Biomechanical Strain Induces Class A Scavenger Receptor Expression in Human Monocyte/Macrophages and THP-1 Cells

A Potential Mechanism of Increased Atherosclerosis in Hypertension

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Background—Although hypertension is an important risk factor for the development of atherosclerosis, the mechanisms for this interaction are incompletely described. Previous studies have suggested that biomechanical strain regulates macrophage phenotype. We tested the hypothesis that biomechanical strain can induce expression of the class A scavenger receptor (SRA), an important lipoprotein receptor in atherogenesis.

Methods and Results—Human monocyte/macrophages or THP-1 cells were cultured in a device that imposes uniform biaxial cyclic 1-Hz strains of 0%, 1%, 2%, or 3%, and SRA expression was analyzed. Mechanical strains induced SRA mRNA (3.5 ± 0.6-fold at 3% strain for 48 hours, \( P < 0.01 \)) and SRA protein in THP-1 cells in an amplitude-dependent manner. This induction was accompanied by augmented expression of the class B scavenger receptor CD36 (2.8 ± 0.3-fold, \( P < 0.001 \)) but not by increased peroxisome proliferator–activated receptor-\( \gamma \) expression. To evaluate this effect in vivo, apolipoprotein E\( ^{2/2} \) mice were randomly assigned to receive standard chow, a high-cholesterol diet, or a high-cholesterol diet with hypertension induced by angiotensin II infusion for 8 weeks. Immunohistochemistry revealed that among macrophages in atherosclerotic lesions of the aorta, the proportion of macrophages with SRA expression was highest in hypertensive animals on a high-cholesterol diet (43.9 ± 0.7%, versus 12.0 ± 2.0% for normotensive animals on a high-cholesterol diet and 4.7 ± 4.7% for animals on standard chow; \( P < 0.001 \)).

Conclusions—Biomechanical strain induces SRA expression by monocyte/macrophages, suggesting a novel mechanism for promotion of atherosclerosis in hypertensive patients. (Circulation. 2001;104:109-114.)

Key Words: strain ■ receptors ■ cells ■ atherosclerosis ■ hypertension

Hypertension is a well-known risk factor for atherosclerosis, but the molecular mechanisms that link elevated blood pressure to atherosclerosis progression remain uncertain. Experimental evidence indicates that hypertension may promote expression of cell adhesion molecules by endothelial cells and production of cytokines in the vessel wall, suggesting a role of hypertension as a proinflammatory stimulus contributing to atherogenesis. Another potential mechanism is that LDL derived from hypertensive patients is more susceptible to lipid peroxidation mediated by angiotensin (Ang) II and to its cellular uptake by macrophages than that obtained from normotensive subjects. Little is known, however, about how the mechanical forces that occur as a result of elevated blood pressure may directly accelerate atherosclerosis.

Monocyte/macrophages play an important role in atherosclerotic lesion formation. Elevated plasma levels of LDL lead to the accumulation of lipoproteins in the arterial wall. In the arterial wall, LDL is chemically modified, and modified lipoproteins activate endothelial cells. Monocytes subsequently adhere to the endothelial cells, cross the endothelial layer to enter the subendothelial space, differentiate into macrophages, and eventually become foam cells. Macrophage-derived foam cells are characterized by accumulation of cholesterol esters, resulting from the uptake of modified lipoproteins, such as oxidized LDL, through macrophage scavenger receptors.

The class A scavenger receptors (SRAs) are trimeric membrane glycoproteins that participate in the deposition of lipids in the arterial wall during atherogenesis. The expression of SRA is confined mainly to tissue macrophages and related cell types. Circulating monocytes express SRA at low levels, and SRA expression increases after migration into the arterial intima and differentiation into macrophages.
Recently, Ricote and colleagues\textsuperscript{12} and Tontonoz and colleagues\textsuperscript{13} demonstrated that peroxisome proliferator–activated receptor-\textgamma{} (PPAR-\textgamma{}), a member of the nuclear receptor superfamily of ligand-dependent transcription factors, mediates inhibition of SRA expression. Our previous experiments suggest that biomechanical strain may regulate macrophage phenotype after adhesion has occurred.\textsuperscript{14} Thus, the differentiation of monocytes to macrophages may be stochastic, with different signals from adhesion, migration, and deformation of monocytes as well as growth factor, cytokine, and biomechanical signals. In this study, we hypothesized that biomechanical deformation of monocyte/macrophages induces expression of the SRA receptor, representing a potential mechanism by which hypertension promotes atherosclerosis.

**Methods**

**Cell Culture**

Human monocyte/macrophages were isolated by Histopaque-1077 centrifugation (Sigma) followed by adherence in culture flasks, as previously described.\textsuperscript{14} Human monocytic cell line THP-1 was purchased from American Type Culture Collection.

**Mechanical Strain**

Mechanical deformation was applied with a device that produces a nearly homogeneous biaxial strain profile; that is, strains that are equal at all locations on the membrane and in all directions.\textsuperscript{15} For the preparation of cells subjected to mechanical strains, autoclaved membranes were coated with 2 \textmu{}g/mL of human serum fibronectin for 16 to 24 hours at 4°C. Primary monocyte/macrophages were plated at a density of 5 000 000 cells/dish in 13 mL of RPMI-1640 containing 10% human serum. THP-1 cells were plated at a density of 10 000 000 cells/dish in 13 mL of RPMI-1640 containing 10% FCS.

**Northern and Western Analysis**

Total RNA was isolated by the guanidinium isothiocyanate–phenol-chloroform method.\textsuperscript{16} Human SRA and the PPAR-\textgamma{} cDNAs were provided by Dr C.K. Glass (University of California, San Diego). The probe for CD36 was generated by polymerase chain reaction. The primer set for the synthesis of CD36 was 5'-AAATGT-ATCCCAAGGAGCCTG-3' sense and 5'-GTCCGACTGACTTTCCCAAT-3' antisense, yielding a 430-bp cDNA. Probes were radiolabeled by the random priming method with [\textalpha{}-\textsuperscript{32}P]dCTP (NEN Life Science Products) and the Klenow fragment of DNA polymerase (Stratagene). Northern analysis was performed as previously described.\textsuperscript{14} Immunoblotting was performed as described previously\textsuperscript{14} with the following primary antibodies: (1) SRA-E5 anti-human SRA monoclonal antibody (mouse IgG1 fraction), a gift from Dr M. Takeya (Kumamoto University, Kumamoto, Japan); (2) anti-human CD36 monoclonal antibody (mouse IgM fraction); and (3) anti-human PPAR-\textgamma{} monoclonal antibody (mouse IgG1 fraction) (Santa Cruz Biotechnology).

**Animal Models**

Apolipoprotein (apo) E\textsuperscript{-/-} mice (Jackson Laboratory), 4- to 6-week-old males, were used in this study. All animals received care in accordance with NIH guidelines. Eleven apoE\textsuperscript{-/-} mice were randomly divided into 3 groups: (1) animals fed standard chow (Purina Certified Rodent Chow 5001, n=4), (2) animals fed a high-cholesterol diet (Research Diets, n=4), and (3) animals fed a high-cholesterol diet with Ang II infusion (n=3) for 8 weeks. The high-cholesterol diet was designed to match the original "Paigen’s Atherogenic Rodent Diet" and contained 1.25% cholesterol and 0.5% cholic acid.\textsuperscript{17} Some mice received Ang II infusions from implanted osmotic minipumps (Alzet model 1002) for 8 weeks. An osmotic pump containing Ang II dissolved in a solution of 0.15 mol/L NaCl and 0.01 mol/L acetic acid at a concentration calculated to deliver \textapprox{}0.7 mg \cdot kg\textsuperscript{-1} \cdot d\textsuperscript{-1} of drug was placed into the subcutaneous space. The osmotic pumps were replaced every 2 weeks to maintain a constant infusion of Ang II during the 8-week experiment. Systolic blood pressure was measured with a computerized, noninvasive tail-cuff system (BP 2000 Visitech systems) during treatment and before euthanization.\textsuperscript{18} Some data from these mice are described in a previous publication.\textsuperscript{19}

**Immunohistochemical Analysis**

Aortic tissues were embedded, frozen in liquid nitrogen, and stored at \textminus{}80°C. Serial 6-\textmu{}m-thick frozen sections were stained with anti-mouse macrophage Mac-3 (PharMingen) and anti-mouse SRA 2F8 (Sero tec). Species-appropriate biotinylated secondary antibodies were applied, followed by avidin–alkaline phosphatase complexes (Vector Laboratories), and the reaction was visualized with fast red dye (Sigma) containing 0.15 mg/mL levamisol. To determine the proportion of SRA-positive macrophages for each animal, the total number of cells positive for Mac-3 or SRA in atherosclerotic plaques of the aorta was counted for each section. To avoid bias, 2 investigators who were unaware of the type of staining or assignment of group were asked to determine the proportion of SRA-positive macrophages. To confirm the visual scoring system quantitatively, a separate computer-based image analysis was also used, as described previously\textsuperscript{20}.

**Results**

**Cell Adhesion Regulates SRA mRNA Expression**

We first investigated whether cell adhesion to the fibronectin-coated membranes modulated gene expression of SRA in THP-1 cells. Untreated THP-1 cells did not express significant amounts of SRA mRNA (Figure 1). After THP-1 cells had been plated onto the membranes, cell adhesion alone resulted in a small induction of SRA mRNA expression in a time-dependent manner. Moreover, 160 \textmu{}mol/L phorbol 12-myristate 13-acetate (PMA), an effective inducer of THP-1 differentiation, led to a markedly additive induction of SRA mRNA expression.

**Strain Induces SRA mRNA Expression by Monocytes/Macrophages and THP-1 Cells**

We next investigated whether mechanical strain increased SRA mRNA expression by primary monocytes/macrophages and THP-1 cells. To eliminate the variable of time-dependent changes due to cell age or effects of adhesion, in each subsequent experiment, all cells were cultured on the membrane for an identical time period, and cells from all samples...
were harvested at the same time. Thus, in a time-course experiment with strain, the time point represents the time before harvest that strain was initiated, such that the strain sample and control sample were cultured, plated, and harvested at the same time. As shown in Figure 2A, 3% cyclic mechanical strain at 1 Hz increased SRA mRNA expression in primary human monocyte/macrophages at 48 hours (mean±SEM, 3.1±0.2-fold, n=3, *P<0.001 by ANOVA with Fisher’s least significant difference test). We further explored the effect of mechanical strain on SRA mRNA expression by THP-1 cells, because THP-1 cells are more readily available in numbers required for strain experiments. Mechanical strain of 3% induced SRA mRNA expression in a duration-dependent manner (Figure 2B). The increase in SRA mRNA at 48 hours was 3.5±0.6-fold (*P<0.01, n=3).

Mechanical strain also induced CD36 mRNA expression with a peak at 48 hours (2.8±0.3-fold, *P<0.001, n=3), whereas strain did not induce PPAR-γ mRNA. In addition, when cyclic biaxial strains of 0%, 1%, 2%, and 3% at 1 Hz for 24 hours were imposed, induction of SRA and CD36 mRNA expression in THP-1 cells was amplitude-dependent (Figure 3A). This effect was less prominent than induction by the positive control, 160 µmol/L PMA. These results demonstrate that the expression of SRA and CD36 may be a stochastic process, with separate signals from adhesion and biomechanical strain.

In studies exploring the potential mechanism of this effect, we tested the hypothesis that mechanical strain increased SRA expression by decreasing the rate of degradation of mRNA. THP-1 cells were incubated in the presence or absence of 3% strain for 24 hours and then incubated further with actinomycin D (5 µg/mL) to inhibit transcriptional activity. The half-life of SRA mRNA was not affected by mechanical strain (data not shown). This experiment suggested that mechanical strain increases the rate of synthesis of SRA mRNA. Transient transfection studies with reporter constructs with 5′ regulatory elements for SRA gene expression (the upstream sequences from −4.5 to +46 bp from the major transcriptional start site) did not demonstrate induction of reporter activity by strain in conditions that successfully induced reporter activity after treatment with PMA. Thus, biomechanical induction of SRA may be mediated by other cis-acting regulatory elements distinct from PMA-responsive elements.

**Effects of Ang II Type 1 Receptor Antagonist**

We studied whether the effect of cyclic mechanical strain on SRA mRNA expression in THP-1 cells is Ang II–dependent. THP-1 cells were subjected to 3% cyclic strain at 1 Hz for 24 hours in the presence or absence of an Ang II type 1 (AT₁) receptor antagonist. As shown in Figure 3B, losartan (1 µmol/L), an AT₁ receptor antagonist, did not inhibit the induction of SRA mRNA by mechanical strain. This suggests that Ang II may not mediate the effect of mechanical strain on SRA mRNA expression in THP-1 cells. In addition, Ang II (1 µmol/L) did not increase SRA expression.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Dependence of SRA mRNA expression in primary human monocyte/macrophages (A) and THP-1 cells (B) on duration of mechanical strain. A. Monocyte/macrophages were plated for 16 to 24 hours, and then no strain or 3% cyclic mechanical strains (1 Hz) were imposed for 0, 6, 24, and 48 hours. Total RNA was isolated at each time point, and SRA was analyzed by Northern analysis. B. THP-1 cells were plated for 16 to 24 hours, and then 0% or 3% cyclic mechanical strains (1 Hz) were imposed for 0, 3, 6, 24, and 48 hours. Total RNA was isolated at each time point, and SRA, CD36, and PPAR-γ were analyzed by Northern analysis. Ethidium bromide–stained 18S ribosomal subunit is also shown. Each figure is representative of 3 similar experiments.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Dependence of SRA mRNA expression in THP-1 cells on amplitude of mechanical strain (A) and effects of AT₁ receptor antagonist on SRA induction by mechanical strain (B). A. THP-1 cells were exposed for 24 hours to 0%, 1%, 2%, and 3% cyclic mechanical strains (1 Hz). Expression of SRA, CD36, and PPAR-γ was analyzed by Northern analysis. B. THP-1 cells were exposed for 24 hours to 0% or 3% cyclic mechanical strain (1 Hz) in presence or absence of losartan (10 µmol/L) or Ang II (10 µmol/L). Losartan was applied to cells 30 minutes before mechanical strain or Ang II treatment. Ethidium bromide–stained 18S ribosomal subunit is also shown. Each figure is representative of 3 similar experiments.
Effects of Mechanical Strain on SRA Protein Expression

We then investigated whether the increase in SRA mRNA by mechanical strain was accompanied by increases in SRA protein. THP-1 cells were subjected to 0% or 3% cyclic mechanical strain (1 Hz) for 24 hours with (+) or without (−) 160 μmol/L PMA. Total cell lysates were then analyzed by Western analysis with anti-SRA, anti-CD36, and anti-PPAR-γ monoclonal antibodies. Mechanical strain induced the expression of SRA protein by THP-1 cells compared with adhesion alone (Figure 4). The induction of SRA by strain was smaller than that by PMA treatment, and there was no additive effect of PMA and strain. Mechanical strain also induced CD36 protein expression, whereas it did not change PPAR-γ protein expression.

Hypertension Increases SRA Expression by Macrophages in Aortic Atherosclerotic Lesions in ApoE<sup>−/−</sup> Mice

We hypothesized that, in the presence of hypertension, the proportion of macrophages expressing SRA would increase. Hypertension was successfully achieved in Ang II–treated apoE<sup>−/−</sup> mice and was persistent for 8 weeks. The average systolic arterial blood pressures at 8 weeks were 104.3±0.5 mm Hg for apoE<sup>−/−</sup> mice placed on normal chow, 103.1±0.8 mm Hg for apoE<sup>−/−</sup> mice on a high-cholesterol diet, and 159.7±2.4 mm Hg for Ang II–treated apoE<sup>−/−</sup> mice on a high-cholesterol diet. The size and number of aortic atherosclerotic lesions were observed to be larger in Ang II–treated apoE<sup>−/−</sup> mice on a high-cholesterol diet than in apoE<sup>−/−</sup> mice on low- and high-cholesterol diets (data not shown). Figure 5A shows representative sections of aortic atherosclerotic lesions that have been stained for mouse macrophage Mac-3 or the SRA protein. Macrophages in Ang II–treated apoE<sup>−/−</sup> mice on a high-cholesterol diet colocalized with SRA protein, whereas macrophages derived from apoE<sup>−/−</sup> mice on low- and high-cholesterol diets did not react with the 2F8 antibody.

Figure 4. Effects of mechanical strain on SRA, CD36, or PPAR-γ protein expression. THP-1 cells were subjected to 0% or 3% cyclic mechanical strains (1 Hz) for 24 hours with (+) or without (−) 160 μmol/L PMA. Total cell lysates were then analyzed by Western analysis with anti-SRA, anti-CD36, and anti-PPAR-γ monoclonal antibodies. This figure is representative of 3 similar experiments.

Figure 5. Expression of SRA proteins by macrophages in atherosclerotic lesions of aortas derived from apoE<sup>−/−</sup> mice. A, Representative examples of staining of Mac-3 and SRA (×400) of atherosclerotic lesions of aorta from apoE<sup>−/−</sup> mice receiving normal cholesterol diet (n=4), high-cholesterol diet (n=4), and high-cholesterol diet with Ang II infusion (n=3) for 8 weeks. B, Proportions of SRA-positive cells to Mac-3–positive cells in atherosclerotic lesions. Data (mean±SEM) represent percentage of macrophages expressing SRA proteins.
By visual scoring, the proportion of SRA-positive macrophages was highest in Ang II–treated apoE−/− mice fed a high-cholesterol diet (43.9±0.7%, n=3, P<0.001 versus other groups, Figure 5B). The percentage of macrophages that were SRA-positive was lower in apoE−/− mice fed a high-cholesterol diet only (12.0±2.0%, n=4) and apoE−/− mice fed normal chow (4.7±4.7%, n=4). By an independent computer-based image analysis, the results were essentially identical. The percentages of SRA-positive macrophage area were 52.3±4.5% for Ang II–treated apoE−/− mice fed a high-cholesterol diet (P<0.001 versus other groups), 15.0±3.6% for apoE−/− mice fed a high-cholesterol diet, and 4.0±4.0% for apoE−/− mice fed normal chow. These data suggest that in addition to promoting increased infiltration by macrophages, hypertension created by Ang II is associated with increased SRA expression.

**Discussion**

**Interaction of Hypertension and Atherosclerosis**

Hypertension may worsen atherosclerosis by several mechanisms. Elevated blood pressure may modulate gene expression of arterial cells by biomechanical forces.22 For example, cyclic strain increases the expression of intercellular adhesion molecule-1 by cultured vascular endothelial cells and enhances adhesion of monocytes to endothelial cells.23 In addition, spontaneously hypertensive rats show an increase in the endothelial expression of intercellular adhesion molecule-1 and the endothelial adhesion of monocytes compared with normotensive rats.3 Patients with hypertension have higher circulating levels of soluble intercellular adhesion molecule-1 than normotensive subjects, and both systolic and diastolic blood pressure influence adhesion molecule levels.24 These findings suggest that hypertension may promote monocyte adhesion to the endothelium and subsequent atherosclerosis.

Ang II may play a role in the hypertension-atherosclerosis interaction through blood pressure–dependent and –independent mechanisms. An animal model of Ang II–induced hypertension showed direct generation of superoxide anions in the vasculature25 and a marked inflammatory response characterized by recruitment of macrophages into the arterial wall.26 Ang II also induces expression of the lectin-like oxidized LDL receptor (LOX-1) by vascular endothelial cells, which enhances LDL clearance by endothelial cells.27 LOX-1 expression increases in spontaneously hypertensive rats and salt-loaded Dahl salt-sensitive rats.28 Thus, the interaction of Ang II and oxidized LDL may provide an additional link of hypertension to angiogenesis.

**Mechanical Strain as a Novel Mechanism of Atherosclerosis**

Our results suggest that augmented levels of SRA and CD36 in response to biomechanical strain is another mechanism whereby increased blood pressure may promote atherosclerosis. SRA is a scavenger receptor that binds with high affinity to modified lipoproteins.29 CD36 is a member of the class B scavenger receptors that recognize oxidized lipoproteins but not acetylated lipoproteins.30 Unlike the classic LDL receptor, high levels of intracellular cholesterol do not suppress expression of scavenger receptors.31,32 The unrestricted uptake of modified lipoproteins by macrophages through scavenger receptors leads to formation of foam cells containing abundant cholesteryl esters.6 Studies of mice lacking both SRA and apoE demonstrate a 60% reduction in atherosclerotic lesion development compared with deletion of apoE alone.33 In addition, mice lacking both CD36 and apoE have a reduction of lesional area by 76% and in the aortic sinuses by 45% after 12 weeks on a high-cholesterol diet.34 These findings suggest a strong proatherogenic role for SRA and CD36.

**Study Limitations**

We used a mouse model of Ang II–induced hypertension to probe the role of elevated blood pressure in the induction of SRA on macrophages and the development of atherosclerotic lesions in vivo. The proatherogenic effects of Ang II infusion may be due not only the mechanical effects of elevated blood pressure but also the humoral effects of Ang II.19 Our experiments showed that Ang II did not induce SRA mRNA expression in THP-1 cells, suggesting that the humoral effects of Ang II do not play a role in the induction of SRA on monocyte/macrophages. Further studies of other hypertension models are necessary to determine whether induction of SRA on macrophages in this mouse model results indirectly from Ang II. Finally, our studies demonstrated that small deformations (as small as 1%) induce SRA expression. Although this amplitude of deformation is well within the range of arterial wall deformation and on the same order as an increase in arterial strain with increased pulse pressure, it is important to recognize that the precise increase in cellular strain experienced by the macrophage itself within the wall is unknown.

In addition to the interactions between hypertension and atherosclerosis, these data also have potential implications for atherosclerotic plaque stability. Unstable plaques frequently have infiltration of mononuclear cells and overexpression of matrix metalloproteinases at sites of increased biomechanical stress. The reasons for this localization of matrix-degrading activity at the precise locations where biomechanical forces are greatest are unclear, although the combination of high stresses and excessive matrix degradation may be particularly detrimental.35 These data suggest that biomechanical activation of monocyte/macrophages participates in the inflammatory cascade at these high-stress locations.

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**References**


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