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Loss of thrombomodulin function modulates the stability of atherosclerotic plaques.

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Cardiovascular diseases will be soon the main cause of death in the western world due to increasing cases of diabetes mellitus and obesity. Epidemiologic studies suggest an important role of altered coagulation in arteriosclerosis in respect to micro- and macroangiopathy. Arteriosclerosis can be viewed as a state of acquired thrombophilia, as arteriosclerosis and endothelial dysfunction are associated with a loss of endothelial thrombomodulin expression.

The overall objective of this thesis was to address the role of loss of thrombomodulin function during atherogenesis. Furthermore, atherogenesis between hypercoagulable diabetic mice and hypercoagulable hyperlipidemic mice (fed a high fat diet) was compared. In addition loss of thrombomodulin as well as a gain of function, increased activated protein C formation, regarding diabetic microangiopathy were ascertained.

For the diabetic nephropathy study wild-type mice and mice of hypercoagulability secondary to a partial loss of thrombomodulin function ($TM^{Pro/Pro}$ mice) and mice with genetically altered in vivo activated protein C formation (APC^{high} mice) were used. For studying macroangiopathy female ApoE-deficient mice (ApoE-/-) were crossed with $TM^{Pro/Pro}$ or APC^{high} mice. Mice were made diabetic or fed a high fat diet (HFD) for 20 weeks (atherosclerosis study) or 26 weeks (only diabetic mice; diabetic nephropathy study). A subset of mice was anticoagulated with daily low molecular weight heparin (enoxaparin) injections. Detailed morphological and morphometric studies were performed to study diabetic glomeruli and atherosclerotic plaques of brachiocephalic arteries, aortic valves and thoracic aortae of hypercoagulable mice or mice with reduced coagulation activation.

During diabetic microangiopathy APC^{high} mice showed significant lower frequencies of apoptosis in glomerular cells compared to diabetic wild-type and diabetic $TM^{Pro/Pro}$ mice, while in $TM^{Pro/Pro}$ mice high frequencies of apoptotic glomeruli was observed. Further analysis identified an endothelial cell protein C receptor (EPCR) and protease-activated receptor-1 (PAR-1) dependent cytoprotective effect of activated protein C formation, while loss of thrombomodulin function lead to hypercoagulability aggravated diabetic renal injury.

In hypercoagulable hyperlipidemic ApoE-/- mice atherosclerotic plaque size was increased but did not result in more pronounced vascular stenosis secondary to positive vascular remodeling in comparison to hypercoagulable ApoE-/- mice on a normal chow diet or hyperlipidemic ApoE-/- mice. The high fat diet model of hypercoagulability showed the typical parameters for a stable plaque. Smooth muscle cell proliferation and migration was increased followed by enhanced extracellular matrix deposition as well as thick fibrous caps guarding the atherosclerotic plaque, while frequency of macrophages was reduced. A plaque destabilizing effect was observed when hypercoagulable hyperlipidemic ApoE-/- mice were

treated with low molecular weight heparin, consistent with the ApoE-/- APC^{high} mouse model of genetically impaired coagulation activation which suffered of sudden death. Further in vitro and in vivo studies did not show an effect of thrombin on monocyte apoptosis or proliferation but revealed a role of thrombin reducing migration of monocytes / macrophages to the site of inflammation. Thus, elevated coagulation activation in hyperlipidemic ApoE-/- mice leads to stable atherosclerotic plaques containing less fragile foam cells.

Comparing the hypercoagulable hyperlipidemic ApoE-/- mouse model with hypercoagulable diabetic ApoE-/- mice smaller but instable, ruptured plaques were observed in the hyperglycemic model. The high frequency of plaque instability hindered detailed analyses of the individual plaque components in diabetic ApoE-/- $TM^{Pro/Pro}$ mice.

Summarizing the data of this thesis, hypercoagulability function is complex and differs in micro- and macrovascular diseases. The opposing effects of atherosclerotic plaque stability in hypercoagulable hyperlipidemic ApoE-/- mice in comparison to less stable plaques in hypercoagulable diabetic ApoE-/- mice demonstrate the severe pathophysiologic differences of these two risk factors for coronary heart disease. In addition, characterizing the role of the thrombomodulin-protein C system in regard to micro- and macrovascular complications in hyperglycemic mice revealed opposing effects. While activated protein C formation protects against apoptosis of endothelial cells in glomeruli during diabetic nephropathy, diabetic ApoE-/- mice with genetically increased activated protein C formation suffered of sudden death.

These data provide for the first time – to our knowledge - experimental evidence that the mechanisms of atherosclerotic plaque formation differ between diabetic and hyperlipidemic individuals and are in part modulated by the coagulation system. Furthermore, these data show for the first time that the endothelial thrombomodulin-protein C system modulates micro- and macrovascular angiopathy through different mechanisms.