ABSTRACT

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Department of Biological and Medical Sciences

Title of Diploma Thesis: Effect of endoglin blockage on inflammation-induced endothelial dysfunction in human aortic endothelial cells

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Aim: The aim of this thesis was to describe the effect of TNF-α and LPS-induced inflammation on the expression of endoglin and its transcription factors, the expression of biomarkers of endothelial dysfunction, the levels of soluble endoglin, and the adhesion and transmigration of monocytes through human aortic endothelial cells. We further investigated the effect of endoglin blockage by the monoclonal antibody TRC105 (carotuximab) on these processes.

Methods: In this thesis, human aortic endothelial cells (HAEC) were used, and they were exposed for 16 hours to TNF-α (10 ng/ml) and LPS (100 ng/ml) and for 12 hours to TRC105 (300 μg/ml). The mRNA expression of endoglin and its transcription factors (KLF6, RelA, NR1H3), mRNA expression of inflammatory adhesion molecules (ICAM-1, VCAM-1, SELE) and mRNA expression of MMP-14 enzyme were measured by real time qRT-PCR. Using flow cytometry, we detected protein levels of endoglin, protein levels of inflammatory adhesion molecules (ICAM-1, VCAM-1), the number of adherent THP-1 monocytes, and the number of transmigrated THP-1 monocytes. We used the ELISA method to measure soluble endoglin levels.

Results: TRC105 alone had no significant effect on mRNA expression of endoglin and its transcription factors but led to a significant decrease in endoglin protein expression levels. TRC105 did not significantly affect protein expression of adhesion molecules or transmigration but was able to reduce monocyte adhesion to endothelial cells. Stimulation of cells by TNF-α and LPS led to a significant decrease in protein expression of endoglin, an increase in soluble endoglin levels and protein expression of inflammatory adhesion molecules, and an increase in monocyte adhesion, but had no effect on monocyte transendothelial migration. Addition of

TRC105 to TNF-α- and LPS-stimulated cells resulted in a significant decrease in protein expression of endoglin but had no significant effect on the expression of adhesion molecules. Only in the case of TNF-α-induced inflammation did the presence of TRC105 led to a decrease in monocyte adhesion. TRC105 did not significantly affect either TNF-α- or LPS-stimulated transmigration.

Conclusion: The results demonstrated that the addition of TRC105 to TNF-α-stimulated cells led to a significant reduction in endoglin protein expression, which may prevent TNF-α-induced monocyte adhesion. These results suggest that endoglin is involved in the development of TNF-α-induced endothelial dysfunction, and thus endoglin could potentially be an interesting pharmacological target in diseases with inflammatory etiology.

Key words: TRC105, endoglin, endothelial dysfunction, inflammation