Inhibition of Progression of Chronic Puromycin Aminonucleoside Nephrosis by Probucol, An Antioxidant

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ABSTRACT

Oxidants have been shown to be involved in the initiation of chronic puromycin aminonucleoside nephrosis in rats, but it is uncertain whether they have a role in the progression of this disease. Rats given a single internal jugular venous bolus of puromycin aminonucleoside (PA) develop early nephrotic syndrome that subsides after about 28 days followed by a 4-wk period of minimal proteinuria and then the development of focal glomerulosclerosis and increasing proteinuria. Fifty-two rats on a high-cholesterol diet were divided into four groups. Two groups of 16 animals each received a single internal jugular venous bolus of PA. One of these groups was started on the dietary antioxidant, probucol (1% wt/wt), 4 days after the PA injection and continued on it until termination. The remaining rats were given an internal jugular venous injection of an equimolar solution of normal saline. Five of these animals were also started on dietary probucol 4 days after the saline injection. At 11 days postinjection all animals given PA, whether on probucol or not, developed marked proteinuria with histologically minimal glomerular change and significant increases in intraglomerular monocyte and proliferating cell nuclear antigen counts. Forty-two days after PA injection all PA-injected rats had minimal urinary protein injection and no glomerular changes. At 98 days postinjection rats given PA without probucol developed focal glomerulosclerosis and significant proteinuria compared with PA-injected rats on probucol and those injected with saline (P < 0.05). The probucol-fed PA-injected rats showed no glomerular disease and their urinary protein excretion rates were very similar to those of the saline controls. The results indicate that probucol inhibits the progression of chronic puromycin aminonucleoside nephrosis and are consistent with the suggestion that oxidants are involved in the progression of this entity.

Key Words: Puromycin aminonucleoside nephrosis, oxidant injury, probucol, monocytes

C hronic puromycin aminonucleoside nephrosis (PAN) is a well-recognized model of human focal glomerulosclerosis (FGS) (1). Recent studies have suggested that oxidants may play an important role in the progression of chronic PAN (2–4). In these studies the use of antioxidants resulted in the amelioration of the renal disease (2–4). However, because the antioxidants were administered over the entire experimental period during which time the animals were given a series of subcutaneous injections of puromycin aminonucleoside (PA) (2–4) and because the initial glomerular damage induced directly by PA has been shown to be mediated by reactive oxygen species (5–9), it is possible that the ameliorating effect of the antioxidants in these experiments may have been the result of their inhibition of the early PA-induced reactive oxygen species-mediated glomerular visceral epithelial cell damage as demonstrated by Ricardo et al. in this model (8). Thus, although it is accepted that reactive oxygen species are involved in the initiation of PA (5–9), it is not yet established that they play a role in the progression of the chronic disease.

The model of chronic PAN which uses a single internal jugular venous bolus of PA to establish the disease (10) appears to be useful in the assessment of the role of oxidants in the progression of the disease, because the inciting agent, PA, is administered only once and an antioxidant can be introduced after the initiation of the disease at a time when it would no longer interfere with the direct PA-induced oxidant generation. In this model the characteristic triphasic response to the PA injection is characterized by severe proteinuria with minimal histologic glomerular change peaking at about 10 to 14 days postinjection and subsiding about 4 wk postinjection. This is followed by a 4-wk period of minimal or no proteinuria and then progression with increasing proteinuria and the development of FGS (10). By using this model of chronic PAN, the effect of probucol (P), a lipophilic antioxidant (11), which was introduced into the diet 4 days after PA injection at a time when much of the PA-induced reactive oxygen species generation had occurred and increased urinary protein excretion and glomerular visceral epithelial cell changes were detectable (8,12,13), was assessed over the long term. The results are reported herein.
METHODS

Experimental Design

The effect of probucol on the development of FGS was assessed by the evaluation of various blood, urine, and tissue parameters at postinjection Days 11 (peak proteinuria), 42 (quiescent phase), and 98 (progressive FGS phase) in the model of PAN induced by a single bolus of PA injected into the internal jugular vein (10). Probucol was added to the diet of the test rats 4 days after the PA injection.

Animals

Sixty 150- to 200-g male Sprague-Dawley rats were used in this experiment. They were housed in a University of British Columbia animal care facility and allowed food and water ad libitum. All animals were fed a 4% cholesterol-1% choline diet (Harlan Teklad, Madison, WI). This diet has been shown to exacerbate glomerular injury in chronic PAN (14).

All animal experimentation described in this report was conducted in accordance with the Canadian Council on Animal Care's Ethics of Animal Experimentation.

Preliminary Studies

Preliminary procedures to determine whether probucol has an effect on blood pressure and to measure the effect of probucol on the resistance to oxidation of low-density lipoprotein (LDL) were conducted. Eight rats on the 4% cholesterol-1% choline diet were divided into two groups of four and observed for 10 days. Probucol (1% wt/wt) was added to the diet of one group only. At the end of the test period blood pressure was measured in all animals by cannulating the carotid artery under anesthesia and measuring the blood pressure with a blood pressure analyzer (Micro-Med, Louisville, KY) every 30 s for 5.5 to 6.0 min. The pressure recorded at 5.0 min was the one used. Serum was then collected from the rats and LDL oxidation resistance was determined by measuring the lag time to increasing conjugated diene formation during copper-induced oxidation (kindly performed by Dr. U.P. Steinbrecher, Department of Medicine, University of British Columbia) (15).

Disease Model

For the main experiment 52 rats were divided into four groups. All animals were injected with either PA (Sigma Chemical Co., St. Louis, MO) dissolved in 3 mL of normal saline at a dose of 5 mg/100 g body weight or an equivolume of normal saline via the internal jugular vein over a 5-min interval under anesthesia at the start of the experiment (Day 0). One group of animals (N = 16) received PA only (PA group). Four days after injection the remaining PA-injected rats (N = 16) began receiving probucol (kindly donated by Hoechst Marion Roussel, Cincinnati, OH) (1% wt/wt), which was added to their diet and continued to termination. At the same time five saline-injected animals began receiving probucol at the same daily dose as the other probucol-fed animals until termination. Finally, 15 saline-injected rats served as dietary controls. The animals from the first three groups were divided into three subgroups each and each subgroup observed for either 11 days (peak proteinuria), 42 days (quiescent phase), or 98 days (FGS phase) postinjection. The saline-injected animals on probucol were observed for 98 days. Food intake was periodically monitored. At the start of the experiment, tail vein blood was taken from 20 animals for baseline hematocrit and monocyte counts. Two days before the end of each experimental period (11, 42, or 98 days) the relevant animals were housed in metabolic cages for 24 h for acclimatization and then for an additional 24 h for 24-h urine collection for protein excretion determination. Before euthanasia by exsanguination under anesthesia each rat was weighed. Subsequently, blood was collected from each animal for hematology and serum chemistry and the kidneys were removed for histology, histochemistry and immunohistochemistry. The kidneys were weighed before sectioning. All animals were weighed at the start of the experiment and at the time of euthanasia.

Histology

Renal tissue taken for histology was fixed in methyl Carnoy's solution, embedded in paraffin, and 3-micron sections were stained with hematoxylin-eosin and periodic acid-Schiff reagent. In each case the mean proportion of glomeruli with foam cells was determined.

Immunohistochemistry

Sections of methyl Carnoy's fixed, paraffin-embedded kidney were stained with the monoclonal antibodies ED1 (Sero- tec, Oxford, UK) for monocytes and PC10 (DAKO, Carpinte- ria, CA) for proliferating cell nuclear antigen by the avidin-biotin complex technique. Six-micron sections of snap-frozen fresh renal tissue were fixed with 3% paraformaldehyde and stained with the monoclonal antibodies L23 and A7 (kindly provided by Dr. U.P. Steinbrecher, Department of Medicine, University of British Columbia) (16). The amount of oil red O material in a glomerulus was scored from 0 to 4+ as follows: 0, no staining; 1+, oil red O material in up to 25% of the glomerular tuft; 2+, oil red O in 26 to 50% of the tuft; 3+, oil red O in 51 to 75% of the tuft; 4+, oil red O in >75% of the tuft. In each kidney 50 glomeruli were evaluated for oil red O accumulation, and a mean oil red O score was obtained.

Hematology and Biochemistry

Blood hematocrit (Hct) and monocyte count, serum creatinine (Cr), albumin (Alb), total cholesterol (TC), LDL, high-density lipoprotein (HDL), and triglyceride (TG) and 24-h urinary protein excretion (Upro) were measured by standard hospital laboratory procedures.
Probucol Inhibition of Chronic PAN

Statistics
Values are expressed as means ± SD. The t test was used when only two groups were being compared. Simultaneous comparisons involving more than two groups were done by analysis of variance (ANOVA) with Tukey's test. P < .05 was considered statistically significant.

RESULTS
Preliminary Studies
There were no significant differences in mean blood pressure between the rats on the high-cholesterol diet only (100 ± 13 mm Hg) and those on the high-cholesterol diet and probucol (101 ± 7 mm Hg) (t test). The serum LDL from the probucol group showed marked resistance to oxidation compared with rats on the high-cholesterol diet alone.

The mean baseline values for Hct and blood monocyte count were 32 ± 10% and 0.11 ± 0.07 × 10⁹/L, respectively.

General
Although the saline- and PA-injected rats gained more weight than the PA animals on probucol at Days 11, 42, and 98, and the saline-injected rats on probucol at Day 98, the differences were not significant (ANOVA). There were no significant differences between the various groups with respect to kidney weight, Hct, and blood monocyte count at any of the time periods (ANOVA).

Biochemistry
Table 1 depicts the serum and urine biochemical results for Cr, Alb, LDL, HDL, TG, and Up,. at 11 days post-PA injection. All PA-treated animals had significantly lower Alb (PA, 25 ± 3 g/L; PA + probucol, 22 ± 4 g/L) than the saline-injected rats (35 ± 2 g/L) (P < 0.05, ANOVA with Tukey's test). There was no significant difference in Alb between the PA-injected rats and the PA + probucol animals. All PA-injected rats developed proteinuria. The mean Up,. of the PA (318 ± 221 mg/24 h) and PA + probucol (348 ± 240 mg/24 h) rats were significantly higher than that of the saline-injected group (7 ± 2 mg/24 h) (P < 0.05, ANOVA with Tukey's test). There was no significant difference between the PA-injected and the PA + probucol rats with respect to Up,. 

Although serum lipid levels in the two PA-injected groups were higher than those of the saline-injected group at Day 11, the differences were not significant except for TG, in which the mean value for the PA-injected group was significantly greater than that for saline controls (P < 0.05, ANOVA with Tukey's test). Also, comparison of the PA animals with the PA animals receiving probucol did not demonstrate any significant differences in serum lipid levels. Mean Cr was similar in all groups.

At Day 42, except for Cr, there were no significant differences between the groups with respect to the serum and urine biochemical parameters measured. The mean Cr values for the PA (48 ± 6 μmol/L) and the PA + probucol (48 ± 3 μmol/L) groups were higher than the mean Cr for the saline controls (40 ± 2 μmol/L) (P < 0.05, ANOVA with Tukey's test). The 98-day postinjection results are presented in Table 2. There were no significant differences between the groups with respect to serum lipids. Although the Alb of the PA group (35 ± 2 g/L) was lower than that of the saline group (36 ± 3 g/L), the difference was not significant. However, the Alb of the PA group was significantly lower than that of the PA + probucol (38 ± 4 g/L) group (P < 0.05, ANOVA with Tukey's test). The mean Up,. of the PA rats (137 ± 83 mg/24 h) was significantly higher than the mean Up,. values of the saline-injected (58 ± 57 mg/24 h) and the PA + probucol (41 ± 29 mg/24 h) animals (P < 0.05, ANOVA with Tukey's test) (Figure 1). The saline + probucol control group was not significantly different from the saline group with respect to the biochemical parameters examined.

Histology, Histochemistry, and Immunohistochemistry
Day 11. The quantitative results at 11 days postinjection are presented in Table 3. The glomeruli of the normal saline (NS) animals were structurally normal. The glomeruli of the PA-injected rats with or without probucol were moderately enlarged and showed variable mild to moderate hypercellularity, which appeared predominantly mesangial but also had an endocapillary component. Mild mesangial matrix expansion was present in occasional glomeruli in these animals. Visceral epithelial cell cytoplasmic protein absorption droplets were observed in numerous glomeruli of the PA and PA + probucol rats but not in the saline controls. There was variable glomerular foam cell infiltration and focal tubular dilation and epithelial cell flattening in the PA-injected animals with or without probucol but not in the saline-injected rats. The foam cell scores of the PA + probucol (16 ± 3%)

<table>
<thead>
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<th>Parameter</th>
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<th>NS</th>
<th>PA</th>
<th>PA + P</th>
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<td>Mean Alb (g/L)</td>
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<td>35</td>
<td>25</td>
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<tr>
<td>Mean TC (mmol/L)</td>
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<td>17.7</td>
<td>33</td>
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<tr>
<td>Mean LDL (mmol/L)</td>
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<td>17.3</td>
<td>32.8</td>
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<tr>
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<td>0.27</td>
<td>0.56</td>
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<tr>
<td>Mean TG (mmol/L)</td>
<td></td>
<td>1.17</td>
<td>3.31</td>
<td>1.15</td>
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<tr>
<td>Mean Up, (mg/day)</td>
<td></td>
<td>7</td>
<td>318×^P</td>
<td>348×^P</td>
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<td></td>
<td>NS, normal saline; PA, purymycin aminonucleoside; PA + P, purymycin aminonucleoside and probucol; Cr, creatinine; Alb, albumin; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; Up,. 24-h urinary protein excretion rate.</td>
<td>P &lt; 0.05 compared with the not significant value (analysis of variance and Tukey's test).</td>
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TABLE 2. Biochemical results at 98 daysa

<table>
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<td>Mean Cr (μmol/L)</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Mean Alb (mmol/L)</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>Mean TC (mmol/L)</td>
<td>7.24 ± 1.07</td>
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<tr>
<td>Mean LDL (mmol/L)</td>
<td>5.58 ± 1.39</td>
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<td>Mean HDL (mmol/L)</td>
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</tr>
<tr>
<td>Mean TG (mmol/L)</td>
<td>0.94 ± 0.58</td>
</tr>
<tr>
<td>Mean Un (mg/day)</td>
<td>58 ± 57</td>
</tr>
</tbody>
</table>

a NS, normal saline; PA, puromycin aminonucleoside; PA + P, puromycin aminonucleoside and probucol; NS + P, normal saline and probucol; Cr, creatinine; Alb, albumin; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; Un, 24-h urinary protein excretion rate.
b P < 0.05 PA compared with PA + P (analysis of variance and Tukey’s test).
c P < 0.05 PA compared with PA + P and NS (analysis of variance and Tukey’s test).

![Figure 1](image-url) Twenty-four hour urinary protein excretion (milligrams per day) in control rats injected with either NS or PA and the test rats injected with PA and receiving probucol (PA + P) at 11 and 98 days postinjection. At Day 11 the PA and PA + P rats showed comparable marked increases in protein excretion which were significantly greater than that of the NS animals (*P < 0.05*). At Day 98 the daily protein excretion of the PA + P rats was similar to the NS rats, and both were significantly lower than the daily protein excretion of the PA rats (*P < 0.05*).

and PA (11 ± 6%) rats were higher than the foam cell score of the saline controls (0 ± 0%), but only that of the PA + probucol rats was significantly higher (*P < 0.05, ANOVA with Tukey’s test*). The tubular changes were often accompanied by a variable interstitial mononuclear cell infiltrate consisting predominantly of monocytes. Small arteries and arterioles showed no significant changes.

The saline controls showed relatively mild, focal glomerular staining for oil red O (score, 0.17 ± 0.15). There was no oil red O staining in the glomeruli of the rats in the PA (score, 0.55 ± 0.32) and PA + probucol (score, 0.74 ± 0.20) groups. However only the oil red O score of the PA-injected rats on probucol was significantly greater than that of the saline controls (*P < 0.05, ANOVA with Tukey’s test*). There was no significant difference in oil red O scores between the PA and PA + probucol rats. Increased intraglo-

merular monocyte infiltration was observed in the PA and PA + probucol animals and the ED1 scores for these two groups (PA, 2.88 ± 0.75; PA + probucol, 3.85 ± 1.17) were significantly higher than that of the saline control group (0.91 ± 0.65) (*P < 0.05, ANOVA with Tukey’s test*). Proliferating cell nuclear antigen scores were also significantly higher in the PA (4.10 ± 1.16) and PA + probucol (3.04 ± 0.96) animals than in the saline-injected rats (1.72 ± 0.90) (*P < 0.05, ANOVA with Tukey’s test*). The difference in proliferating cell nuclear antigen scores between the PA and PA + probucol rats was not significant.

Oxidized lipid products were observed focally in the glomeruli of PA rats only. There was little, if any, staining for oxidized lipid products in the kidneys of the other animals. The staining appeared to be intracellular. Oxidized lipid products were stained by both the L23 and A7 antibodies. The scoring for L23 and A7 was significantly higher in the PA animals (L23, 0.26 ± 0.05; A7, 0.27 ± 0.08) than in the rats from
the other groups (saline control: L23, 0 ± 0.01; A7, 0 ± 0.01; PA + probucol: L23, 0.02 ± 0.03; A7, 0 ± 0) (P < 0.05, ANOVA with Tukey’s test). Oxidized lipid products were not observed in extraglomerular sites.

**Day 42.** Glomeruli were normal in all groups. Occasional foam cells were observed in the glomeruli of some of the rats from all three groups. The foam cell scores were relatively low in all groups. Of interest was the fact that the foam cell score of the PA group (2.0 ± 1.4%) was significantly higher than that of the other groups (saline control, 0.4 ± 0.5; PA + probucol, 0.2 ± 0) (P < 0.05, ANOVA with Tukey’s test). Only minor degrees of tubular atrophy were observed focally in the PA and PA + probucol groups.

Minor amounts of neutral lipid were observed focally in the glomeruli of the rats from all groups. No significant differences in oil red O score were noted.

No significant differences between the groups with respect to ED1 and proliferating cell nuclear antigen scores were observed. Oxidized lipid products were not detected in the kidneys of any of the animals.

**Day 98.** The quantitative results at 98 days postinjection are presented in Table 4. Segmental sclerotic glomerular lesions (Figure 2) were observed in about 1 to 14% of glomeruli in PA rats (mean percent of glomeruli with FGS, 5.3 ± 4.9%) (Figure 3). In one saline-injected animal a single segmental sclerotic lesion was present in 1 of 243 glomeruli assessed. No segmental sclerosis was seen in any of the PA + probucol or saline + probucol rats. Occasional glomeruli in PA-injected rats contained foam cells (1.2 ± 1.3%) whereas these cells were rarely if ever observed in the glomeruli of the other groups. The glomeruli of the PA rats appeared mildly enlarged and their tubules showed focal atrophy accompanied by interstitial mononuclear cell infiltrates.

Minor amounts of neutral lipid were present focally in some glomeruli of rats from all groups but the differences in scores were not significant.

The PA animals showed significantly greater intraglomerular monocyte infiltration (ED1 score, 1.82 ± 0.30) than did the rats from the other groups (ED1 scores: saline controls, 1.35 ± 0.36; PA + probucol, 1.22 ± 0.34) (P < 0.05, ANOVA with Tukey’s test). There was no significant difference between the PA + probucol and saline control animals with respect to the ED1 score. The proliferating cell nuclear antigen score for the PA rats (1.90 ± 0.81) was higher than that of the other groups (saline controls, 1.08 ± 0.76; PA + probucol, 1.23 ± 0.84), but the differences were not significant.

Intraglomerular oxidized lipid products were only occasionally observed in the PA- and saline-injected rats. The saline-injected rats and saline-injected with probucol rats showed no significant differences with respect to any of the parameters assessed.

**DISCUSSION**

The results of this experiment show that an antioxidant introduced after the initiation of the PA-induced oxidant-mediated glomerular visceral epithelial cell damage can inhibit the progression of chronic PAN. This was not caused by the antagonism of the initial oxidant-generating action of PA as indicated by the observations in the acute phase that (1) PA-injected rats on probucol had levels of proteinuria and hypoaalbuminemia no different than those of the PA animals, (2) that the glomerular, tubular, and interstitial changes detected histologically were similar in both groups, and (3) that the degree of intraglomerular monocyte infiltration and proliferative activity were similar in both groups.

**TABLE 4.** Histologic, histochemical, and immunohistochemical results at 98 days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>NS</th>
<th>PA</th>
<th>PA + P</th>
<th>NS + P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % FGS$^b$</td>
<td></td>
<td>0.08 ± 0.16</td>
<td>5.3 ± 4.9c</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Mean FC score$^d$</td>
<td></td>
<td>0.08 ± 0.18</td>
<td>1.2 ± 1.3c</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Mean ED1 score$^a$</td>
<td></td>
<td>1.35 ± 0.36</td>
<td>1.82 ± 0.30a</td>
<td>1.22 ± 0.34</td>
<td>1.24 ± 0.40</td>
</tr>
<tr>
<td>Mean PCNA score$^a$</td>
<td></td>
<td>1.08 ± 0.76</td>
<td>1.90 ± 0.81</td>
<td>1.23 ± 0.84</td>
<td>1.51 ± 0.95</td>
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<tr>
<td>Mean ORO score$^a$</td>
<td></td>
<td>0.1 ± 0.1</td>
<td>0.27 ± 0.26</td>
<td>0.28 ± 0.20</td>
<td>0.04 ± 0.05</td>
</tr>
<tr>
<td>Mean L23 score$^a$</td>
<td></td>
<td>0.02 ± 0.03</td>
<td>0.05 ± 0.07</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Mean A7 score$^a$</td>
<td></td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
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</tbody>
</table>

$^a$ NS, normal saline; PA, puromycin aminonucleoside; PA + P, puromycin aminonucleoside and probucol; NS + P, normal saline and probucol; FGS, focal glomerulosclerosis; FC, foam cell; ED1, Immunohistochemical marker for monocytes; PCNA, proliferating cell nuclear antigen; ORO, oil red O; L23 and A7, immunohistochemical markers for oxidized lipid products.

$^b$ Percent glomeruli with FGS or FC.

$^c$ P < 0.05 PA compared with NS and PA + P (analysis of variance and Tukey’s test).

$^d$ Score, mean number of positive cells per glomerulus.

$^* P < 0.05$ PA compared with PA + P (analysis of variance and Tukey’s test).
In the model of chronic PAN induced by a single internal jugular venous bolus of PA it has been demonstrated that attenuation of the monocyte infiltration of glomeruli in the acute phase ameliorates the glomerular injury of the late phase, which suggests a key role for monocytes in the progression of this disease (23,24). Thus the inhibiting effect of probucol may be caused by an action of this drug on monocytes. Probucol has been shown to inhibit interleukin-1 (IL-1) secretion by macrophages (25) and, because IL-1 stimulates the production of the monocyte chemotactic factors monocyte chemotactic protein-1 and RANTES (regulated on activation, normal T cell expressed and secreted) (26), it is possible that probucol’s ameliorating effect on the progression of chronic PAN may be a result of its inhibition of monocyte infiltration of the glomeruli through its effect on IL-1 secretion. However, probucol did not reduce the level of intraglomerular monocyte infiltration in the acute phase, which suggests that its functional importance may be related to a property (or properties), such as its antioxidant one, which interferes either with monocyte activity or effect. The precise mechanism(s) by which monocytes contribute to progression in this model are uncertain because intraglomerular counts are not elevated in the quiescent phase preceding the development of FGS.

Oxidants may act as mediators of glomerular injury in both immune and nonimmune renal disease (27–30). Infiltrating neutrophils and monocytes and resident glomerular cells have been shown to be sources of reactive oxygen species (31–38). There is evidence that reactive oxygen species can cause glomerular damage (39,40) resulting in proteinuria (41–43), altered glomerular filtration rate (44,45), and morphologic changes (32,41,46) by a variety of mechanisms (30). The administration of reactive oxygen species scavengers in a variety of models of immune glomerulonephritis has been demonstrated to have an ameliorating effect on the development of glomerular disease (47–50).

Monocytes produce a number of cytokines and growth factors that have a significant effect on glomerular structure and function (reviewed in Reference 51). Among the cytokines elaborated by monocytes are tumor necrosis factor-α and IL-1α (52), both of which have been detected immunohistochemically in the acute phase of chronic PAN (53). Because both of these peptides induce human mesangial cells to generate H₂O₂ and superoxide anions in amounts similar...
to those of monocytes in a time- and dose-dependent manner (37) and reactive oxygen species (1) have chemoattractant properties (30), (2) can activate the expression of the monocyte chemotactic factors, monocyte chemotactic protein-1 and RANTES, and intracellular adhesion molecule-1 in mesangial cells (54), and (3) can cause glomerular damage (39,40). It has been proposed that cytokine-induced reactive oxygen species production by mesangial cells can propagate glomerular injury and lead to the FGS observed in the late phase of chronic PAN (28). The results of this experiment are consistent with this concept, which suggests that probucol's antioxidant property may account, at least in part, for its inhibition of progression of the glomerular disease in chronic PAN.

It has been postulated that FGS is analogous to atherosclerosis (55). There are considerable data to suggest that oxidized LDL plays a significant role in the pathogenesis of atherosclerosis (56,57) and some evidence that it may be involved in FGS (17,58). Although the results of this experiment are consistent with a pathogenetic role for oxidized LDL in FGS, oxidized lipid products could not be demonstrated immunohistochemically in the glomeruli of the PA animals with FGS in the chronic phase. This is contrary to the results of a previous experiment in which oxidized lipid products were demonstrable in the glomeruli of rats with FGS induced by a series of seven subcutaneous injections of PA over a 10-wk period by using the same monoclonal antibodies (17). However, the present results are similar to those of a preliminary study in human renal disease in which oxidized lipid products were not detected immunohistochemically in the glomeruli containing foam cells in patients with FGS (59). The failure to demonstrate oxidized lipid products in the glomeruli of PA animals in the chronic phase suggests that either oxidized lipid products are not present in the glomeruli or that they are there but either in a form or an amount that cannot be detected with the monoclonal antibodies used. That oxidized lipid products were detected in the acute phase of PAN and in the glomeruli of chronic PAN rat kidneys harvested 4 wk after the last PA injection suggests that the detection of oxidized lipid products by the monoclonal antibodies may depend on a relatively high level of glomerular oxidant activity (acute phase), a very high serum LDL level to result in a sufficient deposition of LDL in glomeruli to be detectable, and length of time between the administration of PA and the harvest of the kidneys. With respect to the latter condition, it is possible that over time a sufficient amount of the specific epitope(s) of the oxidized lipid-protein adducts were degraded within monocytes making immunohistochemical detection impossible.

In conclusion, the results of this experiment indicate that probucol, an antioxidant, inhibits the progression of chronic PAN. The elucidation of the precise mechanisms involved require further investigation.

ACKNOWLEDGMENTS

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