Cholesterol Accumulation in Podocytes: A Potential Novel Targetable Pathway in Diabetic Nephropathy

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Diabetic nephropathy is a major complication of both type 1 and type 2 diabetes and the leading cause of end-stage renal disease worldwide. Strict glycemic control and renin-angiotensin system inhibition delay the progression of diabetic nephropathy but do not prevent end-stage renal disease. Thus, identification of novel pathogenic mechanisms and targetable pathways is needed to change the outcome of the disease.

In this issue, Merscher-Gomez et al. (1) report that cholesterol accumulation in podocytes plays a pathogenic role in diabetic nephropathy and provide evidence of the beneficial effect of targeting this pathway. Using a cell assay, the authors show that serum from patients with diabetic nephropathy (as opposed to that of diabetic patients without nephropathy) induces abnormal accumulation of cholesterol in human podocytes in culture, which is associated with downregulation of the cholesterol transporter *ABCA1*, increased apoptosis, cell blebbing, and loss of caveolin phosphorylation. Notably, these changes are prevented by exposure to methyl-β-cyclodextrin (methyl-β-CD), an oligosaccharide that mobilizes cholesterol from lipid rafts. Microarray and PCR analysis of human biopsies from a cohort of type 2 diabetic patients confirmed *ABCA1* downregulation in diabetic nephropathy. In addition, Merscher-Gomez et al. used a type 2 diabetic mouse model carrying homozygous leptin mutation on insulin-resistant genetic background (BTBR: *ob/ob*) (2) to target this pathway, and found that long-term parenteral methyl-β-CD administration improves albuminuria and mesangial expansion, as well as insulin resistance and obesity (Fig. 1).

Cholesterol is synthesized from acetyl-coenzyme A by most cells and imported from circulating LDL by receptor-mediated endocytosis. Unlike fatty acid metabolism, cholesterol is not oxidized completely to carbon dioxide; instead, it is actively transported out of the cell by ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1). ABCA1, a major regulator of cholesterol homeostasis, mediates cholesterol efflux from endothelial cells, β-cells, adipocytes, and podocytes to circulating apolipoprotein acceptors (HDL) (1,3,4). HDL completes the so-called reverse cholesterol transport by delivering cholesterol to the liver, where it can be secreted into bile and excreted in the feces (3). Loss-of-function mutations of *ABCA1* cause Tangier disease, an extremely rare recessive disorder associated with HDL deficiency and accelerated atherosclerosis (5). Humans with heterozygous *ABCA1* mutations have HDL deficiency and abnormal β-cell function due to impaired glucose-stimulated insulin secretion (6). *ABCA1*-deficient mice mimic the human phenotype and show defects in caveolae processing and budding from the Golgi network, where caveolin 1 is retained (7). *ABCA1* deficiency in β-cells leads to impaired insulin granule exocytosis and impaired proinsulin processing (8,9). ABCA1 localizes to caveolae and interacts with caveolin 1 (10). Moreover, caveolin 1 is a positive regulator of ABCA1 function that increases cholesterol trafficking to the plasma membrane, thereby enhancing cholesterol efflux (11); in turn, ABCA1 induces caveolin 1 phosphorylation. The scaffolding domain of caveolin 1 interacts with multiple signaling proteins anchored in caveolae: nitric oxide synthase, G-coupled protein receptors, *Src* family kinases, and receptor tyrosine kinases, including the insulin receptor (12). Caveolin-1 amplifies insulin signaling by preventing proteasomal degradation of the insulin receptor and modulating GLUT transporter function (12). Caveolin-1–deficient mice develop insulin resistance, illustrating its important role in energy metabolism (13).

Abnormalities in lipid metabolism and lipid accumulation in the diabetic kidney are well described, including cholesterol accumulation and *ABCA1* downregulation (14,15). These changes are thought to contribute to diabetic nephropathy progression through ROS, vascular endothelial growth factor, and transforming growth factor-β pathways (14). Downregulation of *ABCA1* has also been detected in leukocytes and macrophages from type 2 diabetic patients, correlating with hyperglycemia, HgA_{1c}, and HDL levels (16). The benefit of cholesterol synthesis inhibition (statins) on diabetic nephropathy progression rate is considered modest at best. Merscher-Gomez et al. (1) targeted cholesterol accumulation in human podocytes by stimulating cholesterol efflux, thereby probably modulating signaling in caveolae, where caveolin-1, ABCA1, and insulin receptors (as well as other signaling molecules) physically and functionally interact, resulting in improved podocyte and diabetic nephropathy phenotype (Fig. 1). Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a relatively hydrophobic central cavity that function as potent artificial cholesterol acceptors. The number of sugar rings ($\alpha = 6$, $\beta = 7$, $\gamma = 8$) determines the hydrophobic cavity size, while hydroxyl and methyl modifications influence their binding kinetics and cytotoxicity (17). β-Cyclodextrins form dimers and inclusion complexes with cholesterol, allowing manipulation of cholesterol in biomembranes (18,19). Methyl-β-CD increases cholesterol efflux from cells rapidly without large changes in the cell membrane bilayer structure, although decreased invagination of clathrin-coated pits and flattening of caveolae,

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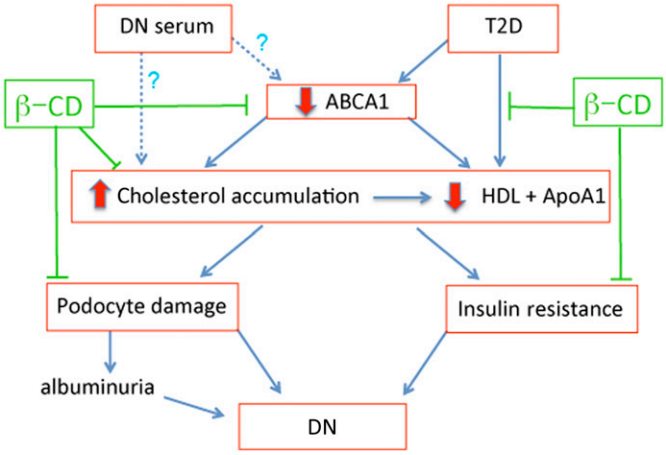

FIG. 1. Exposure to serum from diabetic nephropathy (DN) patients or type 2 diabetic (T2D) patients induces *ABCA1* downregulation, cholesterol accumulation, podocyte damage, and insulin resistance leading to albuminuria and DN progression. Methyl-β-CD (β-CD) causes cholesterol efflux, prevents podocyte damage, and rescues the insulin secretion defect. ApoA1, apolipoprotein A1.

which were reversible, were detected by electron microscopy (20). Exposure of pancreatic β-cells to methyl-β-CD increases glucose-induced insulin exocytosis without altering insulin sensitivity (21), as shown by Merscher-Gomez et al. (1) using human islets. Moreover, cholesterol depletion with methyl-β-CD rescues the insulin exocytosis defect in β-cells lacking *ABCA1*, suggesting that cholesterol accumulation is directly responsible for the β-cell insulin secretion defect (9). Cyclodextrins are used in the food industry as emulsifiers and by the pharmaceutical industry as excipients aimed to increase aqueous solubility, oral absorption of hydrophobic drugs, and drug delivery, although they may also reduce the apparent permeability of the drug, a so-called solubility–permeability interplay (22). Hydroxy- and methyl-β-CDs are considered nontoxic for rodents, dogs, and humans; are well tolerated (23); and have received FDA approval for the treatment of Niemann-Pick and Tangier diseases. α-Cyclodextrin was shown to induce modest weight loss in obese type 2 diabetic and in overweight nondiabetic patients, associated with modest but significant decreases in total cholesterol, LDL, apolipoprotein A, and insulin levels without change in glycemia (24).

Although the findings by Merscher-Gomez et al. (1) suggesting that methyl-β-CD may be a useful treatment for diabetic nephropathy are promising, identification of the diabetic nephropathy patients' serum component that elicits cholesterol accumulation in podocytes (β-cells and others) remains elusive. Further mechanistic studies are needed to understand how methyl-β-CD–mediated cholesterol removal alters specific cell functions, signaling pathways, caveolae-dependent internalization, and clathrin-dependent endocytosis. Such studies will be informative on pleiotropic effects of cyclodextrins, potential toxic effects, and for novel drug designs. Given that methyl-β-CDs may remove cholesterol from both raft and nonraft domains of cell membranes, as well as alter intracellular membranes and plasma cholesterol distribution (19,25), in vivo pharmacodynamics and functional effects should also be determined in acute and long-term settings, including advanced diabetic nephropathy, for safety concerns.

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