UltraRapid Communication

Transcriptional Repression of ATP-Binding Cassette Transporter A1 Gene in Macrophages
A Novel Atherosclerotic Effect of Angiotensin II


Abstract—Angiotensin II (Ang II) is a powerful accelerator of atherosclerosis. Herein, we describe a novel transcription mechanism through which Ang II inhibits macrophage expression of the ATP-binding cassette transporter A1 (ABCA1), a key regulator of reverse cholesterol transport. We demonstrate that chronic Ang II infusion substantially promotes macrophage infiltration, foam cell formation, and atherosclerosis in low-density lipoprotein receptor–deficient mice and significantly reduces ABCA1 expression in peripheral macrophages. Administration of the Ang II type 1 receptor blocker valsartan inhibited Ang II–induced ABCA1 mRNA repression, macrophage cholesterol accumulation, and atherosclerosis. Ang II treatment reduced ABCA1 promoter activity of in vitro cultured mouse peritoneal macrophages, inducing fos-related antigen 2 (Fra2) protein binding to an ABCA1 promoter E-box motif, a site known to negatively regulate macrophage ABCA1 transcription. Valsartan pretreatment blocked Fra2 binding to the ABCA1 promoter, and Fra2 small interfering RNA pretreatment attenuated Ang II–mediated ABCA1 transcriptional inhibition, confirming the role of Fra2 in this process. This new evidence suggests that Ang II, a well-known proinflammatory and pro-oxidative factor, alters macrophage cholesterol homeostasis by repressing ABCA1 to promote foam cell formation and atherosclerosis. (Circ Res. 2005;97:e88–e96.)

Key Words: angiotensin II • ATP-binding cassette transporter A1 • macrophage cholesterol accumulation • fos-related antigen 2

Ang II-mediated repression of ABCA1 was underscored recently by studies in which bone marrow from ABCA1-null mice was transplanted into either apolipoprotein E–deficient (apoE−/−) or low-density lipoprotein receptor–deficient (LDLR−/−) atherosclerosis-prone mice. Macrophage ABCA1 deficiency markedly increased atherosclerosis in these mice.5,6 In contrast, systemic or macrophage-specific ABCA1 overexpression attenuated atherosclerotic lesion area.7 It is now well established that activation of liver X receptor (LXR), which binds to a DR4 site in the ABCA1 promoter, is a critical step in the upregulation of ABCA1 expression and cholesterol efflux.8,9 However, little is known about the mechanisms responsible for the repression of ABCA1 expression.

Endotoxin and cytokines have been reported to decrease ABCA1 expression and cholesterol efflux from cholesterol-loaded macrophages.10–12 Angiotensin II (Ang II) has also
been reported to decrease cholesterol efflux in mouse peritoneal macrophages (MPMs)\textsuperscript{11,12}, however, this study did not find that Ang II suppressed macrophage ABCA1 expression or provide a mechanism for the reduced cholesterol efflux. In the present investigation, we demonstrate that chronic Ang II infusion strongly promotes macrophage foam cell formation and atherosclerosis in LDLR\textsuperscript{-/} mice through an Ang II type 1 (AT1) receptor--dependent pathway. Quantitative real-time RT-PCR (QRT-PCR) analyses, which targeted cholesterol transport--related genes, showed that Ang II infusion reduced peripheral macrophage ABCA1 expression. Previous studies demonstrated that an early response gene, fos-related antigen 2 (Fra2), binds to an E-box in the ABCA1 promoter to repress expression. LOX-1, apoE, and ZNF202 primer/probe sets were purchased from Applied Biosystems. Sequences of the ABCA1, ABCG1, LXR\textalpha, CD36, CD68, Fra2, Mac-2, and SREBP1c primers/probe sets are listed in the online supplement, available at http://circres.ahajournals.org.

### Northern and Western Blot Analyses

Northern blot analyses were performed with 30 μg of total RNA as described previously\textsuperscript{22} using a 400-bp mouse ABCA1 cDNA probe amplified by PCR with sense (5\textsuperscript{'-}TCTCTGTATCTCACCACATCGTAC-3\textsuperscript{'-}) and antisense (5\textsuperscript{'-}CATTTGGGATCACAGCATCA-3\textsuperscript{'-}) primers. Protein extracts from aortae were generated as described previously\textsuperscript{23} and subjected to Western blot analyses (12% SDS-PAGE; 30 μg protein per lane) using mouse ABCA1 (1:1000; NB400-105; Novus Biologicals\textsuperscript{-}), ABCG1 (1:500; NB400-132; Novus Biologicals\textsuperscript{-}), CD68 (1:200; sc-9139; Santa Cruz Biotechnology\textsuperscript{-}), and β-actin (1:3000; ab8227-50; abcam)--specific antibodies. MPM membrane protein extracts were generated as described previously\textsuperscript{24} and subjected to Western blot analyses (6% SDS-PAGE; 30 μg protein per lane) using mouse ABCA1 antibody. Sample equivalence was determined by staining Western blot membranes with BLOT-FastStain (Chemicon International) before blocking.

### ABCA1 mRNA Stability Assay

Subconfluent MPMs on 6-well tissue culture plates were treated for the indicated periods with Ang II (1 μmol/L) or vehicle in the presence of actinomycin D (5 μg/mL) in DMEM medium (Invitrogen) supplemented with 0.5% bovine serum. QRT-PCR for ABCA1 and GAPDH mRNA was performed as described.

### Luciferase Assays

MPMs were seeded into 6-well tissue culture plates the day before transfection. After overnight culture, cells were transiently cotransfected for 15 minutes at 37°C with a DNA/DEAE--dextran mixture containing 2.5 μg per well ABCA1 (~990/110) promoter-luciferase plasmid (kindly provided by Dr Tall, Columbia University, NY\textsuperscript{*}) and 0.125 μg per well pCMV renilla (Promega), then supplemented with complete growth medium. After 18 hours, culture medium was replaced with DMEM (0.5% FBS) supplemented with or without 1 μmol/L valsartan. Cells were pretreated with valsartan or vehicle control for 3 hours, then supplemented with or without Ang II (100 nmol/L) and 22-hydroxycholesterol (1 μmol/L) for an additional 24 hours. Luciferase activity in cell extracts was assayed using a dual-luciferase reporter assay system (Promega).

### Electrophoretic Mobility Shift Assay and Supershift Assays

Nuclear extracts were isolated from MPMs treated with or without 1 μmol/L Ang II for 1 hour, and electrophoretic mobility shift assay (EMSA) analyses were performed as described previously\textsuperscript{29} using double-stranded [\textgamma\textsuperscript{32P}]--labeled probes containing the ABCA1 promoter E-box sequence (5\textsuperscript{'-}CGGCCCTCAAGTGCCTGATGG-3\textsuperscript{'-}). For supershift assays, nuclear extracts were preincubated on ice for 30 minutes with specific antibodies for upstream stimulatory factor (USF)-1 (SC-8983; Santa Cruz Biotechnology\textsuperscript{-}), USF2 (SC-604; Santa Cruz Biotechnology\textsuperscript{-}), or Fra2 (sc-8983, sc-861, and sc-604, respectively; Santa Cruz Biotechnology\textsuperscript{-})

### Chromatin Immunoprecipitation Assays

Soluble chromatin was prepared from MPMs treated with or without Ang II (1 μmol/L) for 0 to 120 minutes with a chromatin immunoprecipitation (ChIP) assay kit from Upstate Biotechnology as described previously\textsuperscript{30} using antibodies directed against USF1, USF2, or Fra2 (sc-8983, sc-861, and sc-604, respectively; Santa Cruz Biotechnology\textsuperscript{-}). Extracted DNA precipitates were subjected to 28 to 32 PCR cycles with ABCA1 primers to amplify the mouse ABCA1 promoter E-box (sense 5\textsuperscript{'-}TCGCCGGTTTAAGGGGC-3\textsuperscript{'-} and anti-sense 5\textsuperscript{'-}GAATTTACTGCTTITTGCGCCG-3\textsuperscript{'-}).
Atherosclerosis

To investigate the effect of Ang II on macrophage foam cell formation in the artery wall, we infused Ang II or PBS into LDLR−/− mice fed a HF diet and treated with or without valsartan. After 2 weeks, Ang II−infused mice on HF diet (HF/Ang II) demonstrated decreased HDL and increased total cholesterol levels relative to PBS−infused mice on HF diet (HF/PBS; P<0.05), but these changes were prevented when Ang II was infused in the presence of valsartan (HF/Ang II/Val; Table 1). However, at 8 weeks, there were no significant lipid differences among the HF diet groups. Ang II infusion significantly increased mean systolic blood pressure at 2 and 8 weeks relative to PBS−infused mice (P<0.05), whereas valsartan treatment attenuated the Ang II−induced blood pressure increase (P<0.05; Table 1).

Mean atherosclerotic lesion area in HF/PBS mice at 8 weeks was ≈1% of the total aorta, which increased to ≈32% in HF/Ang II mice. Valsartan treatment inhibited the development of Ang II−induced lesion area by 50% (∼16% of aortic surface; Figure 1A and 1B). After 2 weeks, aortae of HF/PBS mice contained few MOMA-2 positive and ABCA1−positive cells (data not shown), whereas the aortae of HF/Ang II mice stained for ABCA1 and MOMA-2, and this staining was associated primarily with foam cells (Figure 1C, arrowheads). By 8 weeks, HF/Ang II aortae had significant numbers of vascular lesions that contained foam cells covered by fibrous caps (Figure 1D, arrowheads). ABCA1 expression strongly colocalized with CD68−positive cells, particularly in foam cells (Figure 1D). These results were consistent with ABCA1 mRNA expression in human cell lines, where we

Figure 1. Ang II accelerates macrophage foam cell formation and atherosclerosis lesion area. A, Sudan IV staining of aortic atherosclerotic lesion area after 8 weeks of treatment in HF/PBS, HF/Ang II, and HF/Ang II/Val mice. B, Measurement of Sudan IV−stained atherosclerotic lesion area in the aortae of HF/PBS, HF/Ang II, and HF/Ang II/Val mice. Mean atherosclerotic lesion surface areas were determined by computer−assisted image analysis of Sudan IV staining and represented as a percentage of total surface area of the aorta. HF/PBS (white bar), HF/Ang II (2.5 μg/k min Ang II; black bar), and HF/Ang II/Val (2.5 μg/k min Ang II 100 mg/kg per day valsartan; gray bar) for 8 weeks (P<0.01 vs HF/PBS), P<0.01 vs HF/Ang II). C, Analysis of serial sections of aorta from a representative 2−week HF/Ang II mouse (frozen section). D, Analysis of serial sections of an aorta from a representative 8−week HF/Ang II mouse (paraffin section). Mouse aortic sections were stained with hematoxylin/eosin (H.E.), anti−CD68 antibodies (CD68), anti−MOMA−2 antibodies (MOMA−2), anti−ABCA1 antibodies (ABCA1), or nonspecific antibody (IgG control). Arrowheads indicate foam cell deposits within the vessel wall.
found that human THP-1 macrophage cells expressed 25-fold more ABCA1 than human aortic vascular smooth muscle cells and 100-fold more than human coronary artery endothelial cells (data not shown), supporting our in vivo observation that, within the aorta, significant ABCA1 expression is primarily restricted to macrophages.

QRT-PCR analyses of aortae from 2-week HF/Ang II mice revealed increased expression of CD68 (2.7-fold) and Mac-2 (5-fold), markers of macrophage infiltration and activation, and increased expression of LOX-1 (16-fold), a scavenger receptor (Figure 2A; \(P<0.01\) for each; Mac-2 data not shown). ABCA1 and ABCG1 expression also increased in HF/Ang II aortae (Figure 2B), although the effect on ABCA1 expression was modest relative to ABCG1. Normalization of aorta ABCA1 and ABCG1 expression against CD68, to correct for aorta macrophage content (Figure 2C), revealed that the ABCA1/CD68 ratio decreased 40% in the HF/Ang II mice \((P<0.05)\), whereas ABCG1/CD68 expression did not change. Valsartan attenuated the Ang II–induced decrease in ABCA1/CD68 mRNA expression but did not affect the ABCG1/CD68 ratio. Similar to results of QRT-PCR, Ang II infusion prominently increased protein expression of ABCG1 (4.4-fold) and CD68 (4.1-fold) but had a much more modest effect on ABCA1 protein expression (2.1-fold). Comparison of the ABCA1/CD68 protein ratios in the aortae of Ang II– and PBS-infused mice demonstrated that ABCA1 expression does not increase proportionally to CD68, resulting in a net 48% decrease of ABCA1/CD68 expression \((P<0.05); \) Figure 2D). Norepinephrine infusion (12.5 \(\mu\)g/kg per minute), resulting in hypertension equivalent to that produced by Ang II infusion, had no significant effect on ABCA1/CD68 mRNA expression in the vessel wall (data not shown), suggesting that ABCA1/CD68 repression was not blood pressure dependent.

**Figure 2.** Ang II infusion increases macrophage infiltration but decreases ABCA1/CD68 expression within the vessel wall. A, QRT-PCR analyses of macrophage and scavenger receptor mRNAs in mouse aortae. RNA was isolated from whole aortae from control mice fed a low-fat diet (chow) or HF/PBS-, HF/Ang II-, and HF/Ang II/Val-treated mice \((n=10\) per group). RNA samples were analyzed for macrophage (CD68) and scavenger receptor (LOX-1) gene expression by QRT-PCR and normalized against reference GAPDH expression. B and C, QRT-PCR analyses of ABCA1 and ABCG1 mRNA expression in mouse aortae. RNA samples were analyzed for ABCA1 and ABCG1 expression by QRT-PCR and normalized against GAPDH expression as a measure of total vascular ABCA1 expression or against CD68 as an estimate of ABCA1 expression relative to vascular macrophage content (see Discussion). D, Western blot analysis of ABCA1, ABCG1, and CD68 expression in aortae from the 2-week PBS- or Ang II treatment groups. Quantification of protein expression was performed by densitometry of three independent experiments and normalized to \(\beta\)-actin and CD68 expression. Normalized values were analyzed by ANOVA to identify statistically significant differences among the groups \((P<0.05\) vs HF/PBS; \(P<0.05\) vs HF/Ang; \(P<0.05\) vs chow).

**Ang II Suppresses ABCA1 Expression in Peripheral Macrophages**

To investigate the effect of Ang II on macrophage lipid accumulation, peritoneal macrophages isolated from mice in each treatment group were stained with Oil Red O. Microscopic examination of Oil Red O staining revealed enhanced lipid accumulation in MPM isolated from 2-week HF/Ang II mice relative to HF/PBS mice (Figure 3A). The cellular cholesterol content of HF/Ang II MPMs significantly increased (2.3-fold) relative to that of HF/PBS MPMs, in agreement with the MPM Oil Red O results; this increase was attenuated in MPMs of mice treated with valsartan (Figure 3B).
To address the genes responsible, we next evaluated the in vivo effect of Ang II on the expression of genes related to cholesterol transport. QRT-PCR (Figure 3C), Northern blot (data not shown), and Western blot (Figure 3D) analyses revealed that Ang II significantly decreased ABCA1 mRNA and protein expression in MPMs. However, Ang II treatment had no effect on MPM expression of LXR\(\alpha\) or other LXR target genes, including ABCG1, apoE, and SREBP1c (data not shown).Valsartan (100 mg/kg per day) inhibited Ang II–induced ABCA1 mRNA repression (Figure 3C). Moreover, treatment of HF/Ang II mice with low-dose valsartan (10 mg/kg per day), which did not lower Ang II–mediated blood pressure, still attenuated Ang II–induced ABCA1 repression in MPMs isolated from these mice (data not shown).

**Ang II Decreases ABCA1 Transcription**

Ang II did not affect macrophage ABCA1 mRNA stability because actinomycin D–treated MPMs cultured with or without Ang II revealed similar ABCA1 mRNA half-life (Figure 4A). However, Ang II significantly reduced basal- and ligand-induced (22R-oxysterol) ABCA1 promoter activity in primary MPMs, and this repression was effectively reversed by pretreatment with valsartan (Figure 4B).

Recent reports demonstrated that an ABCA1 promoter E-box motif can mediate ABCA1 transcriptional repression, and three transcription factors, USF1, USF2, and Fra2, have been reported to bind to this ABCA1 E-box motif. ABCA1 promoter activity mediated through this E-box motif was enhanced by USF1 and USF2 and repressed by Fra2.\(^{14,15}\) We found that, after 2 weeks of Ang II infusion, aortae of HF/Ang II mice demonstrated increased Fra2 mRNA expression when assayed by QRT-PCR (1.6-fold; \(P<0.05\) versus HF/PBS; data not shown). EMSA and ChIP assays were performed with MPMs to determine potential Ang II–mediated transcription factor binding to this ABCA1 E-box motif. EMSA analyses revealed that 1-hour Ang II exposure increased complex formation on an ABCA1 E-box probe, and that the major complex could be shifted by antibodies specific for USF1-, USF2-, and Fra2-specific antibody (Figure 5A). ChIP assays demonstrated that Ang II treatment significantly induced Fra2 binding to the ABCA1 promoter E-box and reduced binding of USF1 but had no effect on USF2 binding. Moreover, pretreatment with valsartan inhibited Fra2 binding to the ABCA1 promoter E-box (Figure 5B). These data indicate that Ang II decreases ABCA1 transcription in macrophages by increasing Fra2 binding and decreasing USF1 binding to the ABCA1 promoter E-box, and that these effects are mediated by the AT1 receptor.
Fra2–siRNA Attenuated the Negative Effect of Ang II on ABCA1

To further define the role of Fra2 in Ang II–mediated regulation of ABCA1 transcription, we used siRNA to inhibit Fra2 expression. In these experiments, transient transfection of MPM with Fra2–siRNA substantially reduced Ang II–mediated inhibition of ABCA1 mRNA expression, whereas a nonsilencing, scrambled siRNA had no effect (Figure 6), indicating that Fra2 expression was required for Ang II–mediated suppression of ABCA1 transcription.

**Discussion**

In this study, we report that Ang II, a key proatherogenic molecule, significantly represses ABCA1 gene expression in vascular macrophages of LDLR<sup>−/−</sup> mice. Ang II–mediated ABCA1 inhibition is associated with increased macrophage...
cholesterol accumulation and foam cell formation and accelerated atherosclerosis. We defined the role of Ang II in ABCA1 expression and report that Ang II represses ABCA1 transcription through a novel regulatory mechanism.

To address the effect of Ang II infusion on ABCA1 expression in peripheral macrophages and vascular tissue, we examined ABCA1 expression in the MPMs as well as the aortae of chow, HF/PBS-, HF/Ang II-, and HF/Ang II/Val-treated mice. ABCA1 mRNA expression differed between aortae and MPMs of the Ang II treatment groups. ABCA1 expression increased in HF/Ang II aortae and decreased in HF/Ang II MPMs, relative to expression in HF/PBS and HF/Ang II/Val aortae (Figures 2B and 3C). These differences can be explained by the fact that ABCA1 is predominantly expressed by macrophages in the vessel wall (Figure 1C and 1D), and that HF/Ang II aortae contain significantly more macrophages than HF/PBS or HF/Ang II/Val aortae (Figure 2A). Although ABCA1 is expressed in endothelial and smooth muscle cells, as well as macrophages, our group and others have shown that ABCA1 expression in the artery wall is predominantly in macrophages.22,31–34 We have also shown that the majority of ABCA1 expression colocalizes with CD68 (or MOMA-2)-positive foam cells (Figure 1C and 1D), particularly during Ang II–accelerated atherosclerosis. Normalization of aorta ABCA1 expression against CD68 to correct for aorta macrophage content produced results consistent with those determined for peripheral macrophages (Figures 2C and 3C).

Activation of LXR by endogenous ligands, such as cholesterol-derived oxysterols, potently induces macrophage ABCA1 gene expression after binding.8,35 Exposure to high dietary cholesterol should therefore increase macrophage oxysterol levels and stimulate macrophage ABCA1 expression, altering the balance between cholesterol influx and efflux, to regulate macrophage cholesterol content. Indeed, compared with chow diet, ABCA1 expression is increased in aortae (Figure 2B, first panel) and peritoneal macrophages (Figure 3C) isolated from animals on HF diet. At 2 weeks, Ang II infusion increased serum cholesterol (Table) and scavenger receptor expression in aorta sections (Figure 5A, LOX-1 16-fold) and MPMs (CD36 1.5-fold; P<0.05 versus HF/PBS; data not shown) of HF/Ang II mice. Therefore, theoretically, macrophages isolated from these animals should demonstrate a corresponding increase in ABCA1 expression because of increased LXR:oxysterol-mediated ABCA1 transcription. However, in marked contrast, we found that ABCA1 mRNA and protein expression were significantly decreased in MPMs isolated from HF/Ang II mice (Figure 3C and 3D). Our data indicate that Ang II greatly enhances macrophage accumulation within the vessel wall but suppresses macrophage ABCA1 expression, reducing the ability of macrophages to efflux cholesterol. Repression of ABCA1 protein expression in HF/Ang II mouse macrophages may appear to be relatively modest but may represent a substantial alteration in the balance of cholesterol influx/efflux to promote foam cell formation. Indeed, HF/Ang II mice had a 2.3-fold increase in the cholesterol content of their MPMs relative to those of HF/PBS mice, and this increase was significantly inhibited by valsartan (Figure 3B). Macrophage ABCA1 suppression by Ang II thus appears to substantially contribute to accelerated atherosclerosis, especially when taken together with the other proatherogenic effects of Ang II, which include increased vascular macrophage infiltration, scavenger receptor expression, and uptake of oxidized LDL.36,37 Ang II treatment had no effect on the expression of LXRα or other LXR target genes, such as ABCG1, apoE, and SREBP1c, strongly suggesting that Ang II suppresses ABCA1 through mechanisms that do not affect LXR/DR4 transcriptional regulation. Recent studies have reported LXR/DR4-independent ABCA1 transcriptional repression by several transcriptional factors, including Fra2, SREBP2, and ZNF202.14,15,38–40 Santamarina-Fojo et al reported that an ABCA1 promoter E-box motif mediates ABCA1 promoter activity through interactions with three transcription factors: USF1, USF2, and Fra2. They report that basal ABCA1 transcription is upregulated when the ubiquitous transcription factors USF1 and USF2 bind this E-Box motif as homo/heterodimers, whereas recruitment of Fra2 to the ABCA1 E-box, through protein–protein interactions with the USFs, results in a marked repression of basal ABCA1 promoter activity.14,15 We now found that Ang II induces Fra2 binding to this ABCA1 promoter E-box motif (Figure 5), providing a mechanism to explain the Ang II–mediated downregulation of ABCA1 transcription in macrophages. Furthermore, we demonstrated a functional role for Fra2 in this process because we determined that Fra2–siRNA attenuates Ang II–mediated ABCA1 repression (Figure 6). In contrast, similar ChIP analyses have demonstrated that Ang II does not affect Fra2 binding to mouse ABCG1 promoter E-box motifs (data not shown). Although the specific mech-
anism of this enhanced binding needs to be explored further, the upregulation of macrophage Fra2 expression by Ang II may contribute to this effect.

Recently, Shy et al have shown that during in vitro culture of endothelial cells in serum-free media, SREBP2 can directly bind the ABCA1 promoter E-box to decrease promoter activity. However, results generated in this model are unlikely to apply to macrophages because macrophages, unlike endothelial cells, accumulate significant amounts of intracellular cholesterol. Serum and cholesterol loading suppress SREBP2, and thus it appears very unlikely that SREBP2 suppresses ABCA1 expression in cholesterol-laden macrophages in the context of atherosclerosis.

Schmitz et al demonstrated that overexpression of the ZNF202 transcription factor in human macrophages inhibits ABCA1 and ABCG1 promoter activity through an interaction with the corepressor KAP1. However, in our study, Ang II infusion did not affect MPM ABCG1 expression (data not shown), increase aorta or MPM ZNF202 expression, or enhance ZNF202-ABCA1 binding to the ABCA1 promoter. These results suggest that ZNF202-ABCA1 is highly unlikely to mediate Ang II–induced repression of mouse macrophage ABCA1. However, unlike the E-Box and DR4 (LXRE) sites, the ZNF202/SRE-like site identified by Schmitz et al is not conserved between the human and mouse ABCA1 promoters, so it is not possible to reliably extrapolate this data to human macrophage ABCA1 expression (supplemental Figure I).

ABCA1 deficiency, found in individuals with Tangier disease and ABCA1−/− mice, is associated with decreased serum cholesterol and HDL levels. In the present study, 2 weeks whole-body Ang II infusion decreased macrophage ABCA1 expression and HDL levels but increased serum cholesterol levels. Ang II infusion for 8 weeks had no effect on serum cholesterol or HDL levels (Table). Similar results have been reported previously for Ang II infusion in LDLR−/− mice. We found that Ang II infusion did not significantly affect ABCA1 expression in all the major tissues involved in reverse cholesterol transport. Ang II significantly decreased intestine ABCA1 expression, an effect that might be expected to increase cholesterol absorption and contribute to plasma cholesterol levels, but did not significantly affect liver ABCA1 expression (data not shown). Thus, Ang II infusion only partially repressed systemic ABCA1 expression, suggesting that this model should not be considered equivalent to an ABCA1 knockout mouse phenotype.

Valsartan, an ARB, attenuates Ang II–mediated ABCA1 repression, suggesting that AT1 receptor signaling is required for ABCA1 repression. This effect occurred independent of the change in blood pressure. Thus, the clinical benefit of ARB treatment may not be limited to blood pressure lowering but may directly impact on foam cell formation by decreasing macrophage cholesterol accumulation through improved cholesterol homeostasis. Valsartan treatment significantly attenuates Ang II–accelerated atherosclerosis; however, multiple Ang II–dependent mechanisms, in addition to ABCA1, are likely involved in this improvement. However, our results suggest that ARBs may have beneficial effects on cholesterol metabolism in the vascular compartment, in addition to their antioxidant and antiinflammatory properties, which may expand the therapeutic profile of ARBs in the prevention of CVD.

In summary, we provide new evidence that Ang II transcriptionally represses ABCA1 gene expression in macrophages by increasing Fra2 interaction with an ABCA1 promoter E-box motif. These observations constitute a novel mechanism through which Ang II, a well-known cardiovascular risk factor, can lead to increased foam cell formation and accelerated atherosclerosis.

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References


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Primer and probe sequences for QRT-PCR

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Figure legends for online supplemental data

Online Figure I.

A, QRT-PCR analyses of ZNF202 mRNA in aortae and MPMs isolated from HF/PBS and HF/Ang II mice after 2 weeks treatment. B, EMSA analysis of Ang II- and AngII/Val-treated MPMs for binding to a putative human ABCA1 ZNF202 site (5’-TCCACCCCAACCCACCCCAC-3’). Nuclear extracts from quiescent (lane 1), Ang II-treated (1µmol/L Ang II for 1 hour; lane 2) and Ang II/Val-treated MPMs (1 µmol/L valsartan for 3 hours, then 1µmol/L Ang II for 1 hour; lane 3) were incubated with an ABCA1 promoter probe corresponding to the human ZNF202 binding site. Non-labeled ABCA1 ZNF202 probe was added as specific competitor (lane 4). B1, specific bands; NS, non-specific band. C, ChIP analyses of KAP1 binding to the mouse ABCA1 promoter. MPMs were pretreated with valsartan (1µmol/L) or vehicle control for 3 hours, and then supplemented with or without Ang II (1 µmol/L) for 0 to 120 minutes. ChIP assay was performed using KAP1 antibody (Novus Biologicals, NB500-159A). Extracted DNA precipitates were subjected to 28-32 PCR cycles with ABCA1 primers to amplify the mouse ABCA1 promoter ZNF202/SRE-like site
(sense 5’-GAACGAGCTTTT CCCCTTTC-3’ and antisense 5’-CCTAAACCGGCGACAGTG-3’). D, Sequence homology analysis of human and mouse ABCA1 promoter ZNF202, E-Box and DR4 (LXRE) sites.