Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 498 (2004) 45–52

www.elsevier.com/locate/ejphar

Andrographolide acts through inhibition of ERK1/2 and Akt phosphorylation to suppress chemotactic migration

Hwei-Ru Tsai[a], Li-Ming Yang[b], Wei-Jern Tsai[b], Wen-Fei Chiou[b,*]

[a]*Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, NO.155, Li-Nung Street, Section 2, Shih-Pai Taipei 112, Taiwan, ROC*
[b]*National Research Institute of Chinese Medicine, No. 155-1, Li-Nung Street, Section 2, Li-Nung Street, Shih-Pai, Taipei 112, Taiwan, ROC*

Received 8 July 2004; accepted 13 July 2004
Available online 12 August 2004

Abstract

We now evaluated the anti-inflammatory mechanisms of andrographolide on complement 5a (C5a)-induced macrophage recruitment in vitro. Andrographolide concentration dependently inhibited cell migration toward C5a with an IC_{50} of 5.6±0.7 μM. With relatively specific kinase inhibitors (PD98059, SB203580, SP600125, wortmannin and LY294002, respectively) the results showed that extracellular signal-regulated kinase1/2 (ERK1/2), p38 mitogen-activated protein kinase (p38 MAPK) and phosphatidylinositol-3-kinase (PI3K) were necessary for C5a-induced migration, whereas c-Jun N-terminal kinase (JNK) was nonessential. Andrographolide significantly attenuated C5a-stimulated phosphorylation of ERK1/2, and of its upstream activator, MAP kinase–ERK kinase (MEK1/2). C5a-activated ERK1/2 phosphorylation was 86±9% inhibited by 30 μM andrographolide. Under the same conditions, however, andrographolide failed to affect C5a-stimulated p38 MAPK and JNK phosphorylation. Andrographolide also strongly abolished C5a-stimulated Akt phosphorylation, a downstream target protein for PI3K. These results indicate that inhibition of cell migration by interfering with ERK1/2 and PI3K/Akt signal pathways may contribute to the anti-inflammatory activity of andrographolide.

Keywords: Andrographolide; Chemotactic migration; MAPK; ERK1/2; Akt

1. Introduction

Complement 5a (C5a) has been shown to play important pathological roles in both acute and chronic inflammatory diseases including sepsis (Mollnes, 2002). Septic shock is the worst-case event in sepsis in which mortality can reach 90% (Bone, 1993). Lipopolysaccharide is often used to mimic septic shock in animal models. Among the several systems activated by lipopolysaccharide are the complement-, the cytokine-, and the chemokine-network. In sepsis, there is evidence that excessive C5a generation leads to compromise immune function associated with a poor outcome (Köhl, 2001; Riedemann et al., 2003). Thus, application of C5a blockade prior to sepsis significantly improved survival rates (Ward et al., 2003). Furthermore, C5a is a potent chemoattractant to induce leukocyte infiltration to infected tissue and may contribute to multiple organ failure in sepsis. Other studies also suggest that the effect of C5a in the sepsis syndrome is mediated by a cellular C5a receptor and that blockade of C5a signals may be the therapeutically valuable effect (Köhl, 2001; Haynes et al., 2000).

Andrographolide is a bicyclic diterpenoid lactone isolated from leaves of *Andrographis paniculata* (Lu et al., 1981), a Chinese official herbal medicine used as an anti-inflammatory drug for the treatment of laryngitis, diarrhea, and rheumatoid arthritis. We have reported that andrographolide may be beneficial in rats suffering from endotoxaemia by ameliorating hypotension and vascular hypoeactivity through inhibiting the expression of inducible nitric oxide synthase and subsequently diminishing the production of nitric oxide (Chiou et al., 1998; 2000). Furthermore, recent study has demonstrated that andrographolide has the

* Corresponding author. Tel.: +886 2 28201999x4481; fax: +886 2 28250743.
E-mail address: wfchiou@cma23.nricm.edu.tw (W.-F. Chiou).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.077

potential to prevent endotoxin-induced multiple organ dysfunction (Chiou et al., unpublished data). Thus, we were interested to clarify whether the beneficial effect of andrographolide against septic syndromes was attributable to interruption of C5-induced leukocyte recruitment.

Cell migration in response to chemoattractants is a crucial determinant of leukocyte trafficking. It has been shown that C5a-induced chemotaxis is mediated by a seven-transmembrane-spanning receptor coupled to G protein, resulting in transduction of signals to the interior of the cells and phosphorylation of multiple proteins (Haribabu et al., 1999). The activation of mitogen-activated protein kinase (MAPK) seems to be one of the key components in signal transduction associated with cell migration (English et al., 1999). Three of the most commonly known mammalian MAPKs are extracellular signal-regulated kinase (ERK1/2 or p42/44 MAPK), p38 MAPK, and c-Jun N-terminal kinase (JNK) (Widmann et al., 1999). Another key pathway is involved in cell migrating signals through phosphatidylinositol-3-kinase (PI3K) and Akt/PKB (Tai et al., 2003; Kawasaki et al., 2003). In some, but not all hemopoietic cells, the pivotal signaling enzyme, PI3K, also plays a key role in governing the chemotactic response (Siddiqui and English, 2000; Stephens et al., 2002). This pathway leads to the activation of Akt, a cytosolic serine/threonine kinase that acts downstream of PI3K (Matsui et al., 2003; Whiteman et al., 2002). In this study, we also attempted to characterize whether andrographolide inhibits C5a-induced chemotaxis by interfering with C5a-activated protein kinase cascades.

2. Materials and methods

2.1. Cell Culture Conditions

RAW264.7 (American Type Culture Collection, TIB 71, Rockville, MD, USA) macrophages were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, USA) supplemented with 10% heat-inactivated fetal calf serum, penicillin and streptomycin (Biological Industries, Israel) at 37 °C in a humidified atmosphere in the presence of 5% CO_2 (Chiou et al., 2003a,b).

2.2. Chemotactic migration

Cell migration was assessed using a 24-well chemotaxis chamber with a membrane pore size of 5 μm (Transwell, Corning Costar). Ninety microliters of cell suspension (2×10^7/ml) was added to each of the upper wells in the presence of 10 μl PBS, andrographolide (Sigma, purity approx. 98%) or inhibitors for 30 min. C5a was added to the lower well of the chamber to assess chemoattractic activity. Then the entire chamber was incubated at 37 °C for 4 h to initiate migration. Non-migrated cells were wiped off with a cotton swab and then the filter was fixed and stained with hematoxylin (Sigma) to define the cell nuclei. Chemotaxis was assessed by counting the number of migrated cells in five (at 400× magnification) random microscopy fields per well (Chiou et al., 2003b). All experiments were performed in triplicate. Complement 5a-induced cell migration minus spontaneous migration in PBS served as control and was designated as 100% migration for each experiment.

2.3. Cell viability

Cell viability was assessed by the mitochondria-dependent reduction of MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma) to formazan. The extent of reduction of MTT to formazan within cells was quantitated by measurement of OD_{570} against OD_{630}.

2.4. Preparation of cell extracts and Western blot analysis of MAPK isoforms

Cells were plated in T75 culture flasks and made quiescent at confluence by incubation in fresh DMEM for 24 h then further stimulated with C5a at 37 °C for 30 min. When andrographolide or inhibitors were used, they were applied 30 min prior to the addition of C5a. After incubation, the cells were rapidly washed with ice-cold PBS, scraped and collected. Cell pellets were lysed with ice-cold lysis buffer containing (mM): 25 Tris–HCl, pH 7.4, 25 NaCl, 25 NaF, 25 sodium pyrophosphate, 1 sodium vanadate, 2.5 EDTA, 2.5 EGTA, 1 phenylmethylsulfonyl fluoride (PMSF), 0.05% Triton X-100, 0.5% lauryl sulfate sodium salt (SDS), 0.5% deoxycholate, 0.5% nonylphenoxy polyethoxy ethanol (NP-40), 5 μg/ml leupeptin, and 5 μg/ml aprotinin. The lysates were centrifuged at 45,000×*g* for 1 h at 4 °C to yield the whole cell extract in the supernatants. Protein concentration was determined using BCA reagents according to the manufacturer's manual.

Protein (40 μg) was separated using 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked by incubating the membrane in TBS-T (20 mM Tris pH7.2; 150 mM NaCl; 0.1% Tween 20) with 5% bovine serum albumin for 1 h at room temperature. The membrane was incubated (overnight at 4 °C) with antibodies that specifically detect the phosphorylated (i.e. activated) form of MAP kinase–ERK kinase (MEK1/2), ERK1/2 (p44/p42 MAPK), p38 MAPK and JNK (Cell Signaling Technology, Beverly, MA, USA) at the indicated dilution. Then it was incubated with HRP anti-rabbit (Amersham, Buckinghamshire, U.K.) antibody and detected by ECL (Amersham). The results were evaluated by densitometry analysis.

2.5. Western blotting analyses for Akt

To examine PI3K downstream signaling events, the expression of phosphorylated Akt was measured. Cells were incubated with lysis buffer [10 mM Tris–HCl at pH 7.6, 140 mM NaCl, 1% Triton X-100, 5 mM EDTA and 2

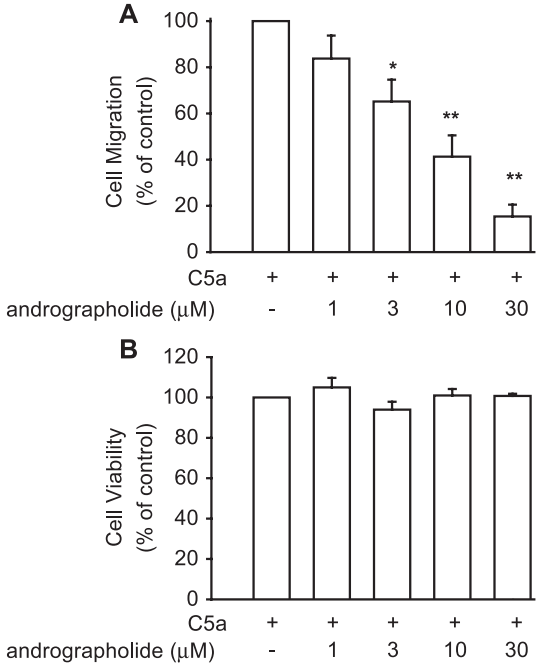

Fig. 1. Effect of andographolide on complement 5a (C5a)-induced chemotactic migration (A) and cytotoxicity (B). Cells pre-incubated with drug for 30 min were plated onto the upper wells of the chamber. C5a (1 μg/ml) was added to the lower wells for 4 h to induce cell migration. Migration was assessed by counting migrated cells in five microscopic fields per well at 400× magnification. C5a-induced cell migration minus spontaneous migration in PBS served as control and was designated as 100%. Data reported are mean±S.E.M. of six independent experiments, each performed in triplicate. *P<0.05 and **P<0.01 indicate significance of difference as compared to samples receiving C5a alone.

mM PMSF] for 30 min. The cell lysates were heated to 90 °C for 10 min then subjected to 8% SDS-PAGE and transferred to a nitrocellulose membrane immunoblotted with antibody against Akt (Cell Signaling, Beverly, MA, USA) or phospho-Akt (within the C-terminus at Ser473) (Cell Signaling).

2.6. Statistical analysis

All values in the text and figures represent means±S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Dunnett's t-test for multiple comparisons. Values of P<0.05 were considered significant.

3. Results

3.1. Effect of andrographolide on C5a-induced chemotactic migration

A 1 μg/ml of C5a was selected as stimulus according to our previous findings (Chiou et al., 2003a; Tsai et al., 2003). Andrographolide (1–30 μM) alone did not influence spontaneous transmigration (data not shown), however, it concentration-dependently reduced chemotactic migration in response to C5a with an IC_{50} of 5.6±0.7 μM (Fig. 1A). None of the concentrations of andrographolide used significantly reduced cell viability (>95%), as measured by MTT assay (Fig. 1B).

3.2. C5a-activated ERK1/2 and p38 MAPK phosphorylation contribute to chemotactic migration

The anti-phospho ERK1/2 antibody recognized the two isoforms at 42 and 44 kDa proteins. In unstimulated cells, only a slight signal appeared, however, stimulation with C5a for 15 min increased the levels of phosphorylated ERK1/2

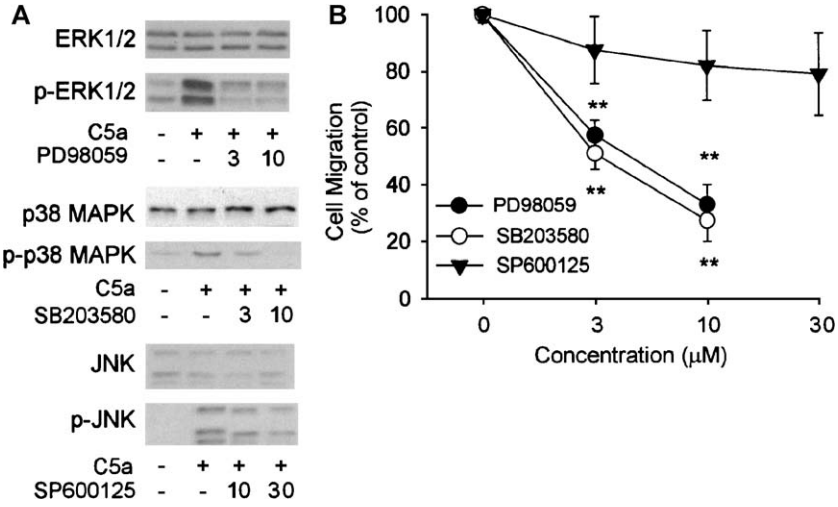

Fig. 2. Effects of MAPK inhibitors (PD98059, SB203580, and SP100625) on C5a-stimulated ERK1/2, p38 MAPK and JNK phosphorylation (A) and on C5a-induced chemotactic migration (B) in RAW264.7 macrophages. The cells were grown to confluence, made quiescent by serum-deprivation for 24 h and incubated with 1μg/ml C5a for 15 min. The cell lysates were subjected to 8% SDS-PAGE and transferred to a nitrocellulose membrane. Western blot analysis was performed as described in Section 2. Similar results were obtained in four independent experiments. C5a-induced cell migration minus spontaneous migration in PBS served as control and was designated as 100%. Data reported are means±S.E.M. of six independent experiments, each performed in triplicate. *P<0.05 and **P<0.01 indicate significance of difference as compared to samples receiving C5a alone.

protein nearly 6-fold as compared to the unstimulated control (Fig. 2A, upper trace). C5a-activated ERK1/2 phosphorylation was clearly suppressed by MEK1/2 inhibitor 2′ -amino-3′ -methoxyflavone (PD98059, 3 and 10 μM) (Hotokezaka et al., 2002). Cell migration toward C5a was also concentration dependently inhibited by 42.5±4.3% and 67.1±5.9% after 3 and 10 μM PD98059 treatment, respectively (Fig. 2B).

Fig. 2A showed that stimulation of the cells with C5a for 15 min increased p38 MAPK phosphorylation 4.3±0.7 times as compared to unstimulated cells, such phosphorylation was significantly inhibited by p38 MAPK inhibitor 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-imidazole (SB203580, 3 and 10 μM) by 43.2±4.5% and 97.6±3.8%, respectively (middle trace). Macrophage migration toward C5a was also markedly inhibited by 49.0±5.7% and 72.8±7.3% after 3 and 10 μM SB203580 treatment, respectively (Fig. 2B).

C5a also activated JNK phosphorylation and anthrax [1, 9-cd] pyrazol-6(2H)-one (SP600125), a specific JNK inhibitor (Vaishnav et al., 2003), attenuated phosphorylation (Fig. 2A, lower trace). However, SP600125 decreased migration in response to C5a to a much lesser degree. As shown in Fig. 2B, only 17.9±12.3% and 20.8±14.6% inhibition was observed when 10 and 30 μM SP600125 was used.

3.3. Role of Akt phosphorylation in C5a-induced chemotactic migration

The results showed that stimulation with C5a for 15 min increased phosphorylation of Akt about 3–6 fold as compared to unstimulated cells (Fig. 3A). It was consistently noted that two PI3K inhibitors, wortmannin and 2-(4-morpholinyl)-8-phenyl-chromone (LY294002), clearly abolished Akt phosphorylation to near the basal level. Furthermore, macrophage migration to C5a was inhibited concentration dependently by wortmannin (0.01–0.1 μM) from the control (100.0%) to 81.3±11.1%, 42.2±9.5% and 23.6±9.2%, respectively (Fig. 3B), but it had no effect upon basal migration (data not shown). Treating the cells with LY294002 (0.01–10 μM) also effectively inhibited C5a-induced cell migration by 3.5±0.7%, 7.8±5.6%, 22.1±10.7%, 47.6±3.9% and 69.8±2.2%, respectively.

3.4. Effect of andrographolide on ERK1/2, MEK1/2, p38 MAPK and JNK phosphorylation activated by C5a

Fig. 4A shows that andrographolide (10 and 30 μM) concentration dependently reduced the C5a-promoted increases in ERK1/2 phosphorylation by 57±3% and 78±9%, respectively. Activation of ERK1/2 is achieved through specific upstream kinases MEK1/2 (Widmann et al., 1999). Similar to the results obtained with ERK1/2, andrographolide was able to suppress MEK1/2 phosphorylation induced by C5a with inhibition percentages of 37±8% and 68±7% by 10 and 30 μM andrographolide treatment, respectively (Fig. 4B). In contrast, andrographolide failed to affect p38 MAPK and JNK phosphorylation activated by C5a (Fig. 4C and D).

3.5. Effect of andrographolide on Akt phosphorylation activated by C5a

Fig. 5 shows a representative immunoblot and pooled data from four experiments examining Akt phosphorylation

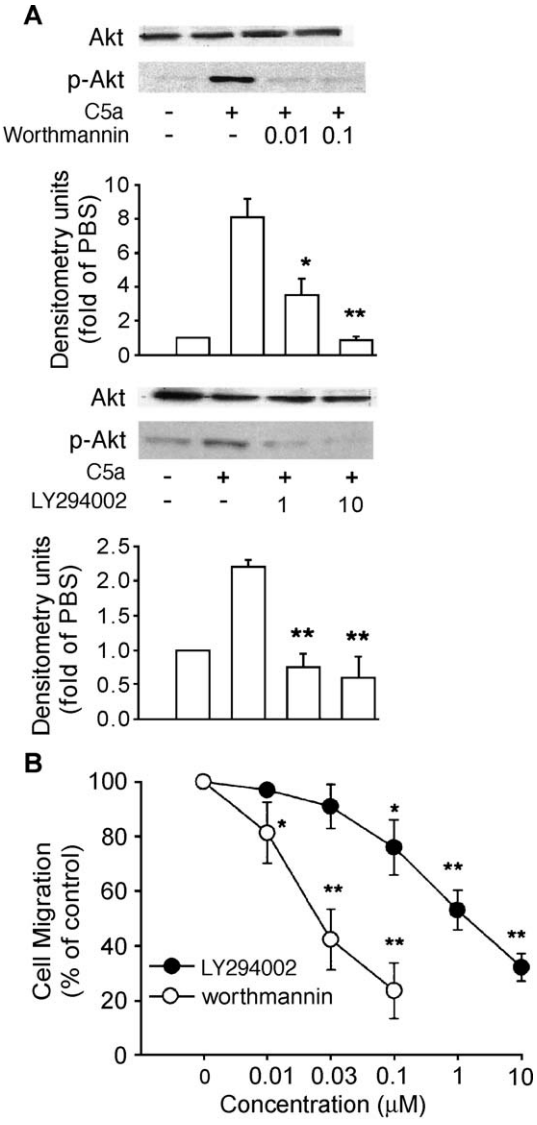

Fig. 3. Effects of PI3K inhibitors (wortmannin and LY294002) on C5a-stimulated Akt phosphorylation (A) and on C5a-induced chemotactic migration (B) in RAW264.7 macrophages. The cells were grown to confluence, made quiescent by serum-deprivation for 24 h and incubated with 1μg/ml C5a for 15 min. The cell lysates were subjected to 8% SDS-PAGE and transferred to a nitrocellulose membrane. Western blot analysis was performed as described in Section 2. Similar results were obtained in four independent experiments. C5a-induced cell migration minus spontaneous migration in PBS served as control and was designated as 100%. Data reported are means±S.E.M. of six independent experiments, each performed in triplicate. *P<0.05 and **P<0.01 indicate significance of difference as compared to samples receiving C5a alone.

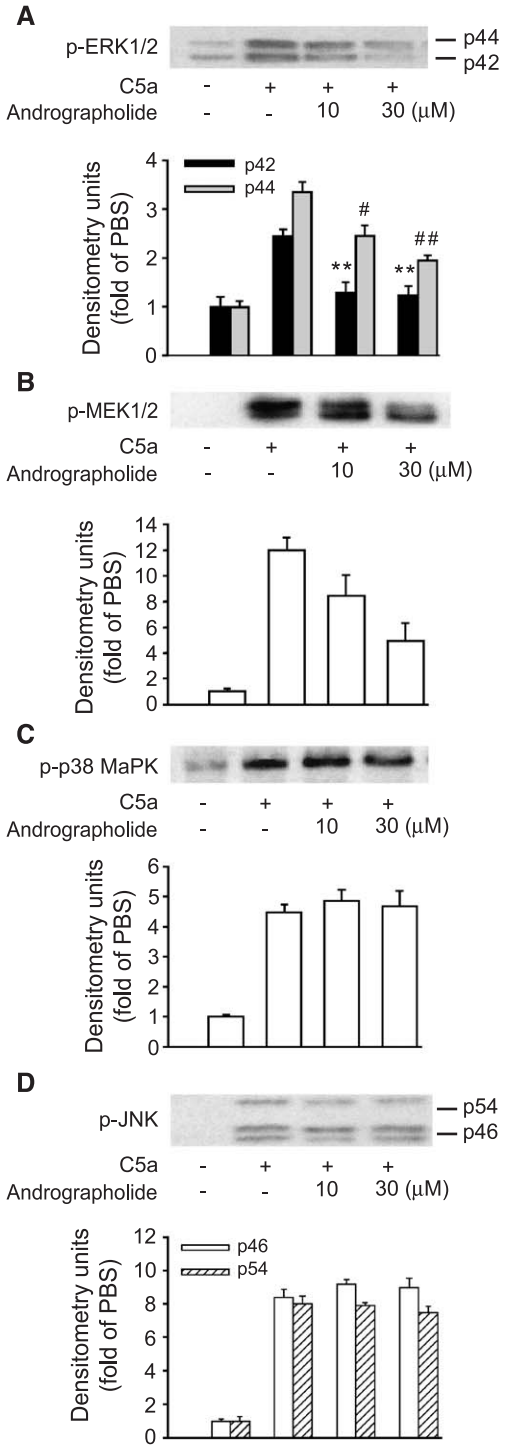

Fig. 4. Effects of andrographolide on C5a-stimulated ERK1/2, MEK1/2, p38 MAPK, and JNK phosphorylation in RAW264.7 macrophages. The cells were pretreated with andrographolide for 30 min then stimulated with 1 μg/ml C5a for 15 min. The cell lysates were subjected to 8% SDS-PAGE and transferred to a nitrocellulose membrane. Western blot analysis was performed as described in Section 2. Similar results were obtained in four independent experiments. Bands were visualized by an ECL method and quantified with a densitometer.

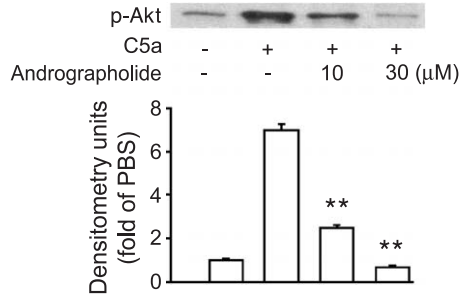

Fig. 5. Effects of andrographolide on C5a-stimulated Akt phosphorylation in RAW264.7 macrophages. The cells were pretreated with andrographolide for 30 min then stimulated with 1 μg/ml C5a for 15 min. The cell lysates were subjected to 8% SDS-PAGE and transferred to a nitrocellulose membrane. Western blot analysis was performed as described in Section 2. Similar results were obtained in four independent experiments. Bands were visualized using an ECL method and quantified with a densitometer.

of C5a-stimulated cells in the absence and presence of andrographolide. The results showed that C5a-stimulated Akt phosphorylation was also significantly attenuated by andrographolide, by 67±3% and 98±6% after 10 and 30 μM andrographolide treatment, respectively.

3.6. Effect of andrographolide on macrophage inflammatory protein-1α (MIP-1α)-induced cell migration

Finally, we examined whether andrographolide could affect the responsiveness of macrophages to agonists representative of different classes of chemotactic agents. Compared with unstimulated conditions (showing spontaneous migration with a total cell number of 68±9), macrophages treated with macrophage inflammatory protein-1α (MIP-1α) evoked significantly chemotactic migration with peak activity occurring at 0.5 μg/ml (total migrated cell number of 274±21). Andrographolide (1–30 μM) concentration dependently inhibited MIP-1α-induced che-

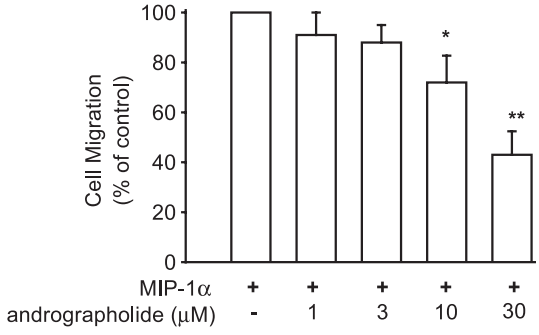

Fig. 6. Effect of andographolide on macrophage inflammatory protein-1α (MIP-1α)-induced chemotactic migration. Cells pre-incubated with drug for 30 min were plated onto the upper wells of the chamber. MIP-1α (0.5 μg/ml) was added to the lower wells for 4 h to induce cell migration. Migration was assessed by counting migrated cells in five microscopic fields per well at 400× magnification. MIP-1α-induced cell migration minus spontaneous migration in PBS served as control and was designated as 100%. Data reported are means±S.E.M. of six independent experiments and each one was performed in triplicate. *$P<0.05$ and **$P<0.01$ indicate significance of difference as compared to samples receiving MIP-1α alone.

motactic migration from 100% to 91.2±7.7%, 88.0±5.9%, 72.4±10.6%, and 42.2±8.3%, respectively. The IC_{50} value was 16.7±2.8 μM (Fig. 6).

4. Discussion

Andrographolide is an active component isolated from anti-inflammatory Chinese herbal medicine *A. paniculata* which has many biological activities (Shen et al., 2000; 2002). Our recent finding also indicated that andrographolide has the potential to prevent multiple organ (liver and kidney) dysfunction in rats suffering from endotoxaemia (Chiou et al., unpublished data). Multiple organ dysfunction syndrome (MODS) (Bone et al., 1992) is a major cause of morbidity and mortality in sepsis. Since MODS is associated with evidence of systemic inflammation (Faist et al., 1993), C5a was postulated to be among the important mediators of this response. Results showed that in a rat sepsis model, leukocytes expressed high levels of C5a on their membrane (Mizuno et al., 1999). Innovative studies now introduce a promising new target for saving lives of sepsis patients: blocking the action of C5a (Czermak et al., 1999; Gurwitz, 1999). Thus, in the present study, we attempted to evaluate whether the beneficial effect of andrographolide against multiple organ failure is attributable to interference with C5a-mediated leukocytes recruitment.

We report here that andrographolide was able to inhibit C5a-induced chemotactic migration of macrophages. Indeed, inhibition was not only observed with C5a-induced migration. Our result indicated that andrographolide also inhibited cell migration in response to macrophage inflammatory protein-1α (MIP-1α), however, with less effectiveness (IC_{50} of 16.7±2.8 μM). Because andrographolide inhibited not only C5a- but also MIP-1α-induced cell migration, it may be postulated that andrographolide may act as a broad inhibitor to treat a variety of inflammatory diseases. Nevertheless, to find whether andrographolide blocked the C5a-receptor binding ability or interrupted receptor-G protein coupling will need further study.

The chemotactic process appears to be highly regulated by MAPKs and ERK1/2, JNK, and p38 MAPK, each with a unique signaling pathway. MAPK were among the first kinases to be implicated in the synthesis of pro-inflammatory cytokines and several inhibitors of cytokine production exert their activity by blocking MAPK activation (Mantovani et al., 2000). Inhibitors of p38 MAPK were shown to suppress the synthesis of pro- and anti-inflammatory cytokines in a nonselective manner (Lee et al., 1994). In contrast, ERK1/2 inhibitors block tumor necrosis factor-α (TNF-α) and interleukin-1β (the two main pro-inflammatory cytokines) (Foey et al., 1998). MAPK inhibitors have been shown to be of significant therapeutic benefit in a number of models of inflammation, including endotoxin shock (Badger et al., 1996; Nick et al., 2000), arthritis (Badger et al., 1996) and pulmonary inflammation (Nick et al., 2000). Activation of MAPK is necessary for chemotaxis, and previous studies showed that MAPK inhibitors decrease cell migration in response to chemoattractants (Boehme et al., 1999; Ayala et al., 2000).

Although the chemotaxis process is the result of multiple signaling pathways (Wenzel-Seifert and Seifert, 2001), it is likely that blocking of the MAPK pathway contributes to the inhibition of monocyte chemotaxis by andrographolide. Our results showed that pre-incubation of macrophages with MEK inhibitor, PD98059, and p38 MAPK inhibitor, SB203580, clearly and concentration dependently suppressed C5a-induced chemotactic migration, whereas JNK inhibitor SP600125 reduced migration to a much lesser degree. These results suggested that C5a might signal through a ERK- and p38 MAPK-dominated pathway, in which JNK played only a very minor role. Moreover, C5a stimulated ERK1/2 and p38 MAPK phosphorylation, and such phosphorylation can be diminished by individual inhibitors. This reconfirmed that ERK1/2 and p38 MAPK play important roles in mediating C5a-induced migration. The results from this study demonstrated that andrographolide specifically abolishes C5a-stimulated ERK1/2 and the upstream MEK1/2 cascade, suggesting that andrographolide acts by blocking this pathway to suppress cell recruitment. Chemoattractants (such as C5a) and chemokines (such as MIP-1α), although acting through different receptors (C5a receptor and C–C chemokine receptors, respectively), can activate intracellular MAPK cascades to mediate cell migration (Lentzsch et al., 2003; Mukherjee and Pasinetti, 2001); hence, it is not surprising that andographolide simultaneously exerts its inhibitory activity against the cell response to C5a and MIP-1α.

PI3K has also been implicated as a signaling enzyme activated by chemoattractant receptors (Siddiqui and English, 2000;). This pathway leads to activation of Akt (also known as PKB), a cytosolic serine/threonine kinase that acts downstream of PI3K (Whiteman et al., 2002). Previous reports revealed that, after binding to the C5a receptor, C5a activates multiple signaling proteins, including ERK and PI3K (Monsinjon et al., 2003; Coffer et al., 1998). In the present study, the possible involvement of PI3K in C5a-induced chemotactic migration was also investigated. Through the use of two specific PI3K inhibitors, wortmannin and LY294002, we demonstrated that these compounds suppressed, not only Akt phosphorylation, but also the cell migration in response to C5a. Thus, we pointed out the importance of this enzyme as part of the C5a receptor activated cascade leading to chemotactic migration of macrophages. Our results showed that C5a-induced Akt phosphorylation was significantly attenuated by andrographolide.

In summary, the results obtained in this study suggest that andrographolide inhibits the migration toward C5a, and that this effect is associated with inhibition of intracellular ERK1/2 and Akt signal transduction pathways. As an effective anti-migratory drug against C5a-attracted leuko-

cytes recruitment, andrographolide may be useful in sepsis by limiting the early phases of macrophage infiltration.

Acknowledgments

This work was supported by grant NSC92-2320-B-077-009 from the National Science Council, Taiwan, and in part by a grant from the National Research Institute of Chinese Medicine, Taiwan.

References

Ayala, J.M., Goyal, S., Liverton, N.J., Claremon, D.A., O'Keefe, S.J., Hanlon, W.A., 2000. Serum-induced monocyte differentiation and monocyte chemotaxis are regulated by the p38 MAP kinase signal transduction pathway. J. Leukoc. Biol. 67, 869–875.

Badger, A.M., Bradbeer, J.N., Votta, B., Lee, J.C., Adams, J.L., Griswold, D.E., 1996. Pharmacological profile of SB203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function. J. Pharmacol. Exp. Ther. 279, 1453–1461.

Boehme, S.A., Sullivan, S.K., Crowe, P.D., Santos, M., Conlon, P.J., Sriramarao, P., Bacon, K.B., 1999. Activation of mitogen-activated protein kinase regulates otaxin-induced eosinophils migration. J. Immunol. 163, 1611–1681.

Bone, R.C., 1993. Gram-negative sepsis: a dilemma of modern medicine. Clin. Microbiol. Rev. 6, 57–68.

Bone, R.C., Balk, R.A., Cerra, F.B., Dellinger, R.P., Fein, A.M., Knaus, W.A., Schein, R.M., Sibbald, W.J., 1992. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 101, 1644–1655.

Chiou, W.F., Chen, C.F., Lin, J.J., 2000. Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW264.7 cells by andrographolide. Br. J. Pharmacol. 129, 1553–1560.

Chiou, W.F., Lin, J.J., Chen, C.F., 1998. Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with LPS. Br. J. Pharmacol. 125, 327–334.

Chiou, W.F., Peng, C.H., Chou, C.J., Chen, C.F., 2003a. Antiinflammatory properties of piperlactam S: modulates complement 5a induced chemotaxis and inflammatory cytokines production in macrophages. Planta Med. 69, 9–14.

Chiou, W.F., Shum, A.Y.C., Peng, C.H., Chou, C.J., Chen, C.F., 2003b. Piperlactam S suppresses macrophage migration by impediment of F-actin polymerization and filopodia extension. Eur. J. Pharmacol. 458, 217–225.

Coffer, P.J., Schweizer, R.C., Dubois, G.R., Maikoe, T., Lammers, J.W., Koenderman, L., 1998. Analysis of signal transduction pathways in human eosinophils activated by chemoattractants and the T-helper 2-derived cytokines interleukin-4 and interleukin-5. Blood 91, 2547–2557.

Czermak, B.J., Sarma, V., Pierson, C.L., Warner, R.L., Huber-Lang, M., Bless, N.M., Schmal, H., Friedl, H.P., Ward, P.A., 1999. Protective effects of C5a blockade in sepsis. Nat. Med. 5, 788–792.

English, J., Pearson, G., Wilsbacher, J., Swantek, J., Karandikar, M., Xu, S., Cobb, M.H., 1999. New insights into the control of MAPK kinase pathways. Exp. Cell Res. 233, 255–270.

Faist, E., Baue, A.E., Dittmer, H., Heberer, G., 1993. Multiple organ failure in polytrauma patients. J. Trauma 23, 775–787.

Foey, A.D., Parry, S.L., Williams, L.M., Feldmann, M., Foxwell, B.M., Brennan, F.M., 1998. Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF-α: role of the p38 and p42/44 mitogen-activated protein kinases. J. Immunol. 160, 920–982.

Gurwitz, D., 1999. Complement depletion or blockade could save lives of sepsis victims. Mol. Med. Today 5, 376.

Haribabu, B., Zhelev, D.V., Pridgen, B.C., Richardson, R.M., Ali, H., Snyderman, R., 1999. Chemoattractant receptors activate distinct pathways for chemotaxis and secretion: role of G-protein usage. J. Biol. Chem. 274, 37087–37092.

Haynes, D.R., Harkin, D.G., Bignold, L.P., Hutchens, M.J., Taylor, S.M., Fairlie, D.P., 2000. Inhibition of C5a-induced neutrophil chemotaxis and macrophage cytokine production in vitro by a new C5a receptor antagonist. Biochem. Pharmacol. 60, 729–733.

Hotokezaka, H., Sakai, E., Kanaoka, K., Saito, K., Matsuo, K., Kitaura, H., Yoshida, N., Nakayama, K., 2002. U0126 and PD98059, specific inhibitors of MEK, accelerate differentiation of RAW264.7 cells into osteoclast-like cells. J. Biol. Chem. 277, 47366–47372.

Kawasaki, K., Smith rs, J.R., Hsieh, C.M., Sun, J., Chao, J., Liao, J.K., 2003. Activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway mediates nitric oxide-induced endothelial cell migration and angiogenesis. Mol. Cell. Biol. 23, 5726–5737.

Köhl, J., 2001. Anaphylatoxins and infectious and non-infectious inflammatory diseases. Mol. Immunol. 38, 175–187.

Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Heys, J.R., Landvatter, S.W., 1994. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 372, 739–746.

Lentzsch, S., Gries, M., Janz, M., Bargou, R., Dorken, B., Mapara, M.Y., 2003. Macrophage inflammatory protein 1-alpha (MIP-1 alpha) triggers migration and signaling cascades mediating survival and proliferation in multiple myeloma (MM) cells. Blood 101, 3568–3573.

Lu, X.L., Zhang, S.L., Wang, Z.S., 1981. Analysis of andrographolide compounds. I. Ion pair high performance liquid chromatographic analysis of andrographolide derivatives. Acta Pharm. Sin. 16, 182–189.

Mantovani, A., Dinarello, C.A., Ghezzi, P., 2000. Pharmacology of Cytokines. Oxford Univ. Press, Oxford, pp. 1–8.

Matsui, T., Nagoshi, T., Rosenzweig, A., 2003. Akt and PI 3-kinase signaling in cardiomyocyte hypertrophy and survival. Cell Cycle 2, 220–223.

Mizuno, M., Nishikawa, K., Okada, N., Matsuo, S., Ito, K., Okada, H., 1999. Inhibition of a membrane complement regulatory protein by a monoclonal antibody induces acute lethal shock in rats primed with lipopolysaccharide. J. Immunol. 162, 5477–5482.

Mollnes, T.E., 2002. C5a in sepsis—a novel link between complement and coagulation. Trends Immunol. 23, 475.

Monsinjon, T., Gasque, P., Chan, P., Ischenko, A., Brady, J.J., Fontaine, M.C., 2003. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. FASEB J. 17, 1003–1014.

Mukherjee, P., Pasinetti, G.M., 2001. Complement anaphylatoxin C5a neuroprotects through mitogen-activated ptotein kinase-dependent inhibition of caspase 3. J. Neurochem. 77, 43–49.

Nick, J.A., Young, S.K., Brown, K.K., Avdi, N.J., Arndt, P.G., Suratt, B.T., Janes, M.S., Henson, P.M., Worthen, G.S., 2000. Role of p38 mitogen-activated protein kinase in a murine model of pulmonary inflammation. J. Immunol. 164, 2151–2159.

Riedemann, N.C., Guo, R.F., Ward, P.A., 2003. Novel strategies for the treatment of sepsis. Nat. Med. 9, 517–524.

Shen, Y.C., Chen, C.F., Chiou, W.F., 2000. Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide. Planta Med. 66, 314–317.

Shen, Y.C., Chen, C.F., Chiou, W.F., 2002. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. Br. J. Pharmacol. 135, 399–406.

Siddiqui, R.A., English, D., 2000. Phosphatidylinositol 3′ -kinase-mediated calcium mobilization regulates chemotaxis in phosphatidic acid-stimulated human neutrophils. Biochim. Biophys. Acta 1483, 161–173.

Stephens, L., Ellson, C., Hawkins, P., 2002. Roles of PI3K in leukocyte chemotaxis and phagocytosis. Curr. Opin. Cell Biol. 14, 203–213.

Tai, Y.T., Podar, K., Catley, L., Tseng, Y.H., Akiyama, M., Shringarpure, R., Burger, R., Hideshima, T., Chauhan, D., Mitsiades, N., Richardson, P., Munshi, N.C., Kahn, C.R., Mitsiades, C., Anderson, K.C., 2003. Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of beta1-integrin and phosphatidylinositol 3′-kinase/AKT signaling. Cancer Res. 63, 5850–5858.

Tsai, J.Y., Chou, C.J., Chen, C.F., Chiou, W.F., 2003. Antioxidant activity of piperlactam S: prevents copper-induced LDL peroxidation and ameliorates free radical-mediated oxidative stress of endothelial cells. Planta Med. 69, 3–8.

Vaishnav, D., Jambal, P., Reusch, J.E.B., Pugazhenthi, S., 2003. SP600125, an inhibitor of c-jun N-terminal kinase, activates CREB by a p38 MAPK-mediated pathway. Biochem. Biophys. Res. Commun. 307, 855–860.

Ward, P.A., Riedmann, N.C., Guo, R.F., Lang, M.H., Sarma, J.V., Zetoune, F.S., 2003. Anti-complement strategies in experimental sepsis. Scand. J. Infect. Dis. 35, 601–603.

Wenzel-Seifert, K., Seifert, R., 2001. Chemoattractant receptor–G-protein coupling. In: Ley, K. (Ed.), Physiology of Inflammation. Oxford Univ. Press, Oxford, pp. 146–188.

Whiteman, E.L., Cho, H., Birnbaum, M.J., 2002. Role of Akt/protein kinase B in metabolism. Trends Endocrinol. Metab. 13, 444–451.

Widmann, C., Gibson, S., Japre, M.B., Johnson, G.L., 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. 79, 143–180.