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Berberine Inhibits HEp-2 Cell Invasion Induced by *Chlamydophila pneumoniae* Infection

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This study investigated the inhibitory effects of berberine on *Chlamydophila* (*Chlamydia*) *pneumoniae* infection-induced HEp-2 cell invasion and explored the possible mechanisms involved in this process. *C. pneumoniae* infection resulted in a significant increase in HEp-2 cell invasion when compared with the control cells (P<0.01) in a Matrigel invasion assay. This enhanced cell invasion was strongly suppressed by berberine (50 μM) (P<0.01). In a cell adhesion assay, the infection-induced HEp-2 cell adhesion to Matrigel was also significantly inhibited by berberine (P<0.01). *C. pneumoniae* infection was found to promote HEp-2 cell migration remarkably (P<0.01), which was markedly suppressed by berberine (P<0.01) in the cell migration assays. There were no statistically significant differences in the expression of matrix metalloproteinase-1 (MMP-1) and MMP-9 in the infected cells and berberine did not change the expression of MMP-1 and MMP-9. These data suggest that berberine inhibits *C. pneumoniae* infection-induced HEp-2 cell invasion through suppressing HEp-2 cell adhesion and migration, but not through changing the expression of MMP-1 and MMP-9.

***Keywords*:** berberine, *Chlamydia pneumoniae*, cell invasion, cell adhesion, cell migration

Chlamydophila (*Chlamydia*) *pneumoniae*, a respiratory pathogen (Grayston *et al*., 1986), has been recognized as a cause of respiratory tract infections and implicated as a potential risk factor rather than an innocent bystander in atherosclerosis (Belland *et al*., 2004; Kern *et al*., 2009). *C. pneumoniae* gains access to the vasculature during local inflammation of the lower respiratory tract, when the organism is disseminated around the body in blood mononuclear cells (Yang *et al*., 1995). Once at the vessel wall, *C. pneumoniae* can be released from the monocytes and subsequently infects the cells relevant to atherosclerosis, such as endothelial cells and vascular smooth muscle cells (Deniset and Pierce, 2010). Some adhesion molecules were found to be upregulated in *C. pneumoniae*-infected endothelial cells, and then enhanced the adhesion of monocytes/neutrophils to endothelial cells (Kaukoranta-Tolvanen *et al*., 1996; Takaoka *et al*., 2008). *C. pneumoniae*-infected endothelial cells can stimulate transendothelial migration of neutrophils and monocytes (Molestina *et al*., 1999; Uriarte *et al*., 2004). Moreover, *C. pneumoniae* may directly trigger the activation of matrix metalloproteinases (MMP)-7 and MMP-9 in monocytes (Schmidt *et al*., 2006). Further, Schmidt *et al*. (2006) also found that *C. pneumoniae* can induce monocytic migration by activating MMP. These studies are considered "pro-atherogenic", but in fact they are immunologic. They show that the natural regulation of adherence, migration and extracellular matrix degradation can be used by *C. pneumoniae*. Cell invasion may work in the same way. Thus, we wonder whether *C. pneumoniae* infection affects HEp-2 cell invasion.

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Berberine, a type of isoquinoline alkaloid isolated from *Rhizoma coptidis*, has long been known for its anti-microbial activity (Schiller, 1995) and anti-inflammation properties (Kuo *et al*., 2004), and been used to treat various infectious disorders in traditional Chinese medicine. Recent studies have indicated that berberine possesses anti-invasion effects. Berberine has been shown to suppress migration and invasion of human SCC-4 tongue squamous cancer cells (Ho *et al*., 2009). It is, however, still unclear whether berberine directly inhibits *C. pneumoniae* infection-induced invasion of HEp-2 cells, and what mechanisms are involved in these processes.

In the present study, we therefore attempted to study the roles of *C. pneumoniae* infection in the invasion of HEp-2 cells infected with *C. pneumoniae*, and then investigate the effects of berberine on *C. pneumoniae* infection-induced HEp-2 cell invasion, and to explore the possible mechanisms of the inhibitory effects of berberine on HEp-2 cell invasion induced by *C. pneumoniae* infection.

Materials and Methods

Reagents and materials

C. pneumoniae AR-39 strain (ATCC 53592) and HEp-2 cells (ATCC CCL-23) were purchased from American Type Culture Collection (USA). Dulbecco's modified Eagle's medium (DMEM) and calf serum were from HyClone company (USA). L-Glutamine, vancomycin, and gentamicin were obtained from Amresco company (USA). Cycloheximide, acridine orange (AO) solution, berberine, dimethyl sulfoxide (DMSO), Giemsa stain, hydroxyurea and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were all purchased from Sigma-Aldrich company (USA). The bacterial DNA kit was from Omega Bio-Tek Inc. (USA). Matrigel was obtained from BD Biosciences

(USA). The Transwell system was from Corning company (USA). ELISA kits were obtained from R&D Systems (USA). All other chemicals were of analytical grade.

Cell culture

HEp-2 cells were maintained in DMEM with 10% calf serum, 2 mM L-glutamine and 10 μg/ml vancomycin/gentamicin at 37°C in a humidified incubator supplemented with 5% CO_2.

***C. pneumoniae* culture and infection of HEp-2 cells**

90% confluent HEp-2 cells in 24-well plates were inoculated with *C. pneumoniae* at a multiplicity of infection (MOI) of 1, centrifuged at 1,700×g at 25°C for 50 min and grown for 2 h in serum-free DMEM without 10 μg/ml vancomycin/gentamicin. Then, the culture medium was replaced with medium containing 2% calf serum, 2 mM L-glutamine, 10 μg/ml vancomycin/gentamicin and 2 μg/ml cycloheximide for *C. pneumoniae* growth. After 72 h, cells were harvested and disrupted with glass beads for 5 min and centrifuged at 2,000×g at 4°C for 20 min. The supernatant containing *C. pneumoniae* was used for experiments.

PCR analysis

The DNA was extracted from the cells inoculated with *C. pneumoniae* at an MOI of 1 for 72 h according to the manufacturer's directions. The primers (from the 5′ to 3′ end) were as follows: HL-1 (GTTGTT CATGAAGGCCTACT) and HR-1 (TGCATAACCTACGGTGTGTT). PCR was performed as described earlier (Fukano, 2004).

AO staining

AO staining was performed as previously described (Pettengill *et al*., 2009). Briefly, HEp-2 cells infected with *C. pneumoniae* at an MOI of 1 for 72 h were fixed with anhydrous ethanol for 10 min and washed twice with phosphate buffered saline (PBS). Subsequently, cells were stained with 1 μg/ml of AO solution for 3 min. The cells were differentiated for 5 min with 0.1 M of anhydrous calcium chloride solution. Then, 0.067 M of potassium dihydrogen phosphate was added to the plates, which were then photographed using a fluorescence microscope.

Transmission electron microscopy (TEM)

HEp-2 cells were infected with *C. pneumoniae* for 72 h at an MOI of 1. Samples were prepared for TEM by standard procedures (Kutlin *et al*., 2001) and were examined with a JEM 1010 electron microscope (JOEL).

Cytotoxicity assay

The cytotoxic effects of berberine were determined by the MTT method (Zhang *et al*., 2007). Briefly, cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to grow to approximately 90% confluence. Then, the cells were incubated with berberine at various concentrations (0, 25, 50, 100, 150, 200, and 250 μM) for 24 h. Thereafter, 10 μl MTT (5 mg/ml) was added to each well and incubated at 37°C for 4 h. Subsequently, the cell supernatants were discarded, and MTT crystals were dissolved with DMSO. The amount of formazan produced was detected by the absorbance at 490 nm. The cytotoxic effects of berberine on cells were assessed as percent cell viability compared with the control cells, which were arbitrarily assigned 100% viability.

Matrigel invasion assay

The Matrigel invasion assay was conducted in 24-well plates by applying the Transwell device, which allows cells to migrate through an 8-μm pore size polycarbonate membrane. After the Transwell filters had been additionally coated on the upper side with Matrigel (1:3 dilution in serum-free DMEM), 600 μl of DMEM containing 50% calf serum was added to the lower chambers. HEp-2 cells were treated with berberine (50 μM) for 24 h and then infected with *C. pneumoniae* at an MOI of 1 for 24 h. Subsequently, 5×10^4 cells in 100 μl of DMEM supplemented with 10% calf serum were added to the upper chambers. After 37 h-incubation at 37°C, the non-migrated cells on the upper side of the membrane were removed by cotton swabs. Then, the migrated cells on the lower side of the membrane were fixed with anhydrous ethanol and stained with Giemsa stain. The cells attached to the lower side of the membrane were counted in nine random fields at 200× magnification.

Cell adhesion assay

HEp-2 cells incubated with berberine (50 μM) for 24 h were infected with *C. pneumoniae* at an MOI of 1. At 24 h postinfection, cells were seeded to 96-well plates precoated with Matrigel (1:3 dilution in serum-free DMEM) and allowed to attach for 2 h at 37°C. Cell adhesion was determined by the MTT method (Zhang *et al*., 2007). The cell adhesion ratio was calculated using the following formula: Cell adhesion ratio = $Absorbance_{490}$ of treated cells / $Absorbance_{490}$ of control cells

Two-dimensional cell migration assay

A two-dimensional cell migration assay was performed by a wound-healing model. HEp-2 cells were treated with 1 mM of hydroxyurea for 12 h to prevent cell proliferation (Sarkar *et al*., 1996). Then, berberine (50 μM) was added to the cells for 12 h. Subsequently, cells inoculated with *C. pneumoniae* at an MOI of 1 for 2 h were wounded by a sterile pipette tip. The time lapse images in a 24 h period were captured by a microscope at 40× magnification. Cell migration was assessed by calculating the average cell migration velocity according to the following formula:

The average cell migration velocity = migration distance (μm) / migration time (h)

Three-dimensional cell migration assay

A three-dimensional cell migration assay was carried out as described above for the Matrigel invasion assay, without Matrigel coating on the filter.

Enzyme-linked immunosorbent assay (ELISA)

To determine the expression levels of MMP-1 and MMP-9, HEp-2 cells pretreated with berberine (50 μM) for 24 h were infected with *C. pneumoniae* at an MOI of 1 for 24 h. Then, the culture supernatants were collected and assayed for MMP-1 and MMP-9 by ELISA according to the manufacturer's directions.

Statistical analysis

All experiments were repeated in triplicate, and all data were presented as Means±Standard Error of the Mean (SEM). The difference of means between multiple groups was assessed by analysis of variance (ANOVA). Statistical analysis was performed using the Student's *t* test. The P values less than 0.05 were considered statistically significant.

Results

Successful infection of HEp-2 cells with *C. pneumoniae*

HEp-2 cells infected with *C. pneumoniae* at an MOI of 1 were swollen and partially desquamated, compared with the polygonal shaped normal cells. More oval-shaped smooth vacuoles, termed inclusions, were found in the cytoplasm of HEp-2 cells at 72 h postinfection (Figs. 1A and B). PCR was then performed to identify the *C. pneumoniae* in HEp-2 cells. A 437-bp *C. pneumoniae*-specific DNA fragment was successfully amplified in the infected cells, whereas no fragment was seen in the control cells (Fig. 1C). Subsequently, AO staining was carried out to further observe the characteristics of *C. pneumoniae* inclusions in HEp-2 cells. AO is a nucleic acid selective metachromatic stain which interacts with DNA and RNA by intercalation or electrostatic attraction respectively. DNA intercalated AO fluoresces green; RNA electrostatically bound AO fluoresces orange. The green grape-like inclusions

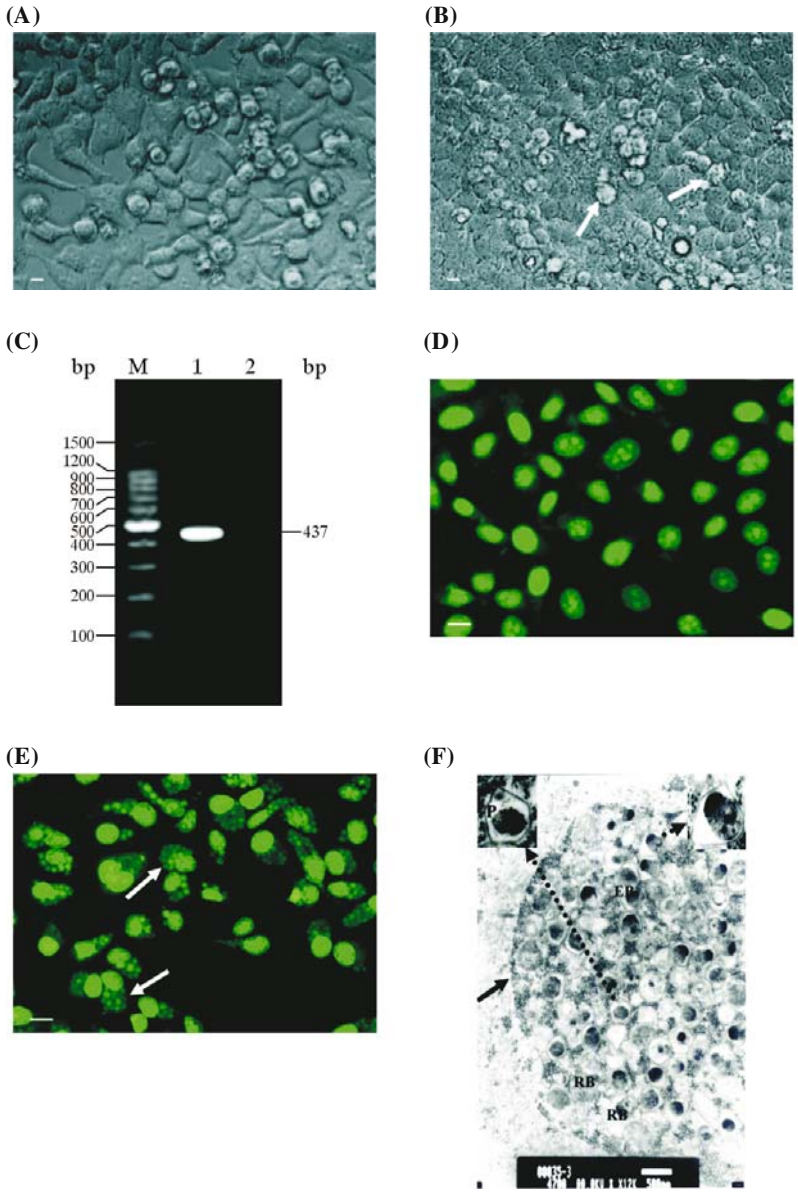

Fig. 1. Successful infection of HEp-2 cells with *C. pneumoniae*. HEp-2 cells were infected with *C. pneumoniae* at a multiplicity of infection (MOI) of 1 for 72 h. (A) Normal HEp-2 cells (200×). (B) *C. pneumoniae*-infected HEp-2 cells (200×). (C) Amplification of *C. pneumoniae* DNA by PCR and agarose gel electrophoresis analysis. Total DNA was extracted from the HEp-2 cells. Samples were amplified by using the parameters described in 'Materials and Methods'. PCR products are as follows: M, molecular size standards. Lanes: 1, *C. pneumoniae*-infected HEp-2 cells; 2, Normal HEp-2 cells. (D) Normal HEp-2 cells stained with acridine orange (AO) solution (400×). (E) *C. pneumoniae*-infected HEp-2 cells stained with AO solution (400×). (F) Ultrastructural characteristics of *C. pneumoniae* inclusions in HEp-2 cells. *C. pneumoniae*-infected cells were processed for transmission electron microscopy. The white arrows indicate *C. pneumoniae* inclusions. The black arrow indicates the *C. pneumoniae* inclusion membrane. EB, elementary body. RB, reticulate body. Scale bar=10 μm.

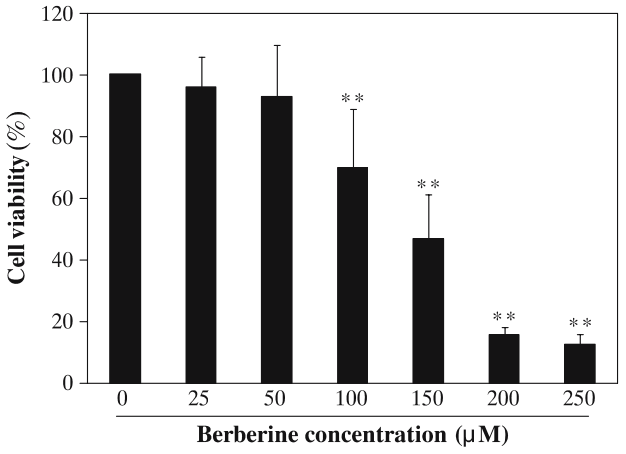

Fig. 2. Cytotoxic effects of berberine on HEp-2 cells. The cytotoxicity of berberine was evaluated using the MTT method. 1×10^4 cells were cultured for 24 h in 96-well plates and then incubated with various concentrations of berberine as indicated. Cell viability values (%) are expressed as Means±SEM. ** P<0.01 vs control.

can be observed in *C. pneumoniae*-infected cells at 72 h after infection (Figs. 1D and E). Ultrastructural characteristics of *C. pneumoniae* inclusions in HEp-2 cells were subsequently examined by TEM. At 72 h postinfection, *C. pneumoniae* inclusions with an increasing percentage of elementary bodies (EBs) were observed in the infected cells. The typical EBs with a large periplasmic space surrounded by a relatively flexible and pleomorphic outer membrane were pear-shaped and electron opaque (Fig. 1F). Reticulate bodies (RBs) surrounded by the membrane were round and electron lucent, as is also shown in the Fig. 1F.

Cytotoxic effects of berberine on HEp-2 cells

In order to determine the cytotoxic effects of berberine on HEp-2 cells, the viability of cells treated with berberine at various concentrations for 24 h was assessed by the MTT method. Treatment with berberine at concentrations of less than 50 μM did not elicit marked cytotoxic effects on HEp-2 cells (Fig. 2). However, at concentrations higher than 50 μM, berberine resulted in a significant reduction in cell viability (Fig. 2). Therefore, berberine was applied at a concentration of 50 μM in all experiments.

Berberine inhibits *C. pneumoniae* infection-induced HEp-2 cell invasion through suppressing HEp-2 cell adhesion and migration, but not through changing the expression of MMP-1 and MMP-9

In the Matrigel invasion assay, HEp-2 cells infected with *C. pneumoniae* at an MOI of 1 exhibited a significant increase in their invasive capacity in comparison with the control cells (32.80±0.75 vs 23.40±2.33, P<0.01, Fig. 3). But, when HEp-2 cells were pretreated with berberine, cell invasion was remarkably inhibited (9.20±0.75 vs 23.40±2.33, P<0.01, Fig. 3). Moreover, *C. pneumoniae* infection did not induce the increase in the invasion of HEp-2 cells pretreated with berberine for 24 h as compared with the *C. pneumoniae*-infected cells (32.80±0.75 vs 13.00±2.53, P<0.01, Fig. 3).

To further explore the possible mechanism of the inhibitory effect of berberine on *C. pneumoniae* infection-induced HEp-2

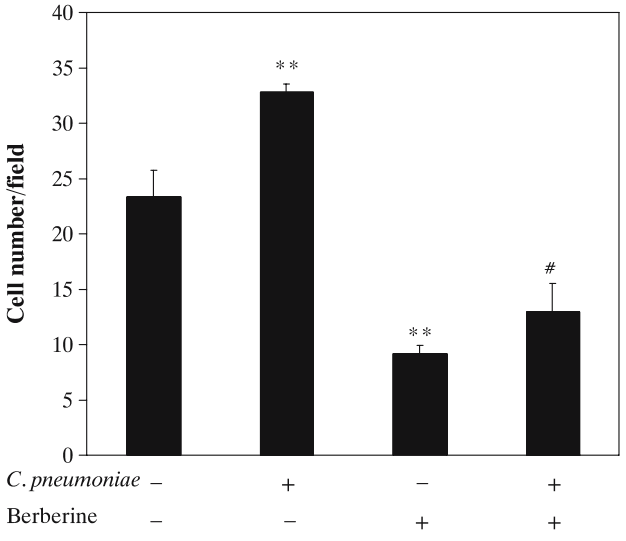

Fig. 3. Berberine inhibits *C. pneumoniae* infection-induced HEp-2 cell invasion. Cells were incubated with berberine (50 μM) for 24 h and then infected with *C. pneumoniae* at an MOI of 1 for 24 h. Cell invasion through a layer of Matrigel was determined by the Transwell device as described in 'Materials and Methods'. The invasiveness was quantified and is presented in the graph. ** P<0.01 vs control; # P<0.01 vs *C. pneumoniae* infection group.

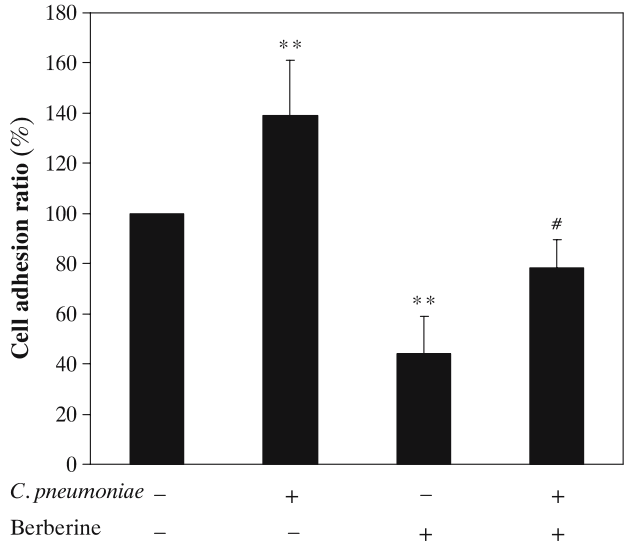

Fig. 4. Berberine suppresses *C. pneumoniae* infection-induced HEp-2 cell adhesion to Matrigel. HEp-2 cells incubated with berberine (50 μM) for 24 h were infected with *C. pneumoniae* at an MOI of 1. At 24 h postinfection, cells were seeded to 96-well plates precoated with Matrigel and allowed to attach for 2 h at 37°C. Cell adhesion was determined by the MTT method. The cell adhesion ratio (% of control cells) is expressed as Means±SEM. ** P<0.01 vs control; # P<0.01 vs *C. pneumoniae* infection group.

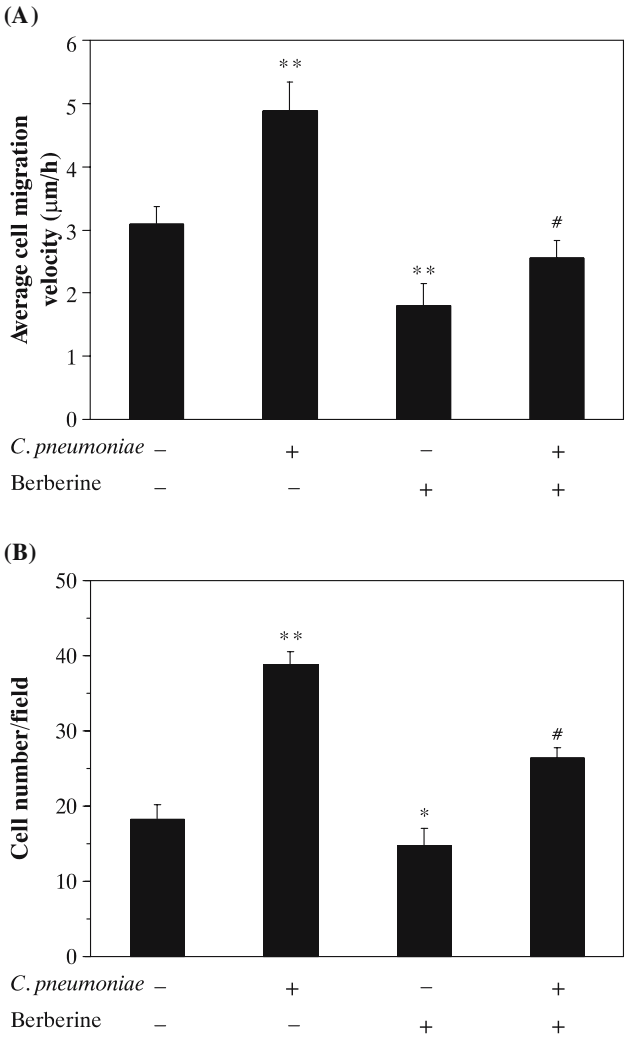

Fig. 5. Berberine decreases *C. pneumoniae* infection-induced HEp-2 cell migration. (A) Berberine inhibits *C. pneumoniae* infection-induced HEp-2 cell migration using the two-dimensional cell migration assay. HEp-2 cells grown to 80% confluency in 12-well plates were pretreated with hydroxyurea (1 mmol/L) for 12 h. Then, cells treated with berberine (50 μM) for 12 h were infected with *C. pneumoniae* at an MOI of 1 for 24 h. A linear injury line was created at the center of the cultured monolayer by scraping with a sterile pipette tip. The average cell migration velocity was calculated as described in 'Materials and Methods'. ** $P<0.01$ vs control; # $P<0.01$ vs *C. pneumoniae* infection group. (B) Effect of berberine on *C. pneumoniae* infection-induced HEp-2 cell migration with the Transwell system. HEp-2 cells treated with berberine (50 μM) for 24 h and then inoculated with *C. pneumoniae* at an MOI of 1 and incubated for 24 h. The cell migration was quantified by counting the number of cells migrated per field of view. * $P<0.05$ vs control; ** $P<0.01$ vs control; # $P<0.01$ vs *C. pneumoniae* infection group.

cell invasion, we investigated whether berberine can suppress the infection-induced HEp-2 cell adhesion and migration, and decrease the expression of MMP-1 and MMP-9 in the infected HEp-2 cells. In the cell adhesion assay, the adhesion ratio of infected HEp-2 cells was 139.00±22.20% compared to the control group ($P<0.01$, Fig. 4). The ratio was reduced to 44.10±14.65%, when preincubated with berberine for 24 h ($P<0.01$, Fig. 4). Furthermore, a *C. pneumoniae* infection-induced increase in the adherence of HEp-2 cells to Matrigel was remarkably inhibited by berberine as well ($P<0.01$, Fig. 4).

Table 1. Effects of berberine on the expression of MMP-1 and MMP-9 during HEp-2 cell invasion induced by *C. pneumoniae* infection

Group	Absorbance values	
	MMP-1	MMP-9
Control	0.661±0.010	0.647±0.076
C. pneumoniae	0.637±0.024	0.581±0.067
Berberine	0.643±0.020	0.540±0.035
Berberine+*C. pneumoniae*	0.623±0.015	0.541±0.043

HEp-2 cells pretreated with berberine (50 μM) for 24 h were infected with *C. pneumoniae* at an MOI of 1 for 24 h. The culture supernatants were collected and assayed for MMP-1 and MMP-9 by ELISA. Each value represents the Mean±SEM (n=3). No significance was observed by the Student's *t* test at $P<0.05$.

In the two-dimensional cell migration assay, *C. pneumoniae* infection accelerated the wound healing of HEp-2 cells compared with the control group at 24 h after the cell monolayer was scratched with a pipette tip. The average cell migration velocity was 4.898±0.443 μm/h, which was much faster than the migration velocity of control cells (3.095±0.271 μm/h) ($P<0.01$, Fig. 5A). Berberine can significantly decrease the cell migration velocity, when compared to control cells (1.803±0.351 μm/h vs 3.095±0.271 μm/h, $P<0.01$, Fig. 5A). The average migration velocity of *C. pneumoniae* infected-HEp-2 cells pretreated with berberine was much slower than that of the infected-cells without berberine pretreatment (2.553±0.284 μm/h vs 4.898±0.443 μm/h, $P<0.01$, Fig. 5A). The three-dimensional cell migration assay showed that HEp-2 cells infected with *C. pneumoniae* at an MOI of 1 migrated more than the control cells (38.80±1.72 vs 18.40±1.85, $P<0.01$, Fig. 5B). The number of HEp-2 cells preincubated with berberine that migrated through the membranes was 14.80±2.32, which was less than that of the control cells (18.40±1.85) ($P<0.05$, Fig. 5B). We also found that berberine can markedly suppress *C. pneumoniae* infection-induced HEp-2 cell migration (26.40±1.36 vs 38.80±1.72, $P<0.01$, Fig. 5B).

To determine the roles of MMPs in the inhibitory effect of berberine on the infection-induced HEp-2 cell invasion, we observed the expression of MMP-1 and MMP-9 by ELISA in the infected HEp-2 cells with or without berberine pretreatment. The absorbance values of MMP-1 and MMP-9 in the infected HEp-2 cells were 0.637±0.024 and 0.581±0.067 respectively, but there were no statistically significant differences compared to control cells ($P>0.05$, Table 1). In the presence of berberine, the absorbance values of MMP-1 and MMP-9 were 0.643±0.020 and 0.540±0.035, which were also not statistically significant ($P>0.05$, Table 1). Moreover, in the infected HEp-2 cells pretreated with berberine, the expressions of MMP-1and MMP-9 were also not changed ($P>0.05$, Table 1).

Discussion

Exposure to *C. pneumoniae* is extremely common and respiratory infections occur repeatedly among many people. However,

C. pneumoniae has always been considered a fastidious organism and difficult to culture and propagate by passage *in vitro*. Improved methods for culture and propagation of *C. pneumoniae* would be valuable in both clinical and research settings. In our study, PCR amplification of a 437-bp *C. pneumoniae*-specific DNA fragment, the green grape-like inclusions by AO staining and the typical pear-shaped EBs under TEM all revealed the successful infection of HEp-2 cells with *C. pneumoniae* strain AR-39.

HEp-2 cells are one of the most sensitive cell lines tested so far for the culture and propagation of *C. pneumoniae* (Roblin *et al*., 1992). However, little is known about the effects of *C. pneumoniae* infection on HEp-2 cell invasion. Our present study showed that *C. pneumoniae* infection can enhance the invasive ability of HEp-2 cells and berberine significantly suppresses *C. pneumoniae* infection-induced HEp-2 cell invasion. Cell-matrix adhesion, cell migration and MMPs-induced extracellular matrix degradation are known to be involved in cell invasion. Therefore, to further elucidate the probable mechanism of the inhibitory effect of berberine on HEp-2 cell invasion induced by *C. pneumoniae* infection, we investigated the roles of berberine in the infection-induced HEp-2 cell adhesion and migration, and whether berberine can decrease the expression of MMP-1 and MMP-9 in the infected HEp-2 cells.

Our data demonstrated that *C. pneumoniae* infection can promote HEp-2 cell adhesion to Matrigel. We also found that berberine can suppress HEp-2 cell adhesion to Matrigel induced by *C. pneumoniae* infection. Liu *et al*. (2008) reported that berberine can significantly decrease the level of intracellular adhesion molecule 1 (ICAM-1) in non-small cell lung cancer patients treated with radiotherapy. Subsequently, Wang *et al*. (2009) also found that berberine can attenuate high glucose-induced expression of ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1), thus suppressing monocyte attachment to endothelial cells. Interestingly, previous reports have shown that *C. pneumoniae* can increase the expression of some adhesion molecules, such as E-selectin, ICAM-1, and VCAM-1 (Kaukoranta-Tolvanen *et al*., 1996; Takaoka *et al*., 2008). Therefore, berberine may inhibit *C. pneumoniae* infection-induced HEp-2 cell adhesion to Matrigel by downregulating the expression of some adhesion molecules.

It has been indicated that *C. pneumoniae* infection can stimulate the transendothelial migration of neutrophils and monocytes (Molestina *et al*., 1999; Uriarte *et al*., 2004; Schmidt *et al*., 2006). These studies demonstrated a pivotal role of *C. pneumoniae* infection in the initiation and progression of atherosclerosis. Unfortunately, these studies were limited to investigating the roles of inflammatory mediators induced by *C. pneumoniae* infection in cell migration, and few studies have focused on the direct effects of *C. pneumoniae* infection on cell migration required for cell invasion. In this study, we first investigated the effect of *C. pneumoniae* infection on the migration of HEp-2 cells, and found that *C. pneumoniae* infection could promote HEp-2 cell migration. Hirono *et al*. (2003) reported that *C. pneumoniae* can induce the production of endogenous HSP 60, and then promotes epithelial cell migration (Zhang *et al*., 2004), suggesting that *C. pneumoniae* can stimulate HEp-2 cell migration possibly by increasing the expression of endogenous HSP 60. Our data also indicated that berberine significantly suppresses the migration of HEp-2 cells induced by *C. pneumoniae* infection. Whether berberine inhibits *C. pneumoniae* infection-induced HEp-2 cell migration by decreasing the expression of endogenous HSP 60 needs further study.

MMPs are known to make an essential contribution to cell invasion. Previous studies have indicated that *C. pneumoniae* can actively contribute to inflammatory, atherogenic processes within the atherosclerotic plaque and, thereby, may promote plaque instability and rupture by increasing the production of MMPs (Rodel *et al*., 2003; Arno *et al*., 2005; Kim *et al*., 2005; Schmidt *et al*., 2006). Recently, selective gene-silencing of either MMP-2 or MMP-9 by recombinant lentivirus mediated RNA interference was found to be able to inhibit HEp-2 cell invasion (Sun *et al*., 2008a, 2008b). However, our data indicated that *C. pneumoniae* infection cannot change the expression of MMP-1 and MMP-9 in HEp-2 cells. Also, we didn't find that the expressions of MMP-1 and MMP-9 were changed in the infected HEp-2 cells pretreated with berberine, although it has been shown that berberine can downregulate the expression of MMPs (Kuo *et al*., 2004). Similarly, Tan *et al*. (2008) demonstrated that chemokine stromal cell-derived factor-1 seems to enhance HEp-2 cell invasion through the upregulation of MMP-13, but not MMP-2 or MMP-9, suggesting that berberine inhibits *C. pneumoniae* infection-induced HEp-2 cell invasion possibly by downregulating other MMPs but not MMP-1 or MMP-9. Therefore, the inhibitory effect of berberine on *C. pneumoniae* infection-induced HEp-2 cell invasion may be closely associated with the suppression of HEp-2 cell adhesion and migration induced by *C. pneumoniae* infection, and involve MMPs other than MMP-1 or MMP-9.

In conclusion, our present investigation has demonstrated that *C. pneumoniae* infection can promote HEp-2 cell invasion and berberine can significantly inhibit *C. pneumoniae* infection-induced HEp-2 cell invasion through suppressing HEp-2 cell adhesion and migration, but not through changing the expression of MMP-1 and MMP-9.

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