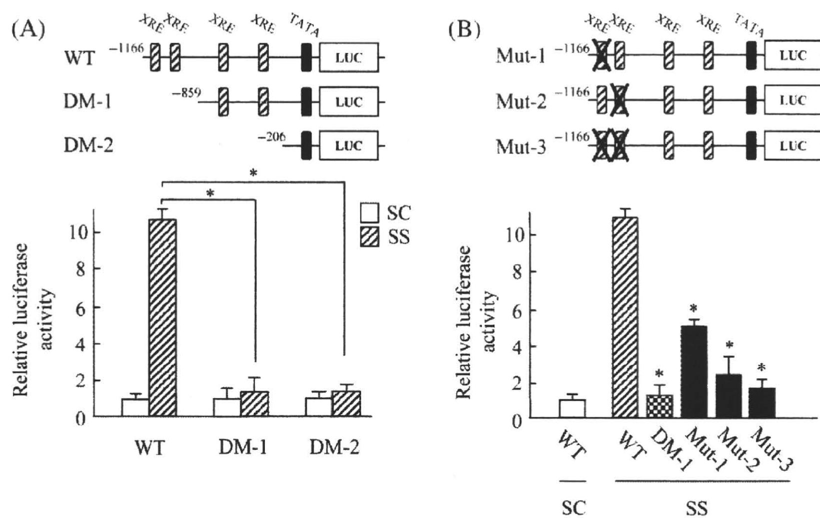

Figure 2 Analysis of the CYP1A1 gene promoter in response to shear stress (SS). (*A*) The wild-type (WT) construct contains −1116/+18 bp of the CYP1A1 promoter, deletion mutant-1 (DM-1) contains −859/+18 bp of the promoter without the distal two xenobiotic response elements (XREs), deletion mutant-2 (DM-2) contains −206/+18 bp of the promoter without all four XREs. TATA, TATA box; Luc, firefly luciferase reporter gene. Bovine arterial endothelial cells transfected with WT, DM-1, or DM-2 together with pRL-SV40 were incubated in static conditions (SC) or exposed to laminar SS (SS) for 8 h. Relative luciferase activity is presented as the fold increase over the value obtained in the control for WT. Data represent means ± SD of four independent experiments. *$P < 0.01$. (*B*) Site-mutated constructs (Mut-1, Mut-2, and Mut-3) were produced as described in 'Methods'. Bovine arterial endothelial cells transfected with Mut-1, Mut-2, or Mut-3, together with pRL-SV40, were exposed to SS for 8 h. Relative luciferase activity is presented as the fold increase over the value obtained in the SC for WT. Data represent means ± SD of four independent experiments. *$P < 0.01$ vs. SS for WT.

3.2 Xenobiotic response element mediates shear stress-induced CYP1A1 transcription

A number of studies have shown that PAHs stimulate CYP1A1 gene transcription through the XREs in the gene's promoter. SS activates the transcription of a variety of genes through several specific sequences; however, the SS-response element in the CYP1A1 promoter has not been identified. Therefore, we attempted to uncover the precise mechanism of SS-induced CYP1A1 expression by analysing the CYP1A1 gene promoter. Since there were four XREs in the rat CYP1A1 gene (−1116/+18 bp), we generated two deletion mutant constructs: DM-1 (−859/+18 bp) and DM-2 (−206/+18 bp). In BAECs transfected with these deletion mutants, the increase in luciferase activity was almost completely suppressed in comparison with the marked elevation in wild type (WT) (*Figure 2A*). This suggests that the main elements responsive to SS were located between bp −1116 and −859 in the 5′-flanking region, where two XREs exist.

Subsequently, we introduced mutations (Mut-1, Mut-2, and Mut-3) into these two upstream XREs to determine whether these sequences are responsible for SS-induced CYP1A1 expression. Mut1 and Mut2 significantly suppressed SS-induced CYP1A1 promoter activity, and Mut3, where both of the XREs were mutated, almost eliminated the response to SS (*Figure 2B*). These results suggest that both of these XREs are essential for CYP1A1 induction by SS.

3.3 Laminar shear stress induced the expression and nuclear translocation of aryl hydrocarbon receptor

The results suggested that SS activates CYP1A1 gene transcription through XREs in a similar way to PAHs. AhR is known to mediate PAH-induced transcriptional activation of the CYP1A1 gene. Therefore, we subsequently examined the effect of SS on AhR expression. As shown in *Figure 3A*, SS significantly increased the expression of AhR protein, as well as CYP1A1, and this induction was sustained over 24 h. Immunoblotting of the subcellular fractions revealed that AhR was predominantly in the cytoplasm with only a small amount present in the nucleus in static ECs; however, it was translocated from the cytoplasm into the nucleus after stimulation with SS (*Figure 3B*). The nuclear translocation of AhR by SS was confirmed by immunofluorescence staining (*Figure 3C*).

We also compared the effects of the physiological level of laminar and turbulent SS on the expression of AhR and CYP1A1, because atherosclerotic lesions tend to develop at arterial bifurcations and curvatures where laminar blood flow is often turbulent. As shown in *Figure 3D*, the physiological level of turbulent SS (average strength: 1.5 dyne/cm^2) induced AhR and CYP1A1, however; the induction was weaker than that by laminar SS (6 dyne/cm^2).

3.4 The aryl hydrocarbon receptor inhibitor alpha-naphthoflavone and aryl hydrocarbon receptor small interfering RNA suppressed laminar shear stress-induced CYP1A1 expression

We examined the effect of AhR inhibition to determine whether AhR is essential for the SS-induced expression of CYP1A1. Alpha-NF (10 μmol/L), an AhR inhibitor, strongly suppressed SS-induced CYP1A1 expression (*Figure 4A*). We also examined the effect of siRNA for AhR (si-AhR) and found that si-AhR significantly suppressed SS-induced expression of AhR, whereas scramble siRNA (si-Scr) did not (*Figure 4B*). When the AhR si-RNA was transfected into BAECs, SS-induced expression of CYP1A1 was strongly suppressed (*Figure 4C*).

Downloaded from cardiovascres.oxfordjournals.org at national cardiovascular center on March 2, 2011

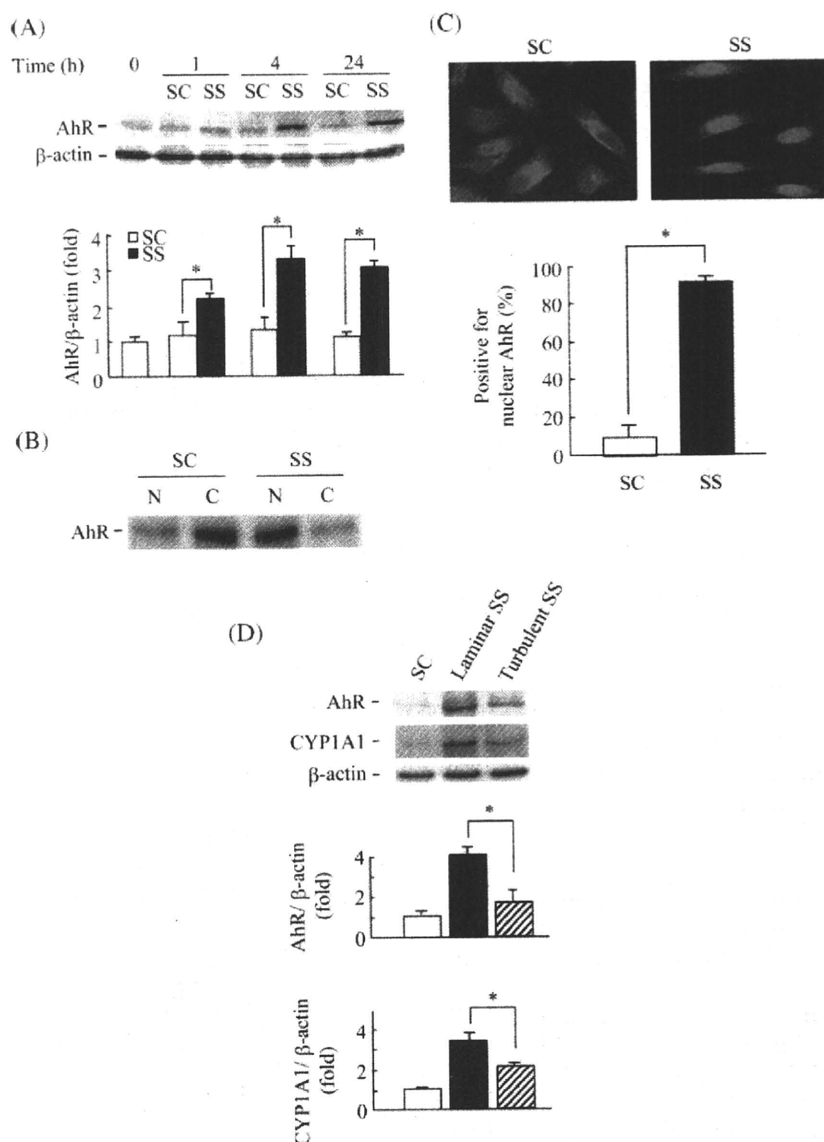

Figure 3 Effects of laminar shear stress (SS) on aryl hydrocarbon receptor (AhR) expression and nuclear translocation of AhR. (*A*) Human umbilical vein endothelial cells (HUVECs) were maintained in static conditions (SC) or exposed to laminar SS (15 dyne/cm^2) (SS) for the periods indicated. Expression levels of AhR protein normalized to those of β-actin protein were standardized to the values obtained at 0 h and shown as means ± SD of four independent experiments in the bar graph. *$P < 0.01$. (*B*) HUVECs were maintained in SC or exposed to laminar SS (15 dyne/cm^2) (SS) for 8 h. Nuclear (N) and cytoplasmic (C) proteins were purified as described under 'Methods'. (*C*) HUVECs were maintained in SC or exposed to laminar SS (15 dyne/cm^2) (SS) for 8 h. After stimulation, immunofluorescent staining with an anti-AhR antibody was performed as described under 'Methods'. Bar graph represents the percentages of cells positive for nuclear AhR staining. *$P < 0.01$. (*D*) HUVECs were exposed to laminar and turbulent SS for 4 h using a cone-plate type apparatus as described in 'Methods'. Expression levels of AhR protein or CYP1A1 protein normalized to those of β-actin protein were standardized to the values obtained from cells maintained in SC, and shown as means ± SD of three independent experiments in the bar graph. *$P < 0.01$.

3.5 Involvement of the mitogen-activated protein kinase pathways in shear stress-induced aryl hydrocarbon receptor expression

Several reports have suggested that MAPKs modulate AhR expression.[24-26] It has also been reported that SS activates MAPKs.[27-29] To elucidate the mechanism by which SS induces the expression of AhR, we examined the effect of SS on the phosphorylation of MAPKs, i.e. ERK, JNK, and p38. As shown in *Figure 5A*, the expression levels of these MAPKs were not altered by SS. However, the levels of phosphorylated JNK and p38 but not ERK were markedly elevated by SS. Importantly, these time-courses were similar to those of AhR and CYP1A1 expression induced by SS. We subsequently examined the effects of the MAPK inhibitors U0126 (ERK inhibitor), SP600125 (JNK inhibitor), and SB203580 (p38 inhibitor) on SS-induced expression of AhR and CYP1A1. Both SP600125 (*Figure 5B*) and SB203580 (*Figure 5C*) markedly reduced SS-induced expression of AhR and CYP1A1, whereas U0126 did not (*Figure 5D*). This suggests that SS induces expression of AhR through phosphorylation of JNK and p38, resulting in an increase in CYP1A1 expression.

Downloaded from cardiovascres.oxfordjournals.org at national cardiovascular center on March 2, 2011

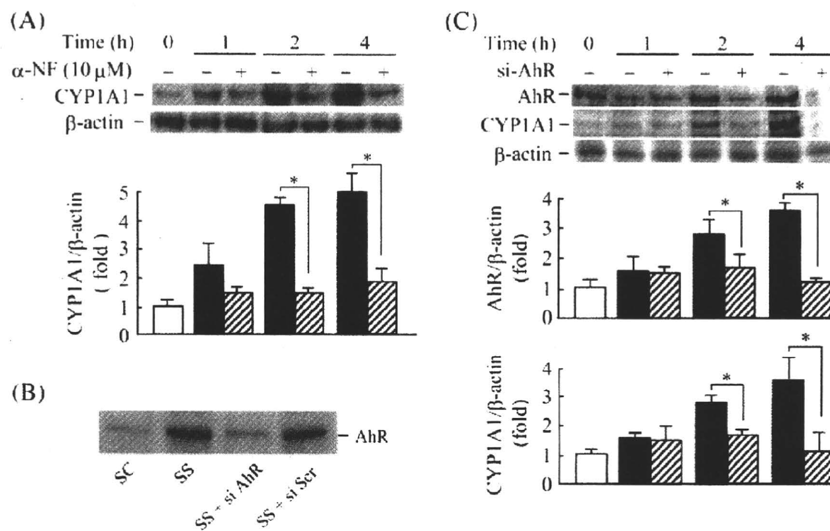

Figure 4 Effects of aryl hydrocarbon receptor (AhR) inhibition on the expressions of CYP1A1 induced by shear stress (SS). (*A*) Human umbilical vein endothelial cells were exposed to laminar SS (15 dyne/cm^2) in the absence or presence of alpha-naphthoflavone (10 μmol/L) for the periods indicated. Expression levels of CYP1A1 protein normalized to those of β-actin protein were standardized to the values obtained at 0 h, and shown as means ± SD of four independent experiments in the bar graph. *$P < 0.01$. (*B*) Bovine arterial endothelial cells (BAECs) transfected with small interfering RNA (siRNA) for AhR (si-AhR) or scramble RNA (si-Scr) were exposed to SS for 8 h. (*C*) BAECs transfected with si-AhR or si-Scr were exposed to laminar SS (15 dyne/cm^2) (SS) for the periods indicated. Expression levels of AhR protein or CYP1A1 protein normalized to those of β-actin protein were standardized to the values obtained at 0 h shown as means ± SD of four independent experiments in the bar graph. *$P < 0.01$.

3.6 Aryl hydrocarbon receptor mediates shear stress-induced cell cycle arrest

Previous studies suggest that there is a relationship between AhR and cell growth inhibition.[22,30] We reported that SS inhibits EC proliferation by inducing expression of the cyclin-dependent kinase inhibitor p21^{Cip1}.[18] To investigate whether AhR is involved in SS-induced cell cycle arrest in ECs, we examined the effects of si-AhR on the arrest. As shown in *Figure 6A*, si-AhR restored the ability to proliferate. SS induced p21^{Cip1} expression and suppressed phosphorylation of the retinoblastoma tumour suppressor protein (pRb) required for G_1/S transition consistent with our previous report.[18] However, both of these changes were reversed by treatment with si-AhR (*Figure 6B*).

4. Discussion

In this study, we showed for the first time that the expression of AhR is induced by laminar fluid SS, probably through activation of the JNK/p38 pathways, and leads to expression of the CYP1A1 gene through XRE-dependent transcription. Furthermore, we found that AhR is also involved in SS-induced cell cycle arrest. The induction of AhR and CYP1A1 by turbulent SS was weaker than that by laminar SS, suggesting that the expression of these proteins is involved in the maintenance of vascular homeostasis.

A physiological level of laminar SS strongly induced CYP1A1 expression in vascular ECs, consistent with previous reports,[12,13] and enhanced CYP1A1 activity. SS increased the activity of the CYP1A1 gene promoter; furthermore, deletion of parts of the 5′-flanking region of the CYP1A1 gene showed that the response to SS depended on the region between bp −1116 and −859, which contains two XREs. Mutations introduced into these two XREs almost completely abolished the SS-mediated response, suggesting that these XREs are essential for SS-induced transcription of the CYP1A1 gene.

Laminar SS induced the expression of AhR and facilitated its nuclear translocation required for binding to XREs (*Figure 3B* and *C*). The inhibition of AhR by α-NF or siRNA suppressed CYP1A1 induction by SS, suggesting that AhR mediates CYP1A1 induction by SS. Importantly, the induction patterns of AhR and CYP1A1 by SS were different from those by PAHs. Previous reports indicated that the expressions of AhR and CYP1A1 are transiently induced by PAHs within 1 h after stimulation, and thereafter expression rapidly declines.[31,32] However, laminar SS showed sustained induction of AhR and CYP1A1 until at least after 24 h in the present study. SS may continuously induce an unknown endogenous ligand that can bind and activate AhR. Considering the very low expression of AhR in ECs in static condition, AhR seems to be mainly regulated by SS in the vascular wall. Furthermore, the sustained induction of AhR and subsequent activation of CYP1A1 by SS may essentially indicate a physiological role, which is distinct from the effects of the vast majority of PAHs.

In addition, SS-induced AhR expression was probably mediated through the activation of the JNK/p38 pathways, since SS induced sustained activation of JNK and p38 (*Figure 5A*) and the inhibition of JNK or p38 suppressed the SS-induced expression of AhR and CYP1A1 (*Figure 5B* and *C*). However, SS did not activate ERK and U0126 failed to inhibit SS-induced expression of AhR and CYP1A1, suggesting that the ERK pathway is not required for this process, although several papers have reported an association between ERK and AhR activation.[33,34]

We previously reported that laminar SS induces cell cycle arrest in vascular ECs by inhibiting the transition from G_1 to S phase.[18] Since frequent cell division seems to increase the vascular wall permeability,[35] restricting the proliferation of EC would achieve the stabilization of ECs. In fact, early

Downloaded from cardiovascres.oxfordjournals.org at national cardiovascular center on March 2, 2011

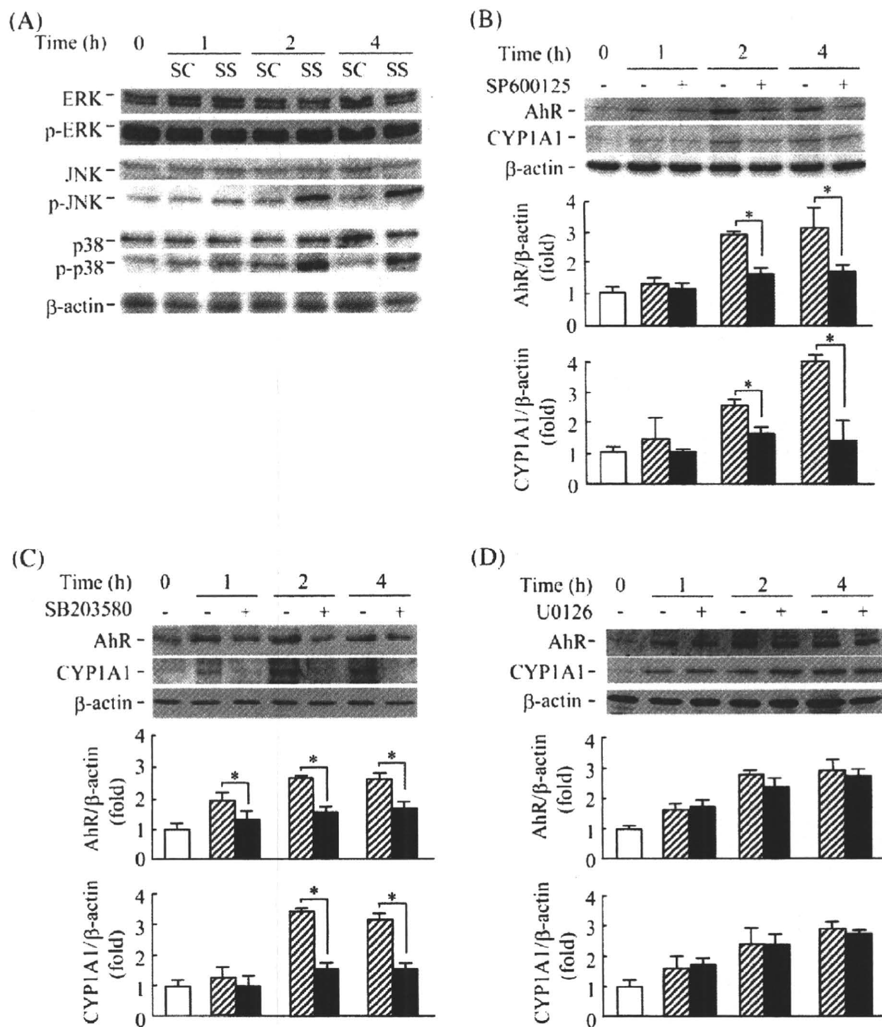

Figure 5 Effect of shear stress (SS) on the phosphorylation of mitogen-activated protein kinase (MAPKs). (*A*) Human umbilical vein endothelial cells (HUVECs) were maintained in static conditions (SC) or exposed to laminar SS (15 dyne/cm^2) (SS) for the periods indicated. Levels of MAPKs and phosphorylated (p-) MAPKs were analysed by western blotting. (*B*) HUVECs were exposed to laminar SS (15 dyne/cm^2) in the absence or presence of SP600125 for the periods indicated. Expression levels of aryl hydrocarbon receptor (AhR) protein or CYP1A1 protein normalized to those of β-actin protein were standardized to the values obtained at 0 h and shown as means ± SD of four independent experiments in the bar graph. *$P < 0.01$. (*C*) HUVECs were exposed to laminar SS (15 dyne/cm^2) in the absence or presence of SP203580 for the periods indicated. Expression levels of AhR protein or CYP1A1 protein normalized to those of β-actin protein were standardized to the values obtained at 0 h and shown as means ± SD of four independent experiments in the bar graph. *$P < 0.01$. (*D*) HUVECs were exposed to laminar SS (15 dyne/cm^2) in the absence or presence of U0126 for the periods indicated. Protein levels of AhR, CYP1A1, and β-actin were analysed by western blotting. Expression levels of AhR protein or CYP1A1 protein normalized to those of β-actin protein were standardized to the values obtained at 0 h and shown as means ± SD of four independent experiments in the bar graph.

studies demonstrated that EC turnover and DNA synthesis are increased in areas around branch orifices where ECs are exposed to turbulent blood flow and atherosclerotic change is often initiated.[36,37] Therefore, the anti-proliferative effect of laminar SS may be essential for the maintenance of vascular homeostasis. In the present study, si-AhR recovered DNA synthesis that had been suppressed by SS, and also prevented SS from inducing expression of the Cdk inhibitor p21^{Cip1} and de-phosphorylating pRb (*Figure 6B*). Recent studies suggested that by directly interacting with pRb,[38,39] AhR inhibits the E2F-dependent transcription that initiates G_1/S transition, resulting in inhibition of the cell cycle. Our results strongly agree with these results. TCDD was reported to induce cell cycle arrest in G_1 phase through AhR-mediated induction of the Cdk inhibitor p27^{Kip1} in rat hepatoma cell line.[8] However, we previously showed that SS did not change the expression level of p27^{Kip1} in ECs,[18] and therefore, the action of AhR may differ among cell species and stimulants.

There are several limitations in this study. We used BAECs instead of HUVECs for the promoter analysis because of their higher transfection efficiency. Furthermore, we used the rat CYP1A1 promoter sequence to examine promoter activity. Although rat CYP1A1 promoter had a high homology with human CYP1A1 promoter, the differences between species in response to SS cannot be denied.

Our results suggested that the constitutive activation of AhR and CYP1A1 in response to blood flow is a normal physiological mechanism to protect the vascular wall from toxic stimuli, distinct from the pathological role of these

Downloaded from cardiovascres.oxfordjournals.org at national cardiovascular center on March 2, 2011

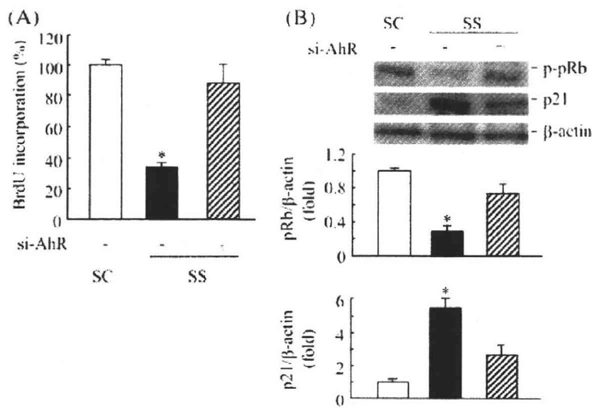

Figure 6 Effects of small interfering RNA for aryl hydrocarbon receptor on shear stress (SS)-induced cell cycle arrest. (*A*) Effects on laminar SS-induced inhibition of DNA synthesis. Once confluent, bovine arterial endothelial cells were immediately exposed to SS (15 dyne/cm^2) for 8 h and thereafter, incubated with BrdU. BrdU incorporation was measured and analysed as described in 'Methods'. The data are presented as values relative to the static control (SC) and shown as means $\pm$ SD of four independent experiments in the bar graph. *$P < 0.01$ vs. SC. (*B*) Effects on expressions of p21 and phosphorylated-pRb. Expression levels of proteins normalized to those of β-actin protein were standardized to the values obtained at 0 h and shown as means $\pm$ SD of four independent experiments in the bar graph. *$P < 0.01$ vs. SC.

molecules as generators of toxic metabolites. Even though, AhR and CYP1A1 expressed in response to PAHs results in the generation of harmful substances, their initially assumed roles may have been different, since few environmental pollutants such as PAHs existed when these molecules first evolved. Further study is needed to precisely determine the physiological and pathological roles of AhR and CYP1A1 expressed in the vascular wall.

Acknowledgement

We are grateful to Kana Oie for her secretarial assistance.

Conflict of interest: none declared.

Funding

Ministry of Education, Culture, Sports, Science and Technology, Japan [a Grant-in-Aid for Young Scientists (B) No.15790137]; Kyushu University Interdisciplinary Programs in Education and Projects in Research Development.

References

1. Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 1982;**22**:517-554.
2. Whitlock JP Jr. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. *Annu Rev Pharmacol Toxicol* 1990; **30**:251-277.
3. Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim Biophys Acta* 2003;**1619**:263-268.
4. Ross JS, Stagliano NE, Donovan MJ, Breitbart RE, Ginsburg GS. Atherosclerosis and cancer: common molecular pathways of disease development and progression. *Ann N Y Acad Sci* 2001;**947**:271-292.
5. Schmidt JV, Bradfield CA. Ah receptor signaling pathways. *Annu Rev Cell Dev Biol* 1996;**12**:55-89.
6. Gonzalez FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. *Drug Metab Dispos* 1998;**26**:1194-1198.
7. Weiss C, Kolluri SK, Kiefer F, Gottlicher M. Complementation of Ah receptor deficiency in hepatoma cells: negative feedback regulation and cell cycle control by the Ah receptor. *Exp Cell Res* 1996;**226**:154-163.
8. Kolluri SK, Weiss C, Koff A, Gottlicher M. p27(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. *Genes Dev* 1999;**13**:1742-1753.
9. Levine-Fridman A, CHne L, Elferink CJ. Cytochrome P4501A1 promotes G1 phase cell cycle progression by controlling aryl hydrocarbon receptor activity. *Mol Pharmacol* 2004;**65**:461-469.
10. Dey A, Jones JE, Nebert DW. Tissue- and cell type-specific expression of cytochrome P450 1A1 and cytochrome P450 1A2 mRNA in the mouse localized in situ hybridization. *Biochem Pharmacol* 1999;**58**:525-537.
11. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci* 2000;**902**:230-239.
12. Eskin SG, Turner NA, McIntire LV. Endothelial cell cytochrome P450 1A1 and 1B1: up-regulation by shear stress. *Endothelium* 2004;**11**:1-10.
13. McCormick SM, Eskin SG, McIntire LV, Teng CL, Lu CM, Russell CG *et al.* DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *Proc Natl Acad Sci USA* 2001; **98**:8955-8960.
14. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev* 1995;**75**:519-560.
15. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arteroscler TbrombVasc Biol* 1998;**18**:677-685.
16. Dimmeler S, Haendeler J, Rippmann V, Nehls M, Zeiher AM. Shear stress inhibits apoptosis of human endothelial cells. *FEBS Lett* 1996;**399**:71-74.
17. Thum T, Haverich A, Borlak J. Cellular dedifferentiation of endothelium is linked to activation and silencing of certain nuclear transcription factors: implications for endothelial dysfunction and vascular biology. *FASEB J* 2000;**14**:740-751.
18. Akimoto S, Mitsumata M, Sasaguri T, Yoshida Y. Laminar shear stress inhibits vascular endothelial cell proliferation by inducing cyclin-dependent kinase inhibitor $p21^{Sdi1/Cip1/Waf1}$. *Circ Res* 2000;**86**:185-190.
19. Kosaka C, Sasaguri T, Masuda J, Zen K, Shimokado K, Yokota T *et al.* Protein kinase C-mediated inhibition of cyclin A expression in human vascular endothelial cells. *Biochem Biophys Res Commun* 1993;**193**:991-998.
20. Sokabe T, Yamamoto K, Ohura N, Nakatsuka H, Qin K, Obi S *et al.* Differential regulation of urokinase-type plasminogen activator expression by fluid shear stress in human coronary artery endothelial cells. *Am J Physiol Heart Circ Physiol* 2004;**287**:H2027-H2034.
21. Takahashi-Yanaga F, Taba Y, Miwa Y, Kubohara Y, Watanabe Y, Hirata M *et al. Dictyostelium* differentiation-inducing factor-3 activates glycogen synthase kinase-3β and degrades cyclin D1 in mammalian cells. *J Biol Chem* 2003;**278**:9663-9670.
22. Willett KL, Gardinali PR, Sericano JL, Wade TL, Safe SH. Characterization of the H4IIE rat hepatoma cell bioassay for evaluation of environmental samples containing polynuclear aromatic hydrocarbons (PAHs). *Arch Environ Contam Toxicol* 1997;**32**:442-448.
23. Miyagi M, Miwa Y, Takahashi-Yanaga F, Morimoto S, Sasaguri T. Activator protein-1 mediates shear stress-induced prostaglandin D synthase gene expression in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2005;**25**:970-975.
24. Tan Z, Chang X, Puga A, Xia Y. Activation of mitogen-activated protein kinases (MAPKs) by aromatic hydrocarbons: role in the regulation of aryl hydrocarbon receptor (AHR) function. *Biochem Pharmacol* 2002; **64**:771-780.
25. Shibazaki M, Takeuchi T, Ahmed S, Kikuchi H. Suppression by p38 MAP kinase inhibitors (pyridinyl imidazole compounds) of Ah receptor target gene activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin and the possible mechanism. *J Biol Chem* 2004;**279**:3869-3876.
26. Yim S, Oh M, Choi SM, Park H. Inhibition of the MEK-1/p42 MAP kinase reduces aryl hydrocarbon receptor-DNA interactions. *Biochem Biophys Res Commun* 2004;**322**:9-16.
27. Jo H, Sipos K, Go YM, Law R, Rong J, McDonald JM. Differential effect of shear stress on extracellular signal-regulated kinase and N-termial Jun kinase in endothelial cells. *J Biol Chem* 1997;**272**:1395-1401.
28. Pearce MJ, McIntyre TM, Prescott SM, Zimmerman GA, Whatley RE. Shear stress activates cytosolic phospholipase A_2 ($cPLA_2$) and MAP kinase in human endothelial cells. *Biochem Biophys Res Commun* 1996;**218**: 500-504.

Downloaded from cardiovascres.oxfordjournals.org at national cardiovascular center on March 2, 2011

29. Tseng H, Peterson TE, Berk BC. Fluid shear stress stimulates mitogen-activated protein kinase in endothelial cells. *Circ Res* 1995;**77**:869–878.
30. Castro-Rivera E, Wormke M, Safe S. Estrogen and aryl hydrocarbon responsiveness of ECC-1 endometrial cancer cells. *Mol Cell Endocrinol* 1999;**150**:11–21.
31. Pollenz RS, Sattler CA, Poland A. The aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator protein show distinct subcellular localizations in Hepa 1c1c7 cells by immunofluorescence microscopy. *Mol Pharmacol* 1994;**45**:428–438.
32. Pollenz RS. The aryl-hydrocarbon receptor, but not the aryl-hydrocarbon receptor nuclear translocator protein, is rapidly depleted in hepatic and nonhepatic culture cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol Pharmacol* 1996;**49**:391–398.
33. Yim S, Oh M, Choi SM, Park H. Inhibition of the MEK-1/p42 MAP kinase reduces aryl hydrocarbon receptor-DNA interactions. *Biochem Biophys Res Commun* 2004;**322**:9–16.
34. Tan Z, Huang M, Puga A, Xia Y. A critical role for MAP kinases in the control of Ah receptor complex activity. *Toxicol Sci* 2004;**82**:80–87.
35. Caplan BA, Schwartz CJ. Increased endothelial cell turnover in areas of in vivo Evans blue uptake in the pig aorta. *Atherosclerosis* 1973;**17**:401–417.
36. Wright HP. Mitosis patterns in aortic endothelium. *Atherosclerosis* 1972;**15**:93–100.
37. Kunz J, Schreiter B, Schubert B, Voss K, Krieg K. Experimental investigations on the regeneration of aortic endothelial cells: automatic and visual evaluation of autoradiograms [in German]. *Acta Histochem* 1978;**61**:53–63.
38. Ge NL, Elferink CJ. A direct interaction between the aryl hydrocarbon receptor and retinoblastoma protein. Linking dioxin signaling to the cell cycle. *J Biol Chem* 1998;**273**:22708–22713.
39. Elferink CJ, Ge NL, Levine A. Maximal aryl hydrocarbon receptor activity depends on an interaction with the retinoblastoma protein. *Mol Pharmacol* 2001;**59**:664–673.

Downloaded from cardiovascres.oxfordjournals.org at national cardiovascular center on March 2, 2011