Renal metabolism of paracetamol: studies in the isolated perfused rat kidney

SANDRA HART, I. CALDER, B. ROSS* AND J. TANGE
Departments of Chemistry and Pathology, University of Melbourne, Parkville, Melbourne, Victoria, Australia

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Summary

1. The metabolism of $[3H]$paracetamol has been studied in the perfused rat kidney. Seventy-four per cent of filtered paracetamol was reabsorbed. Paracetamol appeared in the urine; 90% was unaltered but 10% appeared as metabolites: the glucuronide, sulphate, mercapturic acid and cysteine conjugates.

2. At concentrations of paracetamol of 1–3 mmol/l no impairment of renal physiological function was observed.

3. The presence of the mercapturic acid and cysteine conjugates in the urine demonstrates the capacity of the kidney for oxidative metabolism of paracetamol and hence the formation of potentially toxic intermediates.

Key words: analgesic nephropathy, high-pressure liquid chromatography, kidney, paracetamol, renal drug handling.

Introduction

Paracetamol is a remarkably safe drug when taken in therapeutic doses, but overdose leads to hepatic necrosis in both man and experimental animals. The hepatotoxicity of paracetamol has been attributed to N-hydroxyparacetamol, which is formed by N-hydroxylation of paracetamol. Decomposition of N-hydroxyparacetamol leads to the formation of N-acetyl-$p$-benzoquinone imine. At therapeutic doses, the N-acetyl-$p$-benzoquinone imine reacts with glutathione and the conjugate is ultimately excreted as the mercapturic acid. However, when glutathione is depleted, as it is at higher doses of paracetamol, the quinone imine reacts with cell macromolecules leading to cell death (Mitchell, Potter, Hinson, Snodgrass, Timbrell & Gillette, 1975).

By contrast, the prolonged abuse of the closely related drug, phenacetin, is associated with renal rather than hepatic damage, with papillary necrosis, renal cortical scarring and, eventually, carcinoma of the renal pelvis. Extensive experimental evidence is available that paracetamol is not nephrotoxic (Calder, Funder, Green, Ham & Tange, 1971). Peters, Baechtold-Fowler, Bonjour, Chomety-Diezi, Filloux, Guidoux, Guignard, Peters-Haefeli, Roch-Ramel, Schelling, Hedinger & Weber (1972) showed that neither paracetamol nor phenacetin was acutely nephrototoxic in Wistar rats, although necrosis of proximal renal tubules was observed in Fischer rats (McMurtry, Snodgrass & Mitchell, 1978). The possibility that paracetamol may be nephrotoxic has been discussed by several authors (Boyer & Rouff, 1971; Duggin, 1977; Nanra, 1979), although it is suggested that paracetamol-associated renal failure may be secondary to the accompanying liver damage after overdose (Wilkinson, Moodie, Arroyo & Williams, 1977).

Paracetamol added in concentrations of up to 10 mmol/l did not depress gluconeogenesis in renal cortical tubules (Tange, Ross & Ledingham, 1977). On the other hand, there is evidence of an increased incidence of kidney disease after paracetamol overdose (Boyer & Rouff, 1971; Master, 1973) and
circumstantial evidence implicates paracetamol in the aetiology of analgesic nephropathy (Kincaid-Smith, 1978).

It has been suggested that de-ethylation of phenacetin to paracetamol, followed by N-hydroxylation to N-hydroxyparacetamol, is responsible for the toxicity of phenacetin (Duggin, 1978). However, N-hydroxyparacetamol is not sufficiently stable to be transported in the blood stream from liver to kidney (Healey, Calder, Yong, Crowe, Funder, Ham & Tange, 1978). Thus, for nephrotoxicity to result, it is likely that N-hydroxyparacetamol is formed directly within the kidney.

Attention has recently been focused on the kidney as a metabolic organ, with the capacity to convert drugs into reactive intermediates (Mitchell, McMurtry, Statham & Nelson, 1977). The metabolism of paracetamol by the microsomal fraction of liver (Thorgeirsson, Felton & Nebert, 1975; Buckpitt, Rollins, Nelson, Franklin & Mitchell, 1977) and of kidney (Duggin, 1978) is similar. In the absence of added glutathione, there is substantial binding of paracetamol to protein, but when glutathione is added, glutathione conjugate is formed possibly via N-hydroxyparacetamol.

Studies in fragmented systems, such as kidney microsomal fraction, do not exclude the possibility of permeability barriers to the drug in the intact kidney, and the systems require supplementation with glutathione for full activity. To determine the ability of the intact kidney to form N-hydroxyparacetamol, we have investigated the metabolism of paracetamol in an isolated rat kidney perfusion, using a sensitive assay system for the detection and measurement of paracetamol and its metabolites.

**Materials and methods**

*Isolated rat kidney perfusion*

Well-fed male albino Sprague-Dawley rats (280–350 g) were anaesthetized with Nembutal (intraperitoneal injection, 5 mg/100 g body weight), the kidneys removed and perfused via the renal artery as described by Ross, Epstein & Leaf (1973). The perfusion medium consisted of fraction V bovine serum albumin (67 g/l) suspended in Krebs–Henseleit medium containing bicarbonate (25 mmol/l) and dialysed for 48 h against the same buffer and gassed with O₂/CO₂ (95:5). Perfusion pressure was 140/120 mmHg and 5 mmol of D-glucose/l was added as metabolic substrate. Glomerular filtration rate (GFR) was determined with §¹⁷Cr-labelled EDTA (Lucas Heights, N.S.W.). This was shown to give the same values as inulin clearance for GFR. Fractional sodium reabsorption was determined from measurements of sodium by flame photometry. The function of this perfused rat kidney model has previously been shown, from measurement of adenine nucleotide content, rates of biosynthesis, oxygen consumption, glomerular filtration and fractional sodium reabsorption, to be stable for up to 90 min. This period was chosen for the present studies. Further, the renal content of glutathione, an important cofactor in the metabolism of paracetamol in liver, was 1200 nmol/g dry weight, about 70% of that in the kidney in vivo (Ross, 1978).

Ring-labelled [¹⁷H]paracetamol was added to the medium after a control period of 30 min of perfusion and the perfusion was continued for a further 60 min. Samples of perfusion medium and urine collections at approximately 15 min intervals were taken and stored frozen (−20°C) until analysed for paracetamol and paracetamol metabolites, and total tritium label was determined.

*Experiments in vivo*

For comparison with perfusion experiments a group of animals (n = 4) was treated by intravenous injection of paracetamol (2 mmol/kg body weight). Urine was collected in metabolic cages for 24 h and paracetamol metabolites were determined.

*High-pressure liquid chromatography for paracetamol metabolites*

Analyses were carried out on a Waters µ-Bondapak-C₁₈ column (3.9 mm internal diameter x 300 m) with two Chromatronix 3500 pumps and gradient programmer, and a Waters U6K injector, and a Variscan u.v. detector set at 254 nm with an 8 ml (× 1 cm) cell. The solvent used was 15% methanol in a potassium phosphate buffer (0.05 mmol/l), pH 2-8, at 2 ml/min. For scintillation counting the eluent was collected in 0.4 ml fractions and counted on a Beckmann LS-133 liquid scintillation counter after the addition of 5 ml of scintillation fluid (0.4% PPO in Brydet X 10/ toluene, 1:2). The identification of metabolites was made on the basis of comparison with synthetic samples in the solvent system above.

The concentration of paracetamol was deter-
mined from a standard injection of paracetamol and the absolute concentrations of metabolites were obtained from the radioactivity of individual peaks obtained by high-pressure liquid chromatography. Subsequent calculations were based on the areas of peaks obtained by high-pressure liquid chromatography and compared with earlier standards. In general, values have been expressed as a percentage of the total paracetamol and its metabolites recovered.

Chemicals

[3H]Paracetamol, chromatographically pure by high-pressure liquid chromatography, was prepared from ring-labelled p-aminophenol (The Radiochemical Centre, Amersham, U.K.) by acetylation and kindly provided for this study by A. C. Yong, Department of Chemistry, University of Melbourne.

Results

Renal handling of paracetamol

Over the range of concentrations used in this study, paracetamol was both filtered at the glomerulus and largely reabsorbed from the filtrate (Table 1). Assuming free filtration of paracetamol, and neglecting the small quantities of paracetamol metabolites which also appeared in the urine (see below), the fractional reabsorption was approximately 74% of the filtered load at all concentrations of paracetamol tested. This value is very similar to that observed for paracetamol reabsorption by the dog kidney in vitro (Duggin & Mudge, 1975). In this range of concentration there was no evidence of net secretion of paracetamol. That is to say, the paracetamol ratio (U/P) never exceeded that for inulin. Higher concentrations of paracetamol were not tested, so that no information has been obtained of a tubular maximum for its reabsorption.

Evidence of renal metabolism of paracetamol

Reabsorption of paracetamol from the filtrate indicates that paracetamol enters renal cells very readily. Evidence that paracetamol is metabolized by the intact kidney was obtained by examining the urine produced after addition of paracetamol to the perfusate. All samples of urine examined showed a similar range of paracetamol metabolites, although their proportions varied with the duration of perfusion. The peaks obtained from high-pressure liquid chromatography (Fig. 1) by ultraviolet monitoring and tritium radioactivity were closely similar. They indicate the presence in the urine of free paracetamol, paracetamol glucuronide, sulphate, cysteine and mercapturic acid conjugates. In the sample shown in Fig. 1, obtained with urine produced 30 min after addition of paracetamol (2.6 mmol/l) to the perfusate, paracetamol accounted for 92%, glucuronide 1.1%, sulphate 0.9%, cysteine conjugate 0.5% and mercapturic acid conjugate 0.5% of the [3H]paracetamol recovered.

An unidentified peak, the first eluted after the void volume, accounted for 4.8%. The peak eluted between sulphate and mercapturic acid contained no 'H, was present in control specimens of urine from kidneys perfused without added paracetamol, and is therefore unrelated to paracetamol. Paracetamol and the principal metabolites were quantified in a series of perfusions with increasing concentrations of paracetamol (Table 2).

Fig. 2 shows the pattern of paracetamol metabolites in urine observed after the administration of paracetamol at a dose of 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of kidneys</th>
<th>Paracetamol concn. (mmol/l)</th>
<th>GFR (ml/min)</th>
<th>Urine volume (ml/min)</th>
<th>Fractional reabsorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Perfusate</td>
<td>Urine</td>
<td></td>
<td>Sodium</td>
</tr>
<tr>
<td>A</td>
<td>2 (8–12)</td>
<td>0.66 ± 0.16</td>
<td>0.82 ± 0.18</td>
<td>0.31 ± 0.20</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>B</td>
<td>4 (19–26)</td>
<td>1.29 ± 0.15</td>
<td>2.13 ± 0.49</td>
<td>0.29 ± 0.19</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>C</td>
<td>2 (10–13)</td>
<td>2.59 ± 0.22</td>
<td>5.79 ± 1.22</td>
<td>0.13 ± 0.06</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>(8)</td>
<td>0.26 ± 0.18</td>
<td>0.038 ± 0.031</td>
<td>89.2 ± 4.7</td>
<td>74.5 ± 4.2</td>
</tr>
</tbody>
</table>

Table 1. Paracetamol handling by the isolated perfused rat kidney

[3H]Paracetamol was added at the doses indicated above after 30 min. Perfusate and urine collections were made over the following 60 min, at 15 min intervals. GFR was calculated from clearance of 51Cr-labelled EDTA. Paracetamol reabsorption (filtered —excreted) was calculated from 3H counts in urine and perfusion medium. The number of observations in all experiments is given in parenthesis.
Paracetamol and its metabolites in urine of the isolated perfused rat kidney. [3H]Paracetamol was added after 30 min of perfusion (2.6 mmol/l). Urine was collected between 65 and 80 min of perfusion. (a) Ultraviolet detection (254 nm) on high-pressure liquid chromatogram of urine (100 μl). (b) Curve constructed from radioactive counts (3H) in samples collected from the column eluent. Metabolites: C, paracetamol-cysteine conjugate; G, paracetamol glucuronide; HA, hippuric acid; M, paracetamol mercapturic acid; P, paracetamol; S, paracetamol sulphate.

mmol/kg. The same metabolites were observed in the perfused kidney (Fig. 1) as in vivo. But glucuronide and sulphate conjugates in vivo accounted for 24% and 53% respectively of total metabolites in urine, whereas free paracetamol excretion was less than 20% and mercapturic acid conjugate accounted for 4% of the total.

The consistent presence of mercapturic acid and cysteine conjugate as metabolites in urine from the perfused kidney was a significant finding. They were found at all concentrations of added paracetamol and together accounted for 0.5–1.0% of all paracetamol excreted.

No metabolites of paracetamol could be detected in the perfusion medium samples at any stage during the 90 min perfusions. The rate of excretion and metabolism of paracetamol was too low to influence the concentrations of paracetamol in the perfusion medium, which was the same at the beginning and end.

Time course and concentration dependence of formation of cysteine and mercapturic acid conjugates

The concentration in the urine of the important paracetamol metabolites, cysteine conjugate and mercapturic acid increased progressively with time, reaching a plateau about 30 min after addition of paracetamol to the perfusate (Fig. 3). The rate of formation of the two conjugates increased with increasing concentrations of paracetamol and was approximately linear over the range tested (Fig. 4).

Effect of paracetamol on renal tubular function

In an attempt to determine whether paracetamol in low-toxic doses has an acute effect on renal tubular function, GFR and sodium reabsorption were measured (Table 1). The mean GFR in these experiments was somewhat lower than that observed in earlier series from this laboratory, but paracetamol was without apparent effect on either GFR or sodium reabsorption. In the first 15 min after addition of the higher doses of paracetamol, urine flow was somewhat increased, but the effect was transient, and not accompanied by any alteration in fractional sodium reabsorption.

Discussion

Paracetamol is filtered at the glomerulus and about 75% of that filtered is reabsorbed in the isolated perfused rat kidney. This rate of paracetamol reabsorption is identical with that observed for the dog in vivo (Duggin & Mudge, 1975). The present experiments show that the kidney is capable of metabolizing paracetamol by both oxidation and conjugation. Because the metabolites were detected only in the urine the possibility cannot be ruled out that some paracetamol metabolism occurs in the tubular lumen by way of enzymes located in the luminal brush border. But reabsorption of paracetamol from the glomerular filtrate means that this metabolism can be an intracellular event. The metabolites of paracetamol found in the urine
Renal paracetamol metabolism

TABLE 2. Measurement of metabolites of paracetamol in urine from the isolated perfused rat kidney

Urine collections were made during four successive 15 min intervals after addition of given amounts of paracetamol to the perfusion medium. Total mercapturic acid and cysteine conjugates (MA & C), as well as free paracetamol, were determined in each sample, and the results are the totals excreted during 60 min of perfusion.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Paracetamol added (mg)</th>
<th>Paracetamol added (mmol/l)</th>
<th>Total excreted (nmol/60 min) MA &amp; C</th>
<th>Free paracetamol MA &amp; C</th>
<th>Fraction of total excreted Free paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.0</td>
<td>0.77</td>
<td>8.0</td>
<td>5717</td>
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<tr>
<td>2</td>
<td>6.5</td>
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<td>6.0</td>
<td>1007</td>
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<td>3</td>
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<td>1.32</td>
<td>15.1</td>
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</tr>
<tr>
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<td>15.0</td>
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<td>0.53</td>
</tr>
<tr>
<td>5</td>
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<td>28.7</td>
<td>5149</td>
<td>0.56</td>
</tr>
<tr>
<td>6</td>
<td>30.0</td>
<td>2.74</td>
<td>27.2</td>
<td>5681</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Fig. 2. Metabolites of paracetamol in urine in vivo. Paracetamol was administered intravenously to male rats (2 mmol/kg body weight) and urine was collected from 0 to 24 h and analysed by high-pressure liquid chromatography by using ultraviolet detection (254 nm). For abbreviations see the legend for Fig. 1.

in the isolated kidney are similar to those seen in the whole rat. But whereas the glucuronide and sulphate predominate in vivo, in the isolated kidney the quantities of paracetamol glucuronide and sulphate were small and the production of the mercapturic acid and cysteine conjugates became significant. Various amounts of these two metabolites appeared in the urine at different times of perfusion, and this has been subsequently attributed to the enzymic conversion of the mercapturic acid formed into cysteine conjugate in the urine (unpublished work in this laboratory). For this reason the two metabolites have been considered together.

The total amount of mercapturic acid-plus-cysteine conjugate formed increased with increas-
out with wider ranges of paracetamol concentration to investigate these points. The formation of potentially toxic paracetamol metabolites, together with those obtained with kidney microsomal fraction (Duggin, 1978), demonstrate that observed for kidney cells (Jones, Sundby, Peters & Orrenius, 1979).

These results with the isolated perfused kidney, together with the intact kidney, is able to perform oxidative metabolism of drugs. Thus the nephrotoxicity of the intact kidney is comparable with that observed for kidney cells (Jones, Sundby, Ormstad & Orrenius, 1979).

It has been shown that the kidney contains adequate concentrations of cytochrome P<sub>450</sub> to effect the oxidation observed (Mitchell et al., 1977). The oxidative potential for oxidative metabolism in the isolated perfused kidney is comparable with that observed for kidney cells (Jones, Sundby, Ormstad & Orrenius, 1979).

In these experiments at the concentrations of paracetamol used there was no impairment of renal function. Nevertheless, the presence of mercapturic acid and cysteine conjugates in the urine indicate the formation of potentially toxic paracetamol metabolites. Further experiments are being carried out with wider ranges of paracetamol concentration to investigate these points.

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References


