Regulation of Vascular Endothelial t-PA Expression in Inflammation

Potential Target for Pharmacological Modulation

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The thesis is based on the following papers,

I Ulfhammer E., Larsson P., Karlsson L., Hrafnkelsdottir T., Bokarewa M., Tarkowski A., Jern S. TNF-alpha mediated suppression of tissue-type plasminogen activator expression in vascular endothelial cells is NF-kappaB- and p38 MAPK-dependent.
Journal of Thrombosis and Haemostasis 2006; 4: 1781-9

II Larsson P., Ulfhammer E., Karlsson L., Bokarewa M., Wåhlander K., Jern S. Effects of IL-1β and IL-6 on tissue-type plasminogen activator expression in vascular endothelial cells.
Thrombosis Research 2008; 123: 342-51

III Larsson P., Bergh N., Ulfhammer E., Magnusson M., Wåhlander K., Karlsson L., Jern S. Histone deacetylase inhibitors potently stimulate tissue-type plasminogen activator production in vascular endothelial cells.
In manuscript

IV Larsson P.*, Ulfhammer E.*, Magnusson M., Bergh N., Lunke S., El-Osta A., Medcalf R.L., Svensson P-A., Karlsson L., Jern S. Role of histone acetylation in the stimulatory effect of valproic acid on vascular endothelial tissue-type plasminogen activator expression.
*Both authors contributed equally to this study.
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Abstract

The endogenous fibrinolytic system is important for preventing occluding thrombosis and subsequent tissue infarction. The main activator of the fibrinolytic system in the vascular compartment is tissue-type plasminogen activator (t-PA). In a clotting situation, this enzyme is acutely released from storage pools in the endothelium and initiates fibrin breakdown. The endothelial release capacity of t-PA can be impaired by genetic or functional means and this impairment has been found to be associated with an increased risk for ischemic vascular disease, including myocardial infarction. Hypertension, smoking, and atherosclerosis are among the conditions associated with reduced t-PA production and release.

Another condition that could potentially be associated with reduced fibrinolysis is inflammation, but the role of inflammation in the regulation of t-PA production has not been clearly established. Thus, we investigated the effect of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) on the production of t-PA in cultured human vascular endothelial cells. We also studied which intracellular signaling mechanisms are of importance. TNF-α and IL-1β both caused a significant reduction of t-PA mRNA and protein production in endothelial cells. This effect was most evident at time points ≥24 h. Pharmacological blocking of selected intracellular signaling pathways revealed a central role of NFκB signaling in mediating the pro-inflammatory cytokine reduction of t-PA. p38 MAPK signaling was also found to be of some importance. IL-6, on the other hand, did not cause an effect on t-PA production. In fact, further experiments showed that the endothelial cell model used does not express a complete receptor for IL-6. However a soluble form of the IL-6 receptor exists in the circulation and when present in the cultures, a suppressive role of IL-6 on t-PA production was detected.

Given the central role of t-PA in the fibrinolytic system and the fact that impaired t-PA production is associated with increased risk of atherothrombotic events, a pharmacological means to increase the production of this enzyme could be desirable. There are indications that t-PA production could be partly regulated by epigenetic mechanisms, mainly histone acetylation. We thus investigated a panel of clinically used histone deacetylase inhibitors (HDACis) to determine their effect on t-PA production *in vitro*. All HDACis tested, irrespective of chemical structural class, potently stimulated endothelial t-PA production indicating that it is indeed their ability to modulate histone acetylation that affects t-PA synthesis. This was further supported by the fact that the HDACi valproic acid affected endothelial histone acetylation status, both globally and also specifically around the t-PA transcription start site, although in initial siRNA experiments we were unable to identify which specific HDAC enzyme(s) were of importance.

In conclusion, these data suggest that t-PA production in cultured vascular endothelial cells is suppressed by prolonged exposure to the inflammatory cytokines TNF-α and IL-1β. Moreover, IL-6, in the presence of its soluble receptor, can also attenuate t-PA production. If these results hold true also *in vivo*, it could be of importance e.g. in the local environment surrounding the inflammatory atherosclerotic plaque where these cytokines are present in high concentrations and where a sufficient t-PA production could be of uttermost importance. Clinically used HDACis potently stimulate t-PA in our experimental *in vitro* model, apparently *via* their effects on HDAC enzymes. As such, these substances could perhaps be considered for pharmacological stimulation of the endogenous fibrinolytic system as a novel prevention strategy for ischemic vascular disease.

Key words: fibrinolysis, tissue-type plasminogen activator, endothelium, inflammation, TNF-α, IL-1β, IL-6, NFκB, histone acetylation, HDAC-inhibitor, valproic acid, myocardial infarction

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