Inhibitory Effect of *Uncaria Sinensis* on Matrix Metalloproteinase-9 Activity and Human Aortic Smooth Muscle Cell Migration

Chang Geun Kwak, Dall Yeong Choi*

Department of Pathology, College of Oriental Medicine, Dongguk University

The migration of vascular smooth muscle cells (VSMC) and the production of matrix metalloproteinases-9 (MMP-9) may play a key role in the development of atherosclerosis. In this study, we have more extensively investigated the inhibitory effect of UR on MMP-9 activity and TNF-α induced human aortic smooth muscle cells (HASMC) migration. The result from gelatin zymography showed that UR inhibited MMP-9 activity in a dose-dependent manner (IC50 = 55 g/ml). In addition, UR strongly inhibited the migration of HASMC induced by TNF- treatment (IC50 = 125 g/ml), although it has very low cytotoxic effect on HASMC (IC50 > 500 g/ml). These results suggest that UR is a potential anti-atherosclerotic agent through inhibition of MMP-9 activity and VSMC migration.

Key words : Cho-Deung-san, atherosclerosis, Uncaria sinensis (Oliv.) Havil

Introduction

The proliferation and migration of vascular smooth muscle cells (VSMC) may play a key role in the development of intimal thickening after arterial wall injury or in atherosclerosis[1]. VSMC in the media has low mitogenic activity. During the early stages of arterial wall injury or atherosclerosis, aortic smooth muscle cells (SMC) may undergo transition from a contractile to a synthetic phenotype and begin proliferating in response to various growth factors, causing intimal thickening of the arterial walls[2-4]. However, in addition to growth factor stimulation, the replication and migration of VSMC may require the degradation or remodeling of extracellular matrix surrounding the cells[5,6]. VSMC synthesizes important components of the extracellular matrix, including collagens, elastin, and proteoglycans[7,8]. An imbalance between the accumulation and degradation of extracellular matrix may be crucial in the development of intimal thickening that forms after vascular wall interventions[8].

Matrix remodeling requires the action of proteinases, among which the matrix metalloproteinases (MMPs) appear to play a key role[9]. MMPs comprise three main groups: the interstitial collagenases (MMP-1), the type IV collagenases or gelatinases (MMP-2 and -9), and the stromelysins (MMP-3)[9]. Among these MMPs, gelatinases degrade denatured interstitial collagens, native basement-membrane collagens; the expression of matrix metalloproteinase-9 (MMP-9) has been implicated in the progression of atherosclerotic lesions[10]. Recent reports from an in vivo study concluded that MMP-9 is critical for the development of arterial lesions by regulating both VSMC migration and proliferation[11]. On the basis of in depth reports from several different laboratories, it has been generally concluded that the basal levels of MMP-9 are usually low, and that its expression can be induced by treatment of vascular smooth muscle cells with tumor necrosis factor- (TNF-α)[7,12,13]. Recent results from our laboratory demonstrated that ERK1/2 mediates TNF-α induced MMP-9 expression in VSMC via the regulation of NF-B and AP-1[12,14,15].

The endogenous MMP inhibitors, known as tissue inhibitors of metalloproteinase (TIMPs), maintain a balance between matrix formation and destruction but have short half-lives[16]. Therefore, synthetic inhibitors of MMPs, such as BB94 (batimastat) and BB2516 (marimastat) have been developed. BB94 has a structure that mimics collagen and facilitates chelation of the zinc ion in the active site of the MMP molecule, thereby causing an inactive protease[17]. During the last few years, substances of this type have been used to study the importance of extracellular matrix remodeling in various types of diseases, including rheumatoid arthritis, cancer and cardiovascular diseases. Thus, synthetic MMP inhibitors have been demonstrated to affect the wound-healing

* To whom correspondence should be addressed at : Dall Young Choi Department of Oriental Medicine The Graduated School of DongGuk University, 707 Sukjang-dong, Kyungju, 780-714, Korea

· E-mail : cdydkom@dongguk.ac.kr, · Tel : 054-770-2367

· Received : 2006/07/27 · Revised : 2006/08/28 · Accepted : 2006/09/23

response in injured rat carotid arteries[18,19], to reduce migration of VSMC from baboon aortic medial explants[20], and to inhibit the macrophage modulation of phenotypic change and DNA synthesis in cultured rabbit aortic SMC[21].

Several natural products have been used for vascular diseases[22], and some traditional herbs and prescriptions have been also employed for treatment of atherosclerosis[23,24]. In previous study, we reported that Cho-Deung-san (CDS), which is a Korean herbal formulation including the hook and stem of Uncaria sinensis (Oliv.) Havil. (UR) as an ingredient, has an inhibitory effect on migration and MMP-9 activity of HASMC[25].

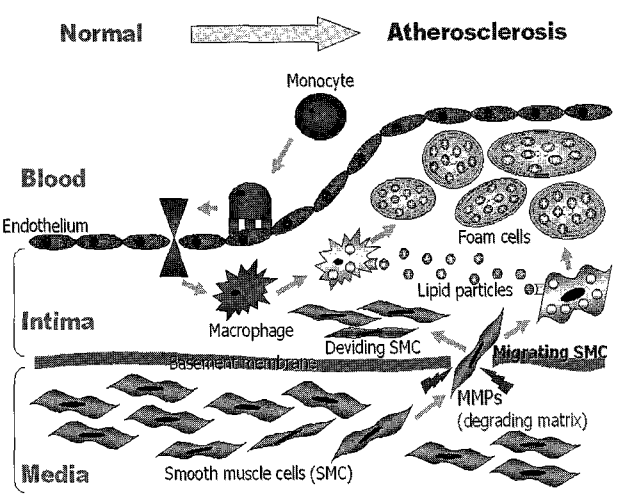
Therefore, we carried out this study to elucidate the inhibitory potential of UR on HASMC migration induced by TNF-α treatment. We also investigated inhibition of UR against MMP-9 activity using gelatin zymography.

Materials and Methods

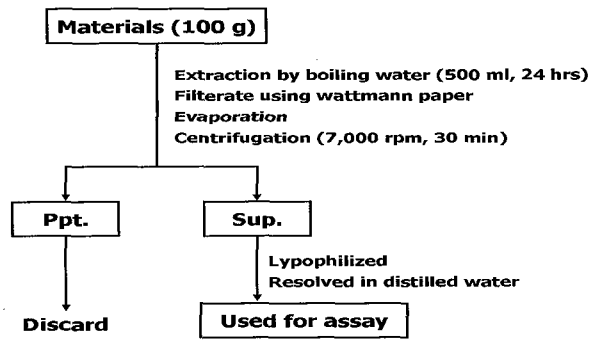
1. Preparation of the plant material extract

The authentic plant material, UR was our deposit[25], which was purchased from local market and identified by the botanical expert at the College of Oriental Medicine, Dongguk University (Gyeongju, Korea).

The dried UR was extracted with boiling water (Scheme 1). Briefly, a 100 g of UR was cut into small sections, boiled in 500 ml of distilled water for 24 hrs. The residues were removed by filtration, and then the filtrate was evaporated to obtain the desired concentration (2 g dry plants equivalent extract/ml). The liquid was centrifuged at 7,000 rpm for 30 min, and the supernatant was lypophilized by freeze-drier to give a powder (yield, 2.35%). The dried extract was resolved in distilled water to appropriate concentrations and the resolved extract was used for analysis.



Scheme 1.The role of migration and MMP production of SMC in development of atherosclerosis.



Scheme 2. Prapatation of the water-extracts of the hook and stem of Uncariae sinensis

2. Cell culture

Human aortic smooth muscle cells (HASMC) were our deposit[14], which were purchased from Bio-Whittaker (CA, USA). HASMC were cultured in smooth muscle cell growth medium-2 containing 10% FBS, 2 ng/ml human basic fibroblast growth factor, 0.5 ng/ml human epidermal growth factor, 50 μg/ml gentamicin, 50 μg/ml amphotericin-B, and 5 μg/ml bovine insulin. For all experiments, early passage of HASMC were grown to 80 - 90% confluence and made quiescent by serum starvation for at least 24 hrs. The serum-free medium, which contains secreted proteins such as MMP-9, was used for gelatin zymography.

3. Gelatin zymography assay

Gelatin zymography was performed as described previously[26] with some modification. Culture supernatants of HASMC treated with or without TNF-α(100 ng/ml) were resuspended in a sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, and 0.00625% (w/v) bromophenol blue and loaded without boiling in 7.5% acrylamide / bisacrylamide (29.2:0.8) separating gel containing 0.1% (w/v) gelatin. Electrophoresis was carried out at a constant voltage of 100 V. After electrophoresis, the gels were soaked in 0.25% Triton X-100 (twice for 30 min) at room temperature and rinsed in distilled pure water.For inhibitory effect of UR on gelatinolytic activity of MMP-9, the samples were freshly solubilized in the Tris-HCl buffer used for incubation of gel to appropriate concentration. The gel slab was cut into slices corresponding to the lanes and was incubated at 37C for 20 hrs in the incubation buffer containing 50 mM Tris-HCl (pH 7.6), 20 mM NaCl, 5 mM $CaCl_2$ and 0.02% Brij-58 with or without the stated concentrations of UR in different tanks. The gel was then stained for 15-30 min in 0.1% (w/v) Coomassie blue R-250 in 30% methanol and 10% acetic acid, and destained in the same solution without the Coomassie blue dye. Proteolysis was detected as a white zone in a dark field and the intensity of the bands obtained from zymogram studies was estimated with

Scion Image (Scion Corp., MA, USA). The values are calculated by percent of control and expressed as means SE.

4. Cell viability assay

The cytotoxic effect of UR on HASMC was investigated using a commercially available proliferation kit (XTT II, Boehringer Mannheim, Mannheim, Germany). Briefly, the cells were plated in 96-well culture plates at a density of 1 104cells per well in DMEM culture medium and allowed to attach for 2 hrs. The UR was added to various final concentrations (and control: 0 g/ml) in triplicates. After 72 hrs of culture, 50 l of XTT reaction solution (sodium 3'-[1-(phenyl-aminocarbonyl)-3, 4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulfonic acid hydrate and N-methyl dibenzopyrazine methyl sulfate; mixed in proportion 50:1) was added to the wells. After the plates were incubated for 4 hrs at the condition of 37°C and 5% CO_2, the optical density at 490 nm was measured by an ELISA plate reader (Molecular device co., CA, USA). All determinations were confirmed using replication in at least three identical experiments. The data were shown mean SE as percent of control.

5. Migration assays

Matrigel migration assay was performed as described previously[27]. Briefly, Matrigel-coated filter inserts (8 m pore size) that fit into 24-well invasion chambers were obtained from Becton-Dickinson (NJ, USA). HASMC cells to be tested for invasion were detached from the tissue culture plates, washed, resuspended in conditioned medium (5×10^4 cells/200 l), and then added to the upper compartment of the invasion chamber in the presence or absence of TNF-α(100 ng/ml) and various concentration of UR (0, 50, 100, 250 and 500 g/ml). The serun-free medium (500 l), which incubated HASMC for 24 hrs, was added to the lower compartment of the invasion chamber. The Matrigel invasion chambers were incubated at 37°C for 24 hrs in 5% CO_2. After incubation, the filter inserts were removed from the wells, and the cells on the upper side of the filter were removed using cotton swabs. The filters were fixed, mounted, and stained according to the manufacturer's instructions (BD bioscience, NJ, USA). The cells that migrated through the Matrigel and were located on the underside of the filter were counted. Three to five invasion chambers were used per condition. The values obtained were calculated by averaging the total number of cells from three filters.

Results

1. Cytotoxicity of UR on HASMC cells

The cytotoxity of the UR on the HASMC cells were evaluated using XTT cell proliferation assay kit. The 5104 cells were incubated for 24 hrs in cultures in 96-well microplates (volume 100 l/well) with various final concentration of UR (0, 50, 100, 250 and 500 g/ml). Dose-dependent cytotoxic effect of UR against HASMC was shown in Fig. 1. UR has a weak cytotoxicic effect on HASMC cells in a typical concentration (IC50 > 500 g/ml).

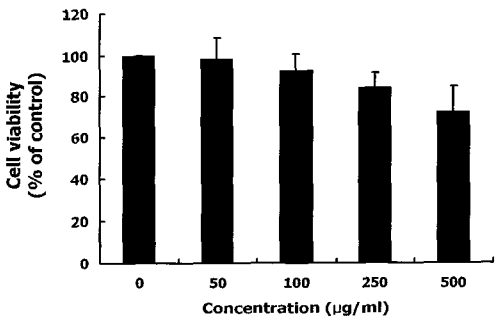


Fig. 1. Effect of UR on HASMC cells viability. The cytotoxity of the UR on the HASMC cells were evaluated using XTT cell proliferation assay kit. The 5104cells were incubated for 24 hrs in cultures in 96-well microplates (volume 100 l/well) with various concentration of UR (0, 50, 100, 250 and 500 g/ml). The values were calculated by % of control and expressed as means SE.

2. Inhibitory effect of UR on the MMP-9 activity

To examine the inhibitory activity of UR against MMP-9 activity, the cultured conditioned media obtained from TNF-α (100 ng/ml) treated HASMC cells have been subjected to the gelatin zymography in the presence of various concentrations of UR (0, 50, 100, 250 and 500 g/ml). As shown in Fig. 2, UR inhibited the MMP-9 activity, which were induced by TNF-α treatment, in a dose-dependent manner (IC50 = 55 g/ml).

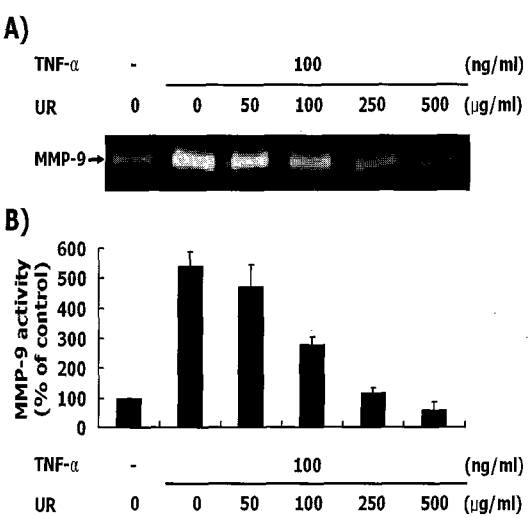


Fig. 2. Effect of UR on the MMP-9 activity of TNF-α induced HASMC cells. (A) The zymography was performed with conditioned media of HASMC cells in the presence or absence of TNF-α and UR (0, 50, 100, 250 and 500 g/ml). The experiments were repeated 3 times and resulted in a similar outcome. (B) The densitometric intensity of the zymographic bands was estimated as described in materials and methods. The values were calculated by % of control and expressed as means SE.

3. Inhibitory effect of UR on HASMC cells migration

HASMC was suspended in conditioned medium (5×10^4 cells/200 l), added to the upper components of Matrigel invasion chamber supplemented with various final concentrations of UR (0, 50, 100, 250 and 500 g/ml) in presence of TNF-α (100 ng/ml). The cells were incubated for 24 hrs at 37C and 5% CO_2. As shown in Fig. 3, the total number of cells that invaded to the underside of the filters was significantly decreased by UR treatment, in a dose dependent manner (IC50 = 125 g/ml).

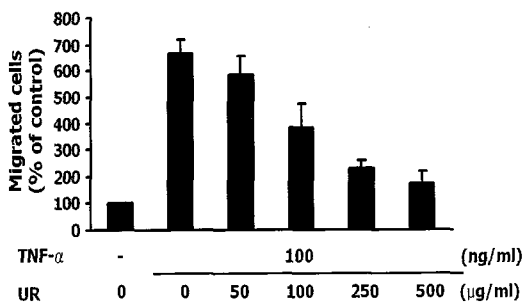


Fig. 3. Effect of UR on HASMC cells migrarion induced by TNF-α treatment. HASMC cells were resuspended in conditioned medium (5104 cells/200 l) and added to the upper components of Matrigel invasion chamber supplemented with or without 100 ng/ml of TNF-α and various concentration of UR (0, 50, 100, 250 and 500 g/ml). After incubating for 24 hrs, underside of Matrigel filter was stained with hematoxylin & eosinand the total number of cells that migrated to the underside of the filters was counted. The values, which were calculated by averaging the total number of cells from three filters, were expressed as % of control.

4. Relative MMP-9 inhibitory activity of UR compared with established MMP-9 inhibitors

The inhibitory effect against MMP-9 activity of UR was compared with established MMP inhibitors such as catechin, caffeic acid and their derivates.

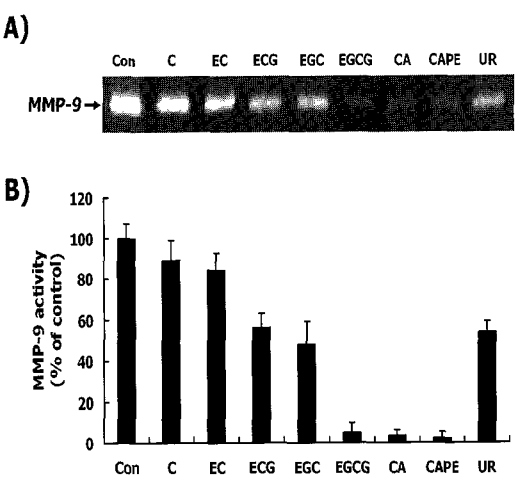


Fig. 4. Comparison of inhibitory effect of UR with established MMP inhibitors on the MMP-9 activity. (A) Gelatin zymography of conditioned media of TNF-α (100 ng/ml) treated HASMC cells were incubated with DMSO (Con) or 50 g/ml of catechin (C), epicatechin (EC), epicatechin galate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), caffeic acid (CA), caffeic acid phenyl ester (CAPE) and UR (UR). The experiments were repeated 3 times and resulted in a similar outcome. (B) The densitometric intensity of the zymography bands was estimated as described in Materials and Methods. The values are calculated by % of control and expressed as means±SE.

As shown in Fig. 4, the inhibitory effect of UR against gelatinolytic activity of MMP-9 was higher than that of catechin and epicatechin (EC), but lower than that of epigallocatechin gallate (EGCG), caffeic acid (CA) and caffeic acid phenyl ester (CAPE). The inhibitory potent of UR against MMP-9 was almost equal to that of epicatechin galate (ECG) and epigallocatechin (EGC).

Discussion

Proliferation and migration of VSMC play an important role in the pathogenesis of atherosclerosis and restenosis after vascular injury[1]. On injury, VSMC migrate from the tunica media to the intima, leading to neointima formation[28]. As VSMC in the media are entirely surrounded by basal lamina and extracellular matrices, VSMC migration requires breakdown of extracellular matrix[29,30]. Among these extracellular proteinases, the matrix metalloproteinases (MMPs) have been shown to play an essential role[30-32]. In the past several years, a number of studies have demonstrated that MMPs, specifically MMP-2 and MMP-9, are important for smooth muscle cell proliferation and migration into the intima[10]. Of these studies, a recent report by Cho et al.[13] showed that TNF-αmarkedly induces the expression of MMP-9. In addition, knock-out study indicated that MMP-9 is critical for the development of arterial lesions by regulating both migration and proliferation of VSMC[11].

Several natural products have been used for atherosclerosis[22]. Especially, baicalein[33] and green tea catechins[34] inhibit proliferation and migration of VSMC, respectively. Some traditional Korean fomulations were employed for treatment of atherosclerosis[24,35]. Previously, we reported that Cho-Deung-san (CDS), which is a Korean herbal formulation including the hook and stem of Uncaria sinensis (Oliv.) Havil. (UR) as an ingredient, has an inhibitory effect on migration and MMP-9 production of HASMC[25].

As shown in Fig. 1, UR has very weak cytotoxic activities on HASMC cells, and the IC50 was higher than 500 g/ml. As our group previously reported, the MMP-9 activity and migratory potential of HASMC was significantly increased by TNF-α treatment[12]. The MMP-9 activity, which was highly increased by 100 ng/ml of TNF-α treatment, was significantly inhibited by UR treatment, in dose-dependent manners (Fig. 2). The IC50 for MMP-9 activity was 55 g/ml. In addition, UR inhibited migration of TNF-α treated HASMC cells in a dose-dependent manner. The IC50 for HASMC migration was 125 g/ml, and the concentration is very lower than that at which it affect the viability of HASMC cells (Fig. 3).

Some synthetic MMP inhibitors are currently in clinical trials but carry undesirable side effects[36-38]. Therefore, understanding the molecular mechanisms by which the natural compound from the herbal drugs inhibits the activity of MMP-9 is important in exploring its properties for prevention and treatment of vascular disease. Among these natural products, catechin and its derivates,which are found in a variety of plants and present particular high amounts in green tea leaves, have been reported for potent anti-atherosclerotic agent[34,39,40]. Among the derivates of cathecin, EGCG caused a strong inhibition of the gelatinolytic activities of MMP-9[41]. In addition, we previously demonstrated that CA and CAPE have very strong inhibitory effect on MMP-9 activity[42]. Therefore, we assayed the inhibitory effect against MMP-9 activity of UR and established MMP inhibitors, such as catechin, cathecin derivates, CA and CAPE, in same condition of gelatin zymography. The inhibitory effect of UR against MMP-9 activity was higher than that of catechin and lower than that of EGCG, CA and CAPE (Fig. 4).

In conclusion, these results showed that UR has an inhibitory effect on TNF-α induced HASMC migration through inhibition of MMP-9 activity.

Conclusion

The migration of vascular smooth muscle cells (VSMC) and the production of matrix metalloproteinases-9 (MMP-9) may play a key role in the development of atherosclerosis. In this study, we have more extensively investigated the inhibitory effect of UR on MMP-9 activity and TNF-α induced human aortic smooth muscle cells (HASMC) migration. The result from gelatin zymography showed that UR inhibited MMP-9 activity in a dose-dependent manner (IC50 = 55 g/ml). In addition, UR strongly inhibited the migration of HASMC induced by TNF-α treatment (IC50 = 125 g/ml), although it has very low cytotoxic effect on HASMC (IC50 > 500 g/ml). These results suggest that UR is a potential anti-atherosclerotic agent through inhibition of MMP-9 activity and VSMC migration.

References

1. Ross, R. The pathogenesis of atherosclerosis: an update. N. Engl. J. Med. 314:488-500, 1986.
2. Chamley-Campbell, J., Campbell, G.R., Ross, R. The smooth muscle cells in culture. Physiol. Rev. 59:1-61, 1979.
3. Schwartz, R.S., Holmes, D.R., Topol, E.J. The restenosis paradigm revisited: an alternative proposal for cellular mechanisms. J. Am. Coll. Cardiol. 20:1284-1293, 1992.
4. Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801-809, 1993.
5. Matrisian, L.M. Metalloproteinases and their inhibitors in matrix remodeling. Trends. Genet. 6:121-125, 1990.
6. Sasaguri, Y., Murahashi, N., Sugama, K., Kato, S., Hiraoka, K., Satoh, T., Isomoto, H., Morimatsu, M. Development-related changes in matrix metalloproteinase expression in human aortic smooth muscle cells. Lab. Invest. 71:261-269, 1994.
7. GalisZ, S., Muszynski, M., Sukhova, G.K., Simon-Morrissey, E., Unemori, E.N., Lark, M.W., Amento, E., Libby, P. Cytokinestimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. Circ. Res. 75:181-189, 1994.
8. Strauss, B.H., Chisholm, R.J., Keeley, F.W., Gotlieb, A.I., Logan, R.A., Armstrong, P.W. Extracellular matrix remodeling after balloon angioplasty injury in a rabbit model of restenosis. Circ. Res. 75:650-658, 1994.
9. Woessner, J.F. Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J. 5:2145-2154, 1991.
10. Newby, A.C., Zaltsman, A.B., Molecular mechanisms in intimal hyperplasia. J. Pathol. 190:300-309, 2000.
11. Galis, Z.S., Johnson, C., Godin, D., Magid, R., Shipley, J. M., Senior, R.M., Ivan, E., Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. Circ. Res. 91:852-859, 2002.
12. Moon, S.K., Cha, B.Y., Kim, C.H. ERK1/2 mediates TNF-α induced matrix metalloproteinase-9 expression in human vascular smooth muscle cells via the regulation of NF-B and AP-1: involvement of the Ras dependent pathway. J. Cell. Physiol. In press. 2003.
13. Cho, A., Graves, J., Reidy, M.A., Mitogen-activated protein kinases mediate matrix metalloproteinase-9 expression in vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 20:2527-2532, 2000.
14. Moon, S.K., Cho, G.O., Jung, S.Y., Gal, S.W., Kwon, T.K., Lee, Y.C., Madamanchi, N.R., Kim, C.H. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2, cellcycle regulation, and matrix metalloproteinase-9. Biochem. Biophys. Res. Commun. 301:1069-1078, 2003.
15. Moon, S.K., Cha, B.Y., Kim, C.H. In vitro cellular aging is associated with enhanced proliferative capacity, G1 cell cycle modulation, and matrix metalloproteinase-9 regulation in mouse aortic smooth muscle cells. Arch. Biochem. Biophys. 418:39-48, 2003.

16. Brew, K., Dinakarpandian, D., Nagase, H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim. Biophys. Acta. 1477:267-283, 2000.
17. Mandal, M., Mandal, A., Das, S., Chakraborti, T., Sajal, C. Clinical implications of matrix metalloproteinases. Mol. Cell. Biochem. 252:305-329, 2003.
18. Zempo, N., Koyama, N., Kenagy, R.D., Lea, H.J., Clowes, A. W. Regulation of vascular smooth muscle cell migration and proliferation in vitro and in injured rat arteries by a synthetic matrix metalloproteinase inhibitor. Arterioscler. Thromb. Vasc. Biol. 16:28-33, 1996.
19. Bendeck, M.P., Irvin, C., Reidy, M.A. Inhibition of matrix metalloproteinase activity inhibits smooth muscle cell migration but not neointimal thickening after arterial injury. Circ. Res. 78:38-43, 1996.
20. Kenagy, R.D., Vergel, S., Mattsson, E., Bendeck, M., Reidy, M.A., Clowes A.W. The role of plasminogen,plasminogen activators, and matrix metalloproteinases in primate arterial smooth muscle cell migration. Arterioscler. Thromb. Vasc. Biol. 16:1373-1382, 1996.
21. Fitzgerald, M., Hayward, I.P., Thomas, A.C., Campbell, G.R., Campbell, J.H. Matrix metalloproteinases can facilitate the heparanase-induced promotion of phenotypic change in vascular smooth muscle cells. Atherosclerosis 145:97-106, 1999.
22. Heber, D. Herbs and atherosclerosis.Curr. Atheroscler. Rep. 3:93-96, 2001.
23. Yoshie, F., Iizuka, A., Kubo, M., Komatsu, Y., Matsumoto, A., Itakura, H., Takeda, H., Matsumiya, T., Kondo, K. Protective effects of Saiko-ka-ryukotsu-borei-to (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang) against atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. Pharmacol. Res. 43:481-488, 2001.
24. Kim, B.J., Kim, Y.K., Park, W.H., Ko, J.H., Lee, Y.C., Kim, C.H. A water-extract of the Korean traditional formulation Geiji-Bokryung-Hwan reduces atherosclerosis and hypercholesteremia in cholesterol-fed rabbits. Int. Immunopharmacol. 3:723-734, 2003.
25. Ha, K.T., Lee, T.K., Kwak, K.H., Kim, J.K., Kim, D.I., Choi, D.Y., Kim, C.H. Inhibitory effect of Cho-Deung-San on human aortic smooth muscle cell migration induced by TNF-αalpha through inhibition of matrix metalloproteinase-2 and - 9 activity. Vascul. Pharmacol. 41:83-90, 2004.
26. Cha, B.Y., Park, C.J., Lee, D.G., Lee, Y.C., Kim, D.W., Kim, J.D., Seo, W.G., Kim, C.H. Inhibitory effect of methanol extract of Euonymus alatus on matrix metalloproteinase-9. J Ethnopharmacol. 85:163-167, 2003.
27. Chung, T.W., Moon, S.K., Lee, Y.C., Kim, J.G., Ko, J.H., Kim, C.H. Enhanced expression of matrix metalloproteinase-9 (MMP-9) by hepatitis B virus infection into liver cells. Arch. Biochem. Biophys. 408:147-154, 2002.
28. Ross, R. Cell biology of atherosclerosis, Annu. Rev. Physiol. 57:791-804, 1995.
29. Dollery, C.M., McEwan, J.R., Henney, A.M. Matrix metalloproteinases and cardiovascular disease, Circ. Res. 77:863-868, 1995.
30. Mason, D.P., Kenagy, R.D., Hasenstab, D., Bowen-Pope, D.F., Seifert, R.A., Coats, S., Hawkins, S.M., Clowes, A.W. Matrix metalloproteinase-9 overexpression enhances vascular smooth muscle cell migration and alters remodeling in the injured rat carotid artery, Circ. Res. 85:1179-1185, 1999.
31. Pauly, R.R., Passaniti, A., Bilato, C., Monticone, R., Cheng, L., Papadopoulos, N., Gluzband, Y.A., Smith, L., Weinstein, C., Lakatta, E.G. Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity and is inhibited by cellular differentiation, Circ. Res. 75:41-54, 1994.
32. Cho, A., Reidy, M.A. Matrix metalloproteinase-9 is necessary for the regulation of smooth muscle cell replication and migration after arterial injury, Circ. Res. 91:845-851, 2002.
33. Huang, H.C., Wang, H.R., Hsieh, L.M. Antiproliferative effect of baicalein, a flavonoid from a Chinese herb, on vascular smooth muscle cell. Eur. J. Pharmacol. 251:91-93, 1994.
34. Maeda, K., Kuzuya, M., Cheng, X.W., Asai, T., Kanda, S., Tamaya-Mori, N., Sasaki, T., Shibata, T., Iguchi, A. Green tea catechins inhibit the cultured smooth muscle cell invasion through the basement barrier. Atherosclerosis 166:23-30, 2003.
35. Kim, D.W., Chung, H.J., Nose, K., Maruyama, I., Tani, T. Preventive effects of a traditional Chinese formulation, Chaihu-jia-Longgu-Muli-tang, on intimal thickening of carotid artery injured by balloon endothelial denudation in rats. J. Pharm. Pharmacol. 54:571-575, 2002.
36. Tonn, J.C., Kerkau, S., Hanke, A., Bouterfa, H., Mueller, J.G., Wagner, S., Vince, G.H., Roosen, K. Effect of synthetic matrixmetalloproteinase inhibitors on invasive capacity and proliferation of human malignant gliomas in vitro. International journal of cancer 80:764-772, 1999.
37. Jiang, M.C., Liao, C.F., Lee, P.H. Aspirin inhibits matrix metalloproteinase-2 activity, increases E-cadherin production, and inhibits in vitro invasion of tumor cells. Biochemical and biophysical research communications 282:671-677, 2001.
38. Kleiner, D.E., Stetler-Stevenson, W.G. Matrix metalloproteinases and metastasis. Cancer chemotherapy and pharmacology 43:42-51, 1999.
39. Hofmann, C.S., Sonenshein, G.E. Green tea polyphenol

epigallocatechin-3 gallate induces apoptosis of proliferating vascular smooth muscle cells via activation of p 53. FASEB J. 17:702-704, 2003.

40. Miura, Y., Chiba, T., Tomita, I., Koizumi, H., Miura, S., Umegaki, K., Hara, Y., Ikeda, M., Tomita, T. Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. J. Nutr. 131:27-32, 2001.
41. Demeule, M., Brossard, M., Page, M., Gingras, D., Beliveau, R. Matrix metalloproteinase inhibition by green tea catechins. Biochimica et Biophysica Acta 1478:51-60, 2000.
42. Chung, T.W., Moon, S.K., Chang, Y.C., Ko, J.H., Lee, Y.C., Cho, G., Kim, S.H., Kim, J.G., Kim, C.H. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J. 18:1670-1681.