NR4A nuclear receptors in atherosclerosis

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Summary

Atherosclerosis is a chronic disease of the arterial vessel wall that results in thickening of the vessel wall and may severely narrow the lumen of a blood vessel. Acute myocardial infarctions or ischemic strokes are caused upon local rupture of such a thickening, also named as atherosclerotic lesion, and the subsequent induction of blood coagulation obstructs the blood flow to vital organs and tissues. Risk factors for atherosclerosis are amongst others obesity, high blood pressure, smoking, and diabetes. When coronary arteries are narrowed due to atherosclerosis, clinical intervention usually involves balloon angioplasty with optional placement of a stent. A major complication of this angioplasty procedure is (in-stent) restenosis, a re-narrowing of the treated vessel which is caused by excessive growth of vascular smooth muscle cells (SMCs). The goal of the research described in my thesis was to further explore the role and therapeutic potential of the NR4A nuclear receptors Nur77, Nurr1, and NOR-1 in atherosclerosis and processes closely related to this process, such as (in-stent) restenosis and lipid metabolism. 6-Mercaptopurine (6-MP) is a metabolite of the immunosuppressive drug Azathioprine, which has recently been shown to enhance the transcriptional activity of NR4A nuclear receptors. The research described in this thesis therefore also focuses on effects of 6-MP in atherosclerosis and related vascular pathologies and the potential clinical application of this drug in vascular disease.

In Chapter 1, a brief introduction on atherosclerosis and related vascular pathologies is provided and the NR4A nuclear receptors are introduced. We describe knowledge that has thus far been obtained in the ‘research field’ of the NR4A nuclear receptors on the topic of their regulation of expression and transcriptional activity, and mechanisms by which the NR4A nuclear receptors modulate gene transcription. In addition, current insights on the NR4A nuclear receptors with respect to vascular disease are reported, and the aim of the research described in this thesis is formulated.

To identify a potential role of Nur77 in hepatic lipid metabolism a gain-of-function approach, involving adenovirus-mediated overexpression of Nur77 in liver of mice, was performed. The results of this study are described in Chapter 2. We report that Nur77 reduces hepatic triglyceride levels and modulates the plasma lipid distribution. Total plasma cholesterol and triglyceride levels remained the same but both low-density lipoprotein (LDL)-triglycerides as well as LDL-cholesterol were increased. Analysis of the expression levels of a selection of >25 key genes involved in hepatic lipid metabolism revealed that Nur77 potently inhibits expression of the transcription
factor sterol regulatory element binding protein (SREBP)-1c. In line with this observation, the SREBP-1c target genes fatty acid synthase, mitochondrial glycerol-3-phosphate acyltransferase, stearoyl-coA desaturase-1, and the LDL-receptor were shown to be downregulated, which is in agreement with the physiological changes observed in mice in response to hepatic expression of Nur77.

We subsequently report in Chapter 3 that the NR4A nuclear receptors are expressed in atherosclerotic lesion macrophages and demonstrate in cultured THP-1 derived macrophages, using lentiviral vectors, that the NR4A nuclear receptors inhibit the expression of a number of pro-inflammatory cytokines and chemokines upon activation with lipopolysaccharide (LPS) or tumor necrosis factor (TNF)-α, notably, interleukin (IL)-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, and MIP-1β. In addition, NR4A nuclear receptors were found to reduce oxidized-LDL uptake in macrophages, which may be explained by a decreased expression of the scavenger receptors CD-36 and SR-A in response to the NR4A nuclear receptors. Furthermore, we show that these nuclear receptors inhibit expression of CD11b, a gene representative for macrophage differentiation, suggesting that NR4A nuclear receptors modulate macrophage differentiation. In agreement with these data, silencing of Nur77 and NOR-1 using lentiviral short-hairpin RNA delivery resulted in increased expression of CD11b, SR-A and CD36, increased oxidized-LDL loading and augmented expression of cytokines.

We demonstrate in Chapter 4 that the three NR4A nuclear receptors are expressed in human in-stent restenosis lesions. Lentivirus-mediated overexpression of Nurr1 in human SMCs allowed us to study the function of this transcription factor and resulted in inhibition of SMC proliferation, consistent with upregulation of the cell-cycle inhibitor p27^Kip1. In line with these observations, silencing of Nurr1 in SMCs, using lentiviral short-hairpin RNA delivery, increased cell proliferation. In addition to modulation of SMC proliferation, we report that overexpression of Nurr1 inhibits expression of the cytokines IL-1β, tumor necrosis factor (TNF)-α, and MCP-1 in these cells, while silencing of Nurr1 increased expression of TNF-α. We demonstrate in the carotid wire injury mouse model, combined with local, lentiviral overexpression, that Nurr1 inhibits SMC-rich lesion formation in vivo, which is consistent with the anti-proliferative and anti-inflammatory properties of Nurr1 observed in cultured SMCs.

In Chapter 5, we challenged the hypothesis that enhancement of the activity of Nur77 with 6-MP is a promising approach to prevent (in-stent) restenosis. First, it was demonstrated that 6-MP indeed enhances the transcriptional activity of Nur77 in primary human SMC. Subsequently we report that 6-MP inhibits proliferation of SMCs, at least partially involving Nur77, as shown in SMCs in which Nur77 is
silenced by small interfering RNA. To study the effect of 6-MP on vascular lesion formation, we applied a mouse model in which a so called cuff is placed around the femoral artery. In this model, Nur77 mRNA expression is induced already 6 hours after injury up to 7 days. Most significantly, it was demonstrated that 6-MP, applied from a drug-eluting cuff, inhibits SMC-rich neointima formation. This inhibition of neointima formation was accompanied by a decreased number of proliferating cell nuclear antigen (PCNA)-positive cells, indicating that 6-MP inhibited SMC proliferation in the lesion area. To substantiate functional involvement of Nur77 in the beneficial effects of 6-MP, it was demonstrated that lesion formation in transgenic mice that overexpress Nur77 in SMCs is inhibited to a greater extent as compared to lesions in wild-type mice, while overexpression of a dominant-negative variant of Nur77 in arterial SMCs blocks the effect of 6-MP. These experiments demonstrate that the inhibitory effect of 6-MP on SMC-rich lesion formation depends on the presence of Nur77.

In Chapter 6, the anti-atherogenic effects of 6-MP on monocytes/macrophages are described. In THP-1 monocytes, 6-MP decreases intracellular purine levels and enhances caspase activity, resulting in monocyte apoptosis and decreased monocyte numbers. The induction of apoptosis by 6-MP is consistent with a strong suppression of 6-MP on the intrinsic anti-apoptotic factors Bcl-xL and Bcl-2. 6-MP also decreases expression of the adhesion integrins VLA-4 and platelet endothelial cell adhesion molecule-1 (PECAM-1) in monocytes, and reduces monocyte adhesion. In LPS-stimulated THP-1-derived macrophages, screening of the expression levels of a wide panel of atherosclerosis-related cytokines revealed that 6-MP robustly inhibits expression and secretion of MCP-1, while viability of these cells was only mildly affected by 6-MP. The effects of 6-MP on apoptosis and MCP-1 expression were shown to be independent of NR4A transcriptional activity, which is in contrast to our observations on SMCs. Significantly, local delivery of 6-MP to the vessel wall, using the drug-eluting mouse femoral artery cuff, inhibits atherosclerosis in ApoE*3-Leiden transgenic mice fed a Western diet. This inhibition of lesion formation was accompanied by decreased lesion macrophage content, which is consistent with our obtained results on cultured monocytes/macrophages.

In Chapter 7, the data presented in the previous chapters, and issues that were not appointed in the previous chapters, are discussed. We place our research in a broader, clinical perspective and propose directions for future research to gain better understanding of the NR4A nuclear receptors in vascular pathologies. Finally, we aim to define the potential clinical implications of this research supporting NR4A nuclear receptors as novel targets for intervention in vascular pathologies.