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IL-8 gene induction by low shear stress: Pharmacological evaluation of the role of signaling molecules

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Abstract. Shear stress can modulate endothelial cell function by regulating gene expression. We have previously demonstrated that low shear stress (4.2 dyn/cm^2) induces the expression of interleukin-8 (IL-8) gene in endothelial cells. The present study was undertaken to further investigate both the effects of shear stress on IL-8 expression and the mechanisms controlling IL-8 mRNA up-regulation in human umbilical vein endothelial cells (HUVEC). We show that shear stress (from 2.23 to 19.29 dyn/cm^2) induces the IL-8 expression at both the mRNA and protein levels by stimulating transcription. In order to determine the possible contribution of G protein, HUVEC were pretreated with an inhibitor of G-protein activation, GDPβS, which abrogated the low shear stress-induced IL-8 gene expression. Such gene expression was also partially inhibited by the tyrosine kinase inhibitor (tyrphostin-25) and in addition by EGTA, BATPA/AM (the intracellular Ca^{2+} chelator), Verapamil (a Ca^{2+} channel blocker), cAMP-dependent protein kinase inhibitor (KT5720) and phospholipase C inhibitor (neomycin). However, the cGMP-dependent protein kinase inhibitor, KT5823, had no effect on such expression. These findings therefore demonstrate the involvement of several signaling molecules, including tyrosine kinase, G protein, calcium, phospholipase C, and cAMP-dependent protein kinase, in the low shear stress-induced IL-8 gene expression.

Keywords: Endothelial cells, interleukin-8, signal transduction

1. Introduction

Vascular endothelial cells, which line the entire cardiovascular system, are normally subjected to biomechanical stimuli resulting from shear stress and from strain associated with the stretching of the vessel wall. Shear stress influences the endothelial cell (EC) structure and function, such as cell orientation,

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migration, cytoskeletal reorganization and macromolecular permeability. When blood flow is disturbed and/or low, shear stress plays an important role in the pathogenesis of the atherosclerotic plaque [21]. Previous studies have shown that atherogenesis is promoted at areas of low shear stress (<5 dyn/cm^2) in the vasculature, such as the inner curvatures of coronary arteries and near bifurcations. In such regions, several vascular wall functions including endothelial nitric oxide synthase (eNOS) production, vasodilatation and endothelial cell repair are reduced. These changes are coupled with increases in reactive oxygen species (ROS), endothelial permeability to lipoproteins, leukocyte adhesion, apoptosis, smooth muscle cell proliferation, collagen deposition [5] and the production of the proatherogenic chemokines monocyte chemotactic protein 1 (MCP-1) and interleukin-8 (IL-8) [19,28].

IL-8 is a pro-inflammatory cytokine with possible atherogenic properties through its multiple actions, such as accelerating the recruitment of neutrophils and T lymphocytes into the sub-endothelial space, triggering monocyte adhesion to the endothelium [10], and regulating the migration of vascular smooth muscle cells [9]. We have previously shown that shear stress induces IL-8 gene expression [3]. Further analyses have revealed that this expression depends both on the exposure time and the shear stress intensity [31,32]. However, the upstream regulatory pathways contributing to the regulation of IL-8 gene expression by shear stress remain to be identified.

A number of studies have shown that shear stress activates many signaling transduction pathways in which a variety of molecules are involved. Among these are Ca^{2+}, GTP-binding protein, adenylate cyclase, integrin and protein kinases [24]. In the present study we therefore investigated the roles of these different signaling molecules in the low shear stress-induced IL-8 gene expression in HUVEC. Our results show that G protein, tyrosine kinase, Ca^{2+}, phospholipase C and cAMP-dependent protein kinase are involved in such gene expression.

2. Materials and methods

2.1. Materials

All reagents used were of analytical grade. Tyrphostin-25, EGTA, BATPA/AM, KT5720, KT5823, neomycin and calphostin C were purchased from Sigma (USA). Verapamil was purchased from Wako (Japan) and GDPβS from Fluka (Switzerland). Recombinant human VEGF and fibronectin were acquired from Roche (Germany).

2.2. Cell cultures

HUVEC were isolated and maintained in M199 medium (GIBCO-BRL) supplemented with 20% FCS (Sigma, USA), 2 mM L-glutamine, penicillin–streptomycin and 20 ng/ml VEGF. Cells of passage 3 or 4 were then seeded onto glass slides that had been pre-coated with fibronectin (FN, 50 μg/ml). All cell cultures were maintained in a humidified 5% CO_2/95% air incubator at 37°C. Confluent cultures of HUVEC exhibited typical cobblestone morphology and most of these cells contained factor VIII-related antigen and Weibel–Palade body.

2.3. Shear stress experiments

Steady laminar shear stress was applied to confluent HUVEC using a parallel flow chamber system. The chamber was incorporated into a closed loop perfusion system containing serum-free M-199

medium driven by a peristaltic pump (Cole-Parmer, USA). Medium was equilibrated with 5% CO_2/95% air, and the temperature was maintained at 37°C. Shear stress was controlled by changing the flow rate of the medium delivered to the cells. Shear stress at 4.2 dyn/cm^2 was used for most experiments, as this has been shown to induce the expression of the IL-8 gene *in vitro* [3]. Static control experiments were performed on HUVEC without any exposure to shear stress. In some experiments HUVEC were pre-incubated with different inhibitors for the indicated time, before being exposed to shear stress.

2.4. Real-time RT-PCR

Total cellular RNA was isolated using TRIzol reagent (invitrogen) according to the manufacturer's instructions. One-step RT-PCR was performed in LightCycler-RNA Amplification Kit SYBR Green I (Roche). Human IL-8 was amplified with the sense primer 5′-GCT AAA GAA CTT CGA TGT CAG TGC-3′ and the anti-sense primer 5′-CTC AGC CCT CTT CAA AAA CTT CTC-3′. β-actin (sense primer 5′-CCA AGG CCA ACC GCG AGA AGA TGA C-3′, anti-sense primer 5′-AGG GTA CAT GGT GGT GCC GCC AGA C-3′) was used to normalize the expression of the IL-8 gene. The reaction mix consisted of 6 mM $MgCl_2$, 0.4 μl LightCycler-RT-PCR Enzyme Mix and 4 μl LightCycler-RT-PCR Reaction Mix SYBR Green I. All primers used were at a final concentration of 0.5 μM. The thermal cycling conditions were as follows: 10 min at 55°C for reverse transcription, 30 s at 95°C for pre-denaturation, 42 cycles at 1 s each at 95°C for denaturation, 10 s each at 55°C for annealing and finally, 13 s each at 72°C for elongation. At the end of each cycle, the fluorescence emitted by the SYBR Green I was measured. The relative abundance of target mRNA in each sample was calculated using the formula suggested by Muller et al. [18]: $2^{-(\text{IL-8 threshold cycle})}/2^{-(\beta\text{-actin threshold cycle})} \times 10^6$.

2.5. ELISA

IL-8 in perfusion was measured using a commercial ELISA kit (R&D Systems) according to the manufacturer's instructions. Briefly, 96-well round-bottom plates were incubated at 4°C overnight with rabbit monoclonal anti-human IL-8 antibody. Plates were washed with the washing buffer, after that they were blocked with 1% (w/v) BSA/PBS for 1 h at room temperature, and then washed as before. IL-8 standard (2.5–500 pg/ml) (rhIL-8, R&D Systems Europe Ltd.), or experimental samples were added to wells in duplicate, and incubated at room temperature for 2 h. Following a further wash, wells were incubated with biotinylated goat anti-human IL-8 antibody (R&D Systems Europe Ltd.) for 2 h at room temperature. After repeating the washing, the wells were incubated with streptavidin-HRP (Sigma-Aldrich Ireland Ltd.) in the dark for 20 min at room temperature. Plates were then washed, and the ELISA was developed for 20 min in the dark with substrate solution containing TMB/DMSO (0.6%, w/v) (Sigma-Aldrich Ireland Ltd.), sodium acetate (0.11 M) and H_2O_2. The reaction was stopped with 1 M H_2SO_4, and the absorbance was measured at 450 nm (ref. 570 nm). The concentrations of IL-8 in the experimental samples were extrapolated from a standard curve.

2.6. DNA plasmids and transfection

The −102 to +61 nt region of the IL-8 promoter (IL8 USCS) was cloned into EGFP reporter plasmid (pEGFP1) [16], which was subsequently transfected into endothelial cells using lipofectamine 2000 (invitrogen). After being selected by G418 (400 μg/ml) for 2 weeks, the endothelial cells were stimulated by 4.2 dyn/cm^2 shear stress for 6 h. Expression of EGFP was analyzed by flow cytometry.

2.7. Flow cytometry

HUVEC were washed with PBS, and detached with 0.25% trypsin (GIBCO-BRL)/0.04% EDTA (Bioprimer, China) for 15 min at 37°C. After centrifugation, the cell pellet was resuspended in 200 μl PBS/10% FCS. Cell viability was monitored using 7AAD (1:400). Cells were analyzed by flow cytometry using the FACS Calibur (Perkin Elmer; CellQuest). In these experiments, around 50,000 events were collected, and analysis was performed using FlowJo (Tree Star Inc.) software. A clearly distinguishable cell population with the background fluorescence level of non-transfected cells was considered to have lost the transfection, and was consequently excluded from the analysis. Relative promoter activity was indicated by the fluorescence intensity normalized to untransfected cells.

2.8. Statistical analyses

Unless otherwise indicated, results are reported as means $\pm$ SD from 3 independent experiments. Statistical analyses were performed by one-way ANOVA followed by Tukey test between various groups, and $p < 0.05$ was considered as statistically significant.

3. Results

3.1. The effects of shear stress on IL-8 expression in HUVEC

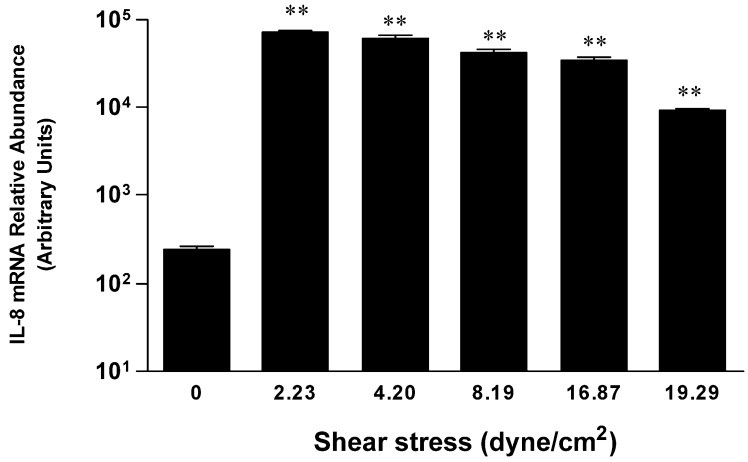
To explore the effects of shear stress on the IL-8 expression, HUVEC were exposed to different levels of shear stress (2.23, 4.20, 8.19, 16.87 and 19.29 dyn/cm^2) for 2 h. In all cases, shear stress was found to induce the IL-8 mRNA expression. However, the gene expression was negatively correlated with the shear stress intensity. Figure 1(a) shows that, as compared to the high shear stress (19.29 dyn/cm^2) treated group, a 52 fold increase in the IL-8 mRNA expression was observed in HUVEC exposed to low shear stress (2.23 dyn/cm^2). After HUVEC were exposed to different levels of shear stress (2.23 dyn/cm^2 and higher) for 6 h, the IL-8 protein in perfusion was measured by ELISA. The induction of the IL-8 protein was detectable at all levels of shear, with maximal protein production observed in the cells subjected to low shear stress (Fig. 1(b)).

3.2. Shear stress stimulates the transcription of the IL-8 gene

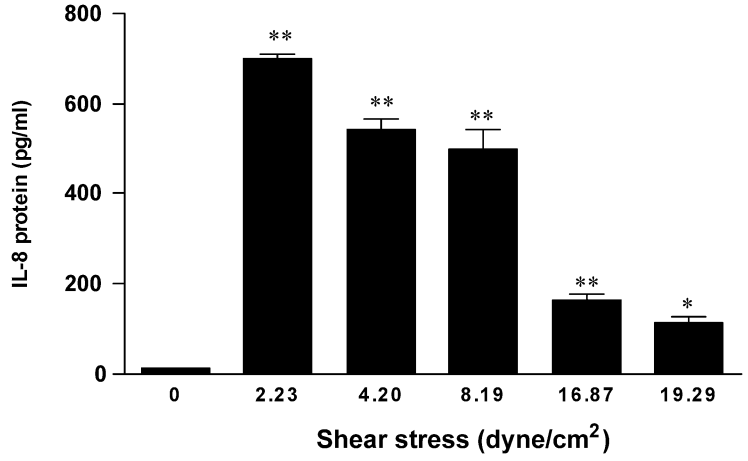
To further investigate the effects of low shear stress on the IL-8 gene expression, a 163-bp region (-102 to $+61$) containing the transcription initiation site of the IL-8 promoter was cloned into an EGFP reporter plasmid, which was then transfected into the endothelial cells. Flow cytometric analysis showed that low shear stress significantly increased the EGFP expression in pEGFP1-IL8USCS-transfected endothelial cells (Fig. 2(a)). Furthermore, pre-treatment with actinomycin D (10 μg/ml, 1 h), an inhibitor of mRNA synthesis, completely inhibited the shear stress-induced IL-8 mRNA expression (Fig. 2(b)). These findings suggest that shear stress-induced IL-8 gene expression is transcriptional.

3.3. Involvement of G protein in the low shear stress-induced IL-8 gene expression

To elucidate the role of the heterotrimeric G protein in the shear stress-induced IL-8 gene expression. HUVEC were treated with GDPβS (300 μM), a membrane-permeable, nonhydrolyzable analogue of



(a)



(b)

Fig. 1. The effects of shear stress on IL-8 expression in HUVEC. (a) After HUVEC were exposed to varying levels of shear stress for 2 h, the expression of IL-8 mRNA was determined using real time quantitative RT-PCR. The bars, representing the mean $\pm$ SD from three separate experiments, show the expression of the IL-8 gene normalized to β-actin. $^{**}(p < 0.01)$ represents the comparison of shear stress groups vs. static control. (b) After HUVEC were exposed different levels of shear stress for 6 h, the IL-8 protein in perfusion was measured by ELISA. The bars, representing the mean $\pm$ SD from three separate experiments, show the production of IL-8 protein. $^{**}(p < 0.01)$ and $^{*}(p < 0.05)$ represent the comparison of shear stress groups vs. static control.

GDP, for 1 h before being exposed to shear stress of 4.2 dyn/cm^2 for 2 h. GDPβS treatment significantly blocked the low shear stress-induced IL-8 gene expression. Under static conditions, however, GDPβS treatment had no visible effect on IL-8 gene expression. These data therefore suggest that the G protein is involved in the low shear stress-induced IL-8 gene expression (Fig. 3).

3.4. Tyrosine kinase (TK) activity is critical for the low shear stress-induced IL-8 gene expression

Shear stress has been shown to induce tyrosine phosphorylation in the endothelium [4]. To determine the possible contribution of TK to the regulation of the low shear stress-induced IL-8 gene expres-

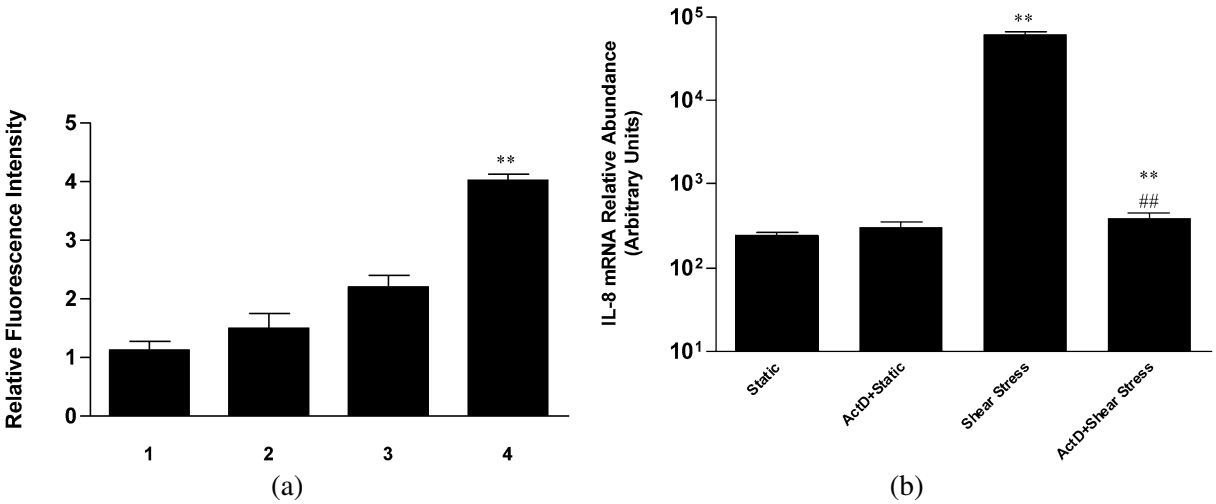


Fig. 2. Shear stress stimulates the transcription of the IL-8 gene. (a) HUVEC were transfected with an EGFP reporter plasmid containing the promoter region of IL-8 gene (IL8 USCS) as described in the experimental procedures section, followed by FACS assay. (1) HUVEC alone; (2) empty pEGFP1 trannsfected cells without stimulation; (3) pEGFP1-IL8USCS transfected cells with no stimulation; (4) pEGFP1-IL8USCS transfected cells stimulated with shear stress ($^{**}p < 0.01$ vs. the 3rd group; $n = 3$). (b) HVUEC were either subjected to low shear stress (4.2 dyn/cm^2) or incubated in a static condition for 2 h, after having been treated with actinomycin D (ActD; 10 μg/ml) for 1 h. The IL-8 mRNA expression was determined using real time quantitative RT-PCR. The bars, representing the mean ± SD from three separate experiments, show the expression of the IL-8 gene normalized to β-actin. $^{**}(p < 0.01)$ represents the comparison of shear stress groups vs. static control, while $^{\#\#}(p < 0.01)$ represents the comparison of shear stress groups vs. shear-stressed EC with ActD pretreatment.

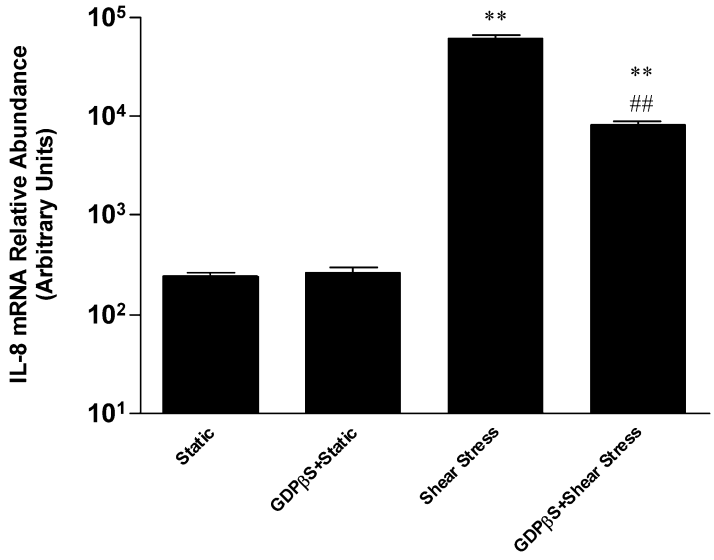


Fig. 3. Involvement of G protein in the low shear stress-induced IL-8 gene expression. After the preincubation of HUVEC with GDPβS (300 μM) for 1 h, cells were subjected to shear stress of 4.2 dyn/cm^2 for 2 h. The IL-8 mRNA expression was determined using real time quantitative RT-PCR. The bars, representing the mean ± SD from three separate experiments, show the expression of the IL-8 gene normalized to β-actin. $^{**}(p < 0.01)$ represents the comparison of shear stress groups vs. static control, while $^{\#\#}(p < 0.01)$ represents the comparison of shear stress groups vs. shear-stressed EC with inhibitor pretreatment.

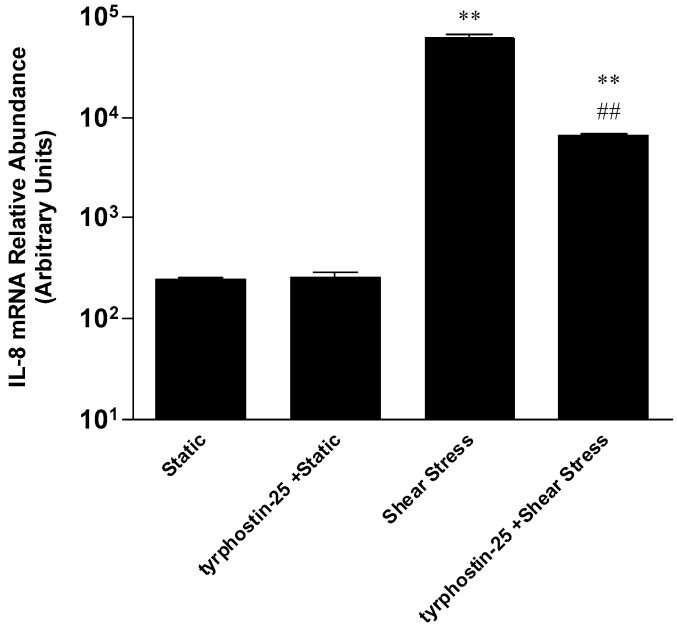


Fig. 4. Tyrosine kinase (TK) activity is critical for the low shear stress-induced IL-8 gene expression. HUVEC were pretreated with tyrphostin-25 (100 mM) for 30 min, after that the cells were either exposed to low shear stress (4.2 dyn/cm^2) for 2 h, or cultured in static condition for the same duration. After this, the IL-8 mRNA expression was determined using real time quantitative RT-PCR. The bars, representing the mean $\pm$ SD from three separate experiments, show the expression of the IL-8 gene normalized to β-actin. $^{**}(p < 0.01)$ represents the comparison of shear stress groups vs. static control, while $^{\#\#}(p < 0.01)$ represents the comparison of shear stress groups vs. shear-stressed EC with the inhibitor pretreatment group.

sion, we pretreated confluent HUVEC monolayers with the tyrosine kinase inhibitor, tyrphostin-25, for 30 min prior to the onset of shear stress. While tyrphostin-25 had no effects on the IL-8 gene expression under static culture conditions, it partially abolished the shear stress-induced IL-8 gene expression (Fig. 4).

3.5. The role of Ca^{2+} in the low shear stress-induced IL-8 gene expression

Previous studies have demonstrated that shear stress stimulates a rapid increase in intracellular Ca^{2+} [27]. In order to determine whether the flow-mediated increase in intracellular Ca^{2+} is necessary for IL-8 gene expression, we used BAPTA/AM to chelate intracellular Ca^{2+}. Low shear stress-induced IL-8 gene expression was partially blocked by 2.5 μM BAPTA/AM, suggesting that the Ca^{2+}-dependent pathway is involved in the regulation of low shear stress-induced IL-8 gene expression. To further investigate the role of Ca^{2+} on the low shear stress-induced IL-8 gene expression, 4 mM EGTA was used to chelate extracellular Ca^{2+}. The results show that EGTA was able to partially block the flow-induced IL-8 gene expression. However, the inhibition of EGTA was not as great as that produced by BAPTA/AM, indicating that the low shear stress-induced IL-8 gene expression is more dependent on the mobilization of Ca^{2+} from internal stores. Additionally, verapamil, a Ca^{2+} channel blocker, partially suppresses the low shear stress-induced IL-8 gene expression (Fig. 5). However, under static conditions, BAPTA/AM, EGTA and verapamil had no visible effects on the IL-8 gene expression (data not shown).

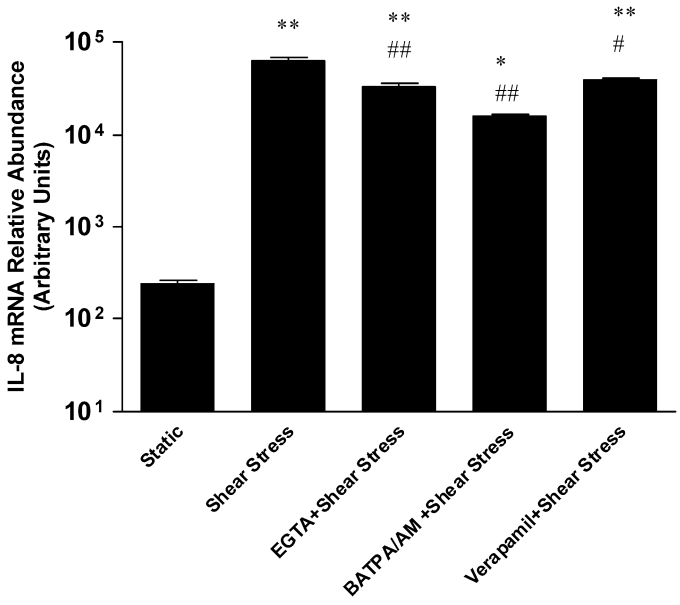


Fig. 5. The role of Ca^{2+} in shear stress-induced IL-8 gene expression. Extracellular calcium was chelated by adding EGTA (4 mM) in Ca^{2+}-free flow buffer. To deplete intracellular calcium, HUVEC were either treated with BAPTA/AM (2.5 μM) for 30 min, or preincubated with verapamil (10 μM) for the same duration. After treatment, the HUVEC were exposed to flow (shear stress $=$ 4 dyn/cm^2) for 2 h. The IL-8 mRNA expression was determined using real time quantitative RT-PCR. The bars, representing the mean $\pm$ SD from three separate experiments, show the expression of the IL-8 gene normalized to β-actin. $^{*}(p < 0.01)$ and $^{**}(p < 0.01)$ represents the comparison of shear stress groups vs. static control. $^{\#}(p < 0.05)$ and $^{\#\#}(p < 0.01)$, represent the comparison of shear stress groups vs. shear-stressed EC with inhibitor pretreatment group.

3.6. Roles of PKA, PKG and phospholipase C in low shear stress-induced IL-8 gene expression

To evaluate the roles of the other cell signaling molecules, we studied the effects of PKA, PKG and phospholipase C using specific inhibitors. Inhibition of the cGMP-dependent protein kinase with 1 μM KT5823 did not produce any visible changes in the flow-induced IL-8 gene expression in HUVEC. On the contrary, inhibition of the cAMP-dependent protein kinase activity with KT5720 (0.5 μM for 30 min) partially blocked the flow-induced IL-8 gene expression. In addition, this expression was also partially inhibited by neomycin, a phospholipase C inhibitor. Taken together, these results suggest that PKA and phospholipase C, but not PKG, are involved in the low shear stress-induced IL-8 gene expression. (Fig. 6). These inhibitors had no effect on the IL-8 gene expression under static culture (data not shown).

4. Discussion

We have previously demonstrated that low shear stress induces IL-8 mRNA expression in endothelial cells [3]. In the present study, we have further investigated both the effects of shear stress on IL-8 expression, and the mechanisms behind the up-regulation of IL-8 mRNA in HUVEC. Our results show that shear stress (from 2.23 to 19.29 dyn/cm^2) induces the IL-8 expression at both the mRNA and protein levels. Moreover, the shear stress-induced IL-8 gene expression is negatively correlated with the intensity of shear stress, i.e. the increase in IL-8 mRNA expression in HUVEC exposed to low shear stress (2.23 dyn/cm^2) is greater than that induced by high shear stress (19.29 dyn/cm^2). These findings,

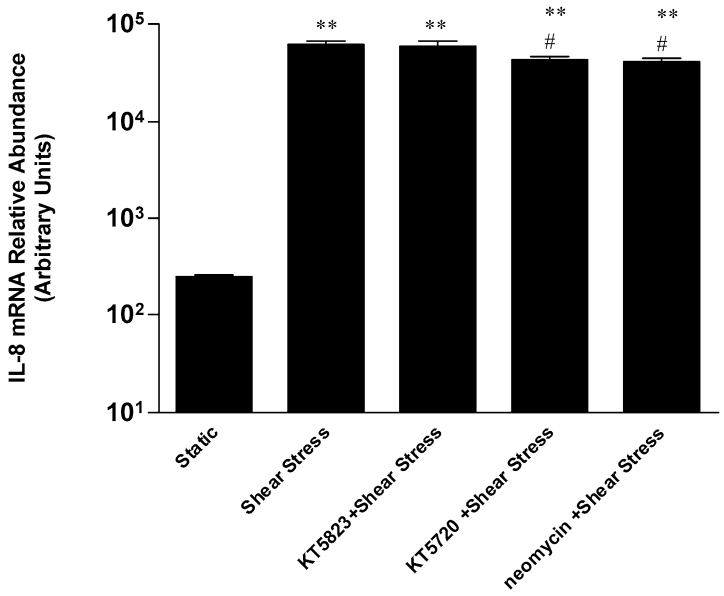


Fig. 6. The roles of PKA, PKG and phospholipase C in shear stress-induced IL-8 gene expression. HUVEC were pretreated with KT5823 (1 μM), KT5720 (0.5 μM) and neomycin (1 mM) respectively for 30 min, after that the cells were either exposed to low shear stress (4.2 dyn/cm^2) for 2 h, or cultured in static condition for the same duration. The IL-8 mRNA expression was then determined using real time quantitative RT-PCR. The bars, representing the mean ± SD from three separate experiments, show the expression of the IL-8 gene normalized to β-actin. $^{**}(p < 0.01)$ represents the comparison of shear stress groups vs. static control, while $^{\#}(p < 0.05)$ represents the comparison of shear stress groups vs. shear-stressed EC with inhibitor pretreatment.

together with data from both animal models and human subjects showing that atherosclerotic lesions occurs predominantly in regions of the vasculature exposed to low shear stress (around 4 dyn/cm^2) [21], led us to subsequently use shear stress at 4.2 dyn/cm^2 to investigate the effects of various signaling pathway inhibitors on shear stress-induced IL-8 gene expression.

Previous studies have shown that expression of the human IL-8 gene is regulated at the transcriptional [8] and/or post-transcriptional levels [10]. In order to identify the level contributing to the low shear stress-induced IL-8 mRNA, the effects of actinomycin D on IL-8 mRNA synthesis were assessed in the present study. Our data demonstrate that pretreatment of cells with actinomycin D results in near total inhibition of low shear stress-induced IL-8 gene expression, indicating that shear stress stimulates transcription of the IL-8 gene. To directly examine the effects of low shear stress on IL-8 gene transcription, a -102 to $+61$ bp $5'$-flanking promoter region of the IL-8 gene (IL8 USCS) containing a NF-κB binding site was cloned into an EGFP reporter plasmid, which was transfected into the endothelial cells. The findings demonstrate that low shear stress induces the expression of EGFP in endothelial cells transfected with pEGFP1-IL8USCS. Thus, the data from these experiments suggest that shear stress up-regulates the IL-8 mRNA expression in HUVEC through promoter, most likely through NF-κB, activation.

It has become increasingly clear that blood flow induced shear stress regulates gene expression through the activation of intracellular signaling networks. For example, shear stress-induced-phosphorylation of p38 MAPK and ERK1/2 has been suggested to regulate MMP9 gene expression [23], while c-Src is involved in the shear stress-regulated endothelial nitric oxide synthase expression [7]. Among the signaling transduction molecules known to be activated by shear stress, G proteins are one of the earliest shear stress-responsive cellular elements. The activation of $\mathrm{G}\alpha_q/\mathrm{a}_{11}$ and $\mathrm{G}\alpha_{i-3}$ protein subunits occurs within

one second of the onset of flow [11,12]. Furthermore, the stimulation of G-proteins triggers a number of signal-transduction cascades, including activation of K^+ channels, phospholipase C, phospholipase A_2 and adenylyl cyclase [25]. In the present study, we have demonstrated the ability of GDPβS to abrogate the low shear stress-induced IL-8 gene expression, indicating that this gene expression is at least in part controlled via G protein. As it is known that the shear stress induced-G protein activation occurs at the cell surface [11,12], it would be interesting to find out how signals are transduced from the cell surface into the cell to control the IL-8 gene expression.

Tyrosine phosphorylation of receptors and regulatory proteins has been shown to play important roles in transducing the chemical and mechanical stimuli received on the cell surface [6,9]. In response to flow, the tyrosine phosphorylation of several proteins is activated. Among these is the focal adhesion kinase (FAK), which has been shown to be localized in the focal adhesion site, and which constitutes a part of the mechanotransduction in EC as a response to shear stress [13]. Activation and autophosphorylation of FAK at tyrosine 397 (Y397) allows it to combine with other intracellular signaling molecules, such as Src and PI3K [20]. FAK/Src association induces FAK binding to the growth factor receptor binding protein 2 (Grb2)/Son of the sevenless (SOS) complex, which activates Ras/MAPK [22]. FAK-F397Y is the dominant-negative mutant of FAK. Previous studies have shown that FAK-F397Y attenuates the shear stress-induced activities of ERK2 and JNK1 [15]. Studies in our laboratory have revealed important roles for the activation of ERK1/2, JNK1/2 and p38 by low shear stress in the regulation of IL-8 gene expression (M. Cheng, J. Wu, H.Q. Chen et al. in review). The present study provides further evidence that tyrosine kinases are also involved in such gene expression. This raises the interesting question whether in this case there is any relationship between the tyrosine kinase and the MAPKs.

Previous studies have also revealed an important role for Ca^{2+} in the response of endothelial cells to shear stress [26]. The application of shear stress causes an influx of Ca^{2+}, inducing an increase in cytosolic Ca^{2+} which may in turn lead to numerous changes in the intracellular activation of molecules and in the production of bioactive agents such as NO and prostacyclin [5]. It has previously been reported that Ca^{2+} is required for IL-8 production in human colonic epithelial cells [30] and in neutrophils [26]. In the present study, the shear stress-induced IL-8 gene expression was observed to be inhibited by both EGTA and BAPTA/AM, suggesting that Ca^{2+} is necessary for the induction of IL-8 gene expression in HUVEC. Furthermore, verapamil (a Ca^{2+} L-type channel inhibitor) also caused partial suppression of IL-8 gene expression, although not as great as the suppression induced by EGTA and BAPTA/AM. This may be due to the presence of other Ca^{2+} channels which are also involved in this process. This hypothesis is supported by a study by Adams et al. [1], which indicated that EC possess a variety of Ca^{2+} channels, including both receptor-mediated channels and Ca^{2+}-leak channels.

In the present paper, we have also demonstrated that neomycin partially inhibits the shear stress-induced IL-8 gene expression. It has been reported that shear stress stimulates phospholipase C (PLC) with increased production of IP3 and intracellular calcium levels [14]. The calcium released from intracellular stores thereby activates calcium/calmodulin-dependent kinases, for example PKC. As Ca^{2+} is involved in the low shear stress-induced IL-8 gene expression, it is therefore not surprising that an inhibitor of phospholipase C partially abrogates this gene expression.

Cyclic AMP/PKA pathways have been reported to be involved in the regulation of the IL-8 gene expression in a number of cell types. For example, increases in intracellular cAMP levels through the activation of the β_2-adrenergic receptor, leads to the induction of IL-8 secretion in monocytes and bronchial epithelial cells [8,17]. Furthermore, recent findings demonstrate that PKA is also activated by shear stress [2]. In our study, we demonstrate that treatment of HUVEC with KT5720 blocks the shear-dependent IL-8 gene expression, suggesting that PKA is also involved in the regulation of such gene expression.

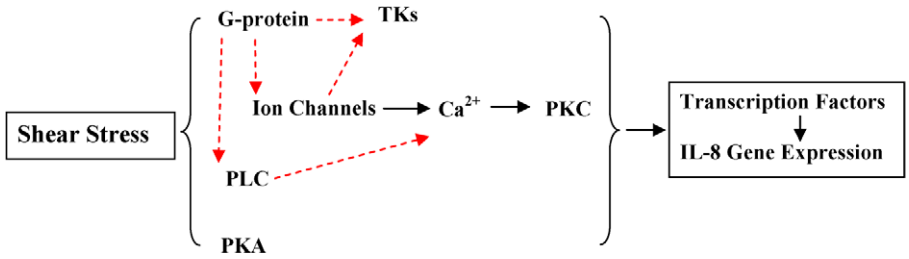


Fig. 7. Schematic summary of the signaling pathways involved in the low shear stress-induced IL-8 gene expression. A diagram summarizing the low shear stress-induced activation of intracellular signaling that leads to the IL-8 gene expression, as discussed in the text.

Shear stress induces several signaling cascades in EC including the opening of Ca^{2+} channels, the activation of heterotrimeric G proteins, the production of tyrosine phosphorylation of proteins such as Shc, c-Src, focal adhesion kinase (FAK) and protein kinase C (PKC), and the activation of transcriptional regulators such as c-fos, c-jun, c-myc, and nuclear factor (NF)-κB [25]. The current study demonstrates that, as summarized in Fig. 7, several signaling molecules play critical roles in regulating the low shear stress-induced IL-8 gene expression. However, the detailed interplays between the various signaling molecules and the crosstalk between signaling pathways remain to be elucidated.

Acknowledgments

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