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Online Publication Date: 16 February 2001

To cite this Article Thanh, Nguyen Thi Kim, Obatomi, David K. and Bach, Peter H. (2001)'INCREASED URINARY URONIC ACID EXCRETION IN EXPERIMENTALLY-INDUCED RENAL PAPILLARY NECROSIS IN RATS', Renal Failure, 23:1, 31 — 42

To link to this Article DOI: 10.1081/JDI-100001281

URL: http://dx.doi.org/10.1081/JDI-100001281
LABORATORY STUDY

INCREASED URINARY URONIC ACID EXCRETION IN EXPERIMENTALLY-INDUCED RENAL PAPILLARY NECROSIS IN RATS

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ABSTRACT

We have evaluated the potential of urinary uronic acid measurement as an early indicator in the development of renal papillary necrosis (RPN). Urinary uronic acid was quantified with a range of other urinary biochemical parameters in rats given multiple doses of N-phenylanthranilic acid (NPAA) or mefenamic acid (MFA), each of which induces a dose-related papillary necrosis. In addition, histological examination was also carried out to confirm the development and presence of RPN. NPAA was administered to male wistar rats at p.o. doses of 100, 250, and 500 mg/kg and MFA at p.o. doses of 75, 150, and 300 mg/kg on days 1–4 and 8–11, and urine samples were collected for 16 hours each day. NPAA increased uronic acid excretion two-fold for both medium and high doses from day four. MFA increased uronic acid excretion to two and a half-fold by day 10 in the highest dose administered. Urinary creatinine was equally elevated in a dose-related manner following treatment with either NPAA or MFA. None of the other routine markers (urinary or serum) of nephrotoxicity showed...
any statistical changes. NPAA produced a dose- and time-related increase in excretion of uronic acid. Evidence of widespread papillary necrosis was seen histologically at the high doses of NPAA or MFA. The significant elevation of uronic acid in urine following treatment with either NPAA or MFA was well ahead of the development of RPN detectable by routine histology, suggesting that uronic acid measurement could serve as an early indicator of RPN. The assessment of urinary uronic acid may therefore provide a novel sensitive and selective marker of identifying the lesion earlier than is currently possible. An increase in urinary uronic acid following NPAA and MFA treatment supports the biochemical basis of these changes as a representative of acid mucopolysaccharides accumulation.

Key Words: Renal papillary necrosis; N-phenylanthranilic acid; Mefenamic acid; Urinary uronic acid; Biomarkers; Rat kidney; Nephropathy; Excretion; Serum; Urinary electrolytes; Histopathology.

INTRODUCTION

The incidence of RPN has been widespread and is one of the causes of end-stage renal failure in developing countries (1,2). At the present, the main diagnosis of RPN is by histological examination. Several urinary parameters including urinary enzymes (3) and urinary lipids (4) have been measured previously to determine whether these parameters could provide an early diagnosis of RPN. While the results with urinary lipids have been encouraging, urinary enzymes have proved not to be useful.

Studies conducted in our laboratory have demonstrated the usefulness of urinary lipid measurement as a sensitive, noninvasive, early marker in the detection and monitoring of the development of RPN due to papillotoxins (4,5). In a recent study (4,5) of rats treated with MFA and NPAA, RPN was clearly demonstrated by early elevation of specific lipids, especially the phospholipids (PLs) into urine, which preceded changes in other renal function parameters.

The high concentration of proteoglycans (PoG) and glycosaminoglycans (GAG) in the medulla, especially the papilla, and the loss of histochemical staining of mucopolysaccharides (6) from those areas of chemically-induced necrosis, suggested that the monitoring of urinary PoG-GAG could also provide a rational and selective approach to non-invasive assessment of RPN. As part of our long-term interest in finding an early marker for this lesion, we have measured the excretion of uronic acid (a representative of GAG) into urine from rats given NPAA and MFA.

NPAA is a chemical agent that has a close structural analogy to MFA (the most commonly used analgesic drug) and are model nephrotoxins for inducing RPN (2). MFA has been found to be the leading single substance (38%) to cause acute renal failure (7). Therefore, they have been selected in the present study to induce mild (NPAA) to widespread (MFA) papillary necrosis.
Histopathology of the kidney was also performed to confirm the development of RPN, while other conventional clinical parameters for assessing renal functions were also determined for comparative sensitivity.

**METHODS**

**Reagents**

All reagents were purchased from Sigma Ltd., Poole, UK except otherwise stated. All solutions were prepared in distilled water redistilled from an all glass still.

**Animal and Treatments**

Male Wistar rats weighing 200 ± 10 g, were obtained from Dr. Karl Thomae GmbH (Biberach/Riss, Germany). The animals housing was as follows: temperature (20–25°C), humidity (45–80%), and light (light/dark cycle 8/16 h, light period 0800 h–1600 h). They were maintained on a standard fortified rodent diet (Altromin®; Altrogge, Lage/Lippe, Germany) and municipal tap water was offered ad lib. All studies were conducted in accordance with German and U.S. National Institutes of Health guidelines for animal welfare, and all experiments were performed according to Good Laboratory Practice (GLP) regulations. At least three days were allowed for the animals to acclimate to the housing conditions in a metabolic cage that separated urine and feces prior to the experiment. They were allowed access to food for only 8 hours per day, and were without food during the period of urine collection.

Following the acclimatization period, control day (day 0) values were obtained. On the following day, rats were divided into six groups consisting of six rats in each group, and were administered with either NPAA or MFA, which was prepared in carboxy-methylcellulose as an aqueous suspension.

NPAA was given repeatedly p.o. at 100, 250, and 500 mg/kg daily for five days, followed by a two-day respite over the weekend, and then four further daily doses. The same dosing procedure was applied with MFA and given repeatedly p.o. but at doses of 75, 150, and 300 mg/kg. For each experimental group, appropriate control groups were given vehicle p.o. using the same volume as was given to the test groups. Animal weights were determined weekly while food and water intake was measured daily.

**Collection of Urine**

Urine samples were collected daily and over a period of 16 h. Samples were collected in plastic universal containers kept at 0–4°C on ice, and total urine volumes were measured and recorded. Urine was centrifuged at 1500 × g for 3 minutes on a bench top centrifuge to sediment the debris and particles. The supernatants
collected were usually stored at $-20^\circ$C and analyzed within one week. All samples were thawed and centrifuged before analysis.

Collection of Blood Samples

A pre- and post-treatment blood sample was obtained from each animal from the retrobulbar venous plexus. The blood was collected into tubes and allowed to clot at $37^\circ$C. Serum was obtained following centrifugation ($300 \times g$, 5 min) and stored at $-20^\circ$C until required.

Uronic Acid Assay

Uronic acids were determined quantitatively as previously described (8). Briefly, a 0.0125 M tetraborate solution (1.2 mL) in concentrated sulphuric acid was added to 0.2 mL of the urine sample. The tubes were refrigerated in crushed ice. The solutions were mixed and the tubes were heated in a water bath at 100$^\circ$C for 5 min. After cooling in a water-ice bath, 20 $\mu$L of 0.15% $m$-hydroxybiphenyl in 0.125 M NaOH was added. The tubes were shaken and within 5 minutes the absorbance was read at 520 nm in a Perkin-Elmer Lambda 5 UV/Vis Spectrophotometer. As carbohydrates produced a pinkish chromogen with sulphuric acid/tetraborate at 100$^\circ$C, absorbance from a blank sample was measured with 20 $\mu$L of 0.125 M NaOH in place of reagent, and subtracted from the total absorbance. The interference from nonuronic acid material has been reported to be less than 4% (9).

Urinary Electrolyte Measurements

The concentration of Na$^+$ and K$^+$ in the urine was measured as previously described (10) using a flame photometer with lithium (Li) used as internal standard. Magnesium concentration in urine was measured by the xylidyl blue reaction (11). The magnesium concentration was measured bichromatically in terms of the decrease in absorbance of xylidyl blue at 505/600 nm. Potential interference by calcium was prevented by 45 $\mu$M EGTA contained in the buffer provided in the kit. Urinary calcium was determined by the $o$-cresolphthalein complexone method (12) using BM/Hitachi$^\text{TM}$ 917 System Pack 1 551 272 obtained also from Boehringer Mannheim (Germany). Ca$^{2+}$ formed a violet complex with $o$-cresolphthalein complexone in alkaline medium, which absorbs at 612 nm.

The concentration of Cl$^-$ was determined using a chloride meter (Corning-EEL 920 Chloride Meter). Urinary phosphate was quantified using the kit purchased from Boehringer, Mannheim.

The excretions of these electrolytes were then calculated from the volume of urine passed and the weight of the rats, and expressed as $\mu$mole excreted per h, per 100 g rat weight (10).
Urinary and Serum Creatinine

Urinary and serum creatinine were determined using the appropriate kit. The method was essentially a modification of the Jaffé method (13). A yellow or orange color forms when creatinine is treated with alkaline picrate. Under acid conditions, the creatinine-picrate color fades faster than interfering chromogens. Thus, kinetic applications were devised in which timed readings were taken during the development of the color.

Measurements of Blood Urea Nitrogen (BUN)

BUN was determined enzymatically as previously described (14) using a kit (Ecoline 25/14855) obtained from Merck (Darmstadt, Germany).

Histological Examination

At the end of the final urine collection, animals were killed by cervical dislocation. Both kidneys were rapidly removed, weighed, and placed in ice-cold formal calcium fixative (4% v/v formaldehyde) for 24 hours. The organ was then washed, paraffined, embedded, sectioned, stained with Haematoxylin and Eosin, and examined by light microscopy.

Statistical Analysis and Upper Limit of Normal

All data are expressed as means ± S.E.M. for at least four separate experiments. Normal ranges were established for all measurements, and the upper limit of normal (ULN) obtained and defined as the mean of the control values for all days plus twice the standard deviation. Differences between treated and control groups were analyzed using one tailed and unpaired Student t-test with two samples having equal variances ($F \leq 2$). Values were taken to be significantly different at $p < 0.05$ and above the ULN.

RESULTS

Urinary Volume

The daily urine volume of rats treated with either NPAA or MFA varied considerably but was not significantly different from the control throughout the collection period (Tabs. 1 and 2).
Table 1. Effect of Repeated Doses of NPAA on the Urinary Volume (ml) of Rats Collected over 16 Hours

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.9 ± 2.7</td>
<td>12.6 ± 1.6</td>
<td>10.9 ± 2.2</td>
<td>12.1 ± 1.8</td>
<td>10.0 ± 2.2</td>
<td>9.9 ± 1.1</td>
<td>8.5 ± 2.1</td>
<td>10.0 ± 1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>16.4 ± 2.2</td>
<td>15.9 ± 1.6</td>
<td>14.5 ± 0.8</td>
<td>13.3 ± 1.4</td>
<td>22.8 ± 1.8**</td>
<td>13.7 ± 3.4</td>
<td>14.7 ± 2.7</td>
<td>13.0 ± 1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>11.8 ± 0.7</td>
<td>10.7 ± 0.5</td>
<td>12.0 ± 0.7</td>
<td>10.9 ± 1.1</td>
<td>14.5 ± 0.8</td>
<td>10.8 ± 0.4</td>
<td>12.0 ± 0.9</td>
<td>11.1 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>11.8 ± 2.2</td>
<td>11.7 ± 1.3</td>
<td>13.6 ± 0.6</td>
<td>11.9 ± 1.0</td>
<td>13.9 ± 0.9</td>
<td>14.4 ± 1.5*</td>
<td>15.1 ± 1.9*</td>
<td>15.0 ± 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5. *p < 0.05 compared to the respective control.

Urinary Uronic Acid

Figure 1 shows the effect of NPAA on excretion of uronic acid during the 11-day treatment period. The low and medium doses produced similar effects, with increased excretion (generally p < 0.05) throughout the treatment period and a notable peak on day 8 (p < 0.01). High dose of NPAA produced a significant linear pattern of uronic acid excretion (two-fold increase from day 3, up to three-fold on day 11, generally p < 0.001). All values of urinary uronic acid following treatment with NPAA were above the ULN.

A somewhat different pattern of uronic acid excretion was observed when animals were treated with MFA (Fig. 2). The low dose of MFA initially reduced uronic acid excretion (p < 0.01), but the medium and high doses caused a progressive increase in excretion up to day 10 (medium dose, p < 0.05, high dose, p < 0.001). The urinary excretion of uronic acid at the medium (after day 3) and high doses (all through the experiment) of MFA were highly significant and were above the ULN.

Urinary Creatinine

There was a significant increase of urinary creatinine excretion between days 2–8 during treatment with NPAA (Tab. 3).

MFA at the low or medium dose increased creatinine excretion up to day 4 (p < 0.01). On days 10–11, the enhanced excretion was less, but still significantly higher (p < 0.05, p < 0.01, respectively) than in the control group. At the high dose, the increase was more variable (Tab. 4).

Table 2. Effect of MFA on the Urinary Volume (mL) of Rats Collected over 16 Hours After 11-Day Treatment

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.9 ± 2.7</td>
<td>12.6 ± 1.6</td>
<td>10.9 ± 2.2</td>
<td>12.1 ± 1.8</td>
<td>10.0 ± 2.2</td>
<td>9.9 ± 1.1</td>
<td>8.5 ± 2.1</td>
<td>10.0 ± 1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>8.7 ± 1.3*</td>
<td>8.5 ± 1.2</td>
<td>8.5 ± 1.2</td>
<td>11.1 ± 1.2</td>
<td>14.2 ± 1.5</td>
<td>11.1 ± 1.4</td>
<td>13.2 ± 1.6</td>
<td>13.2 ± 1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>10.7 ± 2.0</td>
<td>11.8 ± 3.0</td>
<td>11.5 ± 1.4</td>
<td>12.5 ± 0.8</td>
<td>11.9 ± 1.2</td>
<td>10.5 ± 1.9</td>
<td>13.4 ± 0.7</td>
<td>13.6 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>15.8 ± 6.6</td>
<td>15.0 ± 2.6</td>
<td>12.0 ± 2.1</td>
<td>9.4 ± 1.8</td>
<td>8.9 ± 2.1</td>
<td>8.5 ± 0.9</td>
<td>8.5 ± 1.3</td>
<td>9.6 ± 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5. *p < 0.05 compared to the respective control.
Table 3. Effect of Repeated Doses of NPAA on the Total Mass (mg) of Urinary Creatinine Excretion per 16-Hour Collection After 11-Day Treatment

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9 ± 0.3</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.5</td>
<td>5.1 ± 0.7</td>
<td>6.2 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>Low</td>
<td>4.7 ± 0.2</td>
<td>4.2 ± 0.4</td>
<td>6.6 ± 0.4*</td>
<td>7.3 ± 1.5</td>
<td>10.8 ± 0.5*</td>
<td>4.4 ± 0.5</td>
<td>5.4 ± 0.7*</td>
<td>6.0 ± 1.1</td>
</tr>
<tr>
<td>Medium</td>
<td>4.0 ± 0.4</td>
<td>4.0 ± 0.4</td>
<td>5.9 ± 0.2*</td>
<td>6.1 ± 0.7</td>
<td>3.4 ± 0.4*</td>
<td>3.0 ± 0.4*</td>
<td>5.0 ± 0.7</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>High</td>
<td>4.5 ± 0.7</td>
<td>5.9 ± 0.2***</td>
<td>5.6 ± 0.6</td>
<td>7.7 ± 0.7*</td>
<td>3.7 ± 0.5*</td>
<td>4.0 ± 0.5</td>
<td>3.7 ± 0.3</td>
<td>4.4 ± 0.4</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5.

*p < 0.05,  **p < 0.01,  ***p < 0.001 compared to the respective control.

Figure 1. Effect of a repeated dose of NPAA on the excretion of uronic acid in the urine. The results are expressed in mg/16 hour collection. (— indicates the ULN). Error bar (S.E.M.) shown for the high dose group. *p < 0.05,  **p < 0.01,  ***p < 0.001 compared to the respective control (6 rats were used for each experiment repeated 5 times).

Figure 2. The excretion of uronic acid in the urine of rats following repeated doses of MFA. The results are expressed as mg/16 h collection (— indicates the ULN). Error bar (S.E.M.) shown for the high dose group. *p < 0.05,  **p < 0.01,  ***p < 0.001 compared to the respective control (6 rats were used for each experiment repeated 5 times).
Table 4. Effect of Repeated Doses of MFA on the Total Mass (mg) of Urinary Creatinine Excretion per 16-Hour Collection After 11-Day Treatment

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9 ± 0.3</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.5</td>
<td>5.1 ± 0.5</td>
<td>6.2 ± 0.7</td>
<td>4.5 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>3.9 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4.7 ± 0.3</td>
<td>5.6 ± 0.5</td>
<td>6.6 ± 0.4*</td>
<td>9.0 ± 1.0**</td>
<td>9.1 ± 0.9*</td>
<td>4.9 ± 0.7</td>
<td>7.0 ± 0.7**</td>
<td>6.0 ± 0.5*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>5.2 ± 0.2**</td>
<td>6.7 ± 0.7*</td>
<td>8.7 ± 1.1**</td>
<td>9.0 ± 0.8**</td>
<td>8.0 ± 0.5</td>
<td>5.0 ± 0.9</td>
<td>6.4 ± 0.4***</td>
<td>7.0 ± 0.4**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>5.6 ± 1.1</td>
<td>5.7 ± 0.4*</td>
<td>8.2 ± 1.5*</td>
<td>6.3 ± 1.0</td>
<td>5.6 ± 0.4</td>
<td>5.5 ± 0.6</td>
<td>4.7 ± 0.5</td>
<td>5.4 ± 1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5.
*p < 0.05, **p < 0.01 compared to the respective control.

**Urinary Electrolytes**

There was no abnormal change in the excretion rate of electrolytes at the low and medium doses NPAA. At the high dose, however, a significant increase in the excretion of K⁺, Cl⁻ (p < 0.05) and Mg²⁺ was observed (Tab. 5).

There was a significant (p < 0.05) increase in Na⁺, K⁺, and Cl⁻ excretion rate at low dose of MFA, and a significant decrease in Na⁺ and Cl⁻ (p < 0.05) at the high dose. No changes were observed at the medium dose (Tab. 6).

**Serum Creatinine and Urea**

No changes were observed in the creatinine concentration in serum following treatment with either NPAA or MFA (Tab. 7).

The concentration of urea in serum increased following treatment with NPAA, but this occurs only at high doses (p < 0.01), whereas the urea concentration in blood following treatment with MFA significantly increased (p < 0.05) at the lower dose (Tab. 7).

**Histopathological Examination**

There appeared to be no abnormal pathology in animals after taking repeated low (100 mg/kg) or medium (250 mg/kg) doses for 11 days. In the high dose...

Table 5. Excretion Rate of Electrolytes (µmole/h/100g Body Weight) in the Urine of Rats After 11-Day Treatment with NPAA

<table>
<thead>
<tr>
<th>Dose</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Cl⁻</th>
<th>PO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.17 ± 3.07</td>
<td>31.82 ± 2.85</td>
<td>0.51 ± 0.16</td>
<td>1.89 ± 0.38</td>
<td>29.60 ± 2.53</td>
<td>2.77 ± 0.62</td>
</tr>
<tr>
<td>Low</td>
<td>29.92 ± 3.06</td>
<td>37.65 ± 4.82</td>
<td>0.56 ± 0.15</td>
<td>2.14 ± 0.36</td>
<td>36.74 ± 3.84</td>
<td>3.32 ± 0.76</td>
</tr>
<tr>
<td>Medium</td>
<td>30.39 ± 1.17</td>
<td>35.09 ± 0.63</td>
<td>0.73 ± 0.11</td>
<td>2.66 ± 0.24</td>
<td>36.02 ± 0.73*</td>
<td>3.46 ± 0.27</td>
</tr>
<tr>
<td>High</td>
<td>31.31 ± 1.71</td>
<td>40.71 ± 2.33*</td>
<td>0.97 ± 0.14</td>
<td>3.82 ± 0.40**</td>
<td>41.68 ± 2.65*</td>
<td>5.05 ± 0.40*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5.
*p < 0.05, **p < 0.01 compared to the respective control.
Table 6. Excretion Rate of Electrolytes (µmole/h/100g Body Weight) in the Urine of Rats After 11-Day Treatment with MFA

<table>
<thead>
<tr>
<th>Dose</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Cl⁻</th>
<th>PO₄³⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.17 ± 3.07</td>
<td>31.82 ± 2.85</td>
<td>0.51 ± 0.16</td>
<td>1.89 ± 0.38</td>
<td>29.60 ± 2.53</td>
<td>2.77 ± 0.62</td>
</tr>
<tr>
<td>Low</td>
<td>35.71 ± 2.05*</td>
<td>41.40 ± 2.58*</td>
<td>0.56 ± 0.17</td>
<td>1.86 ± 0.41</td>
<td>41.65 ± 3.07*</td>
<td>3.77 ± 0.72</td>
</tr>
<tr>
<td>Medium</td>
<td>30.57 ± 2.16</td>
<td>32.89 ± 2.74</td>
<td>1.09 ± 0.21</td>
<td>3.22 ± 0.56</td>
<td>37.19 ± 2.37</td>
<td>4.58 ± 0.98</td>
</tr>
<tr>
<td>High</td>
<td>11.62 ± 4.31*</td>
<td>21.66 ± 5.33</td>
<td>1.11 ± 0.28</td>
<td>3.28 ± 0.83</td>
<td>15.20 ± 5.16*</td>
<td>8.34 ± 1.71</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5. *p < 0.05 compared to the respective control.

(500 mg/kg) group, only a single animal showed interstitial edema (+++) in the papilla while others appeared normal. In a separate study, 500 mg/kg NPAA caused mild and severe papillary necrosis respectively after 11 days of treatment, and fatalities at higher doses (Tab. 8).

From the histopathology data, each dose of MFA (75, 150 or 300 mg/kg) caused some renal lesions in the papilla and the medulla, with no cortical involvement, apart from one animal in the high dose. Animals had more frequent and severe lesions at high dose (Tab. 8). In a separate study, when a higher dose of MFA (800 mg/kg) was given, the rats had more severe renal lesions and eventually died.

DISCUSSION

The main objective of this study was to determine whether the excretion of uronic acid in urine following the development of RPN by well known papillogens could provide a selective and noninvasive means of identifying the lesion at an early and reversible stage. The two papillogens used were NPAA and MFA, which have been widely considered to be target selective nephrotoxins that cause necrosis of the medulla in animals within 24–48 hours of administration (2).

The histopathology findings in this study have shown that repeated doses of NPAA at 100 mg/kg or 250 mg/kg p.o. for 11 days did not produce any renal lesion, and at 500 mg/kg, there was modest edema in the papilla. This is in marked contrast to previously published studies (15) where NPAA was shown to cause necrosis of

Table 7. Effect of NPAA or MFA on Serum Creatinine and Urea of Rats After 11-Day Treatment

<table>
<thead>
<tr>
<th>Dose</th>
<th>NPAA Creatinine (µmol/L)</th>
<th>UREA (mmol/L)</th>
<th>MFA Creatinine (µmol/L)</th>
<th>UREA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.30 ± 1.42</td>
<td>8.40 ± 0.24</td>
<td>46.30 ± 1.42</td>
<td>8.40 ± 0.24</td>
</tr>
<tr>
<td>Low</td>
<td>46.70 ± 0.83</td>
<td>8.50 ± 0.38</td>
<td>45.80 ± 1.33</td>
<td>9.41 ± 0.31*</td>
</tr>
<tr>
<td>Medium</td>
<td>47.00 ± 1.47</td>
<td>8.78 ± 0.34</td>
<td>47.10 ± 1.95</td>
<td>9.00 ± 0.34</td>
</tr>
<tr>
<td>High</td>
<td>46.80 ± 1.12</td>
<td>9.88 ± 0.19**</td>
<td>39.90 ± 3.92</td>
<td>8.73 ± 0.45</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., n = 5. *p < 0.05, **p < 0.01 compared to the respective control.
Table 8. Lesions in the Kidney of Rats After 11-Day Treatment with High Doses of Different Chemicals

<table>
<thead>
<tr>
<th>Compound</th>
<th>Papilla</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPAA</td>
<td>++ necrosis</td>
<td>++ focal necrosis</td>
</tr>
<tr>
<td>(medium dose)</td>
<td>++++ total necrosis</td>
<td>++++ cellular debris</td>
</tr>
<tr>
<td>NPAA</td>
<td>++++ dilation of distal tubuli and collecting ducts</td>
<td>(partially calcified) in the lumina of cortical tubuli</td>
</tr>
<tr>
<td>(high dose)</td>
<td>mf tubular casts (protein)</td>
<td>++ calcification of tubular epithelial cells</td>
</tr>
<tr>
<td>MFA</td>
<td>+++ necrosis</td>
<td></td>
</tr>
<tr>
<td>(high dose)</td>
<td>+++ dilation of distal tubuli and collecting ducts</td>
<td></td>
</tr>
</tbody>
</table>

++: mild; +++: moderate; ++++: severe; mf: multi-focal.

The renal papillary tip at a very low dose. The discrepancy might conceivably be due to the use of routine histopathology, which often fails to section the papilla in such a way that focal lesions are identified. The papilla is a very small fraction of the kidney, and the sectioning procedure can easily miss the whole papilla. In contrast to this is the effect of MFA administration (75, 150, or 300 mg/kg), which caused some renal lesions in the papilla and the medulla, with no cortical involvement, apart from one animal in the high dose group where the lesion extended to the cortex. Thus, the characteristic of abnormalities induced by NPAA and MFA as defined histologically showed that the latter caused a more widespread papillary necrosis at all dosages while the former caused only subtle papillary lesions.

Based on the fact that routine histological examination, apart from being very cumbersome and time consuming, could on various occasions appear not to be reliable, so that an alternative noninvasive means of diagnosing RPN at an early stage was highly desirable. The changes observed in the excretion of urinary uronic acid as obtained in the present study suggest that it could be useful as markers for the early diagnosis of RPN. Both NPAA and its analogue MFA caused similar changes in urinary uronic acid excretion. Both papillotoxin-induced increases in urinary excretion were evident within 24 hours (day 1) and increased in a time-dependent manner during 11 days of administration. The enhanced excretion of uronic acid after a single dose of NPAA supports the concept of early renal toxicity involving breakdown of interstitial ground substance, leading to rapid loss of cellular phospholipids. It has been shown previously that the loss of histochemically stainable renal mucopolysaccharide was reflected in increased uronic acid excretion. This tissue degradation may well occur before any gross pathology is detectable. In addition, the increased urinary uronic acid suggests a loss of renal acid mucopolysaccharides following NPAA and MFA treatment. Thus, these observations support the concept that urinary uronic acid may be markers of renal damage even earlier than can be identified by histological evaluation, and may also have clinical implications if biochemical assays for early renal damage become.
available. However, comparison of the pathological data with urinary uronic acid changes in individual animals did not show any clear relationship.

There were no changes in urinary volume at any dose of NPAA and MFA. Both compounds did not seem to cause diuresis as observed in the use of another classical RPN-induced compound, BEA (4). A significant increase in urinary volume and electrolyte excretion has been reported at a very high dose (1000 mg/kg) of NPAA administered to rats for 14 days (16). The little changes seen in the parameters (urinary volume, electrolytes, serum products) commonly used to assess renal function, some of them transiently associated with RPN, showed that these changes did not clearly identify the lesion, which underlines the need for a better diagnostic marker for RPN.

Although NPAA caused some rather variable changes (less than 50%) in creatinine excretion, these changes were much less than that of urinary uronic acid (200–fold). Therefore, creatinine could be a good base unit for expressing the data. MFA also caused some changes in creatinine excretion. These changes were very much less in range than the increase in the urinary uronic acid, which were at least 200–fold. Once again, conventional parameters of renal dysfunction (urinary electrolytes and serum products) failed to give any indication of RPN induced by MFA.

No changes in the serum parameters were observed apart from an increase of urea nitrogen at 500 mg/kg NPAA in agreement with earlier published data (16), indicating a fall in the glomerular filtration rate.

The lack of any significant elevation in serum parameters following treatment with either NPAA or MFA points to the fact that this invasive method of investigation may not be useful in detecting the onset of RPN. This justifies the search for noninvasive and sensitive means of detecting RPN, and the results of this study suggest the usefulness of urinary uronic acid, and, to some limited extent, that of urinary creatinine.

In conclusion, this study has established that chemically-induced papillary necrosis causes a comparative increase in urinary uronic acid excretion. Their enhanced excretion is the product of a progressive complex degeneration process whose mechanisms are not well understood. Although we might conclude that the measurement of uronic acid could in future be used as a definitive diagnostic criterion for the early diagnosis of the presence or severity of RPN, it remains to be established whether the changes found in animals parallel those in humans. Thus, the clinical validation of monitoring changes in urinary uronic acid in the diagnosis of RPN in humans should be intensified.

ACKNOWLEDGMENTS

The authors would like to acknowledge Barry Tylee for providing the densitometer; Dr. Greg Stevenson for valuable discussions; Berhnard Aicher, Manfred
Baumeister, Karl Thomae GmbH, Biberach an der Riss, Germany, for supplying all the urine and tissue samples.

REFERENCES
