Canals of Hering: Recent Insights and Current Knowledge

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ABSTRACT

The canals of Hering (CoH) begin in the lobules, are lined partially by cholangiocytes and partly by hepatocytes, and conduct bile from bile canaliculi to terminal bile ducts in portal tracts. They are not readily apparent on routine histological staining but are highlighted by the biliary cytokeratins CK19 and CK7. There is on average 1 CoH per 10 μm of bile duct length. The canals represent the true hepatocytic-biliary interface that thus lies within the lobule and not at the limiting plate. The CoH are destroyed early in primary biliary cirrhosis, perhaps explaining lobular "hepatitis" in this disease. They may also be the primary sites of scarring in methotrexate toxicity. Most intriguingly, the CoH have been speculated to harbor intraorgan stem cells of the liver, perhaps forming the hepatic stem cell "niche" and have been demonstrated to proliferate in disease states.

KEYWORDS: Canals of Hering, stem cells, primary biliary cirrhosis, methotrexate

Objectives: On completion of this article, the reader should be able to summarize (1) the microscopic anatomy of the canals of Hering, (2) the role of the canals of Hering in pathological processes, and (3) the role of the canals of Hering in liver proliferation.
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Bile flow begins at the apical membrane of hepatocytes, apposing apical membranes of adjacent hepatocytes forming bile canaliculi that are, therefore, 1- to 2-μm wide tissue spaces lined by microvilli between these hepatocytes. Bile canaliculi drain into a larger channel that acquires a partial lining of cholangiocytes, the other half being lined by hepatocytes. This structure, lined partially by cholangiocytes and partly by hepatocytes was described in 1866 by Ewald Hering and bears his name.[1] Like the bile canaliculi, CoH cannot be easily visualized on routine light microscopy; both structures are, however, seen distinctly with the electron microscope. Because the CoH have a partial lining of cholangiocytes, they can be highlighted on immunohistochemical staining by biliary type cytokeratins, i.e., CK7 and CK19 (Fig. 1).

Recognized as bonafide anatomic structures for more than a century, the CoH have nevertheless not been the subject of systematic study. This may be related largely to the fact that they are not readily visible on routine sections and therefore are not easily amenable for study. Oddly enough, historical documents on Hering

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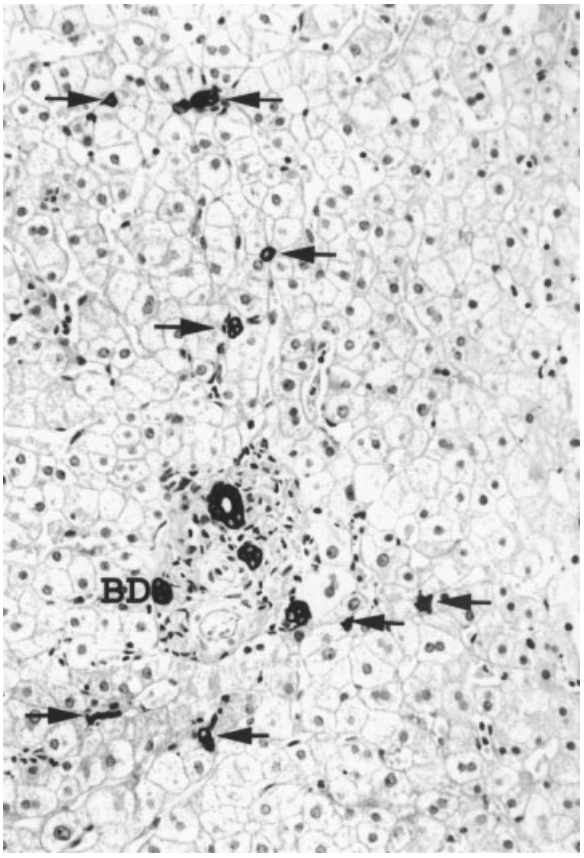

Figure 1 Immunohistochemical staining with CK19 highlights CoH (arrows), which are identified as either single cells or small groups of cells within the hepatic lobule. BD denotes a bile ductule that lies at the periphery of the portal tract and is lined circumferentially by cholangiocytes. (Reproduced with permission from Saxena et al.[14])

himself show a similar lack of enthusiasm about his contribution to the microscopic anatomy of the human liver. The Dictionary of Scientific Biography published under the auspices of the American Council of Learned Societies outlines Karl Ewald Konstantin Hering's theory of visual perception, a treatise that defied the color vision theory of a great scientist of the time, Hermann von Helmholtz. There is ample reference to "Hering's waves" or rhythmical variations in blood pressure; to the vagal "Hering-Breuer" respiratory reflexes; and to "Hering's nerve," which carries signals from the baroreceptors in the carotid sinus to the glossopharyngeal nerve. No space is wasted, however, on the little canals in the liver that bear his name.[2] On the same note, Hering's bibliography in historical texts is an impressive list of works in these aforementioned fields.[3] This is not entirely surprising because Hering spent a large part of his professional career as a physiologist and anatomist studying the physiology of vision. It is therefore a quirk of fate that Hering's theory of visual perception that brought him recognition in his lifetime is hardly mentioned today, challenged by sophisticated modern methods of electrophysiological and biochemical investigation.[2] His name, on the other hand, lives on in current works on hepatic pathology as his namesake biliary channels come under intense investigation and scrutiny leading to insights into their anatomic characteristics, their role in physiological processes, and their responses to pathological states. Hering's publication on liver microanatomy, however, consisted of work on numerous animals—snakes, tree frog, rabbit, and salamander, to name a few—but does not mention the human liver. This article focuses on three aspects of CoH that have been the best elucidated: microscopic anatomy of the CoH, their involvement in pathologic processes, and the proliferative activity associated with these structures.

MICROSCOPIC ANATOMY

Small ovoid cells with scant cytoplasm, lying singly, in small clusters, or in short chains, have long been observed in the hepatic lobule.[4,5] These cells stain for a variety of markers, including markers of biliary differentiation and markers of oval stem progenitor cells and have been thought to represent stem cells of the liver, cells of the CoH, or cells of the ductal plate.[5] That these cells did indeed represent the CoH was demonstrated by us in a three-dimensional reconstruction of serial sections of normal liver tissue stained for the biliary cytokeratin, CK19. Stacking of these serial sections revealed that these seemingly isolated cells within the lobules linked up to form continuous linear arrays of cells that connected hepatocytes within the lobule to bile ducts within the portal tracts. Rarely, these formed complete circular arrays of cells, but by and large they just formed linear arrays, which led us to believe that the CoH have a trough-like structure that conducts bile from the biliary canaliculi in the lobules to the portal tract (Fig. 2).[6,7] Indeed, channels lined partly by hepatocytes and partly by cholangiocytes have been demonstrated by electron microscopy in the hepatic lobule,[8] and intravital microscopy has shown that multiple bile canaliculi converge into a single CoH.[9] Our study demonstrated conclusively that the CoH extend into the hepatic lobule, thus dispelling some confusion in the literature whether these structures extended beyond the vicinity of portal tracts.

CoH are not randomly arranged within the lobule; they have a definite periodic distribution within the lobule. In our study, we found 6 to 15 CK19-positive profiles per 100 μm of bile duct length with a mean of 10.8 ± 3.0 and a median of 10; in other words, there is 1 CoH per 10 μm of bile duct length.[6] This is in agreement with Matsumoto's reconstruction of the primary and secondary liver lobules in which he shows frequent entry points of CoH into the bile ducts.[10] CoH bear close anatomic relationship to inlet venules that arise from terminal portal venules and, together with an inlet venule, form the smallest functional subunit of the liver, the hepatic microcirculatory subunit (HMS).

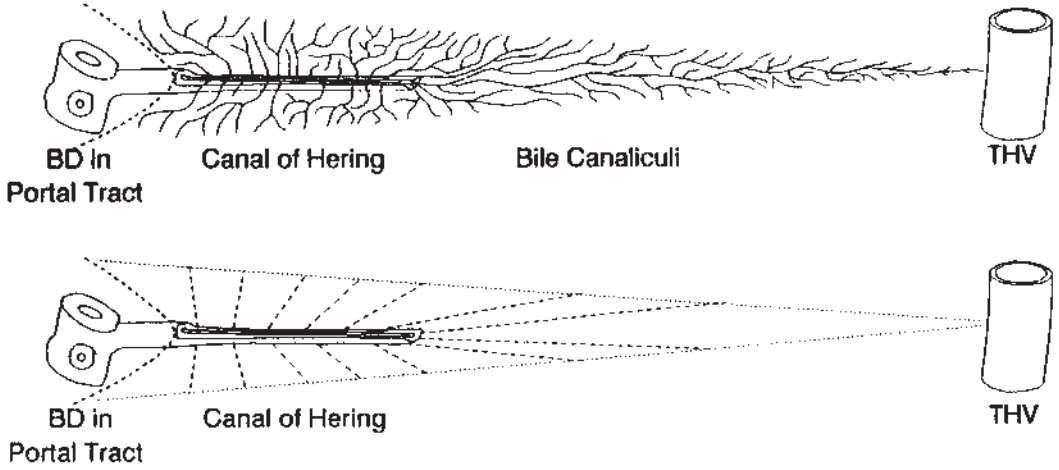

Figure 2 Upper panel shows a schematic of CoH forming troughlike structures that arise within the lobule and drain bile from the bile canaliculi into the terminal bile ductule. The lower panel shows that CoH are not arranged randomly but are organized to drain a leaflike area of parenchyma, one CoH entering every 10 μ of bile duct length. (Reproduced with permission from Saxena et al.[7])

Transmission electron microscopy has shown that CoH wind tightly around the inlet venule.[11] Using double injection of colored media, Ekataksin, demonstrated that the pair consisting of inlet venule and CoH defines a conical territory of sinusoids and hepatocytes with its base toward the portal tract that is fed with a single inlet venule and drained by the corresponding CoH. The CoH, in this schema of strictly streamlined blood flow and bile drainage through the HMS is responsible for collecting and conducting bile to the portal tracts. In vivo studies show accumulation of blood-borne substances like fluorescein isothiocyanate (FITC)–dextran and sodium fluorescein by hepatocytes followed by secretion of the dye into bile canaliculi and CoH, a sort of "countercurrent" mechanism in the flow of blood and bile.[9,11] In vivo microscopy in the rat demonstrates that CoH possess contractile activity so that bile moves unidirectionally toward the portal tract.[12]

The relationship, if any, of CoH to the terminal branches of the hepatic arterial branches is not known. Ekataksin revealed a twig of the hepatic arterial system only occasionally in association with the inlet venule; this is in keeping with the belief that the hepatic artery is a stromal artery that plays a very minor role, if any, in perfusion of the hepatic lobule.[11] There are insufficient data documenting the exact anatomic relationship of nerves to CoH. However, there are abundant indirect data suggesting both neuroendocrine features of CoH and neural influences on these structures,[13] which are most evident in states of regeneration and will be discussed later in this article.

ROLE OF COH IN PATHOLOGICAL PROCESSES

As the smallest channels containing cholangiocytes, the CoH represent the true hepatocytic-biliary interface of the liver that thus lies within the lobule and not at the limiting plate or edge of the portal tract. Furthermore, CoH represent the smallest, most proximal tributary of the biliary tree containing cholangiocytes. It would then not be illogical to assume that CoH are involved in diseases of small bile ducts. We tested this hypothesis in primary biliary cirrhosis (PBC), an autoimmune disease that affects small bile ducts while sparing those of medium and large size.[14] We found that CoH, just like small ducts, express human leukocyte antigen (HLA)-DR in PBC. Furthermore, like small bile ducts, CoH are destroyed in PBC as evidenced by a reduction in number of CoH at all stages of the disease (Fig. 3). The presence of a small cholangiocyte-lined channel in the lobule that expresses HLA-DR in PBC helps to explain lobular inflammation, and indeed lobular granulomas in PBC. We found that in biopsies of PBC, lobular inflammation was associated with CK19-positive CoH profiles in 60% of cases and that 75% of CoH in biopsies with PBC are associated with an inflammatory infiltrate.[15] These studies suggest that CoH play an active part in liver diseases, acting to a good extent like small bile ducts, sharing with them antigens and pathologic reactions.

CoH may be also subject to effects of drugs and medications. The utility of methotrexate as a therapeutic agent in the treatment of chronic diseases such as psoriasis and rheumatoid arthritis is marred by its effects on the liver. Hepatocytic damage is evidenced as anisonucleosis, multinucleation, and steatosis on light microscopy and as mitochondrial and lysosomal alterations on ultrastructural examination. The therapy-limiting factor, however, is the development of fibrosis, which is seen as thin fibrous septa extending out from portal tracts. In a study using concurrent staining for collagen (trichrome stain) and immunohistochemical staining for CK19, we found that the number of CoH was reduced in patients

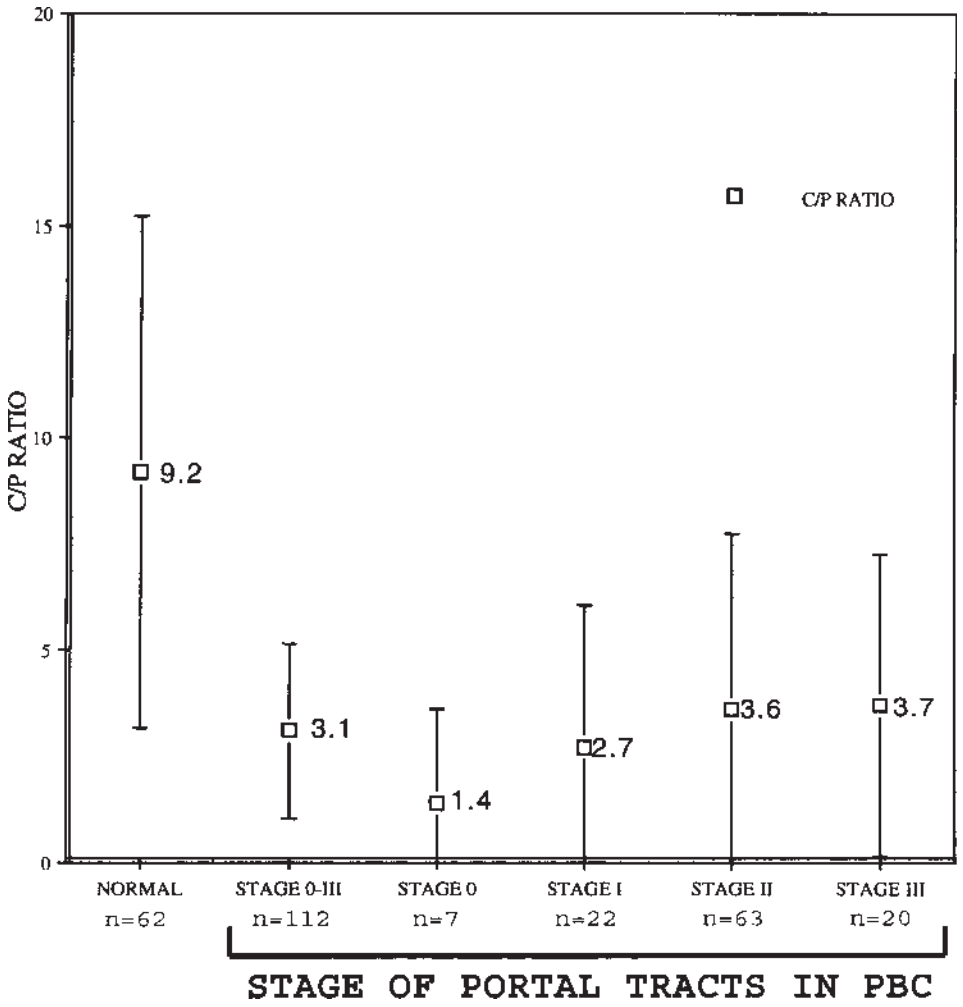

Figure 3 Graph showing a reduction in the number of CoH at all stages of PBC as defined by the ratio of number of CoH to number of portal tracts (c/p ratio). In this study, each portal tract was assigned an individual stage. The difference in the c/p ratio between normal controls and patients with PBC was statistically significant ($p < 0.0001$). The c/p ratio increases slightly in stages II and III, perhaps reflecting a compensatory proliferation of CoH. (Reproduced with permission from Saxena et al.[14])

who had received methotrexate. Furthermore, the fibrous septa seemed to align themselves along the tracks of CoH.[16]

ROLE OF COH IN LIVER PROLIFERATION AND REGENERATION

It has been speculated by some that if stem cells do exist in the human liver, they might do so in the CoH. Because CoH form the biliary-hepatocytic interface, it makes biological sense that any progenitor cell with the potential for biphenotypic differentiation should be located at this interface. Stem cells in the most widely used animal model of regeneration, the rat, have an oval shape and are called "oval" cells. By analogy, the human liver has been extensively investigated for oval cells that may represent the stem cells of the human liver. Such cells have been described in normal and diseased liver, and, although morphologically somewhat different from the oval cells, they do comprise hepatobiliary cells with dual phenotypes of hepatocytic and cholangiocytic antigens, much as do oval cells in rodents. Various publications have focused on varying aspects of these cells, and a considerable body of literature exists documenting this dual phenotype as well as the presence of stem cell markers on these cells.[17–20] The frequent proliferation of these intermediate hepatobiliary cells, expressing biliary or stem cell markers, or both, lends credence to the concept that progenitor cells in the human liver reside in the CoH.

In our study of massive hepatic necrosis due to paracetamol toxicity, we found that there is massive proliferation of CK19-positive hepatobiliary cells in the periportal region that is recognized on the hematoxylin-eosin stain as a ductular reaction. On three-dimensional reconstruction, these cells link up to form large, complex arborizing structures that consist of single linear arrays in the lobule and branching tubes and cords at the portal end. Furthermore, there is approximately one structure per 10 μm of bile duct length, conforming to our belief

that there is 1 CoH per 10 μm of bile duct length. In this study, we showed that CoH, like bile ducts, expressed c-kit, the receptor for stem cell factor in normal and diseased liver.[6]

Similarly, in our study of the destruction of CoH in PBC, we found that, although there was an overall decrease in the number of CoH at all stages in PBC, there were more CoH in stage II PBC than there were in stage I PBC, and the number decreased again in stage III. This indicates a proliferative response, perhaps compensatory to destruction, in stage II PBC, before succumbing to continued immune attack as the disease progresses to stage III (Fig. 3). CoH proliferation in stage II is concurrent with ductular proliferation, the histological hallmark of this stage of the disease, also considered to be a compensatory attempt to restore bile flow.[14]

The small hepatobiliary cells in the liver demonstrate positivity for numerous neuroendocrine markers such as parathyroid hormone–related peptide, chromogranin A, neural cell adhesion molecule, neurotropin 4/5, and the neurotrophin receptor tyrosine kinase B.[18,21–23] Expression of these markers is especially pronounced in states of activation and proliferation under conditions of stress. In addition, these cells express receptors for neurotransmitters, suggesting an active neural control of these cells. It has been shown in rat models of galactosamine-induced hepatic necrosis followed by hepatic vagotomy that there was a decrease in the number of hepatic progenitor cells when compared with galactosamine-intoxicated rats that underwent a sham operation. In the normal human liver, hepatic progenitor cells along with cells lining the small and large bile ducts contain the muscarinic receptor M3. M3 is also present in intermediate hepatobiliary cells in diseased livers. The receptor is not present on heptocytes or sinusoidal cells.[13] It is also known that hepatocytes produce and secrete cholinesterase and that acetylcholinesterase is present on cell membranes of hepatocytes. It has been postulated that in normal livers and in partial hepatectomy, CoH are surrounded by normal numbers of hepatocytes that secrete cholinesterase and inhibit the binding of acetylcholine to the muscarinic receptor, thus inhibiting proliferation of CoH. However, when there is massive loss of hepatocytes in the innervated liver, this inhibitory effect of hepatocytes is lost, leading to a ductular reaction by binding of acetylcholine secreted by the vagus nerve to the M3 receptor. In the denervated liver, this proliferation does not occur because of a lack of acetylcholine.[13,24] It has further been demonstrated that inhibition of the sympathetic nervous system allows accumulation of hepatic progenitor cells in diseased liver.[25]

The previous findings together with the anatomic structure of CoH may help explain the two distinct types of proliferative responses of the liver to injury and cell loss. The liver may regenerate entirely by division and proliferation of mature, committed hepatocytes, or proliferation of oval or progenitor cells may contribute to repair. The latter is evident when metabolites or toxins block the division of hepatocytes. It appears that when injury involves the inner third of the lobule, the response is predominantly one of proliferation of mature hepatocytes. However, when injury reaches the biliary-hepatocytic interface at the CoH, the second response is triggered; CoH acting as a trip wire mechanism to elicit a proliferation of hepatic progenitor cells.[6,13]

It must be conceded, however, that the literature is confusing as to the relationship between cholangiocytes of CoH and oval/progenitor cells. The same small, ovoid cell is variously referred to as a stem/progenitor cell or CoH, depending on the focus of the study. The finding of small morphologically "oval" cells in the liver that stain both with markers of oval cells and biliary cells and proliferate in disease states nevertheless lends credence to the proliferative capacity of cells of, or within, the CoH. Whether these cells represent true stem cells of the liver, a complicated story in itself, or whether the cholangiocytes of the CoH represent facultative progenitor cells is open to question.

ABBREVIATIONS

CK	cytokeratin
CoH	canals of Hering
HMS	hepatic microcirculatory subunit
FITC	fluorescein isothiocyanate
PBC	primary biliary cirrhosis

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