Deformed Liver with Prominent Proliferation of Bile Ducts in a Pig

Akihiko SUGIYAMA1, Kiyokazu OZAKI2 and Isao NARAMA3*

1Osaka City Meat Inspection Center, 5–2–48 Nankominami, Suminoe-ku, Osaka 559–0032 and 2Research Institute of Drug Safety, Setsunan University, 45–1 Nagaotoge-cho, Hirakata, Osaka 573–0101, Japan

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ABSTRACT. A deformed liver characterized by remarkable ductular proliferation was encountered in a 6-month-old pig and examined histopathologically. The most conspicuous histopathologic change was a mild to severe ductular proliferation in the interlobular areas without any degenerative changes of cholangiolar epithelial cells or hepatocytes. Fibrotic changes and reconstruction of the lobule were not found. Morphological evidence of intrahepatic and extrahepatic cholestasis was lacking. Other characteristics were deformity with displacement of the gall bladder, irregular shape and size of lobules, and structural abnormality of large-sized vessels. The severe ductular proliferation was considered to be due to structural malformations of the excretion channel of bile.

KEY WORDS: deformed liver, ductular proliferation, swine.

Non-neoplastic proliferation of intrahepatic bile ducts or hyperplasia of the cholangiolar epithelial cells are usually observed in association with various diseased conditions causing direct damage to the bile duct epithelium, or intrahepatic and extrahepatic cholestasis in humans and animals [2, 3, 10]. Congenital defects of the biliary system such as extrahepatic biliary atresia is also one of the causes of bile duct proliferation [4]. In some rodents such as rats and mice, spontaneous nodular proliferation of the bile duct has been observed in the liver of animals of advanced age [1, 8]. Among these proliferative changes, pathogenesis of the bile ductule proliferation associated with cholestasis in a cirrhotic liver is thought to be caused by an obstructed passage of bile [7]. However, the true pathogenesis of proliferative changes of bile ducts in various diseased conditions is still unknown.

Recently we found a deformed liver characterized by remarkable ductular proliferation in a slaughtered pig. We describe the histopathological features of the liver in this paper, since the proliferative changes in the bile ducts were very severe, and the pathogenesis of ductular proliferation is thought to be unique.

The animal was a 6-month-old, neutered male pig of mixed-breed, apparently healthy in outer appearance, and slaughtered at Osaka City Abattoir. The affected liver was condemned due to gross morphological abnormality. Macroscopically the liver was markedly reduced in size and round in shape. The medial sinister, medial dexter and quadrate lobes were fused to be a cluster lacking in apparent interlobular notches (Fig. 1). The lateral dexter and caudate lobes were also markedly reduced in size, but the thickness of the fused lobes and lateral sinister lobe was increased. Hepatic lobes were coalescent one another, and their margins were distinctly blunt. Several grayish-white areas were present on the serosal surfaces as well as in the subserosal parenchyma, especially near the triangular ligament. The common extrahepatic bile duct was elongated, and the gall bladder was displaced to the diaphragmatic surface (Fig. 2).

The parenchyma of the liver appeared normal. Throughout the liver, the interlobular connective tissues were conspicuous, and whereby hepatic lobules were more clearly seen. No other organs or tissues showed obvious gross lesions.

Several pieces of liver tissue were fixed in 10% neutral phosphated-buffered formalin solution, trimmed, dehydrated in a graded series of ethanol, and embedded in paraffin. Sections for microscopic examination were cut at a thickness of 4 to 5 µm and stained with hematoxylin and eosin. Some representative sections were also stained with azan.

For immunohistochemistry, we used anti-human Ki-67 monoclonal antibody (diluted at 1:50, DAKO, Glostrup Denmark) as a primary antibody for a cell proliferation marker. Deparaffinized sections were autoclaved for 20 min for antigen retrieval. The sections were incubated with bovine serum for 5 min then the primary antibody overnight at 4°C. N-histofine MAXPO(M) system (Nichirei, Tokyo, Japan), including a labeled polymer prepared by combining amino acid polymers with peroxidase and goat anti-mouse immunoglobulin was applied. The sections were colorized using diaminobenzidine solution, and then counterstained with Mayer’s hematoxylin. The area of the hepatic lobule was measured using an image processing and analysis software (Ultimage Pro Ver. 2.6.4, Alliance Vision, Mirmange, France).

Hepatic lobules varied in size and the shapes were round to polygonal (Fig. 3). The measurement of areas of hepatic lobules in this case revealed the evidence of much smaller or larger lobules than a normal pig liver (Fig. 4). Expanded interlobular spaces among remarkably small lobules were filled with proliferated bile ductules (Fig. 5). Neither hyperplastic nor hypertrophic hepatocytes were observed in larger lobules.

The proliferation of bile ductules in the perilobular area...
Fig. 1. Macroscopic feature of the liver markedly reduced in size and round in shape. Several grayish-white areas were present on serosa surfaces. The gall bladder was displaced to the diaphragmatic surface.

Fig. 2. Each hepatic lobe agglutinated and their margins were distinctly blunt. The common extrahepatic bile duct was elongated.

Fig. 3. Hepatic lobules varied in size and the shapes were round to polygonal. Bar=200 µm.

Fig. 4. Histogram of the areas of hepatic lobules from the liver of a normal pig and the present case. The measurement of areas of hepatic lobules in this case revealed the evidence of much smaller or larger lobules than a normal pig.

Fig. 5. Expanded interlobular spaces among remarkably small lobules were filled with proliferated bile ductules. Bar=100 µm.
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varied from mild to severe adenomatous change. Ductular proliferation was most prominent in the grayish-white areas detected by macroscopical observation. In the areas, hepatocytes remained like islets. Masses of proliferated bile ductules were often seen within the hepatic lobule. Nuclear atypia and mitotic figures were not observed in the ductular epithelial cells. Epithelial cells of bile ductules and hepatocytes were negative for Ki-67. In some perilobular areas, vascular lesions consisting of an increased interlobular arteries with medial hypertrophy as well as dilated interlobular veins varying in size were detected together with proliferated bile ductules.

The most conspicuous histopathologic change in this case was a mild to severe proliferation of bile ducts in the interlobular area without any fibrosis or degenerative changes in the cholangiolar epithelial cells and hepatocytes. Morphological evidence of intrahepatic and extrahepatic cholestasis indicated by bile thrombi, dilatation of bile ducts or accumulation of pigment-laden macrophages was also lacking in any part. Other characteristics were severe hepatic deformity with displacement of the gall bladder, the irregular shape and size of the lobule, and structural abnormality of the wall of the large vessel running through the liver parenchyma.

A drastic loss of hepatocytes is thought to be a factor for proliferation of bile ducts, since the cells originating from the duct of Hering, thin connecting channels located between the bile duct system and bile canaliculi, have the ability to differentiate to hepatocytes and provide new hepatocyte population in the damaged liver. However, in the present case degenerative changes or necrosis of the hepatocytes were never observed although many bile ducts were formed in the interlobular area. The diversity of size and shape of hepatic lobules might not be caused by regenerative nodules or reconstruction of destroyed lobules like those in a cirrhotic liver, since neither focal necrosis nor replacement fibrosis were seen [5]. Moreover, the proliferative changes in bile ducts in the present case were not progressive despite extensive replacement by the proliferated ductal structure because of the low proliferative activity of hepatocytes. These observations suggest that the structural abnormality of hepatic lobules was not caused by a replacement reaction to hepatic injury but by a developmental anomaly.

Bile duct proliferation might also be induced by the agents that are directly toxic to bile duct epithelium or that mediate damage by interfering with bile flow [15]. Among the many chemicals highly toxic to bile duct epithelium, α-naphthyl-isothiocyanate (ANIT) is one of the best known,
and has been shown to induce severe damage and subsequent proliferation of bile ducts in rats [11, 12]. However, it is unlikely that bile duct proliferation is a reactive hyperplasia to primary damage of bile duct epithelium, because of the different degrees of proliferative change in bile duct epithelium in different areas of the liver, as well as lack of evidence of any damage to cholangiolar epithelium.

Bile ductular proliferation in the present case was also thought to be non-neoplastic and non-progressive, since there was no massive nor invasive proliferation, and the immunoreactivity to Ki-67 antigen was negative. Furthermore, the structural abnormality of the blood vessel walls along with the many portal veins under medial hypertrophy suggested the possibility of congenital vascular abnormality or arterio-portal shunt.

In bile passage disturbance of intra- and extrahepatic bile ducts, bile ductular proliferation and metaplasia from hepatocytes to bile ductules occurred, connecting bile canaliculi to interlobular bile ductules to avoid the degeneration and necrosis of hepatocytes; thus, such metaplasia was an adaptation for the survival of hepatocytes [13, 14]. In serious cholecystitis and cholestasis of chickens, bile ductules proliferated following an insufficient passage of bile. It was suggested that ductular proliferation was a reactive change to avoid bile passage disturbance [6, 9]. However, we did not see severe hepatic or cholecystic injury causing prominent ductular proliferation in the present case. Though the exact mechanism of bile ductular proliferation in this case is unclear, it is possible that a structural abnormality is attributable to the abnormal formation of bile excretion channels. To our knowledge this appears may be the first report of such kind of liver lesion.

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REFERENCES