NOTE

Electron Microscopic Findings of Vitamin D₂-Induced Glomerular Lesions in Rats

Kunio DOI, Akira YASOSHIMA¹⁾, Hitoshi OKAWA¹⁾, and Azusa OKANIWA¹⁾

Department of Biomedical Science, Faculty of Agriculture, University of Tokyo, Bunkyo, Tokyo 113 and

¹⁾Pharmacological Research Laboratory, Tanabe Seiyaku, Co., Ltd., Kawagishi, Toda, Saitama 335

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Among many reports on the pathology of vitamin D (VD)-induced nephrocalcinosis in rats [2–4, 7–9, 11], the description of glomerular lesions was quite rare. Previously, Okawa *et al.* [7] showed that calcific deposition in the glomerular basement membrane and necrosis were found in the kidneys of rats loaded with VD₂ and cholesterol. This paper deals with electron microscopic findings of such glomerular lesions.

Five male rats of Sprague-Dawley strain weighing about 340 g were orally administered with 320,000 IU of VD₂ in 2 ml of olive oil per kg of body weight per day for 4 consecutive days. Another 5 rats served as untreated controls. On the next day of the last administration, all animals were sacrificed and subjected to perfusion-fixation as previously described [12]. Then, small tissue blocks for electron microscopy were collected from the left renal cortex of each animal, and the right kidney was used for light microscopic examination.

Light microscopic pictures of renal lesions were, as a whole, similar to those reported previously [7]. By electron microscopy, glomeruli with mild changes were exclusively examined to detect the changes in the early stage. The most apparent change in such glomeruli was

observed in the visceral epithelial cells, i.e. podocytes. Namely, they showed swelling of mitochondria and dilation of cisternae of rough-surfaced endoplasmic reticulum (ER) (Figs. 1 and 2). Some of the markedly dilated cisternae of ER contained amorphous material with moderate electron density (Figs. 1 and 2). Most of the podocytes lost their normal foot process arrangement, and broad sheets of their cytoplasm covered directly the outer surface of the capillary basement membrane (Fig. 1). Some podocytes exhibited marked sign of degeneration (Fig. 3), and necrotic debris were found in Bowman's space (Fig. 1). Deposition of electron-dense and needle-like crystallites, believed to be those of calcium salt, was usual finding to these highly degenerated or necrotic cells (Fig. 3 and its insert). A small number of parietal epithelial cells were also involved in such lesions as those in podocytes. Alterations in the glomerular tissue elements other than epithelial cells were minimal except for slight calcareous precipitations in the basement membrane and mesangial matrix.

As mentioned above, the early glomerular lesions in VD₂-induced nephrocalcinosis were characterized by regressive changes in epithelial cells, especially

in visceral ones. Glomerular changes characterized by damage in podocytes have also been reported to occur in rats given *n,n'*-diacetylbenzidine [1] and aminonucleoside [5] up to the present time. In these cases, it has been shown that cysts [1] or absorption droplets [5] possibly due to either excessive passage of protein and other material across the cell or obstruction of such transcellular passage were conspicuous in the cytoplasm of podocytes [1, 5]. Except for the dilated ER, there were no cysts and absorption droplets in the present cases. In addition, concerning the electron microscopic findings in VD-induced nephrocalcinosis in dogs, Spangler *et al.* [10] proposed that shrunken or collapsed glomeruli, associated with hyperplastic juxtaglomerular apparatus, might be explained by renal ischemia resulting from vasoconstriction of afferent arterioles. There was, however, no significant change in juxtaglomerular apparatus in rats. It is therefore more reasonable to consider that the above-mentioned changes in glomerular epithelial cells in the present materials, as well as those in tubular epithelial cells [9], might be caused by direct toxic effect of glomerular filtrate rich in calcium ions as already discussed by Yasoshima *et al.*

[12] on the relationship between pathogenesis of aortic smooth muscle cell alteration and hypercalcemia in rats loaded with VD₂. In this connection, it is worthy to note the recently clarified fact that the podocyte is the target cell of 1 α -25(OH)₂D, i.e. calcium binding protein-producing cell [6].

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EXPLANATION OF FIGURES

Fig. 1. Glomerular basement membrane (BM) in the lower part of the figure is covered by broad sheets of epithelial cytoplasm. Swollen mitochondria (M) and dilated endoplasmic reticulum (ER) in glomerular epithelial cell and small clusters of cellular debris in Bowman's space. $\times 13,000$.

Fig. 2. Glomerular epithelial cell similar to that in Fig. 1. Markedly dilated ER is noticed.

$\times 13,000$.

Fig. 3. Autophagic vacuoles (AV), swollen and vacuolated mitochondria (M), and electron-dense and needle-like crystallites of calcium salt (arrow) in necrotic glomerular epithelial cell. $\times 13,000$.

Insert: Higher magnification of crystallites. $\times 36,000$.

要 約

ビタミン D₂ 負荷ラットの糸球体病変の電顕所見 (短報): 土井邦雄・八十島 昭¹⁾・大川 仁¹⁾・岡庭 梓¹⁾ (東京大学農学部実験動物学教室, ¹⁾田辺製薬薬理研究所)——ビタミン D₂ (320000 IU/kg/day ×4 days) を負荷した SD 系雄ラットの腎糸球体を電顕的に検索した。糸球体病変は被蓋細胞の変性 (足突起消失, ミトコンドリア腫大, 粗面小胞体槽拡張) で特徴づけられ, 変化の顕著な細胞の細胞質には電子密度の高い針状のカルシウム塩の結晶が沈着していた。これらの変化は, カルシウムイオンに富む糸球体沝過液の被蓋細胞に対する直接的な障害作用によるものと考えられた。