Pharmacologic Treatment of Hyperlipidemia Reduces Glomerular Injury in Rat 5/6 Nephrectomy Model of Chronic Renal Failure

Bertram L. Kasiske, Michael P. O'Donnell, William J. Garvis, and William F. Keane

The role of lipid abnormalities in the pathogenesis of focal glomerulosclerosis was investigated in the rat remnant kidney model of chronic renal failure. Rats subjected to right nephrectomy and two-thirds segmental infarction of the left kidney (5/6 nephrectomy) were treated for 10 weeks with the lipid-lowering agent clofibrate acid. Both serum cholesterol and urine albumin excretion were significantly reduced by clofibrate acid. At 10 weeks, the percent of glomeruli with focal glomerulosclerosis was 5 ± 2% in clofibrate acid–treated and 24 ± 5% in untreated 5/6 nephrectomy rats (p<0.01). Inulin clearance was greater in clofibrate acid–treated than in untreated 5/6 nephrectomy rats (0.28 ± 0.02 versus 0.22 ± 0.02 ml/min 100 g body wt, p<0.05). Body weight, kidney weight, and systemic blood pressure were not significantly altered by clofibrate acid. Micropuncture studies, performed in separate groups of clofibrate acid–treated and untreated 5/6 nephrectomy rats, demonstrated elevated single nephron glomerular filtration rates and glomerular capillary pressures 4 weeks after surgery. However, clofibrate acid did not significantly alter single nephron glomerular filtration rates (95 ± 2.1 nl/min in treated versus 97.0 ± 6.2 nl/min in untreated, p>0.05) or glomerular capillary pressures (56.6 ± 1.5 mm Hg in treated versus 57.8 ± 0.8 mm Hg in untreated, p>0.05) in 5/6 nephrectomy rats. In a separate set of experiments, 5/6 nephrectomy rats were treated with the specific cholesterol synthesis inhibitor, mevinolin. Mevinolin improved serum lipid levels and reduced albuminuria in 5/6 nephrectomy rats without causing significant alterations in blood pressure. Focal glomerulosclerosis was also reduced by mevinolin (11 ± 2% versus 30 ± 3%, p<0.01). These results suggest that lipid abnormalities may be important in the pathogenesis of focal glomerulosclerosis in the rat 5/6 nephrectomy model of chronic renal failure. (Circulation Research 1988;62:367–374)
Experiments, group 1 (n = 10) consisted of rats subjected to 5/6 nephrectomy (see below). Group 2 (n = 11) rats were subjected to 5/6 nephrectomy and were treated with clofibrate acid (Sigma Chemical, St. Louis, Missouri). Clofibrate acid dissolved in propylene glycol (50–125 mg/ml) was injected subcutaneously. Rats in group 2 received 50 mg clofibrate acid/kg body wt daily for 3 weeks, followed by 125 mg/kg body wt daily for the remainder of the study period. These doses of clofibrate acid were chosen to parallel anticipated changes in renal elimination of the drug. Six normal, two-kidney rats served as a third untreated control group (group 3) for this first set of experiments.

In the second set of experiments, micropuncture was performed in six additional untreated (group 4) and six clofibrate acid–treated (group 5) 5/6 nephrectomy rats. Micropuncture studies were carried out 4 weeks after surgery in both groups. Micropuncture studies were also carried out in eight two-kidney controls matched for body weight (group 6).

In the third set of experiments, an additional group of 13 5/6 nephrectomy rats (group 7) were injected with vehicle and compared with 15 5/6 nephrectomy rats (group 8) treated with mevinolin (Merck Sharp & Dohme, West Point, Pennsylvania). Using propylene glycol as the vehicle, 4 mg mevinolin/kg body wt was injected subcutaneously each day. To examine the possibility that differences in food intake could account for some of the observed effects of mevinolin, one half of the rats in groups 7 and 8 were pair fed. An additional group of eight ad libitum–fed, two-kidney, untreated rats were also used as controls (group 9).

In all of these investigations, renal ablation (5/6 nephrectomy) was carried out using the same technique. The surgical procedure included isolation of the left renal artery branches and ligation of the inferior and superior branches, leaving only the posterior branch intact. The right kidney was removed simultaneously.

All rats were fed standard laboratory chow (Ralston Purina, St. Louis, Missouri) and were allowed free access to water. Pair-fed mevinolin-treated and untreated rats from groups 7 and 8 were housed individually. All other rats were housed two per cage and were fed ad libitum.

Table of Abbreviations

- BW, body weight
- Cto, inulin clearance
- LKW, left kidney weight
- MAP, mean arterial pressure
- Ppr, free-flow tubular pressure
- Pst, stop-flow tubular pressure
- PE, efferent pressure
- Pt, free-flow tubular pressure
- MAP, mean arterial pressure
- Cta, inulin clearance
- BW, body weight
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- Pt, free-flow tubular pressure
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group to which the rat belonged. A semiquantitative scoring system was used to assess the amount of mesangial matrix expansion as previously described. In each tissue specimen, a minimum of 50 glomeruli were examined. For each glomerulus, the amount of mesangial matrix expansion was graded from 0 to 4+ according to the percent of the glomerular tuft involved. A mesangial expansion score was obtained for each tissue specimen by multiplying the degree of mesangial expansion (0—4+) by the percent of glomeruli with the same degree of injury. The amount of mesangial expansion for the specimen was then obtained by the addition of these scores. In addition, the percent of glomeruli with focal glomerulosclerosis was determined for each tissue specimen by dividing the number of glomeruli with any focal glomerulosclerosis by the total number of nephrons in the specimen.

The extent of tubular dilation, tubular epithelial cell flattening, and interstitial fibrosis was qualitatively assessed. A score of 0—4+ was assigned to reflect the severity of these tubulointerstitial changes for each specimen.

**Single Nephron Function Studies**

Rats were not fasted prior to micropuncture. Studies were carried out in euvoletic rats using techniques previously described. Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg body wt) and placed on a heated table to maintain body temperature between 36.5° and 37.5° C. A tracheostomy was performed, the left femoral vein was cannulated (PE-50), and a solution of lactated Ringer’s containing 25 μCi [3H]inulin/ml (New England Nuclear) was infused at a rate of 0.5 ml/100 g body wt/hr. The femoral artery was cannulated with PE-50 tubing, and MAP was monitored with a digital display pressure transducer (model 91, Western Laboratories, Morrison, Colorado). A PE-50 catheter was placed in the bladder. The remnant kidney was exposed by a subcostal incision, dissected free of perirenal tissue, immobilized in a plastic holder, and continuously bathed with mineral oil at 37° C.

After a 45-minute stabilization period, urine was collected under mineral oil in preweighed tubes for a period of 20—30 minutes. During this interval, three timed (2—3-minute) proximal tubular fluid collections were obtained from superficial nephrons using sharpened glass pipettes (tip diameter 8—10 μm) filled with Sudan black-colored mineral oil. These collections were made just proximal to an oil column of several tubular diameters in length. Arterial blood samples were also collected during this period. Urine volumes were determined gravimetrically. Samples of urine, plasma, and tubular fluid were added to 10-ml scintillation cocktail (SCINT-A, Packard, Downers Grove, Illinois), and radioactivity of the samples was measured in a liquid scintillation spectrometer (model LS230, Beckman Instruments, Fullerton, California).

Pf was measured under free-flow conditions in one group of tubules and after blockage of the tubular lumen (Pf) with Sudan black-colored mineral oil in a different
Table 1. Effects of Clofibrate Add on 5/6 Nephrectomy Rats

<table>
<thead>
<tr>
<th></th>
<th>Group 1 5/6 nephrectomy (n = 10)</th>
<th>Group 2 5/6 nephrectomy clofibrate acid (n = 11)</th>
<th>Group 3 two-kidney controls (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>401 ± 15*</td>
<td>416 ± 16*</td>
<td>484 ± 20†</td>
</tr>
<tr>
<td>Left kidney weight (g)</td>
<td>2.03 ± 0.08*</td>
<td>1.88 ± 0.12*</td>
<td>1.47 ± 0.06†</td>
</tr>
<tr>
<td>Blood pressure, early (mm Hg)</td>
<td>197 ± 10*</td>
<td>183 ± 7*</td>
<td>142 ± 2†</td>
</tr>
<tr>
<td>Triglycerides, early (mg/dl)</td>
<td>71 ± 6*</td>
<td>77 ± 6*</td>
<td>52 ± 12*</td>
</tr>
<tr>
<td>Cholesterol, early (mg/dl)</td>
<td>76 ± 4*</td>
<td>45 ± 3†</td>
<td>40 ± 2†</td>
</tr>
<tr>
<td>Cholesterol, late (mg/dl)</td>
<td>94 ± 7*</td>
<td>53 ± 6†</td>
<td>43 ± 3†</td>
</tr>
<tr>
<td>Albuminuria, early (mg/24 hr)</td>
<td>28.0 ± 7*</td>
<td>3.3 ± 0.8†</td>
<td>0.8 ± 0.2†</td>
</tr>
<tr>
<td>Albuminuria, late (mg/24 hr)</td>
<td>62.5 ± 10*</td>
<td>11.0 ± 4.0†</td>
<td>0.6 ± 0.1†</td>
</tr>
</tbody>
</table>

*Mean ± SEM; shared superscripts indicate p>0.05.

Hypercholesterolemia associated with 5/6 nephrectomy (Tables 1 and 2).

Urine Albumin Excretion
Both clofibrate acid and mevinolin caused significant reductions in urine albumin excretion (Tables 1 and 2). The reduced albuminuria was evident both early and late in the experimental periods.

Renal Function
Five weeks after surgery, mean serum creatinine in group 1 and group 2 5/6 nephrectomy rats was approximately twice that of normal group 3 rats (Table 3). Creatinine levels were similar in untreated group 1 and clofibrate acid–treated group 2 rats at 5 weeks (Table 3). By 10 weeks, however, serum creatinine had decreased in the treated rats. As a result, at 10 weeks serum creatinine was significantly less in clofibrate acid–treated rats (group 2) compared with untreated (group 1) 5/6 nephrectomy rats (Table 3).

Results of Cto measurements paralleled changes in serum creatinine. Ten weeks after 5/6 nephrectomy, Cto in clofibrate acid–treated group 2 rats was greater than that of untreated group 1 rats (Table 3), but this difference was of borderline significance (p = 0.056). When Cto was normalized for body weight, however, Cto/100 g body wt in the clofibrate acid–treated rats was significantly greater than that of untreated 5/6 nephrectomy rats (Table 3).

Histology
Clofibrate acid caused a fourfold reduction in the percent of glomeruli with sclerosis (Figure 1). Similarly, the extent of mesangial matrix expansion was markedly reduced by treatment with clofibrate acid. The mesangial matrix score in group 1 untreated 5/6 nephrectomy rats was 175 ± 14 compared with 89 ± 11 in group 2 clofibrate acid–treated rats (p<0.05) and 32 ± 3 in group 3 two-kidney controls (p<0.05 versus groups 1 and 2). Tubulointerstitial injury paralleled the degree of glomerular damage. Mean tubulointerstitial injury scores were 2.6 ± 0.3 and 0.7 ± 0.2 for groups 1 and 2, respectively (p<0.05). No tubulointerstitial damage was seen in group 3 two-kidney controls.

Mevinolin caused a reduction in glomerular injury comparable to that seen in clofibrate acid–treated 5/6 nephrectomy rats. Focal glomerulosclerosis, for example, was reduced by more than 50% in mevinolin–treated 5/6 nephrectomy rats (Figure 1). Similar reductions in mesangial matrix and tubulointerstitial injury were seen in mevinolin–treated rats.

Superficial Nephron Function
No statistically significant differences in body weight, kidney weight, or blood pressure were ob-

Table 2. Effects of Mevinolin on 5/6 Nephrectomy Rats

<table>
<thead>
<tr>
<th></th>
<th>Group 7 5/6 nephrectomy (n = 13)</th>
<th>Group 8 5/6 nephrectomy mevinolin (n = 15)</th>
<th>Group 9 two-kidney controls (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>422 ± 8*</td>
<td>386 ± 9†</td>
<td>487 ± 16†</td>
</tr>
<tr>
<td>Left kidney weight (g)</td>
<td>1.80 ± 0.1*</td>
<td>1.72 ± 0.05*†</td>
<td>1.51 ± 0.2†</td>
</tr>
<tr>
<td>Blood pressure, early (mm Hg)</td>
<td>185 ± 5*</td>
<td>169 ± 7*</td>
<td>114 ± 6†</td>
</tr>
<tr>
<td>Triglycerides, early (mg/dl)</td>
<td>130 ± 9*</td>
<td>97 ± 7†</td>
<td>85 ± 11†</td>
</tr>
<tr>
<td>Cholesterol, early (mg/dl)</td>
<td>132 ± 6*</td>
<td>89 ± 5†</td>
<td>66 ± 10†</td>
</tr>
<tr>
<td>Cholesterol, late (mg/dl)</td>
<td>138 ± 8*</td>
<td>92 ± 8†</td>
<td>56 ± 11†</td>
</tr>
<tr>
<td>Albuminuria, early (mg/24 hr)</td>
<td>37.9 ± 8.1†</td>
<td>13.8 ± 3†</td>
<td>0.5 ± 0.3†</td>
</tr>
<tr>
<td>Albuminuria, late (mg/24 hr)</td>
<td>83.8 ± 8.6*</td>
<td>43.6 ± 7†</td>
<td>0.8 ± 0.6†</td>
</tr>
</tbody>
</table>

*Mean ± SEM; shared superscripts indicate p>0.05.
Table 3. Renal Function in Untreated and Clofibric Acid–Treated 5/6 Nephrectomy Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum creatinine at 5 weeks (mg/dl)</th>
<th>Serum creatinine at 10 weeks (mg/dl)</th>
<th>Inulin clearance at 10 weeks (ml/min)</th>
<th>Inulin clearance per 100 grams body weight (ml/min/100 g body wt)</th>
<th>Mean arterial pressure (anesthesia) (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: 5/6 nephrectomy (n = 10)</td>
<td>1.11 ± 0.03*</td>
<td>1.09 ± 0.13*</td>
<td>0.90 ± 0.09*</td>
<td>0.22 ± 0.02*</td>
<td>157 ± 11*</td>
</tr>
<tr>
<td>Group 2: 5/6 nephrectomy + clofibric acid (n = 11)</td>
<td>0.99 ± 0.05*</td>
<td>0.76 ± 0.04†</td>
<td>1.20 ± 0.11*</td>
<td>0.28 ± 0.02†</td>
<td>151 ± 12*</td>
</tr>
<tr>
<td>Group 3: two-kidney controls (n = 6)</td>
<td>0.55 ± 0.07†</td>
<td>0.53 ± 0.04‡</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Mean ± SEM; shared superscripts indicate p > 0.05.

Discussion

Clofibric acid is the pharmacologically active form of the lipid-lowering agent clofibrate. Clofibric acid inhibits hepatic release of lipoproteins, interferes with albumin binding of free fatty acids, inhibits cholesterol biosynthesis, improves fatty acid metabolism, and enhances lipoprotein lipase activity. In the present study, clofibric acid substantially reduced the marked glomerular, tubulointerstitial, and vascular injury that
glomerulosclerosis. Prostaglandin production may be altered by alterations in glomerular capillary pressures.

Whether the protective effects of clofibric acid seen in the present study were related to glomerular injury seen in the clofibric acid study resulted from marked reductions in food intake. Platelet inhibition with acetylsalicylic acid and dipyridamole has been shown to reduce blood pressure and ameliorate glomerular injury in the rat remnant kidney model. It is possible that clofibric acid directly or indirectly altered platelet function and that platelet inhibition reduced the amount of glomerular injury.

Although clofibric acid has been reported to alter platelet function in vitro, the significance of these findings is controversial. Clofibric acid has no known effects on coagulation factors affected by heparin and warfarin, agents also shown to ameliorate glomerular injury in this model of renal disease.

Recent studies in the 5/6 nephrectomy model have shown that dietary and pharmacologic maneuvers that lower glomerular capillary pressure retard the development of focal glomerulosclerosis. Indeed, normalization of glomerular capillary pressure with the angiotensin-converting enzyme inhibitor enalapril reduced glomerular size and diminished renal injury in this model. In the present study, glomerular area was reduced, but glomerular capillary pressures were unaltered by clofibric acid in 5/6 nephrectomy rats. Thus, it is unlikely that the beneficial effects of clofibric acid resulted from alterations in glomerular capillary pressures.

Similarly, experimental maneuvers that alter prostaglandin metabolism appear to preserve renal structure and function in the 5/6 nephrectomy model. The specific thromboxane synthesis inhibitor, OKY 1581, increased glomerular filtration rate and ameliorated glomerular injury. Also, a linoleic acid–enriched diet augmented renal production of vasodilatory prostaglandins, ameliorated proteinuria, and reduced focal glomerulosclerosis. Prostaglandin production may be affected by alterations in cholesterol and triglyceride metabolism. Whether the protective effects of clofibric acid seen in the present study were related to alterations in prostaglandin synthesis is unknown.

To further investigate the possibility that the reduction in glomerular injury associated with clofibric acid treatment was specifically related to improved lipid metabolism, a separate set of experiments was carried out using a structurally unrelated lipid-lowering agent. Mevinolin is a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis. Mevinolin has been shown to lower serum cholesterol and increase low-density lipoprotein receptors. In the present studies, mevinolin caused significant reductions in both cholesterol and triglycerides in 5/6 nephrectomy rats (Table 2). Mevinolin also decreased urine albumin excretion and reduced the amount of glomerulosclerosis. The reduction in glomerulosclerosis appeared to be somewhat less with mevinolin than with clofibric acid. There was 5 ± 2% sclerosis in clofibric acid–treated 5/6 nephrectomy rats compared with 11 ± 2% in the mevinolin-treated rats. However, the substantial variability in the amount of glomerulosclerosis within each group and the fact that the two sets of experiments were not carried out at the same time make it difficult to conclude that the protective effect of clofibric acid was greater than that of mevinolin.

Table 4. Single Nephron Function In Untreated and Clofibric Acid–Treated Rats 4 Weeks After 5/6 Nephrectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>LKW (g)</th>
<th>MAP (mm Hg)</th>
<th>SNGFR (ml/min)</th>
<th>SNPF (ml/min)</th>
<th>SNFF</th>
<th>CA (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 4</td>
<td>325 ± 17</td>
<td>1.38 ± 0.14</td>
<td>138 ± 5</td>
<td>97.0 ± 6.2*</td>
<td>305 ± 17*</td>
<td>0.32 ± 0.02</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>Group 5</td>
<td>337 ± 20</td>
<td>1.42 ± 0.13</td>
<td>152 ± 6*</td>
<td>95.3 ± 2.1*</td>
<td>291 ± 16*</td>
<td>0.33 ± 0.02</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>Group 6</td>
<td>351 ± 9</td>
<td>1.15 ± 0.03</td>
<td>124 ± 4</td>
<td>35.5 ± 1.9</td>
<td>111 ± 11</td>
<td>0.33 ± 0.02</td>
<td>5.2 ± 0.1</td>
</tr>
</tbody>
</table>

*p < 0.05 versus group 6.

Group 4, untreated 5/6 nephrectomy rats (n = 6); Group 5, clofibric acid–treated 5/6 nephrectomy rats (n = 8); BW, body weight; LKW, left kidney weight; MAP, mean arterial pressure during micropuncture; SNGFR, single nephron glomerular filtration rate; SNPF, single nephron plasma flow; SNFF, single nephron filtration fraction; CA, arterial plasma protein concentration; Pxx, arterial plasma oncotic pressure; ΔP, mean transcapillary hydraulic pressure difference; Kf, glomerular ultrafiltration coefficient.
there were important changes in triglyceride metabolism that were not detected by measuring fasting triglyceride levels.

Clofibrate and mevinolin may have reduced renal injury by decreasing the amount of lipid deposition in remnant nephrons. Altered cholesterol and/or triglyceride metabolism have been shown to influence the development and progression of focal glomerulosclerosis in a number of animal models. Dietary cholesterol supplementation, for example, exacerbated the extent and severity of age-associated focal glomerulosclerosis in rats, rabbits, and guinea pigs. Although the mechanism of injury is unknown, increased levels of cholesterol in renal tissue have been measured, and glomerular lipid deposits have been observed.924

Investigations using the puromycin aminonucleoside rat model of the nephrotic syndrome have also suggested a potential role for lipids in the pathogenesis of focal glomerulosclerosis. Rats given multiple injections of puromycin aminonucleoside developed proteinuria, focal glomerulosclerosis, and a significant deposition of lipids in the mesangial. Halofenate, a clofibrate-like compound, reduced focal glomerulosclerosis in puromycin aminonucleoside–treated rats fed a lipogenic diet. The results of these and other studies are consistent with the hypothesis that alterations in lipid metabolism may have an important role in the development and progression of renal injury in animal models of focal glomerulosclerosis.

Results of experiments using the remnant kidney model suggest that several factors may be important in the pathogenesis of glomerular injury in chronic renal failure. These mechanisms are not necessarily mutually exclusive. It is possible that increased glomerular capillary pressure, coagulation factors, and lipid abnormalities interact synergistically in the pathogenesis of focal glomerulosclerosis. Indeed, a similar relation between these factors has been shown to be important in the development of atherosclerosis.95 The glomerulus has many structural features that resemble arteries commonly involved in atherosclerosis. Mesangial cells, for example, are structurally similar to arterial smooth muscle cells important in the pathogenesis of atherosclerosis.96 Lipid-laden macrophages are frequently found in both early atherosclerosis lesions and focal glomerulosclerosis.97 Thus, factors important in the pathogenesis of focal glomerulosclerosis may be similar to those that influence the development of atherosclerosis. The results of the present study suggest lipid abnormalities, together with increased glomerular capillary pressure and coagulation factors, may be important in the pathogenesis of focal glomerulosclerosis.

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