World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/2150-5330office
wjgp@wjgnet.com
doi:10.4291/wjgp.v1.i2.38

World J Gastrointest Pathophysiol 2010 June 15; 1(2): 38-49
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

Sharon DeMorrow, PhD, *Series Editor*

Histamine regulation of hyperplastic and neoplastic cell growth in cholangiocytes

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Supported partly by Dr. Nicholas C Hightower Centennial Chair of Gastroenterology from Scott and White, the VA Research Scholar Award, a VA Merit Award and the NIH Grants DK58411, and DK76898 to Dr. Alpini, and by University funds to Dr. Onori and PRIN 2007 and Federate Athenaeum Funds from University of Rome "La Sapienza" to Professor Gaudio
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Telephone: +1-254-7427055
Received: February 9, 2010 Revised: April 3, 2010
Accepted: April 10, 2010
Published online: June 15, 2010

Abstract

Histamine has long been known to be involved in inflammatory events. The discovery of antihistamines dates back to the first half of the 20th century when a Swiss-Italian pharmacologist, Daniel Bovet began his work. In 1957 he was awarded a Nobel Prize for his production of antihistamines for allergy relief. Since that time, histamine has been found to play a role in other events besides allergic reaction. Possibly unbelievable to Bovet and his peers, histamine has now been marked as playing a role in liver pathologies including hepatobiliary diseases.

Key words: Histamine; Receptors; Cholangiocytes; Cholestasis; Cholangiocarcinoma

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Onori P, Gaudio E, Franchitto A, Alpini G, Francis H. Histamine regulation of hyperplastic and neoplastic cell growth in cholangiocytes. *World J Gastrointest Pathophysiol* 2010; 1(2): 38-49 Available from: URL: http://www.wjgnet.com/2150-5330/full/v1/i2/38.htm DOI: http://dx.doi.org/10.4291/wjgp.v1.i2.38

INTRODUCTION

The liver is a dynamic organ that is able to repair and regenerate itself after injury. Numerous resident liver cell types are involved in maintaining a homeostatic state of the liver as well as playing a role during liver injury and disease. Cholangiocytes play a critical role in the regulation and overall function of the liver. These cells are hormone-responsive and much effort has been put

forth to delineate the mechanisms behind cholangiocyte function in liver disease. An emerging role for histamine and liver diseases has begun to be investigated.

In this review we will begin with a brief overview of the intrahepatic biliary tree, cholangiocyte function, hepatic vasculature, cholangiocarcinoma and histamine. After, we will discuss the latest findings regarding histaminergic activation in the liver as well as the most recent findings in regards to cholangiocyte regulation by histamine and histamine receptors. Finally, we will conclude with a section on histamine and cholangiocarcinoma and our speculation for the future of histamine in the progression of liver diseases.

INTRAHEPATIC BILIARY TREE

The biliary tree is the common term used for the pathway where bile is secreted from the liver that begins with initiation of bile from the hepatocytes[1]. It starts with numerous small branches that terminate in the common bile duct, also known as the trunk of the biliary tree[2]. It is here that the common duct comes together with branches of the hepatic artery and portal vein forming the central axis of the portal triad[2]. Lining the intrahepatic biliary tree are epithelial cells known as cholangiocytes[1,3,4]. Once defined as "simple" epithelia, cholangiocytes are now regarded as key players in liver pathophysiology[3-7]. Cholangiocytes are responsible for the modification and release of bile from the liver and the transport of bile acids[6,8-12]. These cells are hormone-responsive, and express a multitude of hormone receptor binding sites that enable them to interact with mediators to induce varying effects on liver pathology[1,6,13-18]. These cells, that are cuboidal by nature, line the three-dimensional network of interconnecting ducts[1]. Cholangiocytes are a heterogeneous population of cells[1-4,16,17,19,20] that are derived from small intrahepatic bile ducts (lined by small cholangiocytes)[3,20,21] and large intrahepatic bile ducts (lined by large cholangiocytes)[3,20,21].

Cholangiocyte proliferation

Heterogeneous cholangiocytes have been identified as the target cells for a number of liver diseases (cholangiopathies) that include primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)[1]. In these diseases there is a destruction of the bile ducts that leads to hyperplastic transformation, termed "typical" or "atypical" proliferation[7,14,22]. "Typical" cholangiocyte proliferation is characterized by an increase in cholangiocyte proliferation that is limited to the portal area, whereas "atypical"[23] is seen in patients with prolonged cholangiopathies (i.e. PBC and PSC) and is noted by irregular proliferation of cholangiocytes (with a well structured lumen) that extend into periportal and parenchymal regions with occasional anastomosing cords with adjacent hepatocytes[7,14,22]. "Oval" cell proliferation is a pre-malignant stage that is seen in early carcinogenesis and characterized by the presence of a disorganized tubular structure without any well-defined lumen that can transform into a full-blown neoplastic condition known as cholangiocarcinoma (CCA)[7,14,22,24].

Models of proliferation

Several animal models of "typical" proliferation have rapidly become an excellent tool for the dissection of the mechanisms behind this event. Bile duct ligation (BDL) is commonly used to mimic "typical" proliferation[19,25-27]. Ligation of the bile duct induces a build-up of bile and increased biliary pressure[8,28] that induce an increase in cholangiocyte proliferation (mostly of large cholangiocytes)[19] and resembles biliary hyperplasia seen in patients with diseases like PBC[7,19,29]. Other models used to mimic "typical" proliferation include 70% partial hepatectomy (PH), acute carbon tetrachloride (CCl_4) treatment and chronic feeding of α-naphthylisothiocyanate (ANIT) or bile salts[7,12,14,16,17,30]. All of these models are found to be associated with an upregulation of secretin receptor (SR) gene expression and secretin-stimulated cAMP levels and ductal secretory activity[7,13,14,16,17,30,31]. Chronic treatment of normal rats with forskolin has also been shown to increase biliary hyperplasia (similar to BDL) and secretin-induced responses in cAMP levels as well as downstream signaling components[32]. While BDL targets mainly the large cholangiocytes[3,29], after CCl_4 treatment, only small cholangiocytes respond and de novo proliferate[16,17]. This occurs with a parallel loss of large ductal mass[16,17]. After 70% PH, small and large cholangiocytes proliferate during liver regeneration[14,24]. These tools are useful in evaluating the mechanisms of cholangiocyte proliferation during liver cholestasis and disease progression.

CHOLANGIOCYTE FUNCTION

Using the tools described above has enabled researchers to uncover important information regarding cholangiocyte function in relation to secretion, proliferation and apoptosis during liver disease[1,4,6,7,13,18].

Secretory function

In BDL rats, there is increased cholangiocyte proliferation coupled with increased bile flow and bicarbonate secretion after stimulation with secretin[3,8,13,19,25,27,33]. This is an excellent tool to use to evaluate functionality of cholangiocytes in normal and diseased states as well as in response to stimulation by numerous factors. Over the years it has been shown that, in addition to secretin, vasoactive intestinal peptide increases bicarbonate secretion, whereas the hormones somatostatin, insulin and gastrin all decrease bile flow and bicarbonate secretion[33-36]. Other factors have been shown to inhibit the secretin-induced increased cholangiocyte response. These include the alpha 2 adrenergic receptor agonist UK,14304[37] and the phenolic compound, caffeic phenethyl ester (CAPE)[38]. The majority of these responses occur through a cAMP-dependent pathway, however other studies have shown

that Ca^{2+} can also play a role in cholangiocyte secretion. Le Sage, *et al*[15] have demonstrated that the alpha-1 adrenergic receptor agonist, phenylephrine, increases secretin-stimulated choleresis through activation of Ca^{2+}/protein kinase c (PKC) mechanisms, whereas the hormone gastrin was found to decrease secretin-stimulated ductal secretion in BDL rats *via* increased expression of Ca^{2+}-dependent PKC isoforms[27].

Proliferative and apoptotic functions

Cholangiocyte proliferation and apoptosis is affected by many hormones and peptides[13]. Recently, Mancinelli *et al*[31] have shown that the sex hormone, follicle-stimulating hormone (FSH) increases cholangiocyte proliferation through cAMP/PKA-dependent phosphorylation of extracellular signal-regulated kinase (ERK1/2) and Elk-1, a member of the ETS oncogene family. The forkhead box proteins A1 and A1 (Foxa1 and Foxa2) have been descried as "terminators" of bile duct expansion by their ability to inhibit interleukin-6 (IL-6) expression[39]. In BDL Foxl1 (-/-) knockout mice it was found that loss of the winged helix transcription factor Foxl1 induced a decrease in cholangiocyte proliferation and loss of bile ductular mass by activation of the canonical Wnt/beta-catenin pathway, suggesting that this transcription factor plays a critical role in cholangiocyte proliferation during cholestasis[40]. After treating BDL rats with CAPE, Mancinelli *et al*[38] demonstrated a large decrease in cholangiocyte proliferation coupled with increased cholangiocyte apoptosis that was reversed after chronic feeding with the bile acid taurocholic acid (TC). TC feeding also led to recuperation in the expression of VEGF proteins and receptors (R2 and R3), implicating bile acids and angiogenic factors in the course of cholangiocyte cholestasis[38]. The endocannabinoid, anandamide (AEA) was found to inhibit cholangiocyte proliferation and increase apoptosis through activation of thioredoxin 1/redox factor 1 and activator protein-1 (AP-1), demonstrating the potential role for endocannabinoids in cholestasis[41]. Genetic knockdown of the transcription factors c-Fos and c-Jun ablated the AEA-induced cholangiocyte death[41]. Tumor necrosis factor-alpha (TNF-α) has been shown to induce cholangiocyte apoptosis and decrease proliferation when coupled with a single injection of actinomycin D[42]. Chronic feeding with TC has been found to reverse this ductopenic effect *via* a phosphatidylinositol-3-kinase (PI3K)-dependent pathway[43]. These studies have provided strong evidence that cholangiocyte function is susceptible to inhibition and stimulation by numerous factors.

Hepatic vasculature

It is pertinent to discuss hepatic vasculature within this article as the microanatomy (including cholangiocytes) of the liver is significantly influenced by blood flow. There is a constant flow of large amounts of blood to and from the liver that is controlled through two separate

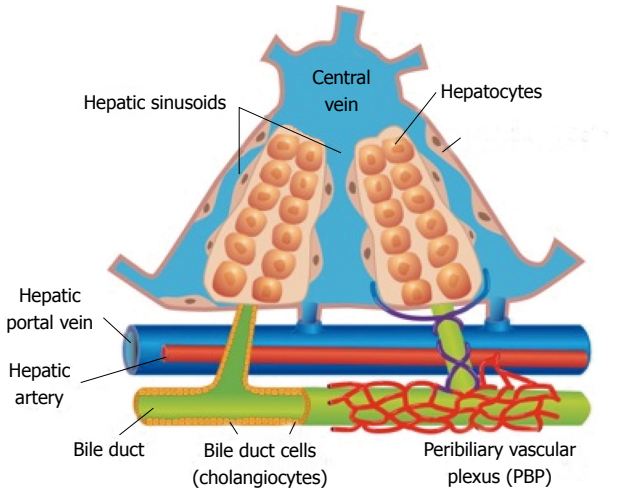

Figure 1 Graphic image of blood flow through the liver and the key cells involved.

blood supplies: the portal vein (PV) and the hepatic artery (HA)[44,45]. There are conflicting views regarding HA distribution and where this artery terminates in the liver parenchyma[46-48]. Recently, a detailed study using scanning electron microscopy of vascular corrosion casts demonstrated that the terminal HA branches do not end directly in the sinusoidal beds[49,50]. The terminal HA give rise to capillaries that form the peribiliary plexus, periportal plexus and single capillaries of the portal space; this implies that only venous blood at a lowered pressure reaches the sinusoids and vena portal branches[49,50].

Structurally, the sinusoids are vascular channels lined with fenestrated endothelial cells (sinusoidal endothelial cells or SEC[51]). It is this fenestrated structure that makes the sinusoids very "leaky", allowing fluid passage to occur[52]. Hepatocytes are found around the sinusoids and are responsible for the initiation, formation and secretion of bile[53]. After secretion of bile by the hepatocytes, bile is modified by cholangiocytes[53]. The hepatic artery is the main supplier of blood to the biliary tract and cholangiocytes within the structure termed peribiliary vascular plexus (PBP)[26,50]. This system has been found to be a source rich in vascular growth factors and other vasoactive substances[50]. Recent work has shown that the PBP has a great influence on cholangiocyte proliferation and/or loss that is mediated by VEGF expression[26]. The bile acid TC has also been shown to influence cholangiocyte growth/loss by regulating VEGF[38]. Please refer to Figure 1 for a graphic representation and diagram of the microcirculation in the liver with regards to blood and bile flow.

Effects of blood flow on liver microanatomy

Significant findings have shown that blood flow in the liver directly influences its microanatomy and how it responds to stress and/or disease. In rats with BDL-induced cholestasis, interruption of the hepatic artery blood supply *via* hepatic artery ligation (HAL) causes a

significant loss of the PBP and increases cholangiocyte apoptosis[26]. Ischemic bile duct injury can occur in association with hepatic artery thrombosis during liver transplantation and can induce the development of biliary casts, bile duct death or chronic disease that mimics PSC[54].

CHOLANGIOCARCINOMA

Description of progression and treatment options

Cholangiocyte hyperplasia that becomes uncontrollable or differentiates into a further neoplastic state can lead to cholangiocarcinoma. Cholangiocarcinoma is defined as a tumor from either intrahepatic or extrahepatic origin[55]. Prominent risk factors of CCA are chronic inflammation, congenital abnormalities of the biliary tree and genetic predisposition[56]. Tumors of CCA progress in a slow and undramatic manner with biliary sepsis, malnutrition and liver failure being typical causes of death associated with this disease[57]. CCA is the second most common type of cancer in the liver after hepatocellular carcinoma and the number of incidences of this disease is on the rise worldwide[55]. Early discovery of cholangiocarcinoma is difficult, resulting in limited treatment options. Long-term survival is only reachable by complete surgical resection of the tumor, which is not feasible for some patients and can be highly unsuccessful. Conventional chemotherapy and radiation have not proven to be successful at prolonging survival rates[55,57]. Photodynamic therapy has recently appeared as a possible treatment option to relieve pain and increase survival, however further studies are needed[56]. A most recent review of the latest advances in CCA diagnosis, treatment and patient care can be found in the reference by Aljiffry, *et al*[56]. Given the current lack of satisfactory treatments, understanding the cellular mechanisms behind the development of CCA will be critical in the development of future curative therapies.

Recent findings

Research to investigate CCA development and mechanism of action has increased in the last decade. A glimpse into the literature reveals thousands of entries including both reviews and original articles. Highlights of some of the latest findings from 2009 are shown here. Most recently, Blechacz *et al*[58] have shown that Sorafenib, an approved treatment for primary renal cancer, inhibits CCA growth in vitro and *in vivo* by sensitizing tumor cells to apoptosis. Blocking the production and secretion of dopamine, over-produced in CCA, has also been shown to decrease CCA growth in both cultured cells and an *in vivo* model of CCA[59]. In liver fluke-associated CCA, inhibition of galectin-3 was found to stimulate apoptosis that was increased by 10 fold by treatment with cisplatin or 5-fluorouracil[60]. Using CCA cell lines, Okada *et al*[61] demonstrated that rapamycin decreased cell proliferation that was synergistic with treatment with gemcitabine. Suppression of the nuclear factor kappa beta (NF-κB) pathway by treatment with CAPE was found to decrease cholangiocarcinoma growth both in culture and *in vivo* by increasing apoptosis[62]. Endothelin (ET-1) has been shown in both *in vitro* and *in vivo* models to decrease CCA growth by inhibiting VEGF and its receptors[63]. Coupled with decreased angiogenic factors, ET-1 also increased apoptosis and collagen tissue deposition[63]. Tamoxifen, the estrogen receptor antagonist, is showing promise as a possible therapeutic agent in CCA through calmodulin targets AKT [protein kinase B (PKB)] and c-FLIP (cellular-FLICE inhibitory protein)[64]. These studies are just the pinnacle of the multitude of studies that have been performed to investigate potential therapies for this devastating tumor.

HISTAMINE

In 1910 the British scientist, Sir Henry Dale identified histamine as a substance that is released and acts as a "mediator" during an allergic responses. Today we know that histamine is involved in many bodily processes in addition to allergic reaction.

Histamine activation and function

This biogenic amine interacts with four G-protein coupled receptors (GPCRs), H1HR, H2HR, H3HR and H4HR[65-68] that exert their actions on various G-proteins[69]. It has been shown that stimulation of the H1 histamine receptor (HR) activates Gαq, inducing a Ca^{2+}-dependent effect in various cell systems[66,70,71]. In contrast, the H2HR appears to signal mainly through Gαs, stimulating a cAMP- dependent action in a variety of cell types[65,71,72]. Coupling of H3 and H4 HR has been linked to Gαi, inducing a negative regulation of cAMP-dependent signaling[65,73] as well as Gαo, mediating cellular regulation through a phospholipase C/Ca^{2+}-dependent pathway[25,73]. Histamine receptors are also able to induce both inhibitory and stimulatory effects in different cell types. In Leydig cells, the H1HR induces an inhibitory effect, whereas the H2HR has a stimulatory effect on steroidogenesis[74]. In cholangiocytes and cholangiopathies, it has been shown that the H1HR stimulates growth, whereas the H3HR inhibits hyperplastic proliferation[65,66]. Histamine and the histamine receptors have been shown to induce a multitude of effects on various cellular pathologies including the H3HR in Alzheimer's Disease[75]; histamine and the H1HR in vascular disease[76]; the H4HR in treatment of chronic pruritus disease[77] and many more. There is not yet a proven direct link between histamine and cancer, however growing evidence suggests that histamine and the histamine receptors may be involved in tumor growth and/or depletion[25,78]. Further, dysregulation of the enzymes that are responsible for histamine synthesis, histidine decarboxylase (HDC) and monoamine oxidase-B (MAO-B), have been implicated in certain cancers[79]. The interaction between cells and the four histamine receptors also complicates our understanding of the regulation of liver function in both normal and diseased (including cancer) states.

Histamine and liver disease

Histamine and the histamine receptors play a role in numerous processes including disease progression in the liver. Histamine, *via* H2HR activation, has been shown to play a protective role in alcohol-induced liver injury by lowering liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] and decreasing inflammation and necrosis in ethanol treated rats[80]. In patients with cirrhotic-related hepatic encephalopathy, evaluation of brain tissue showed an upregulation of histamine and H1 receptors and decreased H3 receptor density, highlighting the importance of histamine regulation in liver pathologies[81,82]. An H2 receptor antagonist, rantidine[83] and an H3/H4 antagonist, thioperamide[84] have both been shown to suppress ischemic reperfusion liver injury[83-85]. Stimulation with dimaprit, an H2 and H4 agonist showed similar results by inducing a protective effect against ischemic reperfusion injury by decreasing cytokine release[85]. The density of H2 histamine receptors is decreased in hepatic tissues from cirrhotic patients with portal hypertension[86]. Furthermore, in H1HR and H2HR knockout mice it was found that the protective effects of histamine on lipopolysaccharide (LPS)-induced liver injury was partially or completely blocked, suggesting that histamine and its receptors have a protective role in endotoxin-induced hepatic injury[87]. It has also been found that histamine levels in plasma from patients with both PSC and PBC are increased compared to healthy controls[88]. Histamine, *via* the H2HR, has been shown to protect against fulminant hepatitis[89].

Histamine and liver cancer

There have been numerous studies involving histamine and histamine receptors in liver cancers. In a study using hepatocellular carcinoma cell lines, histamine stimulation was found to induce a differential effect by decreasing the growth of one line while increasing the growth of another[90]. Histamine-induced effects were attenuated by inhibition of either H1 or H2 HRs[90]. The H2HR antagonist, cimetidine is a common drug used for treatment of gastric ulcers and has also been shown to reduce liver metastasis *via* activation of selectins in liver sinusoids[91]. Both gastrin and histamine H2 receptor antagonists may play a synergistic role in helicobacter-induced gastric cancer[92].

HISTAMINE AND THE BILIARY TREE

The above studies have alluded to the role of histamine in a broad range of liver pathologies from hepatitis to hepatocellular carcinoma. Here we will reflect on the recent studies involving histamine and the histamine receptors in specific cholangiocyte pathologies ranging from cholestasis to cholangiocarcinoma.

Biliary hyperplasia and histamine

To date, only a handful of articles have addressed the direct role of histamine in biliary diseases. Though limited, these studies have provided evidence that there is a strong link between histamine and its receptors in the regulation of cholangiocyte function. Using a BDL-induced model of cholestasis, we have shown that chronic treatment of BDL rats with an H3HR agonist, (R)-(α)-(-)-methylhistamine dihydrobromide (RAMH), results in a significant decrease in biliary hyperplasia[65]. Numerous techniques revealed that liver tissue and RNA from both normal rats and BDL rats express all four of the histamine receptors[65]. Figure 2 depicts expression of the H3 receptor in proliferating cholangiocytes (induced by BDL) by immunohistochemical analysis. This finding is in accordance with other studies showing that cholangiocytes express a wide array of hormone receptors[13]. Biliary hyperplasia was reduced *in vivo* and also *in vitro* in freshly isolated cholangiocytes stimulated with RAMH *via* cAMP/PKA/ERK/Elk1 signaling. By downregulating this signaling pathway, the H3HR is able to decrease and manage biliary hyperplasia that could be a useful therapeutic tool in cholestatic liver diseases like PBC where cholangiocyte proliferation is prevalent.

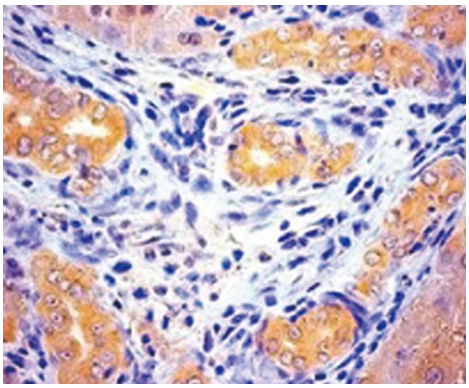

Figure 2 Immunohistochemistry for the H3 histamine receptor in proliferating cholangiocytes (induced by bile duct ligation). Original magnification × 40.

In contrast, the H1HR agonist, HTMT dimaleate, increases the growth of small murine mouse cholangiocytes, but not large[66]. This study has great implication on the effects of histamine and histamine receptors in the heterogeneous population of cholangiocytes where cholangiopathies are likely to occur. These findings show that the H1HR elicits its effects by activation of Gαq, mobilization of calcium and increased intracellular inositol triphosphate receptor (IP_3) levels. This was coupled with increased (1) phosphorylation of calmodulin-dependent protein kinase I (CaMKI) and ERK and (2) activation of the transcription factor CREB (cAMP response element binding protein). It was further shown that knockdown of CaMKI resulted in the loss of CREB activation and H1HR-induced increased proliferation in small cholangiocytes[66]. The importance of calcium-dependent signaling in cholangiocyte function and regulation is demonstrated by this study and is in accordance with other studies[93-95]. Taken together, these studies implicate histamine and the histamine receptors in cholangiocyte regulation.

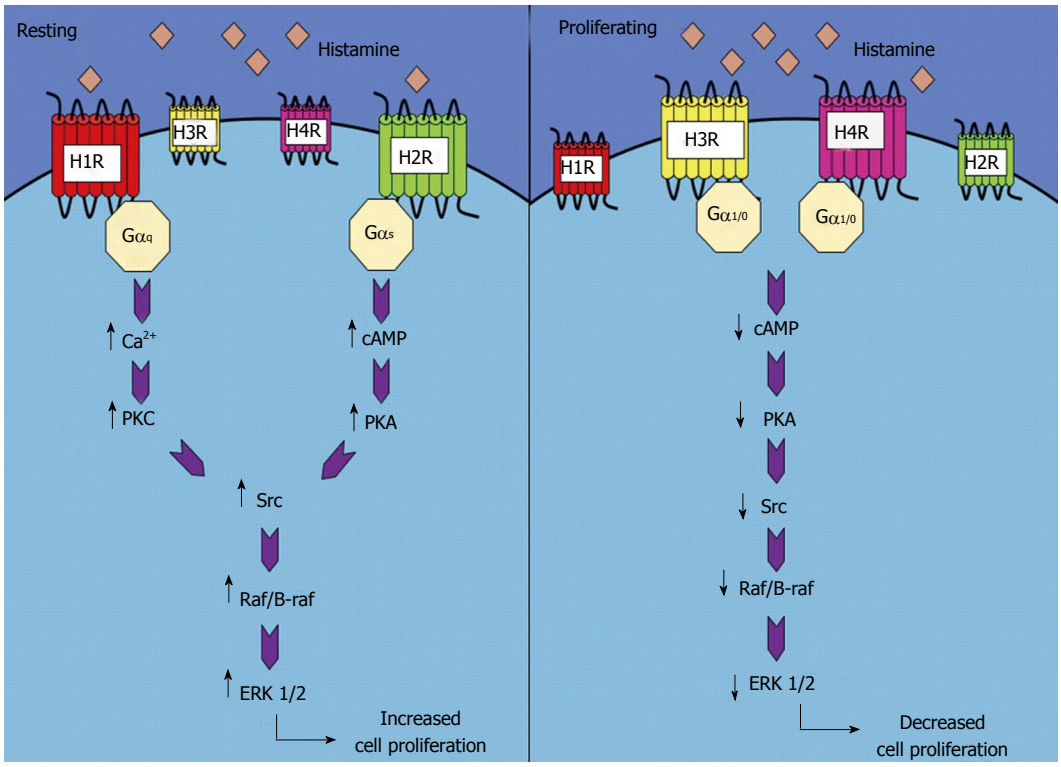

Figure 3 Differential signaling of histamine receptors in resting and proliferating cholangiocytes. Reprinted with permission from Demorrow S, Francis H, Alpini G, Biogenic Amine Actions on Cholangiocyte Function. *Exp Biol Med (Maywood)* 2007; **232**: 1005-1013.

Other recent studies have demonstrated that treatment with histamine; the H1HR agonist or the H2HR agonist (but not the H3HR agonist) stimulates the proliferation of cholangiocytes in normal rats[96]. Preliminary data suggests that there is a differential effect induced by the H1 and H2 HRs where the H1HR appears to signal predominantly through calcium-dependent mechanisms, whereas the H2HR is more prone to work through a cAMP/PKA-dependent signaling pathway[96]. Histamine is able to utilize both signaling pathways, but may have a preference for the H1HR-mediated path[96]. Figure 3 illustrates the differential signaling induced by histamine and histamine receptors in both normal and proliferating cholangiocytes[5]. It appears that histamine receptors may also mediate the VEGF-regulated cholangiocyte response during liver regeneration after 70% partial hepatectomy[96]. After 3 d post PH, treatment with the H1HR agonist, HTMT, induced a 50x fold increase in cholangiocyte proliferation that was coupled with increased VEGF expression[96]. This study has huge potential to lend a therapeutic "hand" to physicians treating patients after liver transplantation by aiding in the acceleration of liver regeneration and healing. Overall, it is clearly apparent that this biogenic amine and its receptors play numerous, critical roles in liver cholangiopathies and regeneration. Further investigations of these events are currently underway.

Biliary neoplasia and histamine

Little is currently known about the possible interaction between cholangiocarcinoma and histamine. Following our study with the cholestatic rat model and the H3HR agonist, RAMH, we evaluated the role and mechanisms of action by which RAMH regulates CCA growth[25]. Using numerous CCA cell lines and normal cholangiocytes, it was found that all four of the histamine receptors were expressed with an upregulation of H3HR expression compared to normal tissue. Treatment with RAMH induced a decrease in growth in all CCA cell lines, but had no effect in normal cholangiocytes. Interestingly, in this study we demonstrated that RAMH was potentially signaling *via* Gαo by inducing an increase in intracellular IP_3 levels, but having no effect on cAMP signaling, thus ruling out any interaction with this pathway. Inhibitors for $[Ca^{2+}]_i$, PKC and PLC all blocked the RAMH-induced inhibitory action in CCA cells. Because PKC signaling has previously been shown to be important in cholangiocarcinoma[11,97,98], the role of this protein was investigated further. RAMH induced phosphorylation of PKC alpha (PKCα) and also caused translocation of PKCα from the cytosolic region into the membrane of CCA cells. Knockdown of PKCα ablated the suppressive effects induced by RAMH as well as reversing ERK phosphorylation. In final studies, *in vivo* examination was performed in a nude mouse model[59,62,63], implanted with CCA cells and treated with RAMH over the course of several weeks. Tumor growth was significantly inhibited by RAMH treatment compared to tumor growth from NaCl-treated mice. This was coupled with decreased

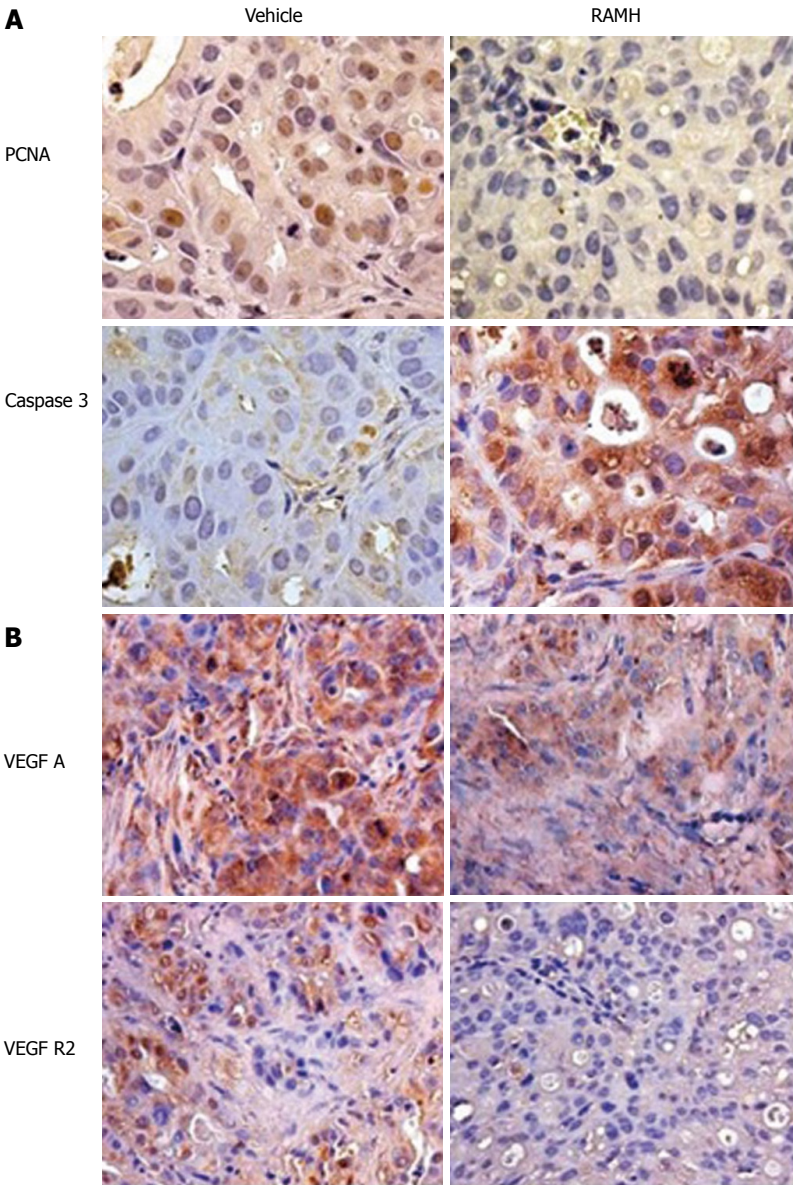

Figure 4 Immunohistochemistry images of tumors excised from vehicle- and RAMH-treated mice (× 40). A: Proliferating cholangiocytes (PCNA) and apoptotic cholangiocytes (Caspase 3). RAMH induced a decrease in PCNA-positive cholangiocytes coupled with an increase in apoptotic cholangiocytes compared to vehicle-treated mice; B: VEGF-A and VEGF-R2. RAMH treatment caused a decrease in the expression of both VEGF-A and VEGF-R2.

proliferation and increased apoptosis. Figure 4A shows a representative image for immunohistochemical staining for both PCNA and caspase 3. Total PKCα expression was also increased in RAMH-treated tumors while VEGF and its receptors were decreased as seen by immunohistochemistry images provided in Figure 4B[25]. This is an important finding related to the regulation of PKCα in cholangiocarcinoma growth and also the ability of histamine to mediate VEGF expression, that plays a critical role in tumor development[63,99-102]. In work that is currently underway, we have found a role for histamine secretion and for other histamine receptor agonists and antagonists. Like other cancers, we have found that HDC and MAO-B are dysregulated in CCA and that CCA cell lines secrete an increased amount of histamine compared to normal cholangiocytes[103]. Further investigation has shown that acute histamine treatment has no significant impact on CCA growth, but that long-term stimulation (up to 2 wk) can increase growth that is coupled with increased VEGF gene expression[103]. *In vivo* treatment with histamine in our nude mouse model also increased tumor growth that was blocked by treatment with an HDC inhibitor[103]. These mechanisms are still being worked out, but it is clear that histamine has a trophic effect in CCA growth. We have also found that the histamine receptors are upregulated in CCA cell lines and have differential effects with regards to changes in proliferation[103]. As stated above, the H3HR agonist decreases CCA growth. However, we have seen that blocking the H2HR with cimetidine has no effect in either the CCA cell lines or *in vivo* (unpublished observations, Alpini and Francis). Stimulation with an H1HR antagonist also decreases CCA growth and increases apoptosis *in vitro* (unpublished observations, Alpini and Francis, 2007). Most recently we have found that clobenpropit, a highly potent H4HR agonist and selective H3HR antagonist can decrease CCA growth by integrin-mediated events including decreasing the invasive capacity of CCA cells[104]. These findings are complex and intricately woven together. Please refer to

Table 1 Effects of histamine and histamine receptors on cholangiocarcinomagrowth

	Expression level	Agonist effect	Antagonist effect	*In vitro*/*in vivo* model	Known or potential signaling mechanism
Histidine decarboxylase (HDC)	↑↑		↓↓ CCA growth	Both	Inhibits histamine synthesis
Monoamine oxidase-B (MAOB)	↓↓	Unknown	Unknown	*In vitro*	Unknown
Histamine	↑↑ secretion by CCA	↑↑ CCA growth	↓↓ CCA growth	Both	↑↓ VEGF expression
H1HR	↑↑	↑ CCA growth	↓↓ CCA growth	*In vitro*	Ca^{2+}-mediated signaling
H2HR	↑↑	↔	↔	Both	Unknown
H3HR	↑↑	↓↓ CCA growth		Both	PKCa-dependent signaling
H4HR	↑↑	↓↓ CCA growth		*In vitro*	Integrin-dependent decreased invasion

CCA: Cholangiocarcinoma; HDC: Histidine decarboxylase; MAOB: Monoamine oxidase-B; HR: Histamine receptor; VEGF: Vascular endothelial growth factor.

Table 1 for a summary of the effects of histamine and histamine receptors in CCA regulation.

FUTURE PERSPECTIVES

While the studies reviewed above are relevant and important, it is also critical to remember that there are numerous other factors that may be involved in histamine regulation of liver disease. *In vivo*, the role of mast cells must be addressed as information regarding the interplay between hepatic mast cells and liver pathologies is increasing. The main source for histamine release is mast cells[105-107] and therefore these cells are likely to play some role in histamine regulation of cholangiocyte function. Bile acids have also been shown to play a major role in cholangiocyte proliferation, secretion and function[9,12,108-113]. We know that bile acids are also able to act on mast cells and can influence the type and amount of a mediator (like histamine) that is being released[114-117]. Not all bile acids induce the same effect. Lipophilic bile acids have been shown to activate mast cells and induce a large amount of histamine release, whereas ursodeoxycholate has almost no effect on histamine secretion from mast cells[118]. Bile acids are currently used as a treatment option for patients with etiologies ranging from gastro-esophageal reflux disease (GERD) to cholestatic liver diseases[119,120]. Histamine receptor agonists and antagonists are also used to treat various digestive diseases. The H2 antagonists, cimetidine and rantidine have both shown promise in treating GERD[121]. Because of the involvement of mast cells in irritable bowel syndrome (IBS), blocking mast cell interaction through either second generation antihistamines or proteinase-activated receptor antagonists could relieve pain in suffering patients[122]. How bile acids interact with hepatic mast cells and possibly cholangiocytes is unknown. However, understanding the interconnecting relationship between *bile acids* ← *mast cells* → *cholangiocytes* could allow us to take our current understanding of histamine-mediated regulation of cholangiocyte function to a new level and thereby allow us to turn translational science into bedside practice.

CONCLUSION

Taking the evidence discussed in this review we can glimpse into the future and see that histamine and histamine receptor regulation can have a major impact on liver diseases. However this information is only useful if we are able to dissect the mechanisms of histaminergic action that occur during disease progression.

ACKNOWLEDGEMENTS

We gratefully acknowledge Bryan Moss, Graphic Services Department S&W Hospital, for his rendering of Figure 1.

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