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Quercetin, a dietary-derived flavonoid, possesses antiangiogenic potential

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Abstract

Quercetin, a dietary-derived flavonoid, suppresses tumor growth in vitro and in vivo, and inhibits the activity of tyrosine kinase. The effects of quercetin on the angiogenic process were examined in this study. Quercetin was found to inhibit several important steps of angiogenesis including proliferation, migration, and tube formation of human microvascular dermal endothelial cells in a dose-dependent manner. Additionally, the effect of quercetin on endothelial cell proliferation was confirmed using human umbilical vein endothelial cells. The activity of quercetin on the proliferation of endothelial cells was stronger than that on A549, BEL-7402, MKN-45 tumor cells and NIH-3T3 fibroblast cells. The chicken chorioallantoic membrane assay revealed that addition of quercetin displayed an antiangiogenic effect in vivo. After exposure to quercetin, a decrease in the expression and activity of matrix metalloproteinase-2, which is involved in the angiogenic process of migration, invasion, and tube formation, was observed by reverse transcription-polymerase chain reaction (RT-PCR) and gelatin zymography. These findings suggest that quercetin has antiangiogenic potential and that this effect may be related to an influence on the expression and activity of matrix metalloproteinase-2.

Keywords: Quercetin; Angiogenesis; Umbilical vein endothelial cell; (Human); Microvascular dermal endothelial cell; (Human); Matrix metalloproteinase-2

1. Introduction

Angiogenesis is characterized by the formation of new vessels from a pre-existing microvascular network. This event involves a sequence of complex steps beginning with the production and release of angiogenic factors by endothelial cells, tumor cells, and matrix cells. These angiogenic factors include vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF). In addition to these cytokines, matrix metalloproteinase is also involved in the angiogenic process (Battegay, 1995; Klagsbrun and Moses, 1999). Angiogenesis is a crucial step in the growth and metastasis of cancers. Expansion of a microscopic solid tumor beyond 1–2 mm in size requires continuous recruitment of new blood vessels. These vessels also serve as gateways for cancer cells to enter the bloodstream and spread to distant organs. Inhibition of angiogenesis has come to be recognized as a promising therapeutic approach for the control of tumor growth, progression, invasion,l and metastasis (Gourley and Williamson, 2000).

Quercetin (3,3′,4′,5,7-penthydroxy flavone) is an important constituent of the flavonoid family and is found in many fruits and vegetables, as well as olive oil, red wine, and tea. Various pharmacological activities of quercetin have been demonstrated including antioxidation by scavenging free radicals, prevention of atherosclerosis, and chronic inflammation (Havesteen, 1983; Corvazier and Maclouf, 1985). Furthermore, it has been well documented that quercetin suppresses tumor growth in vitro and in vivo. Quercetin has various characteristics that make it a potential anticancer compound. These functions include cell cycle regulation, interaction with type II estrogen binding sites, reversal of multidrug-resistance, and induction of tumor cell apoptosis (Yoshida et al., 1990; Scambia et al., 1993; Kim et al., 1998; Xiao et al., 1998). Additionally, quercetin effectively inhibits tyrosine kinase activity (Levy et al., 1984). Accordingly, the effect of quercetin on the angiogenic process attracted our attention. When screening tumor angiogenesis inhibitors, we found that quercetin showed significant antiangiogenic

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activity. In this report, we describe the potential of quercetin as an inhibitor of angiogenesis.

2. Materials and methods

2.1. Compound, cell lines and culture

Quercetin (Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO). The concentration of DMSO was kept below 0.1% in quercetin-treated groups. DMSO 0.1% (v/v) was used as a vehicle control throughout the study.

Human umbilical vein endothelial cells were isolated from human umbilical cord veins by 0.1% type-I collagenase digestion at 37 °C for 15 min and checked by immunofluorescence for von willebrand factor (Sigma). Human umbilical vein endothelial cells were cultured in M199 medium (Gibco, Grand Island, NE, USA) supplemented with 20% heat-inactivated fetal bovine serum and 30 μg/ml endothelial cell growth supplement (Sigma). Cells at three to seven passages were used in the experiments (Jaffe et al., 1973). The human microvascular dermal endothelial cell line was obtained from American Type Culture Collection (ATCC) and propagated in Dulbecco's Modified Eagle Medium (DMEM; Gibco) with 15% heat-inactivated fetal bovine serum. The human gastric adenocarcinoma cell line MKN-45, hepatocellular carcinoma cell line BEL-7402, lung adenocarcinoma cell line A549, and fibroblast cell line NIH-3T3 were obtained from the cell bank of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. These tumor and fibroblast cells were maintained in RPMI-1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml). All cells were cultured in a highly humidified atmosphere of 5% CO_2 at 37 °C (Qing et al., 1999).

2.2. Measurement of growth inhibition of cells

The growth inhibitory effect of quercetin on cells was measured using the Sulforhodamine B (SRB, Sigma) assay. Briefly, cells in 90 μl of medium were seeded in a 96-well plate and allowed to attach for 24 h before addition of quercetin. Cell densities were selected based on preliminary tests to maintain control cells in an exponential growth phase during the period of the experiment and to obtain a linear relationship between optical density and number of viable cells. Each cell line was exposed to quercetin at the desired final concentrations for 72 h and each concentration was tested in triplicate. After exposure, the cells were fixed with 10% trichloroacetic acid (100 μl) (Xiao et al., 2001) and incubated at 4 °C for 1 h. The cells were then stained by exposure to 100 μl of SRB solution [0.4% SRB (w/v) in 1% acetic acid (v/v)] for 15 min, after which the plates were washed with 1% acetic acid to remove any unbound dye. Bound dye was solubilized with 10 mM Tris base (pH 10.5). The plates were measured using a multiwell spectrophotometer (VERSAmax, Molecular Devices, USA) at a wavelength of 515 nm. The rate of inhibition of cell proliferation was calculated using the following formula: Growth inhibition=$[1-(A_{515\ \text{treated}}/A_{515\ \text{control}})]\times 100\%$.

The results were expressed as IC_{50} (the drug concentration reducing by 50% the absorbance in treated cells compared with that in untreated cells). IC_{50} was calculated using the Logit method and the mean IC_{50} was determined from three replicate tests.

2.3. Cell migration assay

Migration of human microvascular dermal endothelial cells was determined in a transwell Boyden Chamber (Costar, MA, USA) using a polycarbonate filter with a pore size of 8 μm (Koyama et al., 1999) which was coated with 0.2% gelatin. In the standard assay, 0.1 ml of cell suspension (2×10^5 cells/ml) with quercetin or 0.1% DMSO (v/v) was added to the upper compartment of the chamber. The lower compartment contained 0.6 ml of DMEM medium supplemented with quercetin or 0.1% DMSO (v/v). After incubation for 8 h at 37 °C, the filter was removed and fixed with ethanol. Cells remaining on the upper surface of the filter (non-migrated) were scraped gently. Then, migrated cells on the lower surface of the filter were stained with hematoxylin and eosin, after which migrated cells from five random fields were counted manually. The rate of inhibition of migration was calculated using the formula: rate of inhibition of migration=$[1-(\text{migrated cells}_{\text{treated}}/\text{migrated cells}_{\text{control}})]\times 100\%$.

2.4. Tube formation determination

The tube formation assay was conducted to investigate the effect of quercetin on angiogenesis in vitro (Ashton et al., 1999). A 96-well plate was coated with 0.1 ml of matrigel (Becton Dickinson Labware, MA, USA) which was allowed to solidify at 37 °C for 1 h. Human microvascular dermal endothelial cells (1×10^4 cells) were seeded on the matrigel and cultured in DMEM medium containing different concentrations of quercetin (25, 50, 100 μM) or 0.1% DMSO (v/v) for 24 h. The enclosed networks of complete tubes from five randomly chosen fields were counted and photographed under a microscope (Olympus, IX70, Japan). The inhibition rate was calculated using the following formula: Rate of inhibition of tube formation=$[1-(\text{tubes}_{\text{treated}}/\text{tubes}_{\text{control}})]\times 100\%$.

2.5. Chicken chorioallantoic membrane assay

Inhibition of angiogenesis in vivo was measured using a modified chicken chorioallantoic membrane assay (Morris et al., 1997). Fertilized chicken eggs were incubated in a humidified egg incubator (Lyon, CA, USA) for 9 days. Following this incubation, a small hole was punched on the broad side of the egg and a window was carefully created

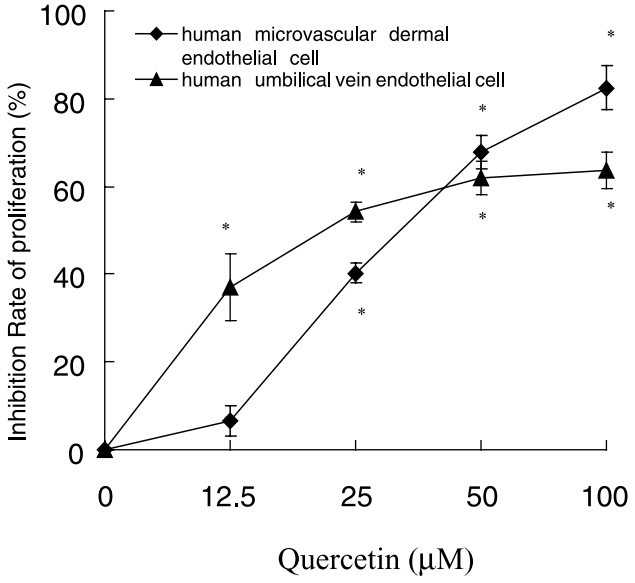

Fig. 1. Rate of quercetin inhibition of growth of human microvascular dermal endothelial cells and human umbilical vein endothelial cells. Values are expressed as $\bar{X} \pm$ S.D. from at least three independent experiments. *$P < 0.001$ vs. control, $n = 3$.

through the egg shell. Filter paper disks saturated with quercetin (50–100 nmol/10 μl/egg) or 0.1% DMSO (v/v) were placed on the chicken chorioallantoic membranes. The eggs were then returned to the humidified egg incubator and chicken chorioallantoic membranes were harvested after 48 h. Non-viable embryos were discarded. At least 10 viable embryos were tested for each treatment. The neovascular zones under the disks were photographed under a stereomicroscope (Leica, MS5, Switzerland).

2.6. Reverse transcription-polymerase chain reaction (RT-PCR) assay

Human microvascular dermal endothelial cells were treated with quercetin (25, 50, 100 μM) or 0.1% DMSO (v/v) for 12 h. Total cellular mRNA was prepared using the TRIzol reagent (Gibco) according to the manufacturer's instructions. RNA yields and purity were assessed by spectrophotometric analysis. Total RNA (1 μg) from each sample was subjected to reverse transcription with random hexamer (Promega, WI, USA), deoxyribonucleoside triphosphates (dNTPs), and 10 U of moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco) in a 20-μl reaction volume. The synthesized cDNA was used immediately for PCR amplification. PCR was carried out using the following primers: 5′-CCATGGAGAAGGCTGGGG-3′ (sense), 5′-CAAATGTGTCATGGATGACC-3′ (antisense) for glyceraldehyde-3-phosphatedehydrogenase (GAPDH); 5′-GCGGATCCAGCGCCCAGAGACAC-3′ (sense), 5′-TTAAGCTTCCACTCCGGG CAGGATT-3′ (antisense) for matrix metalloproteinase-2. Each PCR reaction was performed in a 50-μl volume containing 2 μl of reverse transcription reaction mixture, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 1 mM $MgCl_2$, 0.2 mM of each of the dNTPs, 0.2 μM of each primer, and 1 μl (3 U) of *Taq* polymerase (Gibco). Each PCR cycle consisted of a heat-denaturation step at 94 °C for 1 min, a primer-annealing step at 55 °C for 30 s, and a polymerization step at 72 °C for 1 min. There were 29 and 23 cycles for matrix metalloproteinase-2 and GAPDH amplification respectively. PCR reaction products (10 μl) were subjected to electrophoresis on a 1.5% agarose gel. After staining with ethidium bromide, the DNA bands were visualized, photographed (UVP, USA) and quantified using the formula: Rate of inhibition of expression=$[1 - (\text{matrix metalloproteinase-2/GAPDH})_{\text{treated}} / (\text{matrix metalloproteinase-2/GAPDH})_{\text{control}}] \times 100\%$.

2.7. Determination of matrix metalloproteinase-2 gelatinolytic activity

Human microvascular dermal endothelial cells at 80% confluence were cultured for 24 h in serum-free medium in the absence or presence of 25, 50, or 100 μM quercetin. Gelatin zymography was performed to visualize the matrix metalloproteinase activity present in the conditioned

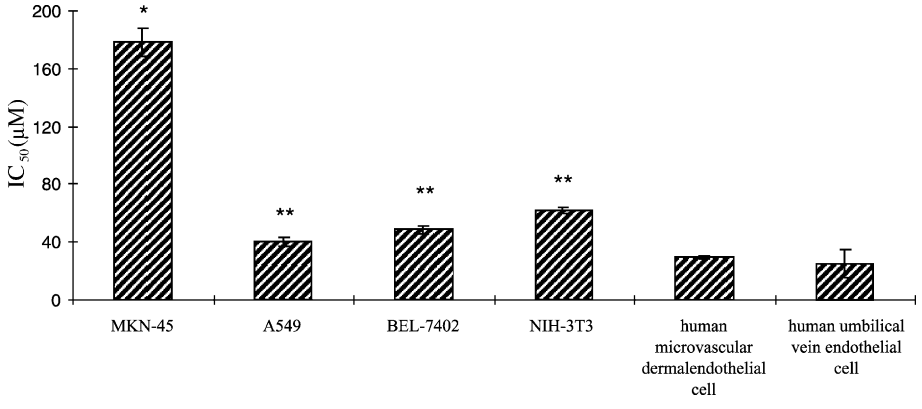

Fig. 2. The IC_{50} (μM) values of quercetin on cell lines. Values are expressed as $\bar{X} \pm$ S.D. from at least three independent experiments. **$P < 0.05$ vs. human microvascular dermal endothelial cells and human umbilical vein endothelial cells; *$P < 0.001$ vs. human microvascular dermal endothelial cells and human umbilical vein endothelial cells.

medium (Vincent et al., 2001). Samples without reducing agents or heating were applied to 7.5% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) copolymerized with 1% gelatin. After electrophoresis, the gel was washed in 2.5% Triton X-100 for 1 h to remove SDS and incubated overnight at 37 °C in developing buffer (50 mM Tris–HCl, 5 mM $CaCl_2$, 0.02% Tween, pH 7.6). The gel was then stained (0.5% Coomassie Blue R-250, 10% acetic acid, 5% methanol) and destained (10% acetic acid, 5% methanol). Gelatinolytic activity was visualized as clear bands against the blue background of stained gelatin. Bands were quantified using Gelworks 1D Intermediate Version 2.01 software. The inhibition rate was calculated using the formula: Rate of inhibition of activity=[1 − (matrix metalloproteinase-$2_{treated}$/matrix metalloproteinase-$2_{control}$)] × 100%.

2.8. Data analysis

Results were expressed as $\bar{X} \pm$ S.D. and significance was assessed with Student's *t*-test.

3. Results

3.1. Cell growth inhibition

Quercetin at 12.5 μM exerted no inhibitory effect on the proliferation of human microvascular dermal endothelial cells but inhibited the proliferation of human umbilical vein endothelial cells with an inhibition rate of 37.0%. After treatment with quercetin at 25–100 μM, proliferation of human microvascular dermal endothelial cells and human umbilical vein endothelial cells was inhibited in a concentration-dependent manner. Following exposure to 25, 50, and 100 μM quercetin for 72 h, the inhibition rate was 40.3%, 67.9%, and 82.5% for human microvascular dermal endothelial cells and 54.2%, 62.0%, and 63.6% for human umbilical vein endothelial cells, respectively (Fig. 1).

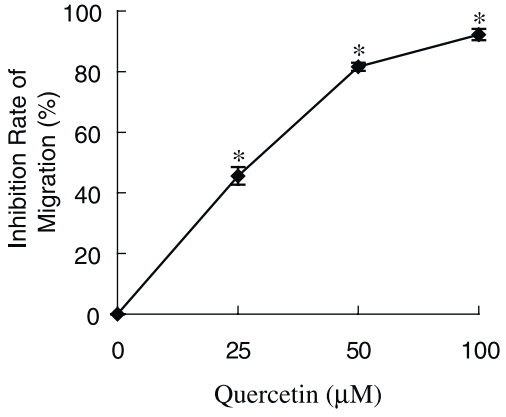

Fig. 3. Rate of quercetin inhibition of migration of human microvascular dermal endothelial cells. The data are shown as $\bar{X} \pm$ S.D. from three separate experiments. *$P < 0.001$ vs. control, $n = 5$.

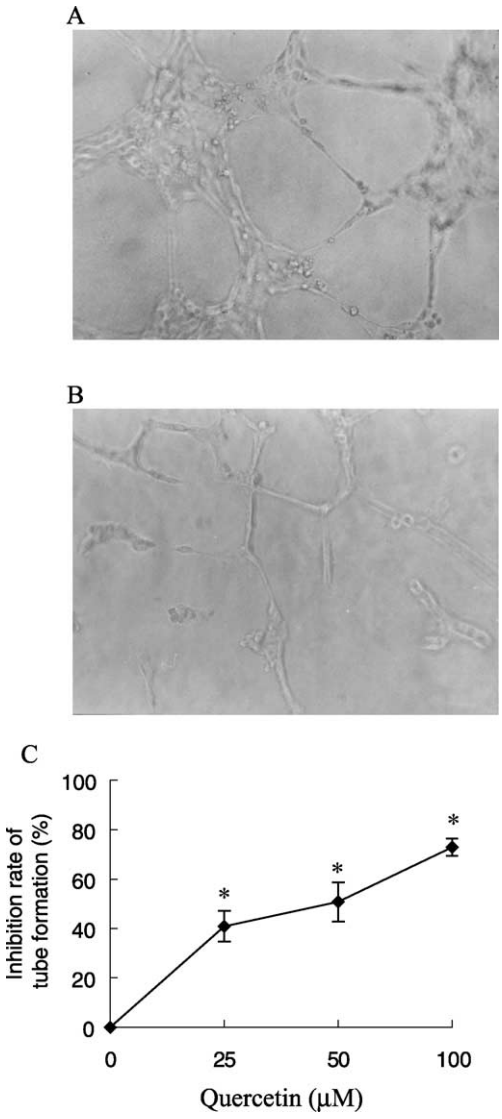

Fig. 4. Inhibitory activity of quercetin on tube formation of human microvascular dermal endothelial cells. Representative photographs of control (A) and treatment with quercetin 50 μM (B) from three independent experiments. (C) Rate of quercetin inhibition of tube formation of human microvascular dermal endothelial cells. Results are expressed as $\bar{X} \pm$ S.D., *$P < 0.001$ vs. control, $n = 5$. Magnification: × 100.

The effect of quercetin on the proliferation of other tumor and fibroblast cells was also tested. The results show that quercetin exhibited a more marked inhibitory effect on the growth of A549, BEL-7402, and NIH-3T3 cells than on MKN-45 cells. On the other hand, the mean IC_{50} values for human microvascular dermal endothelial cells (29.2 μM) and human umbilical vein endothelial cells (25.4 μM) were lower than those for MKN-45 (178.0 μM), A549 (39.8 μM), BEL-7402 (48.4 μM), and NIH-3T3 (61.9 μM) cells, indicating that the growth inhibition activity of quercetin against endothelial cells was stronger than that against tumor and fibroblast cells (Fig. 2).

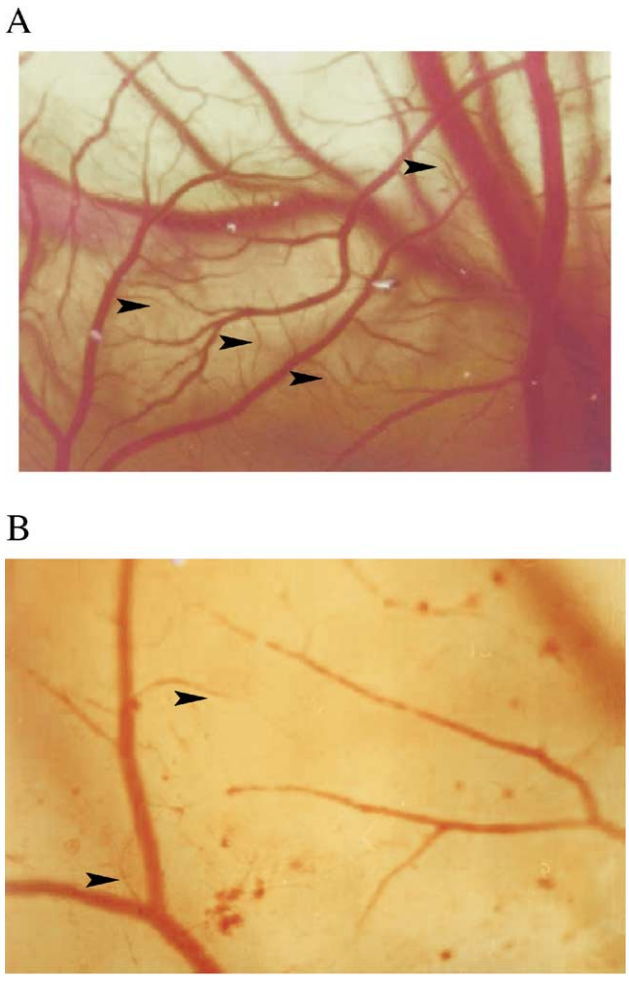

Fig. 5. Angiogenesis inhibited by quercetin in the chicken chorioallantoic membrane assay (arrows show neovasculars forming in the chicken chorioallantoic membrane); similar results were obtained from three independent experiments ($n = 10$). (A) Control, (B) treatment with quercetin at 100 nmol/10 μl/egg. Magnification: × 2.4.

3.2. Inhibition of cell migration

After culture in the Boyden Chamber for 8 h, a large number of human microvascular dermal endothelial cells migrated to the lower side of the filter. In the presence of quercetin at concentrations above 25 μM, the migratory process was significantly inhibited in a concentration-dependent manner. Treatment with 25, 50, and 100 μM quercetin resulted in 45.6%, 81.6%, and 92.2%, respectively, inhibition of the human microvascular dermal endothelial cell migration compared with the control group (Fig. 3).

3.3. Reduction in tube formation

Human microvascular dermal endothelial cells incubated on matrigel substratum for 24 h differentiated into an extensive and enclosed network of tubes in the control group, whereas human microvascular dermal endothelial cells exposed to quercetin formed incomplete and sparse tube networks (Fig. 4A and B). Quercetin at concentrations of 25, 50, and 100 μM reduced tube formation by 47.1%, 58.8%, and 76.5%, respectively, compared with the control group (Fig. 4C).

3.4. Antiangiogenic activity in chicken chorioallantoic membrane assay

The control chicken chorioallantoic membranes showed well developed neovascular zones under the filter saturated with carrier buffer after 9 days culture, whereas the neovascularization in chicken chorioallantoic membranes was significantly suppressed by addition of quercetin (50–100 nmol/10 μl/egg). The antiangiogenic effect was seen in all the viable embryos treated with quercetin at 50 or 100 nmol/10 μl/egg. Quercetin at 25 nmol/10 μl/egg showed no inhibitory effect on neovascularization in chicken chorioallantoic membranes (Fig. 5).

3.5. Decrease in matrix metalloproteinase-2 expression

The effect of quercetin on the expression of matrix metalloproteinase-2 at the transcription level was tested by the RT-PCR method. Quercetin treatment for 12 h produced a marked decrease in expression of matrix metalloproteinase-2 at the transcription level (Fig. 6A). Quantification of mRNA intensity indicated a 26.0% decrease of mRNA expression at 25 μM, a 62.9% decrease at 50 μM, and a 55.8% decrease at 100 μM (Fig. 6B).

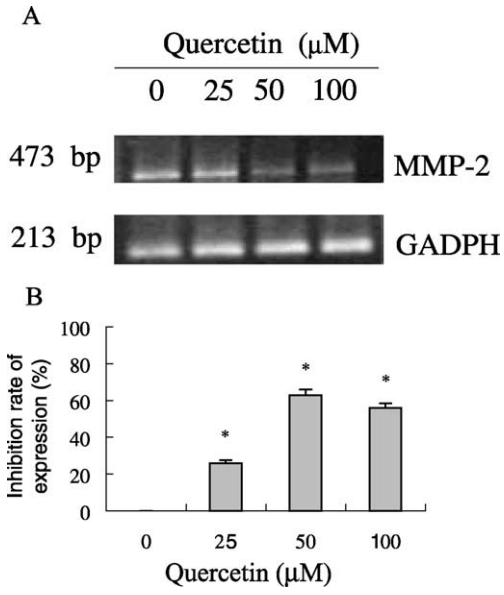

Fig. 6. Inhibition of matrix metalloproteinase-2 expression detected by RT-PCR. (A) Representative photographs from three separate experiments, (B) inhibition rate of matrix metalloproteinase-2 expression, the data are expressed as $\bar{X} \pm$ S.D., $^*P < 0.001$ vs. control.

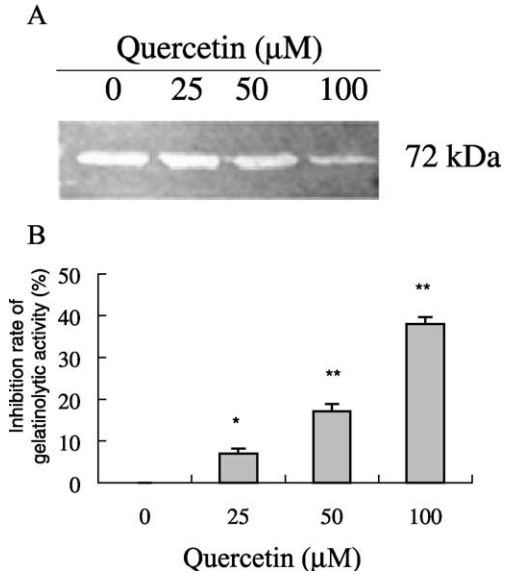

Fig. 7. Reduction in matrix metalloproteinase-2 gelatinolytic activity by quercetin determined by gelatin zymography. (A) Representative photographs from three separate experiments, (B) inhibition rate of matrix metalloproteinase-2 gelatinolytic activity, the data are expressed as $\bar{X} \pm$ S.D., $^{*}P < 0.05$ vs. control, $^{**}P < 0.001$ vs. control.

3.6. Suppression of matrix metalloproteinase-2 gelatinolytic activity

To further assess the effect of quercetin on the activity of matrix metalloproteinase-2, we applied the gelatin zymographic assay to visualize the activity of matrix metalloproteinase-2 expressed by human microvascular dermal endothelial cells. After exposure to quercetin (25–100 μM) for 24 h, the band corresponding to matrix metalloproteinase-2 (molecular weight 72 kDa) was dose dependently suppressed compared with the control (Fig. 7A). Quercetin at concentrations of 25, 50, and 100 μM reduced the gelatinolytic activity of matrix metalloproteinase-2 by 7.0%, 17.1%, and 38.0%, respectively (Fig. 7B).

4. Discussion

The process of angiogenesis can be divided into the following four main steps (Klagsbrun and Moses, 1999): (i) degradation of the basement membrane of existing blood vessels by matrix metalloproteinases secreted by activated endothelial cells; (ii) migration of these endothelial cells toward the angiogenic stimulus; (iii) proliferation of the endothelial cells leading to the formation of solid endothelial cell sprouts in the stromal space; and (iv) organization of endothelial cells into capillary tubes and vascular loops with the formation of tight junctions and the deposition of new basement membrane. In the present study, we found that quercetin inhibited several important steps of angiogenesis including proliferation, migration, and tube formation of endothelial cells in vitro and exerted antiangiogenic activity in vivo. All these effects were concentration-dependent. Moreover, the growth inhibitory effect of quercetin was verified on two human endothelial cell lines and the activity of quercetin was stronger on endothelial cell proliferation than on the proliferation of the tumor or fibroblast cells. This study confirms the recently published data of Igura et al. (2001) that demonstrated the antiangiogenic effect of quercetin in vitro including the effect on proliferation, migration, and tube formation of bovine aorta endothelial cells. Treatment with 25 μM quercetin for 24 h inhibited tube formation in our study. When the concentration of quercetin reached 100 μM, tube formation was reduced by 75%. However, Igura et al. reported that quercetin at concentrations of 6.5–25 μM had no inhibitory effect on the tube formation of bovine aorta endothelial cells and that tube formation was inhibited by 40% when the concentration of quercetin reached 100 μM. One of the reasons for this discrepancy may be the different endothelial cell lines investigated, i.e., human endothelial cells were used in our study whereas bovine aorta endothelial cells were used by Igura et al.

Since quercetin significantly inhibited the angiogenic processes of migration and tube formation, we investigated the activity of quercetin on matrix metalloproteinase which involves these two steps. Matrix metalloproteinases, a family of zinc-containing endopeptidases, are implicated in angiogenesis and tumor metastasis. Matrix metalloproteinases selectively mediate proteolytic degradation of the extracellular matrix that is required for migration and invasion of endothelial cells at the start of angiogenesis. Endothelial cells are shown to produce a variety of matrix metalloproteinases, and of these, matrix metalloproteinase-2 (gelatinase A, 72 kDa collagenase type IV) plays a critical role in angiogenesis (Nguyen et al., 2001; Mignatti and Rifkin, 1996). When endothelial cells are cultured on matrigel, the formation of tubular networks is increased by the addition of recombinant matrix metalloproteinase-2 and decreased when neutralizing antibody or tissue inhibitor of matrix metalloproteinase-2 is added (Schnaper et al., 1993). Fang et al. (1999) have demonstrated, using a tumor nodule model, that matrix metalloproteinase-2 is required for a tumor to switch to the angiogenic phenotype. Experimental data also reveal that tumor angiogenesis is reduced in matrix metalloproteinase-2 deficient mice (Itoh et al., 1998). These findings led us to focus on matrix metalloproteinase-2. Our study verified that quercetin treatment at 25–100 μM for 12–24 h decreased the expression and activity of matrix metalloproteinase-2. It is conceivable, therefore, that the observed suppression by quercetin of both migration of human microvascular dermal endothelial cells and their differentiation into tubular networks on matrigel is closely related to the inhibition of matrix metalloproteinase-2 expression and activity. We consider that this inhibitory effect of quercetin may be, at least in part, responsible for its antiangiogenic potential. However, Morrow et al. (2001)

recently reported that supplementation of the diet with quercetin did not alter the matrix metalloproteinase-2 or tissue inhibitor of matrix metalloproteinase-2 gene transcription or plasma protein levels of healthy subjects, whereas the levels of tissue inhibitor of matrix metalloproteinase-1 were significantly decreased. The discrepancy between our result regarding the effect of quercetin on matrix metalloproteinase-2 and that of Morrow et al. may be due to the differences in the duration of treatment, the dosage of quercetin, and the objectives of the two studies. Tissue inhibitors of matrix metalloproteinases have antiangiogenic activity either because of their ability to inhibit the activity of matrix metalloproteinases or through a direct effect on endothelial cell proliferation (Jiang et al., 2002). Thus, the effect of quercetin on tissue inhibitor of matrix metalloproteinases expressed by endothelial cells warrants investigation to further elucidate its antiangiogenic mechanisms. Additionally, recent studies have shown that nuclear factor-kappa B significantly contributes to tumorigenicity, angiogenesis, and metastasis of human melanoma cells implanted in nude mice, and that inhibition of nuclear factor-kappa B activity can suppress angiogenesis (Huang et al., 2000; Sunwoo et al., 2001). It has been reported that quercetin can inhibit nuclear factor-kappa B activity (Mouria et al., 2002). Hence, the influence of quercetin on nuclear factor-kappa B may also be involved in the antiangiogenic mechanisms of quercetin.

Quercetin is the major bioflavonoid in the human diet. It has been reported that quercetin possesses antiproliferative activity in vitro against ovarian, breast, and stomach cancer cells (Scambia et al., 1990; Yanagihara et al., 1993). Our study also showed weak activity of quercetin on MKN-45, A549, and BEL-7402 tumor cells. Experiments in vivo provide evidence that quercetin inhibits carcinogen-induced tumors in rodents. Tumor growth in mice bearing ascites tumors or abdominal tumors treated intraperitoneally with quercetin was significantly inhibited and the lifespan of the mice was increased (Molnar et al., 1981; Castillo et al., 1989). In a population-based case- control study of lung cancer, researchers found inverse associations between lung cancer risk and intake of foods containing quercetin (Marchand et al., 2000). A trend toward lower risk of stomach cancer with higher intake of quercetin has also been demonstrated (Garcia et al., 1999). In addition, it has been found that a single oral dose of up to 4 g of quercetin has no side effects in humans (Gugler et al., 1975). In a phase I clinical trial of quercetin, researchers found that quercetin could be safely administered as bolus at a dose of injection (60–1400 mg/m^2), and evidence of antitumor activity was seen. The authors recommended 1400 mg/m^2 quercetin as the bolus dose for Phase II clinical trial (Ferry et al., 1996). These studies collectively demonstrate that oral use of quercetin appears safe and possibly useful for cancer treatment. Our present results in vitro and in vivo support these results regarding the antiangiogenic effect of quercetin. Therefore, we suggest that constant consumption of foods rich in quercetin can be beneficial for preventing and controlling some types of cancers.

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