**CONSTITUTIVE EXPRESSION OF CYCLOOXYGENASE-2 BY FLUID SHEAR STRESS IN VASCULAR ENDOTHELIAL CELLS**

Hiroyasu Inoue¹ and Toshiyuki Sasaguri², ¹Department of Pharmacology, National Cardiovascular Center Research Institute, Osaka and ²Department of Clinical Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Cyclooxygenase (COX), a rate-limiting enzyme for prostaglandin (PG) biosynthesis, comprises two isozymes, COX-1 and COX-2. With development and clinical prevalence of COX-2-selective inhibitors as novel NSAIDs, their safety is attracting increasing attention. However concerns have recently been raised over the cardiovascular risks of selective COX-2 inhibitors [1].

Vascular endothelial cells are exposed to a wide variety of biomechanical stimuli including fluid shear stress caused by blood flow. Shear stress modulates several endothelial functions, such as control of vascular tone, maintenance of antithrombotic surfaces, regulation of inflammation, protection against oxidative stresses, and regulation of endothelial cell proliferation and apoptosis.

Laminar shear stress upregulates COX-2 gene expression [2-4]. We found that COX-2 is involved in lipopolysaccharide (LPS)-stimulated production of prostacyclin (PGI₂) in endothelial cells [5], and that shear stress promotes the production of prostaglandin D₂ (PGD₂) in endothelial cells by stimulating the expression of lipocalin-type PGD₂ synthase (L-PGDS), whereas PGI₂ synthase is constitutively expressed in the presence or absence of the shear stress [6]. Therefore, it is necessary to evaluate the roles of COX-2 and L-PGDS expression by shear stress, which will be distinctly involved in the production of PGI₂ and PGD₂ in endothelial cells.

In the present study, we investigated the molecular mechanism for the shear stress-induced expression of COX-2 in vascular endothelial cells [7]. Exposure of human umbilical vein endothelial cells (HUVECs) to laminar shear stress in the physiological range (1 to 30 dyne/cm²) upregulated the expression of COX-2 but not COX-1. The expression of COX-2 mRNA began to increase within 0.5 hours after the loading of shear stress and reached a maximal level at 4 hours. Therefore, the gene expression of
COX-2 was more rapid and more sensitive to shear strength than that of L-PGDS (Table 1).

Table 1. Comparison of induction of COX-2 and L-PGDS mRNAs by fluid shear

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<tr>
<td>Lower shear stress</td>
<td>Yes</td>
<td>No</td>
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<td>(1 dyne/cm²)</td>
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<tr>
<td>Higher shear stress</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>(15 dyne/cm²)</td>
<td>Rapid (within 30 min)</td>
<td>Time lag (6 hours)</td>
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<td>Maximal level at 4 hours</td>
<td>Maximal level at 18 hours</td>
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Our result suggested that COX-2 but not COX-1 is mainly involved in PGI₂ formation in blood vessels loaded with laminar shear stress in the physiological range. This assumption is consistent with recent reports:

1) Selective COX-2 inhibitors suppress systemic biosynthesis of PGI₂ in healthy humans [8].
2) Glucocorticoids do not reduce excretion of urinary PGI₂ metabolites [9].
3) COX-2 expression is involved in PGI₂ formation, but is not suppressed by dexamethasone in vascular endothelial cells [5].
4) COX-2 cooperates with PGI₂ synthase more preferentially than COX-1 [10].

Concerning distinct regulation of COX-2 and L-PGDS expression by fluid shear stress, we hypothesize that there are two steps in COX-2-mediated arachidonate metabolism in endothelial cells (Figure 1). PGD₂ may also play a role in preventing the formation of atherosclerotic lesions by being converted to 15-deoxy-Δ12,14-PGJ₂, which has been reported to display several antiatherogenic effects on cultured vascular cells [11]. It should be examined whether changes in the pattern of blood flow, such as turbulence, influence the PG species produced in endothelial cells.
Figure 1. Possible roles of COX-2 and L-PGDS in endothelial cells

Three cis-acting elements, namely the NF-κB binding site, NF-IL6 binding site, and cyclic AMP response element, reside in the region between base pairs -327 and +59 in human COX-2 gene promoter. Their involvement in COX-2 gene transcription varies among cell species [12]. COX-2 gene expression is post-transcriptionally regulated through its 3′-untranslated region (3′-UTR) containing 17 copies of the “AUUUA” motif, which is assumed to promote mRNA degradation.

Roles of the promoter region and the 3′-UTR in human COX-2 gene were evaluated by the transient transfection of luciferase (Luc) reporter vectors into bovine arterial endothelial cells. Shear stress elevated Luc activity via the region (-327/+59 bp) in the COX-2 promoter. Mutation analysis indicated that cAMP responsive element (-59/-53 bp) was mainly involved in the shear stress-induced COX-2 expression. Moreover, shear stress selectively stabilized COX-2 mRNA in HUVECs. When a 3′-UTR containing 17 copies of the AUUUA mRNA instability motif was inserted into downstream of the Luc coding region, shear stress elevated the Luc expression. These results suggested that shear stress induces COX-2 expression not only at the transcriptional level but also at the post-transcriptional level through the 3′-UTR, which would make it possible to rapidly and persistently induce COX-2 expression in response to shear stress.

Finally, macrophages have been reported to express augmented levels of COX-2 in atherosclerotic lesions. This abnormally elevated COX-2 expression in macrophages may be related with inflammation in the lesions. Since the PGs produced in macrophages are different from those produced in endothelial cells, the regulation of COX-2 and downstream enzymes should be different between endothelial cells and macrophages. We have reported that COX-2 expression is negatively regulated by nuclear receptor PPARγ and its ligand candidate 15-deoxy-Δ^{12,14}-PGJ_{2} in macrophages but not in
endothelial cells [12]. Taken together, 15-deoxy-Δ12,14-PGJ2 produced in atherosclerotic lesions may inhibit inflammatory responses mediated by COX-2 in macrophages without inhibiting endothelial cell PG synthesis. The reason that high doses of rofecoxib, a COX-2 selective inhibitor, increased the rate of cardiovascular incidence [1] may be non-selective inhibition of macrophages and endothelial cells to produce PG.

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References


Correspondence should be addressed to:
Hiroyasu Inoue
E-mail: inoue@ri.ncvc.go.jp