Homocysteine redox receptor and regulation of extracellular matrix components in vascular cells

SURESH C. TYAGI
Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi 39216

Tyagi, Suresh C. Homocysteine redox receptor and regulation of extracellular matrix components in vascular cells. Am. J. Physiol. 274 (Cell Physiol. 43): C396–C405, 1998.—Dynamic changes in the reduction-oxidation (redox) state of the tissue lead to the pathophysiological condition. Reduced homocysteine causes dysfunctions in endothelium. The proliferation of smooth muscle cells may lead to occlusive vascular disease, ischemia, and heart failure, but whether fibrosis and hypertension are a consequence of smooth muscle proliferation is unclear. Redox changes during hyperhomocysteinemia may be one of the causes of premature atherosclerotic heart disease. To examine the effect of homocysteine on human vascular smooth muscle cells (HVSMC), we isolated HVSMC from idiopathic dilated cardiomyopathic hearts. Coronaries in these hearts were apparently normal. HVSMC numbers in culture were measured by hemocytometer in the presence and absence of homocysteine. Results show that homocysteine induced cellular proliferation. This proliferation was reversed by the addition of the antioxidant N-acetylcysteine (NAC). Homocysteine induces collagen expression in a dose- and time-dependent manner, as measured by Northern blot (mRNA) analysis. The 50% inhibitory concentration of 5 µM for collagen was estimated. The induction of collagen was reversed by the addition of NAC and reduced glutathione. To localize the receptor for homocysteine on HVSMC, we synthesized fluorescamine-labeled homocystine conjugate. Incubation of labeled homocysteine with HVSMC demonstrated membrane and cytosol localization of homocysteine binding. The receptor-ligand binding was disrupted by NAC. Based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis fluorography, we observed a 40- to 25-kDa homocysteine redox receptor in HVSMC. Our results suggested that the redox homocysteine induces HVSMC proliferation by binding to the redox receptor and may exacerbate atherosclerotic lesion formation by inducing collagen expression.

Collagen; actin; vascular occlusive disease; gene expression; signal transduction; vascular remodeling

The physiological role of reduction-oxidation (redox) in maintaining the metabolic hemostasis and cardiovascular function is a poorly understood phenomenon. Homocysteine is a sulfur-containing amino acid generated during the metabolism of methionine. Homocysteine is two homocysteine residues linked by a disulfide bond. Extracellular homocysteine is readily oxidized to disulfide homocystine. Homocyst(e)ine is composed of a reduced form, oxidized form, and oxidative mixed disulfide form (mixture of reduced, oxidized, and thiolacetic acid), both free and protein bound.

Homocysteinemia is one of the causes of coronary atherosclerosis and ischemic heart disease (31). Abnormalities in the homocystine levels have been found in 40% of patients presenting with premature peripheral vascular disease or stroke (7). Recent data suggest that increased plasma homocystine levels are an independent risk factor for the development of arterial disease (12, 22). This may be related to the lack of reducing power and/or conversion of homocystine to homocysteine. Homocysteine readily oxidizes to homocystine in human plasma. A high level of homocysteine is associated with endothelial cell damage and an increase in oxidative tension (12, 22), leading to smooth muscle cell (SMC) proliferation.

Previously, high levels of collagen and proteoglycans in the primary atherosclerotic and secondary restenotic lesions (28) have been shown. The level of elastin was reduced (28). This indicated differential regulation of extracellular matrix (ECM) components during the development of atherosclerotic lesions (27–29). The loss of elasticity, or “hardening,” of arteries has long been thought to be an inevitable consequence of aging and an early sign of atherosclerosis. However, Demer et al. (5) reported that this is no more an inevitable degenerative change than is the development of other components of the inflammatory reaction (e.g., oxidative stress). Hypoxia, homocyst(e)inemia, acute coronary occlusion, and ischemic/reperfusion conditions increase oxidative homocysteine and oxidized glutathione (GSSG) in the coronary vasculature (20), which leads to a change in the redox state of tissue surrounding the vascular SMC. Endothelium is the innermost layer of vessel wall between blood and the basement membrane that resides on the top of the inner elastic laminae and communicates signals generated from blood to the interstitium (10). Detachment of endothelium exposes vascular SMC to the oxidative conditions in blood. This may, in turn, lead to SMC proliferation and ECM...
induction. An oxidative environment induces normal fibroblast cell proliferation, and a reducing agent decreases normal fibroblast cell proliferation by inducing matrix proteinase and repressing tissue injury indicator gene transcription (25). It is possible that changes in the oxidative environment around human vascular smooth muscle cells (HVSMMC) induce expression of ECM components.

Whether the effects of oxidative disulfide homocysteine are due to redox reactions involving direct modification by an oxygen radical or to intracellular glutathione-dependent oxidation-reduction reactions is not yet known. The determination of cellular permeability of antioxidants and their half-lives under tissue culture conditions and determination of redox-receptor are the critical parameters to be determined to develop a relationship between redox-sensitive ECM remodeling in vivo versus in vitro. The binding of antithrombin III to endothelial cell membrane was decreased after preincubation with homocysteine, cysteine, or 2-mercaptoethanol (19). This suggested the possibility that reducing agents decrease the disulfide exchange reaction between membrane receptor and antithrombin. However, this raises the possibility that oxidative disulfide homocysteine may bind to receptor directly by a disulfide exchange reaction. To this end, an identification of a redox receptor may be an ideal development. Nothing is known about the redox receptor(s) in vivo or in vitro. Here we report that the HVSMMC are sensitive to redox state and there is a redox-sensitive receptor on HVSMMC.

MATERIALS AND METHODS

Materials. Reduced glutathione (GSH), GSSG, trishydroxymethylaminomethane (Tris) base, N-acetylcysteine (NAC), homocysteine, homocysteine, cysteine, cystine, and trypsin were obtained from Sigma (St. Louis, MO). Fetal calf serum (FCS), normal rabbit serum, minimum essential medium (MEM) with Earle's salts, collagen- and laminin-coated culture plates, and Hanks' balanced salt solution were all obtained from Collaborative Research (Bedford, MA). Elastase was obtained from Elastin Products (St. Louis, MO). Bacterial collagenase was obtained from Worthington (Freehold, N J). N-terminal propeptide (TMRKPRCGNPDVAN) of matrix metalloproteinases (32) was synthesized at the Protein Core Facility at the University of Missouri-Columbia and was kept under vacuum conditions before use.

Human arterial tissue. Normal arteries were isolated from idiopathic dilated cardiomyopathic explanted heart (24). The reasons for heart failure in idiopathic patients were other than coronary. The hearts with idiopathic dilated cardiomyopathy have apparently normal coronary arteries. A waiver for using explanted human tissue was obtained from the Institutional Review Board before collecting the tissue. The coronaries were stripped of adventitial and external tissue before isolating the cells.

Isolation of HVSMMC. HVSMMC were isolated by a modification of the combined collagenase and elastase digestion. The endothelium was removed by gently scraping the luminal side of the vessel with a cotton swab and washing the swab in MEM. Medial SMC were isolated by mincing the rest of the vascular tissue in the presence of collagenase and elastase (10 µM) and were grown in 10% FCS (26). Isolated cells were free of endothelial cell contamination, as determined by positive staining with human anti-smooth muscle actin-related anti-
steady-state mRNA was carried out by Northern blot analysis.

Northern blot (mRNA) analysis of collagen, \( \alpha \)-actin, and 18S R gene. Total RNA was isolated from 1 × 10^6 cells using 4 M guanidine thiocyanate buffer. RNA was quantitated at 260 nm absorbance. The purity of total RNA was assessed by an absorbance ratio (260/280 nm) of 1.9. Twenty micrograms of total RNA were denatured in a 1% formamide-formaldehyde solution at 65°C for 15 min, and samples were then resolved on denaturing 1% agarose gel. The gel was transferred to a nitrocellulose filter where it was prehybridized in a buffer containing 50% formamide, 5× standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), 5× Denhardt’s solution, 50 mM NaHPO₄, and 100 µg/ml denatured sperm DNA at 42°C for 4 h. Blots were then hybridized for 16 h at 42°C with \([^32P]dCTP\) random prime labeled cDNA. The membrane containing collagen type I cDNA was obtained from American Type Culture Collection (Rockville, MD). The collagen probe was a 3.55-kb EcoRI fragment from a type I collagen \( \alpha \)1-chain cDNA. The \( \alpha \)1-protein was a 1.1-kb EcoRI fragment. A 4.5-kb EcoRI fragment of 18S R gene (a gift from Dr. R. Guntaka, University of Missouri-Columbia) was used as an internal control. Bands on the autoradiographs were scanned. The relative intensity level of transcripts of collagen I and \( \alpha \)-actin was normalized with 18S R, an internal control.

Fluorescence-labeled homocystine. Homocysteine contains one disulfide and two primary amines, \( \text{N}H_2 \) groups. To label homocysteine at the primary amines, homocysteine (1 mM) was incubated with fluorescein (4 mM) at room temperature in 0.5 ml of 50 mM Tris-Cl (pH 7.4). Neither homocysteine nor fluorescein fluoresces (30). However, after complexation of homocysteine with free amine, a fluorescent product is generated (30). The reaction product was passed through a Sephadex G-50 column equilibrated with 50 mM Tris-Cl (pH 7.4). The fluorescent product was collected. The concentration of fluorescein-homocysteine was estimated using an extinction coefficient of 6,000 M⁻¹cm⁻¹ at 380 nm (3, 30). This product was used to label the receptor for homocystine in HVSMS. A similar protocol was employed for synthesis of the fluorescein-cysteine-cystine complex, using 1 mM cystine.

Homocysteine receptor in HVSMS. Confluent HVSMS grown on coverslips in six-well plates were washed three times with 1 ml binding buffer (Hank's balanced salt solution containing 10 mM Tris-Cl (pH 7.6), 0.1 mM EDTA, 100 µg/ml bacitracin, 0.1% bovine serum albumin, 1 × protease inhibitor mixture, containing 1 µM each leupeptin, aprotinin, and pepstatin, and 0.1 mM phenylmethylsulfonyl fluoride; Sigma Chemical) and incubated with 0.5 ml binding buffer, with or without fluorescein-homocysteine for 4 h at 37°C. After incubation, unbound ligand was washed three times with binding buffer without protease inhibitors. The coverslips were removed from the wells. The fluorescence labeling was measured with a fluorescence microscope (Leica).

Cross-linking of fluorescein-homocysteine to receptors. The affinity cross-linking SDS-polyacrylamide gel electrophoresis (PAGE) and fluorography were performed (9, 17). Binding of fluorescein-homocysteine was performed in six-well plates, with and without homocysteine in 0.5 ml final binding mixture, and was incubated at 37°C for 120 min. Each incubation was carried out in duplicate [one set each for 0.1 and 1.0 mM disuccinimidyl suberate (DSS) cross-linker (Pierce Chemicals, Rockford, IL)]. The incubates were exposed to ultraviolet light by transluminator. DSS was dissolved in dimethyl sulfoxide. Cell supernatants were aspirated, and cells were washed three times with 1 ml of ice-cold saline.

Washed cells were detached, homogenized, and analyzed by SDS-PAGE fluorography.

Statistical analysis. Data are expressed as means ± SD. Difference between experimental condition of cell numbers treated versus untreated was assessed by paired t-test. Northern blot data were scanned, and the intensity was analyzed. All experiments were carried out in triplicate. A value of \( P < 0.01 \) was considered significant.

RESULTS

Proliferation of HVSMS by homocysteine and inhibition by NAC. HVSMS were isolated from normal vessels. The spindle phenotype characteristic of SMC and growth in close contact to each other is shown in Fig. 1A. To examine whether oxidative mixed-disulfide homocysteine induces cellular proliferation, HVSMS were seeded at day 0 with 2.5 × 10⁴ cells in 2% serum. At day 1, 2% serum, serum plus homocysteine, or serum plus homocystine plus NAC was added and changed every day (Fig. 1B). The number of cells in 2% serum increased 140% to 6 × 10⁴ in 7 days. However, in the presence of serum plus 10 µM homocysteine, the number of cells increased 680% to 19.5 × 10⁴ over the 7-day period. The addition of 50 µM NAC inhibited 62% of the homocysteine-induced growth of HVSMS. To determine whether the growth induced by homocystine (10 µM) is mediated by disulfide or thiol, we cultured HVSMS in the presence of homocysteine plus homocysteine (50 µM). We observed that homocysteine has some inhibitory effect on homocystine-induced proliferation (Fig. 1, B and E; \( P < 0.005 \)). The dose dependence of homocysteine treatment on VSMC growth and DNA synthesis suggested a maximal effect at 10–12 µM (Fig. 1C). The treatment of cystine, cysteine, and NAC demonstrated no significant proliferation on HVSMS (Fig. 1D; \( P > 0.01 \)).

To determine the actual mitogenic effect of homocysteine in HVSMS, incorporation into the HVSMS in the presence and absence of homocysteine of radiolabeled precursor of DNA, \(^{3} \text{H}\) thymidine, was carried out. The radioactivity incorporated into the cells was 210 ± 30 and 380 ± 35 disintegrations/min in the absence and presence of homocysteine, respectively. The results suggested that homocysteine stimulated the de novo DNA synthesis.

Collagen and \( \alpha \)-actin induction in HVSMS by homocysteine. ECM collagen is the primary component of atherosclerotic plaque structure. To determine whether homocysteine induces both collagen and contractile \( \alpha \)-actin expression simultaneously, confluent HVSMS were cultured in the presence and absence of homocysteine at various time intervals (Fig. 2). Incubation of HVSMS with 10 µM homocysteine induces time-dependent expression of collagen and \( \alpha \)-actin. Treatment with cysteine (Fig. 2, lane 5), cysteine, and homocysteine has no effect on HVSMS collagen and actin expression. The scanned intensity data for collagen and actin mRNA were normalized with the 18S R gene. From the plots of normalized data and time, the half-time (t₁/₂) for the homocysteine effect on HVSMS was estimated to be 1 and 2.5 h for actin and collagen expressions, respectively. Based on the concentration of homocysteine (10
µM) and $t_{1/2}$, a second-order rate constant for homocysteine action was calculated. The results suggested induction of actin with a rate of $\sim 1 \times 10^4 \text{min}^{-1} \cdot \text{M}^{-1}$. The rate of collagen expression was $\sim 6 \times 10^2 \text{min}^{-1} \cdot \text{M}^{-1}$ (Fig. 2B). This suggested that the rate of collagen synthesis is slower than the synthesis of actin. To further evaluate that homocysteine induces a contractile phenotype before its expression of ECM components, we assessed protein levels of collagen and actin after homocysteine treatment (Fig. 2C). Results suggested significant ($P < 0.001$) induction of actin at 6 h compared with collagen expression at 6 h, suggesting that homocysteine induces a contractile phenotype before its expression of ECM components.

To determine the effective dose and affinity of homocysteine to HVSMC and its induction of collagen in HVSMC, we incubated cells with various concentrations of homocysteine. The mRNA for collagen was
Fig. 2. Time course of effect of homocystine on gene transcription of collagen type I and α-actin in HVSMC. Cells were grown to confluence (10^6 cell) in 10% fetal calf serum (FCS). Cell growth was arrested by depriving serum for 24 h before treatment with 10 µM homocystine, and cells were harvested at various time intervals in serum-free Dulbecco's modified Eagle's medium (DMEM). Lanes 1-4 are 0, 6, 12, and 24 h treatment, respectively. Lane 5, cells were treated with 50 µM cysteine (Cys) alone.

A: Northern blot analysis on isolated RNA of collagen I and α-actin. 18SR was used as an internal control.

B: Graphic presentation of scanned data normalized with 18SR RNA gene. Size of transcript is 3.5 kb for collagen and 1.1 kb for α-actin. All quantitative data were compared on a single blot.

C: Enzyme-linked immunosorbent assay of protein levels of collagen and α-actin in HVSMC after activation by homocystine at different time intervals.
normalized with the 18SR gene (Fig. 3A). The plot between homocystine concentration and mRNA level of collagen demonstrated a 50% effective concentration of ~5 µM for homocystine on HVSMC (Fig. 3B). Disulfide homocystine interacts with thiol to produce disulfide exchange. This may reduce the homocystine concentration. Therefore, thiol-containing reagents may reduce homocystine binding to HVSMC. To determine whether reducing agent, glutathione, GSH, inhibits the homocystine effect on HVSMC, various concentrations of GSH were added to homocystine-activated HVSMC. The collagen mRNA normalized with the 18SR gene was measured (Fig. 3B). Results suggested dose-dependent inhibition of homocystine-induced collagen expression. At a concentration of 40 µM, GSH inhibited collagen expression by 85%. From the plot between the decrease in collagen mRNA levels and the concentration of GSH (Fig. 3B), a 50% inhibition constant of ~15 µM was estimated (Fig. 3B).

To determine whether other reducing agents inhibit homocystine-induced collagen expression, we cultured HVSMC in the presence of cysteine, NAC, and the thiol-containing propeptide (prop) of matrix metalloproteinase. The propeptide keeps matrix metalloproteinase in its inactive/latent form. The results suggested that cysteine, NAC, and propeptide at a concentration of 50 µM completely inhibited the homocystine-induced collagen expression in HVSMC. On the other hand, thiol-containing homocysteine at a concentration of 100 µM partially inhibits the collagen expression (Fig. 4). The GSH, NAC, and propeptide alone do not induce collagen expression (data not shown). This suggested a differential effect of oxidative thiols on the homocystine-induced collagen expression in HVSMC. Inhibition of collagen expression by propeptide may also suggest the following dual effects of activation of matrix metalloproteinases: 1) active metalloproteinase will degrade collagen, and 2) liberated propeptide will inhibit collagen synthesis. These events will increase ECM turnover during remodeling. However, the reverse of these events will lead to ECM accumulation and atherosclerotic stage. The results (Fig. 4) further
suggest that reducing agent can reverse the effect of homocystine on HVSMC as well as collagen expression. The binding of homocystine was displaced by GSH and NAC. This raised the possibility that HVSMC contains the receptor for homocystine.

Receptor of homocystine on HVSMC. To localize the receptor for homocystine, I labeled homocystine with fluorescamine. Disulfide homocystine contains no free thiol. It is therefore not possible to label at thiol. However, homocystine contains two primary amine, NH₂, groups. Fluorescamine conjugates with primary amines to produce an amide linkage and fluorescent product (Fig. 5). Free fluorescamine has no intrinsic fluorescence. We synthesized homocystine-fluorescamine conjugate by a 1:4 stoichiometry reaction between homocystine and fluorescamine. The reaction product gives fluorescence at 480 nm when excited at 380 nm. Therefore, we used fluorescamine to label homocystine and used fluorescence-labeled homocystine as the probe to measure receptor labeling on the HVSMC. Also, to determine the specificity of binding to HVSMC, we synthesized another fluorescamine analog, the fluorescamine-cystine complex.

In Fig. 6, binding of labeled homocystine to HVSMC is demonstrated. The fluorescence labeling of homocystine in HVSMC was localized at the membrane. Incubation of homocystine with fluorescamine-homocystine produced bright crispy fluorescent granules in and around the cell membrane (Fig. 6A). Some of the homocystine appears to have entered into the cytoplasm as well. Whether this diffusion or transport of homocystine into the cell is mediated through receptor internalization or redox channel mechanisms remains to be elucidated. On the other hand, fluorescamine-cystine has minimal binding under these conditions (Fig. 6B). Pretreatment of homocysteine has no effect on receptor labeling. However, in the presence of NAC, the homocystine ligand binding was abolished (Fig. 6C). These results suggested that NAC was able to prevent the homocystine binding to HVSMC.

Affinity cross-linking. The study was conducted to determine the homocystine distribution, protein/pep-
tide subunits, and their apparent molecular weights in SMC. Fluorography comparing SDS-PAGE patterns obtained after affinity labeling homocystine receptors, with fluorescamine-labeled homocystine as reporter, with and without unlabeled fluorescamine is shown in Fig. 7. A major fluorogenic 40-kDa band and a less fluorogenic band at 25 kDa were observed, suggesting cross-reactivity of homocystine with SMC membrane proteins. This labeling was displaced by NAC, suggesting a disulfide nature of the interactions between the receptor and homocystine.

**DISCUSSION**

The mechanisms by which homocystine induces coronary atherosclerotic and fibrotic lesions are largely unknown. ECM collagen is the primary component of atherosclerotic lesions (31). Here, we demonstrated that oxidative disulfide homocystine induces collagen expression in HVSMC. This induction of collagen was blocked by GSH and NAC. Homocystine interacted with HVSMC via a membrane receptor. The binding of homocystine to receptor was reversed by reduced NAC.

Oxidative mixed disulfide and GSSG induce a proliferative stage in the vessel wall (16). Hypertension, but not total serum lipoproteins, cholesterol, and fibrinogen, was significantly associated with atherosclerosis in homocysteine (e)mic subjects (2). This may also suggest a role of intimal SMC proliferation after homocyst(e)inemia. Homocyst(e)line has been shown to promote SMC growth and cause dysfunction in endothelium (22). The SMC growth was linked to cyclin expression and kinase activity (23), suggesting a role of homocysteine in signal transduction. Previously, we demonstrated that oxidative serum conditions induce cellular proliferation and that a reducing condition inhibits cell proliferation (25). Furthermore, we demonstrated that redox state around the cell modulates ECM expression by regulating its turnover (25). In this study, we determined that oxidative disulfide homocystine induces HVSMC proliferation and ECM expression (Figs. 1 and 2). These data suggested that oxidative homocyst(e)line induces the redox state around the vascular SMC, which in turn induces cellular proliferation and ECM expression.
Thiol autooxidation of reduced homocysteine forms stable homocystine, and it had a direct cytotoxic effect on the cells (14). Cell-detachment studies indicated that sulphydryl-containing amino acids may have marginal relevance to endothelial cell detachment and mechanisms of atherosclerosis in homocystinuria (6). However, oxidative mixed disulfide may induce cell detachment by inducing expression of ECM components. In fact, a role of ECM components in endothelial cell detachment has been suggested (8). In vivo atherosclerotic lesions contain high levels of collagen (31). It was demonstrated that homocysteine induces collagen expression in HVSMC in a dose-dependent and time-dependent manner (Figs. 2 and 3). This is the first study that shows a role of homocystine in ECM collagen expression and suggests a link to the development of atherosclerotic-fibrotic lesion. Our data further suggest that the effect of homocysteine can be reversed by the excess amount of GSH or cysteine (NAC; Fig. 4). To our surprise, we did not observe the reverse effect of reduced homocystine over homocystine. This may in part be due to the fact that, at ambient conditions, most of the free thiols are autooxidized and form stable disulfides and, therefore, decrease the potency of free thiols. In fact, in plasma, most of the homocysteine is in the form of oxidative mixed disulfide and is protein bound, and only a very small fraction is in the reduced form.

Homocysteine detaches endothelial cells by its cytotoxic effect and therefore gets access to the SMC (6, 8, 14). Thereafter, homocysteine induces proliferation in HVSMC. We have shown induction at the transcription level and the role of signal transduction (i.e., actin expression) in matrix metalloproteinase and their inhibitor expression in cardiac fibroblast cells by serum (26). It is not clear whether homocysteine induces cellular migration/contraction before synthesizing ECM components. We show that contractile actin expression is induced before the expression of ECM collagen in HVSMC (Fig. 2). These data suggested that homocysteine may also induce migration in HVSMC by inducing α-actin. This induction may be associated with the transformation of SMC to myofibroblast-like cells. In fact, in the atherosclerotic lesion, SMC are transformed and differentiated to myofibroblast-like cells (21), and myofibroblasts synthesize collagen (1).

Receptor(s) of homocystine on HVSMC have not been demonstrated previously. However, one study has suggested that a membrane-associated assembly of plasminogen and tissue plasminogen activator (tPA) involved a membrane protein of 40 kDa in ternary complex (11). The addition of homocysteine to ternary complex and endothelial cells dissociated 40-kDa protein, and tPA and has no effect on tPA plasminogen interaction (11), suggesting that 40-kDa membrane protein may be involved in homocysteine binding. However, this study does not reveal directly the receptor of homocystine on SMC. We have synthesized and purified fluorescent homocysteine as a probe for binding to the HVSMC membrane (Fig. 5). Incubation of labeled homocystine with HVSMC localized homocystine receptors on HVSMC (Figs. 6 and 7). Based on photo cross-linking experiments, we observed the molecular weight of homocystine receptor on vascular SMC of 40–25 kDa (Fig. 7). This receptor binding was sensitive to the reducing agent (NAC), suggesting reversibility of this binding. The redox cycle plays an important role as an endogenous antioxidant defense mechanism in cultured endothelial cells (13). To our knowledge, this is the first report on the receptor of homocystine. Furthermore, it is the first receptor that is sensitive to the redox state on the cell membrane (i.e., redox receptor). It is of great interest to identify and to characterize this receptor.

In early atherosclerotic lesions, oxidative stress is manifested by an elevated production of reactive oxygen species by neutrophils, macrophages, and endothelial cells that results in the oxidative modification of low-density lipoprotein. Oxidized low-density lipoprotein is a potent stimulator of inflammation. In this oxidative environment, vascular cell adhesion molecular expression and monocyte accumulation have been observed in the early atherosclerotic process (18). We demonstrated that oxidative homocystine induces the metabolic changes in HVSMC. In normal HVSMC, homocystine induces the expression of collagen. After the treatment with homocystine, the α-actin level was induced at the gene transcription level. The mechanism for collagen induction by homocystine in HVSMC is mediated through the redox-sensitive cell membrane receptor. The effect of homocystine was reversed by the treatment of cells with reducing agent. These results suggested that an antioxidant therapy may benefit against atherogenesis.

Perspective. In normal HVSMC, collagen was induced by redox homocystine. Although an effect on a redox mechanism is inferred, this study does not identify the specific redox biochemistry mediating the antioxidant regulatory effect. However, our studies indirectly suggest that thiol-antioxidants function through the mechanism related to its thiol group properties. It will be of great interest to elaborate on other ECM components and the signal transduction mechanisms involved in response to homocystine-induced collagen and other ECM components. For example, transforming growth factor-β1 is a strong stimulator of collagen (4), and decorin, a physiological transforming growth factor-β1 antagonist, neutralizes its effect (15). It is not known whether homocysteine induces transforming growth factor-β1 and represses decorin expression in the cardiovascular system. These studies are in progress.

The author acknowledges the expert technical assistance of Susan Borders and Suresh Kumar in this work. A part of this study was carried out at the University of Missouri, Columbia, MO.

This work was supported in part by National Institutes of Health Grants GM-46366 and HL-51971 and by a Grant-in-Aid from the American Heart Association.

A preliminary account of this study was presented at the 69th conference of the American Heart Association on November 9–13, 1996, New Orleans, LA, and at Experimental Biology '97 on April 5–9, 1997, New Orleans, LA.
REFERENCES


