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Simvastatin Reduces Secretion of Monocyte Chemoattractant Proteins and Matrix Metalloproteinase-9 in Human Abdominal Aortic Aneurysms

Junichi Kudo,[1] *Koichi Yoshimura*[2] *and Kimikazu Hamano*[1]

[1] Department of Surgery and Clinical Science, Yamaguchi University, Graduate School of Medicine, 1-1-1, Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[2] Department of Molecular Cardiovascular Biology, Yamaguchi University School of Medicine, 1-1-1, Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
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Abstract Recent studies have suggested that HMG-CoA reductase inhibitors (statins) attenuate the growth of human abdominal aortic aneurysm (AAA), which is characterized by chronic inflammation and degradation of the extracellular matrix by metalloproteinases (MMPs). While the effects of statins on inflammatory proteins have been reported, the molecular mechanism for the effect of statins on human AAA is largely unknown. Thus, the aim of this study was to elucidate the direct effect of statin on proinflammatory mediators in human AAA walls. Among 79 molecules analyzed by cytokine array, monocyte chemoattractant protein (MCP)-1 and MCP-3 were identified as markedly increased chemokines in human AAA. Both MCPs were mainly localized in inflammatory cells. MCP-1 was also localized in vascular smooth muscle cells in AAAs. In *ex vivo* cultures of human AAA walls, simvastatin significantly suppressed the secretion of MCP-1, MCP-3 and MMP-9 under both basal and tumor necrosis factor (TNF)-α-stimulated conditions. Moreover, application of exogenous MCP-3 increased secretion of MMP-9 in AAA culture. These data indicate that simvastatin reduces secretion of MCPs and MMP-9 in human AAA walls, suggesting one of the mechanisms for the inhibitory effect of statins on human AAA progression.

Key words: aortic aneurysm, statin, MCP-1, MCP-3, MMP-9

Introduction

Abdominal aortic aneurysms (AAA) are associated with chronic inflammation and degradation of the extracellular matrix by proteolytic enzymes such as matrix metalloproteinases (MMPs), leading to abnormal dilatation of the aortic wall and eventual fatal rupture.[1-3] AAA is one of the top 10 causes of death among elderly men.[4] Currently, patients with large aneurysms are regarded as at high risk of rupture, and are therefore treated by open or endovascular repair. Patients with small aneurysms are recommended for close observation,[5] because of the current lack of effective non-surgical options for these patients. Pharmacologic therapy for AAAs has been much awaited.

A class of cholesterol-lowering drugs, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), have gained a great deal of attention because of their pleiotropic effects, which may be beneficial in various vascular diseases.[6][7] Interestingly, recent clinical studies suggested that statins attenuate the growth of human AAAs.[8][9] It has been reported that statins inhibit the secretion of MMP-9, a major proteolytic

enzyme in the pathogenesis of AAA, in the human AAA walls *in vitro*[10] and *in vivo*.[11] In addition, simvastatin, a lipophilic statin, suppresses the development of experimental AAAs.[12)13)] However, the precise mechanisms by which statins affect the growth rate of human AAA have not been elucidated.

Because statins are reported to reduce several inflammatory mediators in vascular cells,[6)7)] we hypothesized that statins inhibit the progression of human AAA not only by inhibiting MMP-9 secretion but also by reducing other proinflammatory mediators, such as cytokines and chemokines. Here, we identified MCP-1 and MCP-3 as prominently upregulated chemokines in human AAA walls. Moreover, we demonstrated that simvastatin significantly suppressed the secretion of these MCPs, as well as MMP-9, from human AAA walls in culture.

Materials and methods

Tissue procurement

AAA walls were obtained from 8 patients undergoing surgical repair. Control abdominal aortae were obtained from 4 patients who died of unrelated causes. Neither the control patients nor the AAA patients took any statins prior to or during the study. The tissue samples of these AAA walls were immediately placed in phosphate-buffered 10% formalin for immunohistochemistry or snap-frozen in liquid nitrogen for protein extraction. For organ culture experiments, fresh AAA walls were placed in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) under sterile conditions. All of the experimental protocols using human specimens were approved by the Institutional Review Board at Yamaguchi University Hospital.

Protein isolation

Proteins were extracted from AAA walls homogenized in 25 mM Tris, pH 7.5, containing 150 mM NaCl, 5 mM EDTA, 1% Triton-X 100, 1 mM phenylmethane sulfonyl fluoride, and 10 μg/ml aprotinin. Protein concentrations were determined with a protein assay kit (Bio-Rad, Hercules, CA, USA).

Cytokine array

Protein samples from 8 AAA patients and from 4 control patients were pooled and applied to the antibody-membrane array for various cytokines and chemokines, as recommended by the manufacturer (Raybiotech, Norcross, GA, USA). Biotinylated antibody was added, followed by HRP-conjugated streptavidine. After treatment with detection reagents, the membranes were developed using a luminoimage analyzer LAS-1000 (Fuji Film, Tokyo, Japan).

Quantification of MCP1 and MCP-3

The concentration of MCP-1 or MCP-3 in the protein samples from the conditioned media of AAA organ culture was quantified using an MCP-1 or MCP-3 ELISA Kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

Immunohistochemistry

Paraffin-embedded sections were stained with antibodies to appropriate antigens. In brief, AAA samples were fixed in phosphate-buffered 10% formalin and processed for paraffin embedding. The slides were deparaffinized, rehydrated, and processed for immunohistochemistry. Antigen retrieval was performed with Antigen Retrieval Solution H (Mitsubishi Kagaku Iatron, Tokyo, Japan) for 20 minutes at 121℃. The slides were incubated with normal serum for 30 minutes before incubation with the appropriate antibodies. MCP-1 and MCP-3 proteins were detected with a mouse anti-human MCP-1 antibody and a mouse anti-human MCP-3 antibody (R&D Systems), respectively. The probed proteins were visualized using the avidin-biotin complex technique with the VECTASTAIN ABC-AP kit (Vector Laboratories, Burlingame, CA, USA).

Organ culture of AAA

Organ culture was performed using a method described previously.[3)] Briefly, each AAA wall obtained during surgery was cut into several pieces and cultured with serum-free DMEM supplemented with penicillin and streptomycin in an atmosphere of 95% air / 5% CO_2 at 37℃. Twenty-four hours after the start of the culture, the conditioned media

were collected and used for internal standardization. The media were then changed, and simvastatin (Merck Biosciences, San Diego, CA, USA) was added at the indicated concentrations. In some experiments, 100 μM mevalonate (Sigma, St. Louis, MO, USA) was added. The culture media were changed every 48 hours after treatment. Conditioned media between 96 and 144 hours after treatment were collected as samples. In some experiments, 50 ng/ml of tumor necrosis factor-alpha (TNF-α, R&D Systems) was added.

Gelatin zymography

Gelatin zymography was performed as described previously.[14] Briefly, an equal volume of conditioned media were electrophoresed in the presence of 0.2% sodium dodecyl sulfate (SDS) in 10% polyacrylamide gels containing gelatin (1 mg/ml) at 4℃ under nonreducing conditions. After electrophoresis, the gels were washed twice in 2.5% Triton-X 100 at room temperature to remove SDS, followed by overnight incubation at 37 ℃ with gentle shaking in developing buffer containing 50 mM Tris, pH 7.5, 200 mM NaCl, 5 mM $CaCl_2$, and 0.02% Briji35. After the incubation, the gels were stained with 0.5% Coomassie brilliant blue (CBB) R-250 in 40% methanol and 10% acetic acid, and then were destained with 40% methanol and 10% acetic acid. Gelatinolytic activity on the gel, which indicates the protein expression levels of gelatinolytic MMPs in the conditioned media, was determined by clear bands against a dark background staining of gelatin in the gel.

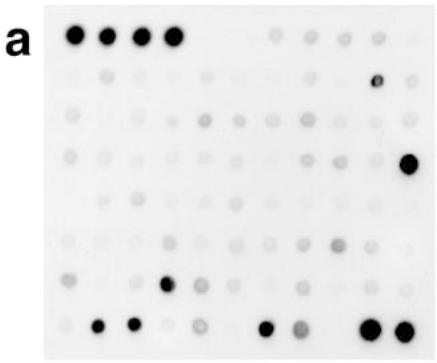
Statistical analysis

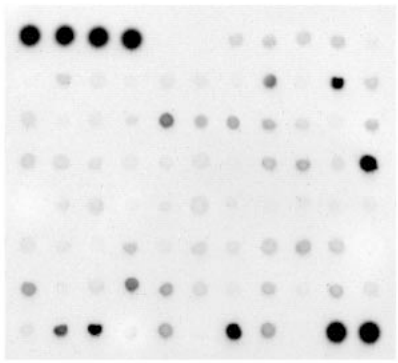
All data are expressed as means ± standard errors (SE). Statistical analyses were performed using Student's unpaired *t*-test or analysis of variance (ANOVA), in which the post-test comparison was performed by the Bonferroni method.

Results

Comprehensive analysis of cytokines and chemokines in human AAA walls

We first analyzed 79 cytokines and chemokines by cytokine array in human aortic walls with or without AAA. As shown in the representative images in Fig. 1, the protein expression levels of interleukin (IL)-6,





c

	A	B	C	D	E	F	G	H	I	J	K
1	Pos	Pos	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α
2	I-309	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10
3	IL-12	IL-13	IL-15	IFN-γ	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1β
4	MIP-1δ	RANTES	SCF	SDF-1	TARC	TGF-β1	TNF-α	TNF-β	EGF	IGF-I	Angio-genin
5	Onco-statin M	Thrombo-poietin	VEGF	PDGF-BB	Leptin	BDNF	BLC	Ckβ8-1	Eotaxin	Eotaxin-2	Eotaxin-3
6	FGF-4	FGF-6	FGF-7	FGF-9	Flt-3 Ligand	Fractalkine	GCP-2	GDNF	HGF	IGFBP-1	IGFBP-2
7	IGFBP-3	IGFBP-4	IL-16	IP-10	LIF	LIGHT	MCP-4	MIF	MIP-3α	NAP-2	NT-3
8	NT-4	Osteo-protegerin	PARC	PIGF	TGF-β2	TGF-β3	TIMP-1	TIMP-2	Neg	Pos	Pos

Fig. 1 Array analysis of cytokines in human AAA
Protein samples of aortic walls were obtained from 8 patients with abdominal aortic aneurysm (AAA) and from 4 individuals without AAA (Control). The pooled samples were prepared from AAA or Control samples and analyzed with a cytokine array. Representative images of developed membranes are shown for Control (a) and AAA (b) samples. A legend for the array explaining the location of controls and probes on the membrane is also shown (c).

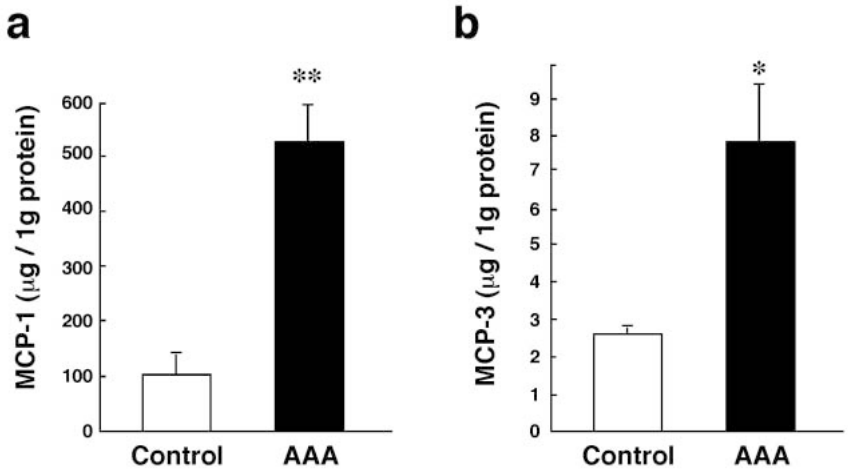


Fig. 2 Expression of MCP-1 and MCP-3 in human AAA
Protein samples of aortic walls were obtained from 8 patients with abdominal aortic aneurysm (AAA) and from 4 individuals without AAA (Control). The protein expressions of MMP-1 and MCP-3 were determined by ELISA. Quantitative analyses are shown for MCP-1 (a) and MCP-3 (b). Data are means ± SE. * and ** indicate $P < 0.05$ and $P < 0.01$ compared to Control, respectively.

monocyte chemoattractant protein (MCP)-1 and MCP-3 were apparently upregulated in human AAA compared to the control. MCP-2 and insulin-like growth factor binding protein (IGFBP)-3 were slightly increased in AAA. All of these proinflammatory mediators are possible mediators that may play a role in the pathogenesis of human AAA.[15-17)22)] No cytokines analyzed were found to be decreased in AAA compared to the control aorta. We focused on MCP-1 and MCP-3 among these five proteins identified by our array analysis, because both MCPs were highly expressed chemokines in human AAA and may participate in the early and critical steps of AAA development by recruiting various inflammatory cells.

Expression of MCP-1 and MCP-3 in human AAAs

We next confirmed the protein expression of MCP-1 and MCP-3 by ELISA. As shown in Fig. 2a, the expression of MCP-1 was markedly higher in the AAAs than in the controls (5.0 ± 0.7 fold, $p < 0.01$). MCP-3 was also significantly higher in the AAAs than in the controls (Fig. 2b, 2.9 ± 0.6 fold, $p < 0.05$).

To investigate the possible roles of these MCPs in the AAA, we examined their tissue localization in AAA walls by immunohistochemistry. As described in previous reports, we observed thin media with few elastic fibers and some smooth muscle cells, and thick adventitia with inflammatory cells surrounding new blood vessels in human AAA walls. In our immunostaining, MCP-1 and MCP-3 were predominantly found in the thick adventitia, and both MCPs appeared to be localized in the oval-shaped cells around neovasculature, which appeared to be inflammatory cell infiltrates (Fig. 3b for MCP-1 and Fig. 3d for MCP-3, respectively). These MCPs were also observed in the outer media of AAA walls. Interestingly, MCP-1 was detected not only in infiltrating cells but also in the elongated and spindle-shaped cells at the thin media, which appeared to be residual vascular smooth muscle cells. In contrast, MCP-3 was not seen in such cells (Fig. 3a for MCP-1 and Fig. 3c for MCP-3, respectively).

Effect of simvastatin on MCP-1 and MCP-3 in human AAAs

We next examined the effect of statins on the expression of MCP-1 and MCP-3 in AAAs. The spontaneous secretion of MCP-1 from AAA walls in culture was significantly inhibited by 10 mM simvastatin compared to the vehicle (Fig. 4a, 18% reduction, $p < 0.01$) as determined by ELISA. Interestingly, the

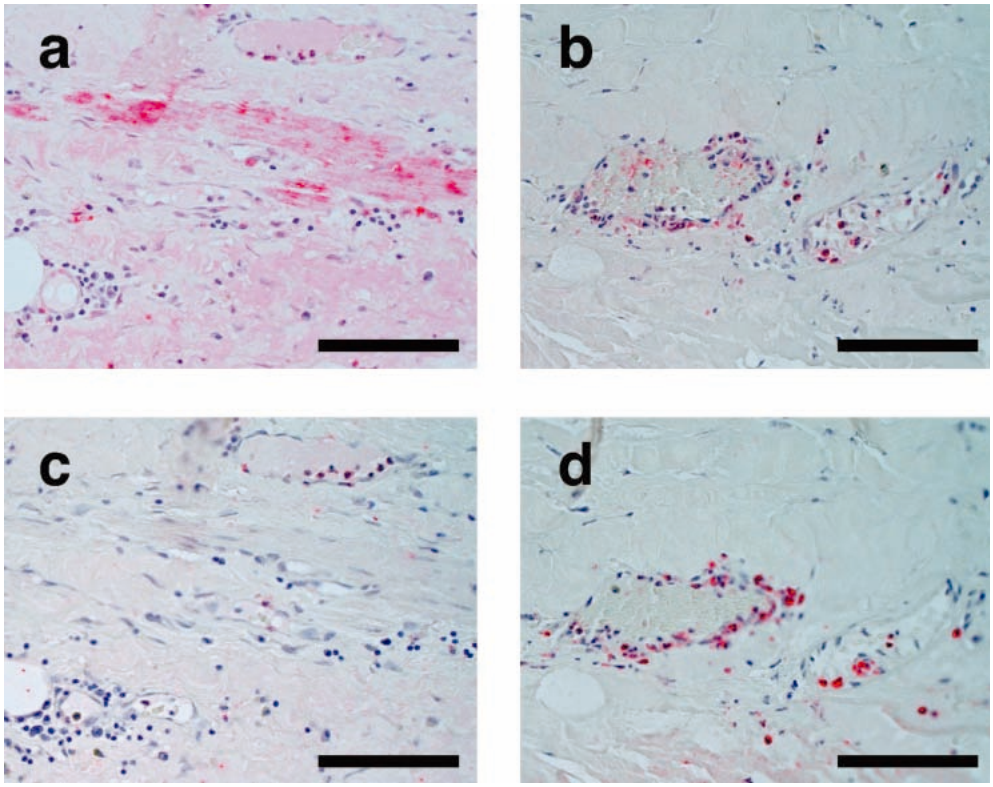


Fig. 3 Localization of MCP-1 and MCP-3 in human AAA
The immuno-localization of MCP-1 or MCP-3 in human AAA is shown by immunohistochemistry as indicated by red staining. Representative images are shown for MCP-1 (a) and MCP-3 (b) at the outer media in the serial sections of human AAA wall, and are also shown for MCP-1 (c) and MCP-3 (d) at the adventitia in the serial sections of human AAA wall. Hematoxylin stains show cell nuclei as indicated by blue counter-staining. Scale bars indicate 100 μm.

MCP-3 secretion in AAA was drastically suppressed by 10 μM simvastatin compared to the vehicle (Fig. 4b, 88% reduction, $p < 0.01$). To determine whether the inhibitory effect of simvastatin was due to inhibition of HMG-CoA reductase and consequent deprivation of mevalonate, we next examined the effect of exogenous mevalonate on the secretion of MCPs. Addition of mevalonate (100 μM) significantly reversed the suppressive effect of simvastatin on MCP-3 protein secretion (Fig. 4b, 96% recovery, $p < 0.01$), and also tended to reverse the effect of simvastatin on MCP-1 (Fig. 4a, 38% recovery, $p=0.10$). These data indicate that simvastatin inhibits the secretion of both MCP-1 and MCP-3 in AAAs in a mevalonate pathway-dependent manner.

It has been reported that TNF-α, a proinflammatory cytokine, is elevated in both the serum and aneurysm walls of patients with AAA.[18)19)] We found that addition of TNF-α (50 ng/ml) caused a large increase in MCP-3 secretion from AAA walls in culture (Fig. 4d, 2.4 ± 0.4 fold, $p < 0.01$ compared to control). Simvastatin (10 μM) dramatically suppressed MCP-3 secretion induced by TNF-α (Fig. 4d, 90% reduction, $p < 0.01$). Simvastatin also inhibited the secretion of MCP-1 under the conditions with TNF-α (Fig. 4c, 18% reduction, $p < 0.05$), although no effect of TNF-α on MCP-1 secretion was detected. These data demonstrate that simvastatin significantly suppresses the secretion of MCP-1 and MCP-3 under both basal and TNF-α-stimulated conditions. In addition, secretion levels of MCP-1 and MCP-3 were not equally changed in response to simvaα statin or TNF-α, suggesting that the expression of two MCPs may be regulated in different ways.

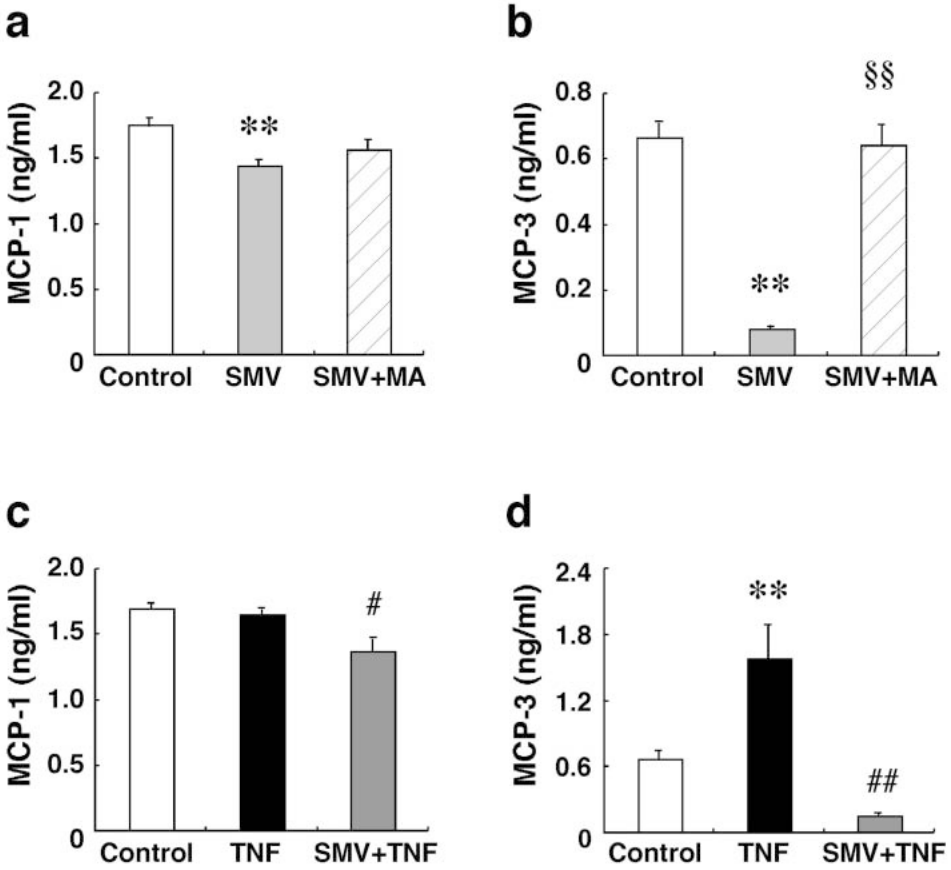


Fig. 4 Effect of simvastatin on secretion of MCP-1 and MCP-3
A fresh human AAA wall was cut into several pieces and cultured with simvastatin (10 μM), mevalonate (100 μM) or vehicle (Control). The amounts of secreted MCP-1 and MCP-3 in the conditioned media were determined by ELISA. Quantitative analyses are shown for MCP-1 (a) and MCP-3 (b). Fresh human AAA wall in *ex vivo* culture was treated by 50 ng/ml of TNF-α with or without simvastatin (10 μM). Quantitative analyses are shown for MCP-1 (c) and MCP-3 (d) in the conditioned media. Data are means ± SE of 4 independent observations. ** and § § indicate $P < 0.01$ compared to Control and to the culture with simvastatin but without mevalonate, respectively. # and ## indicate $P < 0.05$ and $P < 0.01$ compared to the culture with TNF-α but without simvastatin.

Effect of simvastatin or MCPs on MMP-9 in human AAAs

Previous reports demonstrated that MMP-9 is upregulated in human AAA and that inhibition of MMP-9 results in the suppression of AAA development in animal models, indicating that MMP-9 is one of the key molecules in the pathogenesis of AAA.[1)] MCP-1 and MCP-3, which were originally identified as chemokines with chemotactic activity for monocytes, have been reported to affect several other functions of monocytes, including the secretion of MMP-9.[20–22)] In addition, MMP-9 has been used as a biomarker of degenerative aneurysm disease in both experimental and clinical studies .[1)10)11)] Therefore, we next examined the effect of simvastatin, MCP-1 and MCP-3 on MMP-9 secreted from human AAA walls in culture.

As shown by the representative gelatin zymogram in Fig. 5a, human AAA walls secreted significant amounts of MMP-9 into culture media without any treatment. This spontaneous secretion of MMP-9 was significantly reduced by 10 μM simvastatin compared to the vehicle (Fig. 5b, 29% reduction, p

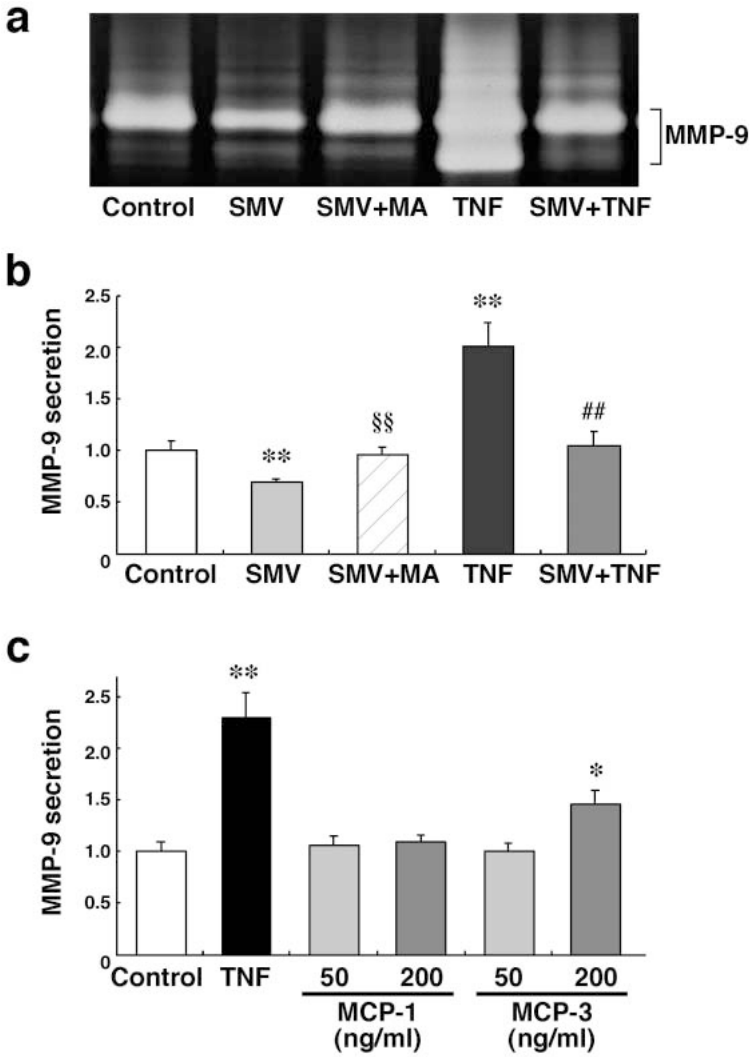


Fig. 5 Effect of simvastatin or MCPs on secretion of MMP-9
A fresh human AAA wall was cut into several pieces, cultured with simvastatin (10 μM), mevalonate (100 μM) or vehicle (Control). AAA in the culture was also treated by 10 μM simvastatin, 100 μM mevalonate, 50 ng/ml TNF-α or vehicle. The amounts of secreted MMP-9 in the conditioned media were determined by gelatin zymography. A representative zymogram is shown for MMP-9 in the media (a). Quantitative analyses of zymograms are shown for the secretion of MMP-9 (b). Fresh human AAA in *ex vivo* culture was treated by recombinant human MCP-1 (50 or 200 ng/ml), MCP-3 (50 or 200 ng/ml), TNF-α (50 ng/ml) or vehicle (Control). Quantitative analyses are shown for the secretion of MMP-9 in the media after experimental treatments (c). Data are means ± SE of 4 independent observations as shown by fold change compared to vehicle (Control). * and ** indicate $P < 0.05$ and $P < 0.01$ compared to Control, respectively. § § indicates $P < 0.01$ compared to the culture with simvastatin but without mevalonate. ## indicates $P < 0.01$ compared to the culture with TNF-α but without simvastatin.

< 0.01). Addition of mevalonate (100 μM) significantly reversed the effect of simvastatin on MMP-9 secretion (Figure 5b, 87% recovery, $p < 0.01$), indicating that simvastatin inhibits MMP-9 in a mevalonate pathway-dependent manner. We found that addition of TNF-α (50 ng/ml) caused a significant increase in MMP-9 secretion in AAA culture (2.1 ± 0.3 fold, p <

0.01 compared to control) and that simvastatin inhibited the secretion of MMP-9 under the conditions with TNF-α (Fig. 5b, 48% reduction, $p < 0.01$). These data demonstrated that simvastatin suppresses the secretion of MMP-9, as well as MCPs, under both basal and TNF-α-stimulated conditions.

Finally, we investigated the effect of exogenous MCP-1 or MCP-3 on the secretion of MMP-9 from human AAA walls. As shown in Fig. 5c, addition of human recombinant MCP-1 (50 or 200 ng/ml) did not affect the secretion level of MMP-9 in our experimental conditions. On the other hand, we found that MCP-3 at 200 ng/ml significantly increased the MMP-9 secretion in AAA walls in culture (Fig. 5c, 1.5 ± 0.1 fold, $p < 0.05$ compared to control). These data suggested that simvastatin inhibits MMP-9 secretion from human AAA walls partly by reducing the MCP-3 level.

Discussion

This study demonstrated two major findings. First, we identified MCP-1 and MCP-3 as highly upregulated chemokines in human AAA walls by a comprehensive analysis using a cytokine array. Both MCPs were expressed in inflammatory cells. MCP-1 was also localized in vascular smooth muscle cells. Second, we clearly showed that simvastatin reduced the secretion of MCP-1 and MCP-3, and MMP-9, from human AAA walls in culture. Coincidentally, exogenous MCP-3 increased MMP-9 secretion.

Human AAA is characterized by disruption of the extracellular matrix, loss of vascular smooth muscle cells and chronic cellular infiltration.[1)2)] Proinflammatory mediators, especially chemokines, cause infiltration of inflammatory cells in AAA walls, which in turn secrete not only proteolytic enzymes to degrade the extracellular matrix, but also proinflammatory mediators to maintain the chronic inflammation. Among 79 mediators analyzed in this study, we demonstrated that IL-6, IGFBP-3, MCP-1, MCP-2 and MCP-3 were elevated in human AAAs compared to non-aneurysmal controls. This is the first report of an upregulation of MCP-3 in AAAs. It has been reported that inhibition of MCP-1 suppresses the development of experimental AAA in mice,[23)] and that serum levels of IL-6 are increased in patients with AAA and correlate with aneurysm size.[24)25)] These reports are consistent with our findings and suggest the importance of these mediators in the pathogenesis of human AAAs. Among these cytokines and chemokines, we focused on MCP-1 and MCP-3, because of the potential diagnostic and therapeutic importance.

Recently, two studies showed the prevention of experimental aneurysms by statin treatment,[12)13)] and two clinical studies showed decreased rates of aneurysm growth and decreased mortality in statin-treated patients with small AAA.[8)9)] However, the mechanism for the beneficial effects of statins has not been elucidated. In this study, we clearly demonstrated that statins have two beneficial effects on human AAA walls. First, simvastatin inhibits the secretion of MMP-9 from human AAA in culture, as supported by a previous report showing the inhibitory effect of cerivastatin on MMP-9 from human AAAs *in vitro*.[10)] Moreover, one recent clinical study showed that preoperative statin treatment decreases the concentration of MMP-9 in tissues from AAA patients.[11)] Second, we demonstrated for the first time that simvastatin inhibits the secretion of MCP-1 and MCP-3 from human AAA in culture. Previous reports showed that statins reduce the expression of MCP-1 in vascular smooth muscle cells,[26)] macrophages,[27)] and endothelial cells[27)] in culture, and the secretion of IL-6 from human AAA tissue in culture.[28)] We revealed MCP-3 inhibition, as well as its importance: recombinant MCP-3 was shown to enhance the secretion of MMP-9 from human AAA as well as recruit inflammatory cells in human AAAs.

Although the concentration of simvastatin in our experimental culture media (10 μM) was much higher than peak plasma concentrations (37 nM, according to the drug information data sheet from Banyu Company in 2005) achieved after a single oral administration of 20 mg simvastatin, the tissue concentration is not known. Since clinical doses of statins have shown their possible inhibitory effect on AAA growth,[8)9)] prolonged treatment with statins may have some effects on

AAA tissues even in low concentrations. On the other hand, statins with greater tissue penetration would have more beneficial effects on human AAA walls.

In conclusion, the present study indicated that statins inhibit not only MMP-9 but also MCP-1 and MCP-3 in human AAA walls, providing one of potential mechanisms for the inhibitory effect of statins on human AAA progression. Because MCPs and MMP-9 are critical for the development and progression of human AAAs, statin may provide a novel therapeutic strategy for AAA.

Acknowledgments

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References

1) Thompson, R. W., Geraghty, P. J. and Lee, J. K.: Abdominal aortic aneurysms: basic mechanisms and clinical implications. *Curr. Probl. Surg.*, **39**：110-230, 2002.
2) Aoki, H., Yoshimura, K. and Matsuzaki, M.: Turning back the clock: regression of abdominal aortic aneurysms via pharmacotherapy. *J. Mol. Med.*, **85**：1077-1088, 2007.
3) Yoshimura, K., Aoki, H., Ikeda, Y., et al.: Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase. *Nat. Med.*, **11**：1330-1338, 2005.
4) Anderson, R. N. and Smith, B. L.: Deaths: leading causes for 2002. *Natl. Vital. Stat. Rep.*, **53**：1-89, 2005.
5) Hirsch, A. T., Haskal, Z. J., Hertzer, N. R., et al.: ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic). *Circulation*, **113**：e463-e654, 2006.
6) Schonbeck, U. and Libby, P.: Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? *Circulation*, **109**：II18- II26, 2004.
7) Takemoto, M. and Liao, J. K.: Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. *Arterioscler. Thromb. Vasc. Biol.*, **21**：1712-1719, 2001.
8) Schouten, O., van Laanen, J. H., Boersma, E., et al.: Statins are associated with a reduced infrarenal abdominal aortic aneurysm growth. *Eur. J. Vasc. Endovasc. Surg.*, **32**：21-26, 2006.
9) Sukhija, R., Aronow, W. S., Sandhu, R., et al.: Mortality and size of abdominal aortic aneurysm at long-term follow-up of patients not treated surgically and treated with and without statins. *Am. J. Cardiol.*, **97**：279-280, 2006.
10) Nagashima, H., Aoka, Y., Sakomura, Y., et al.: A 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, cerivastatin, suppresses production of matrix metalloproteinase-9 in human abdominal aortic aneurysm wall. *J. Vasc. Surg.*, **36**：158-163, 2002.
11) Wilson, W. R., Evans, J., Bell, P. R., et al.: HMG-CoA reductase inhibitors (statins) decrease MMP-3 and MMP-9 concentrations in abdominal aortic aneurysms. *Eur. J. Vasc. Endovasc. Surg.*, **30**：259-262, 2005.
12) Steinmetz, E. F., Buckley, C., Shames, M. L., et al.: Treatment with simvastatin suppresses the development of experimental abdominal aortic aneurysms in normal and hypercholesterolemic mice. *Ann. Surg.*, **241**：92-101, 2005.
13) Kalyanasundaram, A., Elmore, J. R., Manazer, J. R., et al.: Simvastatin suppresses experimental aortic aneurysm expansion. *J. Vasc. Surg.*, **43**：117-124, 2006.
14) Zempo, N., Kenagy, R. D., Au, Y. P., et al.: Matrix metalloproteinases of vascular wall cells are increased in balloon-injured rat carotid artery. *J. Vasc. Surg.*, **20**：209-217, 1994.
15) Koch, A. E., Kunkel, S. L., Pearce, W. H., et al.: Enhanced production of the chemotactic cytokines interleukin-8 and monocyte chemoattractant protein-1 in human abdominal aortic aneurysms. *Am. J. Pathol.*, **142**：1423-1431, 1993.
16) Szekanecz, Z., Shah, M. R., Pearce, W. H., et al.: Human atherosclerotic abdominal aortic aneurysms produce interleukin (IL)-6 and interferon-gamma but not IL-2

and IL-4:the possible role for IL-6 and interferon-gamma in vascular inflammation. *Agents Actions*, **42** : 159-162, 1994.
17) Panek, B., Gacko, M. and Palka, J.: Metalloproteinases, insulin-like growth factor-I and its binding proteins in aortic aneurysm. *Int. J. Exp. Pathol.*, **85** : 159-164, 2004.
18) Juvonen, J., Surcel, H. M., Satta, J., et al.: Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. *Arterioscler. Thromb. Vasc. Biol.*, **17** : 2843-2847, 1997.
19) Hamano, K., Li, T. S., Takahashi, M., et al.: Enhanced tumor necrosis factor- alpha expression in small sized abdominal aortic aneurysms. *World J. Surg.*, **27** : 476-480, 2003.
20) Van Damme, J., Proost, P., Lenaerts, J. P., et al.: Structural and functional identification of two human, tumor-derived monocyte chemotactic proteins (MCP-2 and MCP-3) belonging to the chemokine family. *J. Exp. Med.*, **176** : 59-65, 1992.
21) Opdenakker, G., Froyen, G., Fiten, P., et al.: Human monocyte chemotactic protein-3 (MCP-3): molecular cloning of the cDNA and comparison with other chemokines. *Biochem. Biophys. Res. Commun.*, **191** : 535-542, 1993.
22) Van Coillie, E., Van Damme, J. and Opdenakker, G.: The MCP/eotaxin subfamily of CC chemokines. *Cytokine Growth Factor Rev.*, **10** : 61-86, 1999.
23) Ishibashi, M., Egashira, K., Zhao, Q., et al. : Bone marrow-derived monocyte chemoattractant protein-1 receptor CCR2 is critical in angiotensin II-induced acceleration of atherosclerosis and aneurysm formation in hypercholesterolemic mice. *Arterioscler. Thromb. Vasc. Biol.*, **24** : e174-e178, 2004.
24) Jones, K. G., Brull, D. J., Brown, L. C., et al.: Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. *Circulation*, **103** : 2260-2265, 2001.
25) Rohde, L. E., Arroyo, L. H., Rifai, N., et al.: Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation. *Arterioscler. Thromb. Vasc. Biol.*, **19** : 1695-1699, 1999.
26) Ortego, M., Bustos, C., Hernandez-Presa, M. A., et al.: Atorvastatin reduces NF-kappaB activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. *Atherosclerosis*, **147** : 253-261, 1999.
27) Veillard, N. R., Braunersreuther, V., Arnaud, C., et al.: Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. *Atherosclerosis*, **188** : 51-58, 2006.
28) Evans, J., Loftus, I. M., Bell, P. R. F., et al.: Simvastatin preserves elastin and reduces the inflammatory response in aortic aneurysmal tissue. *Br. J. Surg.*, **89** : 22, 2002.