Case Report

Spontaneous cholangiofibrosis adjacent to a dilated common bile duct with intestinal metaplasia in a Royal College of Surgeons rat

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Abstract: A 130-week-old male Royal College of Surgeons rat kept as a non-treated animal in a long-term animal study presented with a mass in the hepatic portal region that adhered to a dilated common bile duct and the duodenum. Histopathologically, the solitary mass showed expansive growth with no apparent compression and continued to dilate the common bile duct, which had a hyperplastic epithelium with intestinal metaplasia. The mass mainly consisted of small to large dilated and/or tortuous ducts with abundant dense connective tissue and many inflammatory cells. The single-layer lining epithelium of the duct changed from cuboidal to columnar. Immunohistochemically, the lining cells were positive for cytokeratin 7, cytokeratin 19, and OV-6, which are bile duct markers. Based on the pathological characteristics, the rat was diagnosed as spontaneous cholangiofibrosis adjacent to a dilated common bile duct with intestinal metaplasia. (DOI: 10.1293/tox.2021-0032; J Toxicol Pathol 2021; 34: 339–343)

Key words: cholangiofibrosis, rat, liver, bile duct, metaplasia

Cholangiofibrosis, as defined by the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND), is a proliferative and metaplastic biliary reaction with fibrosis extending into the hepatic parenchyma1. Various xenobiotics such as dioxins, furans, thioacetamides, and Fusarium moniliforme can induce cholangiofibrosis2–4. Spontaneous cholangiofibrosis in untreated rats is extremely rare and has only been reported in a case of a Wistar rat and characteristic lesions of a Long-Evans Cinnamon (LEC) rat5, 6. However, the LEC rat was a model of Wilson’s disease, and cholangiofibrosis was caused by a genetic predisposition to hepatotoxicity6. Recently, we encountered a case of cholangiofibrosis in a male Royal College of Surgeons (RCS)/Kyo rat with no genetic predisposition to hepatotoxicity. This report describes the histological characteristics of cholangiofibrosis in this RCS rat.

RCS rats are an inbred strain and are well known for their predisposition to inherit retinal degeneration; they have no genetic predisposition to hepatotoxicity7, 8. RCS/Kyo rats were maintained at the National BioResource Project-Rat (NBRP-Rat) facility of Kyoto University (Japan). They were maintained as an inbred strain by full-sibling mating. Among them was a 130-week-old RCS male rat that was reserved as a non-treated animal in a long-term study and fed a standard diet (Charles River Formula 1; Oriental Yeast Co., Ltd., Tokyo, Japan) with chlorinated water ad libitum. It was kept in an animal room maintained at a temperature of 24 ± 2 °C with a relative humidity of 60 ± 20%, a 12 h light/dark cycle, and ventilation at least 12 times/h. Apart from coarse hair, no clinical signs were apparent until the scheduled sacrifice at 130 weeks of age. The rat was deeply anesthetized using ketamine (40 mg/kg, intramuscular [IM]; Ketalar, Sankyo, Tokyo, Japan) and xylazine (2.0 mg/kg, IM; Seractal, Bayer, Tokyo, Japan) and euthanized by exsanguination. The study was approved by the Committee for Animal Experiments of Setsunan University.

A gross mass of 20 × 10 × 10 mm was observed in the hepatic portal region. The mass adhered to a dilated common bile duct and the duodenum. The mass was soft, and the cut surface was composed of multiple small to large cysts (Fig. 1). Specimens were fixed in 10% neutral phosphorlated formalin (pH 7.4), dehydrated in a graded series of ethanol, and embedded in paraffin. Sections (4 µm) were subsequently stained with hematoxylin and eosin, Alcian blue (AB) stain, and periodic acid-Schiff reaction (PAS). Immunohistochemical staining using a labeled polymer method was performed using N-Histofine MAX PO rat (M or R) (Nichirei, Tokyo, Japan) and xylazine (2.0 mg/kg, IM; Seractal, Bayer, Tokyo, Japan) and euthanized by exsanguination. The study was approved by the Committee for Animal Experiments of Setsunan University.

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The Ki-67 positivity index was estimated as the percentage of Ki-67-labeled nuclei/1,000 lining epithelial cells.

Histopathologically, the solitary mass showed expansive growth with no apparent compression of the liver (Fig. 2A). On the opposite side of the liver, the peripheral area of the mass, the hepatic parenchyma was randomly retained and retracted (arrows). The mass consisted of small to large dilated and/or tortuous ducts with abundant dense connective tissue. The dilated ducts were filled with mucus and cell debris. B: The peripheral area of the mass continued to the submucosa of the dilated common bile duct (asterisk). Inlet: The dilated common bile duct had a hyperplastic epithelium with intestinal metaplasia. HE stain.
In the hepatic parenchyma adjacent to the mass, bile duct hyperplasia with/without fibrosis was observed with lymphoplasmacytic cell infiltration, but cholangiofibrosis of the hepatic parenchyma was not observed simultaneously.

Xenobiotic-induced cholangiofibrosis occurs in the hepatic parenchyma of multiple lobes in the liver. It is characterized by retraction of the surrounding parenchyma, dilated and/or tortuous bile ducts with mucus, cuboidal to columnar lining epithelium with intestinal metaplasia, and abundant fibrous stroma with variable inflammatory cell infiltrates. Cholangiofibrosis in LEC rats involves multiple lobes of the liver and has histological characteristics similar to those of xenobiotic-induced cholangiofibrosis. The histopathological characteristics of our case were consistent with those of xenobiotic-induced cholangiofibrosis, and cholangiofibrosis observed in LEC rats. However, solitary mass formation and extracapsular proliferation differentiated our case from xenobiotic-induced cholangiofibrosis, and cholangiofibrosis observed in LEC rats. The spontaneous cholangio-

**Fig. 3.** A: High-magnification image of mass showing small to large dilated and/or tortuous ducts with abundant dense connective tissue and many inflammatory cells. B: Image showing the single-layer lining epithelium of the ducts. The lining epithelium contained many goblet cells (arrows) with mucus.

**Fig. 4.** Image showing mucus of the ducts and the goblet cells of the lining epithelium of dilated ducts with A: Alcian blue (AB)- and B: periodic acid–Schiff reaction (PAS)-positive mucus.

**Fig. 5.** Immunohistochemical image showing lining cells positive for cytokeratin 7.
fibrosis reported by Chen was a solitary mass located at the hilus of the liver and had histopathological features similar to those of xenobiotic-induced cholangiofibrosis\(^1\). Our case resembled Chen’s case of spontaneous cholangiofibrosis, except for continuity to a dilated common bile duct with intestinal metaplasia and hepatic parenchyma retained in the peripheral area. Recently, in the proceedings of the 2019 National Toxicology Program Satellite Symposium, spontaneous cholangiofibrosis in the liver and pancreas of untreated Hsd:Sprague Dawley (SD) rats was reported\(^8\). This case had histopathological descriptions similar to those of xenobiotic-induced cholangiofibrosis, but was different owing to the singular distribution and close proximity to a large bile duct. Hence, the hepatic and pancreatic lesions were termed “periductal cholangiofibrosis” to differentiate them from xenobiotic-induced cholangiofibrosis\(^10\). In our case, the cholangiofibrosis was characterized by its continuity to the large extrhepatic bile duct, similar to that observed in SD rats. Furthermore, the hyperplastic epithelium of the large bile duct showed intestinal metaplasia and cholangiofibrosis. These findings suggested that the primary site of the cholangiofibrosis in our case might be the extrhepatic bile duct. However, since no clear continuity from the extrhepatic bile duct epithelium could be confirmed, the primary site could not be identified. Based on the pathological characteristics, our case was diagnosed as spontaneous cholangiofibrosis adjacent to a dilated common bile duct with intestinal metaplasia.

The most important differential diagnosis was cholangiogibroma. The distinction between cholangiogibroma (neoplastic lesion) and cholangiofibrosis (non-neoplastic lesion) is difficult because of gradual transition\(^11, 12\). Bannasch stated that Chen’s case of spontaneous cholangiofibrosis should be diagnosed as cholangiogibroma because of the expansive growth and protrusion of the liver surface\(^12, 13\). In contrast, Chen stated that the two terms need not be separated because a unique term for a cholangiogibroma is not available in INHAND and is dependent on the duration of the lesion\(^13\). Macroscopically, the mass formation in our case seemed to be neoplastic. However, since the lesion did not compress the liver parenchyma, and the mass randomly contained residual liver tissue, it was considered to have the characteristics of cholangiofibrosis, which is a non-neoplastic lesion. Other differentiations included cholangiocarcinoma and cholangioma. Carcinoma is differentiated by cell and structural atypia, whereas adenoma is characterized by no compression and frequent intestinal metaplasia.

The ducts in our case were positive for three types of bile duct markers, demonstrating that they originated from the bile duct. The characteristics of the three markers were as follows: cytokeratin 19 was present in all biliary structures\(^14\), cytokeratin 7 was present in the large bile ducts, but absent in the small branch\(^14\), and OV-6 was present in the bile ducts, including the Herring duct\(^5\). In our case, the positivity rate of cytokeratins 7 and 19 was high, whereas the positivity rate of OV-6 was low. Compared with Chen’s case, ours was dominated by dilated bile ducts and a low positivity rate for OV-6, suggesting a long duration of the lesion\(^9\).

We have reported cholangiofibrosis showing proliferation different from that of induced lesions; our case was diagnosed as spontaneous cholangiofibrosis adjacent to a dilated common bile duct with intestinal metaplasia.

**Disclosure of Potential Conflicts of Interest:** The authors declare that they have no competing interests.

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