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Growth hormone promotes glomerular lipid accumulation in bGH mice

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Growth hormone promotes glomerular lipid accumulation in bGH mice.

Background. Bovine growth hormone (bGH) transgenic mice develop progressive glomerulosclerosis and exhibit abnormalities in hepatic lipid metabolism. We have previously shown that growth hormone up-regulates the low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) in mouse mesangial cells. However, a role of lipid abnormalities in bGH kidney disease has not yet been demonstrated.

Methods. Groups of bGH mice (5 and 11 months old) presenting with, respectively, moderate and severe degrees of glomerulosclerosis were compared to age-matched controls. Neutral lipid content in kidney cortex was determined by oil red-O staining, serum cholesterol, and triglycerides by enzymatic assays, relative mRNA expression of LDL receptors, HMGR, and scavenger receptor by real-time reverse transcription-polymerase chain reaction (RT-PCR), and HMGR protein expression by immunoblotting. Two younger (5 and 12 weeks old) groups of mice were used to study scavenger receptor expression at earlier time points.

Results. Serum cholesterol was significantly increased in bGH mice at 5 months, but triglycerides were lower than control levels at both 5 and 11 months. Renal cortex HMGR expression was elevated at the mRNA but not at the protein level in the 11-month-old bGH group compared to controls. However, glomerular neutral lipid staining and scavenger receptor mRNA expression were markedly increased in all bGH mice, including those at 5 weeks of age compared to respective controls.

Conclusion. The bGH mouse exhibits an increased mesangial lipid content and elevated scavenger receptor mRNA expression as early as at 5 weeks of age, suggesting that an increased kidney uptake of oxidized LDL could play a role in the development of glomerulosclerosis in this mouse model.

Key words: glomerulosclerosis, cholesterol, triglycerides, HMG-CoA reductase, low-density lipoprotein receptor, scavenger receptor, real time RT-PCR.

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Glomerulosclerosis is characterized by glomerular lesions, which include partial or total replacement of the glomerular tuft by a fibrous scar, resulting in progressive loss of renal function [1]. It constitutes a common feature of most chronic kidney diseases, including diabetic nephropathy. Of the several animal models of glomerulosclerosis in widespread use, the bovine growth hormone (bGH) transgenic mouse is of particular interest as it develops glomerular lesions that bear a marked resemblance to those present in human diabetic nephropathy [2]. Because of the progressive nature of the disease, the bGH mouse has become a model to study the pathophysiology of glomerulosclerosis. Furthermore, the clinical observation that in acromegaly, abnormally high circulating levels of growth hormone are accompanied by an increase in albuminuria and urinary excretion of glycosaminoglycans, early markers of glomerular injury, strongly supports the involvement of elevated growth hormone in the pathogenesis of glomerular disease [3]. The role of growth hormone in the development of glomerulosclerosis has also been demonstrated in dwarf models of diabetes and subtotal nephrectomy, where growth hormone deficiency was shown to markedly attenuate the severity of glomerular injury [4–7].

It has been reported recently that excess growth hormone in the bGH transgenic mouse leads to hypercholesterolemia and several abnormalities in hepatic lipid metabolism [8, 9], but the involvement of lipids in the development of glomerulosclerosis has not yet been studied in this model. Histopathologic similarities between

glomerulosclerosis and atherosclerosis suggest that subsequent to the initial hormonal, metabolic or hemodynamic insult, the development of glomerulosclerosis follows a course analogous to that of lipid-induced vascular disease [10–13]. As in atherosclerosis, an increased presence of macrophages in the glomerular area, followed by increased glomerular lipid deposition, mesangial cell proliferation and extracellular matrix expansion have been demonstrated in several types of experimental and clinical nephropathies [10, 11, 14]. Furthermore, the demonstration that glomerular low-density lipoprotein (LDL) deposits induce both mesangial cell proliferation [15–18] and extracellular matrix accumulation [19] supports the idea that lipid deposition may underlie the development of glomerulosclerosis.

In the presence of hyperlipidemia, an increased scavenger receptor–mediated uptake of oxidized LDL by glomerular macrophages leads to the development of glomerular foam cells [12, 20]. In addition, cultured human mesangial cells have been shown to accumulate lipids in the presence of high concentrations of LDL through both class A scavenger receptor [21, 22] and LDL receptor [23–25].

Despite the recognized association between hyperlipidemia and both atherosclerosis and glomerulosclerosis, there is evidence that mesangial lipid accumulation in glomerular diseases may occur independently of serum lipid levels, related instead to the renal up-regulation of LDL receptor and scavenger receptor [26, 27].

We have previously shown that growth hormone increased LDL receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) transcript levels in mouse mesangial cells cultured in the absence of lipoprotein [28]. These results suggest a model in which growth hormone mediates the development of glomerulosclerosis in part by a direct mesangial up-regulation of genes involved in lipid metabolism. In the present study, we extended our in vitro studies to an in vivo model of glomerulosclerosis (bGH transgenic mouse) to examine the influence of growth hormone on the renal expression of genes involved in the regulation of lipid synthesis and uptake during the course of glomerulosclerosis. We first measured the renal expression of LDL receptor, HMGR, and scavenger receptor at both moderate and severe stages of chronic kidney disease, respectively, 5- and 11-month-old animals. In addition, the levels of serum creatinine, cholesterol, and triglycerides, as well as accumulation of intraglomerular lipid were compared between bGH and age-matched control animals. In a second study, four additional groups of animals (bGH and nontransgenic at 5 and 12 weeks of age, five animals/group) were included specifically to ascertain the earliest occurrence of a difference in glomerular lipid accumulation and scavenger receptor expression between the bGH and nontransgenic groups.

METHODS

Animals

bGH transgenic mice and nontransgenic littermates were provided by Dr. John J. Kopchick (Edison Biotechnology Institute, Ohio University, Athens, OH, USA). The animals were housed one per cage, with free access to water and mouse chow (rat/mouse standard diet) (Harlan Teklad, Madison, WI, USA) in alternating 12-hour periods of light and dark. A total of 28 male mice were divided into age group I (5 months old) (nine nontransgenic and 10 bGH) and age group II (11 months old) (five nontransgenic and four bGH), representing moderate and severe stages of glomerulosclerosis, respectively [29]. In a second study, four groups of animals were euthanized at a younger age (bGH and nontransgenic mice, 5 and 12 weeks of age, five animals/group). All animal procedures were carried out in accordance with the regulations of the USUHS Laboratory Review Board and with the Guide for the Care and Use of Laboratory Animal, Institute of Laboratory Animal Resources, National Research Council, 1996.

Animals were weighed and euthanized by decapitation following sedation with intraperitoneal injection (12 μL/g body weight) of Avertin (25 g tribromoethanol/12.5 mL tertiary amyl-alcohol) at 1/40 dilution in saline [30]. All mice were euthanized in the morning to avoid the effects of diurnal variation. Total blood was collected and allowed to clot, followed by separation of serum, which was kept at −80°C until analysis. Kidneys were quickly excised and weighed. Samples of kidney cortex were collected for RNA extraction and for preparation of histology sections (frozen and paraffin-embedded). In the second study, prior to euthanasia the 5-week-old nontransgenic and bGH animals were grouped in separate metabolic cages (five animals per group) for a period of 3 hours in 3 consecutive days for urine collection and further assessment of proteinuria.

Light microscopy

A coronal section of the right kidney was fixed in 10% buffered formalin and embedded in glycol methylacrylate. Sections (3 μm) were stained with periodic acid-Schiff (PAS) and examined by an independent investigator blinded to the study to determine the degree of glomerular injury, indicated by a glomerulosclerosis score. The scoring system was based on the modified Banff classification [31] using data collected from the analysis of 20 glomeruli/mouse that were randomly scanned proceeding from the outer cortex to the juxtaglomerular zone in a serpentine fashion. First, each glomerulus was graded (0 to 4) according to the percentage of sclerosis/glomerular area, grade 0 being given to glomeruli with no sclerosis and grade 4 to obsolescent glomeruli. Subsequently, a final score was attributed