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SCOPE OF THE THESIS
Liver fibrosis is characterized by the accumulation of excessive amounts of scar tissue in response to chronic liver injury. Important causes of chronic liver injury are viral hepatitis, metabolic disorders such as Wilson’s disease, autoimmune diseases and chronic exposure to certain chemicals, alcohol or drugs. It is also becoming increasingly clear that obesity is a risk factor for steatohepatitis, finally leading to fibrosis. Although initially the synthesis of scar tissue in the injured liver serves as a repair mechanism to allow proper healing of the damaged tissue, liver fibrosis can be viewed upon as a derailed healing response, in which the deposition of scar tissue is disproportionate to the extent of tissue damage. In its advanced stages the disease is characterized by its self-perpetuating nature, reflected by the fact that fibrosis progresses even after withdrawal of the inciting stimulus. Eventually, fibrosis may develop into an irreversible condition referred to as liver cirrhosis, in which regenerative nodules of still functional liver parenchyma are encapsulated in fibrotic septa, resulting in complete liver failure and, ultimately, death. As outlined in chapter 2, for many patients no effective pharmacological treatment is available and to date a liver transplantation is the only curative option.

Extensive research on the pathophysiology of liver fibrosis has identified the hepatic stellate cell (HSC) as the key fibrogenic cell of the liver. HSC proliferation appears to be a crucial step in fibrogenesis, and attenuating the proliferation of this cell type therefore seems a relevant strategy to inhibit liver fibrosis pharmacologically. However, a serious problem of systemic treatment with antiproliferative drugs would be the unacceptable toxicity in organs other than the liver. Yet, even within the liver systemic treatment with antiproliferative drugs will undesirably affect other cell types. Inhibition of hepatocyte proliferation, for example, may result in an impaired regenerative capacity of the liver, while particularly this process is of great importance for the renewal of the liver parenchyma after massive hepatocyte injury. Because of these serious adverse effects, the use of cytostatics and other potent antiproliferative drugs has not been
examined. One possible approach to reduce the adverse effects is to increase the specificity of antiproliferative agents for activated HSC by cell-selective targeting. With the recent development of the first generation of HSC-selective drug carriers, this has now become an option.

The work described in this thesis focuses on the use of the HSC-selective drug carrier mannose-6-phosphate-modified human serum albumin (M6PHSA). This carrier has been designed to specifically bind to mannose-6-phosphate/insulin-like growth factor-II (M6P/IGF-II) receptors, which are upregulated on the cell surface of activated HSC.

In *chapter 3* the expression of the target receptor is investigated in bile duct-ligated (BDL) rats, since to date no data is available on the M6P/IGF-II receptor expression in this animal model for liver fibrosis. In addition, we evaluated whether the M6P/IGF-II receptor is expressed in fibrogenic cells in a rat model of hypertension-induced fibrotic vascular lesions. We also describe in this chapter how mycophenolic acid (MPA) can be coupled to M6PHSA in such a way that uptake via this receptor is achieved and a cell-selective pharmacologically active construct is obtained. MPA is a known immunosuppressive drug, but we pursued recent observations that suggest a direct antiproliferative effect of this drug on fibrogenic cells as well.

In *chapter 4* the antiproliferative effect of MPA and its mechanism of action are studied for the first time in HSC. Additionally, the pharmacological effects of MPA coupled to the HSC-selective drug carrier, were studied in these cells. *In vivo* experiments with this MPA-containing construct in BDL rats, examining the organ distribution, the intrahepatic distribution over the various liver cell types, as well as the *in vivo* effects on hepatic inflammatory and fibrosis parameters are also reported.

*Chapter 5, 6 and 7* focus on the experiments that we have performed with doxorubicin (DOX), a more potent antiproliferative drug than MPA. In *chapter 5*, experiments on the antifibrotic effects of untargeted doxorubicin are described.
These investigations were performed in order to provide evidence for the potential antifibrotic effect of this antiproliferative drug in experimental liver fibrosis in the rat. Additionally, we report in this chapter on the synthesis of a DOX-containing HSC-selective construct, its organ and cellular distribution pattern \textit{in vivo}, as well as its pharmacological effects on HSC \textit{in vitro}. \textbf{Chapter 6} focuses in more detail on the pharmacokinetics of the M6PHSA-DOX conjugate in fibrotic rats, whereas in \textbf{chapter 7} the effects of this drug targeting preparation on experimental liver fibrosis are investigated.

In summary, the aim of the work presented in this thesis is to investigate the application of two antiproliferative drugs for the treatment of liver fibrosis, with special emphasis on the targeted delivery of these drugs to the hepatic stellate cells. To our knowledge, it is the first study that explores the HSC-selective delivery of antiproliferative drugs as a strategy to treat this chronic disease.