Case Report

Spontaneous Accumulation of Globotriaosylceramide (Gb3) in Proximal Renal Tubules in an ICR Mouse

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Abstract: This report describes spontaneous cytoplasmic vacuolation in the proximal renal tubules of a 7-week-old male ICR [CrJ:CD1(ICR)] mouse. The contents of vacuoles were positively stained with periodic acid-Schiff (PAS) and Sudan black, and the membranes were positive on immunohistochemical staining for lysosomal-associated membrane protein-2 (LAMP-2), a marker of lysosomal membrane. Electron microscopy revealed electron-dense lamellar bodies in the proximal tubular epithelial cells. These histopathological features are similar to those in α -galactosidase A-deficient mice, in which globotriaosylceramide (Gb3), a glycosphingolipid, accumulates in lysosomes. When we performed immunohistochemical staining for Gb3, the contents of vacuoles were positively stained. From these results, spontaneous cytoplasmic vacuolation in the proximal renal tubules in the mouse was identified as lysosomal accumulation of Gb3. (DOI: 10.1293/tox.2013-0029; J Toxicol Pathol 2013; 26: 429–432)

Key words: globotriaosylceramide (Gb3), mouse, proximal renal tubules, spontaneous cytoplasmic vacuolation

It is well known that many chemicals induce cytoplasmic vacuolation in renal tubular epithelial cells, which might be an early sign of irreversible necrosis or represent a reversible change¹. In addition, certain drugs could induce lysosomal accumulation of phospholipids, phospholipidosis, and this is microscopically observed as vacuoles^{2,3}. Furthermore, vacuolation in renal tubules occurs spontaneously with various incidences in some experimental animals, but little is known about their pathogenesis^{4–13}. In this study, we observed the histopathological details of spontaneous cytoplasmic vacuolation in proximal renal tubules in a male mouse.

The animal was a 7-week-old male ICR mouse [CrJ:CD1(ICR); Charles River Laboratories Japan, Inc., Yokohama, Japan] orally administered a vehicle, 0.5% methyl cellulose solution containing 0.4% Tween 80, for 5 days in a toxicity study. The mouse was housed in a plastic cage with softwood chip bedding under controlled conditions (12 h light/dark cycle, 40–60% humidity at 19–25°C) and fed a standard diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. The animal experiments were conducted according to the Guidelines for Animal

Experiments, Research & Development Division, Toray Industries Inc. The animal showed no marked changes in clinical signs, body weight and the results of hematology, blood chemistry and necropsy, but the weight of the kidneys was 1.3-fold that in our background data. The kidneys were fixed in 10% neutral-buffered formalin. Subsequently, hematoxylin and eosin (HE) staining of paraffin sections, periodic acid-Schiff (PAS) staining of frozen sections and Sudan black staining of osmium tetroxide-fixed paraffin sections were performed in the usual manner, respectively. Immunohistochemical staining for lysosomal-associated membrane protein-2 (LAMP-2) of formalin-fixed paraffin sections was performed with a rat monoclonal IgG antibody against mouse LAMP-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and a HRP-labeled polymer-conjugated secondary antibody (Nichirei Biosciences Inc., Tokyo, Japan)³. Immunohistochemical staining of formalin-fixed frozen sections for globotriaosylceramide (Gb3) was performed with a rat monoclonal IgM antibody against Gb3/CD77 (Beckman Coulter Inc., Brea, CA, USA) and an Alexa Fluor 488-conjugated secondary antibody (Life Technologies, Carlsbad, CA, USA)¹⁴, and tissue preparations were examined under a confocal laser scanning microscope (Olympus IX70, Olympus, Tokyo, Japan) to detect Alexa Fluor 488 (excitation filter, BP 470–490 nm; absorption filter, BA 510–550 nm). For electron microscopy, formalin-fixed kidneys were refixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Then, ultrathin sections were double-stained with 2% uranyl acetate and lead citrate, and examined under a transmission

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Fig. 1. HE staining in the kidney. Many small clear vacuoles are seen in the apical cytoplasm of the proximal renal tubules (B), but not in the distal renal tubules (C, arrowhead), Henle's loop (D), collecting ducts (E) and glomerulus (C). A, B, C: cortex. D: outer medulla. E: inner medulla.

Fig. 2. PAS staining, Sudan black staining, and immunohistochemical staining for LAMP-2 in the proximal renal tubules. The contents of vacuoles are positive for PAS and Sudan black staining (A, B), and the membranes are positive for LAMP-2 immunohistochemical staining (C). A: PAS staining. B: Sudan black staining. C: immunohistochemical staining for LAMP-2.

electron microscope (H7000; Hitachi High-Technologies Corporation, Tokyo, Japan).

Many small clear vacuoles were observed in the apical cytoplasm of proximal renal tubules in the mouse under a light microscope, but not in the distal renal tubules, Henle's loop, collecting ducts and glomerulus (Fig. 1). The contents of vacuoles showed positive PAS and Sudan black staining (Fig. 2A, 2B), and the membranes showed positive immunohistochemical staining for LAMP-2, a marker of the lysosomal membrane (Fig. 2C), indicating the lysosomal accumulation of glycolipids. Electron microscopy revealed

electron-dense lamellar bodies in the proximal tubular epithelial cells (Fig. 3). These histopathological features are similar to those in α -galactosidase A (α -Gal A)-deficient mice, known as the Fabry disease model, in which Gb3, a glycosphingolipid, accumulates in lysosomes^{15,16}. On immunohistochemical staining for Gb3, the content of vacuoles showed a positive reaction (Fig. 4). In other organs in this mouse, immunohistochemical staining for Gb3 was not conducted, since no remarkable change such as vacuolation was observed. From these results, the spontaneous cytoplasmic vacuolation in proximal tubular epithelial cells

Fig. 3. Electron microscopy in the proximal renal tubules. Electron-dense lamellar bodies are seen in the proximal tubular epithelial cells.

Fig. 4. Immunohistochemical staining for Gb3 in the proximal renal tubules. The contents of vacuoles are positive for Gb3 immunohistochemical staining.

in the ICR mouse was identified as lysosomal accumulation of Gb3.

Spontaneous cytoplasmic vacuolation in renal tubules has been reported in several strains of mice, although the incidence has been reported to be low. In some cases, they were considered to be accumulations of apolipoprotein B and glycoprotein^{6–13}. However, spontaneous vacuolation in renal tubules resulting from lysosomal accumulation of glycolipids, including Gb3, has not been reported except in

α -Gal A-deficient mice. Therefore, to the best of our knowledge, this is the first report demonstrating spontaneous accumulation of Gb3 in lysosomes of proximal tubular epithelial cells in normal mice.

In the vehicle-administered mice in our study, excluding the present case, neither vacuolation nor immunohistochemically Gb3-positive findings were observed in renal tubules (data not shown). Additionally, the cytoplasmic vacuolation in this case would be beyond the level of spontaneous findings usually observed in normal mice. Therefore, we did not consider that the lysosomal accumulation of Gb3 in this case might occur under physiological conditions. Fabry disease is an X-linked inherited disease caused by a mutation in α -Gal A, which is responsible for catalyzing glycosphingolipids such as Gb3^{17,18}. Fabry disease is classified into 2 categories, the typical and atypical types, depending on α -Gal A activity¹⁵. Lysosomal accumulation of Gb3 is observed in various organs in the typical type, in which α -Gal A activity is completely inactivated, whereas it is observed only in the heart or kidney in the atypical type, in which α -Gal A activity is partly reduced¹⁹. In α -Gal A-deficient mice, accumulation of Gb3 is observed in various organs, such as the kidney, liver, spleen, heart and skin^{20,21}. As for the present case, spontaneous cytoplasmic vacuolation due to the accumulation of Gb3 was observed only in proximal tubular epithelial cells; therefore, histopathological changes might be caused by the reduction of α -Gal A activity.

In conclusion, this single case of spontaneous cytoplasmic vacuolation in proximal renal tubules in an ICR mouse was confirmed to be lysosomal accumulation of Gb3.

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