carrageenan rat paw edema. As shown in Fig. 9C, injection of carrageenan in hand paw stimulated the release of TNF-α in inflamed tissue, which was significantly inhibited by the treatment of coptisine (3.87 mg/kg) or dexamethasone. Similarly the release of NO in inflamed tissue was also significantly reduced by coptisine (3.87 mg/kg) or dexamethasone in the carrageenan-treated rat paws (Fig. 9D). These results suggest that coptisine can exert its anti-inflammatory effect in vivo.

4. Discussion

* C. chinensis is frequently used in Traditional Chinese Medicine prescriptions for the treatment of inflammatory diseases. It has been reported that the anti-inflammatory effect of *C. chinensis* positively correlates with the five alkaloids contents (berberine, coptisine, epiberberine, palmatine and jatrorrhizine) (Li et al., 2015). However, it is still not clear whether their effect and mechanism for anti-inflammation are same or not. We found that, apart from epiberberine and jatrorrhizine, only berberine, palmatine and coptisine exhibited inhibitory effects on NO production in LPS-stimulated RAW 264.7 cells but with varying degrees of potency. This finding suggests that berberine, palmatine and coptisine are the active constituents in *C. chinensis* responsible for the use of this plant in inflammatory diseases. Coptisine has been found to exhibit antispasmodic (Hiller et al., 1998), anti-fungal
Kong et al., 2009) and anti-oxidative (Gong et al., 2012) activities, as well as protection of gastric–mucous membranes (Hirano et al., 2001), however, its effect and mechanism for anti-inflammation are still unknown. In the present study, we, for the first time report that coptisine exhibits potent anti-inflammatory effects by inhibition of LPS-stimulated inflammatory mediators production in macrophages. Interestingly, epiberberine and jatrorrhizine had no effect on NO production in LPS-stimulated RAW 264.7 cells even though they share a very similar structure with berberine, palmatine and coptisine. Berberine and coptisine have a dimethoxy group between R2 and R3, whereas palmatine has methyl groups at the same position. In addition, berberine and palmatine have methyl groups at R9 and R10, whereas coptisine has a dimethoxy group between R9 and R10. Comparison of the structural characteristics of the three protoberberinium compounds suggests that substitution with a dimethoxy group between R2 and R3, or

Fig. 8. Effect of coptisine on TLR-4 signaling. (A and B) RAW 264.7 cells were pretreated with different concentrations of coptisine for 12 h before treatment with 1 µg/ml LPS for 24 h. Cell lysates were immunoblotted with anti-TLR-4 and anti-MyD88 antibody. GAPDH staining is shown as a loading control. Quantitative results are depicted. (C) RAW 264.7 cells were incubated with Alexa Fluor 488-conjugated LPS for 20 min in the presence of coptisine (1–30 µM) or berberine (30 µM), then analyzed with confocal microscopy using an anti-TLR-4 antibody.
substitution with a methyl group at R9 and R10, contribute to anti-inflammatory potency.

NO, IL-1β, IL-6 are pro-inflammatory cytokines implicated in the occurrence of many inflammatory diseases such as diabetes and rheumatoid arthritis (Kristiansen et al., 2005; Nishimoto et al., 2006). Here, coptisine was found to potently inhibit production of these pro-inflammatory mediators not only in mouse macrophages induced by microbial components such as LPS, but also in human macrophages induced by sterile condition such as IFN-γ. This observation further supports the function of *C. chinensis* in clinical use for the treatment of inflammatory diseases. Coptisine decreased the transcription and protein expression of iNOS, which supports the observation that coptisine inhibits NO production in macrophages induced by LPS (Ma et al., 2009). Expression of these pro-inflammatory mediators was found to be suppressed by coptisine at the transcriptional level, which is regulated by the transcription factor NF-κB. Previously, berbereine was found to exert its anti-inflammatory effect by inhibition of the NF-κB signaling pathway (Jiang et al., 2011). In the present study, coptisine was found to inhibit IκBα degradation, suggesting that it may decrease expression of these pro-inflammatory mediators by inhibition of NF-κB activation, similar to the action of berberine.

Berberine was found to inhibit activation of ERK, p38 and JNK MAPKs and PI3K/Akt/NF-κB pathway in a variety of inflammatory models, which partly contributes to its inhibitory effect in LPS-stimulated TLR-4 signaling (Wang et al., 2014; Xiao et al., 2014; Lin et al., 2013b). In this study we found that coptisine had no effect on expressions of TLR-4 and MyD88 as well as LPS binding to TLR-4, which could be inhibited by berberine (Chen et al., 2014; Lee et al., 2010b; Jeong et al., 2014). These results suggest that coptisine may not be inhibiting proinflammatory responses through TLR-4 signaling. Berberine is also found to suppress the ERK, p38 and JNK phosphorylation in LPS-stimulated macrophages by activating AMP-activated protein kinase (Jeong et al., 2009). Thus, it will be of interest to further examine whether coptisine activates AMPK to suppress inflammatory responses in macrophages. *C. chinensis* is one of the herbal plants formulated for Huang-Lian-Jie-Du decoction (HLJDD) and Xiexin decoction (XXD), which have been used to treat inflammation for nearly two thousand years in the clinical practice of Traditional Chinese Medicine. HLJDD and XXD have been reported to attenuate inflammatory responses in carrageenan-induced mice paw edema and LPS-exposed rats (Lo et al., 2005a, 2005b; Lu et al., 2011). In this study, coptisine was also found to suppress carrageenan-induced rats paw edema and production of NO and TNF-α in inflamed tissues, suggesting that coptisine is one of major constituents for the anti-inflammatory effects of HLJDD and XXD. The synergistic action of multiple compounds in herbal medicinal plants is believed to play the key role to exert the health beneficial functions. For example, the antimicrobial effects of berberine *Berberis fremontii* were enhanced > 100-fold when combined with an inactive component, 5′-methoxyhyd-nocarpin, isolated from the same plant *Berberis fremontii* (Sternitz et al., 2000). Therefore, coptisine may suppress the inflammatory responses in vivo with berberine possibly through synergistical effect in the clinical use of *C. chinensis*.

In summary, we demonstrated that coptisine has potent anti-inflammatory properties as demonstrated by the inhibition of LPS-stimulated expression of inflammation-associated genes in mouse and human macrophages and in carrageenan-induced rats paw edema. These effects were exerted by blocking of NF-κB, MAPK, and PI3K/Akt activation, but not through TLR-4. Our findings suggest that coptisine is an anti-inflammatory constituent of *C. chinensis*, which can be further developed as a pharmaceutical agent for the prevention and treatment of inflammatory diseases.

**Fig. 9.** Effect of coptisine on carrageenan-induced rat paw edema. Coptisine at the indicated doses, dexamethasone (20 mg/kg) and vehicle were intravenously administrated after carrageenan (1%) injection. The skin temperature (A) and paw edema (B) were monitored at 0.5-h intervals for 4 h after carrageenan injection. The paw tissues were homogenized and concentration of TNF-α (C) and NO (D) in the supernatants was determined by ELISA and Griess reagent respectively. *P < 0.05, **P < 0.01 compared with the carrageenan group.
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