Focal Glomerular Sclerosis in Aminonucleoside Nephropathy

TAKAO SAITO, TAKASHI FURUYAMA, YOSHIO KYOGOKU, KEI YAMAKAGE, MASAHIRO ARAKAWA and KAORU YOSHINAGA

The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980

Saito, T., Furuyama, T., Kyogoku, Y., Yamakage, K., Arakawa, M. and Yoshinaga, K. Focal Glomerular Sclerosis in Aminonucleoside Nephropathy. Tohoku J. exp. Med., 1981, 133 (3), 349-360——In an attempt to establish a model of focal glomerular sclerosis (FGS), 17 male Sprague-Dawley rats were i.m. injected with aminonucleoside of puromycin (AN) in a dose of 1 mg/100 g body weight for 8 consecutive days as one series. Seven rats were treated through the one series only, and followed up for 4 to 8 months after AN loading (Group I). Two months after the first series, the other 10 were given the same injections as the second series. Four rats were killed within one week after the second series because of their intratable debilitation (Group II-a), and the remaining 6 were kept for 4 to 6 months after the second series (Group II-b). Glomeruli involving sclerotic lesions and/or hyaline deposits were 3.8 to 50.7% (mean±s.E., 23.7±6.3%) for Group I, 3.3 to 41.6% (21.6±8.2%) for Group II-a and 9.3 to 25.0% (17.5±2.7%) for Group II-b. Glomerular sclerosis was closely associated with irreversible proteinuria in the early stage. But, in Group II-a, hyaline deposit was less frequently observed than that in other long maintained groups. Thus, hyaline deposits in this disease usually appeared lagging in time behind the formation of glomerular sclerosis.

In modern nephrology attention has increasingly been directed to focal glomerular sclerosis (FGS), for it is often accompanied by steroid resistant nephrotic syndrome. Histologically it represents singular sclerotic lesions with hyaline deposits and vacuoles associated with foam cells in the glomerulus. Strenuous clinical investigations have been made to clarify its etiology, but nothing has yet been uncovered regarding the pathogenesis of FGS.

On the other hand, it has long been known that the rat administered with aminonucleoside of puromycin (AN) can develop a symptom resembling lipoid nephrosis (Frank et al. 1955; Fiegelson et al. 1957). Recently, Glasser et al. (1977) and Velosa et al. (1977) reported that the AN nephropathy in rats has quite similar histological changes to human FGS, and that this type of nephropathy is an excellent experimental model of FGS. It is the purpose of this paper to argue whether chronic AN nephropathy could be an experimental model of FGS or not.

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Seventeen male rats of the Sprague-Dawley strain, 10 to 12 weeks old, were used. AN, 2% in physiological saline solution, was i.m. injected daily in a dose of 1 mg/100 g body weight for 8 consecutive days (first series). Seven animals were treated through the first series only. After the end of the series, these rats were sacrificed, each one at 4, 5 and 8 months, and the remaining 4 at 6 months (Group I). Two months after the first series, the other 10 rats were considered to be less damaged by AN in regard to the proteinuria and other parameters, and given the same injections of AN for 8 days (second series). Of these 10 rats, 6 were able to withstand the two series. But 4 rats were so debilitated after the second series of AN administration that they were killed within one week (Group II-a). The remaining 6 rats, still vigorous after the second series, were sacrificed, one 4 months, the other five 6 months after the completion of the second series (Group II-b). Untreated Sprague-Dawley rats were used as controls and they were sacrificed — 2 at the age of 7 months, and 4 at 11 months.

All rats were maintained on a diet containing 0.24 g% sodium and 24.1 g% protein; water was given ad libitum. Urinary protein and urinary glucose were checked and followed using Labstix (Ames Co.) on a semiquantitative basis. Blood was sampled from the tail vein several times before and after AN administration and during feeding and finally at the time of sacrificing. Serum protein was measured using the hand protein refractometer (Hitachi Co.), serum creatinine by Folin-Wu’s method, BUN by the diacetylmonoxime method, and serum total cholesterol by the o-phthalaldehyde method.

The kidney removed at sacrificing was fixed in Zenker-formalin solution and the sections were stained separately by hematoxylin-eosin (HE), Azan-Mallory (AM), Masson-elastic (ME), periodic acid-Schiff (PAS) and periodic acid-methenamine silver (PAM) for light microscopy. In some animals colloidal iron-PAS combination stain was performed according to Mowry’s method (1958). Further, immunological investigations were carried out on the sections obtained by snap frozen technique and on paraffin sections made by fixing the specimens in 95% alcohol at 4°C according to Sainte-Marie’s method (1962). On these sections was applied a direct immunofluorescence technique employing fluorescein isothiocyanate (FITC) labeled rabbit anti-rat C3 antiserum prepared in our laboratory, and FITC labeled rabbit anti-rat IgG antiserum (Miles-Yeda Co.), and an indirect immunofluorescence technique using rabbit anti-rat IgM antiserum (Miles-Yeda Co.) and FITC labeled goat anti-rabbit IgG antiserum (DAKO Immunoglobulin Co.). These antiserums had undergone tests with immunoelectrophoresis for their specificity. The kidney specimens were also subjected to double fixation with 2.5% paraformaldehyde-2% glutaraldehyde and 1% osmium tetroxide for electron microscopy.

In some of the rats, renal biopsy with a Vim-Silverman needle was performed at laparotomy once or twice to observe the time course of their histological changes.

The percentage of the affected glomeruli was determined by observing 200 glomeruli on a PAS-stained section of the removed kidney. The affected glomerulus was defined by the presence of sclerotic lesions and hyaline deposits (Hd), or at least either of them. The proportion of hyaline deposits in the affected glomeruli was also determined.

**RESULTS**

*Laboratory findings on urine and blood.* In rats administered with AN, the level of urinary protein was around 1+ before administration, and rose to 4+ by 2 weeks after the first series. The proteinuria was classifiable into two according to the subsequent course; one, gradual decrease toward the pre-administration level in 2 months after the AN administration (reversible proteinuria) and the other, no change (irreversible proteinuria). Almost all rats of Group I treated with only the first series showed irreversible proteinuria. The rats of Group II had either
reversible or irreversible proteinuria after the first series, but irreversible rats were exclusively in Group II-a. On the other hand, all the reversible rats turned irreversible after the second series. In the control group, there was a rat with urinary protein rising up to 3+, but no others showed elevation in the urinary protein. The AN treated rats showed no remarkable fall in the level of serum protein, all remaining short of developing nephrotic syndrome.

The means and s.e. of serum protein, BUN, serum creatinine and serum total cholesterol for each group are shown in Table 1. As for serum creatinine, somewhat high levels were found in some rats in Group II-b, but in any group, no rat developed a level so high as allowing suspicion of renal failure, except one case of Group II-a at the time of sacrificing. However, in each group, there were some rats that showed a transitory rise in levels of BUN and serum cholesterol one week after the AN administration. In Group II-a, only one rat could have its blood sampled immediately before death. In this case, abnormally high levels were observed, 3.3 mg/100 ml for serum creatinine and 202 mg/100 ml for BUN, suggesting that the rat had fallen into renal failure.

### Table 1. Laboratory data in each experimental rat group

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>1 week after AN 1 series</th>
<th>2 months after AN 1 series</th>
<th>1 week after AN 2 series</th>
<th>4 months after AN 1 series</th>
<th>On nephrectomy*</th>
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<tr>
<td>I† (n=7)</td>
<td>Serum protein (g/100 ml)</td>
<td>6.9±0.1</td>
<td>7.1±0.3</td>
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<td>7.0±0.2</td>
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<td>Serum creatinine (mg/100 ml)</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
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<td>1.0±0.2</td>
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<td>Blood urea N (mg/100 ml)</td>
<td>20±1</td>
<td>29±4</td>
<td>21±1</td>
<td>23±2</td>
<td>25±2</td>
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<td></td>
<td>Serum cholesterol (mg/100 ml)</td>
<td>59±6</td>
<td>179±40</td>
<td>84±11</td>
<td>87±12</td>
<td>93±13</td>
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<td>II-a‡ (n=4)</td>
<td>Serum protein (g/100 ml)</td>
<td>6.4±0.1</td>
<td>5.6±0.2</td>
<td>7.0±0.1</td>
<td>5.7</td>
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<td>Serum creatinine (mg/100 ml)</td>
<td>1.0±0.2</td>
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<td>1.3±0.03</td>
<td>3.3</td>
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<td>Blood urea N (mg/100 ml)</td>
<td>19±1</td>
<td>64±20</td>
<td>21±3</td>
<td>202</td>
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<td>Serum cholesterol (mg/100 ml)</td>
<td>63±5</td>
<td>336±24</td>
<td>70±4</td>
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<td>—</td>
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<td>II-b (n=6)</td>
<td>Serum protein (g/100 ml)</td>
<td>6.1±0.2</td>
<td>6.5±0.1</td>
<td>7.0±0.1</td>
<td>6.4±0.2</td>
<td>6.9±0.1</td>
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<td>Serum creatinine (mg/100 ml)</td>
<td>1.2±0.1</td>
<td>1.2±0.2</td>
<td>1.7±0.1</td>
<td>1.5±0.2</td>
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<td>Blood urea N (mg/100 ml)</td>
<td>11±1</td>
<td>15±2</td>
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<td>35±11</td>
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<td>Serum cholesterol (mg/100 ml)</td>
<td>51±3</td>
<td>87±21</td>
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<td>212±31</td>
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<th>7 months old</th>
<th>11 months old</th>
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<tr>
<td>Control§</td>
<td>Serum protein (g/100 ml)</td>
<td>6.7±0.3</td>
<td>6.8±0.1</td>
<td>7.1±0.3</td>
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<tr>
<td></td>
<td>Serum creatinine (mg/100 ml)</td>
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<td>0.7±0.03</td>
<td>1.3±0.3</td>
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<tr>
<td></td>
<td>Blood urea N (mg/100 ml)</td>
<td>19±2</td>
<td>22±0.3</td>
<td>20±2</td>
</tr>
<tr>
<td></td>
<td>Serum cholesterol (mg/100 ml)</td>
<td>57±4</td>
<td>60±3</td>
<td>56±5</td>
</tr>
</tbody>
</table>

Values are means±s.e. AN, aminonucleoside of puromycin.
* Rats were nephrectomized from 5 to 8 months after one series of AN administration.
† One rat was sacrificed 4 months after one series.
‡ Serum of only one rat was measured one week after two series of AN.
§ Two rats were sacrificed at 7 months old.
Histological findings of affected glomeruli in the removed kidney. As shown in Fig. 1a, focal glomerular sclerotic lesions were observed in the removed kidneys from AN administered rats. In high power views most of the lesions appeared segmental, where hyaline deposits and vacuoles were frequently observed (Fig. 1b). These lesions and the surrounding parts in the glomeruli in Groups I and II-b were not stained with colloidal iron procedure, whereas the light-microscopically intact glomerular basement membranes were clearly colored with Prussian blue (Fig. 1c). In all glomeruli of Group II-a, the staining pattern was indistinct.

Fig. 2 shows the percentage of affected glomeruli appearing in the removed kidney specimen plotted against days after the AN administration up to the nephrectomy. (In control rats, ages are shown in months corresponding to days for the rats with AN administration.) The affected glomeruli were 3.8 to 50.7% with a mean±s.e. of 23.7±6.3% for Group I, 3.3 to 41.6% with a mean±s.e. of 21.6±8.2% for Group II-a, and 9.3 to 25.0% with a mean±s.e. of 17.5±2.7% for Group II-b. There was no significant difference in the rate among the 3 groups. Nor was

Fig. 1. Light micrographs of the kidney of Group I at nephrectomy 6 months after AN administration. (a) In some glomeruli segmental sclerotic lesions are demonstrated, but tubular atrophy and vascular abnormality are absent. PAS stain, ×100. (b) A sclerotic lesion of the glomerulus, accompanied by hyaline deposits and vacuoles, is seen with adhesion to Bowman’s capsule. PAS stain, ×300. (c) With colloidal iron-PAS combination stain. Prussian blue along the basement membrane (arrow) is demonstrated in a half of the glomerulus, but the other half involving a sclerotic area is negative. ×300.
there any particular relationship between glomerular changes and the interval from AN administration to nephrectomy. In the control group, the incidence of affected glomeruli was 3.7% at the highest, with a mean±s.e. of 1.5±0.6%, apparently lower than any of AN administered groups.

**Relation between sclerotic glomeruli and urinary protein.** The glomerular sclerosis seems to be severe in degree, independent of the amount of AN administered, in cases showing an irreversible increase in urinary protein from the early stages after AN loading. Fig. 3 shows the incidence of the affected glomeruli (ordinate) plotted against the level of urinary protein 2 months after the initial AN administration (abscissa).

**Relation between glomerular sclerosis and hyaline deposit.** As can be seen in Group II-a, it is evident that glomerular sclerotic lesions appeared relatively early at the portion of adhesion of the capillary loop to Bowman’s capsule, characterized by augmentation of the mesangial matrix and fibrosis of Bowman’s capsule, but there were no signs of Hd (Fig. 4a). By contrast, in rats maintained for more than 6 months after the AN administration, Hd was present not only in most of the sclerotic glomeruli but in some of the non-sclerotic glomeruli. The
incidence of Hd in the affected glomeruli is plotted against the days after the first AN administration in Fig. 5, which evidently shows the incidence of Hd increasing with the lapse of time after AN administration. (In the tabulation, affected glomeruli less than 5% are excluded.)

Such qualitative changes in glomerular sclerotic lesions were similarly observed in renal biopsy specimens obtained in the same rat at different times after AN administration. In the rat of Group II-b, its renal biopsy specimen obtained 50 days after initial AN administration showed augmentation of the mesangial matrix in some glomeruli, in a degree yet short of forming glomerular sclerotic lesions (Fig. 4b). In its second biopsy specimen, taken 50 days after the second series of AN administration, a sclerotic lesions was identified in 3 of 7 glomeruli, of which 2 were global in pattern, but no sign of Hd could be detected (Fig. 4c). The histological specimen of its removed kidney obtained 6 months after AN administration showed 25% of affected glomeruli, of which 71% contained Hd (Fig. 4d).

Histological findings in the tubulus, interstitium and blood vessels. In histological sections of the removed kidney, enlarged tubular lumina, casts and mild interstitial edema were found in the rats of Group II-a. But in animals of
the other groups, any instances of tubular atrophy or interstitial proliferation could not be detected in all but a very small percentage of them. Nor was there evidence of abnormality in the blood vessels (Fig. 1a).

**Immunohistological and electron microscopic findings.** The immunofluorescence technique disclosed focal segmental depositions of IgM and C3 in the glomeruli of the rats in Groups I and II-b (Fig. 6a), but deposits of IgG were less distinct in these groups. It must be noted, however, that such deposits could not always be found in all sclerotic portions of the glomeruli, and their coexistence with Hd was not certain either. Since the control rats, 11 months old, also demonstrated mild deposits of IgM, C3 and IgG, the deposits mentioned above could not be defined as indicative of a feature specific to AN nephropathy. On the other hand, the removed kidney sections (Group II-a) and renal biopsy specimens obtained soon after AN administration revealed abundant deposits of IgM, C3 and IgG,
Fig. 5. Relationship between percentage of glomeruli involving hyaline deposits and days from the initial AN administration to nephrectomy. Rats with affected glomeruli less than 5% are excluded. ○, Group I; ●, Group II-a; •, Group II-b.

Fig. 6. Immunofluorescence micrographs from a kidney of the AN administered rat. (a) A glomerulus from a rat of Group II-b 6 months after the second AN loading with segmental deposition of C3. ×300. (b) A glomerulus from a rat of Group II-a one week after the second AN loading with diffuse granular deposition of IgM. ×300.
Fig. 7. Electron micrograph of a glomerulus obtained from a rat of Group II-b characterized by wrinkling of basement membrane (BM), swelling of epithelium (Ep) and adhesion to fibrous Bowman’s capsule (BC). ×3,000.

Fig. 8. Electron micrograph of a glomerulus from the same rat as in Fig. 7 characterized by hyaline like deposit (H) enclosing cytoplasmic substances in contact with adhesion of the capillary loop to Bowman’s capsule (BC). MC, mesangial cell. ×10,000.
granular or lumpy in pattern, in some of the glomeruli (Fig. 6b).

On electron micrographs of Groups I and II-b, evidence of so-called ischemic changes was observed including prominent wrinkling of the glomerular basement membrane, fibrosis of Bowman’s capsule and adhesion of the capillary tuft to Bowman’s capsule (Fig. 7), together with gigantic subendothelial and mesangial electron dense deposits which often looked like hyaline (Fig. 8).

**DISCUSSION**

In the present experiments, rats administered with AN developed focal and segmental glomerular sclerosis. This glomerular sclerosis begins at the portion of the glomerular tuft adhering to Bowman’s capsule and it is accompanied by Hd and vacuoles, bearing a close resemblance to human FGS in many respects. Also the wrinkling of the glomerular basement membrane and gigantic electron dense deposits observed through electron microscopy are similar to FGS in man. Thus, as far as the glomerular changes are concerned, AN nephropathy may be regarded as an experimental model of FGS.

In FGS, the occurrence of nephrotic syndrome is considered to promote progression of the disease (Churg and Grishman 1973; Ettenger et al. 1977). The finding that irreversible increases in urinary protein beginning soon after the administration of AN were closely related to the formation of glomerular sclerotic lesions is certainly of interest in considering AN nephropathy as an experimental model of FGS. Yet it still remains an unsolved problem whether the change in glomerular basement membrane, vitally related to the permeability, plays a leading role in inducing proteinuria, and the latter is a principal factor causing the sclerotic changes, forming a “circulus viciosus” as often debated in nephrotic syndrome. In favor of such a mechanism, Velosa et al. (1977) have recently proposed a hypothesis that sialoprotein covering the glomerular basement membrane has fallen off for some reason, causing polyanion loss, with eventual increase in permeability for protein leading to onset of glomerular sclerosis. Polyanion loss has been proved by the failure of the colloidal iron method to stain the glomerular basement membrane (Michael et al. 1970). Certainly the staining pattern was indistinguishable between human FGS and AN nephropathy of rat in the present experiment (Fig. 1c), but since a similar finding has been obtained in instances of minimal change type nephrotic syndrome, it seems unreasonable to explain the sclerotic change by polyanion loss alone. Nevertheless, considering the alteration of urinary protein excretion in AN nephropathy, it is likely that irreversible exfoliation of sialoprotein is one of the etiologic factors of glomerular sclerosis. Regarding the hemodynamic factors, it is doubtful that glomerular sclerotic changes are caused by renal ischemia in AN nephropathy as known in nephrotic syndrome of man (Suwa and Takahashi 1971). Rather, the previous experimental results show that unilaterally nephrectomized rats (Striker et al. 1969; Elema et al. 1971; Glasser et al. 1977) or animals infused with physiological saline (Elema and Arends 1975), the states known to be accompanied by an increased renal blood flow, tend to have more
sclerotic changes. But, under these conditions it is thought that blood flow and filtration rate in juxtamedullary glomeruli are reduced on the contrary to those of superficial glomeruli (Horster and Thurau 1968; Barger and Herd 1971). Thus, the mechanisms controlling the distribution of renal blood flow are complex. Although it can hardly be thought that renal ischemia indeed induces sclerotic changes in AN nephropathy, it is undeniable that abnormality in hemodynamics is in some way related to the development of glomerular sclerosis.

Although the appearance of Hd is considered important in both diagnosis and prognosis of FGS, so far no reports have been presented persuasively explaining their relations. In the present experiments, the appearance of Hd, not always coincident with the formation of glomerular sclerosis, was likely to be more prominent in the animals long maintained after AN administration. This suggests that Hd and glomerular sclerosis have different causes. It is unknown if AN nephropathy and human FGS are the same in etiologic mechanism, but the inconsistency in time of appearance of Hd and formation of glomerular sclerotic lesions in AN nephropathy may be suggestive of something helpful in the study of etiology of FGS.

Depositions of IgM and C3 in the glomeruli could be observed by the immunofluorescence technique in AN administered rats, but the same changes were found also in the control rats. So it is unreasonable to use this finding alone for surmising how much the immunological mechanism was concerned with the etiology of AN nephropathy. However, as pointed out in the previous reports (Okuda et al. 1965; Shimizu 1971), the depositions of immunoglobulins and complements, granular or lumpy in pattern, found on the glomeruli soon after AN administration may suggest their contributions in some way to glomerular sclerosis, apart from their possible representation of a simple “entrapping” due to excessive excretion of protein (Sherman et al. 1977).

In performing the present experiments, no particular step was taken to exclude the so-called spontaneous glomerular sclerotic lesions which were very similar in histology to those of AN nephropathy (Striker et al. 1969; Elema et al. 1971; Couser and Stillmunt 1975; Elema and Arends 1975). But the control rats developed no evidence of glomerular sclerosis up to 11 months of age. Nevertheless, the potential spontaneous development of nephropathy should be taken into consideration in carrying out experiments with AN nephropathy in rats.

Acknowledgments

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References