Original

**Apoptosis and Origin of Proliferated Bile Ductules
in Fulminant Hepatitis
—Pathologic and Electron Microscopic
Examination of Hepatic Tissue—**

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Abstract: Proliferation and subsequent loss of bile ductules is a common pathologic disorder in the course of fulminant hepatitis. A pathologic and electron-microscopic study was undertaken to determine the mechanism of bile ductular proliferation and loss, using seven autopsy cases of fulminant hepatitis with different times of onset. The proliferation in bile ductules was mild at 6 days from onset, but had become more severe by 37 days, and by 75 days from onset ductules were deleted. Electron microscopic findings of proliferated bile ductules resembled those of hepatocytes, but at 75 days they showed bile ductular metaplasia. Apoptotic cells were observed in bile ductules between 26–37 days, but could not be found between 6–10 days or at 75 days after onset. From these findings, the proliferated bile ductules of fulminant hepatitis were found to resemble hepatocytes, and the subsequent loss of bile ductules is due to apoptosis.

Key words: apoptosis, bile ductule, fulminant hepatitis

Introduction

Proliferation of bile ductules is a common pathological disorder in the recovery phase of fulminant hepatitis, but the origin of bile ductules remains unclear. In the course of fulminant hepatitis, especially in cases with a favorable prognosis, the proliferated bile ductules are eventually deleted, but the mechanism of deletion of bile ductules is not known. In this study, we examined proliferated bile ductules in autopsy cases of fulminant hepatitis by electron microscopy to determine the origin of the bile ductular epithelium and to evaluate the participation of apoptosis in deletion of proliferated bile ductules.

Materials and Method

Hepatic tissues from seven autopsy cases of hepatic tissues of fulminant hepatitis were obtained. These cases were clinically diagnosed as fulminant hepatitis, and the cause of the

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Table 1. Clinical background and histologic findings

Clinical background							Histologic findings				
case	age	sex	T-bil (mg/dl)	PT (%)	virus	course (days)	PM (hours)	bile ductule	hepatocytic regeneration	bile stasis	apoptosis
1	67	F	6.0	8	B	6	7	(+)	(-)	(-)	(-)
2	0	F	13.9	3	B	10	2	(+)	(+)	(-)	(-)
3	20	F	10.2	20.7	B	10	2	(++)	(++)	(+)	(+)
4	0	M	21.0	6.2	nAnB	26	2.5	(++)	(++)	(+)	(+)
5	1	M	17.0	21	nAnB	27	11	(+)	(+)	(+)	(+)
6	6	M	33.9	23.9	nAnB	37	10	(++)	(++)	(+)	(+)
7	63	F	ND	18.6	nAnB	75	1	(+)	(++)	(++)	(-)

T-bil; serum total bilirubin on admission

PT: serum prothrombin time on admission

virus: B when serum HBs antigen was detected

nAnB when serum IgM HA antibody or HBs antigen was not detected

HCV antibody could be assessed, because the cases died before 1990.

Course: survival periods from the onset to the death

PM: post mortem time

bile ductule: (+) when bile ductular increase is mild

(++) when bile ductular increase is severe

hepatocytic regeneration: (-) when no regenerated hepatocytes were seen

(+) when regenerated hepatocytes were focal

(++) when regenerated hepatocytes were diffuse

bile stasis: (-) when no bile plugs are seen in bile ductules

(+) when bile plugs are focal in bile ductules

(++) when bile plugs are diffuse in bile ductules

apoptosis: (-) when apoptosis can not be seen in bile ductular epithelium

(+) when apoptosis can be seen in bile ductular epithelium

death was hepatic failure in all cases. Hepatitis B virus infection was observed in three cases, but the presence of hepatitis C virus infection could not be determined, because all of the cases had died before 1990. Alcoholic hepatitis or drug-induced hepatitis was clinically excluded. Survival periods from the onset of hepatitis in each case were from 6 days to 75 days. A clinical summary is given in Table 1.

Light microscopic examination

Tissues were fixed in 10% formalin. Macroscopic findings of the cut surface of the liver were dark reddish, and the hepatic parenchyma had collapsed in cases 1-6, and yellowish small regenerated nodules were observed in case 7. More than four specimens were obtained from the largest cut surface of the hepatic left lobe and right lobe and embedded in paraffin wax for hematoxylin-eosin (HE) sections. Proliferation of bile ductules and regeneration of hepatocytes were examined, and the presence of bile plugs in bile ductules was estimated in each case. Proliferation of bile ductules was estimated as mild when there were less than ten bile ductules in the high power field, and as severe when there were more than ten.

Electron microscopic examination

Seven cases of hepatic tissues were fixed with 2.5% glutaraldehyde in 0.05 mol/L

phosphate buffer, and more than five specimens were obtained in each case for electron microscopic examination. The specimens were post-fixed in 2 % osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin. The thin sections were stained with uranyl acetate and lead citrate, and examined with an electron microscope.

The ultrastructure of proliferated bile ductules was examined in each case, and the presence of apoptosis in bile ductular epithelium was recorded.

Results

Light microscopic findings

In case 1, which was 6 days from onset of fulminant hepatitis, most of the hepatocytes were collapsed, and small numbers of proliferated bile ductules were observed near the Glisson's capsules on a necrotic background. The bile ductules were irregular shaped, with an eosinophilic cytoplasm like hepatocytes. No bile plugs were seen in the lumen, and there were no apparent mitotic figures or evidence of apoptosis in the bile ductular epithelium (Fig. 1).

In case 2 (10 days from onset), a small number of proliferated bile ductules were seen, and there were small nodular foci of regenerated hepatocytes. There was continuity between bile ductules and hepatocytes. In case 3 (also 10 days from onset), there were diffuse proliferated bile ductules on a necrotic background, but no regeneration of hepatocytes. Bile ductules in cases 2 and 3 showed small canalicular structures, and the cytoplasm was still eosinophilic. Bile plugs were still not observed in the lumen. Mitotic figures were not seen in bile ductular epithelium, but were evident in hepatocytes. No apoptosis was seen in bile ductular epithelium (Fig. 2).

In cases 4 and 6 (26 and 37 days from onset, respectively), there was increased proliferation of bile ductules and regeneration of hepatocytes. However in case 5 (27 days from onset), bile ductular proliferation was mild and regeneration of hepatocytes was diffuse. Bile ductules in cases 4, 5 and 6 showed almost the same findings as in cases 2 and 3. Bile plugs were occasionally seen in the lumen in cases 4, 5 and 6 (Fig. 3). Mitotic figures were seen in hepatocytes, but not in bile ductular epithelium. Apoptosis was recognized in an acidophilic body with some nuclear fragments among the bile ductular epithelium in cases 4 and 6 (Fig. 4).

In case 7 (75 days from onset), the numbers of bile ductules were lower than in cases 3, 4 and 6, and there was a fibrotic background. Diffuse regeneration of hepatocytes was seen, and the liver showed hepatic fibrosis. Many bile plugs were observed in the lumen. No mitotic figures were seen in either bile ductular epithelium or hepatocytes, and there was no evidence of apoptosis in the bile ductular epithelium (Fig. 5).

Electron microscopic findings

In case 1, bile ductule-like structures were observed, and the epithelium showed severe degeneration. Swelling of mitochondria and intra-cellular vacuolation were noted, and intercellular spaces were dilated. Condensation of chromatin was not severe. The origin of these epithelial cells was uncertain because of degeneration. No apoptosis was seen in the bile ductular epithelium or other isolated hepatocytes.

In cases 2 and 3, proliferated bile ductular epithelium resembled regenerated hepatocytes. The nuclei were irregular-shaped and the nucleoli were small. The numbers of mitochon-

Fig. 1. Light microscopic findings of case 1. Small numbers of proliferated bile ductules are observed near Glisson's capsule (G). HE stain, $\times 50$

Fig. 2. Light microscopic findings of case 3. Numbers of bile ductules are increased. HE stain, $\times 50$

Fig. 3. Light microscopic findings of case 4. Bile plugs (arrow) are observed in bile ductules. HE stain, $\times 33$

Fig. 4. Light microscopic findings of case 4. Apoptotic cell (arrow) can be seen in bile ductular epithelium. HE stain, $\times 200$

Fig. 5. Light microscopic findings of case 7. Many bile plugs are seen in bile ductules on a fibrotic background. HE stain, $\times 33$

dria and endoplasmic reticulum in these proliferated bile ductules were smaller than in mature hepatocytes, but greater than in bile duct epithelium. In contrast to bile duct epithelium, many glycogen granules were seen in the cytoplasm of bile ductular epithelium. The junction between the epithelia was often straight, but other junctions were irregular and frequently contained bile canaliculi. In contrast to hepatocytes, the epithelium was lined with a thin basement membrane (Fig. 6). No apoptosis was evident in bile ductular epithelium, but many isolated hepatocytes in collapsed stroma were undergoing apoptosis. Evidence of apoptosis included condensation of chromatin against the nuclear membrane, the presence of rounded clumps of electron-dense granules, convolution of the nuclear outline, and a sharp line of demarcation between the condensed chromatin and electron-lucent nuclear interior (Fig. 6).

In cases 4, 5 and 6, proliferated bile ductular epithelium showed almost the same findings

Fig. 6. Electron microscopic findings of case 2. Bile ductular epithelia are lined with a thin basement membrane, and many mitochondria are seen in the cytoplasm. Apoptosis is seen in isolated hepatocytes (arrow). C: bile canaliculi, bar=3 μ m

Fig. 7. Electron microscopic findings of case 4. Apoptotic cell (arrow) lies between bile ductular epithelia and the basement membrane. bar=4 μ m

Fig. 8. Electron microscopic findings of case 5. Apoptotic cell (arrow) lies in the ductular lumen. bar=5 μ m

Fig. 9. Electron microscopic findings of case 7. Bile ductular epithelia are lined with a thick basement membrane, and they resemble the epithelium of Hering's duct. C: bile canaliculi, bar=4 μ m

as in cases 2 and 3. Some proliferated bile ductular epithelium was undergoing apoptosis, and isolated hepatocytes showed the same evidence of apoptosis as cases 2 and 3, including multiple nuclear fragments with margined condensed chromatin. Apoptotic cells lay between bile ductular epithelial cells and the basement membrane (Fig. 7) or were seen in the ductular lumen (Fig. 8).

In case 7, proliferated bile ductular epithelium showed the metaplastic changes of regenerated hepatocytes. They contained small rounded nuclei and fewer intracellular organelles than before. The numbers of mitochondria, endoplasmic reticulum and glycogen granules were decreased, and the basement membrane was thicker than before. But the bile canaliculus was still observed. No apoptosis could be found in the bile ductular epithelium or other isolated hepatocytes (Fig. 9).

Discussion

Proliferation of bile ductules is a common reactive change in many hepatic diseases¹⁻³⁾, especially in primary biliary cirrhosis⁴⁾, biliary atresia⁵⁾ and fulminant hepatitis^{6,7)}. Bile ductules are thought to originate from hepatocytes or interlobular bile ducts or immature stem cells^{2,8,9)}. But the origin may differ according to the hepatic disease²⁾. In some hepatic diseases, bile ductules are subsequently deleted, although this process is still poorly understood.

In fulminant hepatitis, proliferation of bile ductules is observed in the recovery phase after massive hepatic necrosis^{6,7)}, and is associated with hepatocyte regeneration and an absence of proliferation of bile ducts. These pathologic findings are commonly seen not only in adults, but also in children¹⁰⁾. Therefore bile ductules in fulminant hepatitis probably originate from hepatocytes²⁾. In our limited examination of seven autopsy cases, including four cases of children, no mitotic figures were observed in the bile ductular epithelium, but were seen in only regenerated hepatocytes. Bile ductular epithelium showed electron microscopic features similar to hepatocytes, especially in the early stage; they were rich in mitochondria and glycogen granules in the cytoplasm and showed bile canaliculus. But in the late stage, they showed bile ductular metaplasia, with a decreased number of mitochondria and glycogen granules. Bile ductular metaplasia of hepatocytes has been reported in many hepatic diseases^{2,8)}, and its pathologic significance is probably to provide bile drainage from hepatocytes to interlobular bile ducts^{11,12)}. But hepatic specimens in the recovery phase of cases of acute hepatic necrosis with a favorable prognosis showed mild hepatic fibrosis without obvious bile ductular proliferation^{6,7)}.

Apoptosis is a potential mechanism of deletion of bile ductules. Many studies have described apoptosis of hepatocytes in fulminant hepatitis¹³⁻¹⁵⁾, but to our knowledge there has only been one report of apoptosis of bile ductular epithelium¹⁶⁾. Apoptosis has been observed in proliferated bile ductules in various other hepatic diseases^{17,18)}, but the method of evaluation of apoptosis in deletion of bile ductules differed. Bhanthall and Gall¹⁶⁾ reported apoptosis of bile ductular epithelium in experimental hepatic injury, and suggested participation of apoptosis in deletion of proliferated bile ductules. Bernuau *et al.*¹⁷⁾ suggested that apoptosis was important in the process of deletion of bile ductules in human primary biliary cirrhosis, although Nakanuma *et al.*¹⁸⁾ considered apoptosis not to be important in this process. Bhanthall and Gall¹⁶⁾ detected apoptosis of bile ductular epithelium in acute hepatic injury throughout a 21-day period, which peaked at 3 days following removal of

proliferative stimulus. In our limited examination of seven autopsy cases of fulminant hepatitis, apoptosis of bile ductular epithelium was observed in the subacute cases at 26, 27 and 36 days from onset, but was not seen in the chronic case at 75 days from onset, in which bile ductules were markedly deleted. Apoptosis was observed in the cases in which bile plugs were present in the lumen of bile ductules. Bile plugs in bile ductules indicate bile drainage from hepatocytes to bile ductules, and in the process of undergoing reconstruction of intrahepatic biliary system in the recovery phase of fulminant hepatitis, excessive proliferated bile ductules might be deleted by apoptosis.

Conclusion

Our limited examination of seven autopsy cases of fulminant hepatitis suggests that bile ductules resemble hepatocytes, and that proliferated bile ductules are deleted by apoptosis.

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