ELSEVIER

International Immunopharmacology 5 (2005) 1029–1039

International Immunopharmacology

www.elsevier.com/locate/intimp

Effects of mycophenolic acid on endothelial cells

Yanfei Huang, Zhihong Liu*, Haidong Huang, Hao Liu, Leishi Li

Research Institute of Nephrology, Jingling Hospital, Nanjing University School of Medicine, Nanjing, 210002, China

Received 18 July 2004; received in revised form 7 September 2004; accepted 28 January 2005

Abstract

Mycophenolate mofetil (MMF) is a potent immunosuppressant that inhibits the activity of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in de novo synthesis of guanosine nucleotides. MMF has been used widely in solid-organ transplantation. Increased evidence indicated that MMF exhibited beneficial effects on various types of vasculitis, for reasons that were not fully understood. Endothelial cells play a pivotal role in the pathogenesis of vasculitis. Endothelium may not only be the main target for injury, but also be able to amplify the inflammatory response by adhesion molecule expression, leukocyte adhesion, cytokine production and angiogenesis. In the present study, the effect of mycophenolic acid (MPA), the active metabolite of MMF, on human umbilical vein endothelial cells (HUVECs) was investigated. MPA markedly inhibited tumor necrosis factor-α (TNFα)-induced intercellular adhesion molecule-1 (ICAM-1) mRNA and surface expression, suppressed TNFα-induced neutrophils adhesion to endothelial cells, and reduced TNFα-induced interleukin-6 (IL-6) secretion. The inhibitory effects of MPA on ICAM-1 surface expression and IL-6 secretion were not attenuated by addition of guanosine, implying that inhibition of these processes were not due to intracellular guanosine nucleotides depletion. MPA also decreased angiogenesis of endothelial cells in three-dimensional collagen gel culture system, reduced the migration in a wounded monolayer of endothelial cells, and inhibited the proliferation of endothelial cells. In conclusion, MPA exhibited multifarious effects on endothelial cells including inhibition of ICAM-1 expression, neutrophil attachment, IL-6 secretion, and the process of angiogenesis, which might contribute to the efficacy of MMF in the treatment of vasculitis.

Keywords: Mycophenolic acid; Endothelial cell; Intercellular adhesion molecule-1; Neutrophil; Interleukin-6; Angiogenesis

1. Introduction

Mycophenolate mofetil (MMF), the morpholinoethyl ester of mycophenolic acid (MPA), is an immunosuppressive agent used for transplant recipients [1]; however, recently it has been tested as a treatment for several types of vasculitis including Takayasu's arteritis, polyarteritis nodosa, ANCA-associated vasculitis, Wegener's granulomatosis, lupus nephritis and skin vasculitis [2–8]. In vivo MMF deesterifies to MPA, which is a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in de novo synthesis of

* Corresponding author. Tel.: +86 25 80860218; fax: +86 25 84801992.

E-mail address: Zhihong@21cn.net (Z. Liu).

1567-5769/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.intimp.2005.01.015

guanosine nucleotides [9]. Inhibition of IMPDH leads to the depletion of intracellular guanosine nucleotides, thereafter suppresses the proliferation of both T and B lymphocytes. In addition, it has been reported [10,11] that MPA down-regulated adhesion molecules expression and reduced lymphocytes and monocytes adhesion, which may also contribute to the efficacy of MMF in the treatment of autoimmune diseases including systemic vasculitis. However, the mechanisms under the effect of MMF on vasculitis are still not fully understood.

Vasculitis is a disease process characterized by the presence of blood vessel inflammation. Endothelial cells are among the most dynamic and biologically active cellular components of blood vessels and thus play a pivotal role in the pathogenesis of vasculitis. The participation of endothelium in the pathogenesis of vascular inflammation is complex. It serves not only as a main target for injury, but also as an active mediator in the process of inflammation. Endothelial cells are able to amplify the inflammatory response by adhesion molecule expression, leukocyte recruitment, cytokine production, and angiogenesis—a process termed "activation". Specific interference in these processes might yield therapeutic benefit in the treatment of vasculitis [12–15].

The aim of our study was to investigate the effect of MPA on the process of endothelial cells activation. Therefore, the effects of MPA on intercellular adhesion molecule-1 (ICAM-1) expression, neutrophils attachment to endothelial cells, interleukin-6 (IL-6) secretion, cell proliferation and migration, and angiogenesis of endothelial cells were assessed in the present study.

2. Materials and methods

2.1. Reagents

Mycophenolic acid (MPA, 27H41105), recombinant basic fibroblast growth factor (bFGF), recombinant vascular endothelial growth factor (VEGF), fibronectin, type I collagen, guanosine, were purchased from Sigma (USA). Recombinant human tumor necrosis factor-α (TNFα) was from R and D Systems (USA). The enzyme-linked immunosorbent assay (ELISA) kit used for the quantitative measurement of human IL-6 was purchased from Jingmei Biotech (China).

2.2. Endothelial cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from American Type Culture Collection (ATCC, No CRL-1998). Cells were incubated at 37 °C under 5% CO_2 in medium F12 (Gibco, USA) containing 10% heat-inactivated fetal bovine serum (FBS) (Sijiqing, China), supplemented with 2 mM glutamine, 1.5 g/l sodium carbonate, 100 kU/l penicillin, and 100 mg/l streptomycin. All experiments were performed on cells between passages 5 and 15.

2.3. Assessment of ICAM-1 mRNA expression

HUVECs cultured in 6-well plates were treated with 10 μg/l TNFα and 10 or 50 μM MPA for 24 h. One microgram of total RNA extracted from the cells was subjected to reverse transcription. Subsequent amplification of the partial cDNA encoding ICAM-1 and β-actin (as a control) was performed with specific oligonucleotide primers as follows: ICAM-1 sense 5′-TATGGCAACGACTCCTTCT-3′antisense 5′-CATTCAGCGTCACCTTGG-3′β-actin sense 5′-CTACAATGAGCTGCGTGTGG-3′antisense 5′-TAGCTCTTCTCCAGGGAGGA-3′. The exact size of polymerase chain reaction (PCR) products for ICAM-1 and β-actin were 220 and 450 base pairs, respectively. The PCR mixtures were subjected to 35 cycles of amplification by denaturation (1 min at 94 °C), renaturation (1 min at 55 °C), and elongation (1 min at 72 °C). The PCR products were electrophoresed by 2% agarose gel with ethidium bromide, and the results were analyzed by using a BF-300 image system (China).

2.4. Assessment of ICAM-1 surface expression

HUVECs were incubated with (1) 10 μg/l TNFα (2) 10 μg/l TNFα + 10 μM MPA (3) 10 μg/l TNFα + 50 μM MPA (4) 10 μg/l TNFα + 50 μM MPA + 100 μM guanosine for 24 h. Cells were harvested with 0.25% trypsin/0.02% ethylenediaminetetraacetic acid (EDTA), washed twice with phosphate buffered saline (PBS), fixed in 1% paraformaldehyde for 20 min, and

then washed and incubated in PBS/1% bovine serum albumin (BSA) for 1 h on ice with mouse anti-human ICAM-1 antibody (1:50, DAKO). The cells were subsequently washed and incubated with fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin G (IgG) (1:40, DAKO) in the dark at 4 °C for 30 min. Background fluorescence was adjusted with cells labelled with mouse control antibodies as the first antibody. Analyses were performed on a coulter flow cytometry (Beckman, USA).

2.5. Adhesion of neutrophils to endothelial cells [16]

Neutrophils were isolated from peripheral blood of healthy human donors. Heparinized blood was diluted 1:1 with PBS and centrifuged in histopaque (Sigma) at 400 g for 15 min. The isolated neutrophils were resuspended in 0.83% ammonium chloride to lyse contaminating erythrocytes. The viability of neutrophils isolated by this method was 95%, as determined by the trypan blue dye exclusion. Monolayers of subconfluent HUVECs were incubated in F12/10% FBS containing 10 μg/l TNFα and 10 or 50 μM MPA for 24 h. HUVECs were washed twice and neutrophils were added to the wells at a ratio of 5:1 (neutrophil/HUVEC) and incubated for 30 min at 37 °C. The cell layers were carefully washed to remove the unadherent cells. The adherent cells were lysed with 0.2 M sodium hydroxide (NaOH). The cellular protein content was determined by Bradford method after centrifugation. The ratio of adhesion was calculated as follows: Adhesion rate = (protein $_{\text{HUVECs and adherent neutrophils}}$ − protein $_{\text{HUVECs}}$)/protein $_{\text{added neutrophils}}$.

2.6. Assessment of IL-6 secretion

HUVECs were incubated in F12/10% FBS containing 10 μg/l TNFα and 10 or 50 μM MPA with or without 100 μM guanosine for 24 h. Supernatant samples were taken and stored at −20 °C until analysis for IL-6 secretion by ELISA. In short, appropriate standard diluents or samples were added to wells coated with anti-human IL-6 monoclonal antibody and incubated at the room temperature for 120 min. The plates were washed and incubated with biotinylated anti-IL-6, followed by streptavidin–horseradish peroxidase (HRP) for 60 min at the room temperature. Substrate solutions were then added and incubated in the dark for 10 min. The reaction was stopped with stop reagent and the optical density (OD) was read at 450 nm on a spectrophotometer (EX800, Beckman, USA).

2.7. In vitro angiogenesis model

A model of in vitro angiogenesis was used in this study as previously described [17,18]. In this model, endothelial cells suspended in three-dimensional collagen culture (3-D culture) in the presence of angiogenic factors (VEGF, bFGF) underwent rapid morphogenesis. Briefly, type I collagen solution (1.5 μg/ml) in 0.1 M acetic acid were stored at 4 °C. Gel solution (pH 7.5) were formed by mixing together ice-cold collagen solution and 10 × F12 medium, and spread on 6-well plates (800 μl/well) followed by incubating at 37 °C for 1 h to gelation. HUVECs were then seeded into the wells and cultured with F12/10% FBS. At confluence, the supernatants were removed and the cells were overlaid with gel solution at a volume of 200 μl/well. Immediately after gelation the gels were covered with F12/1% FBS supplemented with bFGF (10 μg/l) and VEGF (15 μg/l) and incubated at 37 °C for indicated periods of time. Ten or 50 μM MPA was added, respectively in the medium simultaneously. Medium was changed every other day. Endothelial cells started to spread and exhibited an elongated morphology, tuber-like structure and numerous intracellular vacuoles within 24 h, subsequently aligned themselves end to end, the sprouts from cells connected to form a net-like work in 2–4 days (Fig. 1). To quantitate angiogenesis, the number of sprouts and the length of sprouts per high power field were determined with a Nikon Optiphot microscope (Nikon, Japan) every 24 h. Five independent fields were assessed for each well.

2.8. Proliferation of endothelial cells

HUVECs cultured at 80% confluence in 96-well plates were incubated in serum-free medium at 37 °C for 24 h. The cells were exposed to F12/10% FBS containing 10 or 50 μM MPA for 24 h. ^{3}H-thymidine (0.5 μCi/well) was added to each culture during the last 12 h. The cells were harvested onto glass fiber filters and washed with distilled water using a semi-automated cell harvester. The rate of ^{3}H-thymidine

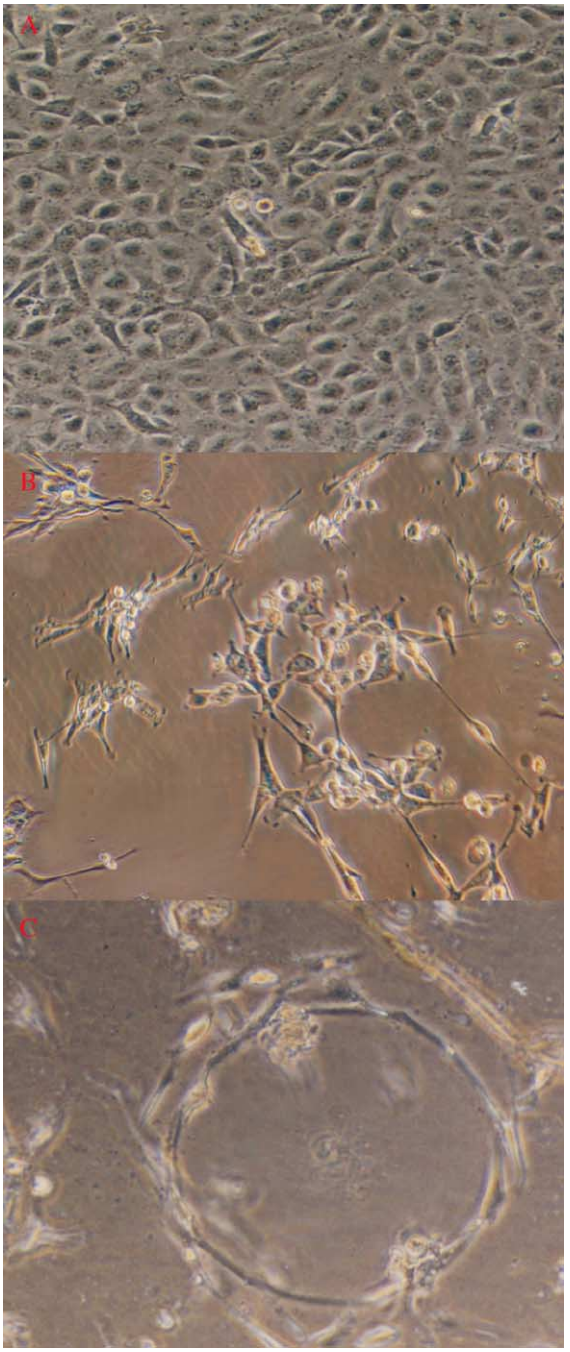

Fig. 1. The process of angiogenesis in 3-D collagen gels culture system. A: HUVECs in general culture; B: HUVECs starting to spread in 3-D collagen gels culture; C: tuber formation by HUVECs in 3-D collagen gels culture.

(radioactive disintergrations per minute, DPM) was determined on a liquid scintillation counter (Beckman, USA).

2.9. Migration of endothelial cells

HUVECs cultured at confluence in 6-well plates were incubated in serum-free medium at 37 °C for 12 h. The endothelial monolayers were wounded [19] by a stroke across the diameter of the well with cell scraper. The media and dislodged cells were aspirated, and the plates were rinsed with PBS. Fresh serum-free medium was added to the plates along with MPA at the dose of 10 or 50 μM. The width of the wound was visualized with a Nikon Optiphot microscope, and the shorten distance (the difference between initial and final width of wound during the treatment of MPA) was calculated. Three random measurements were analyzed for each wound every 24 h.

2.10. Cytotoxic effects of MPA

The activity of lactate dehydrogenase (LDH) in supernatants of HUVECs cultures after different treatment was measured by automated sample processing (Beckman, USA). The activity of LDH maintained in normal range in all experiments, suggesting that MPA did not have cytotoxic effect on endothelial cells (data not shown).

2.11. Statistics

All experiments were performed three times. Results from one representative experiment were expressed as the mean ± SEM. Student's *t*-test was used when comparison was performed between two groups. Results were deemed significant if $p < 0.05$ and very significant if $p < 0.01$.

3. Results

3.1. MPA down-regulates TNFα-induced ICAM-1 mRNA and surface expression

ICAM-1 mRNA and surface expression were markedly increased in the HUVECs after treatment with 10 μg/l TNFα for 24 h. The incubation with

10 μM MPA reduced the TNFα-induced ICAM-1 mRNA and surface expression by 19.3% and 28.2%, respectively. The incubation with 50 μM MPA led to 33% and 44.6% reduction of TNFα-induced ICAM-1 mRNA and surface expression, respectively (Figs. 2 and 3). Since the known mechanism of action of MMF is the inhibition of IMPDH, the key enzyme of guanine nucleotide synthesis, identical experiment was performed in the presence of 100 μM exogenous guanosine [shown to restore intracellular guanosine nucleotide to normal or supranormal levels in MPA-treated HUVECs]. As also shown in Fig. 3, addition of guanosine throughout the duration of the assay did not reverse MPA-mediated suppression of ICAM-1 surface expression ($p > 0.05$).

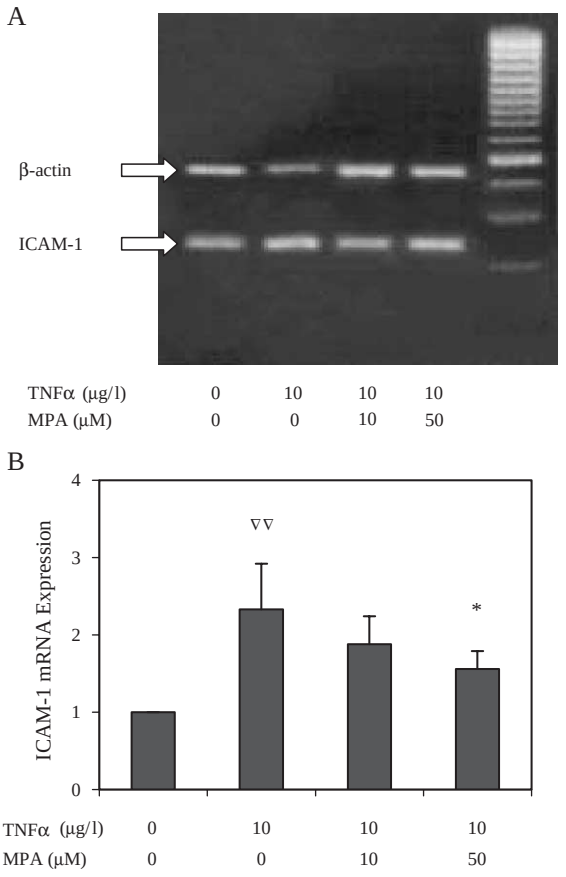

Fig. 2. Influence of MPA on TNFα-induced ICAM-1 mRNA expression. HUVECs were treated with 10 μg/l TNFα with or without 10, 50 μM MPA for 24 h. RT-PCR was performed to measure mRNA expression in HUVECs (result see A). ICAM-1 mRNA expression was calculated by the ratio of ICAM-1 to β-actin (B). TNFα markedly up-regulated ICAM-1 mRNA expression ($^{\nabla\nabla}p < 0.01$ vs. control), and this effect was attenuated by MPA treatment ($^{*}p < 0.05$ vs. TNFα-stimulated group).

3.2. MPA inhibits TNFα-induced adhesion of neutrophils to HUVECs

Incubation of HUVECs with TNFα for 24 h resulted in a significant increase of neutrophils adhesion to HUVECs as compared with control cells. Addition of MPA (10 or 50 μM) markedly decreased the TNFα-induced adherence of neutrophils to HUVECs by 55.2% or 66.2%, respectively (Fig. 4).

3.3. MPA inhibits TNFα-induced IL-6 secretion in HUVECs

As shown in Fig. 5, TNFα markedly induced IL-6 secretion, while incubation with MPA (10 or 50 μM) for 24 h significantly reduced TNFα-induced IL-6 secretion by 63.7% or 70.3%, respectively. Addition of guanosine throughout the duration of the assay did not attenuate the inhibitory effect of MPA on IL-6 secretion ($p > 0.05$).

3.4. MPA inhibits the process of angiogenesis in HUVECs

The impact of MPA on the process of angiogenesis was observed using a model of in vitro angiogenesis. The changers in morphologic characteristic of angiogenesis in a 3-D culture system were analyzed. As shown in Fig. 6, incubation with 10 μM MPA markedly decreased the number and the length of sprouts compared to the control. This inhibitory effect appeared within 24 h and lasted for 120 h. Incubation with 50 μM MPA led to a more reduction of the number and the length of sprouts.

3.5. MPA inhibits the proliferation of HUVECs

As shown in Fig. 7, incubation with 10 or 50 μM MPA for 24 h markedly inhibited the proliferation of endothelial cells by 70.3% or 77.8%, respectively.

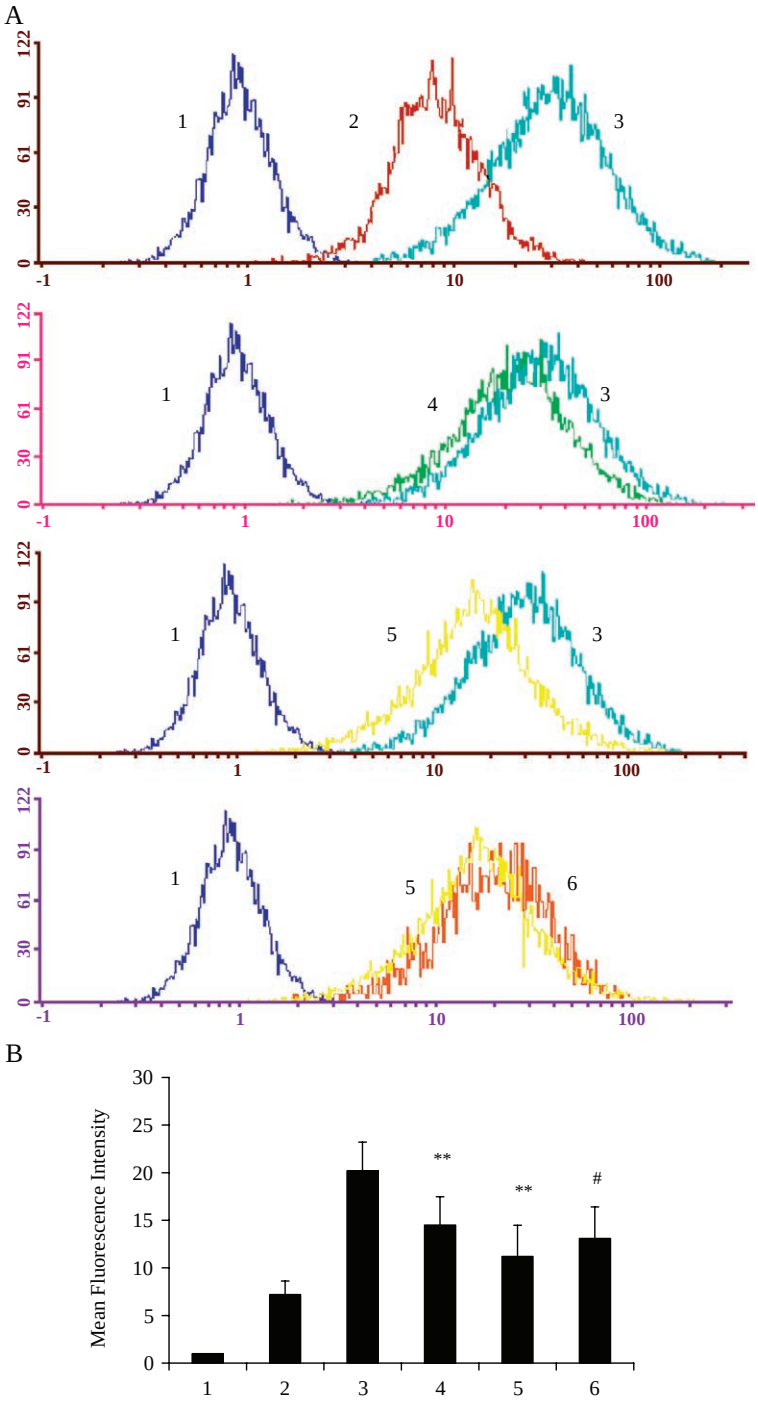

Fig. 3. Influence of MPA on TNFα-induced ICAM-1 surface expression. The *X*-axis of A is fluorescence intensity, and the *Y*-axis the cell number. Flow cytometry was performed to determined ICAM-1 surface expression in HUVECs undergoing different treatments. TNFα markedly induced ICAM-1 surface expression ($^{\nabla\nabla}p<0.01$ vs. group 2), while MPA significantly inhibited ICAM-1 expression induced by TNFα ($^{**}p<0.01$ vs. group 3). The inhibitory effect of MPA was not attenuated by addition of guanosine ($^{\#}p>0.05$ vs. group 5). 1: Negative control; 2: unstimulated control; 3: 10 μg/l TNFα stimulated; 4: TNFα + 10 μM MPA; 5: TNFα + 50 μM MPA; 6: TNFα + 50 μM MPA + 100 μM guanosine.

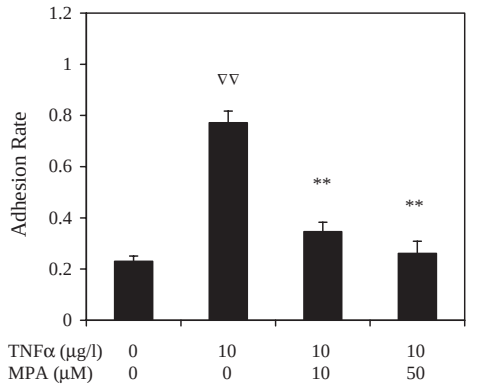

Fig. 4. Influence of MPA on TNFα-induced adhesion of neutrophils to HUVECs. HUVECs were treated with 10 μg/l TNFα with or without 10, 50 μM MPA for 24 h, and adhesion of neutrophils to HUVECs was analyzed. TNFα markedly increased neutrophils binding to HUVECs ($^{\nabla\nabla}p<0.01$ vs. control), while MPA significantly attenuated the induction effect of TNFα (**$p<0.01$ vs. TNFα-stimulated group).

3.6. MPA suppresses the activity of HUVECs migration

To evaluate cell migration in vitro, plate-wounding assay was used as described previously. A defined "wound" was scraped across a HUVEC monolayer cultured in serum-free medium on plastic. Cells cultured with 10 or 50 μM MPA achieved less wound closure than did cells without MPA (Fig. 8),

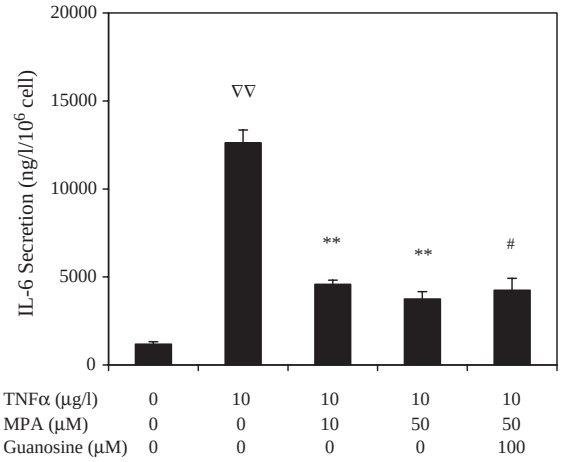

Fig. 5. Influence of MPA on TNFα-induced IL-6 secretion. IL-6 levels in the supernatants of cultured HUVECs undergoing different treatments were analyzed by ELISA. TNFα markedly induced IL-6 secretion ($^{\nabla\nabla}p<0.01$ vs. control), while MPA significantly inhibited IL-6 secretion induced by TNFα (**$p<0.01$ vs. TNFα-stimulated group). The inhibitory effect of MPA was not attenuated by addition of guanosine ($^{\#}p>0.05$ vs. TNFα and 50 μM MPA co-treated group).

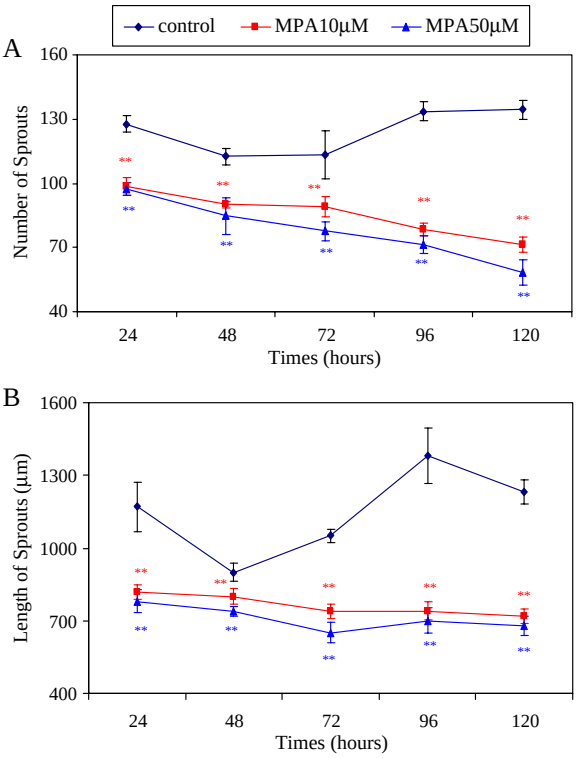

Fig. 6. Effect of MPA on angiogenesis of HUVECs. Cells were cultured in 3-D collagen gels in the absence or presence of 10, 50 μM MPA for 120 h. The number and the length of sprouts were quantified every 24 h. The number of sprouts (A) per high field was markedly decreased by MPA treatment, and the length of sprouts (B) was significantly reduced simultaneously. Compared with control, **$p<0.01$.

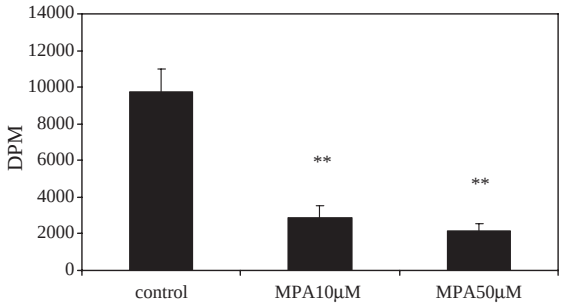

Fig. 7. Effect of MPA on cell proliferation determined by ^{3}H-thymidine incorporation study. Incubation with 10 or 50 μM MPA for 24 h markedly inhibited the proliferation of HUVECs. Compared with control, **$p<0.01$.

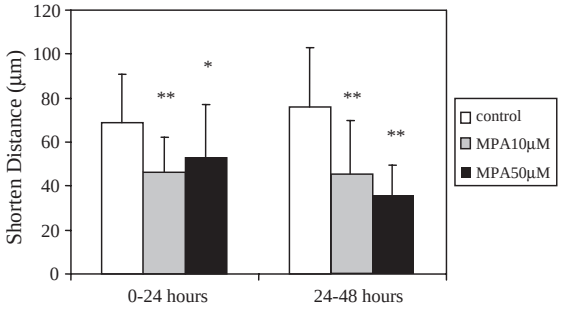

Fig. 8. Effect of MPA on cell migration by plate-wounding assay. HUVECs monolayer with wound was treated with 10 or 50 μM MPA for 48 h. The shorten distance (the difference between initial and final width of wound during the treatment of MPA) was analyzed every 24 h. MPA markedly reduced the shorten distance of the wound compared with control (**$p < 0.01$), indicating that MPA might inhibit the activity of HUVECs migration.

indicating that MPA can inhibit the activity of HUVECs migration.

4. Discussion

MMF has been shown to be effective in the treatment of several types of vasculitis [2–8]. MMF may inhibit the activity of IMPDH and interfere the de novo guanosine nucleotides synthesis therefore suppress lymphocytes proliferation [9]. Moreover, MMF was found to suppress adhesion molecules expression in endothelial cells and inhibit leukocytes adhesion to endothelial cells [10,11]. These mechanisms may attribute to its efficacy of MMF in treatment of vasculitis, however, its mechanism of action remains not well known. Since the endothelium plays a crucial role in the pathogenesis of vasculitis, we presumed that MMF might have multiform effects on endothelial cells which contributed to the efficacy of MMF on vasculitis. The present investigations, therefore, focused on observing the effect of MPA, the active metabolite of MMF, on the process of endothelial cells activation. Incubation with MPA at the dose of 10 or 50 μM had no influences on LDH activity in supernatants of HUVECs, implying that the effects of MPA observed in this study were not unspecifically toxic.

Multistep process involved in endothelial cells activation, includes increased expression of adhesion molecules, leukocytes adhesion to endothelial cells, cytokines production and the progress of angiogenesis. Leukocyte adhesion to the endothelial lining and recruitment to extravascular tissue is a fundamental and pivotal event in endothelial cells activation and the progression of vascular inflammation. The steps in the recruitment cascade are orchestrated by cell-adhesion molecules (ICAM-1, VCAM-1, E-selectin) [20]. In the progress of vasculitis, the expression of VCAM-1 and E-selectin by endothelial cells can be detected at some point and constitutive expression of ICAM-1 is usually up-regulated [12]. Additionally, E-selectin and ICAM-1 expression of endothelial cells in fresh vasculitic lesions are considered to be important for neutrophil recruitment [13,21]. Thus, we first investigated the effect of MPA on ICAM-1 expression on endothelial cells. HUVECs were treated with TNFα in order to mimic inflammatory condition. Our results demonstrated that stimulation with TNFα markedly enhanced the expression of ICAM-1 at both mRNA and surface levels while addition of MPA markedly inhibited TNFα-inducedICAM-1 expression. These results are partly in agreement with the finding of Raab M et al. [10] who demonstrated an inhibitory effect of MPA on TNFα-stimulated expression of ICAM-1, VCAM-1 and E-selectin in HUVECs. In contrast, Hauser IA et al. [22] showed that MPA increased TNFα-stimulated expression of VCAM-1 and E-selectin, but not of ICAM-1 in HUVECs.

The antiproliferative effect of MMF on lymphocytes is mediated by the selective inhibition of IMPDH, and leads to the depletion of guanosine nucleotides. Guanosine nucleotide is additionally involved in the synthesis of membrane glycoproteins, some of which are adhesion receptors. It is considered that inhibition of glycosylation by MPA may contribute to its inhibitory effect on adhesion molecule expression in endothelial cells, although the reasons are not fully understood [23]. We thus added exogenous guanosine into MPA-treated HUVECs to restore intracellular guanosine nucleotide to normal or supranormal levels. However, we found that the reduced expression of ICAM-1 after treatment with MPA was not reversible by exogenous guanosine, implying that inhibition of this process was not due to intracellular guanosine nucleotides

depletion. Our previous work showed that MPA significantly suppressed nuclear factor-κB (NF-κB) activity and prevented IκBα from degradation [24]. Since NF-κB plays a pivotal role in the regulation of the expression of cytokines and adhesion molecules [25], the inhibition of ICAM-1 expression by MPA might be partly through the suppression of NF-κB activity. However, more experiments will be performed to test this hypothesis.

Expression of adhesion molecules quickly recruited the inflammatory cells to endothelium including neutrophils and mononuclear cells such as monocytes and T cells, in which neutrophils play a pivotal role in the initiation of vascular inflammation especially small vascular inflammation [14,26]. We then observed the effect of MPA on neutrophils adhesion to endothelial cells. It was found that TNFα treatment markedly induced the attachment of neutrophils to endothelial cells, while pretreatment of HUVECs with MPA significantly inhibited TNFα-induced neutrophils attachment. As to other inflammatory cells, it was reported that lymphocytes and monocytes adhesion to HUVECs were markedly reduced after treatment with MMF.

Endothelial cells have the potential to produce a variety of cytokines in an inflammatory microenvironment which play different roles on the progression of different disorders. IL-6 was originally identified as a B-cell differentiation factor, but it is now known to be a multifunctional cytokine that regulates the immune responses and inflammation [27]. Through IL-6, endothelial cells may contribute to the systemic acute-phase reaction which is characteristically prominent in many systemic vasculitis compared with other immune-mediated diseases [12]. Moreover, IL-6 was been described to have the potential to promote angiogenesis [28]. Studies of this molecular over the past decade have implicated it in the pathogenesis of a variety of vasculitis including giant-cell arteritis, Takayasu's arteritis, rheumatoid vasculitis, ANCA-associated vasculitis, Wegener's granulomatosis and lupus nephritis. In general, IL-6 concentrations parallel disease activity in these disorders. Inhibition of IL-6 production might result in an improvement of vasculitis [2]. Data from this study showed that TNFα rapidly and significantly induced IL-6 secretion while MPA treatment markedly inhibited TNFα-induced IL-6 secretion in a dose-dependent manner. The effect of MPA on IL-6 secretion was not reversed by addition of guanosine, indicating that the inhibition of this process was not contributed to intracellular guanosine nucleotides depletion. As mentioned above, IL-6 is also the downstream element of NF-κB activation [29], it is possible that the inhibitory effect of MPA on IL-6 production may via the pathway of NF-κB activation.

Angiogenesis, new vessel formation, is a relevant phenomenon in systemic vasculitis. Immunohistochemical studies have shown that, in vasculitis, extensive neovascularization occurs in inflammatory lesions [12]. The molecular mechanisms of angiogenesis, both physiological and pathological, remain to be fully resolved; however, it has been revealed that initiation of blood vessel formation involves several steps, beginning with enzymatic degradation of the associated basement membrane. Vascular endothelial cells then migrate into the stromal space, proliferate, and align. The cells form tubular structures, undergo significant remodeling, and finally reestablish a new basement [30]. We found that MPA decreased the formation of capillary-like networks by HUVECs in 3-D culture system, reduced the ability of migration, and inhibited the proliferation of cells, indicating that MPA might inhibit the process of angiogenesis. The report from Wilasrusmee C et al. [31] demonstrated a moderately inhibitory effect of MPA on angiogenesis. They found that MMF and cyclosporin A (CsA) had injurious effects on in vitro capillaries formation, and suggested that the effect of CsA and MMF on endothelial cells could be relevant to immunosuppressive drug-mediated renal injury in clinical transplantation. It is proposed that angiogenesis may play a dual role in vasculitis. On one hand, angiogenesis may be an important compensating mechanism to avoid ischemia especially in small-vessel vasculitis. On the other hand, new blood vessels may maintain a chronic state of inflammation by transporting inflammatory cells to the affected site and supplying nutrients and oxygen to the proliferating inflamed tissue. The increased endothelial surface area also creates enormous capacity for the production of cytokines, adhesion molecules, and other inflammatory stimuli. The increased endothelial surface area and inflammatory cells induce the secretion of growth factors, such as

FGF, VEGF, and TNFα, which promote tissue fibrosis [12,14]. Thus, whether the inhibition of angiogenesis by MPA will result in clinical improvement or worsen ischemic complication in patients with vasculitis is an intriguing question.

In conclusion, results of our experiments indicated the multifarious effects of MPA on endothelial cells. MPA inhibited the expression of ICAM-1 and the adhesion of neutrophils to endothelial cells, suppressed the secretion of IL-6, decreased the formation of endothelial tube, and inhibited the migration and the proliferation of endothelial cells, which may contribute to the efficacy of MMF in the treatment of vasculitis.

References

[1] Halloran P, Mathew T, Tomlanovich S, Groth C, Hooftman L, Barker C. Mycophenolate mofetil in renal allograft recipients. Transplantation 1997;63:39–45.

[2] Uthman I. Pharmacological therapy of vasculitis: an update. Curr Opin Pharmacol 2004;4(2):177–82.

[3] Thomas-Golbanov C, Sridharan S. Novel therapies in vasculitis. Expert Opin Investig Drugs 2001;10(7):1279–89.

[4] Nowack R, Birck R, van der Woude FJ. Mycophenolate mofetil for systemic vasculitis and IgA nephropathy. Lancet 1997;349(9054):774.

[5] Daina E, Schieppati A, Remuzzi G. Mycophenolate mofetil for the treatment of Takayasu arteritis: report of three cases. Ann Intern Med 1999;130(5):422–6.

[6] Langford CA, Talar-Williams C, Sneller MC. Mycophenolate mofetil for remission maintenance in the treatment of Wegener's granulomatosis. Arthritis Rheum 2004;51(2): 278–83.

[7] Waiser J, Budde K, Braasch E, Neumayer HH. Treatment of acute c-ANCA-positive vasculitis with mycophenolate mofetil. Am J Kidney Dis 1999;34(3):e9.

[8] Hu WX, Liu ZH, Chen HP, Tang Z, Li LS. Mycophenolate mofetil treatment for refractory severe lupus nephritis. J Nephrol Dialy Transplant 1998;7:511–4.

[9] Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 2000;47(2–3): 85–118.

[10] Raab M, Daxecker H, Karimi A, Markovic S, Cichna M, Markl P, et al. In vitro effects of mycophenolic acid on the nucleotide pool and on the expression of adhesion molecules of human umbilical vein endothelial cells. Clin Chim Acta 2001;310:89–98.

[11] Glomsda BA, Blaheta RA, Hailer NP. Inhibition of monocyte/endothelial cell interactions and monocyte adhesion molecule expression by the immunosuppressant mycophenolate mofetil. Spinal Cord 2003;41:610–9.

[12] Cid MC. Endothelial cell biology, perivascular inflammation, and vasculitis. Clevel Clin J Med 2002;69(Suppl 2): SII45–49.

[13] Savage CO. The evolving pathogenesis of systemic vasculitis. Clin Med 2002;2(5):458–64.

[14] Cid MC, Segarra M, Garcia-Martinez A, Hernandez-Rodriguez J. Endothelial cells, antineutrophil cytoplasmic antibodies, and cytokines in the pathogenesis of systemic vasculitis. Curr Rheumatol Rep 2004;6(3):184–94.

[15] Vos IHC, Briscoe DM. Endothelial injury: cause and effect of alloimmune inflammation. Transpl Infect Dis 2003;4: 152–9.

[16] Li LX. Assessment of monocytes adhesion to endothelial cells. In: Xu SY, Bian RL, Chen X, editors. Techniques in pharmacology. Peijing: Peoples Medical Publishing House; 2002. p. 595–6.

[17] Xin XX, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator activated receptor γ ligands are potent inhibitors of angiogenesis in vitro and in vivo. J Biol Chem 1998;274: 9116–21.

[18] Satake S, Kuzuya M, Ramos MA, Kanda S, Iguchi A. angiogenic stimuli are essential for survival of vascular endothelial cells in three dimensional collagen lattice. Biochem Biophys Res Commun 1998;244:642–6.

[19] Morales DE, Mcgowan KA, Grant DS, Maheshwari S, Bhartiya D, Cid MC, et al. Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. Circulation 1995;91:756–63.

[20] Hahne M, Jager U, Isenman S, Hallmann R, Vestweber D. Five tumor necrosis factor-inducible cell adhesion mechanism on the surface of mouse endothelioma cells mediated the binding of leukocytes. J Cell Biol 1993;121:655–64.

[21] Taekema-Roelvink ME, Kooten CV, Kooij SV, Heemskerk E, Daha MT. Proteinase 3 enhances endothelial monocyte chemoattractant protein-1 production and induces increased adhesion of neutrophils to endothelial cells by upregulating intercellular cell adhesion molecule-1. J Am Soc Nephrol 2001;12:932–40.

[22] Hauser IA, Johnson DR, Thevenod F. Effect of mycophenolic acid on TNF alpha-induced expression of cell adhesion molecules in human venous endothelial cells in vitro. Br J Pharmacol 1997;122:1315–22.

[23] Allison AC, Kowalski WJ, Muller CJ, Waters RV, Eugui EM. Mycophenolic acid and brequinar, inhibitors of purine and pyrimidine synthesis, block the glycosylation of adhesion molecules. Transplant Proc 1993;25(3 Suppl 2):67–70.

[24] Huang HD, Liu ZH, Zhu XJ, Zhu XJ, Chen ZH, Li LS. Mycophenolate mofetil inhibits endothelial cell NF-κB activity. Acta Pharmacol Sin 2002;23:649–53.

[25] Margaret A, Maryann Z, Williams Amy J, Collins T. NF-κB and IκBα: an inducible regulatory system in endothelial activation. J Exp Med 1994;179:503–12.

[26] Savill J. The fate of the neutrophil in vasculitis. Clin Exp Immunol 1993;93(Suppl 1):2–5.

[27] Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002;13:357–68.

[28] Motro B, Itin A, Sachs L, Keshet E. Pattern of interleukin 6 gene expression in vivo suggests a role for this cytokine in angiogenesis. Proc Natl Acad Sci U S A 1990;87(8):3092–6.
[29] Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Mol Cell Biol 1990;10(5):2327–34.
[30] Majno G. Chronic inflammation: links with angiogenesis and wound healing. Am J Pathol 1998;153:1035–9.
[31] Wilasrusmee C, Da Silva M, Singh B. Morphological and biochemical effects of immunosuppressive drugs in a capillary tube assay for endothelial dysfunction. Clin Transplant 2003;17(Suppl 9):6–12.