Urinary endothelin-1-like immunoreactivity in young male patients with testicular cancer treated by cis-platinum: comparison with other urinary parameters

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1. Urinary excretion of endothelin-1-like immunoreactivity and urinary excretion of other parameters (β2-microglobulin, N-acetyl-β-D-glucosaminidase and microalbumin) were measured before, within 1 week after and 2 weeks after the administration of cis-platinum in five young male patients with testicular cancer (mean age 33.0 years) and were compared.

2. Urinary endothelin-1-like immunoreactivity/creatinine during, 1 week after, and 2 weeks after cis-platinum treatment was significantly higher than before cis-platinum. There was no difference in urinary endothelin-1-like immunoreactivity/creatinine during, 1 week after and 2 weeks after cis-platinum.

3. Among the four parameters, urinary endothelin-1-like immunoreactivity/creatinine showed the highest level after cis-platinum treatment. Urinary β2-microglobulin/creatinine most rapidly returned to normal levels after cis-platinum.

4. Although urinary endothelin-1-like immunoreactivity/creatinine did not show any significant correlations with urinary N-acetyl-β-D-glucosaminidase (r = 0.291, not significant) or urinary microalbumin/creatinine (r = 0.076, not significant), it showed a significant correlation with urinary β2-microglobulin/creatinine (r = 0.475, P < 0.05).

5. These results suggest that endothelin-1 may be a sensitive urinary parameter in detecting cis-platinum-induced renal tubular injury.

INTRODUCTION

Endothelin-1 (ET-1) is a vasoconstrictor/pressor peptide composed of 21 amino acids, which was originally isolated and sequenced from porcine aortic endothelial cells [1]. Very recently, ET-1 production was found in the kidney [2-4] and urinary ET-1-like immunoreactivity (U-ET-1) was measured by a specific r.i.a. [5], but its physiological roles are still unclear. ET-1 is produced by mesangial cells, distal tubules and collecting duct and possibly not by proximal tubules in the kidney [6-8]. Because the U-ET-1 level is higher than the serum ET-1 level, and is higher than estimated ET-1 clearance, it is believed that U-ET-1 is excreted from the kidney, particularly from the collecting duct or renal tubule [6]. Thus, it is hypothesized that U-ET-1 may be a marker for renal tubule or collecting duct injury. The U-ET-1 level in patients with renal diseases (except for urological renal diseases) is higher than that of normal subjects and is correlated with urinary (U-) β2-microglobulin (β2-MG), N-acetyl-β-D-glucosaminidase (NAG) and albumin [9].

Renal tubular injury frequently occurs under several circumstances, including therapy with aminoglycosides, cis-platinum and heavy metals, and in patients with reflux nephropathy. Although cis-platinum causes renal tubular injury, multi-drug chemotherapy with cis-platinum is a very popular treatment in several kinds of malignancies [10-12], including testicular cancer [13]. In the treatment of testicular cancer, higher doses of cis-platinum are used compared with other types of cancers; renal toxicity is therefore one of the important rate-limiting factors [14]. Both NAG and β2-MG [15] are urinary markers for proximal renal tubular injury, and are in widespread use for diagnosis of cis-platinum-induced renal tubular injury; however, no urinary markers for diagnosing distal renal tubular or collecting ductal injury have been available.

Thus, the aims of this study were to measure the changes in U-ET-1 after the administration of cis-platinum, to compare these findings with the changes in U-NAG and U-β2-MG, and to determine whether distal renal tubular or collecting duct injury also occurs after cis-platinum treatment.

Key words: cis-platinum, renal tubular injury, urinary endothelin-1, urinary parameters.

Abbreviations: Alb, microalbumin; Cr, creatinine; ET-1, endothelin-1; β2-MG, β2-microglobulin; NAG, N-acetyl-β-D-glucosaminidase; PE, cis-platinum and etoposide; PEVB, cis-platinum, vinblastine, etoposide and bleomycin; U-Alb, urinary albumin; U-ET-1, urinary endothelin-1-like immunoreactivity; U-β2-MG, urinary β2-microglobulin; U-NAG, urinary N-acetyl-β-D-glucosaminidase.

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Fig. 1. Patients and regimens of multi-drug chemotherapy for testicular cancer. Abbreviations: P, cis-platinum; V, vinblastine; E, etoposide; B, bleomycin. Each patient was numbered consecutively. For 1 day before and 1 day after cis-platinum treatment, the daily volume of urine was maintained at not less than 3000 ml by diuretics and intravenous infusion.

<table>
<thead>
<tr>
<th>PVEB regimen</th>
<th>Days</th>
<th>Patient no. 1, 31 years old</th>
<th>Patient no. 4, 32 years old</th>
<th>Patient no. 5, 30 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>75 mg/day⁻¹ m⁻²</td>
<td>1 2 3 4 5 8 15</td>
<td>P 75 mg/day⁻¹ m⁻²</td>
<td>E 100 mg/day⁻¹ m⁻²</td>
</tr>
<tr>
<td>E</td>
<td>100 mg/day⁻¹ m⁻²</td>
<td>1 2 3 4 5 8 15</td>
<td>1 2 3 4 5 8 15</td>
<td>1 2 3 4 5 8 15</td>
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</table>

<table>
<thead>
<tr>
<th>PE regimen</th>
<th>Days</th>
<th>Patient no. 2, 33 years old</th>
<th>Patient no. 3, 39 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>75 mg/day⁻¹ m⁻²</td>
<td>1 2 3 4 5</td>
<td>P 75 mg/day⁻¹ m⁻²</td>
</tr>
<tr>
<td>E</td>
<td>100 mg/day⁻¹ m⁻²</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
</tbody>
</table>

EXPERIMENTAL

Patients

Five male patients with testicular cancer who were treated at Niigata University Hospital from 1 August 1992 to 30 October 1992, ranging in age from 30 to 39 years (mean 33.0 years), were examined (Fig. 1). Each patient was numbered consecutively. All patients were treated with a cis-platinum, vinblastine, etoposide, and bleomycin (PVEB) regimen or a cis-platinum and etoposide (PE) regimen because of the presence of metastatic lesions (Fig. 1). No patients had urinary tract obstruction due to metastatic lesions, and none had been treated with nephrotoxic drugs, including cis-platinum, previously. Both regimens included 5 days consecutive administration of 75 mg of cis-platinum/m² body surface area. In the PVEB regimen, vinblastine was administered on day 1, and bleomycin was administered on days 1, 8, and 15 (Fig. 1). For 1 day before and 1 day after cis-platinum treatment, daily volumes of urine were maintained at not less than 3000 ml by diuretics and intravenous infusions. No patients showed impairment of renal function, as measured by serum creatinine (Cr) concentration and 24 h Cr clearance.

In addition to these five patients, 10 age-matched male control subjects were also examined (Table 1). The nephrotoxicities of the other drugs (etoposide, vinblastine and bleomycin) were usually so low that they could be neglected.

Methods

Twenty-four hour urine samples were collected without infection, 1 week before, 1 week after, 2 weeks after and during cis-platinum treatment. To examine the effect of diuresis, urine was also collected during the deliberate diuresis before cis-platinum. In some patients, urine was collected for 2 consecutive days. Consequently, the number of samples at each time was seven. In addition, 11 samples were taken during this period in patient no. 4, to examine the details of change. Aliquots of urine were frozen at -80°C until assessment, and were used for measurement of urinary parameters within 1 month after collection.

Urinary ET-1 was measured by r.i.a. using a rabbit polyclonal anti-ET-1 antibody [5]. Urine samples were serially diluted and assayed. Urine samples with Spec-8 cartridges (J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.) were subjected to r.i.a. Separation of the bound from free ligand was accomplished by the double-antibody method. The minimal detectable quantity in the present ET-1 r.i.a. was 0.4 pg/cartridge. β₂-MG was measured by an enzyme immunoassay using a commercial kit. NAG was measured by the NAG-cresolsulphonphthaleinyl method [16] using a commercial kit, and microalbumin (Alb) was measured by an enzyme immunoassay using a commercial kit (Kitsato Bristol Laboratories, Sagamihara, Japan). Urinary and serum Cr was measured by Jaffé's method. To exclude the effect of the change in daily urine volume, all parameters were divided by the urinary Cr value. Normal values of U-ET-1/Cr, U-β₂-MG/Cr, U-NAG/Cr and U-Alb/Cr are <120 pg/mg of Cr, <0.33 mg/g of Cr, <3.12 units/g of Cr and <21 mg/g of Cr in 10 age-matched normal control subjects. Mean±2SD was considered as an upper limit (Table 1).

Statistical analysis

All data were expressed as means±SD. Statistical analysis was performed by one-way analysis of variance [17], and a P value of less than 0.05 was considered to be significant.
Table 2. Changes in urinary parameters before and after administration of cis-platinum. Values are means ± SD (n = 7). Statistical significance (one-way analysis of variance): *P < 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>With diuresis</th>
<th>During</th>
<th>1 week after</th>
<th>2 weeks after</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-ET-1/Cr</td>
<td>61.3 ± 29.8</td>
<td>0.51 ± 0.21</td>
<td>62.1 ± 32.8</td>
<td>0.81 ± 0.33</td>
<td>0.50 ± 0.19</td>
</tr>
<tr>
<td>U-β2-MG/Cr</td>
<td>51.5 ± 33.2</td>
<td>0.4 ± 0.14</td>
<td>39.28 ± 11.56</td>
<td>21.54 ± 6.71</td>
<td>5.41 ± 3.34</td>
</tr>
<tr>
<td>U-NAG/Cr</td>
<td>3.12 ± 1.53</td>
<td>2.90 ± 1.28</td>
<td>90.5 ± 34.12</td>
<td>172.46 ± 48.52</td>
<td>9.81 ± 3.26</td>
</tr>
<tr>
<td>U-Alb/Cr</td>
<td>4.65 ± 2.54</td>
<td>5.21 ± 2.18</td>
<td>6.21 ± 3.28</td>
<td>21.54 ± 6.71</td>
<td>9.45 ± 3.26</td>
</tr>
</tbody>
</table>

RESULTS

Change in urinary parameters

Table 2 summarizes the results. U-ET-1/Cr during, 1 week after and 2 weeks after cis-platinum treatment were significantly higher than values before cis-platinum therapy without diuresis. There was no difference in U-ET-1 during, 1 week after and 2 weeks after cis-platinum (Table 2).

U-β2-MG/Cr during cis-platinum therapy was significantly higher than that before (without diuresis) and after cis-platinum. There was no difference, however, between that before without diuresis, 1 week after and 2 weeks after cis-platinum (Table 2).

U-NAG/Cr during cis-platinum therapy was higher than both before (without diuresis) and after cis-platinum, and gradually decreased after cis-platinum. However, U-NAG/Cr 2 weeks after cis-platinum was not different from that before cis-platinum (without diuresis) (Table 2). Of the three parameters measured, U-ET-1 did not decrease after cis-platinum therapy (Table 2).

U-Alb/Cr during cis-platinum therapy was higher than before (without diuresis) and 2 weeks after cis-platinum, and that 1 week after cis-platinum was higher than before treatment (without diuresis) (Table 2).

Before cis-platinum, each parameter showed normal levels. Comparing urinary parameters when urine volume was normal with those under the deliberate diuresis before cis-platinum showed no significant difference (Table 2).

Correlation between ET-1 and other parameters

Figs. 2, 3 and 4 summarize the results. There were values: before (n = 7), during (n = 7), 1 week after (n = 7) and 2 weeks after (n = 7) cis-platinum therapy, as previously described in the Experimental section. Although U-ET-1/Cr did not show any significant correlation with U-NAG/Cr (n = 28, r = 0.291, not significant, Fig. 2) or U-Alb/Cr (n = 28, r = 0.076, not significant, Fig. 3), it did show signifi-
U-ET-1 showed a rapid increase during *cis*-platinum therapy, and decreased after *cis*-platinum, but increased again 8 days after the beginning of *cis*-platinum therapy. The peak in U-ET-1 was 2 days after the beginning of *cis*-platinum therapy.

U-β₂-MG/Cr showed a rapid increase during *cis*-platinum therapy, and a similar pattern to U-ET-1/Cr. However, U-β₂-MG/Cr decreased more rapidly than U-ET-1/Cr. After day 8, U-β₂-MG/Cr was stable and within normal limits.

U-NAG/Cr showed a somewhat slower increase during *cis*-platinum therapy, and slower decrease after *cis*-platinum.

U-Alb/Cr gradually increased during *cis*-platinum therapy, and showed its highest level at day 8 (3 days after *cis*-platinum). After day 9, levels returned almost to normal (Fig. 5).

Serum Cr concentration and 24h Cr clearance were measured simultaneously with urinary parameters, and no significantly changes were found during this study.

**DISCUSSION**

Our study clearly demonstrates the changes in U-ET-1 in young male patients with testicular cancer, who were treated with combination chemotherapy including a high dose of *cis*-platinum. Ohta et al. [18] first reported that the U-ET-1 level increased after administration of 70–160mg of *cis*-platinum and changed in parallel with U-β₂-MG and U-NAG in six patients with various cancers (mean age 50.0 years). They indicated that U-ET-1 levels showed significant correlations with U-NAG and U-β₂-MG. Our results, however, showed that the U-β₂-MG/Cr levels more rapidly returned to pretreatment levels than U-ET-1/Cr; U-ET-1/Cr levels after *cis*-platinum therapy were higher than the other two parameters. Although the results of Ohta et al. [18] are different from our data, the correlation coefficients were similar. According to Ohta et al. [18], both U-ET-1 and U-β₂-MG were higher after *cis*-platinum than before, but U-NAG returned to pretreatment levels 1 week after *cis*-platinum. These kinetics of urinary parameters were different from our data. The possible causes of these difference are: (1) differences in age and sex, and (2) differences in treatment protocol (amount and duration of *cis*-platinum treatment and combination of other drugs). In both studies, however, U-ET-1 showed a rapid increase and a prolonged higher level after *cis*-platinum treatment. According to these data, and the localization of ET-1 in the kidney, U-ET-1 may be an indicator of both distal and proximal renal tubular injury, and *cis*-platinum may cause not only proximal renal tubular injury, but also distal renal tubular or collecting duct injury. In patient no. 4, U-ET-1/Cr showed a rapid increase and prolonged higher value with a gradual decrease, whereas it increased again after day 8 (3
days after cis-platinum). The possible cause of this late increase may be that from day −1 (1 day before cis-platinum) to day 6 (1 day after cis-platinum) deliberate diuresis was induced to avoid renal injury, and U-ET-1/Cr decreased during this period of intended diuresis; however, it increased after day 8. Thus, renal injury which was prevented by the deliberate diuresis occurred after day 8. Actually, the true cause of this late increase is unknown, but this may mean that U-ET-1 is a more sensitive marker than the others for the detection of renal tubular injury, since the other parameters did not change at this time.

In our results, Alb showed different changes to the other three parameters. Because Alb is considered to indicate glomerular injury, a gradual increase during cis-platinum therapy could have been expected if the main nephrotoxic action of cis-platinum is tubular injury, and it is still possible that glomerular injury occurs after 5 days of consecutive high-dose cis-platinum therapy even in young male patients.

It is tempting to speculate that ET-1 of renal origin may function as a local modulator of ion transport/water permeability in renal tubules in an autocrine and/or paracrine fashion [4–20].

The mechanism of action of U-ET-1 is unclear, and the exact role of U-ET-1 in patients receiving cis-platinum remains to be determined.

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REFERENCES