Unilateral Ureteroperitoneostomy in the Management of Hypoproteinemia in Nephrotic Rats with Normal Renal Function

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Kavukçu, S., Soylu, A., Türkmen, M., Kuralay, F., Yılmaz, O. and Sarıoğlu, S. Unilateral Ureteroperitoneostomy in the Management of Hypoproteinemia in Nephrotic Rats with Normal Renal Function. Tohoku J. Exp. Med., 2003, 201 (2), 67–73 — Maintenance of serum albumin levels within normal limits is difficult to achieve in nephrotic children with normal renal functions who are unresponsive to specific treatment. One approach in such children is unilateral nephrectomy with rapid progression to renal failure. Peritoneal membrane is permeable to fluids, electrolytes and proteins, and peritoneal space has been used for total parenteral alimentation. Experimental ureteroperitoneostomy has been reported not to cause any significant side effect. The aim of this study was to evaluate the effects of unilateral ureteroperitoneostomy on serum and urine protein levels in rats with adriamycin-induced nephrosis. Adriamycin nephrosis was induced in 45 male Wistar rats. After two weeks, unilateral nephrectomy (Nx), unilateral ureteroperitoneostomy (Up) and sham operated (Sh) groups, each including 15 rats were formed. Serum creatine (S_Cr) and albumin (S_alb), and daily urinary protein excretion (U_pro) were determined before adriamycin injection (week 0), before operations (week 2) and at the end of 6th week in all rats. In addition, percent change in serum albumin (∆S_alb) and urine protein levels (∆ U_pro) between weeks 0–2, 0–6 and 2–6 were calculated for each group (e.g.: ∆ S_alb 0–2 = [S_alb week 2 − S_alb week 0]/S_alb week 0 × 100). Then, these parameters were compared within and between the groups. Furthermore, peritoneal tissue samples were obtained from the rats in Sh and Up groups to be examined for pathological changes. S_Cr did not change within and in between the groups during the study period. S_alb decreased significantly at weeks 2 and 6 with respect to week 0 in all three groups. In addition, although S_alb tended to decrease at week 6 with respect to week 2 in all groups, this was significant only in Sh group. U_pro increased significantly at weeks 2 and 6 with respect to week 0, and at week 6 with respect to week 2.
in all groups. However, $S_{alb}$ and $U_{pro}$ were not different between the three groups at weeks 0.2 and 6. On the other hand, $\Delta S_{alb}$ and $\Delta U_{pro}$ were not different between Sh vs. Nx and Nx vs. Up rats, but $\Delta S_{alb}$ 0–6, $\Delta S_{alb}$ 2–6 and $\Delta U_{pro}$ 0–6 were significantly lower in Up group compared to Sh group. Histopathological examination of peritoneal samples revealed significantly higher fibrosis score in Up group compared to Sh group. In conclusion, unilateral ureteroperitoneostomy may one important therapeutic selection in the treatment of intractable nephrotic syndrome. However, peritoneal fibrosis could be a concern for further use of peritoneum in case of end stage renal failure. —— adriamycin; hypoalbuminemia; nephrosis; unilateral nephrectomy; ureteroperitoneostomy

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Nephrotic syndrome (NS) is characterized by heavy proteinuria, hypoalbuminemia and edema. Hypoalbuminemia is mainly due to renal loss and increased catabolism. Management of NS includes specific therapies to prevent glomerular protein leakage (e.g., glucocorticoids, alkylating agents) and nonspecific measures (e.g., salt and water restriction, provision of adequate protein intake) to decrease edema (Holmberg et al. 1999). Main goal of the clinicians is to keep serum albumin levels within normal ranges until the renal protein loss ceases.

Continuous ambulatory peritoneal dialysis results in protein loss as fluid moves out of intravascular compartment by osmotic gradient of dialysate. In other words, intravascular protein could easily pass into peritoneal cavity. On the other hand, increased dialysate urea concentration does not cause peritoneal damage (Kaysen 1998).

Peritoneal cavity has been used for parenteral alimentation in humans and experimental animals previously (Giordano et al. 1980; Stabile and Borzatta 1997). Ureteroperitoneostomy procedure, leading the urine to drain into peritoneal cavity, has been reported not to cause any complication other than anorexia in experimental animals (Gittes and Gittes 1984).

It is usually not possible to prevent the decrease in serum protein levels in nephrotic children with massive proteinuria until specific therapy becomes effective or when there is no possibility for specific therapy. Albumin given intravenously is both expensive and is lost rapidly through the kidneys. Although renal functions are normal, protein lost through urine is the main problem in these patients (Holmberg et al. 1999).

The aim of this study is to evaluate the effects of unilateral ureteroperitoneostomy on serum and urine protein levels in rats with adriamycin-induced nephrosis for a treatment modality for persistent hypoalbuminemia in pediatric patients with resistant NS.

**MATERIALS AND METHODS**

Male Wistar rats ($n = 45$) were injected a single dose of adriamycin (7.5 mg/kg) to induce NS (Korzets et al. 1997). Two weeks later, three groups each including 15 rats were formed: 1. Unilateral nephrectomy group (Nx), 2. Unilateral ureteroperitoneostomy group (Up), and 3. Sham operated group (Sh) (Gittes and Gittes 1984). The study was approved by the local ethical committee.

Serum albumin ($S_{alb}$), creatinine ($S_{cr}$) and daily urinary protein excretion ($U_{pro}$) levels of all rats were determined before
adriamycine injection (week 0), before operation (week 2), and on 42nd day (week 6).

Furthermore, per cent change in $S_{\text{ah}}$ and $U_{\text{pro}}$ ($\Delta S_{\text{ah}}$ and $\Delta U_{\text{pro}}$, respectively) between the weeks 2–0, 6–0 and 6–2 were calculated as in the following example:

$$\Delta S_{\text{ah}} 2–0 (\%) = \frac{[S_{\text{ah}} \text{ week 2} - S_{\text{ah}} \text{ week 0}]}{S_{\text{ah}} \text{ week 0}} \times 100$$

In addition, peritoneal membranes of the Sh and Up groups were evaluated for the presence of inflammation, fibrosis and thickness at the end of the sixth week to determine the effect of urine on peritoneum. Intestinal and abdominal wall samples along with liver tissue were examined histopathologically. Vertical sections of 3 $\mu$m thickness of intestinal and abdominal samples were taken after formalin fixation. Livers were also sampled including the cortical area. Each specimen was processed, paraffin embedded and cut in sequential 4 $\mu$m sections and stained with Hematoxylin and eosin (H & E) and Van Gieson stain. The H & E sections were evaluated by light microscopy for inflammation and fibrosis.

Acquisition and preprocessing of digital images

As the serosa and capsule of the intestine and liver were sparse and there was not a deviation from the morphology of the control group, image analysis was not performed.

The peritoneal images were performed through a 3CCD colour video camera, connected to a light microscope at an original magnification of 20x. Images were processed with Mediscope Image Analysis Software (Mediscope, Dokuz Eylül University, Clinical Engineering Department, Izmir, Turkey). In each case 10 fields of peritoneum were selected and acquisition and digitalization were performed.

Image analysis

For each captured area the percentage of fibrotic fiber area was determined semi-automatically. First the area stained red was marked with multiple clicking by mouse depending on visual observation. Then the system selected the area with the same configuration of color according to the clicked areas and determined the percentage of the selected area to the measured. The mean of 10 fields was the accepted final value.

The thickness of the peritoneum was also determined by the same software. The thickness was measured at 10 random areas and the mean value was accepted as the thickness.

Then, all these parameters were compared within and between the groups by Mann-Whitney’s U-test.

![Graph](image)

Fig. 1. Serum creatinine ($S_{\text{Cr}}$) levels (mg/100 ml) of sham (Sh), unilateral nephrectomy (Nx) and unilateral ureteroperitoneostomy (Up) groups at weeks 0 (black column), 2 (dotted column) and 6 (white column). $S_{\text{Cr}}$ was not different within and between the groups throughout the study.
Table 1. Serum creatinine (SCr), albumin (Saib) and urinary protein (Upro) levels in sham (Sh), unilateral nephrectomy (Nx) and unilateral ureteroperitoneostomy (Up) groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh</td>
<td>S&lt;sub&gt;Cr&lt;/sub&gt; (mg/100 ml)</td>
<td>0.8±0.2</td>
<td>0.9±0.2</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;aib&lt;/sub&gt; (g/100 ml)</td>
<td>4.2±0.5</td>
<td>3.1±0.6</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td></td>
<td>U&lt;sub&gt;pro&lt;/sub&gt; (mg/kg/24 h)</td>
<td>13.9±7.8</td>
<td>64.6±30.5</td>
<td>88.8±14.1</td>
</tr>
<tr>
<td>Nx</td>
<td>S&lt;sub&gt;Cr&lt;/sub&gt; (mg/100 ml)</td>
<td>0.8±0.2</td>
<td>0.6±0.3</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;aib&lt;/sub&gt; (g/100 ml)</td>
<td>4.0±0.5</td>
<td>2.8±0.9</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td></td>
<td>U&lt;sub&gt;pro&lt;/sub&gt; (mg/kg/24 h)</td>
<td>16.3±6.8</td>
<td>61.2±40.9</td>
<td>107.5±53.5</td>
</tr>
<tr>
<td>Up</td>
<td>S&lt;sub&gt;aib&lt;/sub&gt; (g/100 ml)</td>
<td>3.8±0.7</td>
<td>3.0±0.7</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td></td>
<td>U&lt;sub&gt;pro&lt;/sub&gt; (mg/kg/24 h)</td>
<td>18.3±9.7</td>
<td>62.3±27.7</td>
<td>109.4±40.0</td>
</tr>
</tbody>
</table>

S<sub>Cr</sub> was not different within and between the groups throughout the study.
S<sub>aib</sub> decreased significantly at 2nd and 6th weeks compared to week 0 in all groups (p<0.05). S<sub>aib</sub> at week 6 was significantly lower than S<sub>aib</sub> at week 2 in only Sh group.
U<sub>pro</sub> increased significantly at 2nd and 6th weeks compared to week 0 in all groups (p<0.05). In addition, U<sub>pro</sub> at week 6 was significantly higher than U<sub>pro</sub> at week 2 in all groups (p<0.05).

Fig. 2. Serum albumin (S<sub>aib</sub>) levels (g/100 ml) of sham (Sh), unilateral nephrectomy (Nx) and unilateral ureteroperitoneostomy (Up) groups at weeks 0 (black column), 2 (dotted column) and 6 (white column). S<sub>aib</sub> decreased significantly at 2nd and 6th weeks compared to week 0 in all groups (p<0.05). S<sub>aib</sub> at week 6 was significantly lower than S<sub>aib</sub> at week 2 in only Sh group (p<0.05).

RESULTS
S<sub>Cr</sub> did not change within and between the groups throughout the study (Table 1, Fig. 1). While S<sub>aib</sub> decreased significantly at weeks 2 and 6 with respect to week 0 in all groups, only Sh group had significantly lower levels at week 6 with respect to week 2 (Table 1, Fig. 2). U<sub>pro</sub> increased significantly at weeks 2 and 6 with respect to week 0, and at week 6 with respect to week 2 in all groups (Table 1, Fig. 3). However, S<sub>aib</sub> and U<sub>pro</sub> levels did not differ between the groups throughout the study.

On the other hand, although ΔS<sub>aib</sub> and ΔU<sub>pro</sub> were not different between Sh vs. Nx and Nx vs. Up groups, ΔS<sub>aib</sub> 0–6 and 2–6 and ΔU<sub>pro</sub> 0–6 levels in Up groups were significantly lower compared to Sh group (Table 2, Figs. 4 and 5).
Fig. 3. Urine protein (U_{pro}) levels (mg/kg/24 h) of sham (Sh), unilateral nephrectomy (Nx) and unilateral ureteropercitoneostomy (Up) groups at weeks 0 (black column), 2 (dotted column) and 6 (white column). U_{pro} increased significantly at 2nd and 6th weeks compared to week 0 in all groups ($p < 0.05$). In addition, U_{pro} at week 6 was significantly higher than U_{pro} at week 2 in all groups ($p < 0.05$).

**Table 2.** Percent (%) change in serum albumin ($\Delta S_{alb}$) and proteinuria ($\Delta U_{pro}$) levels between the weeks 0–2, 0–6 and 2–6 in sham (Sh), unilateral nephrectomy (Nx) and unilateral ureteropercitoneostomy (Up) groups.

| %   | Weeks 2–0 |  | Weeks 6–0 |  | Weeks 6–2 |  |
|-----|-----------|  |-----------|  |-----------|  |
|     | Sh        | Nx | Up        | Sh        | Nx | Up        | Sh | Nx | Up |
| $\Delta S_{alb}$ | -28.4 | -32.0 | -18.1 | -55.4 | -42.9 | -32.7 | -43.2 | -25.0 | -24.6 |
|     | $\pm 12.7$ | $\pm 20.2$ | $\pm 23.5$ | $\pm 6.5$ | 15.8 | 18.7 | $\pm 10.6$ | $\pm 18.3$ | $\pm 7.9$ |
| $\Delta U_{pro}$ | 808.9 | 395.3 | 374.7 | 1467.2 | 702.0 | 646.8 | 93.94 | 133.7 | 297.3 |
|     | $\pm 694$ | $\pm 411$ | $\pm 435$ | $\pm 565$ | $\pm 583$ | $\pm 389$ | $\pm 15.6$ | $\pm 149.8$ | $\pm 707$ |

$\Delta S_{alb}$ and $\Delta U_{pro}$ were not different between Sh vs. Nx and Nx vs. Up groups. $\Delta S_{alb}$ 0–6 and 2–6, and $\Delta U_{pro}$ 0–6 were significantly lower in Up group compared to the Sh group ($p = 0.002$, $p = 0.002$, and $p = 0.043$, respectively).

Fig. 4. Percent (%) change in serum albumin levels ($\Delta S_{alb}$) of sham (Sh, black column), unilateral nephrectomy (Nx, dotted column) and unilateral ureteropercitoneostomy (Up, white column) groups between the weeks 0–2, 0–6 and 2–6. $\Delta S_{alb}$ was not different between Sh vs. Nx and Nx vs. Up groups. $\Delta S_{alb}$ 0–6 and 2–6 were significantly lower in Up group compared to the Sh group ($p = 0.002$ for both parameters).
Pathologic examination of peritoneal membranes revealed no inflammation in both the Sh and Up groups. However, fibrosis score as the percent area of peritoneal membrane showing fibrosis was significantly higher in Up group than that of Sh group (10.03 ± 17.3% and 6.66 ± 0.81%, respectively; p = 0.002). Although being thicker in Up group, peritoneal membrane thickness did not show significant difference between the two groups (263.42 ± 114.86 and 206.45 ± 63.05 μm, respectively; p = 0.535).

**DISCUSSION**

It is usually necessary to fight against hypoproteinemia while waiting for specific therapy to be effective in nephrotic syndrome. Utility of nonspecific therapies for the management of hypoproteinemia is controversial up to date. In this study, we aimed to evaluate whether unilateral ureteroperitoneostomy leading to passage of protein rich urine of one kidney into the peritoneal cavity and then to reabsorption of this protein into the circulation could result in alleviation of the degree of hypoproteinemia by causing recirculation of protein between the urine and systemic circulation. We could not see any study with similar objective in the literature. S_{cr} levels did not differ within and in between the groups throughout the study. In other words, renal functions were not effected by any the procedures performed in three groups of rats.

While S_{all} decreased significantly at 2nd and 6th weeks with respect to week 0 in all groups, its level at 6th week was significantly lower compared to 2nd week in only Sh group. However, S_{all} did not differ between the groups. Thus, the degree of hypoproteinemia did not differ statistically after the operations in Nx and Up groups.

Although U_{pro} increased significantly at 2nd and 6th week compared to week 0, and at 6th week compared to 2nd week in all groups, it did not differ between the groups throughout the study. Absence of statistical difference in S_{all} between the 2nd and 6th week in Nx and Up groups in spite of significant increase in U_{pro} at the same period could be related to the sample size of the groups.

While ΔS_{all} and ΔU_{pro} at 0–2, 0–6 and 2–6 weeks did not differ between Sh vs. Nx and Nx vs. Up groups, ΔS_{all} at 0–6 and 2–6 weeks and ΔU_{pro} at 5–6 weeks were significantly lower in Up group compared to Sh group. The insignificance of ΔU_{pro} at 2–6 weeks in Up group compared to Sh group might result from the small sample size.

The results of this study implicate that the severity of hypoproteinemia in nephrotic syndrome could be decreased by unilateral
ureteroperitoneostomy. There was no inflammation in peritoneal membranes in both Sh and Up groups. On the other hand, diversion of urine into the peritoneal cavity resulted in significantly increased amount of fibrosis in peritoneal membrane compared to Sh group, although thickness of peritoneal membrane was not different between the groups. These findings show that use of peritoneum for renal replacement therapy could be limited when end stage renal failure ensues.

Histopathologic changes in the contralateral kidneys in both Nx and Up groups were not examined in this study. Since we aimed only to evaluate the effects of unilateral ureteroperitoneostomy on proteinuria and hypoproteinemia in nephrotic rats, we did not performed nephrectomy to evaluate histopathologic changes at the end of the study. Furthermore, renal histopathologic changes of the contralateral kidneys have been evaluated in a previous study using the same methodology in a non-nephrotic model. In that study, it has been demonstrated that while glomerular hypertrophy and sclerosis in addition to hemodynamic changes are seen in unilateral nephrectomy group, only hemodynamic changes without glomerulosclerosis (increased glomerular blood flow rate, glomerular hypertension and glomerular filtration rate) are observed in unilateral ureteroperitoneostomy group. Based on these findings, it has been claimed that while nephron loss leads to sclerosis in the contralateral kidney, loss of clearance in one kidney without nephron loss leads only to increased glomerular hemodynamic parameters in the other kidney (Yoshida et al. 1989). Our findings along with the results of the mentioned study suggest that unilateral ureteroperitoneostomy procedure performed in resistant nephrotic syndrome could both decrease the severity of hypoproteinemia and do not lead to glomerulosclerosis in the contralateral kidney. This is important in case of the primary pathology leading to nephrosis goes into remission.

In conclusion, unilateral ureteroperitoneostomy results in decreased severity of hypoproteinemia, but leads to increased severity of peritoneal fibrosis in rats with adriamycin induced nephrosis.

References