Atrial fibrillation and hypercoagulability

Summary

Atrial fibrillation (AF) is the most common form of sustained cardiac tachyarrhythmia with an estimated prevalence of approximately 3%. AF is characterized by high-frequency, irregular activation of the atria, leading to hemodynamic impairment. AF often starts with short, self-terminating episodes. However, the progressive nature of the arrhythmia is characterized by prolongation of the AF episodes until they become persistent.

Epidemiologically, advancing age is the predominant risk factor for AF. AF is associated to increased cardiovascular mortality due to sudden death, heart failure (HF) and stroke. The risk of developing thromboembolic stroke increases by five-fold after patients have developed AF.

Recently, hypercoagulability has been described to play a role in the progression of AF. Activated coagulation factors, such as thrombin and coagulation factor (F) Xa, are able to modulate physiological and pathological processes, such as inflammation and fibrosis, which may contribute to cardiac remodeling and progression of AF. These extravascular (non-hemostatic) functions affect different cell types via activation of protease activated receptors (PARs).

The general aim of this thesis was to evaluate how AF progression is linked to hypercoagulability by activated blood coagulation (through thrombin and FXa) and consequent PAR activation. Furthermore, we have investigated the effect of the risk factor age, and the AF comorbidity HF, on AF substrate development and coagulation activity.

In **chapter 2** we reviewed the most important and recent findings regarding the tissue factor (TF):Factor (F)VIIa complex, an essential coagulation trigger, with the emphasis on the heart and blood vessels. TF facilitates the activation of FVII into activated FVII (FVIIa), thereby initiating the extrinsic coagulation pathway followed by the activation of FX (FXa) and thrombin formation. The so-called non-hemostatic functions of TF:VIIa play a role in diverse processes such as inflammation, atherosclerosis and remodeling of vessels and myocardium. We also described some of the pleiotropic effects elicited by thrombin and FXa, and their cellular signaling through activation of PAR.

Thrombin and FXa have been implicated in the activation of cardiac fibroblasts (CF) and promotion of cardiac remodeling. In **chapter 3** we showed that these coagulation factors upregulated the gene expression of pro-fibrotic genes in CF, supporting the link between pathological activation of the coagulation system and cardiac remodeling. Moreover, we found that FXa induces overexpression of proinflammatory genes in human CFs via PAR-1, which was found to be the most abundant PAR isoform in this cell type.

The central hypothesis of this thesis is that the hypercoagulable state during AF contributes to the development of an AF substrate through activation of PAR. To test this hypothesis *in vivo*, we made use of the goat model of AF. This model allows the investigation of changes in the heart and blood that occur within days to months of AF.

In **chapter 4** we described how we customized the Calibrated Automated Thrombography (CAT) assay to provide a global view of the goat coagulation profile by assessing thrombin generation (TG) in goat plasma. Since TG assessment with human reagents appeared not suitable for goat plasma, we reported on two distinct methods using either goat brain proteins (GBP) or Russell's viper venom-factor X activator (RVV-X) as successful triggers of TG in goat plasma. Moreover, we showed that both methods were able to detect the decrease in clotting potential induced by FXa-inhibition.

In **chapter 5** we investigated the effect of FXa-inhibition, by rivaroxaban treatment, on AF substrate development in the goat model of AF. Although rivaroxaban treatment did not prevent the progression of AF, we found that four months of AF led to atrial myocyte hypertrophy, which was fully prevented by FXa-inhibition. Interestingly, AF did not cause a hypercoagulable state, as assessed by the TG assay. One possible explanation is that AF without the presence of other comorbidities, or risk factors for stroke ("lone AF"), might not be sufficient to trigger a thrombotic response in goats. As age is the most important risk factor for AF, as well as for stroke in patients with AF, we studied the effect of advanced age in the goat model of AF. In **chapter 6** we reported that age and AF synergistically increased coagulation potential, early AF stabilization and promoted atrial structural remodeling.

Another important comorbidity of AF is heart failure (HF), which can promote the onset and progression of AF and can increase the risk for stroke during AF. In **chapter 7** we demonstrated that HF created a favorable substrate for the onset of AF, which was associated with a decrease in coagulation activity and increased atrial fibrosis. Moreover, HF led to a strong increase in complex fibrillatory conduction.

Taken together, the results of this thesis lay the basis for future experimental investigations and important translational considerations. Our findings confirm the presence of a crosstalk between AF and hypercoagulability, whose causal link can be described as a multidirectional network between AF, stroke and their shared comorbidities.