EFFECT OF COLCHICINE ON THE THREE-DIMENSIONAL ORGANIZATION OF ELONGATED LYSOSOMES (*NEMATOLYSOSOMES*) IN RAT PANCREATIC EXOCRINE CELLS

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The effect of colchicine on the organization of lysosomal system in rat pancreatic exocrine cells was three-dimensionally examined by 2 μ m semithin section electron microscopy combined with acid phosphatase (ACPase) cytochemistry. In control rat pancreatic exocrine cells, ACPase activity was localized in *trans* Golgi cisternae, usual spherical lysosomes, and elongated lysosomes (nematolysosomes). The distribution of lysosomes was geographically characteristic. Most spherical lysosomes were located around the Golgi complex. The nematolysosomes elongated from near basolateral plasma membrane to the Golgi area. In colchicine-treated rats which were intraperitoneally injected with 5 mg/kg B. W. of colchicine 4 hr before fixation, the normal distribution of lysosomes was strikingly disorganized. The spherical lysosomes increased in number and size. The nematolysosomes appeared to be fragmented or shrunk, and change their form to spherical. The normal appearance of lysosomes was recovered at 12–24 hr after colchicine injection. The changes in distribution and shape of lysosomes by colchicine seemed to be correlated with the integrity of microtubules. The morphological association of microtubules with nematolysosomes was also revealed by electron microscopy. These results suggest that the microtubule is essential for nematolysosomes in pancreatic exocrine cells.

Recently, the presence of elongated lysosomes, termed "nematolysosomes" or "tubular lysosomes", has been increasingly known in variety cell types, such as macrophages (2, 3, 8–10, 22, 23, 25), hepatocytes (14), exocrine cells (4–6, 15–18), smooth muscle cells (20) and others (12, 21, 24). The characterization of nematolysosomes has been studied most extensively in macrophages (2, 3, 8–10, 22, 23, 25), although the physiological role of the lysosomes in other cell types is still unknown. In macrophages, nematolysosomes could be induced by pinocytosis (2, 3, 8–10, 22, 23), but not by phagocytosis (10, 22, 23). The formation of nematolysosomes was dependent on the integrity of microtubules (2, 3, 22, 23), and kinesin is a candidate for microtubule-based motor translocating nematolysosomes (9).

In exocrine cells, Oliver (16) initially found

elongated lysosomes in the basal portion of the cell, and termed them basal lysosomes. Similar to nematolysosomes in macrophages, the basal lysosomes in pancreatic exocrine cells were associated with endocytic activity (15–17). Nevertheless, in earlier reports (6, 16), the basal lysosomes had been distinguished into a unique population of lysosomes by the cytochemical characterization, i.e., they lack acid phosphatase activity (ACPase), but not trimetaphosphatase activity. Most recently, however, we demonstrated ACPase activity in the basal lysosomes, as well as nematolysosomes in macrophages, by an improvement of fixation (4). Therefore, we proposed that the basal lysosomes also could be categorized in a member of nematolysosomes. This proposition gives rise to the next question of whether microtubules are an essential factor for the regulation of nematolysosomes both in exocrine cells, polarized cells, and in macrophages, non-polarized cells. In this paper, to address the question, the effect of colchicine on the organization of lysosomes in pancreatic

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exocrine cells has been examined by three-dimensional electron microscopy combined with ACPase cytochemistry. The morphological relationship of microtubules to nematolysosomes has also been examined by conventional electron microscopy. A preliminary report of our result has been published in an abstract form (5).

MATERIALS AND METHODS

Animals and Drug Treatments

Male and female Wistar rats, weighing 200–400 g, were used for experiments. Colchicine (5 mg/kg body weight) freshly dissolved in saline was intraperitoneally injected to rats. Control rats were untreated or received an intraperitoneal injection of saline. Rats were sacrificed at 0.5, 1, 4, 8, 12 and 24 hr after the injection.

ACPase Cytochemistry and Electron Microscopy

Pancreas was perfused via the left ventricle with a fixative consisting of 2% paraformaldehyde and 0.25% glutaraldehyde in 30 mM PIPES buffer, pH 7.2, containing 10% dimethyl sulfoxide (DMSO) and 8% sucrose. For ACPase cytochemistry, removed pancreas was minced and immersed in the same fixative for 60 min. In order to avoid the depolymerization of microtubules by a low temperature treatment, the process of fixation was carried out at room temperature (above 22°C). Fixed tissues were rinsed in 30 mM PIPES buffer, pH 7.2, containing 10% DMSO and 8% sucrose and cut into 50 μ m sections with a cryostat or a Microslicer (Dosaka EM, Kyoto). The tissues were incubated in a Gomori-type medium for 30 min at room temperature. After incubation, the tissues were rinsed in the buffer without DMSO, and post-fixed with PIPES-buffered 1% osmic acid. The tissue processing and the preparation of the ACPase incubation medium were previously described in detail (1).

For conventional electron microscopy, perfused tissues were minced and immersed in a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde for 90 min. Then, the tissues were rinsed in a buffer and post-fixed with 1% osmic acid and blocked with uranyl acetate. Then, these tissues were dehydrated and embedded in Spurr's resin. Ultrathin sections were cut with an LKB Ultratome, and stained with uranyl acetate and/or lead citrate, and observed under a TEM (Hitachi H-800) at 100 kV. For three-dimensional electron microscopy, 2 μ m-thick sections of pancreatic tissues which were cytochemically reacted for ACPase activity were observed under a TEM at 200 kV. Stereo-paired elec-

tron micrographs were taken at tilting angles of $\pm 8^\circ$ with a goniometer.

Immunofluorescence Cytochemistry

Rat pancreas was fixed with 2% paraformaldehyde and 0.25% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.4, for 1 hr at room temperature. After rinse in PBS, successively in PBS containing 10, 15 and 20% sucrose (2 hr each), 4–5 μ m frozen sections were cut with a cryostat. The sections were adhered to poly-L-lysine coated slide glasses, covered by 1% gelatin in PBS, and reacted with monoclonal antibody against alpha-tubulin (Bio-Makor) or polyclonal rabbit anti-tubulin (a gift from Dr. T. Fujimoto of Kyoto University). After rinse in PBS, the sections were reacted with fluorescein isothiocyanate (FITC)-labeled secondary antibody. In controls, incubation with the primary antibody was omitted. These specimens were examined using an epifluorescence microscope (Olympus BH2-RFK).

RESULTS

In control rats, ACPase activity was demonstrated on usual spherical lysosomes, nematolysosomes, trans-Golgi cisternae and condensing vacuoles in pancreatic exocrine cells (Fig. 1a). As mentioned in our previous reports (4), the three-dimensional electron microscopy combined with ACPase cytochemistry manifested that nematolysosomes were generally elongated from near basolateral plasma membrane to Golgi area (Figs. 1b, 2a). Some nematolysosomes run crossways at the basal portion of the cell (Fig. 2b). They often branched and anastomosed with each other to make a network system in the cytoplasm. (Figs. 2a, b). Continuity to spherical lysosomes and the adjacency to Golgi complex were also frequently observed (Fig. 2c).

At 0.5 hr after colchicine injection, alteration of lysosomes was negligible. One hour after the colchicine injection, the number of nematolysosomes decreased. Glomeration of nematolysosomes was occasionally seen in the cytoplasm (Data not shown). At 4 hr after colchicine injection, the colchicine effect on the pancreatic exocrine cells was most striking. Morphologically, disorganization of Golgi complex and increase in number of autophagosomes were seen. These morphological findings were coincident with earlier data using colchicine (19) or vinblastine (7, 11). The three-dimensional distribution of lysosomes was markedly altered by the colchicine treatment. Nematolysosomes disappeared in the cell. In contrast, spherical lysosomes increased in number and size. (Fig. 3). Eight hours after colchicine injection, a

FIGS. 1a, b. Cytochemical localization of ACPase activity in control rat pancreatic exocrine cells. a. In the ultrathin section, ACPase activity was seen in *trans*-Golgi cisternae, condensing vacuoles, spherical lysosomes and nematolysosomes (arrows). $\times 9,500$ b. In the $2\ \mu\text{m}$ thick section, the elongation of nematolysosomes is notably demonstrated. $\times 11,000$ G, Golgi complex; N, nucleus.

FIGS. 2a-c. Stereo-paired electron micrographs of 2 μ m thick sections showing three-dimensional distribution of ACPase-positive lysosomes in control rat pancreatic exocrine cells. a. Longitudinal section of the cells. Most nematolysosomes are elongated from near basolateral surface toward Golgi complex. $\times 3,300$ b. Cross-cut section of the basal part of the cell. Some nematolysosomes run crossways in the cell. $\times 4,500$ c. Continuity of the nematolysosome to spherical lysosomes (arrow) and the adjacency to Golgi complex (G) (arrowhead) are observed. $\times 9,100$ S, secretory granules; N, nucleus.

few nematolysosomes reappeared in the cells. After 12 to 24 hr of colchicine injection, the number, size, shape and distribution of lysosomes appeared to recover to almost normal (Fig. 4).

Immunofluorescence microscopy using anti-tubulin antibody showed that microtubules exhibited radial or reticular distribution predominantly at the apical side in control pancreatic exocrine cells. This finding coincides with a report by Kurihara and Uchida (13). A small amount of microtubules was also seen at the basal part of the cell. Colchicine injection caused reduction of microtubules. At 4 hr after

the colchicine treatment, no microtubules were seen in the exocrine cell. The almost normal profiles of microtubules were observed again in the cell after 12 to 24 hr of colchicine treatment (Data not shown).

Morphological relationship between microtubules and nematolysosomes in control rats was examined by conventional electron microscopy. It was revealed that nematolysosome-like structures were elongated along microtubules, and short cross-bridge structures were present between them (Fig. 5). On the section of conventional electron microscopy without ACPase cytochemistry, we could not make sure that the struc-

FIG. 3. Stereo-paired micrographs of 2 μ m thick section of pancreatic exocrine cells after 4hr of the colchicine injection. Localization of ACPase activity exhibits that nematolysosomes are absent from the cell, but spherical lysosomes and autophagosomes (arrow) increased in number and size. $\times 4,500$

FIG. 4. Stereo-paired micrographs of 2 μ m thick section of pancreatic exocrine cells after 12 hr of the colchicine injection. Distribution of nematolysosomes is recovered to normal arrangement. $\times 5,200$

FIG. 5. Conventional electron micrograph of nematolysosome in the control rat pancreatic exocrine cell. The close association of microtubule (large arrows) with the nematolysosome (NL) is seen. Note the cross-bridge structures (small arrows) between microtubule and nematolysosome. A mitochondrion (M) is also associated with the nematolysosome and the microtubule. $\times 75,000$

ture was nematolysosomes, however, the structure had a morphological feature of lysosome, i.e. "halo" beneath the limiting membrane. A mitochondrion was closely associated with the microtubule. Thus, the microtubule appeared to be sandwiched between the two (Fig. 5).

DISCUSSION

In our previous reports (1, 4), ACPase activity could be cytochemically demonstrated in nematolysosomes of rat pancreatic exocrine cells by an improvement of fixation. The three-dimensional microscopy combined with ACPase cytochemistry seemed to be an useful tool to investigate the morphological profiles of nematolysosomes in a whole cell. Therefore, in the present paper, we extended the previous report and examined the three-dimensional organization of nematolysosome system in pancreatic exocrine cells and its dependence on intact microtubules.

In macrophages, the nematolysosomes elongate radially from perinuclear region to periphery (2, 3, 8-10, 22, 23), and the elongation of nematolysosomes seems to result from bi- or unidirectional lysosomal movement based on microtubules (2, 3, 9, 22). In polarized cells such as exocrine cells, however, the distribution pattern of nematolysosomes appeared to be different from that in non-polarized cells such as macrophages. Our findings indicated that nematolysosomes were present only at the lower (basal) half part of rat pancreatic exocrine cells. Most nematolysosomes extended longitudinally from near the basolateral plasma membrane to Golgi area of the exocrine cells. Some nematolysosomes also run crossways in the cell along the basolateral plasma membrane. These nematolysosomes connected with each other forming a network system at the basal half of the cell. The continuity or adjacency of nematolysosomes to Golgi complex and spherical lysosomes was also revealed by three-dimensional electron microscopy. Such organization of nematolysosomes including the relationship with Golgi complex and spherical lysosomes seemed to be considerably characteristic and meaningful. Oliver (15-17) reported that horseradish peroxidase was endocytosed from basolateral surface and sequestered in basally located tubular structures in pancreatic exocrine cells. If the tubular structures are correspondent to nematolysosomes, the nematolysosomes may be involved in the endocytosis, degradation and intracellular transport of macromolecules taken up from basolateral surface. Physiological significance of the nematolysosome pathway from basolateral surface to Golgi area would

be investigated in further studies.

Thus, distribution pattern of nematolysosomes in exocrine cells was different from that in macrophages. However, the effect of colchicine on the organization of nematolysosomes might be the same in both cell types. After colchicine injection, nematolysosomes decreased in number with time and then disappeared completely from the exocrine cells at 4 hr, in contrast to the increase of spherical lysosomes in number and size. Glomeration of nematolysosome which was occasionally seen at 1 hr of colchicine treatment may be an earlier change than the disappearance of nematolysosomes. Possible interpretation is that the nematolysosomes were initially glomerated as a result of the loss of microtubule support, then the glomerated nematolysosomes shrank more and changed their form to spherical. After 12 to 24 hr of colchicine injection, the three-dimensional organization of nematolysosomes recovered to normal. This result indicates that the effect of colchicine is reversible. The change of nematolysosome with lapse of time after colchicine treatment was correlated with the change of microtubules revealed by immunofluorescence microscopy. The correlation supports the hypothesis that the disorganization of nematolysosomes results from microtubule depolymerization, although colchicine may have other effects on several cellular metabolisms. In addition to the change of nematolysosomes by colchicine, we observed a morphologically close relationship of microtubules to nematolysosomes in normal exocrine cells. Similar findings have also been reported in macrophages (2, 22). The nematolysosomes may be moved along microtubules using ATP-dependent microtubule-based motors such as kinesin (9). The cross-bridge structures between nematolysosomes and microtubules are presumed to be microtubule-based motors. The association of mitochondria with microtubules which attached to nematolysosomes may suggest that the mitochondria supply ATP to the microtubule-based motors, which have ATPase activity. It was known that microtubules in upper (apical) side of exocrine cells were involved in the intracellular protein transport and the translocation of secretory granules (12). The role of microtubules basally located in exocrine cells has not been investigated till now. Our results revealed that basal microtubules, at least some of them, participate in the organization of the nematolysosome system in exocrine cells.

In conclusion, the present study pronounces that the common mechanism, by which the intracellular movements of nematolysosomes are regulated, is present both in non-polarized and polarized cells.

Surveying common characterization of nematolysosomes in various cell types to resolve their physiological significance, it seemed to be of particular interest that nematolysosomes in both cell types have a common nature.

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