Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 18
Sofia • 2012

Study on the Origin of "Newductules" Appearing in the Rat Liver in Several Hours After Common Bile Duct Ligation

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It is considered that biliary hypertension represent the proliferative trigger for biliary structures in biliary occlusion setting; Appearance of new ductules is evident on the second/third days after common bile duct ligation (CBDL) and is associated with increased mitotic activities of biliary epithelia. We have demonstrated that after CBDL in male Lewis rats the increased amount of ductular profiles is evident already at several hours after biliary obstruction. It is shown that these structures are not related to mitotic activity of existing biliary epithelia and/or bile ductules proliferation, but mediated by widening of the existed finest biliary ramifications - biliary ductules and periportal biliary plexus.

Key words: rats, biliary obstruction, biliary proliferation, bile ductules, periportal biliary plexus

Introduction

Common bile duct ligation (CBDL) represents well-established in vivo model for the study of biliary proliferation including both - cholangiocyte and bile ductular proliferation. This proliferative response of biliary structures in bile retention is regulated by the complex interaction of several factors, including gastrointestinal and neuroendocrine hormones as well as autocrine or paracrine signaling mechanisms . It is considered that biliary hypertension represent the proliferative trigger for biliary structures in biliary occlusion setting, however, dynamics, as well as the mechanisms of this process require further comprehensive investigation.

According to several studies the appearance of new ductular is evident on the second or third days after BDL and is associated with increase mitotic activities of biliary epithelia . It has been demonstrated that "neoductules" originated from proliferated biliary epithelial cells even in vitro. Initially ductular proliferation appears in the periportal areas and then gradually advances intralobulary, what is more notable at one week after biliary obstruction . However, we have previously demonstrated that amount

of ductular profiles increases in the liver of CBDL rats already at several hours after biliary obstruction . The origin of "new" ductular profiles and mechanisms which can be involved in such early ductular reaction (DR) is currently unknown and needs to be elucidated. It is conceivable that such an early DR after CBDL is not related to mitotic activity of existing biliary epithelia and bile ductules proliferation.

The aim of this study was to identify the origin of "new" ductular structures appeared within 24 hours in the rats' liver in response to biliary obstruction.

Materials and Methods

Animals and Experimental Protocol: Inbred male Lewis rats were obtained from Charles River Deutschland (Sulzfeld, Germany). Experimental protocols and use of animals were approved by the Institutional Animal Care Committee. Animals were housed in individual cages at a standard temperature of 24°C and a 12 hour light/dark cycle and fed ad libitum on standard rat chow, with free access to water.

Male Lewis rats (n=36) weighting 250-300 g were subjected to common bile duct ligation (n=24) or sham operation (controls, n= 12) under ethyl-ether narcosis. The animals were killed on 3, 6, 12 and 24 hours after BDL (6 animals in each group); part of the dissected liver samples (median lobe) were frozen at -80°C for immunohistochemistry and part fixed in 10% neutral buffered formalin, embedded in paraffin and used for routine histological examination.

Histology and immunohistochemistry: Liver sections of 5 μm thickness were stained with haematoxilin and eosin (H&E). Fixed in 10% neutral formalin and/or frozen liver samples undergone the standard histological (H&E stained slides) and immunohistochemical investigations. Frozen liver samples were sectioned on cryostat microtome (Leica 800 Cryostat Microtome CM1800) and immunostained for Ki67 (1:150) (ab16667, Abcam plc, Cambridge, UK), pan-Cytokeratin antibody [AE1+AE3] (neat) (ab961, Abcam, plc, Cambridge, UK) and OV-6 (1:100) (MAB2020 R&D Systems, Inc) using appropriate protocols provided by antibody suppliers. Sections were counterstained with haematoxilin.

Statistical Analysis: Data for the average number of ductular profiles were analyzed by the one-way analysis of variances (one-way ANOVA). In ANOVA for the average number of ductular profiles the factor was time. Planned comparisons between the average numbers of ductular profiles were made by using *t*-tests. Statistical tests for the average number of ductular profiles were two tailed.

Results

Histology and immunohistochemistry

Changes in number of bile ductules: CBDL was accompanied with significant (2 - 4 folds) increase of ductular profiles ($p<0.05$) while in sham operated animals statistical analysis revealed no significant effect of the time factor for number of ductular profiles.

Biliary epitheliocytes and "Reactive ductules' cells" were strongly positive for CK and OV-6 at all time points. "New" ductular profiles appeared around the portal tracts of all sizes: from large to the finest (Fig. 1 a, b), also intralobulary - sometimes in several hundreds microns away from the portal area (Fig. 1 c). Intralobular "neoductules" were located in the spaces of Disse (Fig. 1 d); the cells composing the neoductules were small and uniform with light, oval, sometimes spindle-shaped nuclei and scant cytoplasm. They are Ki67-negative, but equally immunopositive for Pan-Cytokeratine and OV6. No Ki67 positive staining of any type of cells was observed.

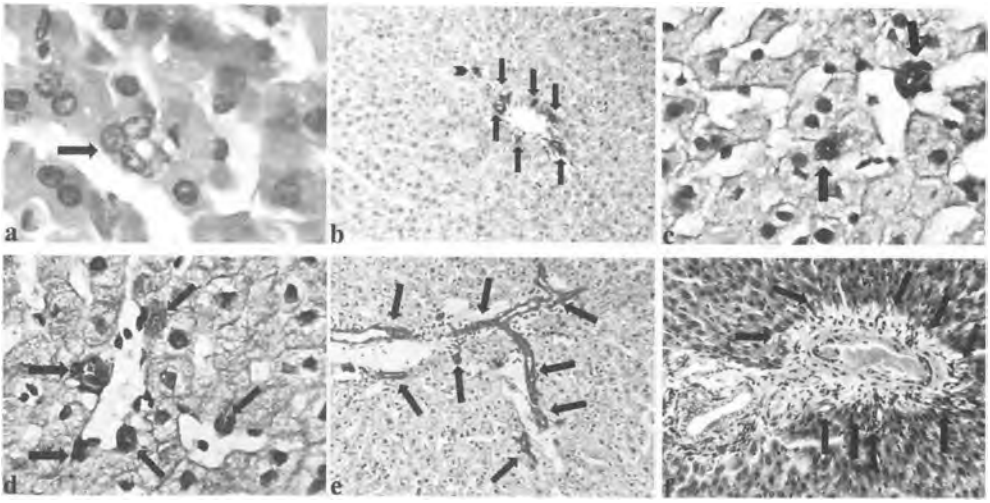

Fig. 1. Histology and Immunohistochemistry of liver tissue after CBDL. a) Intralobular bile ductules (arrows), H&E, 6 h after BDL b) Bile ductules in the small portal area (arrows), intralobular bile ductules (arrowhead), CK,, 6 h after BDL; c) "Newductules" surrounding widened sinusoids (arrows), CK, 12 h after BDL; d) "Newductules" surrounding widened sinusoids (arrows) OV6, 12 h after BDL; e) Bile ductules surrounding portal vein (arrows), CK 24 h after BDL; f) Bile ductules surrounding portal vein (arrows), H&E 24 h after BDL;

Discussion

Our results demonstrate the appearance of "newductules in 3-24 hours after CBDL. However, negative reaction of cells covering the lumens of these newductules towards Ki67 antibodies indicates that this phenomenon cannot be associated with proliferation of existed biliary epithelial or any other cells. These data are in correlation with the results of our previous investigation showing that activation of mitoses of cholagiocytes and hepatocytes begins from the second/third days after CBDL (2). It confirms that the increase of number of ductular profiles just in several hours after CBDL is not associated with cell proliferation. Thus, it has to be related with widening of existed tubular structures covered by epitheliocytes (or epitheliocyte-like) cells.

One of the possible sources of increased ductular profiles can be the lumens of biliary mucosal glands, which are widened due to raised CBDL-induced biliary pressure (as it was described on autopsy liver specimens and in dogs after CBDL . However, in rats (unlikely to dogs, cats and guinea pigs) the amount of mucosal biliary glands is much smaller and majority of them are attached to the extrahepatic but not intrahepatic bile ducts . The topography of the newly appeared ductules (they appeared along the perimeter of portal vein lumen remote but not adjacent to bile ducts, also intralobulary) excludes the possibility of their glandular origin.

Next epithelial-lined tubular structures located at the portal areas, which can be a source of DR is periportal biliary plexus described by Scanning Electron Microscopy (SEM) of biliary corrosion casts in rats . In these plexus the bile ductules are anastomosing with each other and forming a plexiform network around the portal vein branch or at the periphery of portal canal. This network of bile ductules form larger ductules to drain into the intrahepatic bile ducts in the portal canal . (Fig 2 a, b).

We suggest that the periportal biliary plexus is the basis for numerous ductular profiles, which appear in periportal area already in the few hours of bile congestion.

But, according to our data, at 6 h after CBDL the ductular profiles appear also inside of lobules, far away from the portal tract. Obviously, the periportal biliary plexus cannot be the source for these structures. They should be a cross- and tangential sections of the finest bile ducts ramifications, bile ductules and Herring's ductules, which are established to pass the limiting plate and spread quite far inside the lobules . These finest biliary structures which lie within the lobule and not at the limiting plate are not readily apparent on routine histological staining , but the increased biliary pressure due to CBDL can be sufficient factor for their dilatation and appearance on microscopical slices. Ductular profiles can also represent the sections of the finest bilio-biliary anastomoses which are evident on SEM of corrosion casts . They can also be intercalated portion of biliary ductules creating the finest plexus at the bordering areas with limiting plates .

Normally there is on average one Herring's ductile per 10 μm of bile duct length . The comparative analysis of the results of SEM of corrosion casts of portal and biliary trees stipulates the conclusion, that frequently the angles of ramifications of the sinusoids from the thinnest portal branches appear different with angles of ramification of biliary ductules from the finest interlobular bile ducts . The above-mentioned peculiarities of the architecture of vascular and biliary structures are schematically presented on Fig. 3. It can explain the suggested mechanism of appearance of ductular profiles inside of liver lobule caused by widening of lumens of biliary ductules due to biliary hypertension. Localization of described "neoductules" in the spaces of Disse confirms this suggestion, because per sinusoidal spaces are the only areas, where the biliary ductules, Herring's ductules and/or the finest biliary-biliary anastomoses can be placed.

In conclusion, our data indicates that appearance of "new biliary ductules" starts in few hours after CBDL and is mediated by widening of the finest biliary ramifications, biliary ductules and periportal biliary plexus and is not associated with proliferation of existed bile ducts/ductules and their epithelial cells. These proliferate reactions are originated later from $2^{nd}/3^{rd}$ days after CBDL.

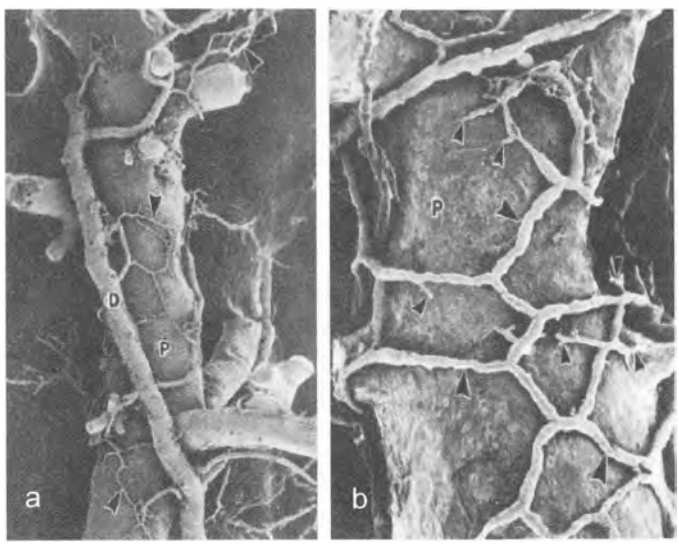

Fig. 2. Periportal biliary ductular plexus (arrowheads) (a, b). In this case, portal vein branches (P) were concomitantly replicated. D - bile duct. X40. (The figures are provided by kind permission of Prof. T. Murakami)

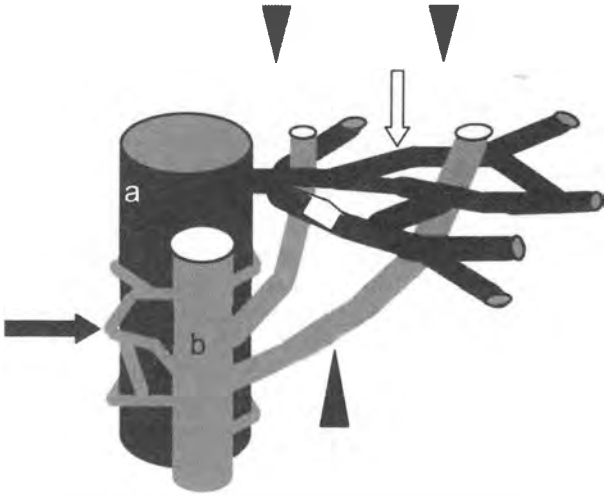

Figure 3. Architecture of the finest vascular and biliary structures.
Interlobular portal vein
(a), interlobular bile duct
(b), periportal biliary plexus (black arrow), Canals of Herring and intralobular ductules (arrowhead), sinusoids (white arrow)

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