Atherosclerosis Amelioration by Moderate Alcohol Consumption Is Associated With Increased Circulating Levels of Stromal Cell-Derived Factor-1

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Background: A moderate intake of alcohol is associated with lower cardiovascular mortality, and the role of circulating progenitor cells in the beneficial effect of alcohol on atherosclerosis is unclear. The hypothesis of this study was that alcohol ameliorates atherosclerosis by modulating the circulating levels of stromal cell-derived growth factor (SDF)-1 and vascular progenitor cells.

Methods and Results: Atherosclerosis was induced by infusion of angiotensin II in apolipoprotein-E deficient mice, which were treated with high and low doses of ethanol for 28 days by intraperitoneal injection. Mice treated with low-dose ethanol had significantly less dilatation and fewer atheromatous lesions than mice receiving the high-dose ethanol. The number of circulating fibrocytes was significantly lower in mice treated with high-dose ethanol compared with mice with atherosclerosis untreated with ethanol. The plasma CXCL12/SDF-1 level was significantly increased in mice treated with low-dose ethanol compared with mice treated with a high dose, and the plasma concentration of transforming growth factor-β1 was significantly increased in mice treated with high-dose ethanol compared with control mice. Ethanol regulated the secretion of SDF-1 and vascular endothelial growth factor from fibroblasts in a dose-dependent and bimodal fashion.

Conclusions: The circulating level of CXCL12/SDF-1 may be involved, at least in part, in the differential effects of alcohol consumption on atherosclerosis. (Circ J 2011; 75: 2269–2279)

Key Words: Alcohol; Atherosclerosis; Chemokines; Mice; Progenitor cells

Atherosclerosis is a chronic inflammatory disease of the vessel walls that may lead to progressive obstruction of coronary, cerebral or peripheral arteries and to acute clinical events caused by plaque rupture and thrombosis.1,2 The disease can be multifactorial and is generally associated with predisposing conditions, including hyperlipidemia, diabetes, arterial hypertension, smoking, obesity or sedentary lifestyle.3 The pathogenesis of atherosclerosis is not completely understood. One mechanism believed to be important in the induction of atherosclerotic inflammation is failure of vascular endothelial repair following vascular injury.4–6 Recent studies have provided evidence that during a physiological host response to vascular damage the number of endothelial progenitor cells (EPCs) increases in the systemic circulation and that they migrate to sites of tissue injury to restore the integrity of the vascular endothelium.7,8 Other studies have also demonstrated that an excessive inflammatory response with abnormal deposition of extracellular matrix proteins occurs at sites of vascular damage when this reparative process of the endothelium is impaired, suggesting a critical role of EPCs in the mechanism of atherosclerosis.9,10

In addition to EPCs, fibrocytes constitute another group of circulating progenitor cells that are associated with tissue repair.11–13 Fibrocytes are mesenchymal progenitor cells derived from bone marrow that are capable of differentiating into fibroblasts, myofibroblasts and adipocytes.12 Fibrocytes can partici-
pate in tissue remodeling by producing and secreting extracellular matrix proteins (eg, collagen), metalloproteinases, growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1, inflammatory cytokines, interleukin-1β, tumor necrosis factor (TNF)-α, and chemokines, including monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α. Besides the role of fibrocytes in physiological fibrosis, they are also implicated in pathological fibrosis associated with fibroproliferative disorders of the lungs, liver, kidneys and vessel walls. Several factors, including stromal cell-derived factor (SDF)-1 and vascular endothelial growth factor (VEGF), have been implicated in the differentiation, migration and tissue homing of both EPCs and fibrocytes.

Alcohol consumption is a common habit, and several studies have shown that light to moderate consumption reduces the mortality of cardiovascular diseases caused by atherosclerosis. The protective effect of alcohol intake on survival was significantly substantial among individuals at high cardiovascular risk. On the other hand, heavy consumption of alcohol has been linked to an increase in mortality among subjects who already had a myocardial infarction. Thus, a U- or a J-shape curve is generally used to describe the relationship between alcohol intake and total mortality.

The ability of alcohol to increase the circulating levels of high-density lipoprotein cholesterol, adiponectin and atrial natriuretic peptide, to improve insulin sensitivity and vascular endothelial function, to inhibit inflammation and to regulate platelet aggregation, fibrinolysis and coagulation system have all been proposed as potential explanations for the beneficial effect of light to moderate intake of alcohol on atherosclerotic cardiovascular disease.

In the present study we hypothesized that regulation of the circulating levels of SDF-1, EPCs and fibrocytes might explain the beneficial effect of a moderate consumption of alcohol on atherosclerotic disease.

**Methods**

**Reagents**

Dulbecco’s modified Eagle’s medium (DMEM) and RPMI were purchased from Sigma (St Louis, MO, USA), fetal bovine serum (FBS) from Bio Whittaker (Walkersville, MD, USA) and penicillin and streptomycin from Nacalai Tesque (Kyoto, Japan).

**Cell Culture**

Mouse lung fibroblasts were isolated from C57BL/6 lung by collagenase digestion, and cultured in DMEM supplement with 10% FBS supplemented with 20% heat-inactivated FBS, 50 μg/ml penicillin and 50 μg/ml streptomycin. The cells were cultured in 5% CO2 and 95% air.

**Stimulation of Fibroblasts With Ethanol**

Fibroblasts were cultured in 12-well plates for 48h, washed with medium containing 1% FBS and then cultured in the same medium for 24h. The cells were then washed and treated with varying concentrations of ethanol in the medium for 24h. Cell supernatants were collected and stored at −80°C until measurement of SDF-1 and VEGF levels. The cells were used for RNA preparation.

**Animals**

Mice deficient in apolipoprotein E (B6:129P2-ApoE<tm1Bal >/J) on a C57/B16 background were provided by Jackson Laboratory. Male mice (9–11 weeks old) weighing 21–23g were used. The animals were maintained in a specific-pathogen-free environment, fed a normal laboratory diet, and subjected to a 12-h light:dark cycle in the animal house of Mie University. Mie University’s Committee on Animal Investigation approved the experimental protocols and all procedures were performed in accordance with the approved institutional guidelines of Mie University.

**Animal Model of Atherosclerosis**

Induction of atherosclerosis was performed as previously described. Angiotensin II (AngII, 1.5 mg·kg−1·day−1) or saline was infused through osmotic minipumps (Alzet, model 2004, Palo Alto, CA, USA) that were implanted subcutaneously in the ApoE knockout mice. The osmotic minipumps allowed continuous infusion of AngII or saline at a constant rate for 28 days. The animals were randomized into 4 treatment groups: (1) Sal/Sal group received sterile saline by osmotic minipump and saline by (ip) intraperitoneal injection, (2) AngII/Sal group received AngII by osmotic minipump and saline by ip injection every day, (3) AngII/Low-ET group received AngII by osmotic minipump and a low dose (10 μg/day) of ip ethanol every day and (4) AngII/High-ET group received AngII by osmotic minipump and a high dose of ip ethanol (30 μg/day) every day. All groups were monitored for 28 days. Mice were treated with ethanol or saline by ip injection to assure that a constant amount was administered.

**Reverse Transcription (RT)-Polymerase Chain Reaction (PCR)**

Total RNA was extracted from fibroblasts by the guanidine isothiocyanate procedure using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was reverse-transcribed using oligo-dT primers and then the DNA was amplified by PCR. RT-PCR was carried out using the Superscript Preamplication System kit (Invitrogen). The sequences of the primers used for mouse SDF-1 cDNA (182bp) amplification were 5′-GCTCTGCATCAGTGACGGTA-3′ and 5′-ATTTCGGTGCTAATGCACACT-3′, for mouse VEGF (818bp) were 5′-CTGTAACGATGAAGCCCTGGAG-3′ and 5′-TGTTGAGGTTTGTATCCGCAT-3′. PCR was performed with 35 cycles, denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and elongation at 72°C for 1 min; at the end of these cycles, a further extension was carried out at 72°C for 5 min. The CDNA (532bp) of the mouse housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (461bp) was amplified using the following primers 5′-CTTTATTGACCCATCACAC-3′ and 5′-GAGGGGGCTCATCCAGCTTCTG-3′. The PCR products were separated on a 2% agarose gel containing 0.1% ethidium bromide, and the intensity of the stained bands was quantified by densitometry using the public domain NIH image program (Wayne Rasband, NIH, Research Service Branch). The amount of mRNA was normalized against GAPDH mRNA.

**Genotyping**

Genotyping was performed by PCR of DNA extracted from the tail. Genotyping was performed using the primers and protocol recommended by the Jackson laboratory.

**Necropsy of Animals**

Mice were killed humanely on day 29 under profound anesthesia by ip injection of pentobarbital (62.5 mg/kg of mouse body weight) to take samples for biochemical and histopathological examinations. Blood was drawn by cardiac puncture into sterilized tubes containing ethylenediaminetetraacetic acid, kept at
4°C until centrifugation, and the plasma was stored at −80°C until analysis.

**Histopathological Examination**

During necropsy of the mice, a laparotomy was performed and the aorta was visualized and perfused through the heart with saline and then with warmed 1.6% solution of low-melting-point agarose (SeaPlaque GTG Agarose, Lonza, Rockland, USA) at a constant pressure of 100 mmHg. After the gel solidified, the complete abdominal aorta was dissected from the surrounding tissues, excised and then fixed in 10% formalin. The aorta wall was longitudinally excised, and expanded and digital images of the arterial surface area were acquired using an Olympus SZ61 microscope with a Plan objective, combined with an Olympus DP25 Digital Camera (Tokyo, Japan). Measurement of the inner surface area of the abdominal aorta was

![Figure 1. Dilatation index of abdominal aorta.](image)

Obvious dilatation of the external surface of the aorta (a) in the AngII/High-ET group compared with the other groups. Quantification of the arterial outer surface (b) showed obvious and significant dilatation of the external surface of aorta in AngII/High-ET mice compared with all other groups. Bars indicate means±SE. The graph is the representative result of 2 independent experiments: Sal/Sal group (n=3) received saline (pump)+saline (ip); AngII/Sal group (n=3) received angiotensin II (pump)+saline (ip); AngII/Low-ET group (n=3) received angiotensin II (pump)+low-dose ethanol (ip); AngII/High-ET group (n=3) received angiotensin II (pump)+high-dose ethanol (ip). ip, intraperitoneal.
Figure 2. Quantification of atherosclerosis. Staining of longitudinally opened aortas with Sudan III (a,b) shows the substantial and significant increase in the number of atherosclerotic plaques in the group of mice that was treated with high-dose ethanol (AngII/High-ET) compared with that receiving a low dose (AngII/Low-ET). Bars indicate means±SE. The graph is the representative result of 2 independent experiments. Sal/Sal group (n=3) received saline (pump)+saline (ip); AngII/Sal group (n=3) received angiotensin II (pump)+saline (ip); AngII/Low-ET group (n=3) received angiotensin II (pump)+low-dose ethanol (ip); AngII/High-ET group (n=3) received angiotensin II (pump)+high-dose ethanol (ip). ip, intraperitoneal.
performed using the WinROOF image processing software (Mitani Corp, Fukui, Japan) for Windows. The samples were randomized and the observers were blinded to the names of the samples. The dilation index was defined as the total inner surface area of the aorta divided by the total artery length.

Quantification of Atherosclerotic Plaques

After removing peripheral tissues, the aorta was opened longitudinally, immersed in Sudan III solution for 40 min (0.2 g Sudan III, 70 ml absolute ethanol, 30 ml distilled water), washed in 2-propanol (99.5%) for 5 min and then mounted for photography. Digital images were taken and quantification of atherosclerotic lesions was carried out with the WinROOF image processing software.

Flow Cytometry of Circulating EPCs and Fibrocytes

Blood (1 ml) was sampled by heart puncture and anticoagulated with ethylenediaminetetraacetic acid. Erythrocytes were lysed with BD Pharmlyse Lysing Buffer (BD Biosciences Pharmingen, San Jose, CA, USA). To identify EPCs, 10^6 blood cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD45.2, Alexa Fluor 647 (AF647)-conjugated anti-mouse CD34 (both from BD Biosciences Pharmingen) and with phycoerythrin (PE)-conjugated anti-mouse VEGFR2 (Flk-1) (Biolegend) for 30 min at 4°C. To identify fibrocytes, 10^6 blood cells were incubated with AF647-conjugated anti-mouse CD34, peridinin chlorophyll protein (PerCP)-conjugated anti-mouse CD45.2 (both from BD Biosciences Pharmingen) and purified anti-mouse collagen I (AbD Serotec) for 30 min at 4°C. As secondary antibody, PE-conjugated anti-rabbit IgG was used and incubated for 30 min at 4°C. For cell permeabilization and intracellular staining, the cells were first fixed with 4% paraformaldehyde, incubated for 2 h at 4°C and then treated with 0.1% saponin for 1 h at 4°C. In order to avoid nonspecific binding, pretreatment with purified anti-mouse CD16/32 (Becton Dickinson) was performed for 30 min at 4°C. The percentage of progenitor cells in relation to the total number of leukocytes was analyzed on FACS CANTO II using the Cell Quest Pro software.

Biochemical Analysis

The concentrations of cytokines in plasma were measured with commercial immunoassay kits specific for mouse cytokines. The immunoassay kits for measuring CCL2/MCP-1 and TGF-β1 were purchased from BD Pharmingen, and the EIA kits for measuring CCL3/MIP-1α, CCL3/MIP-1β, CCL19/MIP-3β, SDF-1, CCL22/macrophage-derived chemokine (MDC), thymus- and activation-regulated chemokine (TARC)/CCL17, VEGF and CD117/stem cell factor (SCF) were purchased from R&D Systems (Minneapolis, MN, USA). The plasma concentration of PDGF was measured by EIA using murine anti-platelet derived growth factor (Genzyme, Boston, MA, USA) antibody in conjunction with the respective biotin-labeled antibody. Thrombin–antithrombin complexes (TAT) were measured using an enzyme immunoassay kit from Cedarlane Laboratories (Ontario, Canada). All cytokines were measured according to the manufacturer’s instructions.

Statistical Analysis

Data are expressed as the mean±standard error of the mean (SE). We chose SE rather than SD to express variance because we were interested in the variance of the mean data, not the variance between individual mice. The statistical difference between variables was calculated by analysis of variance with post hoc analysis using Fisher’s predicted least significant difference test. Statistical analyses were performed using the StatView 4.5 package for Macintosh (Abacus Concepts, Berkeley, CA, USA). P<0.05 was considered significant.
Results

Weight Loss During AngII Infusion
Mice treated with AngII lost weight during the first week of the experiment, but afterward recovered to weights similar to those of the Sal/Sal group (data not shown). There was no significant difference in weight loss between mice treated with high (AngII/High-ET) and low (AngII/Low-ET) doses of ethanol during the entire experiment (data not shown).

Analysis of Morphological Parameters
There was obvious dilatation of the external surface of the aorta in the AngII/High-ET group compared with the other groups. Quantification of the arterial outer surface showed significant dilatation of the external surface of the aorta in the AngII/High-ET mice compared with all other groups (Figure 1). Staining of the aorta with Sudan III showed a substantial and significant increase in the number of atherosclerotic plaques in the group of mice treated with high-dose ethanol (AngII/High-ET) compared with those receiving low-dose ethanol (AngII/Low-ET). There was also enhanced deposition of atheromatous lesions in the AngII/Sal group compared with the Sal/Sal group (Figure 2). No difference was found between the AngII/Low-ET and Sal/Sal groups.

Circulating Progenitor Cells
The percentage of mesenchymal (fibrocytes) (CD45+/CD34+/collagen+) and endothelial (CD45+/CD34+/VEGFR2) precursor cells in relation to the total number of leukocytes in peripheral blood was calculated. There was no significant difference in the percentage of all precursor cells among the Sal/Sal, AngII/Sal and AngII/Low-ET groups. Mice that were

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Data are means±SD.
Sal, saline; AngII, angiotensin II; ET, ethanol; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; TARC, tumor necrosis factor-β1-mediated regulation of thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine; SCF, stem cell factor; PDGF, platelet-derived growth factor.

Figure 4. Circulating levels of CXCL12/SDF-1 and growth factors. CXCL12/SDF-1 (a) was significantly decreased in AngII/Sal and AngII/High-ET mice compared with the Sal/Sal group, and it was significantly increased in AngII/Low-ET compared with AngII/Sal and AngII/High-ET mice. The concentration of TGF-β1 in plasma was significantly increased in the AngII/High-ET group compared with the control group and tended to increase compared with the other groups of mice. (c) The plasma concentration of VEGF (b) was significantly increased in the AngII/Sal group compared with the other groups. Bars indicate means±SE.

The graph is the representative result of 2 independent experiments. Sal/Sal group (n=4) received saline (pump)+saline (ip); AngII/Sal group (n=3) received angiotensin II (pump)+saline (ip); AngII/Sal group (n=3) received angiotensin II (pump)+saline (ip); AngII/High-ET group (n=5) received angiotensin II (pump)+low-dose ethanol (ip); AngII/High-ET group (n=5) received angiotensin II (pump)+high-dose ethanol (ip). ip, intraperitoneal; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.
Figure 5. Regulation of stromal cell-derived growth factor (SDF-1) secretion by alcohol in murine fibroblasts. Fibroblasts were cultured and treated with varying concentrations of ethanol as described in the Methods. The effects of ethanol on protein secretion (a) and mRNA expression (b,c) of SDF-1 in fibroblasts were biphasic and dose-dependent. Bars indicate means±SE. The graph is the representative result of 2 independent experiments.
Figure 6. Regulation of vascular endothelial growth factor (VEGF) secretion by alcohol in murine fibroblasts. Fibroblasts were cultured and treated with varying concentrations of ethanol as described in the Methods. The effects of ethanol on protein secretion (a) and mRNA expression (b, c) of VEGF from fibroblasts were biphasic and dose-dependent. Bars indicate means±SE. The graph is the representative result of 2 independent experiments.
treated with high-dose ethanol (AngII/High-ET) had 3- to 4-fold lower percentages of fibrocytes and EPCs compared with those not treated with ethanol (AngII/Sal). In particular, the number of fibrocytes was significantly decreased in the AngII/High-ET group compared with the AngII/Sal group (Figure 3). The number of EPCs and fibrocytes also tended to be low in the group receiving high doses of ethanol. Based on these data, there appears to be an optimal concentration of alcohol for preserving the number of circulating progenitor cells at a high level.

Circulating Levels of Chemokines

As chemokines are involved in the homing and migration of progenitor cells, we measured the levels of CCL2/MCP-1, CCL3/IP-1α, CCL19/MIP-3β, CCL22/MDC, CCL17/TARC, SCF, VEGF and CXCL12/SDF-1 in plasma from each group of mice. No significant differences were observed among the groups in the plasma concentrations of CCL2/MCP-1, CCL3/IP-1α, CCL19/MIP-3β, CCL22/MDC and CCL17/TARC and SCF (Table). The plasma concentration of CXCL12/SDF-1 was significantly decreased in the Ang/sal group compared to Sal/Sal group but it was significantly increased in the AngII/Low-ET group compared with the AngII/Sal and AngII/High-ET groups (Figure 4).

Alterations in the Coagulation System

The concentration of TAT is a surrogate for activity of the coagulation cascade. The plasma concentration of TAT was not different among the different groups of animals (data not shown).

Circulating Levels of Growth Factors

Because there was vascular wall remodeling in the mice, we investigated whether there were increased levels of growth factors that promote vascular remodeling, vascular smooth muscle cell growth and extracellular matrix deposition. The plasma concentration of VEGF was significantly increased in the AngII/Sal group compared with all other groups but no difference was found between the AngII/Low-ET and AngII/High-ET groups (Figure 4). The concentration of PDGF in plasma was not significantly different between groups (Table). However, the circulating level of TGF-β1 was significantly elevated in the AngII/High-ET group compared with the control (Sal/Sal) group. No significant difference was observed between the other groups of mice (Figure 4).

Regulation of SDF-1 and VEGF Secretion by Alcohol

To evaluate whether alcohol can regulate secretion of SDF-1 and VEGF, we cultured fibroblasts with varying concentrations of ethanol and measured the protein levels and mRNA expression of SDF-1 and VEGF. The secretion of SDF-1 and VEGF from mouse lung fibroblasts gave a bell-shape curve, which dose-dependently increased up to 0.1% ethanol and then decreased (Figures 5a, 6a). Similar results were obtained with the PCR analysis of SDF-1, but the mRNA expression of VEGF increased up to 0.2% of ethanol and then dose-dependently decreased (Figures 5b, 6b). These observations suggest a dual effect of alcohol on SDF-1 and VEGF expressions.

Discussion

This study showed that moderate doses of alcohol protect against the development of atherosclerosis, increase the level of SDF-1 and maintain the number of progenitor cells, particularly fibrocytes, at relatively high levels in the systemic circulation.

Alcohol Intake and Inhibition of Atherosclerosis

Light to moderate intake of alcohol has been shown to reduce the mortality for heart failure, a disease that affects more than 5 million Americans. In contrast, excessive consumption of alcohol is toxic and has been associated with the development of cardiomyopathy and premature death. Thus, most previous studies have reported a J-shaped association curve in which moderate drinkers have less risk of cardiopathy than abstainers, and heavy drinkers have more risk of developing the disease. Moderate intake of alcohol is ≤30 g/day, whereas heavy alcohol consumption is defined as >60 g/day. In the present study, we prepared models of atherosclerosis in mice and treated them with low (10 mg/day) or high (30 mg/day) doses of alcohol. The results showed less dilatation of the aorta and less atherosclerotic plaques in mice treated with low-dose ethanol than in those treated with high dose, corroborating previous epidemiological studies undertaken in humans. The precise mechanistic pathway leading to the beneficial effect of low-dose alcohol is unclear, but, based on the results of the current investigation, increased secretion of SDF-1 with subsequent enhanced mobilization of progenitor cells appears to be involved.

Circulating Progenitor Cells and Alcohol Intake

Several mechanisms have been proposed to explain the beneficial effects of alcohol intake on vascular events, including its inhibitory effects on hypercholesterolemia, insulin intolerance, oxidative stress and inflammation. Higher circulating levels of progenitor cells have been shown to reduce the progression of atherosclerosis. However, the effect of alcohol on peripheral blood cells is unclear. Previous studies have shown that alcohol consumption induces cytopenia, reduces the number of hematopoietic stem cells and of some dendritic cell subsets, and affects the balance of thymus-derived and bone marrow-derived natural killer cells. In the present study, we evaluated the effects of different levels of alcohol intake on the number of circulating fibrocytes and EPCs in mice. We found that both high and low doses of alcohol are associated with a decreased number of circulating fibrocytes and EPCs as compared with untreated atherosclerotic (AngII/Sal) mice. The number of both EPCs and fibrocytes tended to be reduced more substantially in mice receiving the low dose, but the difference did not reach significance because of the low number of mice in each experimental group. However, the number of fibrocytes was significantly lower in mice treated with high-dose ethanol but not in mice treated with a low dose, compared with untreated atherosclerotic (AngII/Sal) mice. Because low consumption is associated with less atherosclerosis, these observations suggest that alcohol intake preserves optimal mobilization of progenitor cells.

Alcohol and Circulating Levels of Chemokines

Several factors have shown to affect the circulating level of progenitor cells, including physical training, estrogen, granulocyte-colony stimulating factor, granulocyte-macrophage stimulating factor and CXCL12/SDF-1. In the present study, to clarify the mechanism by which alcohol affects the number of circulating progenitor cells, we measured the plasma concentrations of several cytokines in mice receiving a high or low dose of ethanol. The circulating level of CXCL12/SDF-1 was significantly increased in the group of mice treated with low-dose ethanol as compared with untreated (AngII/Sal)
mice and those receiving a high dose of ethanol. Although the present study showed no straightforward evidence, based on the results it is conceivable that alcohol, at least in our mouse model, maintains a relatively high number of circulating progenitor cells by augmenting the concentration of CXCL12/SDF-1 in the peripheral blood. CXCL12/SDF-1 may stimulate the mobilization of EPCs by enhancing the activity of protein kinase B (akt) and endothelial nitric oxide synthase or by increasing the expression of VEGF.4,16 To confirm the effect of alcohol on SDF-1 secretion, we conducted an in vitro study in which fibroblasts were stimulated with varying concentrations of ethanol. This experiment demonstrated for the first time that alcohol exerts a biphasic effect on SDF-1 secretion; low percentages of ethanol increase SDF-1 secretion whereas high percentages decrease it. In addition to fibroblasts, several other types of cells, including smooth muscle cells, endothelial cells, macrophages and platelets, may be the source of SDF-1 in an atherosclerotic microenvironment.39 The molecular mechanism of ethanol-mediated increased SDF-1 expression is unknown. The finding that ethanol stimulates protein kinase C activation, and that this pathway promotes SDF-1 production, suggests that activation of the protein kinase C pathway could play a role in mediating the effect of alcohol.40,41

**Circulating Level of Growth Factors**

Vascular remodeling is the hallmark of atherosclerosis, which is demonstrated by the presence atherosclerotic plaques and vascular obstructive changes.2,4,15 Tissue remodeling of vessel walls may result from disruption of the balance between synthesis and degradation, which is regulated by several growth factors, such as TGF-β1 and PDGF.44 PDGF favors vascular remodeling by promoting the proliferation of smooth muscle cells and fibroblasts, and TGF-β1 by stimulating the secretion of extracellular matrix proteins, including collagens.42,43 In our study, the plasma concentration of PDGF remained unchanged but the concentration of TGF-β1 was significantly increased in mice with high levels of aortic atherosclerosis compared with the control group. The role of TGF-β1 in the pathogenesis of atherosclerosis and aneurysm is still controversial.46 Recent studies have shown that TGF-β1 limits the progression of atherosclerotic disease and prevents the development of aneurysm.42,47 However, TGF-β1 may indirectly induce less mobilization of progenitor cells by decreasing the expression of SDF-1.48

VEGF is another humoral factor that plays a critical role in vascular remodeling and vasculogenesis, as well as in the mobilization of EPCs from the bone marrow.49,51 VEGF enhances the mobilization of EPCs from the bone marrow by activating matrix metalloproteinase-9, which increases the concentration of SCF by cleaving the membrane-bound kit ligand; SCF stimulates ckit-positive stem cells to migrate to the vessels.49,50,52 In the present study, the circulating level of VEGF was significantly increased in Angio/Sal mice compared with control mice and mice treated with low or high doses of ethanol, and there was no difference in the circulating level of VEGF in mice receiving low- or high-dose ethanol. However, the in vitro study showed that ethanol increases the secretion of VEGF from fibroblasts at low doses but decreases it at higher doses, suggesting that alcohol may also regulate the expression of VEGF in a dose-dependent manner. Further in vivo studies using lower doses of ethanol may be necessary to clarify the discrepant results in VEGF expression between our in vivo and in vitro experiments.

**Conclusion**

In brief, the results of this study suggest that stimulation of an increased circulating level of SDF-1 and maintenance of circulating progenitor cells at a relatively high level may constitute another explanation for the inhibitory effect of light to moderate consumption of alcohol on the progression of atherosclerosis.

**Acknowledgment**

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**Disclosure**

None of the authors declare a financial conflict of interest regarding this study.

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