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Globotriaosylceramide, the Shigatoxin receptor, contributes to tubular protein absorption and toxic acute kidney failure

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Glycosphingolipids (GSLs) are essential components of lipid microdomains in eukaryotic plasma membranes and, together with cholesterol, form platforms for the attachment and function of glycosylphosphatidylinositol-anchored proteins. Although ubiquitously expressed and involved in cellular processes such as cell adhesion and absorption, it is still a great challenge to attribute a defined physiological role to a specific GSL. Although, it is known that Globotriaosylceramide (Gb3, CD77), a neutral GSL, is the receptor for the Shiga toxins (STx) produced by bacteria such as E.coli and Shigella, its physiologic function in the kidney has not been characterized. The holotoxin is formed by a catalytic (A) and a binding domain (B), the first representing the toxic portion and the latter, the non-toxic, is composed of B fragments that form a pentamer, able to bind the carbohydrate chain of Gb3. The STx-Gb3 complex is internalized via both clathrin-dependent or -independent endocytosis, followed by retrograde transport of the A toxin subunit to the endoplasmic reticulum (ER). The A domain is, then, retro-translocated into the cytosol, inhibiting protein biosynthesis of the host cell and, potentially, inducing cell apoptosis. In recent years, the non-toxic B-subunit of STx (STxB) has been used experimentally as a drug delivery tool for those malignant tumors in which Gb3 is found overexpressed such as Burkitt lymphoma, breast, ovarian and colorectal carcinomas. An important aspect is that many types of cells express Gb3 constitutively, such as renal tubular epithelial cells and erythrocytes. As a consequence, these cells might also be affected by STxB with chemotherapeutic drugs, especially in the kidney, where Gb3 is abundantly expressed. Interestingly, the physiologic function of Gb3 in the kidney is still unknown and clarification of a potential role is of crucial importance because it may provide a better understanding of the molecular mechanisms responsible for those kidney disorders in which this lipid serves a pathophysiologic function. Since it is unlikely that complex organisms such as mammals might produce a molecule to be solely a receptor for a bacterial toxin, my hypothesis is that Gb3 may serve a physiologic role in

the mammalian kidney. The purpose of this study was to elucidate a potential homeostatic and pathophysiologic function for renal Gb3 with the help of genetic Gb3 deficient mice. In this study, I showed that a high fat diet induced a selective upregulation of Gb3 in murine kidneys, specifically in proximal tubules, compared to mice fed with chow diet, an effect that was likely due to a fatty acid-induced increase in Gb3 synthase mRNA levels; this effect served to more easily localize Gb3 synthase and Gb3 within the proximal tubules, with no alteration in other segments of the renal tubule. A higher urinary albumin excretion was measured in $Gb3s^{-/-}$ mice compared to WT. This finding suggested that Gb3 could be involved in reabsorptive processes of the proximal tubules, perhaps via modulation of the megalin/cubilin receptor complex, involved in the uptake of filtered proteins and molecules such as albumin, myoglobin and gentamicin. To verify this hypothesis, two experimental mouse models of glycerol-induced AKI and gentamicin-induced AKI were employed, where the kidney tubular damage is directly correlated with the uptake of myoglobin and gentamicin by the proximal tubules, respectively. Both molecules have been reported to be endocytosed via the megalin/cubilin receptor complex. In both models, Gb3 deficiency showed renoprotective effects against myoglobin- and gentamicin-induced tubular injury in $Gb3s^{-/-}$ mice compared to WT, both at 24h and 7 days (glycerol model) or 6d (gentamicin model), from glycerol and gentamicin administration, respectively. In summary, my results showed for the first time evidences of a physiological role for renal Gb3 in reabsorptive processes by renal proximal tubules, in part via involvement of the megalin/cubilin receptor complex activity and reveal a new potent therapeutic option for the treatment of AKI in clinical conditions such as rhabdomyolysis in crush syndrome and gentamicin nephrotoxicity, administered to treat bacterial infections. Gb3 might therefore be considered as a potential therapeutic target since its removal may have beneficial effects in protecting the kidney from nephrotoxic compounds such as myoglobin and gentamicin and in addition, by providing protection against STx infections, without any relevant adverse effects.