

## Melatonin inhibits cholangiocyte hyperplasia in cholestatic rats by downregulation of CLOCK genes

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*Background and aims.* In the experimental model of bile duct ligated (BDL) rats, the growth of large cholangiocytes is regulated by activation of cAMP-dependent signaling pathway 1-2. Melatonin, which is secreted from pineal gland as well as extrapineal tissues, regulates cell proliferation by interacting with its receptors (MT1 and MT2) via the modulation of cAMP levels and the expression of the CLOCK genes 3. We studied the mechanism by which melatonin regulates the growth of the biliary epithelium.

*Methods.* Normal and BDL rats were treated in vivo with melatonin for 7 days before evaluating: (i) intrahepatic bile duct mass (IBDM) in liver sections by immunohistochemistry for cytokeratin-19 (CK-19), a specific marker of cholangiocytes; and (ii) expression of MT1, MT2 and CLOCK genes by immunohistochemistry and real time PCR. In vitro, large cholangiocytes were stimulated with melatonin in the absence/presence of luzindole (a MT1/MT2 antagonist) and 4-P-PDOT (a specific MT2 antagonist) before assessing cellular growth by MTS proliferation assay, cAMP levels by a RIA kit and ERK1/2 phosphorylation by western blots.

*Results.* Cholangiocytes express MT1, MT2 and CLOCK genes that were all upregulated following BDL. Administration of melatonin to BDL rats decreased IBDM and the expression of all CLOCK genes. In cells, melatonin decreased the proliferation, cAMP levels and the activation of ERK2, decreases that were blocked by luzindole.

*Conclusion.* Supporting other studies we found that melatonin-inhibition of biliary hyperplasia is associated with downregulation of CLOCK gene expression. These findings have important clinical implications since melatonin may be an important therapeutic tool for managing cholangiocyte hyperproliferation in biliary disorders.

Keywords: cAMP pathway, cholestasis, gastrointestinal hormones