

Increased Microfilaments in Hepatocytes and Biliary Ductular Cells in Cholestatic Liver Diseases

To assess the extent of microfilaments in cholestatic liver diseases we examined the cytoplasmic microfilaments in intrahepatic and extrahepatic cholestasis in man by electron microscopy. Study subjects were two patients with drug-induced intrahepatic cholestasis, three patients with intrahepatic cholestasis due to viral hepatitis, four patients with extrahepatic cholestasis due to stones of the common bile duct and two patients with primary biliary cirrhosis. Two biopsied specimens from patients without clinical or histological evidence of liver disease served as non-cholestatic controls. The microfilaments in hepatocytes and biliary ductular cells were significantly increased in cholestasis compared with those in non-cholestatic controls. Well developed bundles of microfilaments were noted around the pericanalicular ectoplasm and seemed to be parallel to plasma membrane of the hepatocytes in cholestasis. In cholestasis, there were increased bundles of microfilaments around the periluminal region, lateral cell wall, and nucleus of biliary ductular cells. Two patterns of microfilaments bundles (fine microfilamentous network and spindle-shaped dense or clusters of microfilaments) were associated with cholestasis. The clustered form of microfilaments also seemed to be clearly associated with intracytoplasmic vacuoles containing bile salts. In conclusion, the increase of microfilaments in hepatocytes and biliary ductular cells may be the consequence of various forms of cholestasis. Further studies are needed to clarify the functional significance of increased microfilaments in cholestasis.

Key Words : *Microfilaments; Hepatocytes; Biliary Ducts cells; Cholestasis*

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INTRODUCTION

Ultrastructure of the liver in intrahepatic and extrahepatic cholestasis in human and animals has been extensively investigated with the use of conventional electron microscopic techniques (1, 2). In cholestasis, an irregular dilation of the bile canaliculus with swollen, stunted, or missing microvilli is accompanied by widening of the pericanalicular ectoplasm and structural alterations of bile canalicular membrane (3, 4), in which the alteration of microfilaments is recently suggested as one of possible causes of cholestasis (5-7). Microfilaments are present in many types of nonmuscular or nonkeratinized cells (8, 9). Microfilaments forming a fine anastomosing network in hepatocytes and biliary ductular cells (10, 11) have been found throughout the cytoplasm but are most concentrated around the bile canaliculi and periluminal region of biliary ductular epithelia.

Hypothetically, it has long been suggested that hypoactive, hypertrophic smooth endoplasmic reticulum in hepatocyte cause impairment of bile acid hydroxylation and result in altered miscelle formation and ductular-hepatocellular circulation of organic anions other than bile salts through ductules

altered by cholestasis (12, 13). However, recently growing evidence suggests that cholestasis may result either from a disturbance of Na^+ , and K^+ -ATPase-mediated canalicular pump (13, 14) or from an alteration of the canalicular membrane associated with microfilament dysfunction (15, 16).

Experimental cholestasis induced by phalloidin showed an increase of microfilaments in hepatocytes mainly around the bile canaliculi (4, 7). An early study revealed microfilament hyperplasia in the pericanalicular hepatocytic region and bile ductular cells in cholestasis in man (17).

In the present study, we observed a striking increase of the microfilamentous network around bile canaliculi and in biliary ductular cells in both intrahepatic and extrahepatic cholestasis. We herein discuss possible causes of microfilament hyperplasia in the pathogenesis of cholestasis.

MATERIALS AND METHODS

Materials

Thirteen liver biopsy specimens were used for this study.

Two biopsied specimens were from patients without clinical or histological evidence of liver disease; two from patients with drug-induced intrahepatic cholestasis; three from patients with intrahepatic cholestasis due to viral hepatitis; four from patients with extrahepatic cholestasis due to stones of the common bile duct; and two from patients with primary biliary cirrhosis.

Electron microscopic study

For electron microscopic study, liver biopsy tissues were fixed in 2% cold glutaraldehyde in 0.1 M Sorensen's phosphate buffer at pH 7.4 at 4°C for 3 hr, washed in the same buffer for 20 min, and then stored in fresh buffer in the refrigerator for 24 to 48 hr.

Tissues were then postfixed for 2 hr in 1% cold osmic tetroxide in 0.2 M Sorensen's phosphate buffer, pH 7.4, routinely dehydrated in graded alcohols and propylene oxide, and embedded in Epon 812.

One-micrometer thin sections were stained with toluidine blue for light microscopic lobular localization, and ultrathin sections were cut with a LKB microtome, and double stained with uranyl acetate and lead citrate, and examined under a HS-8F electron microscope with a 50-kv acceleration voltage.

RESULTS

Distribution of microfilaments

The distribution of microfilaments in various cholestatic diseases was investigated (Table 1). The results showed that microvilli in hepatocytes microfilaments were absent or mildly present in normal control, intrahepatic cholestasis (IHC), extrahepatic biliary obstruction (EHBO), and primary biliary cirrhosis (PBC). The pericanalicular hepatocyte microfilaments were increased in IHC, EHBO, and PBC, compared with normal controls. The intracytoplasmic hepatocyte microfilaments were increased in IHC and PBC, compared with normal controls. The microvilli in bile ductular cells were normal in IHC, EHBO, and PBC. Microfilaments in the peri-

luminal, perinuclear, and intracytoplasmic portions of bile ductular cells were markedly increased in EHBO and PBC compared with the non-cholestatic normal controls. There were no differences of increased microfilaments between various causes of cholestatic liver diseases.

Electron microscopic findings of hepatocytes and bile canaliculi

In hepatocytes of normal controls, bile canaliculi were normal in shape with abundant microvilli protruding into the canaliculi lumen. Microfilaments were found mainly around bile canaliculi where they formed the pericanalicular ectoplasm as a microfilamentous network (Fig. 1). Microvilli contained microfilaments extending from pericanalicular networks and appearing as linear or granular structures. Cytoplasmic microfilaments were hardly seen in normal hepatocytes in this study. In patients with cholestasis, however, bile canaliculi were irregularly dilated and canaliculi lumens contained bile thrombi. Microvilli were lost or distorted. There was an increase of microfilaments in hepatocytes and around bile canaliculi. The pericanalicular ectoplasm contained well developed bundles of microfilaments. Numerous loosely arranged microfilaments were present in the peribiliary cytoplasm near the pericanalicular ectoplasm (Fig. 2). Loose bundles of microfilaments were noted near the intracytoplasmic vesicles containing filamentous lamellar materials, a type of lysosome usually found in cholestasis. Some of them seemed to arise from Golgi vesicles or to be associated with vesicles (Fig. 3, 4).

Findings of biliary ductules and ducts

In normal controls, the luminal surface of biliary epithelia had microvilli that were shorter and farther apart than in hepatocytes. A very fine microfilamentous network extending from the periluminal region into microvilli (Fig. 4) and fine or loosely arranged bundles of microfilaments were noted around the lateral wall, intracytoplasmic and perinuclear regions (Fig. 5).

A striking increase of microfilaments was seen in biliary epithelia of patients with both intrahepatic and/or extrahep-

Table 1. Distribution of microfilaments in hepatocytes and bile ductular epithelial cells

	Hepatocytes			Bile ductular cells			
	Microvilli	Pericanalicular	Intracytoplasmic	Microvilli	Periluminal	Perinuclear	Intracytoplasmic
No cholestasis (n=2)	0/+	+	0	+	+	+	+
Intrahepatic cholestasis (IHC) (n=5)	0/+	++	++	0/+	++	+++	+++
Extrahepatic biliary obstruction (EHBO) (n=4)	0/+	+++	+	0/+	+++	+++	+++
Primary biliary cirrhosis (PBC) (n=2)	0/+	+++	++	0/+	+++	++	++

0=absent; + = present; ++ = increased; +++ = markedly increased.

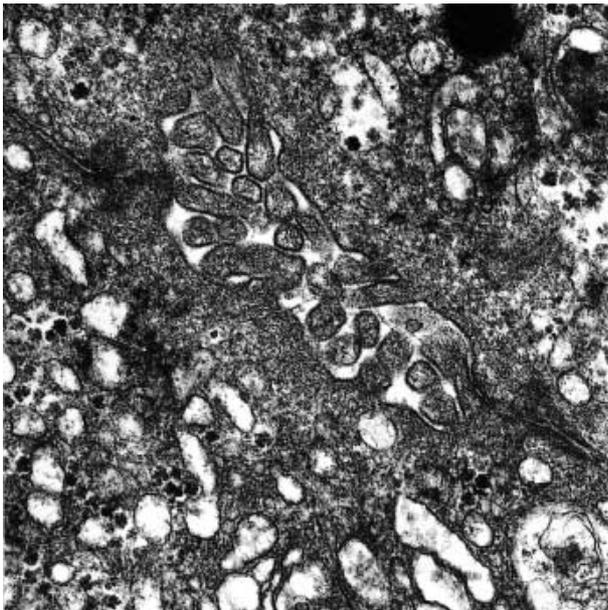


Fig. 1. Normal bile canaliculus in the liver of a patient without cholestasis. Microvilli contain fine microfilaments in their core and occupy the canalicular lumen ($\times 30,000$).

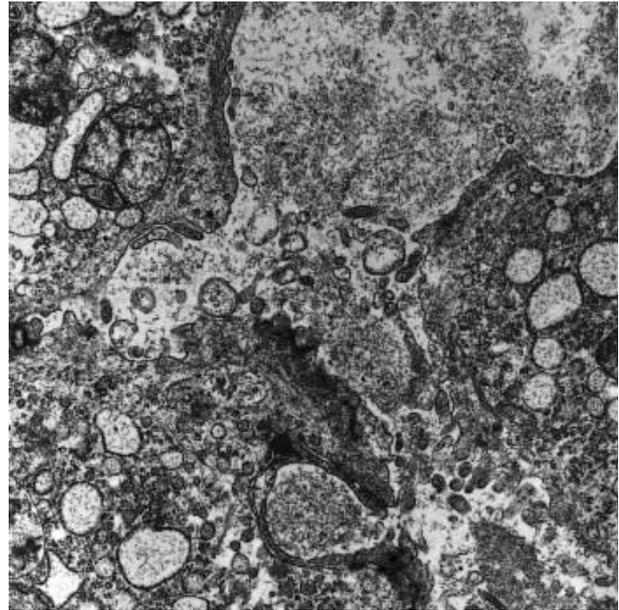


Fig. 2. Irregularly dilated bile canaliculus in a patient with cholestasis, showing thickened pericanalicular ectoplasm containing microfilaments. Only a few microvilli are noted ($\times 30,000$).

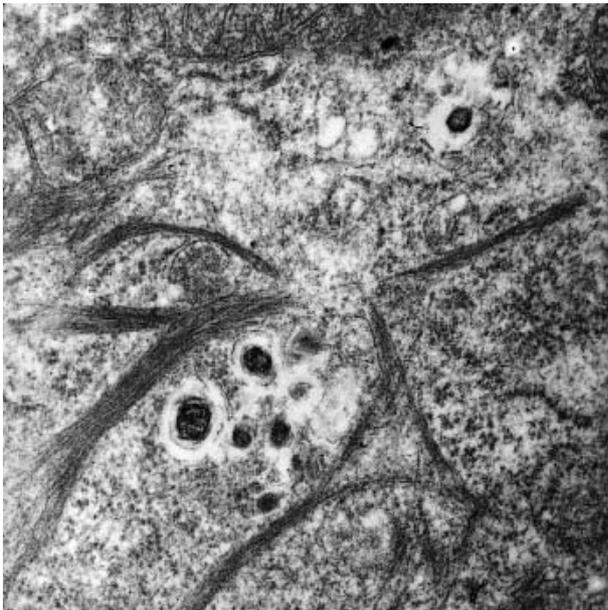


Fig. 3. Thick bundles of microfilaments in hepatocytic cytoplasm in a patient with cholestasis. In the central vacuolization (feathery degeneration), a form of lysosome is seen in cholestasis and surrounded by long microfilament bundles ($\times 30,000$).

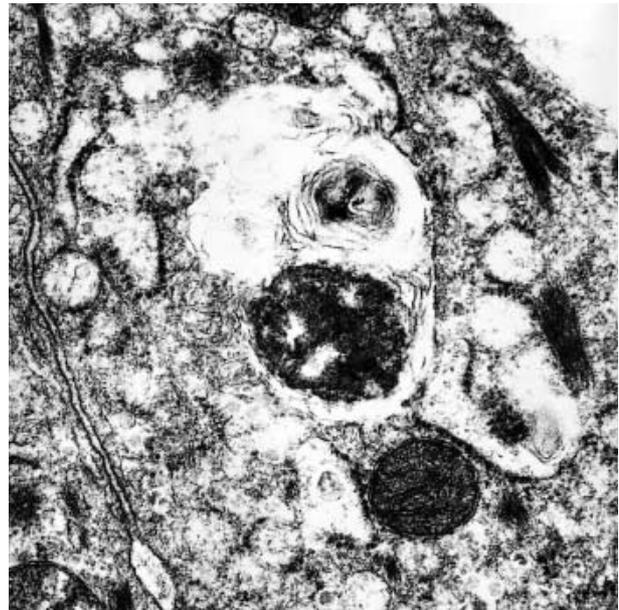


Fig. 4. Feathery degenerated vacuoles near plasma membrane of hepatocytes in a patient with cholestasis. Note the short and thick bundles of microfilaments ($\times 30,000$).

atic biliary obstruction. Microvilli were diminished, lost, or greatly distorted in cholestasis with occasional formation of blebs partially filling the ductular lumen. The microfilaments network was greatly increased around the periluminal area of biliary ductular cells and the lateral cell wall, where they

seemed to be more or less parallel to cell membrane (Fig. 6).

We found two forms of microfilaments in the cytoplasm of hepatocytes and biliary ductular cells of patients with cholestasis morphologically; one was bundles of microfilaments composed of fine microfilamentous networks and the other was spindle-shaped dense bundles or rather clusters of microfila-

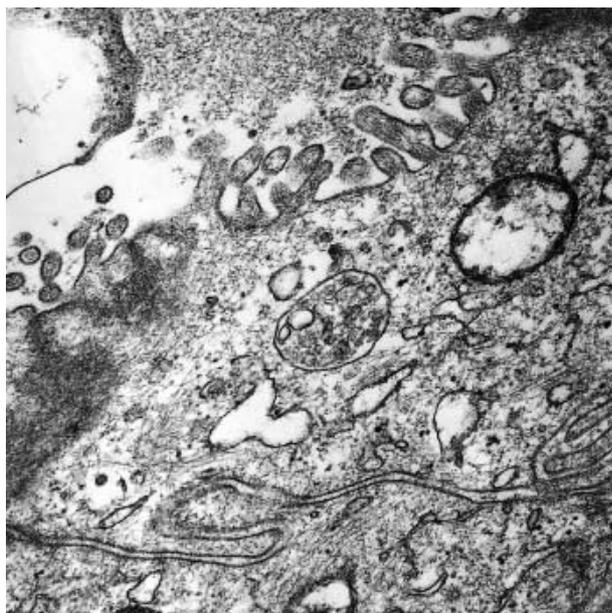


Fig. 5. Bile ductular cells in the liver of a case without cholestasis, showing a few microfilaments in microvilli and a scattered fine network of microfilaments around plasma membrane on the left ($\times 30,000$).

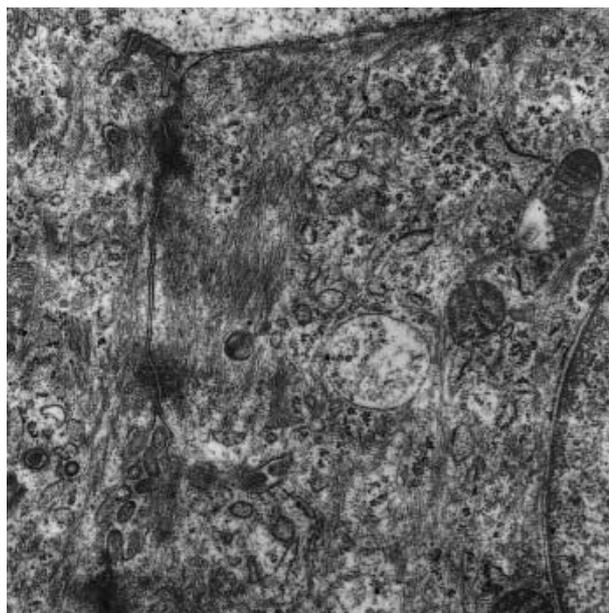


Fig. 6. Luminal surface of a bile ductule in a patient with severe cholestasis and EHBO. Abundant fine microfilament bundles are observed in cytoplasm around plasma membrane on the left and at luminal surface on upper border ($\times 30,000$).

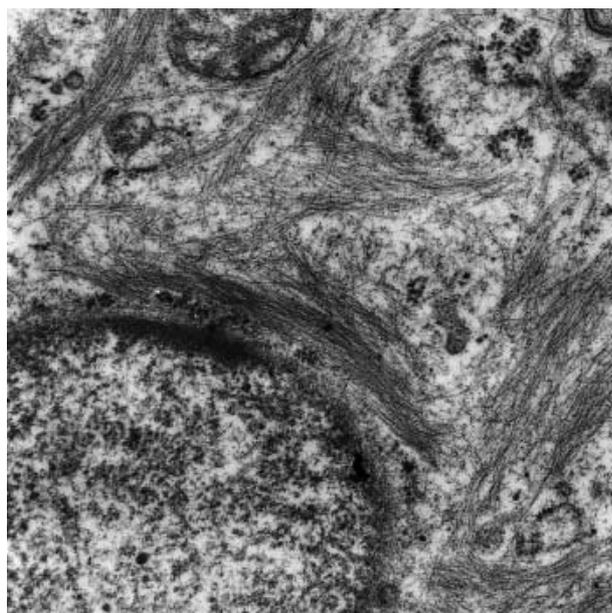


Fig. 7. Abundant bundles of microfilaments around the nucleus of ductular epithelial cell in a patient with cholestasis. Mitochondria are observed on upper and a nucleus on lower left portion ($\times 30,000$).

ments (Fig. 3, 4). The latter could be seen in patients with severe cholestasis and primary biliary cirrhosis. These specimens contained many intracytoplasmic vesicles closely related to the microfilamentous networks. The more severe the cholestasis was, the more the clusters or spindle-shaped microfilamentous

bundles appeared in association with the intracytoplasmic vesicles probably containing unexcreted biliary components. The perinuclear region of ductular epithelial cells in cholestasis also had big bundles of microfilaments extending in all directions (Fig. 7).

DISCUSSION

Microfilaments in epithelial cells are important for the structural and functional integrity of tight junction. Pericanalicular microfilaments might provide a contractile force that serves to facilitate the generation of bile secretory pressure and bile flow in the canalicular system (6). Subsequent support for this hypothesis was provided by experiments with cytochalasin B in which pericanalicular microfilament alteration and intrahepatic cholestasis were observed (5, 22). In addition, chronic administration of phalloidin to rats resulted in an increase of microfilaments in hepatocytes, mainly around bile canaliculi (4, 7). These cytoplasmic microfilaments contain actin as shown by the binding of anti-actin antibody (18) or heavy meromyosin binding (20), and by immunofluorescence and immunoelectron microscopic techniques (7, 21). Both sets of experiments showed that the combined alterations of pericanalicular microfilaments and canalicular membranes were closely related to cholestasis.

The results of this study suggest that the changes in intrahepatic and extrahepatic cholestasis may be present in both the canalicular and ductular epithelial membranes and their

associated microfilaments. The various forms of cholestasis we studied showed a striking increase of microfilaments in the pericanalicular region and biliary ductular cells and no difference was seen between intrahepatic and extrahepatic cholestasis. The extent of distribution of microfilaments was greatly increased in hepatocytes and biliary ductular cells in cholestatic liver diseases than normal controls. But there were no differences in distribution of microfilaments between intrahepatic cholestasis, extrahepatic biliary obstruction, and primary biliary cirrhosis. Regardless of its cause, microfilaments in cholestatic liver diseases is thought to be the consequences of cholestasis, but this also can be related to the pathogenesis of cholestasis. Further studies will be needed to resolve this issue.

Other parallel morphologic changes of cholestasis were also found in hepatocytes and bile ductular cells including luminal dilation, reduction or loss of microvilli, and the formation of blebs in both types of cholestasis. Elias et al. reported that disruption of microfilaments leads to intrahepatic cholestasis. In our study, however, there was no disruption of microfilaments in intrahepatic cholestasis (4). In extrahepatic cholestasis, microfilaments, which serve as a structural support of the bile canalicular system, may alter as a consequence of mechanical disturbance (24). After prolonged extrahepatic cholestasis, the potential of hepatocyte to generate actin filaments was disrupted and atrophy of microfilaments ensued (11).

Electron microscopy was applied for the assessment of structural alterations of microfilaments. Electron microscopically, two patterns of microfilaments were observed in hepatocytes and biliary ductular cells in various forms of cholestasis: one was fine microfilaments bundles and the other was spindle-shaped dense bundles or clusters of microfilaments. The latter findings were especially seen in chronically cholestatic patients with primary biliary cirrhosis whose hepatocytes and ductular cells showed intracytoplasmic vesicles probably containing retained components of bile, and seemed to be closely associated with them. The relation with the severity of cholestasis is uncertain.

The same findings were noted in experimental animals by Oda et al. (21). They speculated that microfilaments surrounding the bile canaliculi not only may help maintain the integrity of the canalicular wall but also may contribute to the contraction and relaxation of the bile canalicular microvilli and to the intracytoplasmic migration of vesicles in the pericanalicular region. Our data demonstrated an increase of microfilaments in human cholestasis, similar to that described in literature in the previous cases.

The morphological findings on the present electron microscopic examination suggest that increased microfilaments in cholestatic liver diseases influence the permeability and microtubular function. A possible explanation for the association between membrane function and the role of microfilaments was proposed by Nicolson (23), who suggested that transmembrane control of cytoskeletal system (microfilaments and microtubules) might be linked to integral membrane com-

ponents on the surface of the membrane. Our study supports the hypothesis that cholestasis may result from an alteration of canalicular membrane and its associated microfilaments (17).

In summary, our results indicate that the increase of microfilaments may be related to the consequences of various forms of cholestasis. Further studies are needed to elucidate the functional significance of increased microfilaments in cholestasis.

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