Glomerular Hyalinosis in Relation to Lipid Deposition in Rats with Reduced Renal Mass¹

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Abstract = Studies have suggested that similarities exist between the pathogenesis of glomerulosclerosis and atherosclerosis. The role of subendothelial accumulation of macromolecular substances, lipids in particular, in the pathogenesis of glomerular hyalinosis and sclerosis was studied in 17 Sprague-Dawley rats with reduced renal mass (1 and 1/3 nephrectomy). Sequential changes of functional and morphologic data were studied three, five and seven months after ablation. Urinary protein excretion, and values for blood urea nitrogen, creatinine, fasting serum cholesterol, and triglycerides all increased by the third month following operation. After the seven months, the rats had a significantly higher daily urine protein excretion than animals at month 3 and greater values for fasting serum cholesterol and triglycerides than animals at month 5. By light microscopy, animals at month 7 showed a significantly greater percentage of glomeruli with segmental hyalinosis/sclerosis than rats at month 3. Glomerular lipid deposition, confirmed by oil red O staining, was noted in 11 animals (65%). Ultrastructurally, a subendothelial accumulation of fat and electron-dense proteinaceous material, which corresponded to the early hyalinosis lesion in glomeruli, was observed more frequently in rats at month 7 than in animals at months 3 and 5. Mesangial deposition of the same substances was seen in nine rats showing no significant difference in each group. These observations suggest that the development of glomerular hyalinosis may be related to the subendothelial deposition of harmful lipids in the circulation, similar to arterial wall damage in atherosclerosis, eventually leading to glomerular sclerosis.

Key Words: Renal ablation, Glomerular hyalinosis, Intraglomerular lipid deposition

Introduction

Focal segmental glomerulosclerosis (FSGS) in humans is thought to represent a final pathologic lesion that may result from a variety of renal disorders (Hostetter et al., 1982; Lee and Spargo, 1985).

Although the pathogenesis of FSGS is as yet unclarified, a number of studies have recently shown that similarities exist between the pathogenesis of glomerulosclerosis and atherosclerosis (Moorhead et al., 1982; Grond et al., 1984, 1986, 1988). Moorhead et al. (1982) hypothesized that chronic progressive glomerulosclerosis, parallel to the pathogenesis of atherosclerosis, may be mediated by abnormalities in lipid metabolism. Grond et al. (1984, 1986, 1988) also postulated that, in chronic aminonucleoside nephrosis in rats, the increased accumulation of lipids in the mesangial cells may stimulate an overproduction of matrix substance eventually leading to glomerulosclerosis, which is analo-
gous to arterial wall damage in atherosclerosis.

In rats with a 5/6 reduction of renal mass, Olson et al. (1985) suggested that endothelial injury was the first indicator of glomerular injury caused by hyperfiltration (Hostetter et al., 1981), which allows plasma constituents to have access to the subendothelial space and to form an early hyalinosis lesion. Hyalinosis is an important lesion that is commonly present in FSGS. Its staining reactions are the same as those of the hyaline material in the vessel wall in arteriolosclerosis (Heptinstall, 1983). Recently, Lee and Spargo (1985) reported a high incidence of afferent hyaline arteriolosclerosis (HA) in human idiopathic FSGS, suggesting that HA and FSGS may be related and may have a similar pathogenesis.

In an attempt to further contribute to this study, I followed the long-term sequence of glomerular damage in rats with a 2/3 reduction of renal mass. When compared to the animals with extreme renal ablation or a 5/6 renal reduction, these with 2/3 renal ablation lived long enough to study the pathogenesis and evolution of the disease.

MATERIALS AND METHODS

Seventeen outbred male albino Sprague-Dawley rats weighing between 140 and 170 g were used in this study. The kidneys were removed through a flank incision under sodium pentobarbital anesthesia (0.45 mg/Kg, intraperitoneally). A silk ligature was placed around the right renal pedicle and the kidney was removed. After five days of recovery, 1/3 of the left kidney was surgically removed by cutting its upper portion, leaving the animal with approximately 1/3 of its normal renal mass. The animals were divided into three groups depending on the time of sacrifice: three months for Group 1, five months for Group 2, and seven months for Group 3. Six rats underwent sham operation and served as controls. The rats were allowed free access to water and normal rat chow (Samyang Food Co., Wonju) with a sodium content of 0.44% and a digestible protein content of 22%.

Every month 24-hour urine was collected from all the rats to determine the daily protein excretion. After two months, fasting blood was obtained monthly by orbita plexus puncture under ether anesthesia. Serum levels of urea nitrogen, creatinine, cholesterol, and triglycerides were determined according to standard methods.

Twenty-four hour urine collections were obtained in the fasting state in individual metabolic cages. Urine protein was measured by the Lowry et al. method (1951) for the 24-hour urine collection, with bovine serum albumin (Sigma Chem. Co., St. Louis, Mo.) as a standard.

On the day of sacrifice, the rats were anesthetized with sodium pentobarbital (0.45 mg/Kg, intraperitoneally). Heart puncture was performed to obtain serum for biochemistry. The kidneys were removed and sectioned coronally. Small pieces of kidney were minced in 2.5% glutaraldehyde in phosphate buffer for overnight fixation, then rinsed in buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in epon. Ultrathin sections were cut on an LKB ultramicrotome and stained with uranyl acetate and lead citrate. Two to four glomeruli were examined in each case in a Hitachi electron microscope. The presence of electron-dense proteinaceous materials and less electron-dense fat substances was evaluated according to their location: subendothelial or mesangial. The amounts of each were graded from 0 to 3 + as follows: ±, trace or questionable deposits; 1 +, small but definite deposits; 2 +, moderate amounts of deposits; 3 +, large amounts of deposits. Particular attention was paid to the earliest changes in the peripheral capillary loops in relation to hyalinosis lesions.

In order to detect lipid deposition within the glomerulus, portions of kidney were snap-frozen in liquid nitrogen. Cryostat sections were stained with oil red O (ORO), then counterstained with hematoxylin. The amounts of total lipids in glomeruli were estimated by semiquantitative grading on a 0 to 3 + scale as described previously (Grond et al., 1986).

The rest of the kidney was processed for light microscopy following a fixation in 10% buffered formalin. Paraffin-embedded tissues were cut in 3 μm thicknesses and stained with hematoxylin and eosin and with periodic acid-Schiff (PAS). Coronal sections of the kidney of each rat were screened at a magnification of x250 by light microscopy to determine the percentage of glomeruli with hyalinosis/sclerosis. At least 200 glomeruli per kidney were studied. Hyalinosis fe-
sion was defined as rounded or crescent-shaped foci of homogeneous PAS-positive material on the inside of the capillary walls. Glomerulosclerosis was defined as loss of cellular elements from the glomerular capillary tuft, collapse of capillary lumina, and folding of the glomerular basement membrane (GBM) with entrapment of amorphous material.

The histologic sections were also scored on a scale of 0 to 3 + for the following changes: tubules (dilatation, casts, atrophy), interstitial (chronic inflammation, acute inflammation, fibrosis), and arteriolar lesions (necrosis, intimal thickening).

Values were expressed as means ± SD. The significance of differences between group means was assessed using a Wilcoxon rank sum test. Differences in the frequency of tubulo-interstitial lesions and ultrastructural changes of glomeruli between groups were tested using a Fisher's exact test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Functional data
The results of 24-hour proteinuria, blood urea nitrogen, creatinine, cholesterol, and triglycerides throughout the course of investigation are shown in Table 1. Urinary protein excretion in nephrectomized rats at month 3 (Group 1) was 143.7 ± 55.2 mg/24 h and in sham-operated rats 27.5 ± 10.5 mg/24 h. This difference was statistically significant. The amounts of proteinuria found to increase further at months 5 (176.8 ± 89.5 mg/24 h) and 7 (312.3 ± 86.4 mg/24 h). The levels of proteinuria in Group 3 rats were significantly greater when compared with those animals in Groups 1 and 2 animals (p < 0.01). The difference of proteinuria between Group 1 and Group 2 was not statistically significant.

Serum concentrations of urea nitrogen and creatinine in Group 1 animals averaged 52.0 ± 18.6 mg/dl and 1.17 ± 0.25 mg/dl, respectively. These values were significantly higher than the corresponding levels of serum urea nitrogen and creatinine in the sham-operated rats being 12.0 ± 2.3 mg/dl and 0.73 ± 0.10 mg/dl, respectively (p < 0.05). Renal functional data were not significantly different in each experimental group.

Values for fasting serum cholesterol and fasting serum triglycerides in Group 1 were 144.0 ± 40.3 mg/dl and 297.5 ± 136.7 mg/dl, respectively. In contrast, those in controls were 52.8 ± 40.2 mg/dl and 64.8 ± 9.5 mg/dl, respectively. The levels for fasting serum cholesterol and fasting serum triglycerides in Group 3 animals were 203.1 ± 65.2 mg/dl and 481.3 ± 232.3 mg/dl, respectively, which were significantly greater than Group 2 animals (p < 0.01), but not different from Group 1 rats.

Morphological studies
Light microscopic features for each group are summarized in Table 2. Glomerular hyalinosis and sclerosis were present in all the nephrectomized animals, affecting 5 to 53% of the glomeruli (Figs. 1 and 2). The glomerular lesions were completely absent in all of the sham-operated animals sacrificed in the seventh month. The percentages of glomeruli with hyalinosis and sclerosis in Group 3 rats were signifi-

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Values are means ± SD.
*P < 0.01; **P < 0.01 vs. Group 2 values
them showed fibrinoid necrosis of the arterioles. The capillary lumens occasionally contained several polymorphonuclear leukocytes, platelet aggregates, and fat vacuoles. Epithelial foot processes were irregularly effaced, affecting 10 to 90% of the areas examined. Epithelial cell cytoplasm often exhibited lipid vacuoles, electron-dense lysosomes, myelin figures, and periodic dense bands composed of cross-striated fibrils.

DISCUSSION

Progressive glomerular sclerosis is a morphologic change that has been studied extensively in the renal ablation model (Stiker et al., 1963; Lalich et al., 1975; Shimamura et al., 1975; Pukerson et al., 1976; Grond et al., 1982; Olson et al., 1982, 1985, 1986, 1987; Hostetter et al., 1981, 1986; Schwartz et al., 1987). The pathologic features of the equally important hyalnosis lesion in the glomeruli were nicely described by Olson et al. (1985). In this experiment, the author confirmed all the morphologic findings described by them. In addition, I observed that an accumulation of lipid droplets in the subendothelial space of glomeruli accompanying electron-dense proteinaceous material was more frequently present in rats at month 7 than in animals at months 3 and 5. This lesion sometimes occurred in glomeruli which appeared normal by light microscopy and corresponded to incipient hyalnosis lesion.

The increased intraglomerular lipid deposition, not only in the segments with hyalnosis/sclerosis lesions but also in normal mesangium of rats with chronic aminonucleoside nephrosis, was well demonstrated by researchers (Grond et al., 1984, 1988; Diamond and Karnovsky, 1987; Ohsawa et al., 1988). Although the presence of

Fig. 5. Electron micrograph of a capillary loop showing widening of lamina rara interna containing medium-density lipid droplets at inset. M, macrophage ×9,800. Inset ×28,000
lipid material in hyalnosis lesion in rats with experimental nephrosis was also described by Olson et al. (1985), its frequency or morphologic detail is not clearly defined. In fact, the foci with the increased accumulation of lipid droplets in the subendothelial regions of glomeruli are often present in a few glomerular loops and can easily be overlooked. The occurrence of lipid vacuoles in the glomerular peripheral loops was only rarely noted, specifically, in a few human cases with congenital lecithin-cholesterol acyltransferase deficiency (Myhre et al., 1977), Alagille syndrome (Chung-Park et al., 1982), hepatic glomerulosclerosis (Sakaguchi et al., 1966), and IgA nephropathy (Shigematsu et al., 1982; Lee et al., 1989), most of which were associated with progressive renal failure.

As in atherosclerosis, damage to the glomerular endothelium seems to be the initiating event in glomerular hyalinosis. There may be a spectrum of endothelial injury, ranging from alterations in cell-surface constituents to loss of endothelial cover (Ross, 1986). In the present study, the glomerular endothelial injury was evidenced by detachment of these cells from the GBM, and the presence of thrombi and of several platelet aggregates adhering to the endothelium, as already noted by others (Olson et al., 1985). In the renal ablation model, adaptive hyperfiltration in residual glomeruli appears to induce endothelial damage (Hostetter et al., 1981). The subsequent interaction of the damaged glomerular endothelium with circulating platelets, monocytes, and lipids might instigate a glomerular atherosclerosis process (Ross, 1986). Moorhead et al. (1982) suggested that an ini-
tial glomerular injury leads to abnormalities in lipid metabolism. This alteration in lipid metabolism may then play a role in the progression of initial glomerular injury to FSGS (Moorhead et al., 1982; Diamond and Karnovsky, 1987). In the present study, the peak levels of hyperlipidemia and proteinuria were noted at the termination of the experiment, in relation to more glomerular involvement with hyalinosis/sclerosis, partly substantiating the above suggestions.

The lamina rara interna of GBM is closely related, histogenetically and phylogenetically, to the mesangium (Morita and Churg, 1983). The plasma constituents or filter residues that penetrated to the capillary endothelium and the underlying lamina rara interna are removed to the mesangium and then phagocytosed by the mesangial cells (Sinniah and Churg, 1983). Alternatively, the macromolecules deposited in the mesangium may be solubilized and return to the capillary lumen (Sinniah and Churg, 1983). I noted that the lamina rara interna, which expanded due to the accumulation of lipids and proteinaceous substances, occasionally blended with the mesangium, which was filled with the same material. Although the factors causing the increase in mesangial matrix are not known, the increased accumulation of filtered lipoproteins in mesangial cells may stimulate the mesangial cell to produce an excess matrix substance (Moorhead et al., 1982; Grond et al., 1984, 1986, 1988).

Arterial fatty streaks in humans, initially formed due to the accumulation of subendothelial macrophages, become enlarged due to the gradual accumulation of smooth-muscle cells that migrate into the intima from the media and also accumulate lipids (Ross, 1986). In this experiment, there was no morphologic evidence that macrophages participate in the formation of hyalinosis lesions in the glomeruli, except for the occasional presence of lipid-containing macrophages in the glomerular capillary lumens close to the hyalinosis lesions. Nevertheless, the early hyalinosis foci with increased accumulation of fat droplets are reminiscent of early fatty streaks. In addition, the fully advanced glomerular sclerotic lesions with foam cell formation have the same appearance as advanced fatty streaks with lipid-filled macrophages, or foam cells.

Depending upon the magnitude and nature of endothelial damage, the morphologic features of the renal ablation model appear to be either segmental hyalinosis or, rarely, fibrinoid necrosis. The rarer occurrence of fibrin material in the glomeruli and the slower development of glomerular sclerosis in this 2/3 reduction model, when compared to those in the very extensive renal ablation model, confirm previous observations (Shimamura et al., 1975; Grond et al., 1982; Hostetter et al., 1981, 1986; Schwartz et al., 1987) that the extent of the initial loss of renal mass appears to dictate the ultimate prevalence of pathological changes.

In summary, the present long-term study suggests that the development of glomerular hyalinosis may be related to the subendothelial deposition of harmful lipids in the circulation, eventually leading to sclerosis, and supports the view that the pathogenetic mechanism of glomerular hyalinosis/sclerosis may be the same as that of atherosclerosis.

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신장 절제술을 시행한 쥐에서 관찰되는 지방 침착과 유관한 하이알리노시스에 관한 연구

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이현순

신장 중첩량의 2/3을 제거한 Sprague-Dawley 천개 17마리의 마우스로 6마리를 대상으로 하여, 신사구체 내막하여 반하려는 지방이 신사구체 하이알리노시스 및 경화증의 발병과 어떠한 연관성은 갖는지에 조사하였다. 기능적 및 형질학적 변화는 수술 후 각각 3개월(N = 6), 5개월(N = 5), 7개월(N = 6)에 걸쳐 연속적으로 조사하였다. 7개월 실험군에서 24시간 요단백 양은 3개월 군에 비해 의미있게 높았고, 천정 플레스테롤 및 트리글리세리드치는 5개월군 모자로 의미있게 높았다. 광활 신장경질자상 7개월 군은 3개월 군에 비해 신사구체 하이알리노시스 및 경화증의 비율이 의미있게 높았다. 신사구체경질자상 신사구체의 초기 하이알리노시스에 인화하는 지방 및 단백질질의 내막과 작용은 3개월 및 5개월 군보다는 7개월 군에서 더 자주 관찰되었다. 예 산화ген 지방 및 단백질질 침착은 9세에서 관찰된 바 실험군간의 의미있는 차이점은 발견되지 않았다.

이상의 결과로 미수어 본 때 신사구체의 하이알리노시스 발생은 동역경화증과 유사하게 신사구체 내막에 침착되는 지방과 유관한 것으로 사료되며 결국 이들 방법은 신사구체 경화증으로 진행될 것으로 생각되어 진다.