Epigenetic influence on cardiovascular protective mechanisms in vivo: explorations of t-PA release and extracellular vesicle genetic content

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet
kommer att offentligen försvaras i Sahlgrens Aula, Sahlgrenska Universitetssjukhuset, Göteborg
fredagen den 5 juni 2015, kl 09:00

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Avhandlingen baseras på följande delarbeten:

I. Svennerholm K, Bergh N, Larsson P, Jern S, Johansson G, Biber B, Haney M. Histone deacetylase inhibitor treatment increases coronary t-PA release in a porcine ischemia model. *PLoS One. 2014;9(5):e97260.*

II. Svennerholm K, Haney M, Biber B, Ulfhammer E, Saluveer O, Larsson P, Omerovic E, Jern S, Bergh N. Histone deacetylase inhibition enhances tissue plasminogen activator release capacity in atherosclerotic man. *PLoS One. 2015;10(3):e0121196.*

III. Svennerholm K, Rodsand P, Hellman U, Lundholm M, Waldenström A, Biber B, Ronquist G, Haney M. Myocardial ischemic preconditioning in a porcine model leads to rapid changes in cardiac extracellular vesicle messenger RNA content. *Accepted for publication in International Journal of Cardiology, Heart & Vasculature.*

IV. Svennerholm K, Hellman U, Rodsand P, Lundholm M, Waldenström A, Biber B, Ronquist G, Haney M. Coronary venous extracellular vesicle DNA content is altered by myocardial ischemic preconditioning in a porcine model. *Manuscript.*

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ABSTRACT

Background: Ischemic heart disease is one of the leading causes of death globally. This thesis explores endogenous mechanisms protecting against myocardial ischemia in context of epigenetics (changes in gene activity not caused by changes in DNA sequences). Epigenetic regulation of vascular thromboprotective mechanism was assessed, as well as the capacity of extracellular vesicle (EV) involvement in mediating epigenetic changes related to cardioprotection in ischemic preconditioning (IPC).

Aims: The aim of Papers I and II was to evaluate if histone deacetylase inhibition, by valproic acid (VPA) treatment, increases stimulated tissue plasminogen activator (t-PA) release capacity and affects plasminogen activator inhibitor-1 (PAI-1) levels *in vivo*, in healthy large animals and in an atherosclerotic cohort. The aim of Papers III and IV was to assess if coronary venous EV genetic content is affected by myocardial IPC *in vivo*.

Methods: In a porcine myocardial ischemia model transcoronary t-PA release was measured and compared between VPA treated (n=12) and untreated animals (n=10). In the clinical cross-over study (n=16), the perfused forearm model was used to measure single and repeated t-PA release capacity by isoprenaline provocation with and without VPA. PAI-1 was also measured. In a porcine model, EV were collected from coronary venous blood before and after myocardial IPC. The EV were isolated by differential ultracentrifugation and the preparation was evaluated by western blot, electron microscopy and nanoparticle tracking analysis. Changes in EV genetic content after IPC were identified by microarray and DNA sequencing.

Results: Animals treated with VPA demonstrated a significantly higher cumulative transcoronary t-PA release compared to controls. In the clinical study, VPA treatment resulted in increased cumulative t-PA release capacity during repeated isoprenaline stimulation, though there was no difference when comparing single stimulation sequences. Levels of PAI-1 were reduced after VPA treatment. Among 11678 mRNA sequences detected in EV, about 10% were up or down regulated after IPC. Among these, over half were increased, including several with association to cardioprotection and IPC. DNA fragments, representing all porcine chromosomes, were identified in EV. The DNA content in EV changed after myocardial IPC.

Conclusions: Intervention of HDACi, by VPA treatment, may improve actions of the fibrinolytic system by enhancing t-PA release capacity and reducing PAI-1 levels *in vivo*. In a future perspective, this may have clinical relevance as novel means of preventive strategies for ischemic heart disease. Myocardial IPC influences the composition of EV genetic content, including increases in gene transcripts associated to cardioprotecion. This may reflect a biological relevance of EV in delivering cardioprotective signals in IPC, although further studies are necessary to confirm such connection.

Keywords: myocardial ischemia, epigenetics, histone deacetylase inhibition, t-PA, extracellular vesicles, ischemic preconditioning

ISBN: 978-91-628-9417-7 (printed)
ISBN: 978-91-628-9418-4 (e-published)

http://hdl.handle.net/2077/38373