Effects of Biomechanical Stress on Gene Regulation in Vascular Cells

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This thesis is based on the following papers:

I **Andersson M**, Karlsson L, Svensson P-A, Ulfhammer E, Ekman M, Jernås M, Carlsson L, Jern S. Differential Global Gene Expression Response Patterns of Human Endothelium Exposed to Shear Stress and Intraluminal Pressure.
Journal of Vascular Research 2005;42:441-52.

II Dourodi R*, **Andersson M***, Svensson P-A, Ekman M, Jern S, Karlsson L. Methodological Studies of Multiple Reference Genes as Endogenous Controls in Vascular Gene Expression Studies.
*Both authors contributed equally
Endothelium 2005;12:215-23.

III Wang L, **Andersson M**, Karlsson L, Watson M-A, Cousens D, Jern S, Erlinge D. Increased Mitogenic and Decreased Contractile P2 Receptors in Smooth Muscle Cells by Shear Stress in Human Vessels with Intact Endothelium.
Arteriosclerosis, Thrombosis, and Vascular Biology 2003;23:1370-76.

IV **Carlström M**, Ulfhammer E, Larsson P, Bergh N, Jern S, Karlsson L. Protective Effect of Laminar Shear Stress on u-PA Expression in Vascular Endothelial Cells Exposed to Inflammatory Stress.
In manuscript

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Effects of Biomechanical Stress on Gene Regulation in Vascular Cells

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Abstract

The vascular vessel wall is constantly exposed to biomechanical forces, such as shear and tensile stress. Biomechanical forces are important for several physiological and pathological processes and have been shown to regulate a number of fundamental vascular functions, such as vascular tone and remodeling processes. The aim of the present thesis was to study the effect of biomechanical forces on the vessel wall.

Intact human conduit vessels were exposed to normal or high intraluminal pressure, or low or high shear stress in combination with a physiological level of the other factor in a unique vascular *ex vivo* perfusion model, developed in our laboratory. Global gene expression profiling was performed with microarray technology of endothelial cells from stimulated vessels. Biomechanical forces were found to regulate a large number of genes. The fraction of genes that responded to both pressure and shear stimulation was surprisingly low, which indicates that the two different stimuli induce distinct gene expression response patterns. Further, these results suggest that the endothelium has the capacity to discriminate between shear stress and pressure stimulation.

Detection and quantification of changes in gene expression require valid and reliable endogenous references genes. Therefore, the appropriateness of ten reference genes for studies of biomechanically stimulated endothelium was evaluated by microarray technology and real-time RT-PCR.

Shear stress plays an essential role in regulation of vascular tone and remodeling, and P2 receptors have been suggested to be mediators of some of these effects. We therefore studied the effects of shear stress on P2 receptor expression in intact human vessels. In the endothelium, no significant regulation of P2 receptor mRNA levels was observed. However, in smooth muscle cells, high shear stress decreased mRNA expression of the contractile $P2X_1$ receptor and increased the mitogenic $P2Y_2$ and $P2Y_6$ receptors. These findings were consistent at the protein level with Western blot analysis and morphologically with immunohistochemistry. This suggests that the shear force can be transmitted to the underlying smooth muscle cells.

The interplay of shear stress and inflammatory stress on urokinase-type plasminogen activator (u-PA) and plasminogen activator inhibitor-1 (PAI-1) expression was studied in an *in vitro* shear stress system. Endothelial cells were exposed to either shear stress, the proinflammatory cytokine tumor necrosis factor-α (TNF-α), or a combination of both. High shear stress markedly reduced u-PA expression whereas TNF-α induced u-PA expression. Combining shear stress and inflammatory stimulation reduced the TNF-α mediated u-PA induction, which suggests that shear stress exerts a strong protective effect. The TNF-α induced expression was proposed to be partly mediated by activation of c-jun N-terminal kinase (JNK). The PAI-1 expression was induced both by shear stress and TNF-α, and the effect was potentiated when the two stimuli were combined.

In conclusion, these findings illustrate that biomechanical forces regulate a large number of genes in the endothelium and that shear stress and pressure induce distinct expression patterns. Shear stress also has the capacity to influence gene expression in smooth muscle cells in intact vessels and protect against inflammatory stress, which illustrates its potency as a regulator of endothelial cell function.

Key words: shear stress, intraluminal pressure, endothelium, gene expression, DNA microarray, real-time RT-PCR , reference genes, smooth muscle cells, P2 receptors, TNF-α, urokinase-type plasminogen activator

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