Combined Antihypertensive and Lipid-Lowering Therapy in Experimental Glomerulonephritis

Rhonda Rubin, Sharon Silbiger, Leonarda Sablay, Joel Neugarten

Abstract We examined the interrelation between systemic hypertension, hyperlipidemia, and progressive renal injury in experimental glomerulonephritis. Induction of nephrotoxic serum nephritis in Sprague-Dawley rats led to systemic hypertension and hyperlipidemia. Four groups of rats were studied over a 16-week period: (1) untreated nephritic rats; (2) nephritic rats treated with hydralazine, reserpine, and lasix (AH); (3) nephritic rats treated with lovastatin (4 mg/kg) (Lova); and (4) nephritic rats treated with combined antihypertensive/lipid-lowering therapy (AH/Lova). Systolic blood pressure rose progressively in untreated rats (152±4 mm Hg at 16 weeks). Blood pressure was reduced by antihypertensive therapy (P<.001) (108±2 mm Hg in the AH group and 111±3 mm Hg in the AH/Lova group) but remained elevated in animals treated with lovastatin alone (P>.05) (156±3 mm Hg in the Lova group). Serum cholesterol rose progressively in untreated rats (2.22±0.41 mmol/L [86±16 mg/dL] at 16 weeks). The rise in serum cholesterol was prevented by lovastatin therapy (P<.001) (2.22±0.41 mmol/L [86±16 mg/dL] in the Lova group and 2.09±0.52 mmol/L [81±2 mg/dL] in the AH/Lova group) but not antihypertensive therapy (P>.05) (2.92±0.65 mmol/L [113±25 mg/dL] in the AH group). Proteinuria was reduced by antihypertensive therapy (P<.001) and lipid-lowering therapy (P<.05) (16-week values: 1.069±0.167 g/d in untreated rats, 0.663±0.164 g/d in the Lova group, 0.392±0.051 g/d in the AH group, and 0.176±0.035 g/d in the AH/Lova group). Glomerular injury score was significantly reduced by antihypertensive therapy (P<.01) and lipid-lowering therapy (P<.05). Glomerular injury score was lowest in animals receiving combined therapy, reflecting an interaction between these therapies (P<.01) (untreated, 173±29; Lova, 128±24; AH, 111±22; AH/Lova, 48±11). Our results suggest that both hypertension and hyperlipidemia accelerate glomerular sclerosis in experimental glomerulonephritis and that combined therapy of these disorders may best limit progressive renal injury. (Hypertension. 1994;23:92-95.)

Key Words • hyperlipidemia • glomerulonephritis • hypertension

Complex interrelations exist between systemic hypertension, hyperlipidemia, and progressive renal injury. Recent evidence indicates that hyperlipidemia may be associated with accelerated renal damage in experimental glomerular disease and that lipid-lowering therapy ameliorates progressive renal injury in many of these models.1-3 Moreover, it has been suggested that pathogenetic mechanisms responsible for the evolution of fatty streak lesions in blood vessels are analogous to those responsible for the development of glomerulosclerosis.1,2 We have previously shown that systemic hypertension exacerbates renal injury in experimental glomerulonephritis.4 Similarly, other investigators have demonstrated acceleration of atherosclerotic vascular lesions when experimental hypertension is combined with an atherogenic diet or superimposed on hereditary hyperlipidemia.5-8 We hypothesize that factors predisposing to atherosclerotic vascular lesions, most notably systemic hypertension and hyperlipidemia, may have similar importance in the development of glomerular sclerosis. In fact, several studies suggest that hypertension and hyperlipidemia may have additive or perhaps synergistic effects on the development and progression of glomerular injury, as reflected by proteinuria and glomerular sclerosis.9-12 In this context, we undertook a study to determine the relative efficacy of antihypertensive and cholesterol-lowering therapy in ameliorating progressive renal injury in nephrotoxic serum nephritis in the rat, a model characterized by both hypertension and hyperlipidemia. Our results suggest that both hypertension and hyperlipidemia accelerate glomerular sclerosis in experimental glomerulonephritis and that combined therapy of these disorders best limits progressive renal injury.

Methods

Eighty-seven male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) weighing 350 to 425 g underwent unilateral nephrectomy under pentobarbital anesthesia. On the second postoperative day, all rats were immunized by subcutaneous administration of 20 mg goat γ-globulin in Freund’s complete adjuvant.9 This was followed on the fifth and eighth postoperative days by intravenous injection of goat γ-globulin with activity directed against rat glomerular basement membrane. Four weeks after induction of nephritis, blood samples and 24-hour urine collections were obtained for determination of blood urea nitrogen, cholesterol, triglyceride, and creatinine in the serum and protein and creatinine in the urine. Animals were randomly divided into four groups. Group 1 (untreated) consisted of 23 rats given normal saline to drink. Group 2 (AH) consisted of 21 rats treated with antihypertensive drugs added to the saline drinking solution (hydralazine, 200 mg/L; reserpine, 2 mg/L; furosemide, 100 mg/L). Group 3 (Lova) consisted of 20 rats given normal saline to drink and treated with daily subcutaneous injections of lovastatin (4 mg/kg in 50% propylene glycol). Group 4 (AH/Lova) consisted of 23 rats given normal saline and treated with both the antihypertensive regimen and daily subcutaneous injections of lovastatin. Groups 1 and 2 received daily subcutaneous injec-
tions of vehicle. Each animal was fed standard rat chow preweighed to provide 20 g/d. All food was consumed. Drinking solution was "pair-fed" to administer identical quantities of saline. At monthly intervals, tail systolic blood pressure was measured, 24-hour urine collections for protein and creatinine measurements were obtained, and blood was collected for creatinine, cholesterol, and triglyceride determinations. Systolic blood pressure measurements were made with rats under light ether anesthesia using a tail-cuff sphygmomanometer (Narco BioSystems, Houston, Tex). Immediately before death at 16 weeks, 24-hour urine collections for protein and creatinine were obtained, and blood was collected for serum creatinine, triglyceride, and cholesterol determinations. Intra-arterial blood pressure was determined immediately before death by previously described techniques.13

The procedures followed were in accordance with institutional guidelines for animal experimentation. Urinary and serum creatinine were determined by the Jaffe-rate method using an automated creatinine analyzer (Beckman Instruments, Brea, Calif). Cholesterol and triglyceride levels were determined enzymatically (Sigma Diagnostic Laboratories, St Louis, Mo). Urinary protein was measured by colorimetric assay (Bio-Rad protein assay, Bio-Rad Laboratories, Richmond, Calif).

At death, one half of each kidney was sectioned in the coronal plane and fixed in 10% buffered Formalin. Coded sections of renal cortex cut at approximately 4 µm were stained with hematoxylin and eosin and examined by one of the investigators (L.S.) without knowledge of the group from which the specimen was obtained. A semiquantitative score was used for assessment of the degree of glomerular and tubulointerstitial damage.14 A minimum of 50 glomeruli in each specimen were examined, and the extent and severity of glomerular injury were rated on a scale of 0 to 4+ as follows: grade 0, no abnormality; grade 1, mild mesangial expansion; grade 2, segmental endocapillary proliferation or moderate mesangial expansion; grade 3, diffuse endocapillary proliferation; and grade 4, endocapillary and extracapillary proliferation. A glomerular injury score was calculated for each kidney as the sum of each grade (0 to 4+) multiplied by the percent of glomeruli assigned that grade. The extent and severity of tubulointerstitial injury tended to be focal, the assigned grade was multiplied by the approximate percent involvement to yield a tubulointerstitial injury score. Additional renal tissue was snap-frozen at death, and cryostat sections were cut at 3 µm. Frozen sections were stained with oil red O by standard techniques and examined for the presence of lipid-laden foam cells.

### Statistical Analysis

All values are summarized as the mean±SEM. Data were analyzed with two-way ANOVA with 95% confidence intervals and Duncan's multiple range testing (STATGRAPHICS software, version 5.2, 1991, STSC Inc, Rockville, Md). Type III sums of squares were used for hypothesis testing of this unbalanced design. Morphological data were subjected to logarithmic transformation before analysis. Statistical significance was defined using an overall type I error of 0.05.

### Results

Final body weight was significantly lower in animals receiving antihypertensive therapy (P<.05) (Table). Lipid-lowering therapy had no effect on body weight, nor was there a significant interaction between the two therapies on body weight (Table). Systolic blood pressure measurements are shown in Fig 1. Antihypertensive therapy significantly lowered blood pressure (P<.001). Lovastatin had no effect, nor was there a significant interaction between the two therapies on blood pressure. Animals that received the antihypertensive regimen either alone (AH) or in com-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Lova</th>
<th>AH</th>
<th>AH/Lova</th>
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<td>Body weight, g</td>
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<td>551±20</td>
<td>501±17*</td>
<td>497±18*</td>
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<td>Serum triglyceride, mg/dL</td>
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<td>102±11*</td>
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<td>Proteinuria, mg/24 h</td>
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<td>663±87*</td>
<td>392±51†</td>
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<td>Heart wt/body wt, ×10⁻³</td>
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<td>2.57±0.13*</td>
<td>2.64±0.06*</td>
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<td>Kidney wt/body wt, ×10⁻³</td>
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<td>5.55±0.31</td>
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<td>Glomerular injury score</td>
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<td>111±22*</td>
<td>48±11†</td>
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<td>Interstitial injury score</td>
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<td>14±6</td>
<td>9±2‡§</td>
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<td>1.4±0.3</td>
<td>0.8±0.1†</td>
<td>0.7±0.1†</td>
</tr>
</tbody>
</table>

Lova indicates rats treated with lovastatin; AH, rats treated with antihypertensive drugs; and AH/Lova, rats treated with combined antihypertensive therapy and lovastatin.

*P<.05; †P<.01 vs untreated.

§P<.01 vs Lova and vs AH.

$P<.05$ vs Lova.

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FIG 1. Line graph shows tail-cuff systolic blood pressure (BP) in nephritic rats treated with lovastatin (Lova), antihypertensive drugs (AH), or combined antihypertensive therapy and lovastatin (AH-Lova). Elevated blood pressure was prevented by antihypertensive (P<.001) but not by lipid-lowering therapy (P>.05).
bination with lovastatin (AH-Lova) remained non-motensive throughout the study. At 16 weeks, blood pressure averaged 108 ± 2 mm Hg in the AH group and 111 ± 3 mm Hg in the AH/Lova group. In contrast, a progressive rise in blood pressure was observed in untreated and lovastatin-treated rats (16-week values: 152 ± 4 mm Hg in the untreated group and 156 ± 3 mm Hg in the Lova group).

As shown in Fig 2, serum cholesterol values were elevated in all groups at the time of randomization and ranged from 2.59 to 3.41 mmol/L (100 to 132 mg/dL) compared with values of 1.03 to 1.55 mmol/L (40 to 60 mg/dL) obtained in our laboratory in normal non-nephritic rats. Serum cholesterol levels rose progressively in the untreated rats andLovastatin-treated rats (16-week values: 152 ± 4 mm Hg in the untreated group and 156 ± 3 mm Hg in the Lova group).

The severity of interstitial injury was not significantly reduced by antihypertensive therapy (P>.05) but not by antihypertensive therapy. Antihypertensive therapy significantly reduced proteinuria (P<.001) and lipid-lowering therapy (P<.05) significantly reduced proteinuria (Table). There was no significant interaction between the two therapies on proteinuria. At death, serum creatinine values did not differ significantly among the groups. Heart weight factored by body weight was significantly reduced by antihypertensive therapy (P<.05) (Table). Kidney weight factored by body weight was significantly reduced by antihypertensive therapy (P<.01) but not lipid-lowering therapy (P>.05) (Table). There was no interaction between the two forms of therapy on heart weight or kidney weight. Glomerular injury score was significantly reduced by antihypertensive therapy (P<.01) and lipid-lowering therapy (P<.05). Combined antihypertensive and lipid-lowering therapy was more effective in ameliorating glomerular injury, reflecting an interaction between these therapies (P<.01) (untreated, 173 ± 29; Lova, 128 ± 24; AH, 111 ± 22; AH/Lova, 48 ± 11) (Table).

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Systemic hypertension and dietary hyperlipidemia each have been shown to accelerate the progression of glomerular injury in several different models of renal disease. Our results demonstrate that combined therapy of these abnormalities in nephrotic serum nephritis provides a better outcome than treatment of either disorder alone. We have previously shown that glomerular capillary pressure is increased in untreated, hypertensive nephritic rats and that antihypertensive therapy lowers intraglomerular pressure. We suggest that enhanced deposition of lipid within glomeruli in the setting of combined hypertension and hyperlipidemia is responsible for enhanced severity of glomerular injury when both abnormalities are present. Consistent with this hypothesis is the marked reduction in lipid-laden foam cells within glomeruli associated with amelioration of renal injury in animals treated with combined antihypertensive and lipid-lowering therapy.

Our study did not evaluate the possibility that lovastatin might impair immunologic responses or directly alter glomerular hemodynamics. However, studies performed in another model of renal injury, the obese Zucker rat, showed no alteration in glomerular capillary pressure in animals treated with mevinolin. Neither can we exclude the possibility that our results were influenced by the lower final body weight observed in animals treated with the antihypertensive regimen.

Hyperlipidemia is a frequent complication of renal disease and has recently been recognized as a possible

Fig 2. Line graph shows serum cholesterol levels in nephritic rats treated with Lovastatin (Lova), antihypertensive drugs (AH), or combined antihypertensive therapy and Lovastatin (AH-Lova). Parallel lines indicate range of normal values. Elevation in serum cholesterol was reduced by lipid-lowering (P<.001) but not by antihypertensive therapy.

Fig 3. Line graph shows proteinuria in nephritic rats treated with Lovastatin (Lova), antihypertensive drugs (AH), or combined antihypertensive therapy and Lovastatin (AH-Lova). Proteinuria was significantly reduced by antihypertensive (P<.001) and by lipid-lowering therapies (P<.05).
participant in progressive glomerular injury.1,2 In nephrotic serum nephritis, hyperlipidemia and enhanced oxidation of low-density lipoprotein cholesterol in the mesangial microenvironment of the diseased glomerulus may lead to lipid-induced renal injury.19 Glomerular injury is accelerated by cholesterol feeding in numerous experimental models of renal disease.12,17 In addition, cholesterol-lowering drugs have been shown to ameliorate renal injury in these models.1,16 Numerous investigators have pointed out similarities between the glomerular lesions of segmental hyalnosis and sclerosis and atherosclerotic lesions of the vessel wall.1,3 These glomerular lesions resemble histologically the early atherosclerotic plaque, with accumulation of lipid, serum proteins, monocytes, and lipid-laden macrophages.1,2 Central to this analogy are similarities in origin, structure, and function that exist between smooth muscle cells and glomerular mesangial cells.1,3 Factors predisposing to atherosclerotic vascular lesions may have similar importance in the development of glomerular sclerosis.19-21

When hypertension is superimposed on dietary or genetic hyperlipidemia, the development and progression of vascular lesions are markedly accelerated.5,8 Hypertension induced by renal artery clipping increases the rate of development of vessel lesions in cholesterol-fed baboons and rabbits and in Watanabe hereditary hypercholesterolemic rabbits.5,7 The contribution of hemodynamic factors to the development of atherosclerotic vascular lesions is further suggested by the reduction in the severity of intimal lesions induced by an atherogenic diet after vessel ligation.19 Similarly, an additive effect of combined hypertension and hyperlipidemia on the progression of glomerular lesions has been proposed.9,10 Grone et al9 induced hyperlipidemia in normal rats by feeding a diet enriched in saturated fat and cholesterol and compared the severity of glomerular injury with that observed in the clipped and unclipped kidneys of hyperlipidemic rats with-two kidney, one-clipped hypertension. Glomerulosclerosis was increased in the kidneys exposed to systemic hypertension and reduced in the clipped kidneys. Similarly, Kasiske et al10 showed exacerbation of proteinuria in two-kidney, one clip hypertension. Glomerulosclerosis was increased in the kidneys exposed to systemic hypertension and reduced in the clipped kidneys. Similarly, Kasiske et al10 showed exacerbation of proteinuria in two-kidney, one clip rats fed a high-cholesterol diet compared with rats subjected to each experimental maneuver alone. Glomerulosclerosis was increased in kidneys exposed to hypertension, whereas morphological protection was observed in the clipped kidney. That hypertension and hyperlipidemia interact to produce glomerular injury is suggested by studies in the corpulent spontaneously hypertensive rat.12 This strain is characterized by hypertension and hyperlipidemia and develops proteinuria and progressive glomerular and tubulointerstitial injury at a faster rate than observed in its normolipidemic counterpart, the spontaneously hypertensive rat.12 Moreover, studies performed in the Dahl salt-sensitive rat indicate that hyperlipidemia is a major factor contributing to the development of glomerular injury in this hypercholesterolemic model of systemic hypertension.11 Lipid-lowering therapy reduced blood pressure, proteinuria, and the severity of glomerular sclerosis.11 Our results in a model of immunologically mediated proliferative glomerulonephritis are in agreement with observations made in other models of renal disease. Our studies extend previous observations by examining the effect of combined therapeutic interventions on renal injury. The findings suggest that hypertension and hyperlipidemia both accelerate glomerular injury in renal disease and that combined therapy yields a more favorable outcome than treatment of either disorder alone. We suggest that enhanced deposition of lipid within glomeruli in the setting of combined hypertension and hyperlipidemia is responsible for enhanced severity of glomerular injury when both abnormalities are present.

References

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