RENIN-SODIUM PROFILE IN EXPERIMENTAL NEPHROSIS INDUCED BY PUROMYCIN AMINONUCLEOSIDE

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The roles of the renin-angiotensin system (RAS) and aldosterone in the pathogenesis of nephrotic syndrome were investigated with rats following subcutaneous injections of puromycin aminonucleoside (PAN) for 7 d.

Prominent reduction in plasma renin activity (PRA) was observed at day-15 after the initial treatment with PAN preceded by the onset of the nephrosis and there was no significant difference in plasma aldosterone concentration (PAC) between the nephrosis and normal control groups despite the higher levels obtained in the nephrosis animals.

Although we could not elucidate the precise mechanism of reduction in PRA, it was suggested that neither the RAS nor aldosterone secretion played a primary role in the pathogenesis of the nephrosis. PAN-induced nephrosis is interesting in studying the pathophysiological mechanism of the lowered activity of plasma renin in some patients with nephrotic syndrome.

Keywords—puromycin aminonucleoside; nephrosis; edema; renin-angiotensin system; angiotensin converting enzyme inhibitor; aldosterone

INTRODUCTION

Administration of puromycin aminonucleoside (PAN) to rats has been reported to result in the development of the nephrotic syndrome with morphologic alterations similar to those observed in minimal lesion nephrotic syndrome. PAN leads to loss of structure of glomerular permeselectivity against macromolecules.1-4 Although there have been several reports concerning the histological changes in the glomerulus, the role of renal hemodynamics and vasoactive substances in the pathogenesis of the nephrosis has not been evaluated in detail.5-7 It is important to establish the relationship between the incidence or progression of the nephrotic syndrome and some humoral factors such as the renin-angiotensin system (RAS) in the experimental model, since there was no consistent finding about this relation in patients with nephrotic syndrome.8-10

We measured plasma renin activity (PRA), plasma aldosterone concentration (PAC), and electrolyte excretion to show the factors that have a key role for the renin-angiotensin-aldosterone profile when the nephrosis due to glomerular impairment was induced following the treatment with PAN.

MATERIALS AND METHODS

Experimental Animals and Induction of Nephrosis — Male rats of Donryu strain weighing 200-250 g were maintained on a regular chew diet (Clea Japan Inc.) throughout the experiment.

Experimental nephrosis was produced by the slight modification of the methods of Caulfields, et al.11) Animals were given puromycin aminonucleoside (PAN; Sigma Chemical Co.) 15 mg/kg subcutaneously. PAN dissolved in physiological saline at 0.75% solution was administered once daily for 7 d. Physiological saline was injected at the same regimen in normal control animals.

Biochemical Measurements of Plasma and Urines — Urine was collected over 24 h in the individual metabolic cages (Yazawa Scientific Mfg. Co., Ltd.) after the initiation of PAN treat-
ment at about 5-d intervals. Protein content of urine was determined with 3% sulfosalicylic acid solution and an aliquot of urine was stored at −30°C until electrolyte measurements.

Electrolytes (sodium and potassium) were measured by the flame photometry (Hiranuma Ind. Co., Ltd. Type. FRF 3A). Blood sample in a volume of 2 ml was withdrawn from the carotid artery into the tubes containing 10 mg of EDTA disodium under pentobarbital anesthesia. Plasma was isolated by the centrifugation and frozen. Plasma total protein and albumin concentrations were determined by biuret reaction and binding of bromocresol green respectively.

Determinations of PRA and PAC — PRA was measured with a commercial kit (Dainabott Radioisotope Laboratories) and expressed in terms of ng of angiotensin I generated per ml of plasma per hour of incubation at 37°C. PAC was expressed as pg per ml of plasma (Dainabott Radioisotope Laboratories).

Histology — Isolated kidney was fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned into 3–5 μm. Each slice was stained by the standard procedure.

Drug Treatment — Captopril (CS-522. Sankyo Co., Ltd.) dissolved in drinking water at a concentration of 0.2 mg/ml and 0.4 mg/ml, was given to the animals from day-0 to day-15.

The mean daily intake of captopril calculated from the water over 24 h and body weight, were approximately 31 mg/kg and 62 mg/kg. Tap water was given in the control group.

RESULTS

Fig. 1 showed the onset, severity, and duration of proteinuria following PAN treatment. Protein excretion in normal animals remained within normal levels (5.2±1.2 mg/24 h at day-0, and 7.0±1.3 mg/24 h at day-53) throughout the experimental period. Animals received PAN began to develop prominent proteinuria within a week, and the proteinuria lasted for more than 3 weeks. Urinary protein contents in PAN-treated rats were still higher than normal value at day-45.

As shown in Fig. 2, urinary sodium excretion was decreased in the nephrotic rat with the onset of proteinuria but the decrease of sodium excretion was transient. Sodium excretion was returned to normal control levels at day-15, when the nephrosis was established. On the other hand, potassium excretion was not significantly affected during the induction period of the nephrosis.

Hyoproteinemia and hypoalbuminemia were manifest at day-15, in the nephrotic rats. However, there was no statistically significant difference in plasma protein levels of the nephrosis group at day-45 compared with those of normal group. PRA in the nephrosis group was markedly decreased at day-15, and returned toward control levels. The time course of plasma protein level resembled that of PRA. PAC tended to increase at day-15, but this increase was not significant due to the individual fluctuations in the nephrosis group.

Light microscopic observations indicated that there were local infiltrations of some inflammatory cells in the glomerulus and that protein casts were found in the tubular lumen. Although moderate dilation of distal convoluted tubules could be identified, apparent alterations in juxtaglomerular area were not observed at day-15.

Captopril, an orally active inhibitor of
angiotensin converting enzyme, was given to study the change in the feedback control process of the RAS, since captopril stimulates renin secretion through interruption of the negative feedback by angiotensin II. Fig. 4 showed the effect of captopril administration on PRA. Captopril produced a dose-related rise in PRA approximately 4 times and 7 times higher than that in the nephrosis control group. Biochemical measurements at day-15 were shown in Table II. Captopril treated groups never showed the significant differences in proteinuria, plasma protein levels

FIG. 2. Time Course of Electrolytes Excretion
Animals were given PAN or saline from day-0 to day-6. Each values represent mean ± S.E.M. of 6 animals. Normal control (○), PAN treatment (●). Statistically different from the normal control: a) p < 0.02.

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<tr>
<th>TABLE I. Alterations in Plasma Protein, PRA and PAC in Normal and in PAN Administered Rats</th>
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<tr>
<td>Normal control</td>
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<tr>
<td>No. of animals</td>
</tr>
<tr>
<td>Total protein concn (g/dl)</td>
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<tr>
<td>Albumin concn (g/dl)</td>
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<td>PRA (ng Al/ml/h)</td>
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<td>PAC (pg/ml)</td>
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Blood in the normal control group was drawn at 15 d after the initial injection of physiological saline. Each values represent mean ± S.E.M. Statistically different from the normal control: a) p < 0.001, b) p < 0.05.
and electrolyte excretion compared with the nephrosis control group.

DISCUSSION

As shown in the present results, it was evident that PAN-treated rats showed massive proteinuria and hypoproteinemia (Fig. 1, Table 1). Nephrotic syndrome with minimal change lesions could be established, since no clear glomerular lesion was observed in the light microscopic features (Fig. 3). The nephrosis improved during the subsequent weeks following the continuous proteinuria, which were in accord with other previous studies.\textsuperscript{12,13}

Table 1 showed that PRA values of the nephrotic rats were markedly lower than those of normal control ones at day-15, when the proteinuria attained the highest levels. Thereafter, PRA inclined to recover to normal values during the recovery period, even though PRA at day-45 was significantly different from that of normal control group. Gross, et al\textsuperscript{[5]} reported that treatment with PAN to rats did not reduce the renin content of the kidney, nor caused hypertension. Although there is no data about the content of renin in the present study, it is certain that an increased activity of the RAS is unlikely to contribute to the incidence of the nephrosis. Some probable mechanisms seem to offer an explanation for the great decrease of activity of plasma renin in PAN-induced nephrosis.

![Image: Light Micrographs of Glomerulus](image1)

**FIG. 3. Light Micrographs of Glomerulus**

a) A glomerulus obtained from the normal control rats 15 d after the first injection of saline. PAS strain (\times 160).

b) Minimal lesions in a glomerulus in the kidney of PAN-induced nephrosis 15 d after the first dose of PAN. PAS strain (\times 160).

![Image: PRA Graph](image2)

**FIG. 4. Influences of Oral Administration of Captopril on PRA in PAN-induced Nephrosis**

- : nephrosis control (drinking water alone), : captopril 31 mg/kg/d, : captopril 62 mg/kg/d. Each values represent mean ± S.E.M. of 6 or 7 animals. Statistically different from the nephrosis control: a) \( p < 0.05 \), b) \( p < 0.001 \).
Medina et al. reported that renin substrate concentrations in plasma were decreased in some nephrotic patients and that lowered renin substrate could impair angiotensin generation during the PRA measurements. Since we have no evidence concerning this point of view in our results, it is uncertain whether the decreased activity of plasma renin resulted from the low substrate level or not. It is well known that plasma albumin is the major component of the urinary protein. Additional analysis of the urine specimen may be necessary to measure the excretion amount of low molecular protein such as renin substrate and to demonstrate the relationship between decrease of substrate level and urinary loss of substrate in the nephrosis.

Captopril, which has no direct effect on renal handling of electrolytes and β-adrenoceptor of the kidney, was given to show the direct inhibitory action of PAN on the renin release from the juxtaglomerular apparatus. Dose-related enhancement of PRA was found in PAN-treated rats as well as normal or hypertensive animals in the previous reports (Fig. 4). However, neither proteinuria nor hypoproteinemia was significantly affected in 2 captopril groups compared with the nephrosis control group (Table II). These results do not support the concept that renin release was greatly suppressed as a result of the nephrotoxic effect induced by PAN.

Fig. 2 showed that sodium balance was almost approached to previous level at day-15 despite the significant reduction in sodium excretion during the first few days of PAN injection. This also denies that electrolyte imbalance may play a primary role for the status of the RAS. Additionally, we failed to show the oversecretion of aldosterone in contrast to the great decrease in PRA. The results suggested that aldosterone level was independent of the renin-angiotensin profile for the incidence of the nephrosis. Kalant, et al. reported that the nephrosis could be sustained in the absence of adrenal gland, if the rats were fed up saline. It is reasonable to assume that aldosterone by itself has no share in the alteration of the RAS, nor the pathogenesis of the nephrosis.

Further studies must be performed to conclude the cause for the marked reduction in PRA of

| TABLE II. Influences of Oral Administration of Captopril on Several Biochemical Measurements of Plasma and Urine |
|---------------------------------------------------------------|-----------------|-----------------|
| PAN nephrosis control | Dose of captopril | 31 mg/kg/d | 62 mg/kg/d |
| No. of animals | 6 | 7 | 7 |
| Total protein concn (g/dl) | 5.04 ± 0.27 | 5.30 ± 0.46 | 4.58 ± 0.20 |
| Albumin concn (g/dl) | 1.90 ± 0.20 | 2.07 ± 0.21 | 1.67 ± 0.04 |
| Alb./Glob. Proteinuria (mg/24 h) | 0.60 ± 0.06 | 0.66 ± 0.09 | 0.58 ± 0.03 |
| Sodium excretion (meq/24 h) | 324.4 ± 27.1 | 305.9 ± 18.5 | 265.1 ± 22.4 |
| Potassium excretion (meq/24 h) | 2.43 ± 0.51 | 3.16 ± 0.63 | 2.85 ± 0.65 |

Urine was collected at day-13 and plasma was at day-15. Each values represent mean ± S.E.M.
PAN treated rats. As conclusion PAN-induced nephrosis appears to be a useful model to understand the pathophysiological features of low renin patients with nephrotic syndrome.

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REFERENCES