Chapter 8

Discussion

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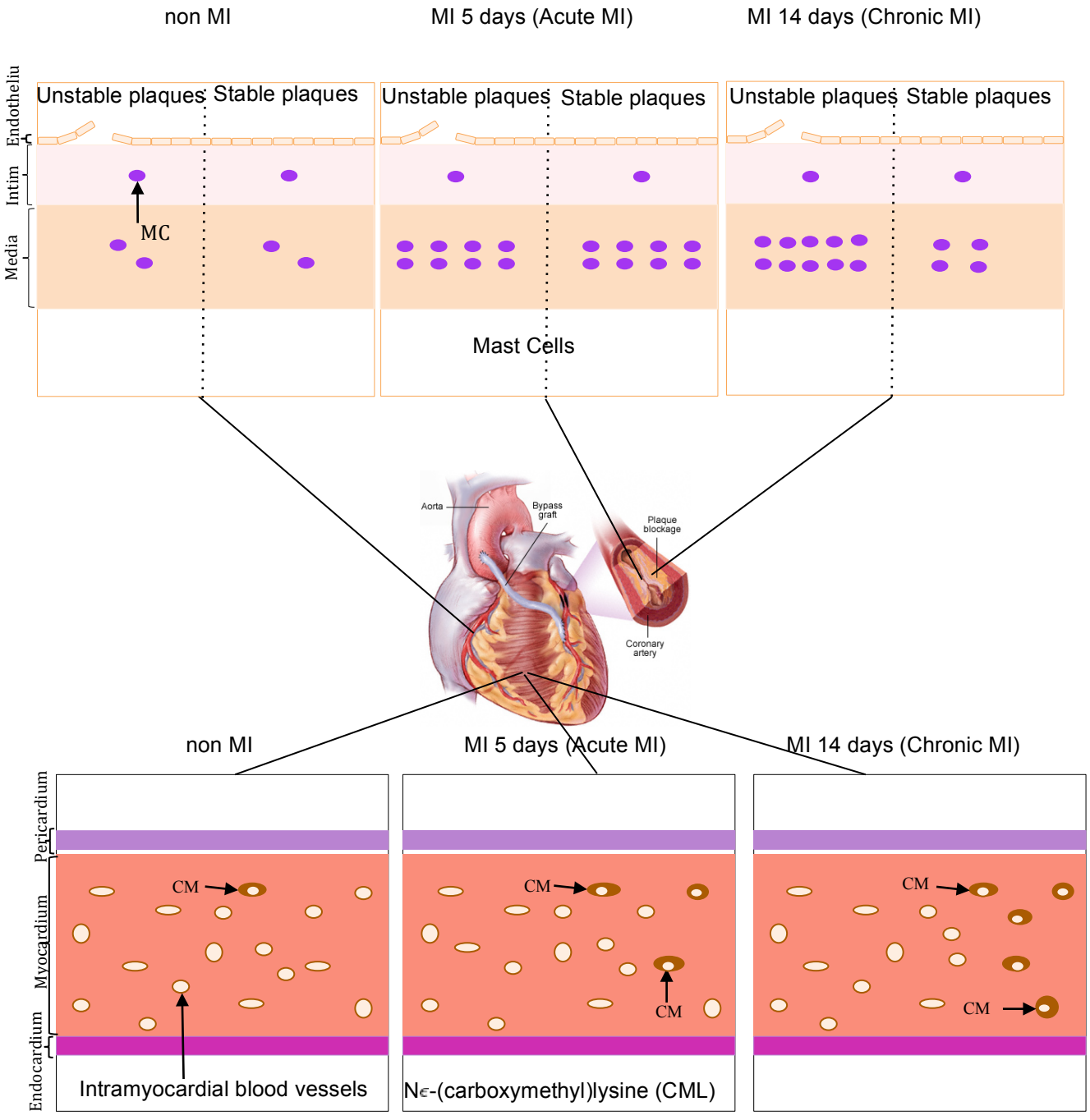
Coronary artery disease

Worldwide cardiovascular disease is a major cause of morbidity and mortality, in which coronary artery disease (CAD) is playing a major role. The pathophysiology of CAD is multifactorial and includes dyslipidemia, hypercoagulability, oxidative stress, endothelial dysfunction, inflammation and/or infection (1). CAD is the most common cause of myocardial infarction (MI) and occurs when an obstructing plaque or thrombus prevents blood flow. It was previously thought that progressive luminal narrowing is the main cause of MI. It is however now evident that inflammation in the atherosclerotic plaque rather than stenosis precipitates ischemia and infarction (2). In recent years it has become clear that MI not only is related to the diseased epicardial coronary arteries. The intramyocardial vasculature is namely also playing an important role, dependent as well as independent of the epicardial coronary arteries (3). From studies in diabetic patients it is known that the advanced glycation endproduct N_{ε}-(carboxymethyl)lysine (CML) accumulates in the intramyocardial vasculature and plays a role in cardiac disease (4). We therefore wondered whether intramyocardial CML might play a role in MI induction in patients without diabetes. We found in patients with different infarct duration that CML depositions were significantly increased in the intramyocardial vasculature, predominantly on endothelial cells, compared with control patients **(Chapter 2**). Additionally we found in a rat MI model, in which MI was induced via ligation of the left anterior descending coronary artery, that CML deposits in the intramyocardial vasculature from day 5 post-MI on, but not before, indicating that the post-MI inflammatory response additionally induces CML accumulation (***Figure 1***). Since increased accumulation of CML was already present in patients who died very early after MI, this suggests that

in MI patients CML accumulates already in advance of MI. Even more, in patients with MI we didn't find differences in CML intensity in the intramyocardial vasculature between infarcted and non-infarcted areas, also indicative for a global ischemia effect of the heart, next to chronic inflammation (3).

Figure 1 Coronary artery



In the epicardial coronary arteries, atherosclerotic plaque complications preferentially occur in plaques wherein the fibrous cap is thin and/or is eroded, mainly at a side where activated immune cells are abundantly present (the so-called unstable plaque) (2) These immune cells however can also infiltrate stable plaques. By producing inflammatory mediators and proteolytic enzymes they can weaken the cap and thus transform a stable plaque into a vulnerable, unstable plaque (5; 6). Next to this, vasospasm of coronary arteries can induce plaque complications, resulting in plaque rupture and/or plaque bleeding. Recent studies point to a role of mast cells (MC) in especially vasospasm (7). The majority of MCs in the coronary arteries are found in the adventitia (6) and the number of degranulated MCs in the adventitia surrounding ruptured plaques in MI was found to be increased in infarct-related coronary arteries (8). It then was suggested that histamine released by degranulation of MCs in the adventitia might reach the media, where it locally provokes coronary spasm, contributing to the onset of MI (8). Next to this, MCs have also been described in shoulder regions in the intima of atherosclerotic plaques, but this did not correlate with MI (5). We wondered whether MCs would be present in the intimal and medial layer and as such may be involved in plaque complications (***Figure 1***). For this we have analyzed MCs in infarct-related and non-infarct-related coronary arteries at different time-points after onset of MI and in control patients **(Chapter 3**). Between the different groups, the number of MCs was similar in the intima, irrespective of plaque stability. No differences were found in the intima of control patients and patients with acute (up to 5 days) or chronic (up to 14 days) MI. The density of MCs in the media however of both acute and chronic MI patients was significantly higher than in controls. Even more, the stable lesions of patients with acute MI contained more MCs than controls. While the number of MCs in the media of stable lesions of

chronic MI patients was significant lower than in patients with acute MI. Remarkably, in contrast to control and acute MI patients, in chronic MI patients the number of MCs in unstable lesions was significantly higher than in stable lesions. This coincided with a significant increase in the relative number of unstable plaques in chronic MI patients compared with acute MI and control patients.

In conclusion, we have shown in patients with MI CML depositions on activated endothelium in the intramyocardial vasculature that might reflect an increased risk for MI rather. We also show that the presence of MCs in the media of epicardial coronary lesions might contribute to the onset of MI through their plaque-destabilizing or spasm-inducing properties. MI in turn contributes to intra-plaque infiltration of MCs especially in unstable plaques, thereby increasing the risk of re-infarction.

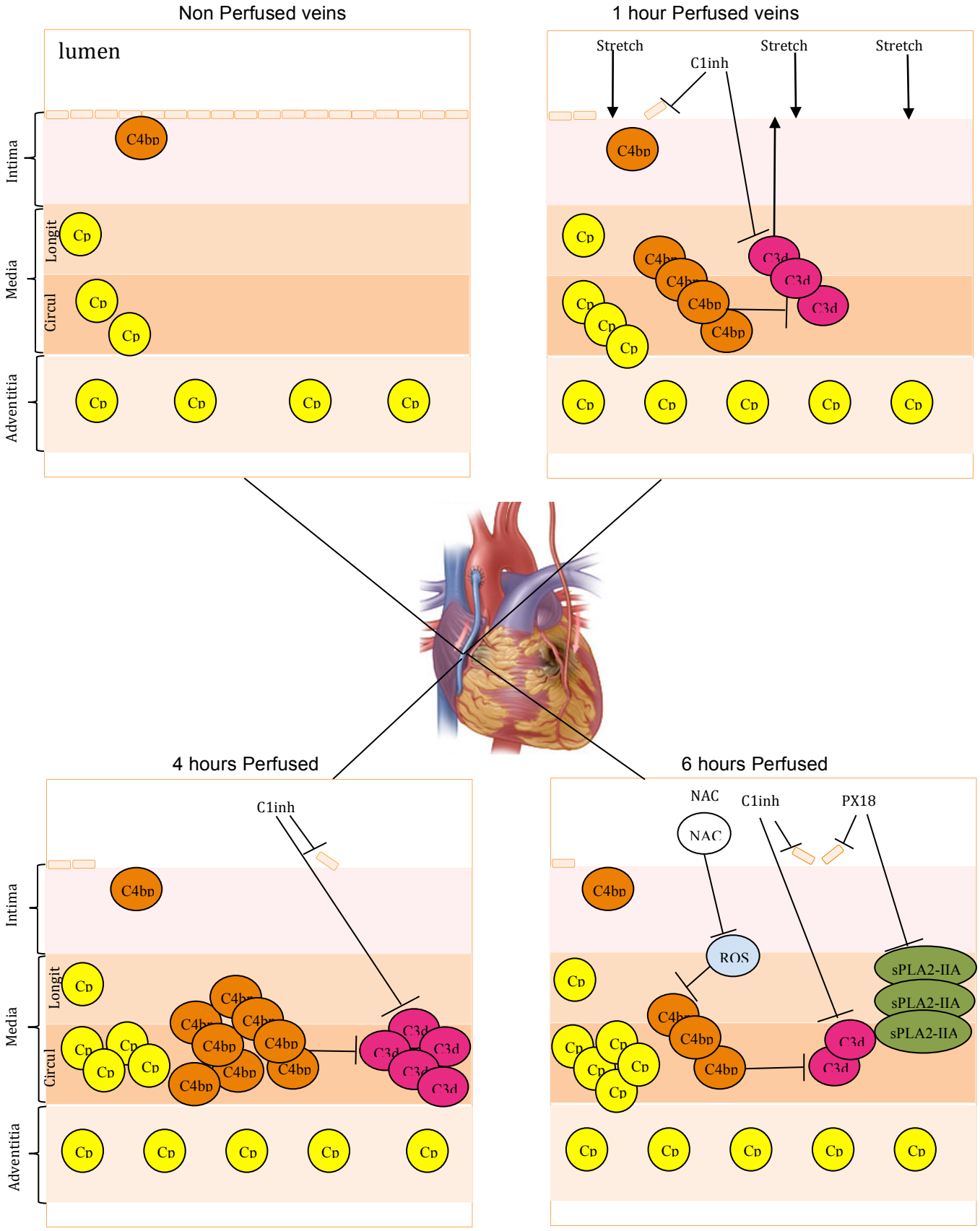
Coronary artery bypass graft surgery

Coronary artery bypass graft surgery (CABG) provides symptomatic benefit for patients with CAD and prolongs life in selected patient groups (9). In CABG the saphenous vein is still frequently used as an aortic coronary bypass graft. However, patency rates are known to be less favourable compared to arterial conduits. Saphenous vein graft patency is 60% at 10 years postoperatively (10-12), whereas arterial conduits like e.g. the left internal mammary artery to the left anterior descending artery (LAD) show patency rates of 95% at ten years. However, due to surgical-technical reasons, limitations or contraindications associated with the use of arterial grafts, saphenous vein bypass grafts are still used in coronary artery bypass surgery (13).

Next to patient-related and surgical technical factors loss of perivascular innervation (14), destruction of vasa vasorum (15), and changes in blood flow velocity (16) play a

role in vein graft failure. When the vein graft is exposed to the higher arterial blood pressure and flow velocity, injury of the vein is also induced. The greater saphenous vein namely has different wall characteristics compared to the arterial wall, both morphologically and functionally (17-20), which makes it less suitable for the arterial circulation system. Kockx et al (21) showed that within 24 hours after grafting the endothelium showed extensive desquamation. Subsequently massive migration of neutrophilic granulocytes occurred in the venous wall. In addition, the circular layer of the media was also severely damaged, resulting in a loss of smooth muscle cells (SMC), initiating the process of fibrosis in the media of vein grafts (21). Arterial pressure-induced vein graft changes can be prevented using perivenous support preventing overdistention (22). Next to this, increasing evidence is pointing to an important role of the immune response also, including complement (23). The involvement of complement in cardiovascular disease is well accepted, including atherosclerosis of arteries (24). However, we have found that complement positivity in perfused veins was quite limited (25). We wondered whether this could be by explained by activation of endogenous complement inhibitors, such as e.g. C4bp within these veins as it was shown that C4bp plays a role in the process of arterial atherosclerosis (26). In perfused veins C4bp was increasingly present in the media of veins perfused up to 4 hours, which coincided with a transient but much more limited increase in the presence of C3d. After 6 hours of perfusion the presence of both C4bp and C3d decreased again (*Figure 2)* (**Chapter 4**). This would suggest that C4bp could be part of an adaptive mechanism that protects grafts against excessive complement-mediated cell damage, at least early after perfusion.

Figure 2 Saphenous vein graft



Interestingly the decrease in C4bp levels after 4 hours of perfusion was inhibited by the Reactive Oxygen Species (ROS) scavenger N-acetyl cysteine (NAC). It was shown before that ROS have a devastating effect in perfusion-induced vein graft failure (27), at least within 24 hours. To determine whether the increase in C3d and especially C4bp in perfused saphenous veins was related to ROS, saphenous veins were perfused for 6 hours in two parallel perfusion systems, with autologous blood with or without the ROS scavenger NAC. In the presence of NAC the C4bp-positive area increased significantly, suggesting that ROS suppress the upregulation of C4bp in perfused veins. Remarkably however, also the C3d-positive area increased in the presence of NAC, suggesting that ROS may suppress the activation of complement also **(Chapter 4).**

Interestingly, it was shown before that interference with C3 activation with complement receptor-related gene y-Ig or cobra venom factor prevented vein graft failure in an APOE3 Leiden mouse model on the long term (28).

We wondered whether C1-inhibitor (C1inh), a complement inhibitor with not only anti-inflammatory but also anti-apoptotic properties and that has been used in patients with hereditary angioedema, sepsis and myocardial infarction already, would also protect vein grafts. Application of C1inh in the in vitro perfusion model resulted in significantly higher C1inh blood levels and significantly increased presence of C1inh in the vein wall. This coincided with a significant reduction in endothelial loss and presence of complement activation products C3d and C4d in the vein wall, especially in the circular layer, compared to vein segments perfused without supplemented C1inh ***(Figure 2***). We subsequently showed that C1inh protected vein grafts against atherosclerotic changes on the long term in mice **(Chapter 5).**

It has previously been shown that in addition to inflammation, apoptosis is also playing a role in vein graft failure. Interestingly we previously studied the role of PX18 in acute myocardial infarction and found that it not only inhibited the acute phase protein $sPLA_2$-IIA, but also prevented apoptosis of cardiomyocytes independent of $sPLA_2$-IIA (29). We therefore wondered whether PX-18 would also have protective effects in veins.

Supplementation of PX18 in the perfusion blood led to a significant reduction in perfusion-induced endothelial cell loss in saphenous veins to 55% compared to 75% in veins perfused without PX18 in human veins (**Chapter 6**). In line with this, $sPLA_2$-IIA-positive smooth muscle cells (SMCs) were observed focally in the longitudinal and circular layers of the media in veins perused without PX18 that was significantly reduced by PX18. We additionally found that PX18 significantly attenuated shear stress-induced caspase-3 activation in HUVECs in vitro, independent of $sPLA_2$-IIA.

The results suggest that PX18 protects the endothelium of saphenous vein graft subjected to prolonged arterial pressures that in part can be explained by inhibition of endothelial cell apoptosis, also independent of $sPLA_2$-IIA.

Finally, infection is also playing a role in vein disease. Several studies have suggested an association between *Chlamydophila pneumoniae* (Cp) infection and atherosclerosis. In saphenous veins, Cp DNA was detected in 12% of all patients before using the veins in bypass grafting, while in 38% of the failed grafts Cp DNA was found (30). Because of the observed association of Cp infection and vein graft failure (31), we performed a study to determine Cp at the protein level in the various cell types of the saphenous vein using a monoclonal antibody against *Cp*-specific membrane protein (RR-402). We therefore analysed veins that were perfused with autologous blood under arterial pressure (**Chapter 7**). In non-perfused veins, Cp was

present in macrophages of the adventitia in 91% of all patients, which indicates that the RR-402 antibody recognised “residual” Cp. While in the media we found Cp in macrophages in both the circular (64%) and longitudinal (23%) smooth muscle layer, no positivity was found in the intima. Perfusion subsequently resulted in a significant increase of Cp positive cells within the circular layer of the media, but not in the longitudinal layer or intima (***Figure 2***). As Cp DNA was not detected by PCR in these veins, this indicates that antigens, rather than viable bacteria, persist in the veins. This suggests that antibiotical therapy in this respect might not be successful.

In conclusion our studies in veins therefore suggest that anti-inflammatory therapy, e.g. C1-inhibitor and/or PX18 rather than antibiotic treatment might be beneficial in patients undergoing vein graft surgery.

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