

Review

The Missing Link - Likely Pathogenetic Role of GM3 and Other Gangliosides in the Development of Diabetic Nephropathy

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Key Words

Ganglioside • GM3 • Diabetic nephropathy • Lipid rafts

Abstract

Despite scientific advances, diabetic nephropathy remains both a therapeutical challenge, and one of the major diabetic complications. Chemical structure of gangliosides, the most complex of glycosphingolipids, is characterised by one or more sialic acids and carbohydrate groups linked to a ceramide structure. Their potential pathogenetic role in a number of disorders linked to diabetes mellitus has recently been conjectured, due to evidence of their negative modulation of the insulin-mediated signaling and general effects on key cell functions like proliferation, differentiation, apoptosis, cellular signaling and adhesion. Elevated levels of advanced glycation products (AGE) usually found in diabetic conditions seem to be responsible for increased concentration of a-series gangliosides in tissues, most notably GM3. GM3 was shown to compromise the renal pericyte and mesangial cell regeneration via the inactivation of VEGF receptor and the receptor-associated Akt signaling pathway. Likewise, the lipid raft theory opened a new research area for GM3 influence, since in the glycosynapse model glycosphingolipids have a key cell-to-cell communication unit with modulating capabilities on signaling receptors. The goal of this review is to provide insight into currently available theories on proposed mechanisms that mark the GM3 as a pathophysiological mediator in the development of diabetic nephropathy.

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Introduction

Diabetes mellitus is a major cause of end-stage renal disease (ESRD) worldwide, in both first-world countries [1] and in developing ones [2, 3]. It occurs in up to 40% of diabetic patients, somewhat more frequently in type I than in type II. Diabetic nephropathy is

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characterized by an onset of microalbuminuria coupled with increased glomerular filtration rate (GFR) in the initial stage, followed by progressive increase of the total urine protein excretion and decrease of the GFR. A fully developed nephrotic syndrome is marked by total urine protein excretion of over 3.5 g/day [4]. According to de Zeeuw et al., albuminuria itself is a reliable predictor of diabetic nephropathy development and various therapeutic antiproteinuric strategies are expected to be adopted [5].

Diabetic nephropathy pathogenesis remains in focus for many research groups [6-11]. It was shown in previous research that the proliferation of the extracellular matrix plays a key role in pathogenesis of diabetic nephropathy. It is most likely induced by the activity of the transforming growth factor β (TGF- β). The stimulation of TGF- β is a focal point where various mediators intercede; glucose, advanced glycation products (AGE), endothelin and angiotensin II [12]. The expansion of extracellular matrix causes thickening of glomerular basal membrane, as well as the tubulo-interstitial and mesangial compartment [13]. This mechanism is responsible for obstruction of the glomerular filtration [14]. Also, inflammatory processes, probably induced by stimulatory effects of hyperglycemia on the nuclear factor- $\kappa\beta$ (NF- $\kappa\beta$) system and macrophages producing IL-12, contribute to glomerulo-tubular damage and further augment signaling pathways facilitating the deposition of extracellular matrix [15]. Inflammatory pathways seem to be interlinked with those of systemic and local hypertensive pathomechanisms, since angiotensin II, through the AT₁ receptor, induces the protein kinase C (PKC) activity, and also rises the intracellular levels of reactive oxygen species (ROS), [16] contributing to the inflammatory process. Activation of Gq alpha subunit-coupled signaling pathways in glomerular podocytes promotes renal injury [17]. AT₁ receptor is G protein-coupled receptor. Commonly, Gq alpha subunit of G protein-coupled receptors activates phospholipase C. Phospholipase C generates DAG as second messenger of AT₁ receptor binding, further activating protein kinase C. PKC induction by AT₁ is synergistically affected by reactive oxygen species, generated by interactions of AGE and corresponding receptors, which also increases the phospholipase C activity producing more molecular units of DAG. This double pathway PKC activation defines its central role in diabetic nephropathy pathogenesis [18]. PKC is responsible for up regulation of NADPH-oxidase expression, additionally increasing ROS quantities and the intensity of the inflammatory process and for induction of the cytokine TGF- β leading to consequential above-mentioned mechanisms [19].

At the present moment, there are no specific therapeutic modalities available for management of diabetic nephropathy. Clinicians resort to maintaining plasma glucose and triglyceride level and arterial blood pressure values within physiological ranges, while plasma urea, creatinine and albuminuria levels serve as prime indicators of disease progression.

Among etiologic factors assumed to be involved in the pathological cause-effect cascade that links hyperglycemia with kidney damage leading to diabetic nephropathy are gangliosides, most notably GM3 [20]. There is a scarce of data concerning relation of PKC activity and GM3 levels. GM3 synthase gene is expressed by PKC activation in human leukemia cells [21]. The goal of this review is to provide insight into currently available theories on proposed mechanisms that mark the GM3 as a pathophysiological mediator in the development of diabetic nephropathy. We focused our interest in early reversible phase of diabetic nephropathy (hyperfiltration and microalbuminuria) and the advanced stage of diabetic nephropathy would not be referred to in this manuscript.

Biochemical structure and functional roles of gangliosides

Gangliosides are the most complex glycosphingolipids. Etymologically, their name reflects the fact that they are mostly present in plasma membranes of neural cells. By their chemical structure, they are characterized by the presence of one or more sialic acids

and carbohydrate groups linked on the ceramide molecular structure. Gangliosides are categorized according to the Svennerholm classification. Letters “M, D, T, Q” and “P” indicate the number of sialic acid groups on molecules (M=1...P=5). Numbers “1-4” designate the number of carbohydrate groups bound to ceramide, derived from the formula; n = 5 - number of carbohydrate groups. When present, lowercase letters “a”, “b” and “c” indicate the number of sialic acids on the inner galactose segment [22]. Therefore, the eponymous GM3 ganglioside contains a single sialic group with 2 carbohydrate groups bound to a ceramide structure. GM3 formation is catalyzed by the GM3 synthase, a lactosylceramide α 2,3 - sialyl transferase, that catalyses the N-acetylneuraminic (sialic) acid transfer on the terminal galactose residue of lactosylceramide [23]. Glycosyltransferases are enzymes that catalyse ganglioside synthesis and determine the key sequence of carbohydrates in the glycosphingolipid chain [24].

GM3 ganglioside is linked with a variety of physiological functions on the molecular level, such as: control of cell proliferation and differentiation, apoptosis, cellular signaling pathways, cell adhesion and mobility [25-28]. It is ubiquitous in almost all organ systems. Highest levels are found in the cerebral cortex, liver, spleen, placenta, thyroid gland, kidneys and skeletal muscles. Thin-layer chromatography (TLC) colorimetric analysis defined the GM3 a dominant ganglioside in kidney tissue, where it comprises three quarters of total gangliosides present in the kidney [29]. Significant body of evidence emphasizes negative modulation effects of GM3 on insulin-mediated signaling pathways, at the same time highlighting its potential role in the development of type II diabetes [30, 31].

Another important nephrologic clinical syndrome has also been linked with GM3 as an underlying cause, namely the polycystic kidney disease (PKD) and more notably the nephronophthisis related-PKD. According to Natoli et al., GM3 and GM3 precursor, glucosylceramide (GlcCer) seem to promote cystogenic activity [32]. Loss of the GM3 synthase gene was reported to have a protective effect on forming cystic structures in rats, while authors highlighted the inhibitory effects of GM3 on cell cycle induction and Akt/mTOR signaling pathway, a pathway linked with cystogenic effects. They also hypothesized the potential GM3 interference with EGF and IGF-1 signal receptors on lipid rafts, which are both cystogenic mediators [32].

GM3 interferes with regenerative capacity of renal cells

AGE are a key biochemical characteristic of diabetes [33]. In a concentration-dependent reaction, surplus sugar molecules bind to protein structures without the mediation of enzymes, causing conformational changes and protein function disruptions. Masson et al., reported a positive correlation between the increase in AGE levels and increased levels of a-series gangliosides in rat renal mesangial cells, with GM3, GM2 and GM1 particularly increased [34]. The effective increase in ganglioside concentrations is coupled by a significant up-regulation of expression of ganglioside synthases responsible for production of respective gangliosides and also by an inhibition of synthase enzymes, that mediate the production of GM3-derived molecules. GM3 levels are subsequently further increased by blocking its usage as a precursor for other ganglioside types. Because of their given influence on cell proliferation, gangliosides are proposed to be a factor responsible for proliferatory inhibition of pericyte and mesangial renal cells [35], particularly through the possible inhibition of vascular endothelial growth factor (VEGF) effects as was proved by addition of GM3 in cell culture [36]. In addition, GM3 in lipid rafts on the surface of human podocytes binds soluble vascular endothelial growth factor receptor FLT1, one of the key regulators of angiogenesis, promoting adhesion and rapid actin reorganization [37].

The *in vitro* studies focusing on renal mesangial cells reveal that Akt signaling pathway, activated by phosphatidylinositol-4,5-bisphosphate 3-kinase in the presence of platelet-

derived growth factor (PDGF), attributes significantly to regenerative processes, due to its blockage of apoptotic induction through the activation of NF- κ B and the phosphorylation of Bad pathways [38]. This proposed model emphasizes ganglioside role in compromising the kidney vascular endothelium cell regeneration processes.

Lipid raft model of GM3 influence

The connection between increased GM3 levels and several diabetic complications is established [39, 40]. The missing link between the abovementioned phenomenon and the diabetic nephropathy could lie in the concept of the so-called „lipid rafts“. Ever since Singer and Nicholson proposed the revolutionary mosaic double-layer model of the cellular membrane structure, numbers of publications and body of knowledge have expanded in an exponential trend [41-43]. Following the evolution of basic biomedical sciences, the physiological and clinical focus in disease research migrated to the molecular level, and diabetology research proved no exception. Among the newer concepts that attempt to contribute to the general understanding of insulin physiology and pathophysiology are caveolae and lipid raft membrane formations. Simons and Ikonen presented a model in which sphingolipids and cholesterol form mobile clusters on the lipid double layer [44]. Caveolae are defined as invaginations 50-100 nm in diameter, placed in cell membranes and formed by special proteins; caveolins (present in three isomers) [45]. Caveolae are rich in lipid raft structures, and serve as placement sites for various receptors, with possible important roles in signal transduction [46]. In the proposed glycosynapse model, glycosphingolipids form cluster formations that have a functional role in cell-to-cell communication, play a part in cellular adhesion systems or modulate certain receptor functions, therefore affecting signal transduction as well [47-49].

In a study of renal Na⁺-glucose cotransporter type 2 (SGLT2) expression and activity in streptozotocin induced diabetic rats, conducted by Albertoni-Borghese et al., authors concluded that altered SGLT2 activity could be a consequence of changes in membrane lipid composition (decrease in sphingomyelin content and an increase in phosphatidylcholine) [50]. Also, published results suggest that the renal Na⁺/K⁺/Cl⁻ cotransporter is lipid raft-dependent [51]. This physiological process causes increased tubular reabsorption with higher hydrostatic pressures in efferent arterioles. It is presumed that such an effect would remodulate the tubuloglomerular balance of the „Guytonian“ kidney increasing the glomerular filtration rate and hydrostatic tubular pressure, eventually damaging the tubular cell system in a manner presented in Figure 1. GM3 is the most abundant renal ganglioside and together with ganglioside GM1 constitute lipid rafts [52]. GM3 rafts are localized predominantly on the peaks of microvillus-like protrusions in the apical membrane of canine kidney cells, whereas GM1 rafts are distributed mainly on the slopes of protrusions or the valleys between protrusions in the plasma membranes [53]. However, due to missing data on direct GM3 influence upon renal SGLT2 and Na⁺/K⁺/Cl⁻ cotransporters, VEGF and Akt signaling pathways remain more likely GM3 targets leading to depletion of functional renal parenchyma (Figure 1).

Maintaining the glomerulo-tubular barrier polarity; an alternate point of view

An interesting and considerably different approach was proposed by Kwak et al. They reported of lowered total ganglioside concentrations in kidney glomerulus in diabetic conditions, with streptozotocin-induced diabetic rats [54]. Among the pathogenetic mechanisms they proposed is the concept in which gangliosides contribute to the total amount of membrane negative electric charge [55]. In renal physiology, the electronegative groups situated on the glomerular basement membrane have a key function in restricting the filtration to

a balanced amount, since the plasma proteins carry electronegative charges from acid groups and are repelled by the glomerular charges of the same polarity, preventing proteinuria and polyuria that would appear due to changes in the glomerulo-tubular oncotic balance [56, 57]. The mentioned results showed that total ganglioside levels decrease during diabetic conditions. Authors presume that consequently the glomerular filtration becomes less selective and permits increased quantity of charged ions to pass through glomerular barrier. The hydrostatic pressure would be increased due to a shift in osmotic and oncotic balance, eventually causing damage to the tubular cells, due to their exposure to increased pressure and mechanical stress.

In addition to gangliosides, negative electric charge of glomerulus belongs to heparan sulfate proteoglycans, constituents of glomerular basement

membrane (GBM) [58]. High glucose level inhibits activity of key enzyme of heparan sulfate synthesis (N-deacetylase: N-sulfotransferase) contributing to the loss of electronegative charge and increased permeability of glomerulus [59]. The glomerulus capillaries are lined by fenestrated endothelial cells and covered by specialized epithelial cells known as podocytes. GBM is between the endothelial and podocyte cell layers [60]. Among three lay-

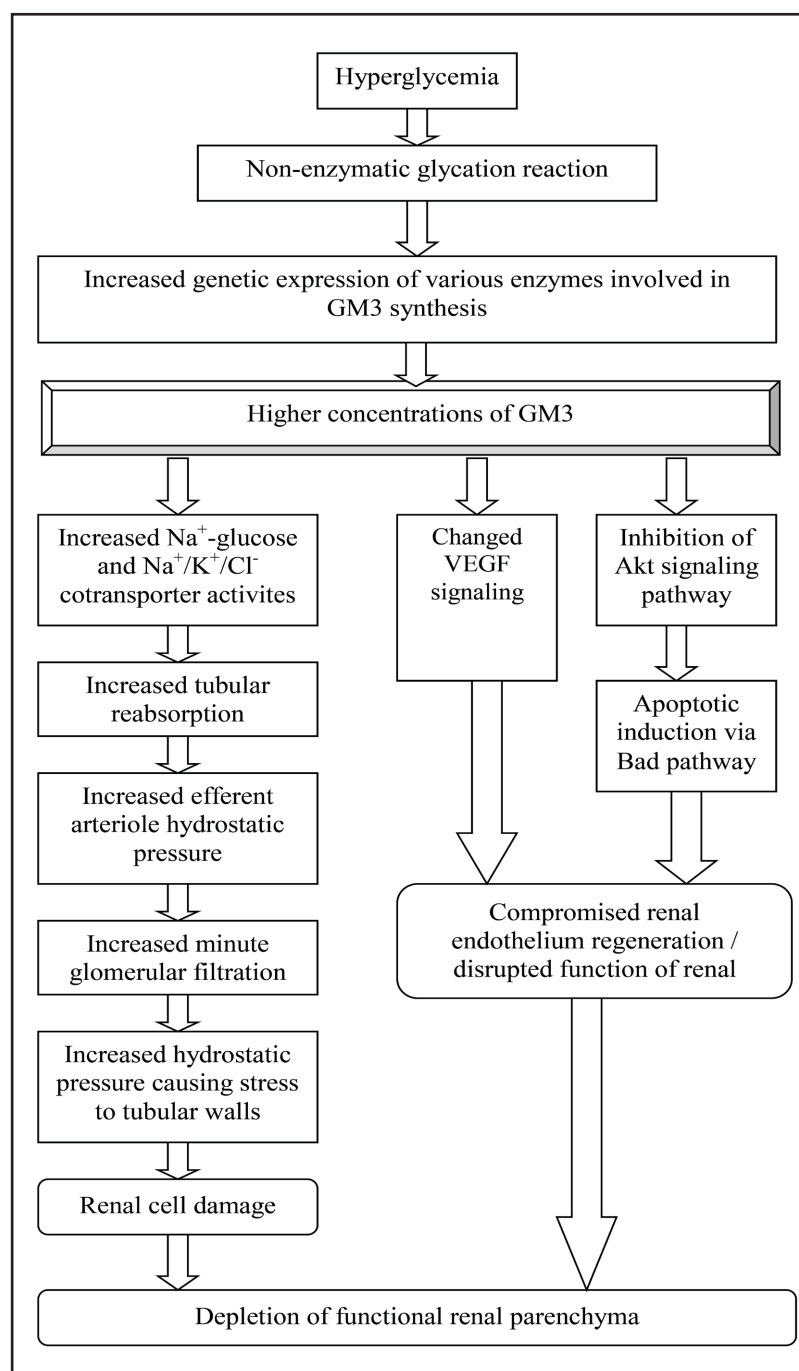


Fig. 1. Proposed mechanisms how hyperglycemia lead to GM3 increase with final consequence of depletion of functional renal parenchyma.

ers of glomerulus, podocyte is now regarded as an important component for controlling the glomerular permeability [61]. Podocyte is adhered to GBM by integrin which is contained in lipid raft [62]. Podocyte detachment from GBM was reported in type1 diabetic nephropathy [63]. Gain of glycosylation in integrin $\alpha 3$ causes nephrotic syndrome [64]. Deletion of vascular endothelial growth factor receptor FLT1 from podocytes, causes reorganization of their cytoskeleton with massive proteinuria and kidney failure, characteristic features of nephrotic syndrome in humans [37]. Knowing that GM3 in lipid rafts on the surface of human podocytes binds soluble vascular endothelial growth factor receptor FLT1 resulting in correct structure of podocyte slit diaphragm that prevents proteinuria [37], we can assume that loss of GM3 will result with nephrotic syndrome too.

Conclusion

Although diabetic nephropathy has been recognized as a major diabetic complication for quite some time, the exact pathogenesis remains *terra incognita* in need of further scientific research. The implications of the proposed model, in which gangliosides are the key pathophysiological mediator, introduce novel molecular interaction pathways that take part in the course of the disease. Since gangliosides are considered to be an important and widespread cellular signal modulators, the totality of their interactions in cellular functions and cell cycle, and therefore also in the titular pathological entity of this paper are numerous and currently difficult to predict. Apart from disorders on the molecular scale, tubular damage from increased luminal hydrostatic pressure is proposed as an important factor. The whole picture of diabetic nephropathy, comparable to the lipid raft model, is in fact a mosaic of various single molecular discoveries, giving possibility to different insights and approaches when dealing with pathogenetic mechanisms in new research. As rafts float on the ocean of a lipid double layer, so does our current knowledge on diabetic nephropathy on the surface of physiology, open to ideas from various domains of thought.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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