Histopathological Characteristics of Kupffer Cells and Ito Cells in the Porcine and Bovine Liver

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ABSTRACT. We previously reported that no Kupffer cells reacted with the antibody against lysozyme, and Ito cells contained a large cytoplasmic vacuole in the feline liver. In this report, we further examined the characteristics of porcine and bovine hepatic non-parenchymal cells. In the liver of both animals, Kupffer cells were positive for lysozyme, and cytoplasmic vacuoles in Ito cells were small. The histopathological characteristics of porcine and bovine hepatic non-parenchymal cells were different from those of the feline liver.

KEY WORDS: cattle, Ito cell, swine.

Hepatic non-parenchymal cells including sinusoidal endothelial cells, Kupffer cells, and Ito cells, play important roles in each process of liver diseases [18] through the production of various kinds of humoral factors and extracellular matrices [4, 12]. In order to better understand the pathophysiology of hepatic diseases, the contribution of non-parenchymal cells to various hepatic conditions should be elucidated.

We have previously reported the histopathological characteristics of Kupffer cells and Ito cells in the feline liver [17]. To compare the characteristics of the liver of felines and other animal species, we histologically and immunohistochemically examined porcine and bovine livers.

The liver materials were obtained from 23 pigs slaughtered in 2005 at the Yokohama City Meat Sanitation Inspection Station and 11 cows slaughtered in 2005 at the Meat Inspection Station, Kanagawa Prefectural Government. The pigs comprised 4 males, 17 females and 2 of unknown sex, ranging from about 6 months to 5 years old. The cows comprised 2 males and 9 females, ranging from 2 to 9 years old. Tissues were fixed in 10% neutral-buffered formalin (NBF), embedded in paraffin, and sectioned in 4 µm thick slices. The paraaffin sections were stained with hematoxylin and eosin (HE), and also used for immunohistochemical analysis. Sudan black staining was performed using frozen 6 µm thick sections prepared from NBF-fixed tissues embedded in a water-soluble compound (Tissue Tek 4583; Miles, Elkhart, IN, U.S.A.).

For immunohistochemical examination, deparaffinized sections were autoclaved or incubated in 1% trypsin solution for antigen retrieval. Then, sections were immersed in 0.3% hydrogen peroxide to block internal peroxidase, and in skimmed milk to block non-specific antibody binding. Primary antibodies were anti-human lysozyme, anti-chicken desmin and anti-human α-smooth muscle actin (SMA) antisera (Dako, Glostrup, Denmark). Biotinylated anti-mouse or -rabbit immunoglobulin G (KPL, Gaithersburg, MD, U.S.A.) was applied as a secondary antibody. The sections were incubated with peroxidase-labeled streptavidin (Dako, Glostrup, Denmark) and visualized in diaminobenzidine-tetrahydrochloride solution. Counterstaining was done with methyl green.

For electron microscopy, NBF-fixed tissues were refixed in 2.5% glutaraldehyde and 1% osmium tetroxide. Then, they were dehydrated through a graded alcohol series, and embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined under a JEM 1200 electron microscope (JEOL Ltd., Tokyo, Japan).

In porcine livers, Kupffer cells were positive for lysozyme (Fig. 1a). The reaction was also observed in the cytoplasm of spindle cells in interlobular connective tissue (Fig. 1b). Small-sized vacuoles about 5 to 7 µm in diameter were observed in the hepatic perisinusoid (Fig. 2a). Flattened nuclei were observed at the edge of vacuoles, indicative of the intracytoplasmic location of the vacuoles. The vacuoles were positive for Sudan black (Fig. 2b). In electron microscopy, the small-sized vacuoles were homogeneously stained (Fig. 2c). These data may suggest that lipid is contained in the cytoplasmic vacuoles. The small vacuole-laden perisinusoidal cells were positive for desmin (Fig. 2d), suggesting that such cells were identical to Ito cells. No positive reaction for α-SMA was observed in porcine Ito cells in all the liver samples in this examination. The number and size of the cytoplasmic vacuoles of porcine Ito cells were constant irrespective of age and hepatic lipid contents.

In bovine livers, the results were almost the same as in porcine liver. Kupffer cells reacted with the antibody against lysozyme (Fig. 3). Small vacuoles about 5 to 7 µm in diameter were observed in the hepatic perisinusoid (Fig. 4a). Such vacuoles were positive for Sudan black (Fig. 4b), and the vacuole-laden perisinusoidal cells were positive for α-SMA.
desmin (Fig. 4c), suggesting that they were Ito cells. No positive staining for α-SMA was observed. The number and size of the cytoplasmic vacuoles were irrespective of age and hepatic lipid contents.

In the present study, porcine and bovine Kupffer cells were positive for lysozyme, which is in accord with those of dogs, humans, and rats [13]. In contrast, feline Kupffer cells were negative for lysozyme; however, the immunohistochemical result does not indicate an absence of lysozyme in feline Kupffer cells. Alveolar macrophages of humans were immunohistochemically negative for lysozyme [7, 10], whereas they have been shown to contain the enzyme by demonstration of their muramidase activity [6]. Therefore, both the amount and activity of lysozyme in Kupffer cells

Fig. 2. Porcine liver. Small lipid-laden vacuoles (arrows) in the cytoplasm of Ito cells are observed in the hepatic perisinusoid. HE stain (a), Sudan black stain (b), Electron micrograph (c), and immunohistochemical stain for desmin (d). Bar = 25 µm (a, b and d) and 5 µm (c).

Fig. 1. Porcine liver. Cells positive for lysozyme are observed. They are Kupffer cells (a) and spindle cells in the interlobular connective tissue (b). Immunohistochemical stain for lysozyme. Bar = 25 µm (a) and 40 µm (b).
should be compared in addition to immunohistochemistry.

In porcine and bovine Ito cells, cytoplasmic fat vacuoles were smaller than those of felines. Ito cells play a major role in vitamin A storage under normal conditions [5], and they also store triacylglycerol and cholesterol ester together with fat-soluble vitamin A. For example, lipid droplets of rat hepatic Ito cells consist of 40% retinoids, 30% triacylglycerol, and 15% cholesteryl ester [14]. The lipid droplets size of Ito cells may be influenced by the total hepatic lipid contents. In this study, we investigated liver samples of both normal and fatty livers; however, in both porcine and bovine livers, there were no apparent differences in the lipid droplet size of Ito cells between normal and fatty livers (data not shown).

Lindberg and Grohn [9] showed a significant negative correlation of fat volume between hepatocytes and Ito cells in severely ketotic cows, although there is a report showing hepatocytic fat decrease without an increase of fat volume of Ito cells in cows after parturition [9]. Therefore, the effect of hepatic total lipid contents on the lipid storage of Ito cells is not clear. In cats, Ito cells contain large lipid vacuoles, and hepatic lipidosis is one of the most frequent liver diseases. We should also compare the lipid droplet size of feline Ito cells between normal and fatty livers.

Some reports [2, 19, 20] mention body fat accumulation in aged rodents and humans due to alteration of the systemic metabolism. In the feline liver, large vacuole-laden Ito cells are observed only in adults, and not in young cats under 2 years old [17]; however, in this study, Ito cells in the porcine and bovine liver did not show such age-dependent changes. It is necessary to examine aged porcine and bovine livers to elucidate age-dependent changes.

Pigs, cows and cats are omnivores, herbivores and carnivores, respectively. They, therefore, have quite different food habitats, and also different metabolic systems, which may affect the size of lipid droplets in Ito cells; indeed, herbivores in nature have relatively low levels of liver vitamin A [11]. It is also reported that hepatocytic enzyme activities were different between carnivores and non-carnivores [1]. It is possible that such interspecies differences also exist in Ito cell morphology and function.

Vitamin A or its derivatives such as retinoids has been used to treat liver fibrosis induced either by hepatotoxic chemicals or heterologous serum in rats. When injecting retinol, both the suppression and acceleration of fibrosis have been observed [15]. A contradictory result has also been reported in porcine serum-induced rat experimental hepatic fibrosis treated with a stable retinoic acid analog [3, 8, 16]. As ECMs in hepatic fibrosis are produced by activated Ito cells, there may be some functional relationship between vitamin A accumulation and Ito cell activation. Although no positive reaction for α-SMA, a marker of activated Ito cells, was observed in porcine and bovine Ito cells in this study, some large vacuole-laden feline Ito cells were shown to be positive for α-SMA [17]; therefore, the amount of lipid in Ito cells may influence the morphology and function.
In conclusion, the histopathological characteristics of Kupffer cells and Ito cells in porcine and bovine liver differed from those of felines in terms of immunohistochemical features and lipid content. To clarify the reason, we should examine the livers of other animal species in addition to aged pigs and cows, and further elucidate the functional characteristics of Kupffer cells and Ito cells.

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REFERENCES