Studies on the Effectiveness of Sairei-to on Puromycin
Aminonucleoside Nephrosis in Rats

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ABSTRACT

In order to assess the effectiveness of sairei-to on nephrosis and to elucidate its mechanism of action, we made a puromycin aminonucleoside (PAN) rat model by a single intra-peritoneal injection of PAN at a dose of 100mg/kg body weight (B.W.) and compared it to the normal controls. Sairei-to was administered in various doses (100, 200, 500mg/kg B.W.) orally for 8 days after the initial injection of PAN. Proteinuria and serum triglyceride levels were significantly reduced in the sairei-to-treated groups which had been pretreated with PAN. Light and electron microscopically sairei-to-treated groups showed a morphological improvement in the kidney over the PAN group. We found 500mg/kg B.W. of sairei-to to be the most effective dose. The superoxide dismutase (SOD)-like activity was significantly elevated in the serum but not changed in the urine of sairei-to-treated groups pretreated with PAN. The normal control fed with 500mg/kg B.W. of sairei-to showed a significant increase in serum SOD-like activity. The urinary prostanoïd levels in the PAN group were lower than those in the normal and sairei-to-treated groups. These results support our hypothesis that sairei-to has effects on the elevation of SOD-like activity and on the synthesis of prostanoïd in PAN-induced nephrosis, and that these effects are responsible for the mechanism of action of sairei-to.

Key words: Sairei-to, Puromycin aminonucleoside, Nephrotic syndrome, Superoxide dismutase, Prostaglandins

Puromycin aminonucleoside (PAN)-induced nephrosis, a model of human nephrotic syndrome, is characterized by the rapid development of high levels of proteinuria and a number of glomerular morphological changes. A significant biochemical difference from the normal control is another characteristic feature. Although the mechanisms of proteinuria in both human nephrotic syndrome and PAN-induced nephrosis in rats are not fully understood, an association with both immunological and non-immunological mechanisms has been suggested. Regarding the treatment of nephrotic syndrome, glucocorticoids, immuno-suppressants and antiplatelet drugs have been reported to be effective in reducing proteinuria to a variable extent. However, a better drug with minimum side-effects is yet to be found.

Over 1000 years, Bupleurum falcatum L. root has been used extensively in oriental medicine for the treatment of renal diseases. Sairei-to (Chai-Ling-Tang in Chinese) is composed of the extracts of Bupleurum falcatum L. roots and some other biologically active components. Recently this drug has been widely used in oriental and South-East Asian countries as a potent antinephritic agent. Until now, there have been only a few studies on the effectiveness of sairei-to. Senaga and Kawashima reported that Chai-Ling-Tang was effective against nephrotic syndrome in children. Abe et al also demonstrated electron microscopically that sairei-to remarkably inhibited histological damage in the kidney of rats with PAN-induced nephrosis. Sairei-to has been reported to have a suppressive effect on intraglomerular cell-mediated immunity and hypercoagulability, both of which are known as renal deterioration factors in human glomerulonephritis. In recent years, the search for the precise patho-biological mechanism responsible for massive proteinuria has been focused on the oxygen radicals which are produced during the metabolic breakdown of PAN and cause glomerular injury. These reactive oxygen products are toxic to a variety of biomolecules and can damage tissues by the peroxidation of membrane lipids. Superoxide dismutase (SOD) catalyses the conversion of superoxide to hydrogen peroxide and oxygen, and hydrogen peroxide is converted to water and oxygen by catalase. Recently, Diamond et al reported that SOD reduced proteinuria and glomerular injury in PAN-induced nephrosis. Other researchers have reported that the increased formation of oxygen radicals suppresses the intrarenal prostaglandin production. Thus, this study was performed to assess the effectiveness of sairei-to on PAN-induced nephrosis and to verify its effect on the elevation of SOD-like activi-
ty that scavenges the superoxide anions and also on the prostanoid synthesis.

MATERIALS AND METHODS

Animals: Male Wistar rats, weighing 180-200g (Charles River, Japan) were used in this experiment. Experimental Protocol: Five different sets of experiments were carried out. Each set was divided into 2-4 groups and each group consisted of 5-6 rats. The first experiment (1st Expt.) was undertaken to establish the PAN rat model and the second (2nd Expt.) to assess the dose-dependent effectiveness of sairei-to on PAN groups. The third (3rd Expt.) was performed to observe the effect of sairei-to on SOD-like activity in PAN groups while the fourth (4th Expt.) was to test if sairei-to has any effect on the elevation of SOD-like activity in normal rats. The fifth (5th Expt.) was to investigate the effect on prostaglandin synthesis. The rats of PAN group in all the experiments except the 4th Expt. received a single i.p. injection of PAN (Sigma Chemical Co., St. Louis, MO.) 100mg/kg body weight (B.W.) dissolved in 1 ml of 0.9% saline, and the normal control received only 1 ml of 0.9% saline i.p. on the day 0. In the 2nd Expt. sairei-to (Tsumura Co. Ltd, Tokyo) was administered orally to the rats of PAN+S-100, PAN+S-200, and PAN+S-500 groups at doses of 100mg/kg (almost the same dose as in humans), 200mg/kg and 500mg/kg B.W., respectively, mixed in 1-2 ml of warm water daily for 8 days after the initial i.p. injection of PAN. The composition of sairei-to is shown in Table 1. The 3rd and 5th Expt. consisted of the normal control, PAN and PAN+S-500 (most effective dose of the 2nd Expt.) groups. In the 4th Expt. the S-500 group was fed with sairei-to 500mg/kg B.W. without pretreatment of PAN and the normal control with 1 ml of warm water daily for 8 days. The oral administration was carried out with the help of a curved steel cannula fixed to a 5 ml plastic syringe. All the rats were fed with standard rat chow ad libitum and given free access to water. Each rat was kept in an individual cage and was sacrificed on day 8.

The sacrifice was carried out under ethyl ether anaesthesia and all the limbs were fixed against a wooden board in a supine position. The abdomen was opened by a longitudinal midline incision from the xiphisternum to the pubis and four lateral incisions on both sides of the flank. The abdominal aorta was dissected from the surrounding tissues and blood was collected with the help of a butterfly needle connected to a 10 ml plastic disposable syringe. Then the aorta was tied with thread above the level of the renal arteries and both the kidneys were slowly perfused with normal saline, using the same butterfly needle channel. The left renal vein was punctured to allow proper drainage of the perfusion fluid. To collect the tissues for electron microscopic study, only the left kidney was perfused with 2.5% glutaraldehyde-cacodylate buffer while the right kidney was clamped or tied at its hilum.

Urine and Blood collections: The 24 hrs urine was collected daily for 8 days by keeping each animal in an individual cage. The urine was centrifuged at 3,000 rpm for 7-10 minutes at 4°C and the supernatant was used to determine proteinuria. Blood was collected from the tail vein immediately after the urine collection on days 0, 2 and 5 for the study of serum SOD-like activity. During the process of sacrifice, blood was collected from the abdominal aorta and was later used for blood chemical analysis, assay of SOD-like activity and for prostaglandin levels.

Biochemical assay: The urinary protein content was determined by using a Tonein-TP kit (Otsuka Pharmaceuticals, Tokyo) and expressed in mg/day. The total protein and albumin were measured according to the method of McCord et al, using a SOD-TEST kit (Wako Pure Chemical Co., Osaka) and a digital Spectrophotometer (UVIDEC-220B, Japan Spectroscopic Co., Ltd). To measure the urinary SOD-like activity, we dialysed the urine sample overnight with phosphate buffered saline (pH 7.2) at 1:100 ratio. Then, the dialysed urine was similarly measured for SOD-like activity with a SOD-TEST kit and a digital spectrophotometer. The SOD-like activity was expressed in U/ml and U/day for serum and urine, respectively. Prostaglandin E₂(PGE₂), 6-keto prostaglandin F₁α (6-keto PGF₁α) and thromboxane B₂(TxB₂) in the serum and urine were measured according to the method of Kawano et al. Using octadecylsilyl silica (ODS) suspension and silica minicolumn (BOND ELUT Si, Analytichem International, Inc., USA), PGE₂, 6-keto PGF₁α and TxB₂ were fractionated.

Table 1. The amounts of crude substances required to obtain 6.0 g of sairei-to extract

<table>
<thead>
<tr>
<th>Crude drugs</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupleurum Root</td>
<td>7.0 g</td>
</tr>
<tr>
<td>Alisma Rhizome</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Pinellia Tuber</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Scutellaria Root</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Atractylodes Lancea Rhizome</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Jujube, Jujube fruit</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Chiling</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Ginseng</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Hoelen</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Glycyrrhiza, Licorice Root, Liquorice Root</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Cassia Cassia Bark Chinese Chinnamom</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Ginger</td>
<td>1.0 g</td>
</tr>
</tbody>
</table>
by a simultaneous extraction system. These fractionated substances were quantitated by radioimmunoassay (NEN, Boston, USA).

Pathology: Light and Electron microscopy: During the process of sacrifice, both the kidneys were slowly perfused with 40-50 ml of normal saline and then the left kidney was infused with 2.5% glutaraldehyde-cacodylate buffer (pH 7.2), keeping the hilum of the right kidney clamped or tied. Tissues from the right kidney were obtained for light microscopic study and those from the left were preserved for electron microscopic study. HE and PAS staining were carried out for light microscopic study. The histological grading was done according to the degree of proliferation of mesangial cells, expansion of the mesangial matrix and adhesion to the Bowman’s capsule from 500 glomeruli in each group. The proliferation of mesangial cells and expansion of the mesangial matrix were scored as 0, 1, 2, 3 and 0, 2, 4, 6, respectively for normal, mild, moderate and severe proliferations. Adhesion to the Bowman’s capsule was scored as 0, 1, 2, 3 for 0%, 0.1-14.9%, 15-29.9%, 30% and over, respectively. According to the total score calculated from the above criteria, each group was graded into : I (0-4.0), II (4.1-8.0) and III (8.1-) for mild, moderate and severe, respectively.

For electron microscopic study, specimens were prefixed in ice-cold 2.5% glutaraldehyde, and then postfixed in 2% osmium tetraoxide (OsO4). After dehydration they were embedded in Epon 812. Semi-thin sections were cut with glass knives. The slides for light microscopy were stained with toluidine blue and those for electron microscopy (H-7000) were doubly stained with 1% uranyl acetate and lead citrate.

Statistical analysis: Statistical analysis was performed by Student’s t-test and chi-squared analysis. One way ANOVA analysis was also used in this experiment. All the values were expressed as means ± S.D.

RESULTS
Proteinuria in the normal control and PAN groups
The urinary excretion of protein in the PAN group was significantly higher (p<0.01) on days 6 and 8 as compared to the normal control. The peak level of proteinuria was markedly high on day 8 while the level of urinary protein concentration in the normal control remained almost at the basal level (Fig. 1).

Blood chemical findings in the normal control and PAN groups
The serum levels of total protein and albumin in the PAN group were significantly lower, while the total cholesterol, triglyceride, blood urea nitrogen, creatinine and uric acid were significantly higher than the corresponding levels in the normal control (Table 2).

Histological grading in the normal control and PAN groups
The light micrographs for different histological grades are shown in Fig. 2. The proliferation of mesangial cells, expansion of the mesangial matrix and adhesion to the Bowman’s capsule of the glomeruli were much more observed in the PAN group than in the normal control (Table 3).

Electron microscopy in the normal control and PAN groups
The electron micrographs for the PAN group and their control is shown in Fig. 3. The PAN group showed fusion of the foot processes, swelling and vacuolization of the epithelial cells. There was also effacement of the podocytes, an increase in the intracellular granules, thickening and thinning of the

Table 2. Chemical findings in the blood of normal control and PAN groups

<table>
<thead>
<tr>
<th>Group</th>
<th>TP (mg/dl)</th>
<th>Alb (mg/dl)</th>
<th>T.Chol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Cr (mg/dl)</th>
<th>UA (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.42 ± 0.36</td>
<td>4.54 ± 0.31</td>
<td>77.0 ± 14.3</td>
<td>234 ± 55.2</td>
<td>21.1 ± 2.88</td>
<td>0.48 ± 0.05</td>
<td>1.5 ± 0.22</td>
</tr>
<tr>
<td>PAN</td>
<td>4.48** ± 0.86</td>
<td>3.04* ± 1.06</td>
<td>298.2** ± 105.4</td>
<td>601* ± 228.0</td>
<td>73.5* ± 28.79</td>
<td>0.82* ± 0.26</td>
<td>2.44* ± 0.72</td>
</tr>
</tbody>
</table>

TP: Total protein; Alb: Albumin; T.Chol: Total cholesterol; TG: Triglyceride; BUN: Blood Urea Nitrogen; Cr: Creatinine; UA: Uric Acid

Fig. 1. Daily urinary protein excretion in the normal control and PAN groups. The level of proteinuria in the PAN group was significantly higher than the normal control on days 6 and 8.

Fig. 2. Histological grading in the normal control and PAN groups.
Fig. 2. Light micrographs (PAS×400) showing different histological grades. The degree of proliferation of mesangial cells, expansion of the mesangial matrix and adhesion to the Bowman’s capsule has been classified into three grades: I (mild), II (moderate) and III (severe).

Fig. 3. Electron micrographs (×8000) showing the ultrastructures of glomeruli of the normal control (a) and PAN (b) group. Fusion of the foot processes, swelling and vacuolization were well-marked in the glomeruli of the PAN group.

Table 3. Histological grades in normal control and PAN groups

<table>
<thead>
<tr>
<th>Grade</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>456</td>
<td>44</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>PAN</td>
<td>198</td>
<td>271</td>
<td>31</td>
<td>500</td>
</tr>
<tr>
<td>Total</td>
<td>654</td>
<td>315</td>
<td>31</td>
<td>1000</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 296.36 \ (p<0.01) \ (n=5) \]

basement membrane and the presence of numerous microvilli. However, the above mentioned changes were absent in the normal control group.

Effect of sairei-to on PAN-induced proteinuria

The daily urinary protein excretion in the PAN+S-100, PAN+S-200 and PAN+S-500 groups remained lower on days 6 and 8 (except for the PAN+S-200 group on day 6) as compared to the PAN group. The urinary protein excretion in the PAN, PAN+S-100, PAN+S-200 and PAN+S-500 on day 8 were 678.9±148.6, 496.1±84.7, 443.8±59.8 and 339.0±80.4 mg/day, respectively. Thus, the effectiveness was most marked in the PAN+S-500 group on day 8 as shown in Fig. 4.
Effects of Sairei-to on Puromycin Aminonucleoside Nephrosis

Table 4. Effect of sairei-to on blood chemical findings in PAN-induced rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TP (g/dl)</th>
<th>Alb (g/dl)</th>
<th>T.Chol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Cr (mg/dl)</th>
<th>UA (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN</td>
<td>4.66±0.67</td>
<td>3.68±0.59</td>
<td>361.4± 33.2</td>
<td>827.0±262.3</td>
<td>54.4±21.6</td>
<td>0.76±0.13</td>
<td>2.3±0.6</td>
</tr>
<tr>
<td>PAN+S·100</td>
<td>4.42±0.28</td>
<td>3.24±0.21</td>
<td>354.4± 17.5</td>
<td>532.2±104.5*</td>
<td>44.0±17.1</td>
<td>0.72±0.11</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>PAN+S·200</td>
<td>4.68±0.52</td>
<td>3.76±0.55</td>
<td>368.8±141.1</td>
<td>511.0±457.1</td>
<td>37.0±6.9</td>
<td>0.70±0.07</td>
<td>2.2±0.6</td>
</tr>
<tr>
<td>PAN+S·500</td>
<td>4.56±0.27</td>
<td>3.60±0.28</td>
<td>287.4± 93.1</td>
<td>451.4±154.3*</td>
<td>40.0±19.9</td>
<td>0.66±0.15</td>
<td>2.7±1.0</td>
</tr>
</tbody>
</table>

S-100, S-200 and S-500 : sairei-to 100, 200 and 500 mg/kg, respectively (n=5)

*: p<0.05; Significant difference from PAN group

Table 5. Effect of sairei-to on histological grades in PAN-induced rat glomeruli

<table>
<thead>
<tr>
<th>Grade</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN</td>
<td>240</td>
<td>208</td>
<td>52</td>
<td>500</td>
</tr>
<tr>
<td>PAN+S·100</td>
<td>272</td>
<td>200</td>
<td>28</td>
<td>500</td>
</tr>
<tr>
<td>PAN+S·200</td>
<td>411</td>
<td>88</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>PAN+S·500</td>
<td>417</td>
<td>80</td>
<td>3</td>
<td>500</td>
</tr>
</tbody>
</table>

S-100, S-200 and S-500: sairei-to 100, 200 and 500 mg/kg, respectively

\[ \chi^2 = 259.12 \text{ p}<0.01 \text{ (n=5)} \]

Effects of sairei-to on chemical findings in the blood

The serum triglyceride, blood urea nitrogen, and total cholesterol levels in the PAN group were higher than in the corresponding sairei-to-treated rats. As shown in Table 4, the serum triglyceride levels in the PAN+S·100 and PAN+S·500 groups were significantly lower (p<0.05) than in the PAN group.

Effects of sairei-to on the pathological findings

Light microscopically, the PAN+S·100, 8·100, 8·200 and 8·500: sairei-to 100, 200 and 500 mg/kg, respectively

\[ \chi^2 = 259.12 \text{ p}<0.01 \text{ (n=5)} \]

Fig. 5. Electron micrographs (×6400) showing the ultrastructure of the glomeruli of the PAN group (a), PAN+S·100 group (b), PAN+S·200 group (c) and PAN+S·500 group (d). The PAN+S·500 (d) group showed a remarkable improvement over the PAN group.
Fig. 6. Serum SOD-like activity in the normal control, PAN and PAN+S·500 groups on day 8. The SOD-like activity was found to be higher in the normal control and PAN+S·500 groups than in the PAN group (p<0.01).

PAN+S·200 and PAN+S·500 groups showed significant improvements over the PAN group as shown in Table 5. The proliferation of mesangial cells, adhesion to the Bowman's capsule and mesangial expansion in the PAN group showed a dose-dependent improvement in the sairei-to-treated groups.

Electron microscopically, the PAN+S·500 group showed a remarkable improvement over the PAN group by reducing the characteristic changes of PAN-induced nephrosis. The other sairei-to-treated groups showed relative improvements over the PAN group (Fig. 5).

Effects of sairei-to on the SOD-like activities in the serum and urine of the PAN group

The serum SOD-like activity in the normal control, PAN and PAN+S·500 groups on day 8 is shown in Fig. 6. The level in the PAN+S·500 group was significantly higher than in the PAN group and reached almost to the same level as in the normal control. The urinary SOD-like activities in all the groups were elevated on days 6 and 8 as compared to days 0 and 3. And the values were significantly higher on days 6 and 8 in the PAN group and on day 8 in the PAN+S·500 group as compared to the corresponding days in the normal control.
**Table 6. Effect of sairei-to on prostaglandin levels in the rat serum and urine**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum PGE$_2$ (pg/ml)</th>
<th>6-Keto PGF$_{1α}$ (pg/ml)</th>
<th>TxB$_2$ (pg/ml)</th>
<th>6-Keto PGF$_{1α}$/TxB$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>29.2 ± 7.1</td>
<td>125.6 ± 39.4</td>
<td>152.0 ± 44.4</td>
<td>0.91 ± 0.49</td>
</tr>
<tr>
<td>PAN</td>
<td>41.4 ± 15.9</td>
<td>350.0 ± 142.6*</td>
<td>346.0 ± 233.4</td>
<td>1.23 ± 0.49</td>
</tr>
<tr>
<td>PAN+S·500</td>
<td>54.8 ± 35.7</td>
<td>266.6 ± 135.2</td>
<td>288.0 ± 141.9</td>
<td>0.93 ± 0.24</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>12320.0 ± 5717.3</td>
<td>4600.0 ± 768.1</td>
<td>354.0 ± 135.0</td>
<td>14.42 ± 5.12</td>
</tr>
<tr>
<td>PAN</td>
<td>898.0 ± 625.1*</td>
<td>590.0 ± 242.1*</td>
<td>272.0 ± 159.6</td>
<td>2.41 ± 0.63**</td>
</tr>
<tr>
<td>PAN+S·500</td>
<td>1784.0 ± 759.8*</td>
<td>1230.0 ± 77.2*</td>
<td>238.0 ± 69.0</td>
<td>5.12 ± 2.32**</td>
</tr>
</tbody>
</table>

*: p < 0.05, **: p < 0.01; Significant difference from normal controls (n=5)

The urinary 6-keto PGF$_{1α}$/TxB$_2$ ratio in the PAN+S·500 group was significantly lower than in the normal control. And the urinary 6-keto PGF$_{1α}$/TxB$_2$ ratio in the PAN+S·500 group on day 8 (p < 0.01) as compared to that on day 0.

The urinary SOD-like activity in the normal control and S-500 groups on days 0, 2, 5 and 8 showed no significant differences throughout the course (Fig. 9).

**DISCUSSION**

It is well established that PAN produces marked proteinuria, hypoalbuminemia, hyperlipidemia and remarkable glomerular morphological changes in rats which are very similar to those of nephrotic syndrome in humans. We have shown similar results in this experiment with a single i.p. injection of PAN in rats.

The present studies have also shown that sairei-to remarkably reduces the proteinuria, and serum triglyceride levels and improves the overall pathological status of the PAN group. And 500mg/kg B.W. of sairei-to was found to be the most effective dose among the other various dose-regimens. Many studies have been performed so far on the mechanisms of proteinuria in PAN-induced nephrosis. The results have been summarized to indicate that proteinuria occurs secondarily due to alterations in both the charge and size-selective barriers of glomerular filtrations. Free oxygen radical is generated from the hypoxanthine, an intermediate metabolite of PAN via the xanthine oxidase system, and can play an important role in proteinuria. Recently, it has been reported that SOD is an effective scavenger of oxygen free radical in the glomerular injury associated with PAN-induced nephrosis. We have shown that the serum SOD-like activity in the sairei-to-treated group was significantly higher than in the PAN group and reached almost to the same level as in the normal control on day 8. The urinary SOD-like activity increased in all the groups on days 6 and 8 as compared to that on days 0 and 3. The levels were significantly higher on days 6 and 8 in the PAN group and on day 8 in the PAN+S·500 group as compared to the corresponding days in the normal control. The low level of serum SOD-like activity in the PAN group may be explained by the fact that much more SOD is used to remove the excess oxygen radicals, while the increased excretion of urinary SOD-like activity may be due to the increased glomerular leaking. Similar findings were reported in children with renal diseases.

The high level of serum SOD-like activity in the PAN rats treated with sairei-to may possibly be explained by the elevated production of SOD-like substances. To clarify whether sairei-to has any effect on the elevation of SOD-like activities in normal rats, we tested this possibility and obtained the result that sairei-to increases the SOD-like activity significantly in the rats fed with 500mg/kg B.W. of sairei-to. Again, the reduced SOD-like activity in the PAN group may explain the active phase of
renal disease and the increased level in the sairei-to-treated group may be due to the state of the healing process of glomerulonephritis. So, SOD-like activity can be considered as a parameter of glomerulonephritis during the progression of the disease. Sairei-to has some effect on the release of corticosterone from adrenal gland. So, it can be used for patients who cannot be treated with steroid. Studies also have shown that sairei-to has some beneficial effects on glomerulonephritis as an antiplatelet agent.

Metabolic studies have shown that the excretion of prostanoids into urine does not result from the circulating levels of these prostanoids. However, urinary excretion predominantly reflects their intrarenal synthesis. In the present study we have discovered that the decreased excretion of urinary prostanoids mimics the reduced production of intrarenal prostanoids. The mechanism responsible for the reduced level may be due to the increased production of superoxide radicals formed during the metabolic breakdown of PAN which in turn suppresses PGE_2 formation. As a result, the decreased production caused a deterioration of the renal function and thus elevated the plasma levels. However, we found an increase in the production of intrarenal prostanoids which was relatively higher in the PAN+S-500 group than in the PAN group. The higher value of urinary 6-keto PGF_1α and the high ratio of PGI_2/TXA_2 in PAN+S-500 may result in vasodilatation and inhibition of platelet aggregation. Thus, sairei-to repairs renal damage and improves function as well. Prostanoids also have hypothetically cytoprotective effects which might ameliorate proteinuria of PAN-induced nephrosis.

These results indicate that sairei-to has a potent antinephritic effect by reducing the excretion of protein remarkably and improving the renal function and pathological damage in the nephrotic syndrome rat model. Our results also ascertain the effects of sairei-to on the elevation of the SOD-like activity that scavenges the oxygen radicals and on the improvement of intrarenal prostan gland synthesis. Further study in vitro is necessary to investigate the exact mechanism of the synthesis of SOD-like substances in sairei-to.

ACKNOWLEDGEMENTS

Parts of this work were presented at the 32nd Annual Meeting of the Japanese Society of Nephrology, November 9-11, 1989, Hamamatsu, Japan; the 20th Western Annual Meeting of the Japanese Society of Nephrology, April 26-27, 1990, Okayama, Japan; the 6th International Congress of Oriental Medicine, October 19-21, 1990, Tokyo, Japan, and the 21st Western Annual Meeting of the Japanese Society of Nephrology, May 31-June 1, 1991, Kobe, Japan.

(Received August 22, 1991) (Accepted November 20, 1991)

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