The Vitamin A-Storing Cells in the Human and Rat Pancreas

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Summary: Vitamin A-storing cells with lipid droplets and fluorescence characteristic of vitamin A were observed in the normal human and rat pancreas, using light, fluorescence, and electron microscopy. Vitamin A-storing cells in the normal human pancreas, as well as in the rat pancreas, were located predominantly in a perivascular area, and had lipid droplets with a fluorescence characteristic of vitamin A. Pancreatic vitamin A-storing cells had more numerous lipid droplets and more distinct fluorescence of vitamin A in vitamin A-loaded rats than in normal rats. In the rat, hepatic vitamin A-storing cells contained significantly more lipid droplets and had more distinct vitamin A fluorescence than pancreatic vitamin A-storing cells. Pancreatic sections from human subjects with chronic alcoholic pancreatitis revealed abundant vitamin A-storing cells surrounded by collagen fibers in an area of fibrosis. These results indicate that vitamin A-storing cells are widely distributed in the normal human and rat pancreas, although pancreatic vitamin A-storing cells have less storage capacity for vitamin A than do hepatic cells. Vitamin A-storing cells, as well as fibroblasts, may participate in the development of pancreatic fibrosis in chronic alcoholic pancreatitis, similar to the way hepatic vitamin A-storing cells participate in hepatic fibrosis.

Key words: vitamin A-storing cell—pancreatic fibrosis—chronic pancreatitis—Ito cell—hepatic fibrosis

Introduction

Fluorescence microscopy and autoradiography have revealed that the Ito cells, fat-storing cells in the liver, contain vitamin A in lipid droplets; and thus, the Ito cells are also called the vitamin A-storing cells (VASCs) in the liver (Popper, 1944; Nakane, 1963; Kent et al. 1976). These VASCs have been known to be distributed not only in the liver but also in many other kinds of tissues, such as, the stomach, intestine, lung, spleen, lymph nodes, and skin (Hirosawa and Yamada, 1980; Wake, 1980). This group of cells has been collectively called the vitamin A-storing cell system (VASCs system) (Yamada and Hirosawa, 1976). Whether the VASCs occur in the pancreas was not determined until Watari et al. (1982) described VASCs in the pancreas of vitamin A-loaded mice; but no study has attempted to identify the VASCs in the human pancreas.

The Ito cell currently receives attention for its potential role in fibrogenesis of the liver (Ballardini et al. 1983; De Leeuw et al. 1984; Clement et al. 1986; Davis et
al. 1987), especially intralobular fibrosis. In contrast, the pathogenic process resulting in pancreatic fibrosis is totally unknown.

In the present study, the fine structures of VASCs in the normal human and rat pancreas were investigated. The storage capacity of vitamin A in VASCs of the rat pancreas was compared with that of the Ito cell in the liver. VASCs, not previously described, were identified in the pancreas of patients with chronic alcoholic pancreatitis.

Materials and Methods

A. Experimental Study

Male Wistar rats, weighing 180 to 200 g (both control and vitamin A-loaded rat groups) were maintained on a chow diet (CE-2, Kyudo, Fukuoka) containing 1000 IU/100 g of vitamin A and water *ad libitum*. The rats were administered 25,000 IU/100 g body weight of vitamin A (retinyl palmitate, Eizai Co., Ltd., Tokyo), intramuscularly (designated vitamin A-loaded rats), daily for eleven days. On the fourth day, three vitamin A-loaded rats, and on the 12th day, three vitamin A-loaded and three control rats were sacrificed under anesthesia with 5 mg/100 g body weight of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) intraperitoneally. The liver and pancreas were examined by the following procedures. Several pieces of the resected liver and pancreatic tissue were fixed for 10 min with 10% formaldehyde solution in distilled water at room temperature, mounted in OCT compound (Tissue Tek II, Miles Laboratories, Naperville) and frozen in acetone at -80°C. Frozen sections of 6 μm thickness were prepared. The sections were mounted on glass slides, and observed with a fluorescence microscope (Leitz) under ultraviolet light (excitation wavelength of 330 nm) (Watari et al. 1982).

The remaining tissue was fixed by perfused fixation from the thoracic aorta, with 2% glutaraldehyde in 0.05 M cacodylate buffer, cut into 1 mm³ blocks, and fixed with glutaraldehyde and cacodylate for two hours at 4°C. The blocks were dehydrated with ethanol, and embedded in Epon 812 (Nissin EM, Tokyo), cut into 1 μm sections, stained with toluidine blue, and observed by light microscopy. Ultrathin sections were stained with lead citrate and uranium acetate, and observed using a transmission electron microscope (Hitachi H-500).

The number of VASCs and their lipid droplets from 6 specimens including 3 controls and 3 vitamin A-loaded rats was counted under a BH-2 Olympus light microscope. The number of VASCs was expressed as the number of cells per mm². The number of lipid droplets was calculated in 32 cells from control rats and 105 cells from vitamin A-loaded rats. The morphometry of hepatic VASCs was also determined as follows: transmission electron micrographs (×500 in magnification, 48 pictures) from 3 control and 3 vitamin A-loaded rats were analyzed for the number of VASCs (converted to cells per mm²) and lipid droplets. All data were expressed as mean ± SD and statistical analyses were performed with the student-t test.

B. Human pancreas

A normal human pancreas was obtained for pathological examination of a metastatic carcinoma during an operation for colon cancer. Pancreatic specimens were taken from three patients with chronic alcoholic pancreatitis (two pancreatic specimens were obtained during operations for chronic pancreatitis, and the other during a postmortem examination of a patient with chronic pancreatitis, who died of hepatocellular carcinoma). These specimens were investigated
Results

In the normal human pancreas, light microscopy showed acinar cells, ductal cells, islet cells, vascular cells, and a few interstitial cells. Some of the interstitial cells in the interacinar or interlobular area demonstrated one or two lipid droplets in the cytoplasm (Fig. 1). With the electron microscope, these interstitial cells were observed to have large lipid droplets, a large and occasionally triangular-shaped nucleus, a prominent rough endoplasmic reticulum (RER), abundant free ribosomes, a few mitochondria, and several cytoplasmic projections (Fig. 2a, b, c). The cisternae of the RER were partially dilated and contained flocculent material (Fig. 2a). Collagen fibers were also observed around the cells (Fig. 2a, b, c). The morphological features of these interstitial cells were very similar to the features of the Ito cells in the liver.

The pancreas of control rats contained interstitial cells with morphological features similar to those of the human pancreas. They have one or two lipid droplets (Fig. 3a) and had the characteristic bright green fluorescence of vitamin A, which rapidly fades under ultraviolet light (Fig. 3b). Electron microscopy also revealed large lipid droplets, well-developed RER with partially dilated cisternae, and numerous free ribosomes in the cytoplasm of these cells.

In the liver of control rats, Ito cells located in the space of Disse possessed two or three lipid droplets (Fig. 4) and showed distinct vitamin A fluorescence. The Ito cells in the liver contained more lipid droplets and had a stronger fluorescence than the interstitial cells of the pancreas.

The number of lipid droplets (1.03±0.18 lipid droplets per cell in controls, n=32) and the intensity of the fluorescence in the pancreatic interstitial cells gradually increased with the load of vitamin A; however, those cells had less than five lipid droplets per cell (1.78±1.22, n=105) even after vitamin A-loading for eleven days (Fig. 5a, b, c, d). In comparison, the Ito cells in the liver had 2.35±1.35 lipid droplets per cell (n=20), four to six lipid droplets after four days of loading with vitamin A (Fig. 6a), and finally 22.2±13.0 lipid droplets after eleven days of administration of vitamin A (n=66) (Fig. 6b, c and Fig. 7a). The fluorescence of the vitamin A in the Ito cells was always greater and more intense than the fluorescence in the pancreatic cells throughout the study (Fig. 8). The rat pancreatic cells with lipid droplets and vitamin A fluorescence after vitamin A-loading appeared to be preferentially distributed in the perivascular, interacinar, and interlobular regions (Fig. 5b, c, d). Furthermore, as observed by light microscopy, these cells were also increased in number after the treatment (4.8±3.2 to 19.5±6.9 cells/mm², n=6, p=0.001) as were the VASCs in the liver (54.3±54.2 to 196.1±120.5 cells/mm², n=23 and 21 respectively, p<0.001) (Fig. 7b).

The pancreas of patients with chronic alcoholic pancreatitis contained several interstitial cells with lipid droplets (Fig. 8a) and the fluorescence of vitamin A (Fig. 8b). The morphological features of these cells were similar to those previously described for interstitial cells in the normal human and rat pancreas. These cells were preferentially distributed in the fibrotic area where collagen fibers seemed to be associated very closely to these cells and were accompanied by fibroblasts. The fine structure of the cells among collagen fibers was very similar to the structure of fibroblasts (Fig. 9c, d).
Discussion

Ito described the cells as a characteristic cell in the human liver, located mainly in the space of Disse and containing lipid droplets in the cytoplasm. Hence, they became known as Ito cells (Ito, 1951). A more detailed morphology of the Ito cells in the liver was revealed by electron microscopy (Nakane, 1963; McGee and Patric, 1972; Tanikawa, 1975).

Recently, studies with fluorescence microscopy and autoradiography demonstrated that Ito cells store vitamin A in lipid droplets in the cytoplasm. The cells have subsequently become known as VASCs (vitamin A-storing cells) (Yamada and Hirosawa, 1976; Wake 1980); since VASCs are distributed in many organs. Ito cells are considered to be VASCs in the liver. VASCs are present in the stomach, intestine, lung, spleen, skin, and lymph nodes (Hirosawa and Yamada, 1980), as well. VASCs in the pancreas were first demonstrated in vitamin A-loaded mice, by N. Watari (1982), with fluorescence and electron microscopy. But VASCs in the human pancreas have not been previously described.

The present study shows that some of the interstitial cells in the pancreas of the normal human and control rats have morphological similarities to Ito cells, including lipid droplets, a large nucleus, well-developed RER, abundant free ribosomes, and extensive cytoplasmic projections. Since these cells demonstrate vitamin A fluorescence, they should be classified as VASCs in the pancreas; and the pancreas can be added to the list of tissues containing VASCs.

After vitamin-A loading, the intensity of the vitamin A fluorescence and the number of lipid droplets in the pancreatic VASCs gradually and significantly increased without any morphological changes of the other cytoplasmic organelles present VASCs. The number of observed VASCs in the rat pancreas also increased with the treatment. Vitamin-A loading may be the reason for the better observation of the VASCs by light and fluorescence microscopy, as an increased number of lipid droplets can be seen and there is a more distinct fluorescence.

Ito cells in the normal rat liver possessed several lipid droplets and vitamin A fluorescence. After accumulation of vitamin A for eleven days, the number of lipid droplets in the Ito cell increased to more than 20, and the fluorescence of vitamin A was also more distinct. The differences between VASCs in the pancreas and Ito cells observed in these studies suggest that the Ito cell may possess a higher capacity for storing vitamin A than the pancreatic VASCs or, that the Ito cell is more closely associated with vitamin A metabolism than the pancreatic VASCs.

The Ito cells have received attention for their potential role in hepatic fibrosis, especially intralobular fibrosis (Schnack et al. 1967; Kent et al. 1976; Senoo et al. 1982; Minato et al. 1983; Ballardini et al. 1983; Clement et al. 1986). The Ito cells are presumed to be resting fibroblasts for several reasons. No fibrogenetic cells, like fibroblasts, are found in the intralobular area of the normal liver. Bundles of collagen fibers are frequently observed in very close association with Ito cells (Tanikawa, 1975). The Ito cells are very similar to the fibroblasts in fine structure, except for the number of lipid droplets. Many Ito cells are present around fibrotic lesions in the liver during chronic hepatitis or experimentally induced fibrosis by CCl₄ (McGee and Patric, 1972). Finally, cultured Ito cells produce collagen and fibronectin (De Leeuw et al. 1984; Davis et al. 1987).

Chronic pancreatitis is always accompanied by fibrosis irrespective of its etiology; however, the mechanisms of pancreatic fibrosis have not been clarified.
The present study reveals that VASCs in the normal human pancreas are associated with a few collagen fibers surrounding the cells, however, VASCs in the pancreas of subjects with chronic pancreatitis are embedded in collagen fibers in the areas of fibrosis. If the analogy of the role of the VASCs in the liver (Ito cells) on hepatic fibrosis can be extended to the pancreas, the VASCs in the fibrotic pancreas might participate in the fibrotic process as well. In the liver, Ito cells are commonly thought to be involved in the development of fibrosis, especially in alcoholic liver disease; and the present study indicates that the pancreatic VASCs should be further investigated in other types of chronic pancreatitis. Since the pancreatic VASCs are distributed in the fibrotic area with fibroblasts, it should further be determined whether the pancreatic VASCs a) are directly involved in fibrogenesis of the pancreas, b) are cellular precursors to the fibroblasts, or c) are converted from fibroblasts by the vitamin A supply.

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References


Fig. 1. Vitamin A storing cells (VASCs) in the normal human pancreas (1 μm section with toluidine blue stain). The VASC (indicated by an arrow) located in the interacinar region possesses a lipid droplet (×450).

Fig. 2. VASCs in the normal human pancreas, a) in the interacinar region (×7,000), b) around a blood vessel (V) (×6,000), and c) in the interacinar region (×8,000). VASCs possess lipid droplets (L), Well-developed RER with partially dilated cisternae, and cytoplasmic projections. Collagen fibers (Co) were observed close to the VASCs.
Fig. 3. VASCs in the pancreas of a control rat. The pancreas of the control rat contained VASCs (indicated by the arrow) with lipid droplets near a blood vessel. 
a) A light micrograph of a section was stained with toluidine blue (×450).  b) Fluorescence microscopy under ultraviolet light demonstrated VASCs (arrows) with a bright green fluorescence characteristic of vitamin A (×1,100).

Fig. 4. An Ito cell in the liver of a control rat, that was stained with toluidine blue and observed with light microscopy (×450). The Ito cell (indicated by the arrow) with two lipid droplets was located in the perisinusoidal area.
Fig. 5. VASCs in the pancreas of rats loaded with vitamin A (25,000 IU/100g body wt/day, intramuscular injection) for eleven days. Each cell contained several lipid droplets (1) (a, b, c, d), as observed in a) a light micrograph (×450); (VASC indicated by the arrow), b) an electron micrograph (EM) (×14,000) of the interacinar region, c) an EM (×12,000) near a blood vessel (IV), d) an EM (×7,000) in the interlobular region.
Fig. 6. The Ito cells (1) in the livers of rats that were loaded with vitamin A for four days (a) or eleven days (b, c) have greater numbers of lipid droplets (L) than do the VASCs in the pancreas as shown in a) an electron micrograph (EM) (×9,000), b) a light micrograph (×450), and c) an EM (×8,000).
Fig. 7. Comparison of the number of lipid droplets (a) and number of cells (b) in controls and rats loaded with vitamin A for eleven days. Note the greater number of both lipid droplets and cells in the liver (hatched columns) relative to the pancreas (open columns), in control as well as in vitamin A-loaded groups.
Fig. 8. Vitamin A fluorescence in VASCs of rats loaded with Vitamin A for eleven days. With fluorescence microscopy, the bright green fluorescence which is characteristic of vitamin A was seen in a) the rat pancreas ($\times1,100$) and, b) the rat liver ($\times1,100$). More intense and more numerous fluorescence was observed in the liver than in the pancreas.

Fig. 9. VASCs in the pancreas of patients with chronic alcoholic pancreatitis. The cells with lipid droplets (L) were embedded in collagen fibers (Co), as shown in a) a light micrograph ($\times450$), b) a fluorescence micrograph where VASCs were indicated by arrows ($\times1,100$), c) and d) electron micrographs: (c. $\times12,000$; d. $\times7,000$).