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# Commonly Used H1 and H2 Histamine Receptor (HR) Blockers Decrease Cholangiocarcinoma Xenograft Tumor Growth, Angiogenesis and EMT

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Background: Cholangiocarcinoma (CCA) is a fatal liver cancer with limited treatment options. We have shown that: (i) mast cells (MCs) infiltrate human CCA tumors via stem cell factor (SCF)/c-kit interaction; and (ii) blocking MC-derived histamine (HA) decreases CCA tumor growth. Chronic treatment with H1 or H2 histamine receptor (HR) antagonists decreases MC number, biliary damage and fibrosis in $Mdr2^{-/-}$ mice. The aim of this study was to examine the effects of chronic treatment with H1 or H2 HR antagonists on CCA. Methods: Nu/nu mice were implanted with Mz-Cha-1 CCA cells into the hind flanks. After tumor development, mice were treated with saline, mepyramine (H1HR antagonist, 10mg/kg/BW) or rantidine (H2HR antagonist, 10 mg/kg/BW) by IP injection for 35 days. Tumor growth was measured 3x/week and tumors were evaluated for the following: (i) proliferation by qPCR and immunoblotting for PCNA; (ii) MC presence by toluidine blue staining and qPCR for chymase, tryptase and c-kit; (iii) angiogenesis by immunofluorescence for vonWillebrand factor and qPCR for VEGF-A; and (iv) EMT by immunohistochemistry for vimentin and e-cadherin and qPCR for s100A4. Cholangiocyte SCF expression was measured by immunofluorescence (co-stained with the biliary marker, CK-19). HA and tryptase serum levels were detected by EIA. Human tissue arrays were used to evaluate MC presence and H1HR and H2HR expression by immunohistochemistry. In vitro, CCA lines were treated with saline, mepyramine (25 mM) or rantidine (25 mM) before evaluating proliferation by MTS assay, and angiogenesis and EMT by qPCR. To demonstrate the effects of MC on CCA cells, MCs were treated with saline, mepyramine or rantidine for up to 96 hours and conditioned medium was collected. CCA cell lines were subsequently treated with the collected MC conditioned medium prior to evaluating CCA proliferation, angiogenesis and EMT by qPCR. Results: Chronic treatment with H1HR or H2HR antagonists decreased tumor growth, proliferation, serum histamine and trypatse levels, angiogenesis and EMT compared to saline. Saline-treated tumors showed increased biliary SCF expression, which was reduced in H1HR and H2HR treated mice. MC presence, H1HR and H2HR expression were increased in human CCA tissue arrays. In vitro CCA cells had decreased proliferation, angiogenesis and EMT gene expression following treatment with H1HR or H2HR antagonists. Further, CCA cells treated with supernatants from MCs treated with H1HR or H2HR antagonists had decreased proliferation, angiogenesis and EMT compared to basal treated CCA. Conclusion: Inhibition of H1HR or H2HR decreases CCA tumor growth, angiogenesis and EMT. Commonly used over-the-counter HR inhibitors may be potential therapeutics for the treatment of CCA.