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PHARMACOLOGICAL ANTAGONISM OF THE CHEMOKINE CCL2 (MCP-1) INHIBITS MACROPHAGE INFILTRATION INTO THE LIVER AND EXPERIMENTAL STEATOSIS *IN VIVO*

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Background and Aims: Recent experimental models of liver fibrosis highlighted the importance of liver-infiltrating macrophages for perpetuating hepatic inflammation by releasing proinflammatory cytokines and activating hepatic stellate cells (HSC). Monocyte/macrophage infiltration into the liver was found to be critically regulated via the chemokine receptor CCR2. In this study, we investigated the pharmacological inhibition of monocyte chemoattractant protein-1 (MCP-1, CCL2), the major CCR2 ligand, using a specific MCP-1 antagonist in two models of murine liver fibrosis in vivo.
Methods: Liver fibrosis was induced in C57BL/6 wild-type mice by intraperitoneal injections of carbon tetrachloride (CCl_4) twice weekly over 6 weeks or by feeding a methionine-choline-deficient diet (MCD) over 8 weeks. Mice received anti-MCP-1 (3×/week, s.c.) or a non-specific control drug during liver injury models. CCR2-deficient mice served as controls. The infiltration of immune cells into the liver was assessed by FACS and immunohistochemistry. The intrahepatic cytokine profile was analyzed by ELISA. Fibrosis and fatty degeneration were analyzed by histology (H&E, Sirius Red, oil red O staining), hydroxyproline content, intrahepatic trigylcerides and mRNA expression of fibrosis-related genes.
Results: Antagonizing MCP-1 inhibited murine monocyte chemotaxis in vitro (Boyden chambers). Upon CCl_4- or MCD-induced liver injury in vivo, the infiltration of macrophages into the liver was significantly decreased in anti-MCP-1 treated mice (by FACS and immunohistological staining; $p < 0.01$). In line with the lower level of intrahepatic macrophages, pro-inflammatory, anti-inflammatory and profibrogenic cytokines (TNFa, MCP-1, IFNg, IL-10, IL-6; all $p < 0.05$) and fibrosis related genes (MCP-1, TNFa, TGFb, alpha-sma; all $p < 0.01$) were significantly regulated. In MCD challenged mice a lower level of fatty liver degeneration was detected (oil red O staining, intrahepatic triglyceride measurement, $p < 0.05$) in anti-MCP-1-treated animals. However, overall fibrosis development was not significantly altered by anti-MCP-1 treatment.
Conclusion: These results demonstrate the successful pharmacological inhibition of hepatic monocyte/macrophage infiltration by blocking MCP-1 during liver damage in vivo. The associated decreased fatty degeneration suggests that inhibition of MCP-1 may be an interesting novel approach for pharmacological treatment in liver inflammation and steatohepatitis.

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ONCOSTATIN M STIMULATES DIRECTIONAL MIGRATION OF HUMAN HEPATIC PROFIBROGENIC CELLS

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Background and Aims: Myofibroblast like cells (MFs) can originate from activated hepatic stellate cells (HSC/MFs), portal fibroblasts and bone marrow-derived mesenchymal stem cells (MSCs). A critical pro-fibrogenic feature of MFs is represented by their ability to migrate towards the site of injury and to align with nascent and established fibrotic septa in response to several polypeptides, hypoxia or reactive oxygen species (ROS). Along these lines, oncostatin M (OSM), a cytokine expressed in cirrhotic liver and belonging to the interleukin-6 family, has been reported to orchestrate hypoxia-modulated processes in the liver (development, regeneration, angiogenesis) involving HIF-1 and to be potentially involved in the progression of chronic liver diseases (CLDs). In this study we investigated signaling mechanisms regulating migration of human HSC/MFs and human, MF-like and bone marrow-derived, MSCs in response to oncostatin M.
Methods: Signal transduction was evaluated by integrating cell and molecular biology techniques, whereas non-oriented migration and chemotaxis were assessed by wound healing assay and the modified Boyden's chambers assay, respectively. Morphological analysis was performed by immunohistochemistry (IHC) on liver specimens from HCV cirrhotic patients (Metavir F4).
Results: Exposure of pro-fibrogenic cell types to human recombinant OSM resulted in increased non-oriented migration and chemotaxis. Moreover, IHC revealed positive stain for OSM in hepatocytes of cirrhotic nodules, mainly in the proximity of fibrotic septa. The following features, common to both HSC/MFs and MSCs were demonstrated by employing specific experimental approaches:

a. early intracellular ROS generation and activation of Ras/Erk JNK1/2 as well as of STAT1 and STAT3 signaling pathways and involvement of hypoxia-inducible factor-1α (HIF-1α);
b. OSM-dependent migration, which was significantly inhibited by apocynin, indicating NADPH-oxidase as a major source of ROS;
c. OSM-dependent motogenic action that, similarly to hypoxia, appeared to be exerted in HSC/MFs and MSCs through a biphasic mechanism requiring early generation of ROS and late HIF1-dependent expression and release of VEGF.

Conclusions: OSM, which is expressed by hepatocytes in cirrhotic parenchyma, may contribute to fibrogenesis by stimulating directional migration of profibrogenic human hepatic MFs through a biphasic, redox- and HIF-1α/VEGF-dependent mechanism.

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THE ANTIFIBROTIC EFFECT OF SORAFENIB IS DUE TO DECREASED HSCS ACTIVATION MEDIATED BY ANGIOGENESIS BLOCKAGE

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Background: Liver cirrhosis represents the pathological response of the liver to all forms of chronic injuries and may lead to hepatocellular carcinoma. Recent evidence have shown that kinase inhibitors are able to interfere with liver cancer development and spreading by inhibiting angiogenesis. Angiogenesis is a key mechanism in both cancer and fibrosis development. Although sorafenib treatment has been shown to reduce liver fibrosis in vitro and in vivo, the role of this molecule in interfering with the mechanisms of fibrogenesis are still under investigation.
Aim: To investigate the role of sorafenib, a multikinase inhibitor, in a mouse model of liver fibrogenesis.
Methods: Wild-type mice were treated by i.p. injection of CCl_4 (0.2 ml/kg three times a week) for five weeks to induce fibrosis. Mice received orally either sorafenib (at the dose of 50 mg/kg of body-weight) or vehicle starting 1 week later than fibrosis-induction.
Results: Liver fibrosis induced by CCL_4 was significantly reduced by sorafenib administration. Morphometry after Sirius red staining showed reduced collagen deposition in CCL_4-treated mice exposed to sorafenib, in comparison to control mice treated with CCL_4 and