Sinusoidal endothelial hyperplasia in pathogenesis of perilobular fibrosis in rabbit after experimental ligation of common bile duct


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Development of perilobular hepatic fibrosis in rabbits after experimental ligation of the common bile duct was studied by microscopy. Twelve hours after the ligation, the lobular boundaries assumed prominence by appearance of row(s) of congested sinusoids around the distended perilobular canals of Hering. Seven days later, the lobular laminae limitans around such canals of Hering revealed myxomatous ballooning degeneration forming well defined inter-lobular bands (ILBs). On day 15 post-ligation, the residual sinusoidal endothelial-lining cells in the ILBs manifested hyper chromasia and occasional mitotic activity. It was followed, on days 25 and 35, by proliferation (hyperplasia) of the sinusoidal endothelium cells forming the portal tract like fibrotic tissue in the perilobular bands. These findings, showed the course of interlobular bridging necrosis along the bile ductules at the lobular circumferences and subsequently indigenous origin of the fibrotic tissue in the ILBs from the residual cells in the affected parenchyma independent of the portal tracts.

Several studies have documented perilobular fibrosis after ligation of the common bile duct (CBD) in various experimental animals. Histologic features in the rat model were comparable to those of human biliary cirrhosis. The cell components in the newly forming interlobular bands (ILB) were reported to have a close resemblance to the hepatic sinusoidal endothelial cells. Early morphological changes in the cells after trauma manifest by 12 h. Granulation tissue or fibrosis occur between 7 and 15 days and 25-35 days is a suitable period for development of appreciable cirrhosis like perilobular fibrosis in the rabbit. We attempted to study the subsequent sequence of events in the process of perilobular fibrosis and its relation to the sinusoidal endothelium during 12 h to 35 days after CBD ligation in rabbit.
Material & Methods

Thirty healthy male rabbits (weighing 1-1.5 kg) were divided into 6 equal groups—5 test and one control. In the test groups, common bile duct was explored under nembutal (30 mg/kg body weight) and ligated close to its duodenal end after excluding anomalies of liver and gall bladder. All the animals were fed ad libitum on green leafy vegetables and tap water. The first experimental group was sacrificed 12 h after the ligation. Remaining four test groups were killed on days 7, 15, 25 and 35 post-ligation, respectively. Two animals which died in the post-operative period were excluded and replaced by new ones. Liver(s) were removed from both control and experimental animals after clamping the hepatic vein, portal vessels and common bile duct. The tissue was fixed in 10 per cent formal-saline after making superficial nicks in the Glisson’s capsule. Multiple pieces in various planes from the different lobes were processed to obtain paraffin sections stained with haematoxylin and eosin. Other stains, methylene blue, von Gieson and trichrome were used wherever required. Morbid anatomy of the inter/peri lobular cell components, hepatic laminae and sinusoidal endothelium were studied under light microscope with a greater emphasis on the appearance of cells and their anatomical relationship. The control group was studied (one animal each on 12 h, and days 7, 15, 25 and 35) for normal structure of the rabbit liver.

Results

In group I (12 h after ligation), the liver parenchyma architecture was well preserved. Interlobular areas showed dilated and congested sinusoids, occasionally in a band along the lobular circumference. These interlobular sinusoids were arranged in a few rows around a circumferentially placed bile ductule or canal of Hering. The single layer endothelium of such sinusoids were seen directly resting on the external surface of the single layer hepatocytic wall of the bile ductule and the inter-sinusoidal laminae joined the ductular wall, between the sinusoids, with no fibrous tissue elements in between. Occasionally, terminal branches of the portal veins were found to be entering into the inter-lobular parenchyma, communicating with the interlobular sinusoids.

The wall of the gall bladder showed oedema, leukocytic (neutrophils) infiltration and vascular congestion.

In group II (7th day after CBD ligation), the parenchyma around the bile ductules (canals of Hering) exhibited myxomatous ballooning (oedematous) degeneration of the hepatocytes in the laminae, obliterating the sinusoids and forming inter-lobular bands (ILB) of connective tissue appearance. The fibrillar cell density in these ILB was very low and uniform and their nuclei were elongated, stout and curved in contrast to the portal fibrous tissue cells. There was no mitotic activity and the nuclei of the fibrillar and those of the ballooned degenerating hepatocytes were vesicular. The canals of Hering, escaped the degeneration and were seen intact in the myxomatous tissue (Fig. 1). Their lining epithelia comprised distinctly of hepatocytes showing vesicular nuclei and indistinct mitotic activity confirming that these channels were not newly formed.

Findings in gall bladder were the same as those found after 12 h of ligation, except for the leukocytic infiltration which was very marked.
Hepatic fibrosis after CBD ligation

Fig. 1 (a and b). Well preserved canals of Hering (†) in an inter-lobular band (ILB) of myxomatous tissue, with balloononed, degenerating hepatocytes and residual sinusoids(S). H and E × 400. Fig. 2. Markedly increased fibrillar cell density in the ILB, clearly visualised residual sinusoids and attenuated hepatic laminae 25 days after CBD ligation. H and E × 200. Fig. 3. Fibrillar cells in the stroma of the ILB are identical to those lining the sinusoids (S). Proliferative activity (hyperplasia) of these cells is marked on 25th day after CBD ligation. H and E × 400. Fig. 4. ILBs on the 35th post ligation day. The sinusoids on the periphery are widely opened but the laminae showed continuing prominence/hyperplasia of sinusoidal lining and atrophy of hepatocytes. H and E × 100.
In group III (15th day after CBD ligation) the nuclei of the fibrillar cells in the ILB showed hyperchromasia similar to those of the endothelial cells in the lining of the occasional residual sinusoids in these bands. Mitotic activity was insignificant. The hepatocytes disappeared from the central parts of the ILBs, but occasional degenerating ones could be found along the margins.

The gall bladder showed excessive leukocytic infiltration which was mainly of eosinophils.

In group IV (25th day after CBD ligation), the oedema was markedly decreased and the fibrillar cell component of the ILB showed proliferation (Fig 2). The outlines of cellular components were well defined, though the density of the fibrillar cells in the ILBs was markedly increased. Many residual sinusoids, isolated degenerating hepatocytes and bits of laminae were clearly defined in the tissue of the ILB. The cells in the fibrillar stroma of the ILB distinctly resembled the endothelial cells of the sinusoids. Hepatic laminae of the margins of the ILBs showed progressive hyperplasia and proliferation of the sinusoidal endothelial lining cells with concomitant atrophy of the hepatocytes (Fig. 3). The sinusoids, as a result, were gradually narrowed, collapsed and in most cases obliterated, thus adding to the dimensions of the ILBs.

Scattered lymphocytes and plasma cells were marked features in the wall of the gall bladder.

In group V (35th day after ligation), in addition to the earlier findings, the deeper parts of the proximal segments in the elderly ILBs, manifested maturation of the tissue by alignment of the fibres and hyalinization like that of the portal tissue, but had several residual patent sinusoids in it. Cell activity in hepatic laminae at the margins and in the distal parts of the ILBs was still progressive (Fig. 4). In some of the lobules, there was massive hyperplasia of the sinusoidal endothelium and concomitant atrophy of the hepatocytes, rapidly progressive inside from periphery of the lobule, severely adding to the connective tissue of the ILB. This resulted in small areas of residual healthy parenchyma in the centre surrounded by extensive ILB. Evidences of gradual endothelial cell prominence and hyperplasia were well marked in the former parenchyma also.

Mononuclear cell infiltration accompanied by fibrosis in the subepithelial and subserosal region, were seen in the gall bladder.

Discussion

In previous studies on various animals, inter-lobular fibrosis has been a constant finding, in the form of non-invasive fibrosis, invasive fibrosis, general intra-and extra-lobular mesenchymal hyperplasia, spread of portal tissue in interlobular and intra-lobular regions, and bridging fibrosis. However, there is no unanimous view as to the timings of recognisable fibrosis after CBD ligation.

In the present study, we found no inter-lobular fibrosis at 12 h after ligation of the CBD in rabbit. The congestion of inter-lobular sinusoids seen by us at 12h may suggest an obstruction of the portal circulation close to the junction of the interlobular with the intralobular sinusoids. This could be due to disruption of the bile canaliculi, at their junctions with the bile ductules obliterating the adjoining sinusoidal spaces by the hepatocytes due to the mechanical and bile toxic injuries. The sinusoidal congestion also explained the congestive dilatation of the portal vessels and occasional
thrombosis of their branches after CBD ligation reported by earlier workers. However, the anoxia of tissue due to portal thrombosis or sinusoidal congestion may not be sufficient to cause degenerative necrosis of the interlobular parenchyma (seen after 7 days) because of the widespread anastomosis between the lobular sinusoids. However, the anaemia of sinusoidal congestion could add to the effects of bile toxicity leading to myxomatous degeneration of the hepatic laminae obliterating the sinusoids as was observed in the present study after 7 days.

The ILB tissue formed 7 days after CBD ligation was thus comparable to the avascular connective tissue in the interlobular areas in cat described by Stewart and Liebar. Although some workers have reported fibrosis directly following CBD ligation, our observations of degenerative necrosis preceding the fibrosis are supported by the findings of Cameron and Kountouras. Evidence of fibrotic proliferation, or granulation tissue within 15 days after CBD ligation was not seen in the present study (groups I, II and III). The present observations further indicate that such myxomatous degeneration/necrosis forming ILBs followed the course of the pre-existing bile preductules on the lobular circumference.

Morphologically, the fibrillar cells in the ILBs (groups II-V) were markedly different from those of the portal fibrous tissue. The former cells resembled the oedematous endothelial cells lining the sinusoids as was also described in the dog. Very low density of cells, vesicular appearance of the nuclei, and insignificant mitotic activity in the ILBs (groups II-III) suggested indigenous origin of the fibrillar cells from the linings of the obliterated sinusoids instead of the portal tissue. This was followed (group IV) by proliferation of the sinusoidal lining cells leading to degeneration and pressure atrophy of the hepatocytes, and thus, adding to the diminution of the inter-lobular bands. The dimension of oedema and myxomatous change replaced by hypertrophic/atrophic degeneration in the later groups (IV and V) suggested that the pathogenesis of the ILB was not the same as initially (groups I-III). Probably, in the former, rupture of canaliculi was no more a factor to make bile available for toxic and myxomatous change.

The findings in the present study further suggested that the proliferative capability was acquired by the cells under consideration at a later stage (groups III and IV) in the rabbit. Thereafter, the endothelial cell proliferation was a continuous process probably because of ischaemia resulting from obliteration/strangulation of the interlobular sinusoids and consequent diversion of the lobular-sinusoidal circulation. The continuation of the process, otherwise, may be due to pooling of bile in the biliary canaliculi and continuous irritation of the hepatic laminae. A fair number of the residual sinusoids, however, were patent in all stages (groups II-V) in the ILB and could be easily differentiated by the pattern of their lining endothelial cells and their relationship with the adjoining sinusoids, residual bits of attenuated hepatic laminae and hepatocytes in the ILB. The prominence of the sinusoidal cells in the necrotic areas after experimental ligation of the common bile duct have been mentioned by the earlier workers but not correlated to the process of perilobular fibrosis. Most earlier workers believed that the fibrotic tissue in the ILBs migrated from the adjoining portal tracts by one or the other mechanisms. The findings in the present study, however, suggested that the
portal-like fibrotic ILBs were formed by in-situ proliferation of the sinusoidal endothelial lining cells after CBD ligation in rabbit. The reticulin pattern of this bridging (ILB) fibrous tissue has been shown to resemble cirrhosis.

It is of interest that in the present study, bile preductules (canals of Hering) of significantly larger size lined by the hepatocytes were observed in the inter-lobular parenchyma in rabbits. These structures require further investigations. Usually the canals of Hering are defined as miniature structures of shorter length lined by a few hepatocytes and biliary ductular cells.

References


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