SHORT COMMUNICATION

Urinary ligandin and glutathione-S-transferase in gentamicin-induced nephrotoxicity in the rat

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Summary

1. Eight rats developed detectable glutathione-S-transferase activity in their urine after three daily injections of toxic doses of gentamicin.
2. Seven of the eight rats had immuno-detectable ligandin in their urine at this time.
3. The level of enzyme activity correlated well with the degree of elevation of serum creatinine.
4. This confirms ligandinuria and urinary glutathione-S-transferase as markers of acute renal proximal tubular injury.

Key words: enzymeuria, gentamicin, glutathione-S-transferase, ligandin, nephrotoxicity.

Introduction

Ligandin is a major organic anion binding protein and a glutathione (GSH)-S-transferase (EC 2.5.1.18) in the cytosol of renal proximal tubule, parenchymal liver and small intestinal mucosal cells [1, 2]. Ligandinuria occurs after nephrotoxic acute renal failure [3] and serves as a marker for tubular cell necrosis [4]. Because gentamicin, a widely used aminoglycoside antibiotic, produces acute tubular cell injury [5], we have studied urinary ligandin and GSH-S-transferase in rats given toxic doses of gentamicin. The purpose of the study was to determine whether this model of acute renal failure leads to ligandinuria.

Methods and materials

Eight adult female Sprague-Dawley rats weighing 170–200 g were housed in metabolic cages and given free access to standard laboratory chow (Teklad) and tap water. The cages were fitted with special tubing to allow for collection of urine in ice-cold containers.

Each rat was given a daily subcutaneous injection of 100 mg of gentamicin/kg; this dose causes proximal tubular injury in this strain of rat [6]. Urine was collected every 24 h beginning 1 day before the injections were started, and kept at 4°C. On day 4, 24 h after the third dose of gentamicin, a 3 ml blood sample was obtained from each animal for serum creatinine determination. Urinary ligandin was measured on 5 ml portions from each 24 h urine specimen which were concentrated 50-fold in Minicon (R) B-50 concentrating chambers (Amicon, Lexington, MA, U.S.A.). Qualitative determination of ligandinuria was performed by radial immunoprecipitation [7] with monospecific antiligandin antibody [1]. The antibody was placed in the centre well of the diffusion chamber; samples of concentrated urine or a positive control solution containing purified rat ligandin (50 ng/ml) were in peripheral wells. A positive test was a precipitin line of identity extending from a positive control well to a well containing a urine sample [3].

Quantitative determination of GSH-S-transferase activity was performed as described by Goldstein, Feinfeld, Fleischner & Elkin [8]. The rate of catalysed conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced GSH was measured in a Pye Unicam SP.1800 double-beam recording spectrophotometer at 344 nm. Concentrated sample (100 μl) was added to 2 ml of a solution of 1-chloro-2,4-dinitrobenzene (1-33 mmol/l; Sigma Chemical Co., St Louis, MO, U.S.A.) and GSH (2.5 mmol/l Eastman...
Chemical Co., Rochester, NY, U.S.A.) in potassium phosphate buffer (0.1 mol/l, pH 6.5). A blank solution containing all reagents but no sample was placed in the second cuvette of the spectrophotometer and permitted subtraction of non-enzymatic from enzymatic conjugation. Activity is expressed as umol of catalysed GSH–CDNB conjugate formed min⁻¹ 100 µl⁻¹ of sample. Sera which were obtained on day 4 were concentrated 20-fold and tested for GSH-S-transferase activity.

Serum creatinine was measured by standard autoanalyser techniques. Linear regression was analysed by the method of least squares.

**Results**

Serum creatinine 24 h after the third gentamicin dose ranged from 97 to 310 µmol/l (Table 1). Normal rats in our laboratory have had serum creatinine concentrations between 25 and 45 µmol/l. These sera had no detectable GSH-S-transferase activity even after concentration.

Urines collected during the 24 h control period were negative for both ligandin and GSH-S-transferase activity, as reported previously [3]. Urine collections for the first 3 days were also negative for ligandin and GSH-S-transferase; however, of samples obtained during the 24 h after the third gentamicin injection, seven out of eight showed immunologically detectable ligandinuria and GSH-S-transferase activity (Table 1). Urine from the rat with the negative immunoaassay had a lower level of enzyme activity and this rat had the lowest serum creatinine concentration in the group.

Linear regression analysis of urinary GSH-S-transferase activity as a function of serum creatinine after three injections of gentamicin revealed a correlation coefficient of 0.79 (P < 0.02).

**Discussion**

The development of acute tubular injury in rats after the administration of high doses of gentamicin is well described [5, 6]. Using the same dosage and strain of rat, Cohen et al. [6] noted renal insufficiency after 4 days. In the present study the rats demonstrated serum creatinine elevation of varying degrees after only three doses of the drug, associated with the appearance of ligandinuria. We previously identified ligandin in the urine of rats given mercuric chloride and a lower level of GSH-S-transferase activity in rat urine after potassium dichromate [3].

In the kidney ligandin is almost entirely, if not exclusively, restricted to the proximal tubular cells. A study of GSH-S-transferase activity in isolated tubule segments from rabbit kidney localized the enzyme to proximal convoluted and straight tubules [9]. Ligandin plays a role in renal organic anion transport [10]. Because such transport is largely confined to proximal tubules [11], it seems reasonable that these cells should contain the greatest amount of ligandin. Gentamicin primarily injures proximal tubules [5], which results in leakage of ligandin into the urinary space [4].

Ligandin may be released into the blood during tubular necrosis and enter the urine by filtration through functioning glomeruli. In a recent paper Bass, Kirsch, Tuff, Campbell & Saunders [12] used radioimmunoassay for ligandin to examine nephrotoxic renal failure in rats. In addition to confirming our earlier findings [3], ligandinaemia and ligandinuria were demonstrated after administration of mercuric chloride or potassium dichromate.

The direct relationship between serum creatinine concentration and GSH-S-transferase activity in the urine in the present study also suggests that ligandinuria reflects acute tubular cell injury. The immunodiffusion assay does not

**Table 1. Results in rats given gentamicin for 3 days**

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Urine ligandin immunoaassay*</th>
<th>Urine GSH-S-transferase (µmol/min)</th>
<th>Final serum creatinine concn. (µmol/l)</th>
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* + Indicates positive test. — Indicates negative test.
yield quantitative results and is less sensitive. Since ligandin is a major GSH-S-transferase, this assay probably estimates the amount of ligandin released from the injured cell. Prospective testing for ligandin and GSH-S-transferase activity in urine from patients taking gentamicin may provide a sensitive early sign of nephrotoxicity.

Urinary levels of β-galactosidase and β-N-acetyl-D-glucosaminidase have been shown to increase during gentamicin nephrotoxicity in the rat and may also provide a marker for tubular injury in this setting [13]. However, these enzymes are normally found in urine, and their urinary excretion is increased in a wide variety of renal disease, including hypovolaemic azotaemia [14, 15]. Ligandin and GSH-S-transferase are undetectable in normal urine, by immuno-diffusion or enzymatic activity, and have not been found in the urine in any renal disease other than acute tubular necrosis [3, 4].

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References