Nephrotic urine prevents increased rat glomerular albumin permeability induced by serum from the same patient with idiopathic nephrotic syndrome

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Abstract

Background. The putative humoral mediator thought to be involved in the pathogenesis of idiopathic nephrotic syndrome has not yet been identified. However, components exist in normal serum that block the permeability activity of FSGS serum in vitro. The potential of FSGS serum to increase glomerular albumin permeability may result from an imbalance between permeability factors and naturally occurring inhibitors. We hypothesized that this imbalance may be favoured by loss of inhibitory factors in nephrotic urine.

Methods. The study population consisted of seven patients with biopsy-proven FSGS, one with IgM nephropathy, and three with idiopathic nephrotic syndrome without biopsy, from whom frozen serum and dialysed and lyophilized urine samples were available. Glomerular albumin permeability (P_{alb}) was determined from the change in glomerular volume induced by applying oncotic gradients across the basement membrane of normal isolated rat glomeruli pre-incubated with patient serum, normal control serum, patient serum mixed with an equal volume of urine from the same patient, or patient serum mixed with normal urine. Serum and urine apolipoproteins J and E were measured by dot-blot, utilizing peroxidase-labelled antibodies. The urinary capacity to scavenge oxygen radicals was determined after exposure of isolated glomeruli to superoxide generated by xanthine and xanthine oxidase.

Results. The mean P_{alb} of the patients was markedly elevated at 0.74 ± 0.08. The addition of urine from the same patient significantly reduced P_{alb} (mean 0.15 ± 0.23) in all but one of the patients with FSGS. Normal urine had no inhibitory effect in the 10 patients in which it was tested (mean 0.71 ± 0.09). Serum apo J was slightly decreased and serum apo E was slightly increased compared with controls. Urine levels of both lipoproteins were significantly decreased compared with controls. Urine from FSGS patients effectively neutralized superoxide, whereas normal urine did not.

Conclusions. Nephrotic urine but not normal urine contains components that block increased albumin permeability in isolated rat glomeruli induced by serum from patients with the idiopathic nephrotic syndrome. The inhibitory function of these components, which appear not to include apolipoproteins J and E, may involve scavenging of superoxide as a final common pathway. Loss in the urine from the serum of naturally occurring inhibitors in the initial stages of the disease may propagate proteinuria and glomerular injury.

Keywords: apolipoproteins; FSGS; glomeruli; nephrotic syndrome; permeability factors; urine

Introduction

Several lines of evidence have suggested that a circulating humoral factor is involved in the pathogenesis of focal segmental glomerulosclerosis (FSGS), including (i) the often rapid appearance of proteinuria and renal failure following transplantation in affected patients [1,2], (ii) the efficacy of ex vivo techniques including plasmapheresis and immunoadsorption in reducing proteinuria following recurrence [3-5], (iii) permeability changes induced by patient serum in isolated normal glomeruli [6,7] and in cell culture [8], (iv) the possible placental transmission of permeability factors from mother to child [9] and (v) the resolution of proteinuria when kidneys with histological evidence of FSGS are transplanted into patients with end-stage
renal disease other than FSGS [10]. Nevertheless, the disease is not easily replicated by direct injection of patient serum in healthy laboratory animals [11]. In fact, normal serum from a variety of species contains factors that block the permeability activity of FSGS serum in vitro [12]. We have recently demonstrated that some apolipoprotein components of the high-density lipoprotein complex in normal serum inhibit the permeability activity of FSGS serum in vitro, and clearing of these apolipoproteins from normal serum by specific antibodies restores the permeability activity of the pathological serum [13]. Thus, the integrity of the glomerular permeability barrier in health and disease may depend on the interplay of various permeability factors and their naturally occurring inhibitors.

The stimulus that upsets the balance of permeability and inhibitory factors and the sequence of events that follows the initial insult remain unknown. We hypothesized that loss of inhibitory factors in nephrotic urine may occur, which would amplify the effect of permeability factors in the production of proteinuria.

Subjects and methods

Test and control sera and urine

Frozen serum and urine samples were available from seven patients with biopsy-proven FSGS, one with IgM nephropathy, and three with idiopathic nephrotic syndrome without biopsy, all of whom had elevated albumin permeability values (P_{ab}, see below) during prior testing. Demographic and clinical characteristics of the patients are shown in Table 1; the majority of patients were in the paediatric age range. Of the 11 patients studied, nine were males. Serum albumin was <15 g/dl in all patients with the exception of patient 3, who had low normal values. Pooled normal serum, frozen at −20°C in 12 ml aliquots, was available from 100 healthy renal allograft donors. Twenty-four-hour urine samples were collected from six healthy subjects without trace proteinuria, to serve as control.

Preparation of urine samples

Urine specimens were dialysed against water for 48 h using membranes with a molecular-weight cut-off of 8000 kDa.

Determination of apolipoprotein J and E levels

Serum and urinary levels of apo J and apo E were determined by dot-blot, utilizing peroxidase-labelled anti-apo J polyclonal

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>Steroid use</th>
<th>Creatinine (mg/dl)</th>
<th>Proteinuria (g/24 h)</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>FSGS</td>
<td>Sensitive</td>
<td>0.9</td>
<td>9.0</td>
<td>CsA</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>FSGS</td>
<td>Resistant</td>
<td>0.9</td>
<td>2.0–3.0</td>
<td>CsA, steroid, PP</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>FSGS</td>
<td>Sensitive</td>
<td>0.7</td>
<td>0.1</td>
<td>CsA</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>FSGS</td>
<td>Sensitive</td>
<td>1.2</td>
<td>4.5</td>
<td>CsA, steroid</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>FSGS</td>
<td>Sensitive</td>
<td>2.8</td>
<td>1.8</td>
<td>Steroid</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>FSGS</td>
<td>Sensitive</td>
<td>2.6</td>
<td>2.0–3.0</td>
<td>Steroid</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>FSGS</td>
<td>Sensitive</td>
<td>0.9</td>
<td>1.0–2.0</td>
<td>CF, PP</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>IgM</td>
<td>Resistant</td>
<td>0.5</td>
<td>4.0–5.0</td>
<td>CsA</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>Idio. NS</td>
<td>Sensitive</td>
<td>0.8</td>
<td>6.0–13.0</td>
<td>CsA, steroid</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>Idio. NS</td>
<td>Resistant</td>
<td>0.5</td>
<td>2.0</td>
<td>Steroid</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>Idio. NS</td>
<td>Resistant</td>
<td>0.3</td>
<td>Trace</td>
<td>CsA</td>
</tr>
</tbody>
</table>

Means ± SD 11.6 ± 10.6 1.10 ± 0.83 3.50 ± 3.21

CsA, cyclosporin; CF, cyclophosphamide; PP, plasmapheresis; Idio. NS, idiopathic nephrotic syndrome.
antibodies (Chemicon, Temecula, CA, USA) and anti-apo E monoclonal antibodies (cell clone 2E11, Roche, Monza, Italy). Apolipoproteins were first adsorbed under vacuum to Hybond C super nitrocellulose (Amersham-Pharmacia, Little Chalfont, UK). Specific antibodies were incubated overnight at room temperature, and visualization was achieved with ECL Plus (Amersham–Pharmacia Biotech, Milan, Italy). Apo J was purified for the standard titration curve according to the method described by Calero et al. [15]. Standards for apo E were purchased from Dauchi Pure Chemicals (Tokyo, Japan). The luminescent signal was acquired with a STORM 860 laser scanner (Amersham–Pharmacia Biotech), utilizing 420 nm and 460 nm as λ excitation and emission, respectively. Normal values for apo J and apo E in serum are represented by the mean value from six healthy subjects.

Urine scavenging capacity

To determine whether oxygen radical scavenging was involved in the urinary inhibitory activity, the responses of isolated glomeruli after exposure to superoxide generated by xanthine and xanthine oxidase were studied after the addition of normal or nephrotic urine, according to the method described by Dileepan et al. [16]. Briefly, isolated glomeruli were incubated in medium containing 0.1 mmol/l xanthine for 5 min at 37°C. Superoxide was generated by the addition of xanthine oxidase 20 U/ml, 10 μl in 1 ml of 5 g/dl BSA, and the incubation continued for 10 min. Parallel incubations were run in which superoxide dismutase 300 U/ml, pooled normal urine, or separate nephrotic urine samples (4% vol/vol) from three patients with FSGS were added to the incubation medium with xanthine/xanthine oxidase.

Statistical analysis

Data are presented as means ± SD. Comparisons of means were performed by Student’s t-test for unpaired data (two-tailed α = 0.05).

Results

Glomerular albumin permeability following incubation with patient serum, patient serum mixed with urine from the same patient, and patient serum mixed with normal urine is shown in Table 2. In all but one case (patient 3) patient urine abrogated the permeability activity of the matched serum, whereas normal urine did not in the 10 cases in which the experiment was performed. It should be noted that the ‘unprotective’ urine of patient 3 was almost protein free at the time of the measurement.

Serum apo J was non-significantly decreased compared with controls (30 ± 13 vs 36 ± 6 mg/dl), and serum apo E was non-significantly increased compared with controls (16 ± 20 vs 5 ± 3 mg/dl). There was no correlation between the degree of proteinuria and serum apolipoprotein levels (r = 0.41). Both apolipoproteins were significantly (P < 0.001) decreased in patient urine compared with controls (apo J, 0.05 ± 0.05 vs 0.71 ± 0.1 mg/dl; apo E 0.0089 ± 0.0105 vs 0.58 ± 0.1 mg/dl).

Isolated glomeruli exposed to xanthine/xanthine oxidase showed increased albumin permeability (P_{ab} 0.64 ± 0.10, median 0.60, range 0.55–0.80). This reaction was abrogated by simultaneous incubation with superoxide dismutase (P_{ab} 0.23 ± 0.21, median 0.29, range 0.00–0.40). Nephrotic urine significantly reduced albumin permeability induced by superoxide exposure (mean P_{ab} 0.17 ± 0.13, median 0.20, range 0.00–0.33; P < 0.001 vs xanthine/xanthine oxidase), whereas pooled normal urine did not (P_{ab} 0.62 ± 0.18, median 0.58, range 0.44–0.85).

Table 2. Glomerular albumin permeability (P_{ab}) in patient serum and in serum mixed with the patient’s own urine or with normal urine.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Serum P_{ab}</th>
<th>Serum + urine P_{ab}</th>
<th>Serum + normal urine P_{ab}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FSGS</td>
<td>0.68</td>
<td>0.37</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>FSGS</td>
<td>0.75</td>
<td>0.06</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>FSGS</td>
<td>0.92</td>
<td>0.74</td>
<td>0.73</td>
</tr>
<tr>
<td>4</td>
<td>FSGS</td>
<td>0.65</td>
<td>0.00</td>
<td>0.94</td>
</tr>
<tr>
<td>5</td>
<td>FSGS</td>
<td>0.74</td>
<td>0.00</td>
<td>0.64</td>
</tr>
<tr>
<td>6</td>
<td>FSGS</td>
<td>0.77</td>
<td>0.00</td>
<td>0.77</td>
</tr>
<tr>
<td>7</td>
<td>FSGS</td>
<td>0.66</td>
<td>0.00</td>
<td>0.60</td>
</tr>
<tr>
<td>8</td>
<td>IgM</td>
<td>0.81</td>
<td>0.00</td>
<td>0.60</td>
</tr>
<tr>
<td>9</td>
<td>Idiopathic</td>
<td>0.68</td>
<td>0.11</td>
<td>0.69</td>
</tr>
<tr>
<td>10</td>
<td>Idiopathic</td>
<td>0.67</td>
<td>0.20</td>
<td>0.73</td>
</tr>
<tr>
<td>11</td>
<td>Idiopathic</td>
<td>0.76</td>
<td>0.15</td>
<td>0.69</td>
</tr>
<tr>
<td>Means ± SD</td>
<td></td>
<td>0.74 ± 0.08</td>
<td>0.15 ± 0.23</td>
<td>0.71 ± 0.09</td>
</tr>
</tbody>
</table>

Discussion

The present study demonstrated that a component of urine is capable of blocking in vitro the serum permeability activity of the patient with idiopathic nephrotic syndrome from whom the urine sample was obtained, whereas non-nephrotic urine lacked this potential. One FSGS patient, 3, demonstrated elevated in vitro permeability activity, but appeared to be in clinical remission at the time of testing, and P_{ab} did not improve with the addition of autologous urine. Personal observations of permeability activity after successful plasmapheresis for recurrent FSGS demonstrate rapid return of elevated P_{ab}, which subsequently decreases with time. Thus, it may be that patient 3 was indeed in remission at the time of testing, and the autologous urine was, in fact, normal. Patient 11 had
between Palb and steroid sensitivity could not be derived.

Unbiased FSGS population to ascertain the correlation.

Regardless of the quantity collected during the 24-h

the permeability testing was equal for all subjects,

may have been protective. It is important to stress that

hypoalbuminaemia present at the time of testing, may

trace protein in the urine but, on the basis of

hypoaalbuminaemia present at the time of testing, may

not have been in remission, and thus his or her urine

may have been protective. It is important to stress that

the quantity of lyophilized urine protein used during

the permeability testing was equal for all subjects,

regardless of the quantity collected during the 24-h

urine samples.

A systematic study of glomerular albumin permea-
bility and steroid sensitivity has yet to be performed. An

unbiased FSGS population to ascertain the correlation

between $P_{\text{m}}$ and steroid sensitivity could not be derived

from the present study, the subjects of which presented

difficult management problems and with important

risk factors for eventual transplantation.

The most likely candidate for the urinary blocking sub-

stance would be a protein filtered through the damaged

glomerular permeability barrier or secreted by the

tubular epithelium, normally restricted to the blood

compartment in conditions of health. In addition to

albumin, a number of plasma proteins have been

shown to be lost in the urine of patients affected with

the nephrotic syndrome, and some of these losses may

have pathological consequences. For example, anti-

thrombin III concentrations may be greatly reduced in

patients with hypoaalbuminaemia, probably because of

urinary losses [17], which may contribute to the

hypercoagulable state characteristic of the nephrotic

syndrome. Decreased serum transferrin levels due to

urinary losses may lead to a microcytic, hypochromic

anaemia resistant to iron therapy [18].

Since apolipoproteins associated with the HDL

complex (namely apo J, apo L, apo E2, apo E4 and

a fragment of apo A-IV isolated from normal serum)

have the ability to block the permeability activity of

FSGS serum in vitro [13], we speculated that the pre-

sence of these apolipoproteins in nephrotic urine may

be responsible for the blocking activity observed in the

present study. However, the results of this study
demonstrated that urinary levels of the apolipoproteins

J and E were, in fact, significantly lower than in control

(i.e. non-proteinuric) urine, and there was no cor-

relation between the degree of proteinuria and the pre-

sence of apolipoproteins. Thus, it is unlikely that these

apolipoproteins are responsible for the protective

effects of autologous urine, since their concentrations

are actually greater in normal urine. However, the

limited number of cases studied at this point does not

allow a firm conclusion.

Few studies have addressed lipid and apolipoprotein

abnormalities both in serum and urine of patients and

laboratory animals with the nephrotic syndrome. To

our knowledge, the present study is the first in which

apo J levels have been measured in serum and urine

from nephrotic patients. Among the investigations of

apo E in nephrotic subjects, Shafrir et al. [19] found

apolipoproteins A-I, A-II, E, and traces of C in control

rat urine, and apo E and large amounts of apo C in

the urine of nephrotic rats previously treated with

puromycin aminoglycoside. Apolipoproteins found in

nephrotic urine were virtually always complexed with

lipids. van Goor and colleagues [20] studied apolipo-

proteins A-I, A-IV, E, and B in plasma and kidney

tissue from nephrotic rats previously treated with

puromycin aminoglycoside or adriamycin.apo A-I and

apo B plasma levels were significantly increased

compared to controls, whereas apo A-IV and apo E

levels were unchanged. The immunoreactivity of

apo A-I, apo A-IV and apo E—those apolipoproteins

frequently associated with HDL—was increased in

glomerular visceral epithelial cells, whereas apo B and

E—frequently associated with VLDL—were found prin-

cipally in the mesangial matrix. It should be noted

that rodents have different serum lipoprotein cha-

racteristics compared to humans, particularly in that

HDL is the major lipid constituent in rodents. Taken

together, however, these data suggest a distinct differ-

eence in the apolipoprotein profile both in the plasma

and in the urine of nephrotic patients and animals

when compared with controls.

In our study, nephrotic urine from FSGS patients

prevented increased albumin permeability induced by

superoxide generated by the action of xanthine oxidase

on xanthine, whereas normal urine did not. Reactive

oxidant metabolites have been shown to be key medi-

ators of proteinuria in a number of pathological set-

tings, also before significant histological evidence of

glomerular damage is observed. Sharma et al. [21] have

recently shown that hydroxyl radicals may mediate

increased albumin permeability in isolated glomeruli

induced by incubation with transforming growth factor

ß1. Chen et al. [22] found a role for reactive oxygen

species in diabetic glomerulopathy, and Gwinner et al.

[23] found increased radical generation in the acute

phase of puromycin aminonucleoside glomerulopathy.

The concentration of reactive oxygen species, and

thus any pathophysiological effects that they may

have at the cellular level, is determined by the balance

between oxidative enzymes and anti-oxidants such as

enzymes (superoxide dismutase, catalase, etc.), metal-

binding proteins (such as transferrin), vitamins (A, E,

C), among others [24]. Thus, nephrotic urine may be

endowed with enhanced anti-oxidant capacity, due to

loss in the urine of potent oxygen radical scavengers.

Further studies are necessary to isolate the protective

factors in urine, to determine the anti-oxidant poten-
tial of apolipoproteins in the serum, and to determine

whether the putative permeability factor in FSGS

initiates a cascade of events which terminates in the

production of radical oxygen species and proteinuria.

In conclusion, the present study demonstrates that

urine from patients with idiopathic nephrotic syn-
drome blocks the serum permeability activity of the

same patient, just as normal serum blocks the per-

meability activity of FSGS serum. However, apolipo-

proteins, which appear to be protective in the serum,
do not appear to play a role in urinary protection. The

scavenging of radical oxygen species may be the mecha-
nism by which protective factors exert their effect. The

loss of protective factors in the urine may lead to a

vicious cycle, in which the putative serum permeability

factor provokes urinary loss of inhibitory factors,
thereby accentuating the permeability defect and eventual glomerular damage.

References


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