FATE OF THE GOLGI CISTERNAE AFTER COLCHICINE TREATMENT

TORU NODA AND KAZUO OGAWA

Department of Anatomy, Faculty of Medicine, Kyoto University, Konoe-Cho, Yoshida, Sakyo-ku, Kyoto 606-01

Received for publication January 29, 1992

A single intra peritoneal injection of colchicine to mice causes both functional and morphological changes on Golgi apparatus in pancreatic exocrine cells. Structural changes included shrinkage of Golgi area in the secretory cell, whirling of the Golgi cisternae, decrease of the Golgi cisternae and increase of small vesicles around the remnants of Golgi cisternae. Cytochemical distribution of Golgi marker enzyme, nicotinamide adenine dinucleotide phosphatase (NADPase) which was found in the middle cisternae of Golgi stack in control, decreased the activity and expanded to the entire Golgi stack and in most small vesicles. Also by zinc-iodide-osmium (ZIO) stain which preferably stained cis-side of Golgi stack in control, the entire Golgi stacks were stained as well as these small vesicles. From these observation, by colchicine treatment, the NADPase and the osmium-reducing substance in the cis Golgi cisternae, first appeared to expand its distribution to all the Golgi components. Then, Golgi cisternae appeared to reduce the size either by vesiculation of the cisternae or by trimming the cisternae as cisternal whirling into autophagosomes.

The Golgi apparatus is a highly polarized organelle which assembles the differentiated subcompartments in the supranuclear cytoplasmic region. This organized structure of Golgi apparatus is known to be disrupted by the microtubule depolymerizing drug, colchicine (7, 11, 12). The main structural changes are an increase of small vesicles and a reduction in the size of the Golgi apparatus. The mechanisms which explain both events caused by colchicine are not well understood. To examine any movements of Golgi components after colchicine treatment, we investigated the fate of the Golgi components using cytochemical techniques; enzyme cytochemical demonstration of NADPase and osmium impregnation by ZIO method. The distribution of two Golgi markers were studied on the of colchicine-treated mouse pancreas.

MATERIALS AND METHODS

Animals

Colchicine dissolved in physiological saline was administered intraperitoneally (0.6–0.7 mg/ 100 g body weight) to ddY-strain mice. The animals were sacrificed by perfusion fixation from the left ventricle, and the pancreas was excised into 1 mm³ pieces and immersed in the same fixative for 2.5 hr. The fixative

contained 2% glutaraldehyde and 5% sucrose in 0.1 M cacodylate buffer, pH 7.4. The tissue blocks were rinsed with 0.1 M cacodylate buffer, pH 7.4, containing 8% sucrose. Then, the tissue was processed in three different ways described as following. After post-fixation, all the specimens were stained *en bloc* in 0.5% uranyl acetate aqueous solution for 30 min at 40°C in dark, dehydrated and embedded in Spurr's resin.

Morphology

The tissue was post-fixed with 1% osmium tetroxide, 1.5% potassium ferrocyanide aqueous solution for 2 hr at room temperature (4).

NADPase enzyme cytochemistry

The tissue was sliced with a Microslicer (Dosaka EM Co., LTD, Japan) with 40 μ m thickness and incubated in the medium for demonstration of NADPase (15), modified by Clermont *et al.* (1) for 40–60 min at room temperature. After the reaction, the sections were post-fixed with 1% osmium tetroxide, 1.5% potassium ferrocyanide aqueous solution for 1 hr in room temperature.

ZIO stain

The tissue was rinsed again briefly with Tris-HCl buffer, pH 7.4. The composition of Tris-HCl buffer was 50 mM Tris, 0.57 M NaCl, 5 mM CaCl₂, and 15 mM MgCl₂, and pH was adjusted to 7.4 with

HCl. Zinc-iodide solution was prepared by filtering the suspension of the mixture of 3 g Zinc powder in 20 ml distilled water and 1 g iodine crystal by filter paper. Zinc-Iodide-Osmium (ZIO) solution was the mixture of Zinc-iodide solution, Tris-HCl buffer, pH 7.4 and 4% osmium tetroxide aqueous solution in volume ratio, 2 : 1 : 1 (9). The tissue pieces were incubated in the ZIO solution for 16–24 hr at 4°C in dark.

Ultrathin section of all the specimens were cut with Ultratome III (LKB, Sweden) and stained with 2% uranyl acetate and 0.4% lead citrate or 0.4% lead citate only. Observation of the ultrathin sections were done with a JEM 1200 EX (JEOL, Japan) or a JEM 2000 EX (JEOL, Japan) electron microscope.

RESULTS

Changes in staining patterns of Golgi markers after colchicine treatment

Structural changes of pancreatic exocrine cells by colchicine treatment were similar to the previous reports (7, 11, 12). Major changes by the drug were induction of autophagic vacuoles, increase of Golgi vesicles, and shrinkage of the Golgi stack (Figs. 1, 2). Although increase of the vesicles occurred in the vicinities of the Golgi stacks at 4 hr after the injection in our experiment, some Golgi cisternae still maintained their stacks at 12 hr after the drug injection (Fig. 5). The intensity of enzyme cytochemical activity of NADPase, which was normally detected in the in-

termediate to *trans* cisternae in control, decreased (Figs. 4, 5). The weak reaction products were observed in most Golgi cisternae. However, the reaction products were not observed in ER compartment including the transitional cisterna facing to *cis*-most Golgi cisterna (Figs. 5, 6).

By ZIO stain, the specificity of osmium reduction in *cis* Golgi cisternae in control was not observed in the pancreatic acinar cell of colchicine-treated mice (Figs. 8–10). The luminal contents of most Golgi cisternae including the *trans* cisternae of Golgi stack and the small vesicles around the Golgi stacks were heavily stained by electron-dense reaction deposits (Figs. 9, 10).

Occurrence of small vesicles with Golgi-markers

From 4 hr after the injection, the number of vesicles around the Golgi apparatus increased. The size and the morphological appearance of the vesicles resembled the transitional vesicles which appeared to bud off from the ER surface to Golgi *cis*-most cisterna (Figs. 1–3). Indeed, the budding images were often observed in the transitional part of ER and the number of transitional vesicles appeared to increase in the cytoplasm between *cis*-Golgi cisterna and the transitional ER after colchicine treatment (Figs. 1, 2, 5–7). However, increased vesicles were found not only in the transitional region, but also in the *trans* Golgi cytoplasm (Figs. 1, 3, 10). In the advanced stage (24 hr after the injection), the fragments of Golgi cisternae were predominantly found in the outside of the

FIG. 1. Morphology of colchicine-treated (6 hr after the injection) mouse pancreatic exocrine cell. The Golgi stack is well maintained despite of the drug treatment. Arrows indicate the nests of accumulated small vesicles. Although some small vacuoles and *trans* Golgi network (TGN) appear to be coated with clathrin-like fuzz (arrowheads), most small vesicles in the nests do not have such an apparent coat. TGN: *trans* Golgi network. Bar=0.5 μ m

FIG. 2. Morphology of colchicine-treated (6 hr after the injection) mouse pancreatic exocrine cell. The Golgi cisternae appear to be folded and rolled up into a myelin-like figure (arrow). The membranes of Golgi cisternae and those of the myelin structure appear to be continuous but show different membrane profiles which suggest degeneration of the Golgi membrane. The occurrence of the rolls of Golgi stacks were often observed in the colchicine-treated mouse pancreatic acinar cells (see Figs. 6, 9). This structure is considered as one nascent form of autophagosomes. Bar=0.5 μ m

FIG. 3. Morphology of colchicine-treated (24 hr after the injection) mouse pancreatic exocrine cell. Numerous small vesicles are found in Golgi area. Remnants or fragments of Golgi cisternae are seen in this area (arrow). Bar=0.1 μ m

FIG. 4. Enzyme cytochemical activity of NADPase in normal mouse pancreatic exocrine cell. The heavy reaction products are found in the intermediate to *trans* cisternae of the Golgi stack. Bar=0.5 μ m

FIG. 5. Enzyme cytochemical activity of NADPase in colchicine-treated (12 hr after the injection) mouse pancreatic exocrine cell. The weak reaction products are found in the most Golgi cisternae. The inhibition and expanded distribution of NADPase was recognized in the Golgi stack including the *trans* and *cis* vesicles. Although the transitional vesicles contain very weak reaction products, no reaction products were found in ER cisternae and the buddings (arrowheads) from transitional ER. Bar=0.1 μ m

FIG. 6. Enzyme cytochemical activity of NADPase in colchicine-treated (12 hr after the injection) mouse pancreatic exocrine cell. The weak reaction products are found in the transitional vesicles, but no reaction products were found in ER cisternae and the buddings (arrowheads) from transitional ER. Bar=0.1 μ m

FIG. 7. Enzyme cytochemical activity of NADPase in colchicine-treated (12 hr after the injection) mouse pancreatic exocrine cell. The weak reaction products are found in the whirling of the Golgi cisternae (arrowhead) and lysosome (LYS). This roll appears to be a nascent form of autophagic vacuole. Bar=0.1 μ m

FIG. 8. Golgi apparatus of normal rat pancreatic exocrine cell stained by ZIO method. *Cis* Golgi cisternae and some transitional vesicles are heavily stained by ZIO stain. Bar=0.5 μ m

FIG. 9. Colchicine-treated (6 hr after the injection) mouse pancreatic exocrine cell stained by ZIO method. Almost all the Golgi components including the *trans* cisternae are heavily stained by this method. A part of Golgi stack shows cisternal whirling and the cisternal organization of the Golgi stack is lost (arrow). Transitional vesicles also stained by this method. Bar=0.1 μ m

FIG. 10. Colchicine-treated (24 hr after the injection) mouse pancreatic exocrine cell stained by ZIO method. Numerous accumulated small vesicles contained the reaction products as well as the fragments of the Golgi cisternae (arrowhead). The fragments of Golgi cisternae tend to localize outside of the vesicular nest. Bar=0.1 μ m

vesicular nests (Figs. 3, 10). The weak reaction products of Golgi marker enzyme, NADPase were observed in these structures. Although the intensity of the enzyme reaction in these vesicles was very weak, the distinction by the deposits of reaction products between the small vesicles and the budding portion of transitional ER appeared to be very strict (Figs. 5-7). Although the morphology of the transitional vesicles and the budding portions of ER were very similar, the reaction products of NADPase was observed in the former, but not in the latter. The similar staining pattern was also observed by ZIO stain. The small vesicles in the transitional regions were heavily stained as well as the Golgi stacks but the ER compartment was, again, devoid of ZIO stain (Figs. 8, 9).

Moreover, among the small vesicles around the Golgi stack, there appeared to exist two types of small vesicles. One type was relatively large and of polymorphic structure with apparent coat which was often found in the *trans* Golgi cytoplasm. The other vesicles which increased by the drug treatment, were uniform in the size (50-60 nm in diameter) and did not have an apparent coat like clathrin (Fig. 1).

Golgi cisternae are trimmed by cisternal whirling

Formation of autophagic vacuoles were dramatically induced by colchicine treatment in pancreatic exocrine cells. The contents of the most autophagic vacuoles did not appear to be targeted to selective organelles. Almost all the organelles in the cell were observed in the autophagic vacuoles. Among the nascent autophagic vacuoles, some degenerative myelin structures often appeared to arise from Golgi cisternae (Fig. 2). As membrane of Golgi cisternae had continuity with membranous component of myelin-like structure, it is apparent that myelin-like structure was formed from Golgi cisternal whirling. Both weak activity of NADPase and heavy deposits of reduced osmium by ZIO stain were observed in these nascent vacuoles as well as the Golgi cisternae (Figs. 7, 9). In addition to that, the reaction products of NADPase were also often found in the lysosomes in the vicinity of the Golgi apparatus (Fig. 7).

DISCUSSION

Expansion of the intermediate- and cis-markers to whole Golgi compartments after colchicine treatment

The existence of the polarity in the organelle is one of the major characteristics in Golgi apparatus, although the mechanism for maintaining the polarity is unknown. With colchicine, the disruption of the polarity was demonstrated in our experiment.

Already before the morphological changes in the Golgi cisternae are noted, the localization of weak NADPase activity and reduced osmium deposits by ZIO stain tended to expand to all the Golgi components. Both markers in control which were observed either in the intermediate to *trans* cisternae for NADPase or the *cis* cisternae for ZIO stain, respectively, changed their localization to all the cisternae and the small vesicles around the cisternae. The cytochemical intensity of NADPase indeed decreased in the intermediate cisternae, but the weak reaction products rather expanded to other compartments which were not observed in control. A similar inhibitory effect of colchicine was reported about the *trans*-Golgi marker enzymes; acid phosphatase, thiamine pyrophosphatase and inosine diphosphatase (12, 16). Although the decrease of the staining intensity by colchicine was not observed in ZIO stain, the homogeneous distribution of reduced osmium was, again, observed in all the Golgi elements (Figs. 9, 10).

The increased vesicles also acquired the Golgi markers, although they had similar morphology to that of the transitional vesicles. One possible interpretation of our cytochemical labelings in the Golgi apparatus is early maturation of pre-Golgi compartments (transitional vesicles) and the remaining of *cis*- or intermediate-specific substances in *trans* cisternae. In general, specific distribution of the Golgi markers could be understood either as expression of the fixed resident substance *in situ* or as that of the transient substance processed *in situ* along the secretory pathway. Although changes in the localization of the Golgi markers by colchicine may occur either by the sorting disorder including intercisternal carrier vesicles or by early maturation of some substances in pre-Golgi compartments, the details are left to be elucidated. The inhibition of the Golgi marker enzyme and the expansion of the distribution may be related to the two major actions of colchicine; depolymerizing action on microtubules which promote the movement along secretory pathway and the direct action on the Golgi membrane where the enzyme may bind (6).

Origin of the accumulated vesicles being associated with Golgi apparatus

Three possibilities could be considered about the origin of the increased vesicles. Increase of the transitional vesicles, vesiculation of the Golgi cisternae, and the vesicle formation from condensing vacuole or zymogen granules. The third possibility would be difficult to conceive, because condensing vacuole and zymogen granules were not labeled with the two Golgi

markers (data not shown). As the entire Golgi stack were labeled with the two Golgi markers, the Golgi cisternae would be the most probable source of the increased vesicles. Moreover, the participation of the transitional vesicles in the increased vesicles also cannot be denied for the same reasons. According to the biochemical study of liver, the transport of secretory protein, pro-albumin, from ER to Golgi apparatus is not inhibited by colchicine treatment (13). Actually the frequent observations of the budding images after colchicine treatment may reflect the biochemical data.

The existence of two different coats were reported; clathrin (2) and non-clathrin (10) on the Golgi vesicles. However, existence of the coat on the increased vesicles was not clear from this experiment. A morphologically apparent clathrin-like coat could be identified on some vesicular structures in the trans Golgi cytoplasm and the part of trans Golgi network, but not on the increased vesicles (Fig. 1). Existence of non-clathrin coat on the increased vesicles was left to be elucidated.

Golgi apparatus reduces its size by the cisternal whirling

Numerous autophagic vacuoles arose in the cytoplasm by colchicine treatment. Similar autophagosome induction was reported with other drugs, vincristine, vinblastine and ethionine (3, 8). Most autophagosomes observed in our experiment contained all the intracellular organelles; ER, mitochondria and zymogen granules. From the various contents, the target organelles appeared to be non-selective.

The involvement of the Golgi component in formation of autophagic vacuole was repeatedly suggested by the presence of reduced osmium deposit in autophagic limiting membrane or the gap between the limiting membrane and the contents (5, 14). In our experiments, similar findings were observed by ZIO technique. But our findings further suggest the possibility of the direct pathway from Golgi apparatus to autophagosome by the cisternal whirling. By the fact that two Golgi markers, NADPase and the reduced osmium were demonstrated in the cisternal rolls as well as the Golgi stacks, the possibility of participation of Golgi element in the formation of autophagic vacuoles was strongly suggested. The co-distribution of the reaction products of NADPase both in the myelin-like degenerating Golgi cisternae and the lysosomes also suggests the involvement of Golgi cisternae for the autophagolysosome formation. However, the number of autophagic vacuoles with these Golgi markers were still small in portion to all the autophagic vacuoles induced by colchicine in the secretory cells. Therefore, at least, two different

mechanisms appear to exist in the autophagosome formation in the colchicine-treated pancreatic exocrine cells in mouse.

REFERENCES

1. Clermont, Y., Lalli, M. and Rambourg, A.: Ultrastructural localization of nicotinamide adenine dinucleotide phosphatase (NADPase), thiamine pyrophosphatase (TPase), and cytidine monophosphatase (CMPase) in the Golgi apparatus of early spermatids of the rat. *Anat. Record*, 201; 613-622, 1981.
2. Croze, E. M. and Morré, D. J.: Distribution of clathrin and spinycoated vesicles on membranes within mature Golgi apparatus elements of mouse liver. *Eur. J. Cell Biol.*, 28; 130-138, 1982.
3. Ekholm, R., Edlund, R. and Zelander, T.: The ultrastructure of the rat exocrine pancreas after brief ethionine exposure. *J. Ultrastruct. Res.*, 7; 102-120, 1962.
4. Karnovsky, M. J.: Use of ferrocyanide-reduced osmium in electron microscopy. *Proc. 14th Annual Meeting Amer. Soc. Cell Biol.*, p. 146, 1971.
5. Locke, M. and Sykes, A. K.: The role of the Golgi complex in the isolation and digestion of organelles. *Tissue and Cell*, 7; 143-158, 1975.
6. Mitranic, M. M., Boggs, J. M. and Moscarello, M. A.: An effect of colchicine on galactosyl- and sialyltransferases of rat liver Golgi membranes. *Biochim. Biophys. Acta*, 672; 57-64, 1981.
7. Moskalewski, S., Thyberg, J., Lohmander, S. and Friberg, U.: Influence of colchicine and vinblastine on the Golgi complex and matrix deposition in chondrocyte aggregates. *Exp. Cell Res.*, 95; 440-454, 1975.
8. Nevalainen, T. J.: Cytotoxicity of vinblastine and vincristine to pancreatic acinar cells. *Virchows Arch. B Cell Path.*, 18; 119-127, 1975.
9. Noda, T. and Ogawa, K.: Golgi apparatus is one continuous organelle in pancreatic exocrine cell of mouse. *Acta Histochem. Cytochem.*, 17; 435, 1984.
10. Orci, L., Glick, B. S. and Rothman, J. E.: A new type of coated vesicular carrier that appears not to contain clathrin: its possible role in protein transport within the Golgi stack. *Cell*, 46; 171-184, 1986.
11. Patzelt, C., Brown, D. and Jeanrenaud, B.: Inhibitory effect of colchicine on amylase secretion by rat parotid gland. *J. Cell Biol.*, 73; 578-593, 1977.
12. Pavelka, M. and Ellinger, A.: Effect of colchicine on the Golgi complex of rat pancreatic acinar cells. *J. Cell Biol.*, 97; 737-748, 1983.
13. Redman, C. M., Banerjee, D., Howell, K. and Palade, G. E.: Colchicine inhibition of plasma protein release from rat hepatocyte. *J. Cell Biol.*, 66; 42-59, 1975.
14. Reunanen, H., Punnonen, E.-L. and Hirsimäki, P.: Studies on vinblastine-induced autophagocytosis in mouse liver V. A cytochemical study on the origin of membranes. *Histochemistry*, 83; 513-517, 1985.
15. Smith, C. E.: Ultrastructural localization of nicotina-

- mide adenine dinucleotide phosphatase (NADPase) activity to the intermediated saccules of the Golgi apparatus in rat incisor ameloblast. *J. Histochem. Cytochem.*, 28; 16, 1980.
16. Thyberg, J., Piasek, A. and Moskalewski, S.: Effects of colchicine on the Golgi complex and GERL of cultured rat peritoneal macrophages and epiphyseal chondrocytes. *J. Cell Sci.*, 45; 42-58, 1980.