6873
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Opposite effects of knocking out MT1 and MT2 melatonin receptor on senescence and fibrosis of cholangiocytes and hepatic stellate cells during cholestatic liver injury

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Senescent cholangiocytes (a key feature in primary sclerosing cholangitis, PSC and primary biliary cholangitis, PBC) contribute to liver fibrosis through activation of hepatic stellate cells (HSCs) by decreased HSCs senescence. Melatonin decreases biliary hyperplasia and liver fibrosis in bile duct ligated (BDL) rats. No data exists on the role of MT1 and MT2 melatonin receptors on biliary homeostasis, cellular senescence and liver fibrosis. Therefore, we hypothesized that there is a differential role of melatonin MT1 and MT2 on biliary proliferation, cellular senescence and liver fibrosis during BDL.

Methods: Studies were performed with Wild-type (WT), $MT1^{-/-}$ or $MT2^{-/-}$ mice with/without BDL for 1 wk. MT1 and MT2 levels were evaluated in liver sections by immunofluorescence (IF). Intrahepatic biliary mass (IBDM) and proliferation were evaluated by immunohistochemistry (IHC) for CK-19 in liver sections. SA-β-gal staining and qPCR for p16 and p21 were used to evaluate biliary senescence. Liver fibrosis was measured by Sirius red staining and qPCR for fibrosis genes (TGF-β1, α-SMA, fibronectin and collagen alpha 1) in total liver, cholangiocytes and Laser Capture Microdissection (LCM)-isolated HSCs. In vitro, human HSCs (hHSCs) were treated with biliary supernatants from all groups of mice before measuring the expression of fibrosis genes by qPCR.

Results: MT1 and MT2 in bile ducts was increased in BDL compared to WT mice. Biliary senescence, proliferation and liver fibrosis were increased in BDL compared to WT mice. Biliary senescence, proliferation and liver fibrosis were decreased in $MT1^{-/-}$ mice during BDL, but there was aggravated biliary senescence, proliferation and liver fibrosis in $MT2^{-/-}$ mice during BDL. In LCM-HSCs from BDL mice, fibrotic genes expression was decreased in $MT1^{-/-}$ but increased in $MT2^{-/-}$ mice. However, expression of senescent genes was increased in $MT1^{-/-}$ but decreased in $MT2^{-/-}$ mice in LCM-HSCs during BDL. In hHSCs treated with biliary supernatants from BDL WT mice, there was an expected increased expression of fibrotic genes compared to controls. Fibrotic gene expression was reduced in hHSCs treated with biliary supernatants from BDL $MT1^{-/-}$ mice and enhanced by biliary supernatants from BDL $MT2^{-/-}$ compared to hHSCs treated with supernatants from BDL WT mice. In contrast with fibrosis, senescence changes in the opposite trend simultaneously in hHSCs.

Conclusion: Biliary proliferation, cellular senescence and liver fibrosis are differentially regulated in MT1 and MT2 knockout mice. Specific targeting the MT1 receptor may be provide a key approach for the treatment of liver fibrosis during chronic liver diseases.

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