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Title of Ph.D Thesis: Rho Kinase Signaling in Animal Model of Portal Hypertension: Role of Hepatic Stellate and Endothelial Cells

Abstract

Portal hypertension (PHT) is a serious consequence of liver cirrhosis that results in life-threatening complications with elevated morbidity and mortality. Intrahepatic vascular resistance and hepatic blood flow contribute to the increased portal pressure (PP). This work focused on the hepatic vascular changes in PHT and cirrhosis and on correcting the abnormal hemodynamic associated with PHT and cirrhosis by targeting the RhoA/kinase pathway. The central objective was to investigate the vasoregulatory role of Rho kinase signaling in pathogenesis and treatment of PHT in animal models of liver diseases with sustained PHT. The specific objectives were (1) Investigation of pharmacological intervention in animal models of portal hypertension, (2) RhoA/Rho-kinase and NO cGMP pathway in animal models of portal hypertension, and (3) The cellular cross-talk of hepatic stellate and endothelial cells in liver diseases.

Hemodynamic and molecular effects of atorvastatin and metformin were investigated in endotoxin induced non-cirrhotic portal hypertensive (EIPH) rabbits and CCl4/BDL cirrhotic portal hypertensive rats respectively. Additionally, we tested whether metformin influences the hemodynamic response to non-selective beta-blockers. Role of Liver sinusoidal endothelial cells (LSEC), Hepatic stellate cells (HSC) and its contribution in pathogenesis of liver disease with PHT was investigated. The role and interaction of these cells with circulating endothelial progenitor cells (EPCs) in progression of liver fibrosis and pathological angiogenesis was also investigated. In this study, we also evaluated the effect of cirrhotic and control EPCs on hepatic angiogenesis and fibrosis *in vivo*.

One week chronic treatment with atorvastatin lowered PP significantly without affecting systemic hemodynamics in three months EIPH animals. These findings open new avenues to further investigate its effect in other animal models of liver diseases. Further, the potentially beneficial effects of statins on human liver disease should be evaluated more thoroughly. In cirrhosis, there

are significant observations which provide novel information about metformin administration to cirrhotic animals, which decreases PP, thus ameliorating PHT. Moreover, the metformin-derived improvement has a synergistic effect with beta-blockers reducing PP, suggesting a new therapeutic approach to treat cirrhotic patients with sustained PHT.

The non cirrhotic animal study data shows that chronic administration of Atorvastatin lowers the PP and this effect was mediated by reduction in dynamic component of liver via hepatic inhibition of Rho kinase signaling pathway. Inhibition of Rho kinase pathway activates the eNOS dependent enhanced intrahepatic nitric oxide production in an experimental model of EIPH. On the other hand, in case of cirrohotics, metformin significantly reduces PP in pre-clinical experimental rat models by decreasing intrahepatic vascular resistance (with a similar and comparable order of magnitude in both cirrhotic models). Reduction in intrahepatic vascular resistance is mediated by inhibition of RhoA/Rho-kinase signaling. Metformin also enhances the nitric oxide bioavailability via reduction in hepatic generation of reactive oxygen species which was achieved by enhanced activity of superoxide dismutase.

Our results clearly demonstrate that the activated phenotype of HSCs modulate the endothelial cell functionality and hepatic microenvironment. Activated phenotype of HSCs lowers the vascular endothelial growth factor stimulated nitric oxide production. Resident endothelial cells interacts with EPC and synergistically contribute in enhancing the intrahepatic angiogenesis in patients with liver fibrosis and cirrhosis. EPCs seems to contribute to the hepatic disease pathogenesis by stimulating resident liver LSECs via secreting paracrine factors. Also, EPCs contribute to in vivo angiogenesis in the liver and cirrhotic EPCs have enhanced angiogenic functions as compared to the healthy control EPCs. Our results also suggest that EPCs may have direct and/or indirect role in liver fibrosis, however may influence it indirectly by the process of angiogenesis. EPC may enhance directly, fibrosis through HSC secreted profibrogenic factors. Even EPC transplantation promotes angiogenesis in chronically damaged livers, however whether this enhanced angiogenesis will lead to fibrosis or regeneration possibly will depend on other microenvironmental factors associated with the type and extent of liver injury involved. A thorough analysis of these factors and clues may provide new leads and specific approaches to augment liver regeneration and in amelioration or regression of liver fibrosis in chronic injury and hence combat liver diseases.